Plant Protection using Arbuscular Mycorrhizal Fungi

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Signed Declaration

I hereby declare that this thesis has been composed by myself and the work described is entirely my own unless stated.

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Contents

Abstract

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The interaction between several species of arbuscular mycorrhizal fungi, micropropagated strawberry plants and *Phytophthora fragariae*, the pathogen which causes red stele disease of strawberry plants, was investigated. The optimum temperature for germination of zoospore cysts of *P. fragariae in vitro* was found to be 15°C, and growth of the emerging germ tube was significantly orientated towards the strawberry root tip. Cyst germination was reduced in the presence of a mycorrhizal strawberry root. The method of inoculation of strawberry plants with *P. fragariae* and assessment of the resulting disease affected the results and the conclusions drawn from the experiments, depending on the virulence of the *P. fragariae* strain used and the susceptibility of the strawberry cultivar. Elsanta was more susceptible to *P. fragariae* than the cultivar Rhapsody. A low level of colonisation of Elsanta with the arbuscular mycorrhizal fungi *Glomus mosseae*, *Glomus intraradices* or *Glomus fistulosum* resulted in a significantly greater amount of total phosphorus in plant shoots compared to non-mycorrhizal plants, although further increases in the percentage of root colonisation by the fungi had no effect on the plants. The presence of these mycorrhizal fungi had no effect on disease due to subsequent inoculation of the plants by *P. fragariae*. Increasing colonisation of Elsanta by *Scutellospora nodosa* was correlated with a significant increase in plant size and additional phosphorus uptake. However, these same plants exhibited greater levels of disease due to the following inoculation with *P. fragariae*. A low level of root colonisation of Elsanta by *Acaulospora scrobiculata* caused significant increases in plant size and phosphorus uptake up to a threshold level of root colonisation beyond which further increases had no affect on the plant. Examination of biochemical markers in the form of isozyme banding patterns extracted from spores of arbuscular mycorrhizal fungi was explored to assess its potential for use in strain identification, and discussed in relation to other available techniques. The results are discussed in relation to the utilisation of specific strains of arbuscular mycorrhizal fungi as inoculants of micropropagated strawberry plants of particular cultivars with the potential to increase plant growth and reduce the level of disease due to soil-borne plant pathogens.

CHAPTER 1: Introduction

1.0 Introduction

Arbuscular mycorrhizal fungi are ubiquitous soil-borne fungi which colonise the roots of most plant species. This root colonisation is in the form of a symbiosis whereby the plant provides the fungus with carbon and the fungus can provide the plant with additional nutrients. The fungus colonises the roots, forming several distinct morphological structures, including intracellular arbuscles which act as the major point of exchange of materials between the plant and fungus.

A considerable amount of published research has provided evidence of the varying and contradictory effects of an arbuscular mycorrhizal colonisation of host plants. These fungi have been reported to affect several different components of plant growth, nutrition and susceptibility to plant pathogenic fungi. These effects have been described as being beneficial to the host plant (Abbot & Robson, 1984), with the benefits depending on the particular plant–fungus interaction (Niemi & Vestberg, 1992), nutrient content of the environment (Ravnskov & Jakobsen, 1995), and growth season (Lapointe & Mollard, 1997).

Much research has concentrated on the additional uptake of phosphorus into mycorrhizal plants, which generally results in an enhancement of growth compared to non-mycorrhizal plants. This effect has frequently been observed when plants are grown in a low-phosphorus environment (Abbot & Robson, 1982; Smith & Gianinazzi-Pearson, 1988), but is also apparent in high-phosphorus soils, particularly when the plant has a high demand for phosphorus, e.g. strawberry plants (Werner *et al.*, 1990; Williams *et al.*, 1992). Therefore the growth conditions can influence the effects of a mycorrhizal colonisation on a plant and must be taken into account when mycorrhizal experiments are performed. Much of the mycorrhizal research performed in glasshouse conditions may produce very different results from a field environment where the mycorrhizal fungi will be subject to varying nutritional and environmental conditions and competition and interaction between different mycorrhizal species and other microorganisms (Ravnskov & Jakobsen, 1995). Therefore one aim of this research was to investigate the effect of colonisation by different mycorrhizal species on two strawberry cultivars.

Problems in research into arbuscular mycorrhizal fungi are compounded by the fact that the fungi are obligate symbionts and therefore maintenance of pure fungal cultures is difficult. Micropropagation of plant material offers a method of producing sterile plants which can be grown in precise nutritional conditions and exposed to colonisation by a mycorrhizal fungus, having had no prior contact with any other microorganisms (Niemi & Vestberg, 1992). Therefore, micropropagated strawberry plants were used in this research as micropropagation provides an ideal environment to study the effects of mycorrhizal colonisation on host plants.

Mycorrhizal associations have also been considered in the presence of certain plant pathogenic fungi. Research has shown that mycorrhizal plants are more resistant to invasion by a fungal pathogen (Davis & Menge, 1980; Graham & Menge, 1982; Guillemin *et al.*, 1994). In contrast, some reports suggest that mycorrhizal plants are more susceptible to some pathogens (Graham, 1988).

Phytophthora fragariae, a soil-borne fungus which causes red stele disease in strawberry plants, is a multi-race pathogen and a major cause of crop losses and disease. *P. fragariae* produces motile zoospores which, in suitable environmental conditions, can encyst and germinate to produce germ tubes which can penetrate plant roots and infect the plant. Infection results in the production of a red stele in plant roots, associated with root infection, plant wilting and dwarfing with resultant crop losses (Maas, 1984). It has been suggested that mycorrhizal roots exhibit decreased root exudation which can reduce the infection of the root by some zoospore-producing pathogens (Graham & Menge, 1982; Meyer & Linderman, 1986).

Sexual reproduction of the fungus results in the formation of spherical oospores within the root. These oospores can overwinter and infect strawberry plants in the following spring. Disease due to *P. fragariae* is manifested as rotting, infected roots and the presence of fungal oospores in infected roots. The disease symptoms can differ with the resistance of the strawberry cultivar (Van der Weg, 1989a), and the virulence of the pathogen strain (Law & Millholland), both of which affect the numbers of rotting oospore-containing roots that may be observed. Therefore the methods of assessing disease resulting from infection of strawberry plants with *P. fragariae* were investigated by observation of both infected roots and oospores.

In vitro and *in vivo* experiments were performed to investigate some aspects of the biology of *P. fragariae* and to determine whether different methods of plant inoculation and disease assessment could influence the observations and conclusions drawn from the inoculation of two strawberry cultivars, Elsanta and Rhapsody, with one of three strains of *P. fragariae* (strain 171, 293 or 372).

The amount of disease resulting from subsequent inoculation of micropropagated mycorrhizal strawberry plants with zoospores of *P. fragariae* was also determined to investigate whether the presence of a mycorrhizal fungus had any effect on infection by the pathogen.

Isozyme analysis of spores of arbuscular mycorrhizal fungi was also attempted to determine whether this technique could be used to distinguish between spores of different species and aid in their identification.

The aim of the work described in this thesis was to investigate the interaction between a host plant (strawberry), arbuscular mycorrhizal fungi and the root-infecting pathogen *P. fragariae* with the purpose of determining the value of the mycorrhizal association in disease reduction. In order to achieve this, the study had the following objectives:

a) Development of reproducible experimental conditions for production and maintenance of plants and fungal inoculum.

b) Investigation of the pathogen *P. fragariae* to evaluate methods of inoculating host plants with the pathogen, and assessing the resulting disease.

c) Determination of the effect of root colonisation by each of several arbuscular mycorrhizal fungi on the growth and phosphorus status of the host plant.

d) Evaluation of the effect of an arbuscular mycorrhizal colonisation on the disease resulting from subsequent inoculation of host plants by *P. fragariae*.

e) Investigate the effectiveness of isozyme banding patterns obtained from the electrophoresis of mycorrhizal spore extracts in distinguishing and identifying different fungal species.

CHAPTER 2: Literature Review

2.1 Types of mycorrhizal associations

Arbuscular mycorrhizal associations exist as endomycorrhizas where the fungus develops in both the soil and the plant root. Mycorrhiza literally means 'fungus-root'. The term was first used by Frank (1885) to describe the long-lived association between plant roots and fungal mycelia. All mycorrhizal associations involve a mutual symbiosis (Isaac, 1992). Several different types of mycorrhizal associations are recognised. They are distinguished by their structural characteristics at maturity, as their morphology may vary during establishment and senescence. Representatives from all major taxonomic groups of fungi are capable of forming mycorrhizal associations (Isaac, 1992).

2.1.1 Ectomycorrhizas

This type of association is common for trees and shrubs, mainly, but not confined to, the families Fagaceae, Pinaceae, Betulaceae and Myrtaceae. The fungi involved are mostly basidiomycetes, some ascomycetes and two zygomycetes from the genus Endogone. Mycorrhizal infection alters root morphology such that short lateral roots become thickened, pigmented, lack root hairs and exhibit characteristic branching. Colonised roots are usually ensheathed by a layer of fungal tissue while intercellular hyphae penetrate between the outer cortex cells to form a characteristic Hartig net around the root (Isaac, 1992). The fungus does not penetrate intracellularly.

2.1.2 Endomycorrhizas

Members of this rather artificial group show a high degree of structural diversity. In contrast to ectomycorrhizas, the hyphae generally penetrate root cortical cells. This group consists of ericaceous, orchidaceous and arbuscular mycorrhizal fungi.

Three types of ericaceous mycorrhizal associations, ericoid, arbutoid and monotropoid, may form between certain fungi and plants of the order Ericales. All three associations involve nutrient transfer to the host which is advantageous because Ericaceous plants frequently grow in nutrient-deficient environments. Ericoid mycorrhizal fungi are mostly Ascomycete fungi of the genus *Pezizella* which exist as intracellular hyphal coils in the root cortical cells, with a weft of hyphae growing over

the root surface. Arbutoid mycorrhizas consist of Basidiomycete fungi associated with plants of the genera Arbutoideae and Pyrolaceae. These fungi form intracellular hyphal coils in root cortical cells, together with a Hartig net around the root surface. Finally, monotropoid mycorrhizas are a specialised group which are associated with plants of the family Monotropaceae (Isaac, 1992).

All orchids form mycorrhizal associations in their early stages of development. Orchidaceous mycorrhizal fungi frequently involve the genus *Rhizoctonia*, a basidiomycete fungus. Such associations involve a fine balance between symbiosis by both partners and parasitism by the fungus (Isaac, 1992).

2.1.3 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi, formerly known as vesicular arbuscular mycorrhizal fungi, are the most common and widely occurring mycorrhizal associations. They are also amongst the more common and widely distributed of the soil-borne fungi (Schenk, 1982). They are geographically and ecologically ubiquitous, being distributed from Tropic to Arctic environments, including areas of grassland, woodland, arid land and swamps (Walker, 1987).

These fungi exist in association with the roots of host plants from most plant families, although some such as Cruciferae and Chenopodiaceae are described as characteristically non-mycorrhizal because they do not usually form mycorrhizas of any obviously recognisable form (Paul & Clark, 1989). However, some members of these families have exhibited mycorrhizal associations (Tester *et al.*, 1987). Similarly, isolated species or genera which have absent, low or atypical mycorrhizal colonisations also occur in families which are typically mycorrhizal. An example of this is *Lupinus* which occurs in the otherwise mycorrhizal Leguminosae (Morley & Mosse, 1976).

Arbuscular mycorrhizal associations are mutualistic symbioses where the fungi are biotrophic parasites with no saprophytic ability (Hayman, 1984). The association involves a nutrient exchange where the fungus provides the host plant with certain mineral and nutrient ions in return for the plant supplying carbon to the fungus.

Examination of plant fossils from the Devonian period, 360–410 million years ago, has shown the presence of *Glomus*-like structures within plant roots (Pirozynski & Dalpé, 1989). Estimation of the substitution rates per year of the nuclear genes encoding for small subunit ribosomal RNA was used to suggest that arbuscular mycorrhizal fungi originated between 353 and 462 million years ago (Simon *et al.*, 1993). It has been suggested that they were involved in the colonisation of land by ancient plants (Simon *et al.*, 1993). Although the limited number of species used to estimate this value casts some doubt on the integrity of this time estimate, examination of fossil records has shown that arbuscular mycorrhizal symbioses identical to those found in the present time existed 400 million years ago (Remy *et al.*, 1994).

2.2 Morphology of arbuscular mycorrhizal root colonisation

The name 'vesicular arbuscular' is derived from the morphology of the fungal colonisation of the host root; structures called vesicles and arbuscles are formed between and within root cortical cells (Powell & Bagyaraj, 1984). Infective propagules of the fungi exist in soil as resting spores, fungal hyphae and colonised root fragments. Mycorrhizal spores are globose structures borne terminally or on lateral hyphal branches. A spore is thick-walled with a dense cytoplasm which contains many lipid globules (Bonfante-Fasolo, 1984). Some of these fungal propagules can survive for several years in soil.

2.2.1 Colonisation of plant roots

Spore diameter is in the range 15–800 μm, depending on the species (Sieverding, 1991). Germination is influenced by physical soil factors such as oxygen levels, temperature and water content, and chemical factors such as soil pH, nutrient content and fungistatic soil effects (Sieverding, 1991). A germ tube may be produced which can grow and contact a host plant root.

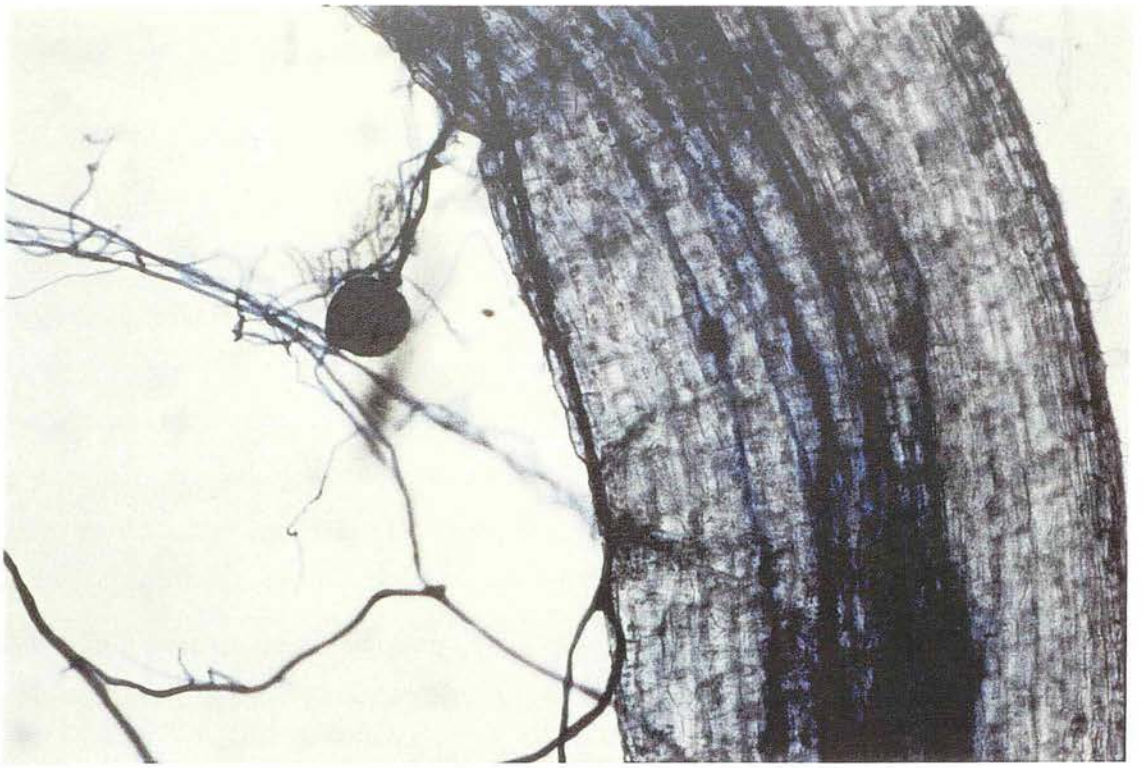

Fig. 2.1. Elsanta root colonised by *Glomus intraradices*. The fungal structures are stained with trypan blue, showing the arbuscular mycorrhizal hyphae around the root and a large spore attached to a subtending hypha.

However, if it does not encounter a root of a possible host, any infection potential will be lost within days or weeks. Mycorrhizal spores are multinucleate (Cooke *et al.*, 1987). Burggraaf and Beringer (1989) reported that nuclei in spores of *Gigaspora margarita* do not divide or undergo DNA synthesis during production of a germ tube. It has been suggested that this is associated with the inability of the fungi to grow continuously in the absence of a host (Gianinazzi, 1991). In contrast, Banciotto and Bonfante (1993) indicated that nuclear DNA is replicated during hyphal elongation of *Gigaspora margarita* in the absence of a host plant, suggesting that more complex mechanisms may influence growth *in vitro*.

The presence of a host plant root can stimulate both spore germination and hyphal growth (Gianinazzi-Pearson *et al.*, 1990). It has been shown that appressoria formation may occur as quickly as 36 h after a germ tube or hypha contacts a host root (Giovannetti & Citernesi, 1993) Penetration of the root will then occur either directly through the wall of a root hair or epidermal cell, or between epidermal cells. The mechanism is not fully known but may involve mechanical and/or enzymatic actions (Bonfante-Fasolo, 1984). It has been shown that pectolytic enzymes may be involved in penetration into root cells (Garcia-Romera *et al.*, 1990).

2.2.2 Intracellular and intercellular hyphae

Fungal hyphae are generally aseptate, 5–20 μm in diameter, and dichotomously branched. Secondary, septate hyphae may develop as lateral branches 1–5 mm in diameter (Bonfante-Fasolo, 1984).

Hyphae penetrate the root from the appressorium. Hyphae may be inter- or intracellular, depending on the plant–fungus combination. Intracellular hyphae are found in the outer root cortical layers, frequently coiled within root cells. As the hyphae spread from cell to cell, the host plasmalemma remains in continuous contact with the hyphae and deposits wall-like material containing proteins and polysaccharides against the wall of the fungus. This isolates the invading hyphae from the host protoplast (Bonfante-Fasolo, 1984), and has been implicated in the lack of a host response to penetration by the fungus.

Intercellular hyphae can subsequently penetrate the root cortex. Fungal growth is restricted to the cortex; the meristematic tissues, endodermis and vascular tissues are not colonised. Hyphal growth along the root extends the fungal colonisation; both the root growth and the rate at which new fungal colonisation units are formed and grow within the root cortex affect the progress of root colonisation (Sieverding, 1991).

2.2.3 Arbuscles

Intercellular hyphae can penetrate parenchyma cells in the inner layers of the root cortex. The hyphae then produce finely divided dichotomous branches which cause the host cell plasmalemma to invaginate. A fungal arbuscle is thus formed within the host cell, forming a large surface area of contact between the host and the fungus. The arbuscle is the preferential site for fungus–host metabolite exchanges (Isaac, 1992).

The arbuscle wall is morphologically distinctive from that of the main hyphae in that it is thin, amorphous and lacking in chitin fibrils. In contrast with the host behaviour towards the hyphae, the host cell plasmalemma deposits very little wall material against the fine arbuscular branches so that the gap between host and fungus is much reduced (Gianinazzi, 1991), thus decreasing the distance across which metabolites are transported. The host plasmalemma is also characterised by the presence of the

enzymes neutral phosphatase and ATPase which are not normally found on the membrane of undifferentiated cortical cells (Gianinazzi, 1991). Plasmalemma-bound ATPases are involved in active transmembrane ion transport in higher plants and their presence around the arbuscular branches implies their involvement in the active transfer of nutrients from the fungus to the host (Bonfante-Fasolo, 1984).

Arbuscles begin to senesce and die after 4–15 days. The host cell then regains its pre-colonised appearance and functioning (Jacquelinet-Jeanmougin *et al.*, 1988). Formation and degeneration of arbuscles are continuous processes which occur simultaneously in a root without affecting the development of the colonising hyphae (Bonfante-Fasolo, 1984). This dynamic process of colonisation along the growing root enables a permanent biotrophic condition to be maintained between the two symbionts (Gianinazzi, 1991).

2.2.4 Vesicles

Vesicles are globose bodies formed by apical or intercalary swellings of hyphae within a root. Vesicles may occur inter- and intracellularly and can be found in both inner or outer layers of the cortical parenchyma (Bonfante-Fasolo, 1984). The presence and number of vesicles formed depends on the fungal species. Some species of genera *Gigaspora* and *Scutellospora*, for example, have never been observed to form vesicles in host roots (Sieverding, 1991). Daft & Nicolson (1974) recognised that some of these mycorrhizal associations resulted in the formation of arbuscles but not vesicles. Therefore the use of the term arbuscular mycorrhiza rather than vesicular arbuscular mycorrhiza has been encouraged (Walker, 1995). Mature vesicles contain large lipid droplets. A low supply of metabolites from the host plant causes the fungus to utilise its reserves and the vesicles may degenerate (Sieverding, 1991). This suggests that vesicles function as resting organs and lipid reserves.

Fig. 2.2. Elsanta root colonised by *Glomus intraradices*. The fungal structures are stained with trypan blue. The intercellular hyphae (H) are clearly visible, as is the intracellular arbuscle (A). The hypha which enters the root cell before dividing to form the arbuscle is indicated (S). The phloem of the root is also visible.

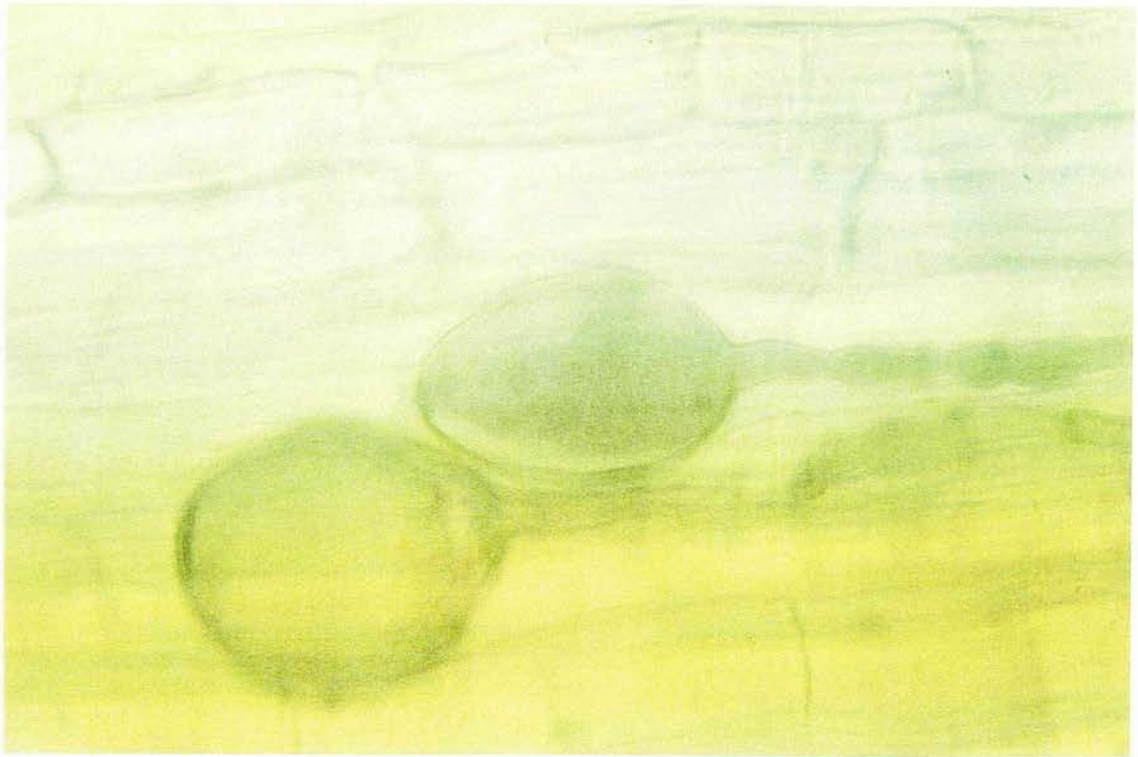

Fig. 2.3. Vesicles of the arbuscular mycorrhizal fungus *Glomus intraradices* within a colonised Elsanta root.

2.3 Identification of arbuscular mycorrhizal fungi

Taxonomic differences within the arbuscular mycorrhizal fungi have been determined primarily on the structure of their spores and sporocarps. The spores show a very high degree of morphological diversity, enabling them to provide sufficient information to identify individual species and define taxonomic groups (Giovanetti & Gianinazzi-Pearson, 1994). The spore wall structures are stable and differentiate concurrently with the growth of the spore (Fig. 2.4). It has been suggested that information from the 'whole fungus' would be more accurate for use in taxonomy than spore morphology alone. However, the difficulties involved in producing axenic cultures of these fungi makes this approach impractical (Walker, 1992).

Previous classification of the order Endogonales to which these organisms belong had a single family, the Endogonaceae, which contained eight genera; seven genera were thought to be arbuscular mycorrhizal fungi with, in addition, the genus *Endogone* which was observed in ectomycorrhizal associations. This classification was revised and all soil-borne fungi known or presumed to form arbuscules in obligate mutualistic associations with terrestrial plants were placed in the new order Glomales (Morton & Benny, 1990). This revision created a more accurate arrangement according to patterns of common evolutionary descent. Two genera, *Endogone* and *Sclerogone*, were retained in the order Endogonales. The remaining genera were transferred to three new families in the order Glomales, shown in Fig. 2.5, p. 38 (Walker, 1992).

A preliminary phylogenetic analysis of sequenced ribosomal DNA genes of selected arbuscular mycorrhizal species appears to support the classification of arbuscular mycorrhizal fungi into three families; Glomoceae, Acaulosporaceae and Gigasporaceae (Simon *et al.*, 1993). However, this analysis should be interpreted with caution as many more species representative of the range of known morphological variability must be examined prior to confirming the validity of using this technique for classification.

The order Glomales is defined by the ability of its members to form arbuscular mycorrhizae. However, the symbiotic status of some species is frequently assumed rather than confirmed, and the difficult task of confirming the nutritional status of the

fungi would have to be performed before accurate classification could be made. Therefore, trophic level may not be a satisfactory characteristic to define an order (Walker, 1992).

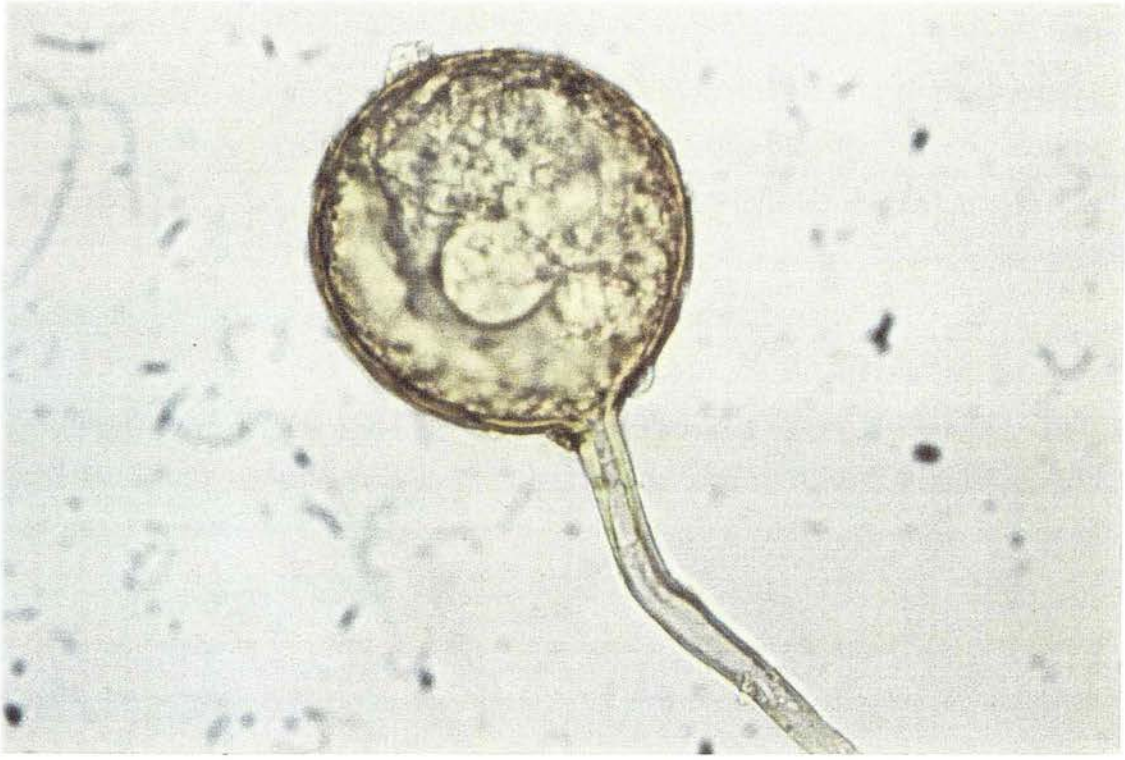

Fig. 2.4. Single spore of the arbuscular mycorrhizal fungus *G. intraradices*. The point of spore attachment to the subtending hypha is clearly visible. The thick outer wall of the spore is also apparent. (Microphotograph ×500).

2.3.1 Isozyme analysis

Present identification of arbuscular mycorrhizal fungi relies on complex terminology based on structure of the spore walls, which may be inconsistently understood and applied. The use of molecular techniques may enhance or supplement a morphological classification which is presently difficult to elucidate without previous experience of the subject.

Isozymes are proteins that have the same enzymatic activity but are coded by either separate genetic loci or by different alleles at the same genetic locus (van Tuinen *et al.*, 1994). The latter are termed allozymes. Isozymes have different tertiary protein

structure and therefore vary in their electrophoretic mobility in gels. Analysis of electrophoretic products reveals different banding patterns that express genetic differences between closely related fungi. Selective staining of isozymes following electrophoresis may be used to distinguish between and identify certain arbuscular mycorrhizal species.

Sen & Hepper (1986) characterised six species of *Glomus* by subjecting spore extracts to polyacrylamide gel electrophoresis and selective enzyme staining. This technique was able to show variations between species and geographically different isolates (Hepper *et al.*, 1988). Hepper *et al.* (1988) used isozyme analysis both to quantify arbuscular mycorrhizal colonisation of leek roots and to identify species colonising roots both individually and with several species colonising the root.

Rosendahl *et al.* (1994) emphasised that care must be taken to identify all bands produced during isozyme analysis, prior to using banding patterns to estimate genetic distances. Putative loci, allelic variation and secondary bands can cause additional bands to occur following electrophoresis. Putative loci arise from gene duplications where more than one locus codes for production of the same enzyme in an organism (Rosendahl & Sen, 1992) as in, for example, the enzyme malate dehydrogenase which has both a mitochondrial and cytoplasmic locus which are each inherited independently (Rosendahl *et al.*, 1994), causing two bands to appear during electrophoresis. Secondary bands arise from non-genetic post-transcriptional processes and are not related to the genetics of the organism and must therefore be identified and disregarded (Rosendahl *et al.*, 1994). Allelic variation is variation in the enzyme produced from a single locus.

Analysis of isozymes has shown variation between species and geographically different isolates (Hepper *et al.*, 1988). This technique can provide information on the taxonomy of arbuscular mycorrhizal species and aid their correct identification, providing care is taken to consider the complete banding pattern before any analysis is made. Therefore, isozyme analysis may provide information on the diversity of arbuscular mycorrhizal spores (Giovanetti & Gianinazzi-Pearson, 1994).

2.4 Culture of arbuscular mycorrhizal fungi

One of the single most-inhibitory factors in arbuscular mycorrhizal research is the fact that the fungi are obligately biotrophic with no saprophytic ability. Attempts to grow the fungi in pure culture have been largely unsuccessful, although some work has been done towards establishing sterile cultures of the fungi on the roots of axenically grown host plants (Hepper, 1981; McDonald, 1981; Mosse, 1959, 1962; St. John *et al.*, 1981). This usually requires surface-sterilised spores to be capable of germinating to produce a germ tube which will penetrate the root of an axenically grown seedling.

Mycorrhizal spores are capable of germinating in the absence of any mineral or organic nutrition as, for example, in water agar (Azcón, 1987; Hepper, 1979; Hepper & Smith, 1976). However, a variety of factors influence spore germination. The addition of 20 mg L^{-1} and 0.1 mg L^{-1} of CaH_2PO_4 and thiamine, respectively, to 1% water agar was reported to significantly increase germination of spores of *Gigaspora margarita* (Siqueira *et al.*, 1982). An optimum pH of 4–5 provides favourable conditions for spore germination (Hepper, 1984) while the germination rate may be increased by the presence of free-living soil microorganisms (Azcón-Aguilar *et al.*, 1986).

Subsequent hyphal growth can be significantly improved by the incorporation of peptone, yeast, thiamine or boiled seed fragments into the agar medium on which the spores are germinated (Hepper, 1979). Siqueira *et al.* (1982) reported that low concentrations of sucrose (4 g L^{-1}) favoured germ-tube growth while the presence of complete bacterial cultures were found to significantly stimulate hyphal growth (Azcón, 1987).

A dormancy factor may also exist in the spores of some species. It has been suggested that seasonal dormancy may exist (Gemma & Koske, 1988) and that dormancy may be broken by cold treatments or storage (Gazey *et al.*, 1993; Tommerup, 1983).

Plant roots transformed using DNA from the Ri plasmid of *Agrobacterium rhizogenes* can be grown and maintained in nutrient medium as root organ cultures. These transformed roots have been successfully inoculated with germinating mycorrhizal

spores, to allow the establishment of long-term dual cultures of the host and fungus (Bécard and Fortin, 1988; Nuutila *et al.*, 1995).

2.4.1 Production of inoculum

The absence of techniques to grow arbuscular mycorrhizal fungi successfully in pure culture means that production of sufficient amounts of inoculum can be difficult. For research purposes, cultures of these organisms are frequently maintained on the roots of host plants grown in non-sterile open pot cultures in the glasshouse. These cultures provide inoculum in the form of spores, colonised root fragments and hyphae that can be used in research and to maintain further cultures (Ferguson & Woodhead, 1982; Gilmore, 1968). However, such pot cultures are frequently colonised by other, non-mycorrhizal microorganisms (Secilia & Bagyaraj, 1988) and they remain sterile for a short time only. The risk of contamination from adjacent pot cultures of different isolates of mycorrhizal fungi must also be avoided.

Frequently, pot cultures have utilised a growth substrate of very low phosphorus content as mycorrhizal colonisation may be reduced by increases in phosphorus availability in the soil. However, recent work has suggested that sand fertilised with bone meal is an effective substrate with which to initiate rapid arbuscular mycorrhizal colonisation of host plants and sporulation of the fungus (Vestberg, 1992a).

2.5 Arbuscular mycorrhizal fungi in field conditions

Laboratory experiments are frequently performed in idealised conditions which are very different from those in the field. Relationships that can be demonstrated between the host and mycorrhizal fungus in the laboratory are rarely shown in field conditions. In general, increases in plant growth resulting from inoculation with arbuscular mycorrhizal fungi will only occur in nutrient-poor soil. If the nutrient status, particularly of phosphorus, is increased to a level where it is not limiting for plants, then there is often no difference in growth between mycorrhizal and non-mycorrhizal plants, i.e. the mycorrhizal plants have no nutrient advtantage over the non-mycorrhizal plants. This suggests that field inoculation may only be worthwhile in situations where phosphorus is limiting for plant growth (Abbott & Robson, 1982).

However, it has been reported that legumes maintain a high mycorrhizal colonisation at all phosphorus levels in an acid infertile tropical soil, while the colonisation of a grass species decreases as soil phosphorus levels increase Arias *et al.* (1991). Legumes require large amounts of phosphorus for nodule formation, and are dependent on mycorrhizal associations to provide this phosphorus. Jakobsen (1986) suggested that this mycorrhizal dependency of legumes causes the mycorrhizal colonisation to remain high even when soil phosphorus levels are high.

In contrast, in a non-tropical soil, McGonigle and Fitter (1988) demonstrated that the effect of mycorrhizal fungi in increasing phosphorus uptake in pots was not observed when the plants were transferred to the field. Although the mycorrhizal colonisation continued to increase in the field, phosphorus inflow rates declined. This led to the suggestion that for large parts of the life cycle, plant demand for phosphorus may be satisfied by the root system without requiring mycorrhizal transport. In contrast, in a temperate field soil low in phosphorus, Merryweather & Fitter (1995) demonstrated a correlation between mycorrhizal colonisation and phosphorus inflow in an obligatory mycorrhizal host plant.

These conflicting opinions may be resolved by Fitter (1991) who suggested that the benefit of the mycorrhizal association to the plant can be measured as the net carbon exchange. A hypothesis was presented that colonisation only yields benefits, thus operating as a mutualistic association, at periods during the plant life cycle when phosphorus demand exceeds the capacity of the root system. At other times, the association may be better described as parasitic or neutral, depending on the phosphorus demand of the host (Fitter, 1991). The fact that the benefits of a mycorrhizal colonisation vary with the requirement of phosphorus by the host implies that mycorrhizal inoculation may only result in a growth response if the host plant is in a phase of its life cycle which requires a large quantity of phosphorus.

The efficiency of a particular combination of plant and mycorrhizal fungus, together with the mineral requirements of the plant, must be considered in any field inoculation. Ravnskov & Jakobsen (1995) indicated that the functional compatibility of a particular mycorrhizal association, measured in terms of direct hyphal phosphorus

transport to the host, varies with the fungus used, the mycorrhizal dependency of the host and the environmental conditions, including season (Lapointe and Mollard, 1997).

In natural conditions it is probable that more than one mycorrhizal fungus colonises the roots of a particular plant. It has been suggested that observations of spore production do not accurately predict the mycorrhizal species which are present (Sanders & Fitter, 1992). Clapp *et al.* (1995) demonstrated a technique to identify the combination of fungal symbionts in roots taken directly from natural communities. This novel method enables the mycorrhizal species present in the roots of plants in the field to be individually identified so that the contribution of each fungus to the plant can be estimated.

It has been suggested that for field inoculation to be worthwhile, the indigenous mycorrhizae must be absent or at a low inoculum potential (Abbott *et al.*, 1983) or must be inefficient in increasing plant growth (Abbott & Robson, 1982). A method for selection of mycorrhizal isolates from the field and screening isolates to test for their effectiveness and ability to compete with indigenous mycorrhizae has been outlined by Dodd & Thomson (1994).

An alternative to field inoculation may be to use crop and management practices to influence both development and efficiency of the indigenous mycorrhizal population. This may be appropriate when increasing the colonisation by indigenous mycorrhizal species does not alter their effectiveness (Sieverding, 1991). Management practices could also be used to reduce the population of less effective, indigenous mycorrhizal species, to encourage colonisation by an introduced, effective arbuscular mycorrhizal fungus (Dodd & Thomson, 1994).

It is recognised that aspects of an arbuscular mycorrhizal symbiosis that are unrelated to phosphorus nutrition can be utilised for field inoculation in certain situations. The ability of mycorrhizal fungi to assist in the recolonisation of soil spills or in land restoration (Rosales *et al.*, 1997) may be a useful application for mycorrhizal inoculum. For example, certain mycorrhizal species can adapt to a high cadmium

concentration in soils (Weissenhorn *et al.*, 1993). The lack of arbuscular mycorrhizal fungi in soils that are contaminated by toxic heavy metals such as cadmium could delay rejuvenation of such soils. The ability of mycorrhizal fungi to adapt to this environment may enable them to play an important role in recolonising such waste soils. Arbuscular mycorrhizas can increase root growth in grasses used to reclaim taconite iron-ore spoils that are low in both water and phosphorus (Noyd *et al.*, 1995). The grasses can then act as inoculum to assist in the establishment of more persistent, mycorrhizal-dependent species. Herrera *et al.* (1993) showed that exotic mycorrhizal species aided in the establishment of native legumes in a desertified ecosystem low in both water and nutrients.

Therefore, the potential exists to utilise several aspects of a mycorrhizal symbiosis for field inoculation of certain host plants using arbuscular mycorrhizal fungi. Laboratory experiments provide valuable information about a mycorrhizal association but experimental work is frequently performed in optimum conditions. Fitter (1985) suggested that the possible effect of an arbuscular mycorrhizal inoculation in the field would be more efficiently determined using experimental conditions more similar to those in the field, particularly soil phosphorus levels. The use of varying soil phosphorus levels would enable growth-response curves to be constructed to give more information about the effect of the mycorrhizal fungi.

2.6 Effect of arbuscular mycorrhizal fungi on the host plant

Colonisation of plant roots by an arbuscular mycorrhizal fungus can have a number of effects on the growth and nutrition of the host plant. These effects are often studied and explained by comparing mycorrhizal and non-mycorrhizal plants in controlled experiments in a laboratory or glasshouse (Cooper, 1984).

2.6.1 Increased nutrient uptake

Arbuscular mycorrhizal colonisation usually increases plant growth by enhancing the nutrient uptake into the plant (Abbott & Robson, 1984). Plant nutrient uptake is determined by the absorption capacity of the root and by diffusion of nutrients and delivery of elements in or to the soil solution (Sieverding, 1991). The uptake of poorly mobile ions such as phosphorus and zinc, which diffuse only slowly in soil, depends

on the root density per unit volume of soil. The presence of external mycelia around a mycorrhizal plant root increases the soil volume from which nutrients may be removed, thus increasing the uptake of some nutrients into mycorrhizal plants (Cooper, 1984).

2.6.1.i Phosphorus

The uptake of phosphorus from soil to a plant is usually limited by the rate of movement of phosphorus to the root rather than the rate of absorption at the root surface (Abbott & Robson, 1982). Phosphorus is poorly mobile in soil and often becomes depleted in the area immediately around the root. The presence of external mycelia around a mycorrhizal root allows the plant to exploit phosphorus from soil which is beyond the normal zone of depletion of a non-mycorrhizal root. In the majority of cases, improved phosphorus uptake is the primary cause of growth and yield enhancement of arbuscular mycorrhizal colonised plants (Abbott & Robson, 1982).

Mycorrhizal plants may have increased growth in soils low in phosphorus compared to non-mycorrhizal plants (Smith & Gianinazzi-Pearson, 1988). However, in general, increases in plant growth resulting from mycorrhizal colonisation can be completely overcome by increasing nutrient supply to a level where it is not limiting for host plants (Abbott & Robson, 1982).

There has been conflicting evidence for the suggestion that mycorrhizal fungi can utilise non-labile forms of phosphorus, such as insoluble rock phosphate, which are usually unavailable to a plant root (Cooper, 1984). Examination of growth-response curves at varying levels of phosphorus supply to plants indicated that mycorrhizal and non-mycorrhizal plants obtain phosphorus from the same soil pools (Pairunan *et al.*, 1980). In contrast, Jayachandran *et al.* (1992) indicated that arbuscular mycorrhizal fungi are involved in mineralisation of organic phosphorus by an unknown method.

The ability of mycorrhizal fungi to increase the uptake of phosphorus into plant roots initially depends on the formation of hyphae in the soil around a plant root. Different arbuscular mycorrhizal species differ in their ability to form external hyphae in soil.

This is also influenced by soil factors such as pH and phosphorus status (Abbott & Robson, 1985).

Studies with isotopically labelled ^{32}P have shown the direct uptake and transport of phosphorus by mycorrhizal fungal hyphae (Cooper & Tinker, 1981). Studies indicate that the rate of phosphorus inflow in hyphae is greater in mycorrhizal than non-mycorrhizal roots (Sanders *et al.*, 1977; Jakobsen, 1986).

2.6.1.ii Other nutrients

Arbuscular mycorrhizal fungi can increase the uptake of several nutrients which move by diffusion to roots (Cooper, 1984). However, investigations into individual nutrient uptake are frequently difficult because the supply of all other nutrients must not be limiting for the growth of either the mycorrhizal or non-mycorrhizal plants, and increasing the phosphorus supply may reduce mycorrhizal colonisation (Abbott & Robson, 1984).

Rhodes and Gerdemann (1978) demonstrated that external hyphae of arbuscular mycorrhizal fungi may translocate sulphur. Concentrations of both copper and zinc have been reported to be greater in mycorrhizal compared to non-mycorrhizal plants. This effect was due to the improved phosphorus status of the mycorrhizal plants because the decreased mycorrhizal colonisation which results from increasing the phosphorus supply to the plants decreased concentrations of these nutrients in the mycorrhizal plants (Lambert *et al.*, 1979; Thompson, 1990). Bürkert & Robson (1994) demonstrated that external arbuscular mycorrhizal hyphae translocate labelled ^{65}Zn towards colonised plant roots, and that this uptake varies with different fungi. It has been reported that external hyphae of arbuscular mycorrhizal fungi transport labelled ^{15}N towards colonised plant roots (Frey & Schüepp, 1993; Johansen *et al.*, 1993). This hyphal transfer may depend on the degree of nitrogen sink in the plant, because it was suggested that more nitrogen is transported to plants which are deficient in nitrogen (Johansen *et al.*, 1994). This implies that the fungus was able to regulate the hyphal uptake of soil mineral nitrogen.

2.6.2 Carbohydrate demand on the host plant

The fungal demand for host photosynthate depends on the degree of fungal colonisation in the plant root and its metabolic rate. Mycorrhizal roots frequently have a higher metabolic activity than non-mycorrhizal roots (Smith & Gianinazi-Pearson, 1988). Short-term $^{14}CO_2$-labelling experiments have shown that arbuscular mycorrhizal colonisation can increase the ability of onion roots to incorporate carbon from both external sources and photosynthetic assimilate (Lösel & Cooper, 1979).

Transfer of carbon is thought to occur via an active sugar transfer system at the arbuscular interface between the host and fungus (Smith & Gianinazzi-Pearson, 1988). It may be possible that this carbon drain from the host to the fungus may initiate growth depressions in some mycorrizal plants. The fungus may act as a respiratory sink which is usually compensated for by additional phosphorus intake into the plant. A lack of any compensation may result in a loss of crop productivity (Cooper, 1984).

2.6.3 Hormonal effects

Plant growth substances can be directly involved in the interaction between the host and mycorrhizal fungus. Allen (1980) demonstrated increased cytokinin activity in a rangeland grass as a result of mycorrhizal colonisation. A combination of increased giberrellic acid and reduced abscisic acid has also been observed in the leaves of mycorrhizal plants (Allen *et al.*, 1982). The altered hormone production in mycorrhizal plants may cause changes in plant physiology that can benefit the association.

The concentration of abscisic acid has been reported to be higher in both shoots and roots of arbuscular mycorrhizal colonised maize plants compared to non-mycorrhizal plants. The fact that this feature persisted throughout development of the plants suggested that it was due to the mycorrhizal colonisation and not just an effect of the improved phosphorus nutrition of the mycorrhizal plants (Danneberg *et al.*, 1992).

2.6.4 Water relations

Mycorrhizal plants have been demonstrated to have higher hydraulic conductivities, i.e. a lower resistance to water flow, than non-mycorrhizal plants (Smith & Gianinazzi-Pearson, 1988). It has been suggested that the effect of mycorrhizal colonisation on the water relations of a host plant is probably due to the improved phosphorus nutrition of the host plant. For example, the lower root hydraulic resistance observed in mycorrhizal plants grown in a nutrient-deficient soil was eliminated if both mycorrhizal and non-mycorrhizal plants were nutrient amended (Safir *et al.*, 1972).

Koide (1985) showed that mycorrhizal colonisation reduces stomatal resistance, thus causing transpiration to increase, resulting in reduced hydraulic resistance of mycorrhizal plants in a soil with low phosphorus availability, probably a result of improved phosphorus nutrition of the mycorrhizal plants because phosphorus amendments to the soil were also observed to reduce stomatal resistance.

In contrast, Augé *et al.* (1986) showed that increased leaf conductance of mycorrhizal plants was correlated with the level of mycorrhizal colonisation and not influenced by supply of phosphorus to the host. It was suggested that mycorrhizal hyphae within the root may be affecting transport of water into the plant. Other work also implicated the involvement of internal mycorrhizal hyphae, suggesting that cortical hydraulic resistance may be bypassed by the hyphae (Kothari *et al.*, 1990).

The concept of non-hydraulic root-to-shoot signals that communicate soil water availability was described by Ebel *et al.* (1994). Augé *et al.* (1995) demonstrated that mycorrhizal colonisation of plant roots altered this signalling process independently of the mycorrhizal effects on plant phosphorus nutrition. Applied phosphorus was found to have no effect on leaf growth response to soil drying but the leaf phosphorus concentration did.

Therefore, there is conflicting evidence whether mycorrhizal colonisation affects plant water relations either indirectly by increasing phosphorus uptake or directly by altering the root response to changing soil conditions. This may be studied more

appropriately by comparing mycorrhizal and non-mycorrhizal plants of equivalent size and phosphorus nutrition (Kothari *et al.*, 1990).

2.7 Interaction of arbuscular mycorrhizal fungi with plant pathogens

There have been several reports that arbuscular mycorrhizal colonisation of a plant can effect the tolerance or susceptibility of the host to infection by soil-borne plant pathogenic organisms. In some cases, inoculation of plants with certain mycorrhizal fungi was found to reduce subsequent infection of the plants by some soil-borne pathogenic fungi (Davis & Menge, 1980; Graham & Menge, 1982). However, it was also suggested that the presence of arbuscular mycorrhizal fungi in plant roots may increase the susceptibility of the host to disease (Davis *et al.*, 1978; Ross, 1972), or have no effect on the infection by a root-invading pathogen (Bååth & Hayman, 1984).

The improved phosphorus nutrition of mycorrhizal plants may have a number of effects on the interaction between the host and soil-borne pathogens. For example, improved phosphorus uptake by mycorrhizas can decrease root membrane permeability which results in a decrease in root exudation from the host. This was reported to reduce the activity of *Gaeumannomyces graminis*, the fungus which causes take-all disease of wheat, by altering the response of pathogenic hyphae towards mycorrhizal roots (Graham & Menge, 1982). A reduction in rhizosphere leachates from mycorrhizal roots of sweet corn (*Zea mays* var. *rogusa*) was also found to decrease sporangium and zoospore production by the pathogen *Phytophthora cinnamomi* (Meyer & Linderman, 1986). It was suggested that the presence of an arbuscular mycorrhizal fungal colonisation of plant roots may exert a selective pressure on the microbial population in the mycorrhizosphere, influencing the presence or activity of fungal pathogens (Linderman, 1988).

The increased uptake of nutrients other than phosphorus in mycorrhizal plants can affect the host's nutritional status, which can increase tolerance of a plant to a pathogen infection. Improved phosphorus nutrition of mycorrhizal plants may result in an increase in root growth, enabling a plant to compensate for the debilitating effect of a pathogen invasion. However, this increased production of roots may also provide more potential sites for soil-borne pathogens to infect the plant (Graham, 1988).

Competition for space, colonisation or penetration site between an arbuscular mycorrhizal fungus in plant roots and an invading fungal pathogen has been demonstrated to contribute to the reduction of invasion of citrus roots due to *Phytophthora parasitica* (Davis & Menge, 1980).

Mycorrhizal association with plants can influence the production of certain compounds which are usually involved in the defence response of a plant to invasion by a pathogen. For example, it was demonstrated that mycorrhizal colonisation increased the arginine content and chitinase production in plant roots (Dehne & Schönbeck, 1978). Production of peroxidase is stimulated by mycorrhizal development, particularly during the early phases of penetration into host roots (Spanu & Bonfante-Fasolo, 1988). The effect of this enzyme on secondary wall formation of epidermal cells in mycorrhizal roots could affect their subsequent resistance to infection by root pathogens. One feature of the mycorrhiza–plant symbiosis is that colonisation with the mycorrhizal fungus induces a very weak host defence response which is retained throughout the fungal association. This permanent activation of host defence mechanisms may be involved in enhanced resistance of mycorrhizal plants to subsequent infection by some plant pathogens (Gianinazzi, 1991).

Guillemin *et al.* (1994) suggested that the combined effect of arbuscular mycorrhizal fungi on plant physiology, root growth and phosphorus nutrition were factors contributing to the tolerance of endomycorrhizal micropropagated pineapple to *Phytophthora cinnamomi*, the cause of root rot. Vestberg *et al.* (1994) studied the effect of a pathogen which causes shoot rot of strawberry plants. In this situation, the mycorrhizal fungi could only indirectly influence infection by the pathogen, possibly by altering plant physiology such that the resistance of the plant to *Phytophthora cactorum* was altered. However, it was found that inoculation with one particular mycorrhizal fungus actually appeared to predispose the plant to infection by the pathogen, illustrating that not all inoculations with arbuscular mycorrhizal fungi may be of benefit to the plant.

Comparisons of mycorrhizal and non-mycorrhizal plants that are of similar size and nutritional status are important in determining the effect of any interaction between

the mycorrhiza and pathogen. This may be achieved by using soils of varying phosphorus levels that approximate to field recommendations. This is more efficient than using soils deficient in phosphorus because non-mycorrhizal plants frequently then have underdeveloped root systems which would affect any infection by a pathogen. Use of equivalent non-mycorrhizal plants will determine whether any increase in host tolerance is a consequence of factors other than improved host vigour that may result from better phosphorus supply (Smith, 1988).

Sharma *et al.* (1992) proposed a working model to explain the interaction between arbuscular mycorrhizal fungi and disease under varied environmental conditions. However, the mechanisms and effects of interaction between a mycorrhizal fungus, host and plant pathogen may vary with different individual combinations of these three. The role and involvement of each component can affect any interaction. In addition, certain environmental conditions may affect one or more component of the system in different ways. Factors such as the growth substrate, timing of both mycorrhizal and pathogen inoculation and the length of time for which a mycorrhizal colonisation must be established in the plant roots can all affect the interaction in a mycorrhiza–host–pathogen system (Caron, 1989). Therefore, more knowledge is required about individual combinations of arbuscular mycorrhizal fungi, host plants and plant pathogens to identify any interactions that may be occurring.

2.8 Effect of arbuscular mycorrhizal fungi on strawberry plants

Several reports indicate that inoculation of strawberry plants with arbuscular mycorrhizal fungi may affect the productivity of the crop. As with most host plants, cultivated strawberry was originally claimed to benefit from such inoculation only when soil phosphorus was limiting for plant growth (Holevas, 1966). However, Dunne and Fitter (1989) found that strawberry has a very high phosphorus demand, particularly during the reproductive phase of the plant. This demand exceeded the capacity of the plant root system but the presence of an arbuscular mycorrhizal colonisation provided the necessary additional phosphorus uptake. This suggests that mycorrhizal colonisation may be beneficial even when soil phosphorus level is high. This may be particularly important because the phosphorus demand may be greater in

strawberry cultivars that have been selected for enhanced fruit production. This agrees with the suggestion by Fitter (1991) that a mycorrhizal association is beneficial when the host has a high phosphorus demand.

It has been shown that mycorrhizal colonisation of strawberry plants can exist and even increase in field soils of high phosphorus levels (Werner *et al.*, 1990). The increased uptake of phosphorus resulting from a mycorrhizal colonisation was also reported to improve the growth of micropropagated strawberry plants receiving commercial rates of phosphorus fertiliser (Williams *et al.*, 1992).

Micropropagated strawberry plants readily lend themselves to inoculation with arbuscular mycorrhizal fungi. These plants are micropropagated and rooted in an agar-based substrate in sterile, controlled conditions. The process of transferring the rooted plantlets into pots of soil-less growth substrate offers an ideal opportunity for inoculation with mycorrhizal fungi. The transplantation into a soil-less, frequently fumigated substrate also avoids the presence of indigenous mycorrhizal fungi with which the inoculating fungi would have to compete (Niemi & Vestberg, 1992). Soil fumigation can severely reduce numbers of mycorrhizal propagules (McGraw & Hendrix, 1986). However, a variety of factors may affect the influence of fumigation on mycorrhizal propagules, but a decrease in the number of propagules will reduce the colonisation of micropropagated strawberry plants that are planted into the soil (Robertson *et al.*, 1988).

The effect of arbuscular mycorrhizal fungi on micropropagated strawberry plants varies with the particular host–fungus combination used. Certain mycorrhizal species may be more effective at colonisation and producing beneficial growth responses than others (Cháves & Ferrera-Cerrato, 1990; Hughes *et al.*, 1978; Niemi & Vestberg, 1992). Early strawberry cultivars may show a greater root colonisation by certain arbuscular mycorrhizal fungi than later maturing cultivars, possibly resulting from differences in root morphology (Vestberg, 1992b). Inoculation with a number of mycorrhizal species has been reported to produce an increased growth response compared to using a single species (Koomen *et al.*, 1987). Therefore arbuscular

mycorrhizal fungi must be screened for their effectiveness in producing beneficial growth responses on selected strawberry cultivars in particular soil conditions.

It has been indicated that arbuscular mycorrhizal inoculation of strawberry plants may produce a significant increase in fruit numbers (Robertson *et al.*, 1988) and shoot dry weight (Holevas, 1966; Kiernan *et al.*, 1984; Vestberg, 1992c). A positive influence on runner formation has also been reported (Hrselová *et al.*, 1989; Niemi & Vestberg, 1992; Vestberg, 1992c).

The benefit of arbuscular mycorrhizal inoculation must be investigated over a long time period, after inoculated strawberry plants have been transferred to field soils. The increased runner production in the first year of field growth of inoculated micropropagated strawberry plants may not exist in the following 2 years. This may be caused by the overexploitation of a limited pool of axillary buds in the first year of growth, or competition between the inoculated mycorrhizal fungi and the natural population of mycorrhizal fungi present in the field soil (Niemi & Vestberg, 1992). However, the increased plant growth resulting from mycorrhizal inoculation of strawberry plants has been reported to persist throughout the first year in the field and even after overwintering into the second year (Vestberg, 1992c).

Therefore the inoculation of micropropagated strawberry plants at the transplantation stage with effective arbuscular mycorrhizal fungi may positively benefit the future productivity of the plants under commercial production conditions.

2.9 The Scottish Strawberry Certification Scheme

Approximately 500 ha of land in Scotland is used for growing strawberries, amounting to an annual production of approximately 4.5 thousand tonnes of fruit. This activity is concentrated in the Tayside and Grampian regions. Seventy-five per cent of fruit is processed via canning, freezing and jam manufacture, with fresh fruit sold both on local markets and in England (Harper *et al.*, 1986). Plantations are cropped for 2–3 years and replanted, usually in the spring (Turner, 1980). This replant of between 200 and 300 ha generates an annual requirement for 5–6 million runners (Harper *et*

al., 1986) which are bought from specialist runner producers in both England and Scotland.

Successful strawberry production relies on the use of disease-free planting stock. Legislative measures are necessary to control the occurrence and spread of disease due to virus infection, fungal pathogens and nematode pests. The Sale of Strawberry Plants and Black Currant Bushes (Scotland) Order was made in 1947. Its aim was the production of healthy planting stock because, at that time, red core or red stele disease, caused by *Phytophthora fragariae*, was present in most strawberry-producing areas (Montgomerie, 1980). The Order prohibited the sale of uncertified plants and the planting in Scotland of uncertified plants from the rest of the United Kingdom and Eire. This was followed in 1952 by the Red Core Disease of Strawberry Plants Order for England and Wales, aimed at preventing further spread of the disease. Red stele disease then became a notifiable disease such that infected areas were identified and the distribution of plants from them was prohibited (Montgomerie, 1980). Therefore, the Scottish and English legislation differ slightly but are both aimed at disease control. Certification is now carried out under the Soft Fruit Plants (Scotland) Order, 1991.

2.9.1 Introduction of the root-tip test

Official inspections to detect *P. fragariae* rely initially on the presence of aerial disease symptoms such as wilting and loss of vigour before analysing roots for the presence of oospores. Detection of the pathogen in planting stocks may be complicated by the fact that certain resistant cultivars may have symptomless infections which can spread red stele disease if planted to areas of clean soil adjacent to susceptible cultivars (Fulton, 1959). Also, susceptible cultivars may be infected with the fungus but soil conditions may not be conducive to symptom development (Maas, 1976). Despite the legislative measures, some certified strawberry runners were found to be infected with *P. fragariae* (Turner, 1980). Therefore, in 1980, the root-tip test was incorporated into the Scottish Strawberry Certification Scheme (Montgomerie, 1980).

The root-tip test involves collecting root tips from certified runner stocks which are then mixed with soil-less compost (Duncan, 1980). Susceptible bait plants are planted into this mixture and their roots examined 2 weeks later for the presence of oospores. This test is sufficiently sensitive to detect low infection levels equivalent to one infected root system per 100 sampled (Duncan, 1980). The test is more reliable, sensitive and reproducible than methods relying on visual symptoms and its incorporation increased the efficiency of the certification scheme (Montgomerie, 1980).

2.9.2 Introduction of micropropagation to the Scottish Strawberry Certification Scheme

Techniques for the micropropagation of strawberry plants have been described (Adams, 1972; Boxus, 1974). In the late 1970s, the Scottish Office: Agriculture and Fisheries Department (SOAFD) performed a feasibility trial of the micropropagation system. This demonstrated that micropropagation of strawberry plants was a convenient method to initiate runner production (Harper *et al.*, 1986). The trial used virus-tested mother plants to generate a foundation stock of several thousand plantlets (Harper *et al.*, 1986).

Four cultivars (Cambridge Favourite, Cambridge Vigour, Saladin and Troubadour) were removed from *in vitro* storage and multiplied to 2000 plants using micropropagation techniques. In the spring of 1981, these were planted as the first micropropagation foundation stocks at the East of Scotland College of Agriculture's Bush Estates near Edinburgh (Harper *et al.*, 1986). This stock generated almost 80 000 Elite runners. These were distributed to four Scottish Nuclear Stock Association growers for production of the next grade. In 1983, 7.4 ha were planted and submitted for Special Stock Certification and the resulting 1.8 million runners were planted in commercial plantations in 1984 (Harper *et al.*, 1986).

2.9.2.i Advantages of micropropagation

There are many advantages to using micropropagated planting stock. The rapid proliferation of strawberry stocks by micropropagation techniques under aseptic conditions provides healthy plants which can meet the commercial demand for strawberries (Boxus, 1981). Micropropagation also reduces the duration of strawberry

production in the field, thus reducing exposure time of the plants to possible pathogens (Harper *et al.*, 1986). It also reduces the time needed to introduce new cultivars by 1–2 years (Swartz *et al.*, 1981). Micropropagation also allows the use of *in vitro* storage of plants in a fridge for several years. This requires less energy and space than traditional runner plant storage, and can be initiated at any stage during the production cycle or year (Swartz *et al.*, 1981).

It was reported that micropropagated strawberry plants have a better resistance to frost compared to standard plants (Rancillac & Nourrisseau, 1989). Regeneration of strawberry plants by meristem cultures has achieved complete and definitive elimination of various viruses in a range of affected strawberry cultivars (Boxus, 1981). The use of micropropagation in combination with site and root-tip testing virtually eliminated the risk of distributing infected runners (Harper *et al.*, 1986).

2.9.2.ii Drawbacks to micropropagation

There are inevitably some negative aspects to micropropagation. Phenotypic changes in both appearance, runnering and fruiting habit have been noted in micropropagated strawberries (Anderson, 1981; Swartz *et al.*, 1981). Changes in performance are due to the increased vigour and axillary bud activity of micropropagated plants (Swartz *et al.*, 1981). This may produce a greater number of crowns per square metre, thus increasing the yield per square metre of matted row although the mean individual fruit weight may be reduced compared to field-propagated runner plants (Swartz *et al.*, 1981).

The occurrence of variants or rogues in any propagation system of sufficient size is not unusual, but in the field any such inadvertently multiplied variants are easily recognised by inspections and removed by rogueing (Harper *et al.*, 1986). Such variants include dwarfs, sectorial chlorotic plants and leaf-spot susceptibles (Swartz *et al.*, 1981). Observations are made of the original virus-tested mother plants and samples from individual clones of micropropagated plants of both are grown on for fruiting in the field. Visual inspections are made over two years of field multiplication (Harper *et al.*, 1986).

2.9.3 The micropropagation process

Micropropagation uses plant tissue-culture techniques and many of the fundamental principles of conventional propagation to achieve more control over the propagation process. In this process, isolated parts of whole plants are grown *in vitro* under aseptic conditions, requiring careful control of the culture environment including light, temperature, nutrients and plant hormones (Pennell, 1987). The growth conditions, media, sterility and manipulation of plant material during this project differ from those employed in commercial production of micropropagated strawberry plants only in scale.

To initiate micropropagation, a meristem is aseptically removed from a strawberry plant and placed in an agar-based growing medium in a sterile container. The medium contains high levels of the plant growth regulators auxin and cytokinin, which encourage the formation of axillary buds. The plant is allowed to proliferate for 4–6 weeks (Boxus, 1981). After this stage, the plant is divided into individual plantlets which are placed into sterile containers containing rooting medium that lacks plant growth regulators. This medium encourages the plants to develop roots. After 4–6 weeks, each plantlet has rooted and can be transferred to a plant pot and allowed to acclimatise from the micropropagation environment to a glasshouse environment (a process known as 'weaning') in a glasshouse in conditions of 100% humidity for 1 week. The humidity is then allowed to fall to ambient levels, and the plants are ready for transfer to the field after 4–5 weeks.

2.9.4 Certification of micropropagated material

Certification is required under the Soft Fruit Plants (Scotland) Order, 1991. The commencement of any propagation program requires prior approval from the Scottish Office: Agriculture and Fisheries Department (SOAFD). This initially involves confirmation of the source and eligibility of the planting material, i.e. pathogen-tested nuclear stock issued by the Nuclear Stock Association. Standards of hygiene and basic laboratory *in vitro* production are also assessed. Once approval is obtained, a propagation program requires suitable laboratory equipment for aseptic handing of plant material. Rancillac & Nourisseau (1989) reported that physiological changes in micropropagated strawberry plants could be avoided if the number of permitted

subcultures is limited to 10. Therefore, the number of permitted subcultures or the time for which plant material is held in culture is restricted, generally being limited to 1 year or 10 subcultures, whichever is reached first.

During weaning, stocks entered for certification are isolated and maintained under protected environment conditions with insect proofing, double doors and disinfectant foot baths at the entrance to the building. Floors are covered with polythene with a sand/gravel topping to ensure there is no contact with the base soil and to allow rapid drainage of surface water. A sterile growing medium or soil-less compost is used, and an approved pesticide treatment regime implemented. Annual sterilisation of all structures and the use of new or sterilised containers for each batch of plants help to maintain the required high standard of hygiene.

Clones and mother plants are accurately labelled and recorded to ensure that stocks are not mixed. All records of propagation, pesticide treatments and sales are inspected annually. Inspection of weaned or *in vitro* plant material occurs once prior to despatch. The standards of inspections are as for Foundation grade (see Fig. 2.5). Micropropagated stocks are inspected for health and vigour only because purity cannot be accurately assessed at this stage. Therefore certificates include the suffix 'M', which is only removed once the material has fruited and trueness to type is confirmed by field inspections. Certificates are valid until the stocks are either entered for a further cycle of propagation or planted out for commercial production. Certified stock can be sold, providing the material has a plant passport issued under the Plant Health (Great Britain) Order 1993. This operates in accordance with the Single Market Regulations of the European Union which require growers to be registered and have stocks inspected and passports attached before material can be moved. All commercial strawberry stocks must then be submitted for inspection and may be certified at the appropriate grades depending on the standard of the crop.

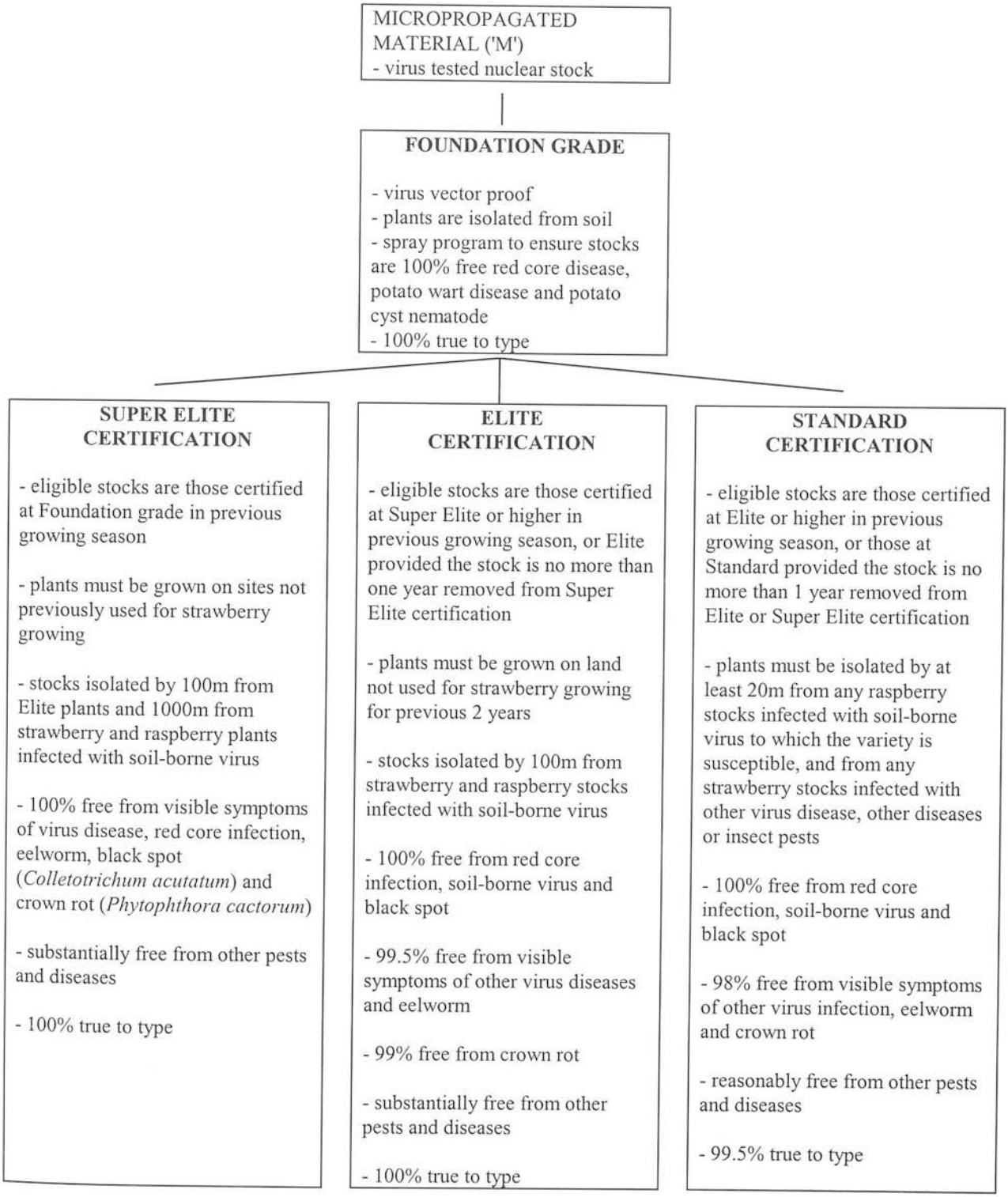

Fig. 2.5. Certification grades of micropropagated strawberry plants.

2.9.4.i Certification grades

Stocks eligible for Foundation Grade are derived from virus-tested nuclear stock which has been produced under approved micropropagation conditions. Stocks which fail to achieve the required standards for Foundation grade may be graded to a lower standard or rejected. Lower certification grades are Super Elite, Elite or Standard, in descending order.

The requirements for certification become less stringent as grades are lowered from Super Elite to Standard, as shown in Fig. 2.5. For example, the isolation of certified stocks from other strawberry plants is reduced and permitted levels of disease become higher. Each certification grade has criteria of purity and disease which must be satisfied at both the spring and autumn inspections for plants to achieve certification. Root-tip samples are removed in November and any stocks infected with *P. fragariae* are refused certification. If inspection shows any one stock in a group to be below the standard for the grade for which it is entered, all the stocks in that group are then graded according to the standard of this stock.

2.10 Red stele disease of strawberries caused by *P. fragariae*

Red stele disease is the most serious root-rotting disease of strawberry in Scotland, causing severe economic losses in affected areas (Harper *et al.*, 1986). The disease originally appeared in Lanarkshire in 1921, and subsequently became known as the Lanarkshire disease (Wardlaw, 1926). This outbreak virtually eradicated the then-important strawberry growing industry of the Clyde valley (Montgomerie, 1980). The causal agent of the disease was first recognised in 1940 as *Phytophthora fragariae* Hickman (Hickman, 1940). Subsequent reports of the disease indicated that it is more frequent in areas with cool, moist soil conditions (Maas, 1984). It has caused heavy losses to strawberry growers on nearly every continent where strawberries are grown (Maas, 1984).

2.10.1 Disease cycle of *P. fragariae*

Development of the fungus in roots is influenced by the resistance or susceptibility of the strawberry cultivar to the pathogen. Healthy strawberry roots may be infected by

motile, biflagellate zoospores of *P. fragariae*. The distribution of zoospores in soil water is a major factor influencing development of red stele disease. Maximum infection occurs under conditions of high soil water which promotes liberation of zoospores from sporangia and their transportation through soil to plant roots. Waterlogging may produce high soil moisture but this is less significant than the actual transport of zoospores through soil in stimulating infection (Hickman & English, 1951a). The optimum temperature for zoospore activity is 13–14°C, at which motility may be observed for 5 h following their release from sporangia. Zoospores have been found to be attracted to the root-tip region where they may encyst and germinate to produce a germ tube (Goode, 1953). Development of the fungus in roots is influenced by the resistance or susceptibility of the strawberry cultivar to the pathogen.

In a susceptible cultivar, the germ tube may penetrate between and through epidermal cells. Mycelium can then develop into and within cortical cells 48 h after the initial penetration (Goode, 1953). In contrast, in a resistant cultivar, a germ tube may penetrate the epidermis but the pathogen will not develop further in the root (Goode, 1953; Milholland & Daykin, 1993).

Sexual reproductive structures may develop in the vascular tissue of diseased roots, in the cortex of smaller laterals and near the tips of new main roots (Hickman, 1940). Oogonia are globose bodies borne on short stalks in the stele or cortex. They possess a thick, smooth wall which becomes golden brown with age. They can expand and mature to produce a thick-walled oospore which lies free within the oogonium (Hickman, 1940).

Oospores may be detected in the root of a susceptible strawberry cultivar 4–6 days after inoculation with encysted zoospores of *P. fragariae* (Law & Milholland, 1991; see Figs 2.6 and 2.7). An oospore may germinate and form a sporangium at the surface of the root. This may be observed 6 days after initial infection of the root, indicating the speed of the pathogen development (Law & Milholland, 1991). The sporangium can germinate asexually (Ribeiro, 1983) to release 40 to 50 biflagellate zoospores which can, in turn, infect more roots as secondary inoculum (Maas, 1984).

A few days after infection, the roots rot from the tip and rotted roots containing oospores become incorporated into the soil (Maas, 1984). Oospores are the primary survival structure of the fungus in the field, capable of over-wintering (Hickman, 1940).

2.10.2 Symptoms of *P. fragariae* infection

Above-ground symptoms depend on the severity of root rotting. Severely diseased plants which are wilted and stunted may occur in soil conditions which favour the fungus (Maas, 1984). A blue-green leaf discolouration may be seen in such dwarf plants. The progressive destruction of the root system by the invasion of the pathogen causes physiologic drought and aerial symptoms (Bain & Demaree, 1945). Diseased plants tend to occur in irregular patches and produce fewer runners and smaller berries than healthy ones, and may eventually die. Plants may be infected to a small degree and show no aerial symptoms (Maas, 1984).

The symptoms in roots are progressive. Young roots begin to rot at the tip with the lateral roots being destroyed as the disease progresses, giving the main root a 'rat-tail' appearance (Fig. 2.8). The stele above the rot is red in colour, giving the disease its name (Bain & Demaree, 1945). However, it has not been shown that the fungus produces a specific substance which induces stelar reddening (George & Milholland, 1986) and it may therefore be assumed that the red colouration originates from the plant.

Aerial and root symptoms of red stele disease can be used to detect the pathogen but are both influenced by soil conditions and resistance of strawberry cultivars to *P. fragariae* (Maas, 1984; Milholland & Daykin, 1993). Although the presence of a red stele is a useful preliminary indication of infection by the pathogen, microscopic confirmation of the presence of oospores in plant roots is required as positive proof of infection by *P. fragariae* (Bain & Demaree, 1945; George & Milholland, 1986).

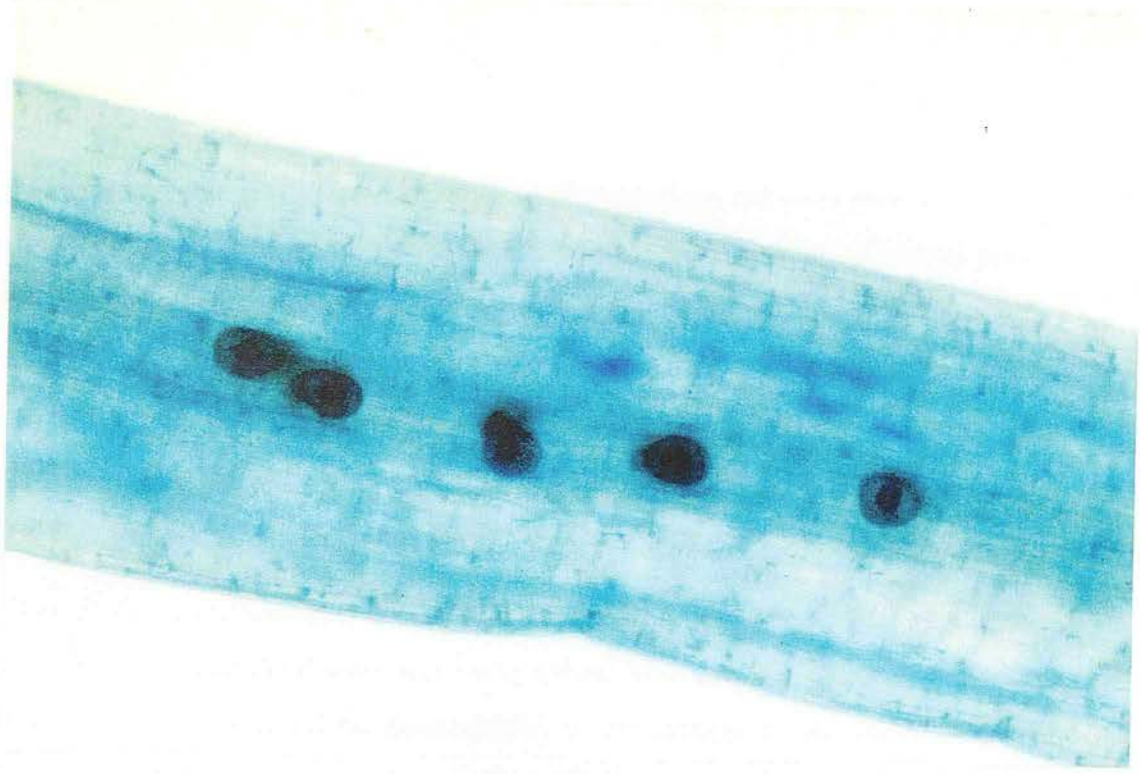

Fig. 2.6. Oospores of *P. fragariae* in an Elsanta root infected with *P. fragariae* and stained with trypan blue. The spherical oospores are seen to accumulate in the vascular region of the root. (Microphotograph ×125).

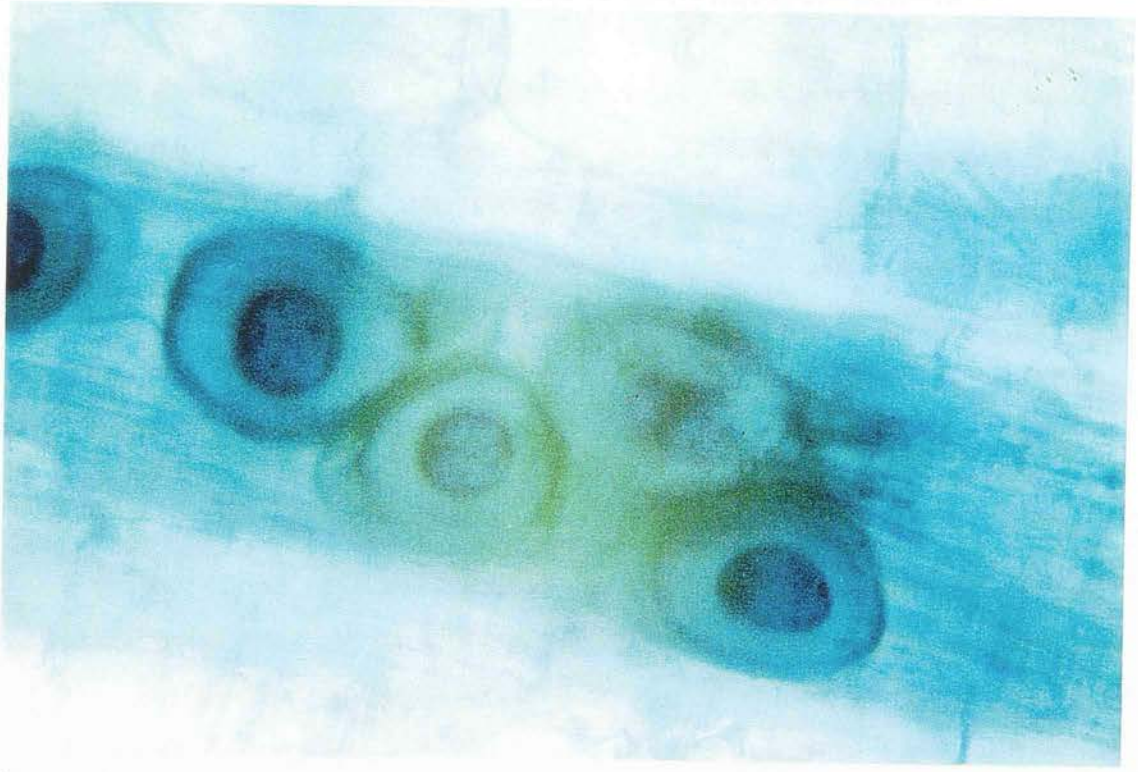

Fig. 2.7. Focus on oospores in an Elsanta root infected with *P. fragariae* and stained with trypan blue. (Microphotograph ×500).

2.10.3 Control of red stele disease

A variety of cultural and chemical means of controlling red stele disease have been suggested but as yet there is no single effective control treatment. Cultural methods which prevent spread of the pathogen are considered to be most important in reducing the incidence and severity of disease due to *P. fragariae*. These include avoiding distribution of infected soil and plants, improving soil drainage and the use of resistant strawberry cultivars.

2.10.3.i Physical control

When *P. fragariae* was first described as the causal organism of red stele disease, it was recognised that the disease was being spread from one area to another (Hickman, 1940). The fungus could be disseminated in contaminated soil which adheres to footwear and machinery, and by planting infected runner plants (Hickman, 1940). Inspections to detect any infected plants were incorporated into the Scottish Strawberry Certification Scheme to prevent their distribution (Montgomerie, 1980).

Various treatments to control the disease were largely unsuccessful and the use of crop rotation to control the disease was suggested. This involved growing crops other than strawberry in the contaminated soil for several years. However, there was still a risk of infection because the thick-walled fungal oospores were able to persist in the soil for at least 10 years (Hickman, 1940; Bain & Demaree, 1945). A hot-water treatment to remove all traces of the pathogen from infected strawberry runners was found to be ineffective as it was unable to kill the pathogen in the plant roots (Bain & Demaree, 1945).

It was generally considered that improved soil drainage would help to reduce the disease if present in the soil because waterlogged soil was thought to encourage the release of zoospores of the fungus which could then infect plant roots. However, it was found that the actual transport of zoospores by water movement through soil was

Fig. 2.8. Micropropagated Elsanta plants removed from the growth substrate and their roots washed under running tap-water. The plant on the left is infected by *P. fragariae*. The symptoms of the infection are clearly visible as red/brown rotting roots. The shoot is wilted due to the presence of the pathogen in the vascular system. The plant on the right is not infected by *P. fragariae*.

very significant in producing infection. High disease levels were possible in soils which, although freely drained, were subjected to high levels of rainfall because water movement could transport this inoculum to roots (Hickman & English, 1951a). However, improved soil drainage is still recommended as a measure to reduce red stele disease in an infected soil.

Planting runners on soil ridges was found to reduce disease severity significantly for 2 years. However, in the absence of disease it was found that plants grown on ridges had a significantly lower yield in their first year than those grown on the flat (Montgomerie & Kennedy, 1982). It was suggested that a combination of deep ploughing and rotovation would expose any oospores in the soil to daylight, thus encouraging their germination and reducing this form of inoculum in the soil. However, this cultivation method was found to have no effect on subsequent disease in an infected soil (Montgomerie & Kennedy, 1987).

2.10.3.ii Chemical control

It was found that *P. fragariae* is resistant to a number of fungicide treatments including copper sulphate, formaldehyde and captan. Although soil treatments of

methyl bromide could eradicate the fungus in field soils, the concentrations required to achieve this were probably phytotoxic (Jeffers, 1957).

Some pot experiments indicated that soil treatments with captan, dichlofluanid and fentin hydroxide could cause significant decreases in the level of disease compared to a standard treatment of fenaminosulf (Montgomerie & Kennedy, 1975). Application of fungicide LS70 1038 as a pre-plant dip, foliar spray or curative soil drench was found to reduce the incidence of red stele disease significantly (Montgomerie & Kennedy, 1975). *P. fragariae* is sensitive to the antibiotics streptomycin (Montgomerie & Kennedy, 1975) and cyclohexamide (Montgomerie & Kennedy, 1977) but such treatments may be phytotoxic and very unlikely to be approved for release on a food crop.

Montgomerie & Kennedy (1987) suggested that a useful control strategy would be a single application of an effective fungicide applied at a time that would protect the majority of new main roots. This time was identified as late September or early October following an April planting of strawberry runners because most new roots would be formed at this time. However, one disadvantage is that several fungicide applications would be uneconomical and difficult to apply to a wet winter soil using machinery.

Fosetyl-aluminium is recommended as a high volume, overall foliar spray to be applied to established crops at a rate of 1000 L ha^{-1} (ADAS, 1989). It is suitable to treat autumn-planted runners 2–3 weeks following planting, while spring-planted crops should be treated in the following autumn. The fungicide is applied to crops, post-picking if established, which have good foliage cover and while root growth is occurring. This is in agreement with the recommendations of Montgomerie & Kennedy (1987). In Scotland only, etridiazole is recommended to control red stele disease in spring-planted strawberries. It may be applied a minimum of 3 months after planting and has a minimum harvest interval of 6 months (A.D.A.S., 1989).

2.10.3.iii Resistant cultivars

Breeding strawberry plants which have resistance to *P. fragariae* is complicated by the presence of physiological races of the fungus (Scott *et al.*, 1984). Development of

resistant cultivars involves screening strawberry plants to determine their resistance to known or unknown races of the pathogen. This is carried out in both the field and the glasshouse. Glasshouse studies have the advantage that plants can be inoculated with known races and grown under controlled environmental conditions. Cultivars showing sufficient levels of field resistance to one or more races are generally chosen to use as parents in breeding programs (Scott *et al.*, 1984). The existence of physiological races of the fungus encouraged the emphasis of breeding to shift from that of plant immunity towards breeding for field resistance against several races (Hickman, 1962).

Van de Weg (1989a) suggested that resistance of strawberry and virulence of *P. fragariae* could be explained by a gene-for-gene system with at least five race-specific resistance and virulence genes. This model was suggested by examining cultivar–race interactions described previously (Converse, 1970; Converse & Scott, 1962). Additional interactions were studied and it was concluded that most could be described by this model (Van de Weg, 1989b). Some inconsistencies have been recognised and attributed to disagreements between authors and their experimental methods. This work could lead to resistance genes being employed in breeding programs to develop resistant strawberry cultivars.

Such programs may also allow for the selection of partial resistance in strawberry plants. Partial resistance is the incomplete resistance through which host plants become invaded by the pathogen but disease development is reduced or delayed. Law & Milholland (1992) indicated the presence of partial resistance to *P. fragariae* in some strawberry cultivars. However, it was unclear whether this was race or isolate specific because only one isolate of the fungus was used. The genetic and epidemiological mechanisms causing partial resistance are unclear. It has been shown that one particular race of *P. fragariae* colonised the roots of a partially resistant cultivar much more slowly than that of a susceptible cultivar and produced fewer reproductive organs (Milholland & Daykin, 1993). It was suggested that the reduced sporangial production would create an absence of secondary inoculum in the form of zoospores which could infect further roots. In the absence of secondary inoculum, plant roots could proliferate enabling the partially resistant cultivar to grow and produce a good crop.

2.11 Isolates of *P. fragariae*

In 1952, Reid (1952) reported the results of a breeding program whereby a range of strawberry cultivars were grown in soil heavily infected with *P. fragariae*. Cultivars which showed some resistance to the red stele disease were selfed and the resulting seedlings tested in the infected soil. It was observed that susceptible cultivars produced progeny which were infected within 1 year, while more resistant cultivars produced a minimum of 50% resistant progeny in the first year. It was concluded that the observed resistance was heritable. However, it was also found that under certain conditions a cultivar which had maintained complete resistance for a number of years could succumb to infection by the pathogen. It was concluded that such breakdown of resistance was due to the existence of a number of strains of *P. fragariae* with special host relationships (Reid, 1952).

P. fragariae is a very variable organism. It is recognised that some isolates are unstable in culture resulting in changes in virulence or even loss of pathogenicity (Kennedy & Duncan, 1993). Isolates belonging to the same race can cause differences in the amounts of disease they cause (Converse & Scott, 1962). Kennedy *et al.* (1986) assessed the pathogenicity of 32 isolates from one field on a series of strawberry cultivars. Cluster analysis was used to separate isolates into clusters that differed in virulence (Kennedy *et al.*, 1986).

2.11.1 Differential strawberry cultivars

Isolates of *P. fragariae* are separated into pathogenic races by their ability to infect certain strawberry genotypes, known as differential hosts. It is difficult to equate races recognised in different parts of the world without a uniform set of differential strawberry hosts and an international numbering system (Converse *et al.*, 1958). Attempts to use British or American sets of strawberry differential hosts or a combination of them to classify isolates of *P. fragariae* from Britain, Canada and America proved unsuccessful (Converse & Scott, 1962; Pepin & Daubeny, 1964). Kennedy and Duncan (1993) proposed that the addition of a British differential to Converse's American differential hosts (1970) would allow both the British and American known physiologic races to be distinguished. These cultivars would also

identify races such as B66-5 to B66-8 which were detected by Mongomerie (1966) but lost due to their instability in culture.

The use of different combinations of strawberry hosts has resulted in conflicting reports of the susceptibility of some strawberry cultivars to certain races of *P. fragariae*. For example, Montgomerie (1966) was unable to distinguish race A4 from A2 or race A6 from A5 whereas they had been clearly distinguished by Converse *et al.* (1958).

It has been indicated that the resistance of strawberry and virulence of *P. fragariae* is based on a gene-for-gene system with at least five race-specific resistance and virulence genes (Van de Weg, 1989a). It has been proposed that a set of strawberry differential hosts should contain cultivars which each have only one of the five resistance genes, and a universally susceptible cultivar (Van de Weg *et al.*, 1993). Research by Van de Weg *et al.* (1997) described some very reproducible classifications of resistance of differential strawberry cultivars which will lead to a formal gene-for gene model of disease being developed.

2.12 Variability in experimental methods

There has been a lack of uniformity in the *P. fragariae* isolates and the strawberry cultivars and the methods of assessing pathogenicity which are used to determine fungus–host interactions.

2.12.1 Inoculation of strawberry plants

Significant variation exists in the method of inoculating strawberry plants with the pathogen. Motile zoospores of *P. fragariae* have been used to inoculate strawberry plants by dipping roots in zoospore suspensions (Goode, 1956), pouring zoospore suspensions over established plants (Kennedy *et al.*, 1986) and spraying roots with a suspension of either motile or non-motile, encysted zoospores (Milholland *et al.*, 1989). Naturally infested soils may contain oospores of the pathogen which are formed in infected strawberry roots. Plants may be inoculated by planting them into such soil (Converse *et al.*, 1958; Montgomerie & Kennedy, 1987) or adding infected roots to the growing medium (Draper *et al.*, 1970).

2.12.2 Disease assessment

Several experiments have relied on the degree of reddening in the stele of infected roots to assess the pathogenicity of isolates (Hickman & English, 1951b; Kennedy *et al.* 1986). However, although this is used as a symptom of the disease, it has never been shown that the fungus produces a specific substance which can cause the red colouration (George & Milholland, 1986). Indeed, the roots of both resistant and susceptible cultivars have been shown to exhibit a red stele in response to challenge by a sterile fungal filtrate, a streptomycin–captan mixture and even distilled water (Otterbacher *et al.*, 1969). Thus although a red stele can be a useful preliminary indicator of infection by *P. fragariae*, microscopic confirmation of the presence of oospores of the pathogen in infected roots is essential for positive proof of infection by the fungus (Bain & Demaree, 1945).

Some work used a combination of the quantitative amount of stelar reddening together with the presence of oospores in infected roots to assess the pathogenicity of isolate–cultivar interactions (Converse *et al.*, 1958; Converse & Scott, 1962; Montgomerie, 1966). In these cases such methods were used to produce a disease rating on a scale which varied from one to 10 (Converse & Scott, 1962), and a classification on a scale of one to three where a score of one indicated an immune cultivar and susceptible plants were scored as three (Montgomerie, 1966).

It was suggested that assessment of pathogenicity would be more accurate if based on the basis of the number of oospores in infected roots and the percentage of roots containing oospores (George & Milholland, 1986). This method of disease assessment was extended to incorporate a disease severity index (DSI) which was used to determine genotype susceptibility (Milholland *et al.*, 1989). The DSI is equal to the number of oospores per root segment multiplied by the percentage of roots containing oospores divided by 100. A value greater than one indicates a resistant cultivar–isolate interaction while a DSI less than one suggests a susceptible interaction. This was found to be particularly accurate in determining resistance of a cultivar because a few oospores often developed in resistant cultivars but the percentage of roots containing oospores in these cultivars would be very low (Milholland *et al.*, 1989). In contrast, Van de Weg *et al.* (1993) showed that cultivars could be accurately classified as

resistant or susceptible on the basis of percentage of infected root without considering numbers or densities of oospores.

Root age is important because different levels of disease can be produced by spraying young roots of plants of different ages. Uniformity in both root age and environmental conditions should be standardised. The use of controlled environmental growth chambers ensures uniform conditions for pathogen development and enables experiments to be initiated at any time of year (Milholland *et al.*, 1989).

Therefore, there is a need for standard criteria to be used in experiments using differential strawberry hosts to separate isolates of *P. fragariae* into pathogenic races. Standardisation of methods of inoculation, growing conditions and disease assessment will improve the uniformity of results and information available concerning the interaction between strawberry cultivars and *P. fragariae*.

CHAPTER 3: Materials and Methods

3.1 Pot cultures of arbuscular mycorrhizal fungi

3.1.1 Isolates of arbuscular mycorrhizal fungi

Three isolates of *Glomus* spp. were initially obtained from Dr Mauritz Vestberg of the Agricultural Centre of Finland, Laukaa, Finland. Additional isolates of mycorrhizal fungi were provided by Dr Christopher Walker of the Forestry Authority, Edinburgh. Isolates of arbuscular mycorrrhizal fungi were previously assigned a code number according to the individual researcher who isolated each fungus. For example, isolates provided by Dr Vestberg were designated by a code number prefixed with the letter 'V'. However, following the deposition of specimens of each isolate in the Bank of European Glomales (BEG), a new BEG number was assigned to each isolate. Table 3.1 illustrates the source and BEG number of each isolate.

Table 3.1. Isolates of arbuscular mycorrhizal fungi used in this study.

Arbuscular mycorrhizal fungus	Code Number	BEG number	Source
Glomus mosseae	V57	BEG 29	M. Vestberg
Glomus intraradices	V98	BEG 48	M. Vestberg
Glomus fistulosum	V128	BEG 31	M. Vestberg
Glomus fistulosum	Gryndler 23A	BEG 23	C. Walker
Acaulospora scrobiculata		BEG 33	C. Walker
Scutellospora nodosa		BEG 34	C. Walker

3.1.2 Maintenance of pot cultures

Arbuscular mycorrhizal fungi are obligate symbionts and are maintained as a living culture colonising the roots of a suitable host plant. Such cultures are open pot cultures maintained under glasshouse conditions (Fig. 3.1). The growing substrate is of great importance because mycorrhizal fungi are particularly involved in the uptake and transfer of nutrients, particularly phosphorus, into the host plant. The growth substrate of pot cultures of arbuscular mycorrhizal fungi frequently has a very low nutrient content, to encourage extensive development of a mycorrhizal colonisation in

the roots of the host plant. However, the substrate must provide sufficient nutrients to allow adequate growth of the host plant; a fine balance must be reached.

The composition of the growing substrate must also be considered; for example, peat-based compost encourages the mycorrhizal fungus to sporulate inside the peat particles which makes the spores very difficult to extract from the culture (personal observation). Sand is a useful alternative, providing the diameter of the sand particles is sufficiently large to allow water to drain through the pot. Very small particles reduce the flow of water through the sand, which often results in water-logging which is very detrimental to the growth of both the host plants and the arbuscular mycorrhizal fungi (Ferguson & Woodhead, 1982). The pH of the growing substrate is a further consideration because certain mycorrhizal fungi require conditions of particular acidity or alkalinity for optimum development in the roots of the host plant (Daft & Nicolson, 1974).

3.1.3.i Sterilisation of growth substrate

Horticultural sand composed of sand particles with diameters in the range of 1–3 mm was placed into autoclavable bags. Each bag contained approximately 8 L of sand as this was a sufficiently heavy weight to lift. The bags were left unsealed and placed inside an autoclave. They were autoclaved at 121°C for 30 min and then removed from the autoclave to cool. Each bag was autoclaved using the same conditions 24 h later and again on the next day. This process of autoclaving the sand on each of three successive days ensured complete sterilisation of the sand.

3.1.3.ii Determination of substrate pH

This was performed according to a method provided by Dr C. Walker (personal communication). A sample of 10 mL of autoclaved sand was removed in duplicate from each autoclave bag and placed into a 100 mL glass beaker. A volume of 20 mL of 0.1 M calcium chloride was added to the beaker and the contents stirred vigorously for 30 s. The sand suspension was allowed to settle for 15 min. The contents of each beaker were vigorously stirred immediately before inserting a pH electrode into the beaker and recording the pH of the solution. The bulb of the pH electrode was rinsed in distilled water and dried prior to recording the pH of the next sample.

Fig. 3.1. Pot cultures of arbuscular mycorrhizal fungi in association with *Zea mays*. Growth of the plants was generally poor, caused in part by the low-nutrient growth substrate, and also due to the low levels of sunlight afforded by the local climate.

3.1.3.iii Addition of dolomitic lime and nutrients

Dolomitic lime was added to the sand at a concentration of 5 g L^{-1} to increase the pH of the substrate to between 6.5 and 7.0. Nutrients were added in the form of powdered bone meal, according to the growth substrate described by Vestberg (1992a,b). Bone meal was added at a concentration of 1.07 g L^{-1}. Lime and bone meal were added by placing 1 L of autoclaved sand in a large plastic tub. The correct quantities of lime and bone meal were weighed out and added to the sand, which was mixed by hand to ensure even distribution of the lime and bone meal. This was repeated with the remaining sand, which was then returned to an autoclave bag until required.

3.1.3.iv Preparation of pot cultures

A circular piece of commercially available net curtain was placed in the base of a plastic plant pot 3 L in volume. Sand to which lime and bone meal had been added was poured into the pot until the sand level reached just below the rim of the plant pot. Each pot was watered thoroughly with distilled water and left for 24 h to allow the water to soak through the sand.

3.1.3.v Host plants

The host plants used were seedlings of maize (*Zea mays* L.), seedlings of onion (*Allium porum*) and onion-sets, which were small onion bulbs that were obtained from a commercial garden centre. Seeds were surface sterilised by placing them in a 100 mL glass beaker containing 50 mL of 1% sodium hypochlorite solution for 10 s. The sodium hypochlorite was drained away and the seeds rinsed in three changes of sterile distilled water. Two sheets of germination paper, 1 m in length, were placed one on top of the other and soaked in distilled water. The sheets were laid flat on the bench. Surface-sterilised seeds were placed individually at 5 cm intervals along the soaked sheet. A further sheet which had been soaked in distilled water was placed on top of the first sheets. The bottom 2 cm of the sheets was folded upwards and, starting at one of the short ends, the sheets were loosely rolled up. The rolled sheet was placed in a 1 L beaker containing distilled water at a depth of approximately 3 cm. The beaker was placed inside a transparent plastic bag which was loosely sealed and placed at room temperature to allow germination of the seeds.

After approximately 7–10 days, sufficient seeds had germinated and the sheets of paper were removed from the plastic beaker and unrolled. The top sheet was removed to reveal the germinated seedlings. Each seedling was carefully lifted from the sheets when it was to be transplanted into a pot.

3.1.3.vi Inoculation of pot cultures

Inoculum of the arbuscular mycorrhizal fungi was in the form of substrate from an established pot culture of the particular fungus. This substrate contained sand and infective propagules of the fungus in the form of spores, hyphae and pieces of host-plant root that are colonised by the mycorrhizal fungus. A planting hole was made in the centre of the plastic pot which had been filled with sand growing medium and watered 24 h previously. The depth of the planting hole varied from 10 cm if seeds or onion sets were to be planted, to 20 cm if a germinated seedling was being planted. Approximately 20 g of inoculum of the required arbuscular mycorrhizal fungus was placed into the planting hole.

Either a surface-sterilised seed or a germinated seedling of onion or maize, or an

onion set was inserted into the planting hole which was filled in with growth substrate. The substrate around the seed or seedling was firmly pressed down and approximately 20 mL of distilled water poured onto the plant material to encourage germination of either a seed or onion set, or establishment of a seedling. A plant label was placed into each pot to provide details of both the fungus and host plant and the date. The pot cultures were allowed to grow in a glasshouse with average conditions of 18°C ± 3°C, with 16/8 h alternating light/dark period for at least 8 weeks to allow establishment of both the host plant and the mycorrhizal fungus. During this time, plants were watered sparingly at daily intervals. Samples of the pot culture substrate were then periodically examined for the presence of spores of the mycorrhizal fungus.

3.1.3.vii Maintenance of additional pot cultures

Following the observation of spores in substrate removed from a well-established pot culture, this substrate was used to provide inoculum for experimental purposes and to establish further pot cultures. The substrate was removed from the plant pot and placed into sealed plastic bags. Each bag was stored at 4°C until required.

When large amounts of inoculum were available, the method of initiating further pot cultures was modified: as an alternative to adding approximately 20 g of inoculum into the planting hole, inoculum was instead used to provide the growth substrate for the middle depth of the new plant pot, i.e. the bottom 8 cm of the plant pot was filled with autoclaved sand to which lime and bone meal had been added. A layer of inoculum 10 cm in depth was placed on top of this sand, followed by a top layer of the autoclaved sand containing lime and bone meal. The addition of a large layer of inoculum encouraged a faster and greater degree of mycorrhizal colonisation because a larger number of infective propagules of the fungus were available.

3.2 Staining plant roots to observe mycorrhizal colonisation

The method described by Koske & Gemma (1989) was modified to improve the staining of arbuscular mycorrhizal structures within colonised plant roots and reduce the time and labour required to stain large numbers of root samples simultaneously. Adaptations were also made to take into account the equipment available. Throughout the procedure, great care was taken to label all pots and boiling tubes with accuracy

and continuity, so that each root sample corresponded to a particular plant and treatment. This was particularly important to enable large numbers of root samples to be stained in batches. Fungal structures were clearly visible in stained roots, as shown in Figs 3.2 and 3.3.

3.2.1 Preparation of the root sample

Fresh root samples were washed in tap water immediately after being removed from the plant, to remove debris attached to the roots. The whole root system or part of the root system was either immediately cleared and stained or, if not used immediately, placed in a 50 mL plastic bottle containing 50% (v/v) ethanol solution for storage. A lid was placed onto each pot. Care was taken to label each pot to correspond to the plant from which the root was removed.

3.2.2 Staining the root sample

3.2.2.i Clearing the roots

The roots were either removed from a fresh plant or removed from storage in 50% (v/v) ethanol. The root sample was placed in a clean glass 9 cm diameter Petri dish and cut into 1–2 cm segments using a scalpel. Forceps were used to place each root sample individually into a boiling tube containing approximately 30 mL of 3% (w/v) potassium hydroxide solution. This was repeated for each root sample. All samples were then left in the boiling tubes at room temperature for 24–48 h, or until the root cortex had cleared sufficiently such that the stele was visible in the centre of each root segment. The contents of a boiling tube were poured into a sieve containing a mesh of pore size 35 μm diameter. The sieve was rinsed under running, cold tap water for 1 min to remove all traces of the potassium hydroxide. This was repeated for each root sample.

3.2.2.i Acidification of fungal structures

Immediately after rinsing with water, a glass rod with a finely rounded tip was used to transfer the root sample back into its original sample pot from which it had been removed. This pot contained 20 mL of 1% (v/v) hydrochloric acid solution. The roots

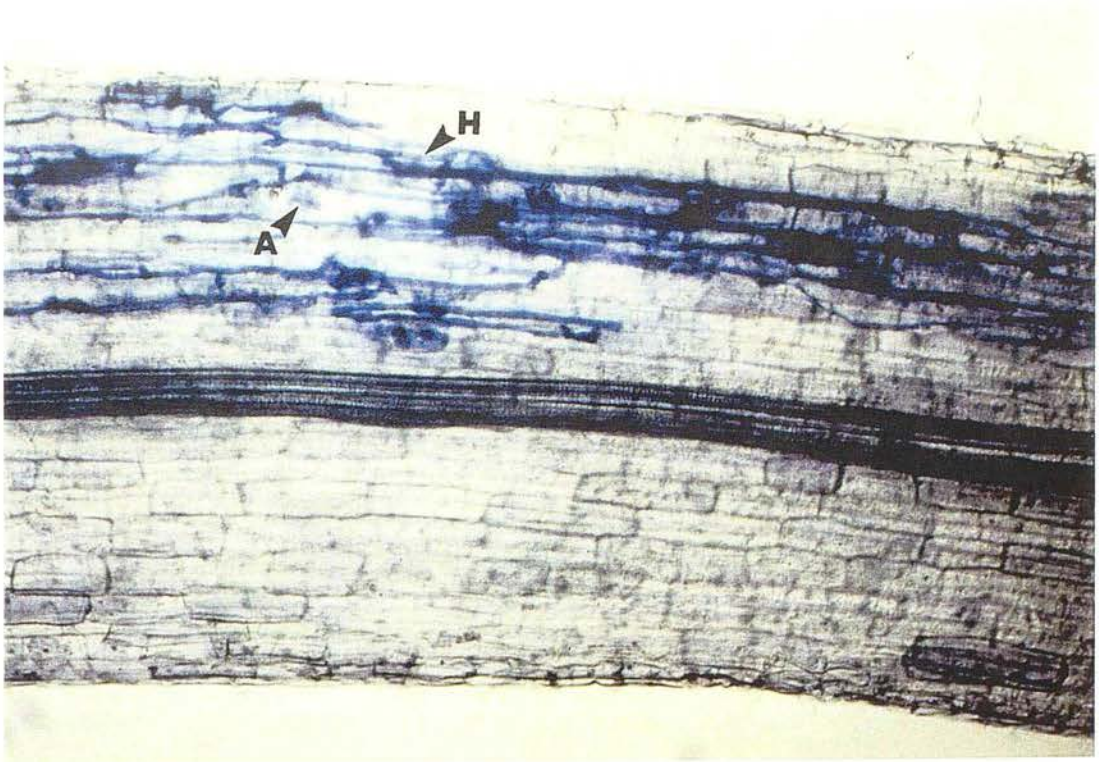

Fig. 3.2. Elsanta root stained with trypan blue to show colonisation by the arbuscular mycorrhizal fungus *G. intraradices*. Inter- and intracellular hyphae (H) are visible. Arbuscles (A) are visible in cortical cells. (Microphotograph ×125).

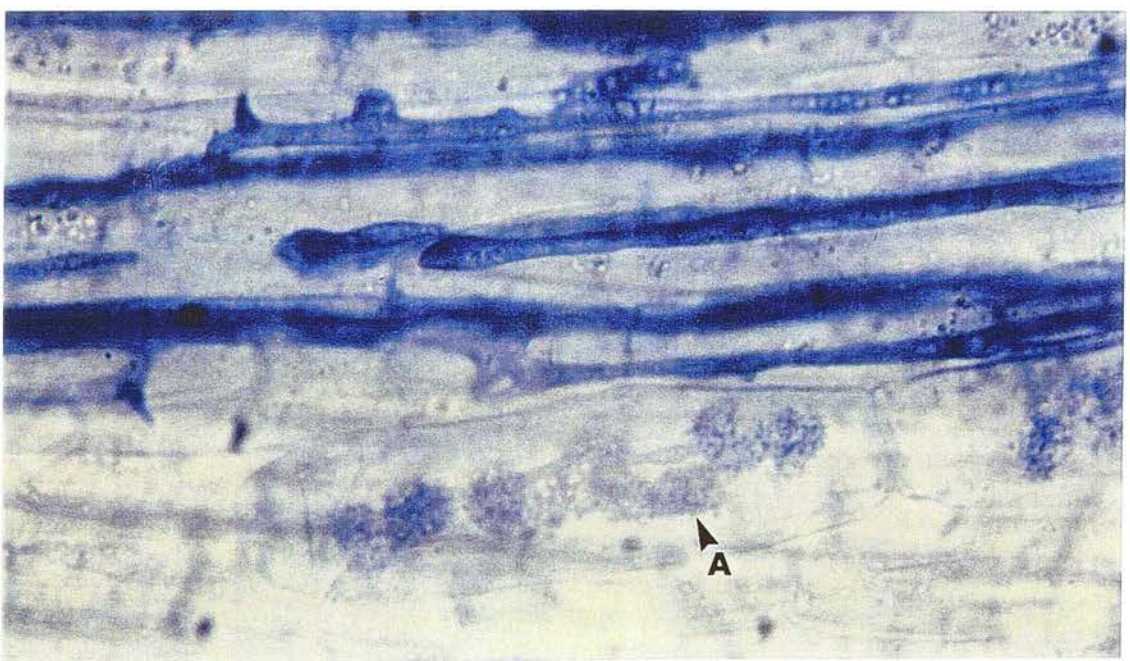

Fig. 3.3. Elsanta root stained with trypan blue to show colonisation by the arbuscular mycorrhizal fungus *G. intraradices*. The heavily branched arbuscular structure is apparent (A), together with the intracellular hypha which enters the cell before forming an arbuscle. (Microphotograph ×500).

were allowed to stand in this solution at room temperature for at least 48 h. This time period was required to acidify the fungal structures to ensure that they would stain with trypan blue.

3.2.2.iii Staining with trypan blue

The 1% (v/v) hydrochloric acid was carefully poured from each pot, leaving the root sample in the bottom of the container. A piece of mesh was placed over the opening of the pot so that any roots which were poured out with the acid could be returned to the pot. Without rinsing the roots, the glass rod was used to transfer each root sample into a boiling tube containing 40 mL of an acidic glycerol/trypan blue solution (500 mL of glycerol, 450 mL of distilled water, 50 mL of 1% (v/v) hydrochloric acid, placed in a 2 L conical flask to which 0.05% trypan blue was added, before mixing).

Each boiling tube was carefully numbered and the number copied onto the appropriate plastic pot from which the root sample had been removed. This ensured that the correct root sample could be returned to each pot after staining. The boiling tubes were placed in numerical order in a rack and placed in a water bath at 90°C for 30 min. After this time, the rack was removed from the water bath and the tubes were allowed to cool for 5 min. A piece of 35 μm diameter mesh was placed over the opening of each boiling tube and the trypan blue solution was then poured off each boiling tube into a glass beaker. The root sample was held in the mesh and the glass rod was used to place the roots into the original, labelled plastic pots containing 30 mL of destaining acidic glycerol solution. This destain solution was identical in composition to the staining solution but without the addition of trypan blue. The root samples were stored in the dark at room temperature until they were required.

3.3 Extraction of spores of arbuscular mycorrhizal fungi

The arbuscular mycorrhizal fungi sporulated when grown under appropriate conditions, colonising the roots of host plants in pot cultures. A reliable method to extract spores from the growth medium of these pot cultures was provided by Dr Walker, as a modification of a density-gradient centrifugation technique.

Approximately 20 g of growth medium was removed from the rhizosphere of a pot

culture and placed in a plastic 1 L beaker. The beaker was filled with tap water and a metal spatula used to vigorously stir the contents. The contents were left to settle for no more than 5 s, to allow sand particles to sink to the bottom of the beaker. The solution was then poured off into a sieve with pores 35 μm in diameter. Any aggregations of peat or substrate were pressed with the back of the spatula to disperse the particles. The contents of the sieve were poured through a plastic funnel into a 50 mL centrifuge tube composed of transparent plastic. A volume of 10 mL of 50% (w/v) sucrose solution was added to the bottom of the centrifuge tube using a 50 mL syringe to which a 20 cm length of flexible, plastic tubing had been attached.

Two centrifuge tubes were prepared simultaneously and each was weighed, with its lid, and distilled water was added until the weights of the tubes were within 0.5 g of each other. The tubes were placed opposite each other to balance the centrifuge (Centaur 2E MSE) and centrifuged at 2000 revolutions per minute for 5 min. The tubes and lids were then removed from the centrifuge.

The syringe with tubing attached was inserted into each tube and the plunger withdrawn to remove the layer of spores and root fragments which accumulated at the interface between the sucrose solution and the water. Debris observed at the surface of the water was composed of light organic matter and dead mycorrhizal spores. A layer of sand particles and peat was visible at the bottom of the centrifuge tube. The spores and root fragments, which were removed using the syringe, were transferred into a 50 μm sieve and rinsed with tap water to remove any traces of the sucrose solution. The contents of the sieve were poured into the lid of a 5 cm diameter Petri dish, using a wash bottle containing tap water to rinse the material into the dish.

3.3.1.i Storage of extracted spores

Spores of each mycorrhizal species were stored in batches of 100 in sealed glass bottles containing 1 mL of distilled water. Each bottle was labelled and stored at 4°C until required, for up to a period of 2 months.

Fig. 3.4. Spores of *G. mosseae* attached to subtending hyphae. The spore contents can be observed. The spores were extracted from the sand substrate of a pot culture. (Microphotograph ×312).

3.3.2 Special requirements for particular mycorrhizal spores

The spore extraction technique successfully removed spores from a sample of substrate from a pot culture. However, it was found that extraction of spores of particular mycorrhizal species required additional techniques to be employed. For example, spores of *G. mosseae* were found to be produced in a sporocarp which is an additional wall enclosing the spore. Debris was found to attach to this wall and the whole wall had to be removed from each spore for the purposes of observation and electrophoresis using the spores. Therefore extraction of these spores was made more time-consuming by the need to use two pairs of fine forceps to remove the sporocarp from each spore. It was found that certain arbuscular mycorrhizal species tended to sporulate within pieces of organic matter. Therefore it was useful to thoroughly agitate the substrate in the sieve to dislodge any spores from inside fragments of organic matter. This was particularly important for spores of *G. intraradices*.

3.4 Analysis of isozymes extracted from arbuscular mycorrhizal spores

This work was based on techniques described by Hepper *et al.* (1988) and Sen and Hepper (1986). Additions to these methods were provided by Dr C. Walker (personal communication).

3.4.1 Sample preparation

3.4.1.i Sample buffer

Fifty millilitres of distilled water was measured into a 100 mL conical flask. The quantity of each compound shown in Table 3.2 was added to the flask.

Table 3.2. Composition of sample buffer.

Compound	Quantity in 50 mL of sample buffer
Tris-HCl	0.158 g
Sodium hydrogen carbonate ($NaHCO_3$)	0.042 g
Magnesium chloride ($MgCl_2$)	0.102 g
Dithiothreitol (DTT)	0.077 g
Ethylenediamine tetraacetic acid Ferric–Sodium salt (FeNA–EDTA)	0.002 g
Sucrose	7.5 g
Bromophenol Blue	0.001 g
Triton X-100	7.5 µL

The buffer was kept on ice at 0°C at all times if it was being used immediately. Alternatively, 400 µL samples of buffer were dispensed into autoclaved 1.5 mL Eppendorf tubes and stored in a freezer at –20°C until required. Storage was limited to 1 month because the activity of the buffer was found to be lost after longer periods of storage in these conditions.

3.4.1.ii Removal of spores from water

Spores were extracted from pot culture substrate and stored at 4°C. A filter unit was

assembled and attached to a vacuum pump (Whatman). A sterile cellulose nitrate filter membrane of 47 mm diameter and a pore size of 0.45 μm (Millipore) was placed into the filter unit and the contents of a single bottle containing spores was carefully poured onto the membrane. Distilled water was used to wash any remaining spores from the bottle onto the membrane.

The lid was replaced onto the filter unit and the vacuum pump switched on to draw the water through the membrane, leaving the spores on the surface of the membrane. The filter unit was then opened and forceps used to lift the membrane into the lid of a 5 cm diameter Petri dish. Each dish was then left on the bench to allow the remaining water to evaporate, leaving the spores to dry on the surface of the membrane. This technique ensured that the spores could be transferred into the sample buffer with minimum transfer of water adhering to the spores or the forceps. The transfer of even small amounts of additional water with wet spores was found to severely adjust the concentration of spores in the sample buffer and was therefore to be avoided.

3.4.1.iii Preparation of spore extracts

Spore samples were transferred into sample buffer to which 10% (v/v) glycerol (electrophoresis grade) had been added. The glycerol was added immediately prior to using the sample buffer, which was then kept on ice at 0°C. A volume of 10 μL of the sample buffer with 10% glycerol was then pipetted into an autoclaved Eppendorf tube and placed in ice. A filter membrane with spores on its surface was observed at a magnification of ×40 using a binocular microscope. Fine forceps were used to transfer 40 spores into the Eppendorf tube. The forceps were wiped with 70% (v/v) ethanol which was then allowed to evaporate between samples. This process was repeated using the appropriate spore samples until the required number of Eppendorf tubes were obtained. All tubes containing sample buffer were stored on ice.

The appropriate safety precautions (i.e. use of goggles, 2 pairs of surgical gloves, long-handled pair of metal forceps) were employed while the base of a single Eppendorf tube was dipped into the surface of a flask containing liquid nitrogen (N_2) for 30 s. This time period was sufficient for the contents of the Eppendorf tube to be frozen. An autoclaved pipette tip was then used to transfer 10 μL of sample buffer

containing 10% glycerol into the frozen Eppendorf tube. The contents of the tube were mixed using a mini-homogeniser, crushing the spores into the sample buffer. The tube was returned to the ice at 0°C. The number of spores per sample was varied.

The tip of both the pipette and the mini-homogeniser was replaced with a sterile tip before repeating the process with the next Eppendorf tube, until all the required spore samples had been crushed. All the Eppendorf tubes were then centrifuged in a mini-centrifuge (Biofuge 13 Heraeus) at 5000 revolutions per minute for 25 s to ensure that all of the buffer had returned to the bottom of each tube. All spore extracts were kept on ice until they were loaded onto the gels.

3.4.3 Electrophoresis conditions

3.4.3.i Gels

Mini-Protean®II Ready Gels (polyacrylamide) were used (Bio-Rad). Either 12% Tris-HCl gels or gradient gels of 4–20% Tris-HCl were used.

3.4.3.ii Running buffer

The buffer in which the electrophoresis was performed was prepared by dissolving 14.4 g glycine and 3.03 g Tris base in 1 L of distilled water. Drops of 1 M hydrochloric acid were added until the pH of the buffer was adjusted to 8.3.

3.4.3.iii Running conditions

The gels were subjected to a voltage of 200 V for 30 min.

3.4.4 Isozyme stains

3.4.4.i α and β esterase (EST)

A volume of 10 mL of 1 M phosphate buffer at pH 6.2 was placed into a 250 mL conical flask to which 90 mL of distilled water was added. A small glass bottle containing 2 mL of acetone was used in which to dissolve 0.04 g of α-naphthyl acetate and 0.04 g of β-naphthyl acetate. Immediately before adding the stain to a gel, 0.1 g of Fast Blue RR salt was added to the flask. The flask was swirled to mix the stain which was then filtered through one layer of tissue paper into a conical flask wrapped in aluminium foil. This filtering operation was performed in the dark. The filtered stain

was immediately added to a gel. If isozyme activity was present, black bands were visible in the gel within 30 min of incubation at room temperature.

3.4.4.ii Glucose-6-phosphate dehydrogenase (GPD)

A volume of 100 mL of 0.1 M Tris at pH 7.5 was placed into a glass conical flask to which 1 mL of 1 M $MgCl_2.H_2O$ was added. Additions of 0.04 g of glucose-6-phosphate and 0.02 g of β-NAD sodium salt were made to the flask, which was wrapped in aluminium foil. Immediately before adding the stain to a gel, 0.02 g of NBT and 0.004 g of PMS were added to the flask and swirled to mix.

3.4.4.iii Glutamate oxaloacetate transaminase (GOT)

A conical flask containing 100 mL of distilled water was used in which to dissolve 0.073 g of α-ketoglutaric acid, 0.266 g of L-aspartic acid, 0.1 g of EDTA, 3.74 g of Na_2HPO_4 and 1.0 g of PVP-40. The pH was adjusted to 7.4, and 50 mL of this solution was placed into a flask wrapped in aluminium foil. Immediately before adding to a gel, 0.05 g of Fast Blue RR salt was placed into the flask and mixed into the stain. The gel was incubated at 37°C in the dark for 1 h or overnight to allow dark bands to form in the gel if isozyme activity was present.

3.4.4.iv Malate dehydrogenase (MDH)

DL-Malic acid (0.6 g) was added to 10 mL of 0.5 M Tris at pH 6.8 in a conical flask. Distilled water (80 mL) was added and the pH adjusted to 8.0 by adding drops of 1 M potassium hydroxide. The solution was made up to 100 mL with distilled water and the flask was wrapped in aluminium foil. Immediately before adding the stain to the gel, 0.02 g of NAD sodium salt, 0.02 g of NBT and 0.02 g of PMS was added to the flask. The gel was incubated at 37°C in the dark for 1 h or overnight to allow purple bands to form in the gel if isozyme activity was present.

3.4.4.v Phosphoglucomutase (PGM)

A volume of 10 mL of 1 M Tris at pH 7.5 was placed in a conical flask to which 2 mL of 1 M $MgCl_2$ was added. Additions of 0.160 g of α-D-glucose-1-phosphate and 0.06 g of β-NADP sodium salt (hydrated) were made to the flask which was wrapped in aluminium foil. Immediately before adding the stain to a gel, 0.02 g of NBT and 0.012 g PMS were added to the flask and mixed. The gel was incubated at 37°C in the

dark for 1 h or overnight to allow dark bands to form in the gel if isozyme activity was present.

3.5 Micropropagation of strawberry plants

Conventional methods of plant tissue culture were used to micropropagate two strawberry cultivars (Boxus, 1974; 1981).

3.5.1 Strawberry cultivars

Two strawberry cultivars were selected which had different properties of growth habit, yield and disease resistance (ADAS, 1989). The cultivars were Elsanta and Rhapsody. Table 3.3 illustrates the major properties of each cultivar.

3.5.2 Preparation of strawberry micropropagation growth medium

The medium which encouraged proliferation of the micropropagated strawberry plants was Murashige and Skoog Basal Salts with Minimal Organics (MSMO; Sigma). This was prepared in batches of 3 L which was sufficient to fill approximately 70 sterilised jars with approximately 60 mL of proliferation medium. Sufficent MSMO (13.62 g) to make 3 L of medium was placed in a conical flask containing 2 L of distilled water. A stirrer bar was placed in the flask and the contents were stirred using a magnetic stirrer. Sucrose was added to the medium at a concentration of 30 g L^{-1}. Therefore, 90 g of sucrose was added to the conical flask. Then 40 mg L^{-1} ethylenediaminetetraacetic acid ferric–sodium salt (EDTA–FeNa) was added to the flask, i.e. 120 mg per 3 L of MSMO. Plant growth regulators were added to this medium to prevent formation of roots and allow proliferation of shoots from the plants.

3.5.2.i Addition of plant growth regulators

The plant growth regulators indole-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) were required in the proliferation medium at a concentration of 1 mg L^{-1}. Twenty milligrammes of IBA were placed into a glass Universal bottle containing 2 mL of 1 M sodium hydroxide, and the bottle gently agitated until the chemical dissolved. The contents of the bottle were poured into a 25 mL measuring cylinder and made up to a volume of 20 mL using distilled water. One millilitre of the IBA

Table 3.3. Properties of the strawberry cultivars Elsanta and Rhapsody.

Feature	Elsanta	Rhapsody
Origin	Institute for Horticultural Plant Breeding, Wageningen, The Netherlands	Scottish Crop Research Institute, Invergowrie, Dundee, Scotland
Fruiting season	Mid-season	Late-season
Appearance	Medium vigour with semi-erect habit. Large leaves	Medium vigour. Pale green 'crinkled' leaves with lighter inter-veinal areas
Runner production	Poor	Good
Disease resistance	Very susceptible to red stele disease	Resistant to red stele disease
Fruit	Large, firm berries, glossy red colour	Large, conical fruit, glossy red colour
Uses	High quality dessert fruit	Dessert variety, particularly grown in Scotland and northern Britain.

solution was added per litre of MSMO medium, i.e. 3 mL of IBA solution was added to the conical flask. This process was repeated to prepare the BAP which was also added to the conical flask at the same concentration and volume.

3.5.2.ii pH of the proliferation medium

A pH electrode was placed in the conical flask to measure the pH of the medium. Drops of 1 M sodium hydroxide were added until the pH was between 5.5 and 5.7.

3.5.2.iii Addition of agar to the proliferation medium

A volume of 1 L of distilled water was poured into an Agarmatic in which the proliferation medium was to be sterilised. Davis standard agar was added at a concentration of 6 g L^{-1}. Therefore 18 g of Davis standard agar was added to the Agarmatic.

3.5.2.iv Sterilisation of proliferation medium

The contents of the conical flask were poured into the Agarmatic which was then switched on and the proliferation medium was sterilised at 121°C for 20 min. The sterilised medium was then held at a temperature of 60°C until it was dispensed into the sterilised jars. The proliferation medium was dispensed from the Agarmaticinto the sterilised jars in a laminar flow cabinet via a Peristaltic pump. Sufficient proliferation medium was dispensed into each jar to a depth of approximately 1.5 cm.

3.5.2.v Preparation of rooting medium

Rooting medium consisted of MSMO without the addition of plant growth regulators, but with the addition of sucrose, EDTA–FeNa, and Davis standard agar at the concentrations described above. Therefore rooting medium was prepared using the same technique as for proliferation medium but with the absence of plant growth regulators.

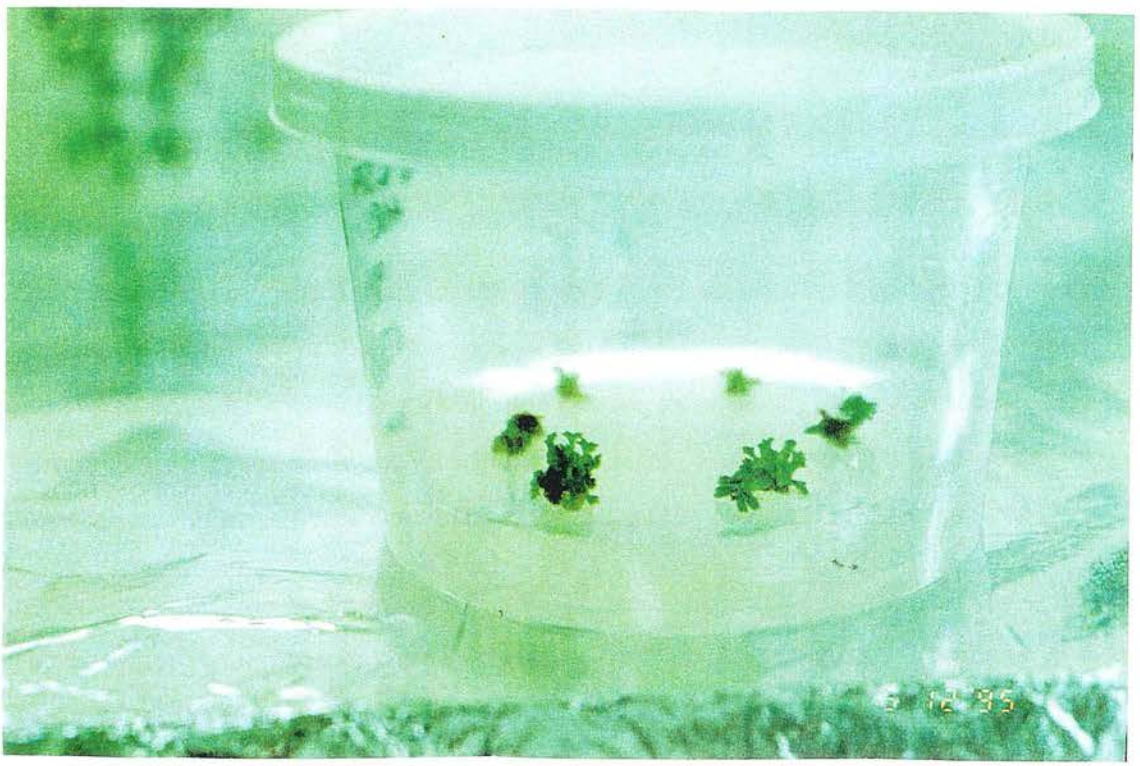

Fig. 3.5. Micropropagated Elsanta plants grown in proliferation medium for a period of approximately 4 weeks. Each plantlet has produced a number of shoot apices that can then be divided under aseptic conditions and inserted into a fresh container of proliferation medium in order to propagate the plants.

3.5.4 Micropropagation of plant material

3.5.4.i Initiation of meristem cultures

Meristem cultures were initiated from micropropagated plants which had been subcultured several times in tissue culture. It has been reported that subculture of micropagated strawberry plants more than 10 times can result in deleterious physiological changes in the plants, including early senesence (Rancillac & Nourrisseae, 1989). Therefore, the number of subcultures was limited to 10 throughout this study.

3.5.4.ii Excision of plant meristems

The plant which was to be excised was removed from its tissue culture jar and placed in a sterile Petri dish lid under a dissecting microscope. A sterilised scalpel blade and forceps were used to cut away the shoots from the plant until only the main, central shoot remained. The leaves were carefully stripped away until the central meristem was covered only by one layer of leaf material. It was possible to remove this layer but this did risk damaging the meristem and was not necessary. The plant would regenerate from the meristem with some leaf layers still attached to it and it was thought to be unecessary to risk damaging the meristem to remove all leaf material from it.

Sterile forceps were used to transfer the excised meristem into a sloped Universal tube containing 10 mL of proliferation medium. All tubes containing meristems were allowed to grow under lights at 22°C for 6 weeks, after which time new shoots were visible. These plantlets were labelled subculture 1 and were then divided for propagation. All procedures were performed aseptically in a laminar flow cabinet.

3.5.4.iii Proliferation and rooting

The strawberry microplants were allowed to grow on proliferation medium for 4–6 weeks by which time a cluster of new shoots had proliferated from the original plant (see Fig. 3.5 above). This cluster was removed, under aseptic conditions in a laminar flow cabinet, and placed into a sterile Petri dish. Sterilised forceps were then used to divide the cluster into individual shoot apices. The leaves were trimmed from each shoot apex to encourage further proliferation of shoots rather than expansion of the

leaves. Each shoot apex was then transferred into a jar containing proliferation medium. A total of six such shoots were placed in each jar and labelled as the second subculture. The number of the subculture was increased by one each time the micropropagated plants were subcultured onto either proliferation or rooting medium. The plants were allowed to grow at 22°C for 4–6 weeks, by which time they would be ready for subculture again.

After 4–6 weeks of growth on proliferation medium, the microplants were of sufficient size to be subcultured onto rooting medium to encourage the formation of roots. Each cluster of plant was removed from the proliferation medium under aseptic conditions and divided into shoot apices. In this case the leaves were not trimmed from each shoot and the larger shoots were preferentially transferred into jars containing rooting medium. The transferred plants were labelled as the following subculture and placed in the culture room for a period of 4–6 weeks. After this time, roots had developed from the micropropagated plants and they could be removed from tissue culture conditions for weaning (see Fig. 3.6).

3.5.5 Transfer of micropropagated material to the glasshouse

Plastic pots containing the appropriate growth substrate were watered thoroughly and allowed to stand in the glasshouse for 24 h. A planting hole was then made in the centre of each pot. The contents of a jar containing rooted micropropagated plants were tipped into a bowl containing tepid water (cold water provides a too rapid change of environment for the delicate plantlets). The agar was removed from the plant roots and each plant was placed into the planting hole of a plant pot. The growth medium was pressed around the base of the plant and a fine mist of water was sprayed over the surface of the plant. Subsequently, the plants were watered using a hosepipe and a weak jet of water.

3.5.5.i Application of fungicide

A solution of Rovral-flo was prepared at a concentration of 2 mL L^{-1} distilled water in a pump-action spray bottle. Each plant received a liberal spray of this fungicide immediately after transplantation from tissue culture, whilst the operator took care to avoid contact with the fungicide (rubber gloves, wellington boots and goggles were

worn). The incidence of mould resulting from colonisation by the fungal pathogen *Botrytis cinerea* was successfully prevented by the application of this fungicide.

Fig. 3.6. Micropropagated Elsanta plants showing both shoot and root growth. The plants had been grown in the proliferation medium for approximately 6 weeks before being transferred to rooting medium. These rooted plantlets are suitable for transfer to a glasshouse environment.

3.5.5.ii Weaning of micropropagated material

The pots containing transplanted strawberry plants were placed in trays which were covered with transparent lids with ventilation holes. The pots were kept in the glasshouse with average conditions of 18°C ± 3°C, with a 16/8 h alternating light/dark period. The ventilation holes were closed for the following 4 days to allow 100% humidity to be achieved around the fragile transplanted material. After this time, the ventilation holes were opened for a period of 4 days and then the transparent covers were removed to progressively reduce the humidity to ambient levels and gradually acclimatise and wean the plants.

3.6 Determination of shoot dry weight (SDW)

The appropriate number of glass 90 mm diameter Petri dishes were labelled with permanent marker and individually weighed so that one labelled Petri dish of known weight was available for each shoot sample. The weight of each dish was recorded. The contents of a plastic bag containing an entire shoot sample were placed into the Petri dish with the corresponding label and the weight of the dish and shoot sample was recorded. This was repeated for each shoot sample. The dishes and shoots were placed in an oven (Gallenkamp Hotbox) at 100°C for 48 h. The dishes were then weighed and the weight of each sample was recorded. The shoot samples were returned to the oven for a further 24 h and then weighed again. The shoot samples were returned to the oven for further drying until the weight remained constant, i.e. all of the water had been dried from the shoot sample. The dry weight of each shoot sample was then calculated by subtracting the original weight of the Petri dish from the weight of the dish and the dried shoot sample. The dry weight of each shoot sample was then recorded.

3.7 Determination of root dry weight (RDW)

Each root sample was first stained to observe mycorrhizal colonisation and assessed for the percentage of colonisation before the sample was dried. The root samples were held in plastic pots containing acidic glycerol destaining solution. This solution was quite viscous and adhered to the roots. Therefore each root sample was washed prior to determining the dry weight.

The root sample was poured from the plastic pot into a 35 μm sieve. The sieve was held under running, tepid tap water and the root sample was swirled around. The sample was then placed into a labelled glass Petri dish, the weight of which had been recorded. The dish and root sample were placed in the oven (Gallenkamp Hotbox) at 100°C and the dry weight of each root sample was determined by weighing the dish and root sample combined again, in a similar method to the shoot dry weight.

3.8 Determination of the phosphorus content of shoots

The dry weight of the strawberry shoots was first determined before they were analysed for phosphorus.

3.8.1 Reagents for phosphorus analysis

3.8.1.i Phosphorus standard solution

A quantity of 0.4394 g of pure dry potassium hydrogen phosphate (KH_2PO_4) was placed in a conical flask containing 1 L of distilled water. A volume of 50 mL of this solution was placed in a 250 mL conical flask to which 150 mL of distilled water was added. The concentration of the phosphorus standard solution was 0.025 mg mL^{-1} of phosphorus. The solution was stored in a stoppered bottle at room temperature until required.

3.8.1.ii Ammonium molybdate solution

A total of 25 g of ammonium molybdate were dissolved in 300 mL of distilled water in a 1 L conical flask. Next, 75 mL of concentrated sulphuric acid was placed into a 500 mL conical flask to which 125 mL of distilled water was added. The sulphuric acid solution was added to the ammonium molybdate solution and stored in a 1 L glass bottle until required.

3.8.1.iii Hydroquinone solution

Hydroquinone (0.5 g) was dissolved in 100 mL of distilled water in a 250 mL bottle. One drop of concentrated sulphuric acid (H_2SO_4) was added to the bottle to prevent oxidation. A lid was placed tightly onto the bottle until the solution was required.

3.8.1.iv Sodium sulphite solution

A total of 200 g of sodium sulphite (Na_2SO_3) was dissolved in 200 mL of distilled water, before mixing the solution in a 1 L conical flask containing 800 mL of distilled water. The solution was stored in a glass bottle with a tightly sealed lid and was prepared fresh for each experiment.

3.8.1.v Magnesium nitrate solution

A total of 950 g of phosphorus-free magnesium nitrate hexahydrate ($Mg(NO_3)_2.H_2O$) was dissolved in 1 L of distilled water in a conical flask. The solution was stored in a glass bottle until required.

3.8.2 Preparation of shoot sample

A shoot sample of known dry weight was removed from the glass Petri dish into a silica crucible. Each crucible was labelled with a number, which was recorded, and placed on a metal grid positioned directly above a water bath at 90°C. The water bath was placed in a fume hood. Sufficient crucibles were placed on the grid so that they covered the surface of the water bath, and 1 mL of magnesium nitrate solution was added to each crucible. After 3 min, two or three drops of concentrated hydrochloric acid were added to each crucible.

Further additions of both magnesium nitrate and hydrochloric acid were made while the crucible was on the water bath, until the shoot sample began to char and turn brown. The crucible was removed using metal tongs and placed in an oven at 100°C before being transferred to the furnace. This was repeated for all shoot samples before placing the samples in the furnace.

3.8.2.i Reduction of shoot sample to ash

Each crucible was placed inside a furnace (Gallenkamp) which was heated to 500°C. The samples were subjected to this temperature overnight for a period of 16 h. This was sufficient to reduce the contents of each crucible to grey ash. The furnace was switched off and allowed to cool slightly. The crucibles were carefully transferred from the furnace into an oven (Gallenkamp Hotbox) at 100°C where they were stored until the phosphorus analysis was performed.

3.8.2.ii Acidification of sample

A digital balance was calibrated to zero against a 100 mL glass beaker which was labelled to correspond to that of the crucible. The contents of a crucible were scraped into the glass beaker and the weight of the sample was recorded. A volume of concentrated hydrochloric acid equivalent to four times the weight of the sample was added to the beaker. The beaker was placed on a metal grid positioned over a water bath at 90°C in a fume hood. Concentrated hydrochloric acid (5 mL) was added to the beaker, which remained on the steam bath until the acid had evaporated. This ensured dehydration of any silicon dioxide that was present.

The residue was moistened with 2 mL of concentrated hydrochloric acid and 50 mL of distilled water was added to the beaker. After a few minutes of heating on the steam bath, the contents of the beaker were poured into a 100 mL volumetric flask with washings. The flask was placed into a cold-water bath to cool the contents. The volume of the flask was then made up to 100 mL with distilled water. The flask was mixed and filtered through a filter paper (Whatman No. 1) into a labelled 100 mL conical flask.

3.8.3 Determination of phosphorus concentration

A 5 mL aliquot was removed from the filtrate and placed in a 10 mL volumetric flask. A volume of 1 mL of ammonium molybdate solution was added to the flask which was rotated to mix and left to stand for a few seconds. A volume of 1 mL of hydroquinone solution was added and the flask again mixed and left to stand for a few seconds, before 1 mL of sodium sulphite solution was added to the flask, and the volume made up to 10 mL with distilled water. The flask was stoppered and inverted three times to mix. The flask was allowed to stand for at least 30 min to allow the blue colour to develop. The absorbance of the solution was then measured at 650 nm using a spectrophotometer (Beckman DU®-65).

3.8.3.i Determination of a phosphorus standard curve

The phosphorus standard solution was serially diluted using distilled water. The 100% standard solution had a concentration of 0.025 mg mL^{-1}. One millilitre of this solution was placed in a 10 mL volumetric flask containing 9 mL of distilled water, in order to produce a 10% solution. This was repeated using the volumes of the phosphorus standard solution and distilled water shown in Table 3.4, to generate a series of dilutions of the standard. The absorbance of each solution was measured at 650 nm.

3.8.3.ii Calculation of phosphorus concentration (mg g^{-1} dry weight of shoot)

Regression analysis was used to calculate the intercept and the gradient of the graph, which were –0.00955 and 53.5818, respectively. The equation below was used to calculate the phosphorus concentration (mg mL^{-1}) of the solution resulting from each shoot sample.

$$y = mx + c$$

where y is the absorbance of the solution at 650 nm; x is the phosphorus concentration of the solution (mg mL^{-1}); m is the gradient of the graph; and c is the intercept on the y-axis. This equation was rearranged such that:

$$x = (y - c)/m$$

This enabled the phosphorus concentration (mg g^{-1} SDW) in the solution to be determined. This value was multiplied by 20 to give a value of the total phosphorus (mg) in the shoot sample and then divided by the shoot dry weight of the sample to obtain the phosphorus concentration in the shoot (mg g^{-1} SDW).

A phosphorus standard curve was constructed as shown in Fig. 3.7.

Table 3.4. Concentration and absorbance of diluted phosphorus standard solutions.

Standard solution (mL)	Distilled water (mL)	Concentration of phosphorus solution (mg mL^{-1})	Absorbance
0	10	0.0000	0.000
1	9	0.0025	0.110
2	8	0.0050	0.205
3	7	0.0075	0.386
4	6	0.0100	0.515
5	5	0.0125	0.637
6	4	0.0150	0.819
7	3	0.0175	0.930
8	2	0.0200	1.051
9	1	0.0225	1.174
10	0	0.0250	1.226

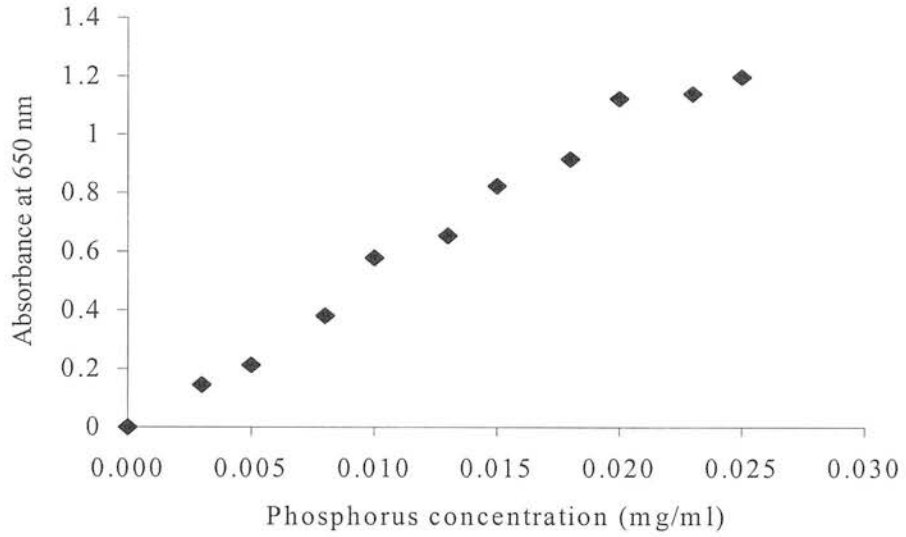

Fig. 3.7. The absorbance of the phosphorus standard solution was measured at 650 nm and plotted against the concentration of phosphorus in the solution.

3.9 Culture of *Phytophthora fragariae*

Phytophthora species require sterols in order to undergo sexual or asexual sporulation. However, the fungi themselves are incapable of producing sterols, which must therefore be provided in the growth medium if sporulation to produce zoospores is required. Sterols were provided by the presence of vegetable extract, in this case French bean extract, in the agar medium.

3.9.1 Strains of *P. fragariae*

Three strains of the fungus were obtained from Dr Diana Kennedy, formerly of the Scottish Crop Research Institute, Dundee. The strains used had been designated 171, 293 and 372, and had previously each been assigned to a different race of *P. fragariae*, as described by Kennedy & Duncan (1988) and Montgomerie (1966). The strains were selected because of their different virulences to the strawberry cultivars used in the experiments. Virulence was assessed by a disease score on a scale of 1 to 5, with 5 being the greatest level of disease, as shown in Table 3.5. This information was provided by Diana Kennedy (personal communication).

Table 3.5. Disease scores on Elsanta and Rhapsody by strains of *P. fragariae*.

Isolate	Race	Elsanta	Rhapsody
171	11A	5	1.3
293	11B	5	3
372	9	5	3.

3.9.2 Preparation of agar growth medium

3.9.2.i French bean extract

A kitchen knife was used to trim both ends of the bean from 500 g of dwarf French beans in their own, unsalted juice (Sainsburys). The remaining beans were cut into 2 cm lengths and placed in a saucepan with 1.5 L of distilled water. The pan was heated until the water boiled, and the contents were simmered for 20 min. The pan was removed from the heat and a pestle used to mash the beans into the remaining liquid. The beans and water were poured into a 2 L conical flask, through a large glass funnel lined with a 0.5 m^2 piece of muslin. Cold distilled water was added to the beans and compressed through the muslin, into the glass funnel until the final volume of the French bean extract was 1.5 L. The flask was rotated to mix the bean extract before pouring the liquid into three glass bottles. The bottles were autoclaved at 121°C for 15 min and the extract allowed to cool before being stored at 4°C until required.

3.9.2.ii Preparation of French bean agar

A volume of 570 mL of distilled water was poured into a 2 L conical flask. Davis standard agar (11.4 g) was added to produce a 2% (w/v) solution. A total of 430 mL of autoclaved French bean extract was added and the flask autoclaved. The agar medium was dispensed, inside a laminar flow cabinet, into 5 mm and 9 mm diameter sterile Petri dishes, and into 30 mL sterile bottles. The bottles were laid on their side around the rim of a plastic tray until the agar solidified to form agar slopes. The filled Petri dishes and bottles were allowed to stand until the agar had solidified. If not used immediately, the dishes and bottles were placed in clean plastic bags sealed with tape and stored at 4°C until required.

3.9.2.iii Aseptic transfer of P. fragariae

A scalpel was sterilised by heating until red hot and then plunging into 70% (v/v) ethanol and then used to cut a 0.5 cm^2 piece of agar from the outside edge of a growing colony of the fungus. The agar piece was placed, agar side downwards, onto the surface of a Petri dish or agar slope of French bean agar. Each dish or bottle was carefully labelled with the isolate of *P. fragariae* and the date of culture. Both dishes and bottles were sealed with laboratory film (Parafilm) and placed in an incubator at 20°C in the dark. Petri dishes were stored upside down while the bottles were allowed to stand upright. *P. fragariae* is relatively slow growing; a period of approximately 7 days of growth at 20°C was required for the colony edge to reach the perimeter of a 5 cm diameter Petri dish.

3.10 Production of zoospores of *P. fragariae*

Asexual reproduction of the fungus results in the formation of sporangia terminally on hyphae. A sporangium is a flask-shaped structure inside which motile, biflagellate zoospores are formed. Each zoospore possesses one tinsel and one whiplash flagellum which allow it to move through water. Upon contact with a strawberry root, a zoospore can encyst and germinate to penetrate the root, enabling the fungus to infect the plant. Zoospores are therefore infective propagules of the fungus and were used throughout the project to inoculate strawberry plants with *P. fragariae*. The method of producing zoospores was modified from one provided by Dr Diana Kennedy.

Strains of the fungus were cultured onto French bean agar in 5 cm diameter Petri dishes. The isolates were allowed to grow at 20°C for 7–10 days until the colony had reached the perimeter of the Petri dish. It was observed that isolates 293 and 372 grew at a slightly faster rate than 171; however, all isolates were grown for a maximum of 10 days to achieve sporulation.

3.10.1 Preparation of peat compost

The compost was a mixture of peat, nutrients and lime. A 300 L bale of medium-grade sphagnum peat (Shamrock) was emptied into a mechanical mixer. The compounds shown in Table 3.6 were added to the compost in the mixer. Water was

Table 3.6. Composition of compost.

Compound	Quantity added to 300 L of peat compost (g)
'Frit' (trace elements)	120
Potassium nitrate	120
Ammonium nitrate	120
Superphosphate	450
Garden lime	675
Dolomitic lime	675

added from a hose pipe as the mixer was switched on to ensure that the nutrients were distributed within the compost.

3.10.1.i Compost filtrate

A piece of glass wool was inserted into the neck of a large glass funnel. Peat compost (75 g) was placed into a plastic 1 L beaker. A volume of 750 mL of distilled water was measured into a glass measuring cylinder and a small quantity of this water was poured into the beaker and mixed into the compost using a metal spatula. The damp compost was poured into the funnel, together with most of the distilled water. The remaining water was used to rinse the contents of the beaker into the funnel. The water was then allowed to leach through the compost and collect in a 1 L glass conical flask. Another glass funnel of similar size was lined with two sheets of tissue paper and the flask was rotated to mix the leachate before pouring the solution through the tissue into a clean flask. This removed the majority of the sediment from the compost leachate. The filtrate was collected and stored in glass bottles at 4°C, or was used immediately. The leachate was only stored for the duration of a single sporulation; compost filtrate was prepared fresh for each sporulation.

3.10.3 Production of a suspension of *P. fragariae* zoospores

A sterilised scalpel was used to cut all except the central part of a colony of *P. fragariae* from a 5 cm diameter Petri dish into 1 cm^2 pieces. The pieces of colony from a single Petri dish were placed in a 9 cm diameter glass Petri dish which contained 25 mL of compost filtrate. The colony pieces were allowed to float either

side up in the filtrate. Each glass Petri dish was carefully labelled according to the appropriate isolate of the fungus and placed in an incubator at 13–15°C in the dark.

Twenty-four hours later, each dish was removed from the incubator and the compost filtrate poured off into the sink. A sieve with 2 mm diameter holes was positioned in the sink to catch any colony pieces that fell from the Petri dish. Any such pieces were rinsed with distilled water and replaced into the Petri dish. The sieve was rinsed in hot tap water and scrubbed to remove pieces of mycelium before being allowed to dry. A separate sieve was used for each isolate of the fungus. A fresh volume of 25 mL of compost filtrate stored at 4°C was poured into the dish, which was returned to the incubator.

After a further 24 h, the dishes were examined. At this stage, a build-up of bacteria around the edge of each colony piece was reduced by pouring the entire contents of each dish into the sieve and rinsing the colony pieces with approximately 500 mL of cold (4°C) distilled water. The compost filtrate was again replaced with fresh filtrate from the refrigerator.

Twenty-four hours later, each dish was individually removed from the incubator and placed on the stage of a binocular microscope which was illuminated from above. A magnification of ×40 was used to examine the edges of each colony piece for the presence of flask-shaped sporangia, located on the terminal ends of mycelia. The compost filtrate was poured from each dish and replaced with 25 mL of cold distilled water which had been placed at 4°C the previous day. Each dish was again replaced into the incubator for a further 24 h.

Each dish was then individually removed from the incubator and placed on the stage of a binocular microscope which was illuminated from below. A magnification of ×10 was used to examine the solution for the presence of motile zoospores. Each zoospore was visible as an elongated rod-shaped structure moving quickly in a spiral path. Zoospores are negatively geotropic and were therefore better observed towards the surface of the solution where they tended to accumulate.

The zoospore suspension was carefully poured off through a small glass funnel into a sterile 70 mL plastic bottle with lid, and returned to the incubator. Zoospores begin to encyst if subjected to more than a few minutes at room temperature, or if the suspension is agitated. Therefore, care was taken to pour off the suspension and agitation of the suspension or a prolonged period at room temperature was avoided.

3.10.4.i Estimating the concentration of the zoospore suspension

A zoospore suspension was removed from the incubator and gently stirred using a Pasteur pipette to mix the zoospores which had gathered towards the surface of the suspension. The pipette was used to remove a drop of solution from the centre of the stirred suspension. The drop of the suspension was placed onto each of two grids on a haemocytometer slide, covered with a glass cover slip.

The slide was examined using a stereoscopic microscope at a magnification of ×100. The counting grid was located and the motile zoospores were observed. Their motility renders the zoospores very difficult to count. Therefore the microscope slide was passed six times through a Bunsen flame and then replaced onto the microscope. The heat caused the zoospores to encyst whereby the flagellae were withdrawn and motility ceased. The encystment was observed down the microscope when the warmed slide was returned to the stage. The number of zoospores over the area of 25 large squares on the whole haemocytometer grid was counted. This figure was multiplied by 10^4 to obtain the number of zoospores per millilitre of the suspension. The mean of six replicate counts was used to estimate the concentration of zoospores in the suspension. Care was taken to ensure that the suspension did not remain at room temperature for longer than was necessary to remove drops for sampling, to avoid excessive encystment of zoospores in the suspension.

3.10.4.ii Calculating the dilution of the zoospore suspension

The zoospore suspension was diluted with cold distilled water to obtain the concentration that was required for the particular treatment or experiment. After the concentration of the suspension was known, the required dilution was calculated. Care was taken to ensure that the final diluted solution was not only of the necessary concentration, but that the volume was also sufficient to inoculate all the plants to be treated. For example, each plant was inoculated with 50 mL of zoospore suspension

and each plant was to receive 10 000 zoospores. Therefore, the plant received 50 mL of a zoospore suspension containing 200 zoospores per mL. However, the number of replicate treatments was also taken into account when preparing the zoospore dilution. For example, if six plants were inoculated with 50 mL of 200 zoospores mL^{-1} each, the final zoospore solution would require a volume of 300 mL of 200 zoospores mL^{-1}, i.e. six plants at 50 mL per plant.

3.10.4.iii Motile zoospore inoculum

A sufficient volume of distilled water was stored in conical flasks at 4°C for 24 h to inoculate all the plants or treatments for the experiment. The zoospore suspension, of which the concentration was known, was removed from the incubator at 13–15°C and stirred using a Pasteur pipette to evenly distribute the zoospores in the suspension. The appropriate amount of cold distilled water was measured into a conical flask. The flask was swirled gently to mix the solution and stored at 13–15°C until required. Storage time was kept to a minimum and the zoospore solution was used as soon as possible after it was prepared.

3.10.4.iv Encysted non-motile zoospore inoculum

The concentration of both the zoospore suspension and the final diluted solution was calculated in exactly the same manner as for motile zoospore inoculum. However, a 5 mL aliquot of the original zoospore suspension was placed into a test tube. The contents of each tube were agitated for 1 min using a Whirlimixer. This agitation caused the zoospores to encyst. The correct volume of the encysted zoospore suspension was measured into a conical flask containing cold distilled water. The flask was swirled to mix the contents and stored at 13–15°C until required.

3.11 Inoculation of strawberry plants with zoospores of *P. fragariae*

In all experiments, the strawberry plants were inoculated with either a motile or non-motile, encysted suspension of zoospores of *P. fragariae*. This inoculation was performed in one of two methods; pouring the zoospore suspension over the weaned strawberry plants, or spraying the plant roots with the non-motile zoospore suspension.

3.11.1 Poured inoculation

Micropropagated strawberry plants had been transplanted into plastic 0.5 L volume plant pots prior to weaning in the glasshouse. Each pot containing a single strawberry plant was placed inside a transparent plastic bag with the entrance open at the top. A plant label, providing information concerning the isolate and concentration of zoospores with which the plant was to be inoculated, was placed inside the plant pot.

A conical flask containing the lowest of the appropriate zoospore concentrations was removed from the incubator at 13–15°C. The flask was swirled to mix the contents and 50 mL of the suspension was slowly poured into a clean 50 mL glass measuring cylinder. This 50 mL was poured over the surface of the plant pot inside the plastic bag. The bag was then loosely sealed with a twist-tie, and placed in a growth cabinet at 15°C with 12 h of alternating light and darkness. This method was repeated for all plants being inoculated with that particular zoospore suspension. Further plants were inoculated in the same manner, using the next highest concentration of zoospores of the same isolate of *P. fragariae*. A clean measuring cylinder was used to inoculate plants with a different isolate of the pathogen. Control non-inoculated plants received 50 mL of cold distilled water. All plants were grown in the growth cabinet for 14 days when the results of the inoculation were assessed. The plants did not require watering during this period.

3.11.2 Spray inoculation

Plants were spray-inoculated with a suspension of non-motile encysted zoospores. This method of inoculation was only suitable for non-motile zoospores, because the procedure of shaking the spray-bottle and forcing the suspension through the atomiser aperture would agitate the zoospores to such a degree that they would encyst. A spray-bottle was calculated to deliver water at a rate of 1 $cm^3 s^{-1}$. This was taken into account when the original zoospore suspension was diluted, as the calculation differed from that for a poured zoospore suspension.

Micropropagated strawberry plants were weaned in 0.5 L volume plastic pots in the glasshouse. Each plant was then gently removed from the plant pot by inverting the pot over a tray and allowing the growth substrate to fall onto the tray. The root system

of the plant was gently washed under a stream of cold tap water. The plant was placed onto a paper towel that had been dampened with cold distilled water. The roots were spread out on the towel. A transparent plastic bag and a plant label were labelled with information concerning the particular plant, and the isolate and concentration of *P. fragariae* with which the plant was to be inoculated.

The appropriate isolate and concentration of the pathogen was poured into the spray-bottle. The spray-bottle was shaken to distribute the zoospores and the plant roots were sprayed with the suspension for 2 s, such that the plant received 2 mL of the suspension. The paper towel was wrapped around the plant roots and the whole plant was placed inside the labelled plastic bag. The neck of the bag was loosely sealed. This process was repeated for each plant. A clean spray-bottle was used for each isolate of *P. fragariae*. Control plants received a 2 s spray of cold distilled water.

All of the plants in bags were placed in a growth cabinet at 15°C, with 12 h of alternating light and darkness, for 48 h. Each plant was then removed from the plastic bag and paper towel and replanted into the original, labelled plant pot from which it had been removed. All plants were returned to the growth cabinet for a further 12 days, when they were assessed for any disease resulting from inoculation with the pathogen.

3.12 Assessment of disease caused by *P. fragariae*

The disease resulting from inoculation of strawberry plants with *P. fragariae* was assessed by one of three methods: (i) a disease score resulting from a visual estimate of the percentage of infected roots; (ii) a physical measurement of the percentage of infected roots, to produce a disease score; and (iii) microscopic observation of the root tips to determine the number of oospores of the pathogen in the root tip. All three methods required the plant to be first removed from the growth cabinet and the loosely tied plastic bag. The pot was upturned and the growth substrate tipped into an autoclavable bag, to be autoclaved prior to disposal of the infected substrate. The root system was gently washed under a stream of tap water to remove all traces of the

Table 3.7. Disease scores (Kennedy *et al.*, 1986).

Disease Score	Symptoms
0	No symptoms or a few necrotic tips
1	Necrotic tips plus rotting and red steles < 3% of the adventitous root
2	4–25% rotting and red steles
3	26–50% rotting and red steles
4	51–75% rotting and red steles
5	76–100% rotting and red steles

growth substrate, before being spread out in a metal tray containing a depth of water of 0.5 cm. The water allowed the root system to spread out, while the metal tray enabled a scalpel to be used to cut pieces of root.

3.12.1 Visual assessment of the percentage of infected roots

With the root system spread out, the amount of necrotic, i.e. brown, rotting root was estimated as a percentage of the total root system. The percentage was given a disease score on a scale of 0 to 5, where 0 indicates no symptoms, and 5 indicates 100% infection (Kennedy *et al.*, 1986), as shown in Table 3.7.

3.12.2 Physical measurement of infected root

Vernier calipers were used to accurately measure the length of each main root and its secondary roots, which were added cumulatively to calculate the total length of the root system. The total length of the red stele-infected root was then measured and the percentage of infected root was calculated as:

Percentage of infected root = (length of infected root ÷ total root length) × 100

3.12.3 Determination of the number of oospores per root tip

After the visual assessment and measurement of the percentage of infected root had

been performed, a scalpel was used to cut ten 1.5 cm length root tips from the plant. The root tips were placed on a microscope slide, covered with a cover slip, and observed for the presence of oospores at a magnification of ×100 using a stereoscopic light microscope. Oospores were clearly visible as spherical structures within the root.

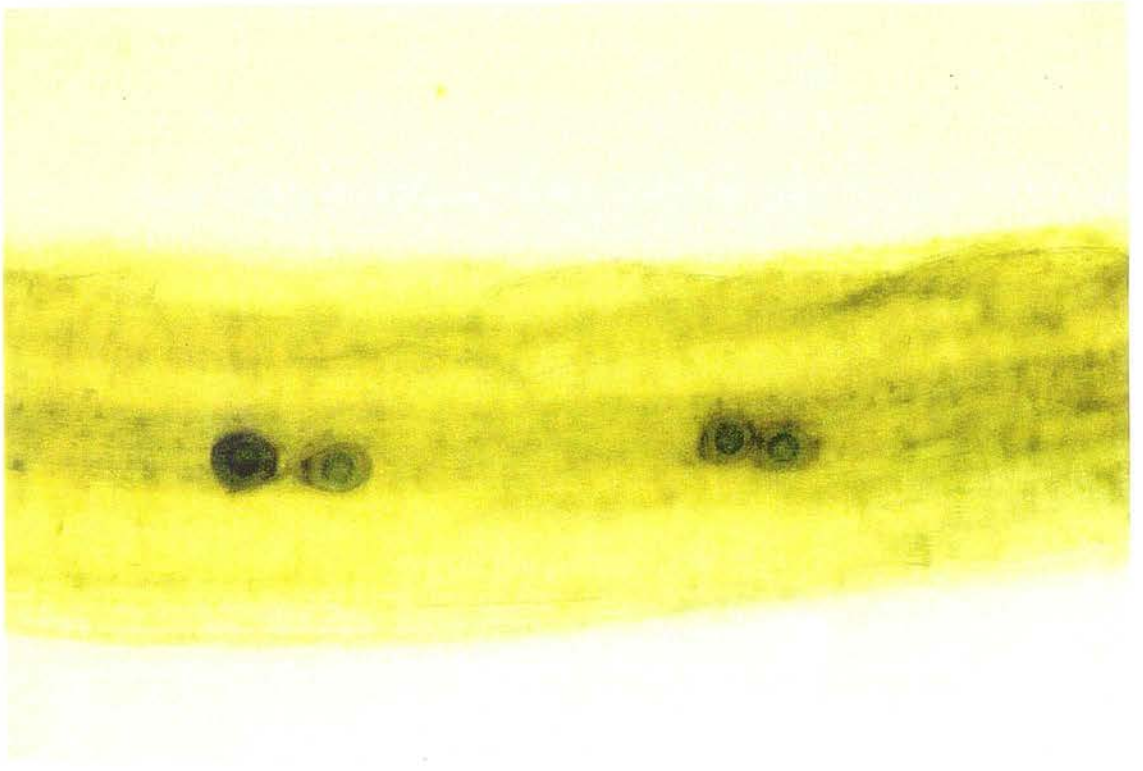

Fig. 3.8. Oospores of *P. fragariae* observed in an Elsanta root. The oospores are spherical structures with a resistant outer wall. They are formed within the root stele. (Microphotograph ×125).

CHAPTER 4: Inoculation of strawberry plants with *Phytophthora fragariae*

4.1 Effect of inoculation with *Phytophthora fragariae* on micropropagated strawberry plants

4.1.1 Introduction

A series of experiments was performed to investigate several aspects of the disease resulting from inoculation of micropropagated strawberry plants with the pathogen *P. fragariae*, which causes red stele disease of strawberry plants. The results obtained were used to develop a controlled method of disease assessment which was used to subsequently explore the effects of arbuscular mycorrhizal colonisation on the infection of micropropagated strawberry plants by *P. fragariae*. First, the germination of cysts of *P. fragariae* was measured at different temperatures and in the presence of different root types. The number of oospores produced in the root tips of strawberry plants in relation to the time period after inoculation of the plants with zoospores of *P. fragariae* was also measured.

Measurement of disease resulting from inoculation with *P. fragariae* was made by assessing the percentage of red/brown infected root by a visual estimate of this percentage. This subjective method was compared with the physical measurement of the length of infected root and the length of the whole root system, and the calculation of the exact percentage of the root system that was infected.

Various methods of inoculating plants with zoospores of *P. fragariae* were compared. Three inoculation methods were used: (i) a suspension of motile zoospores or (ii) non-motile, encysted zoospores being poured over the plants, or (iii) a suspension of non-motile cysts being sprayed onto the plant roots.

4.1.2 Germination of cysts of *P. fragariae* at different temperatures

4.1.2.i Experimental procedures

A suspension of 2000 zoospores per millilitre of each of three strains of *P. fragariae* was prepared as described in the Materials and Methods. The strains were 171, 293 and 372. The zoospore suspension was sprayed onto clean glass microscope slides such that there were 3 replicates for each pathogen strain. A total of 3 slides could fit into one plastic box and one slide of each strain was inserted into every box, with the slides placed on a layer of moist tissue paper and a lid put on the box. The boxes were then incubated in the dark at temperatures of 10°C, 15°C and 20°C for 24 h, with all

boxes containing slides being positioned on the same shelf in each incubator. The slides were then removed from the incubator and boxes and a maximum of 100 cysts were counted per microscope slide (i.e. a maximum of 300 cysts per strain) and the percentage of germinated cysts of each strain was calculated at each incubation temperature.

4.1.2.ii Results

A chi-square test (Appendix I, section 10.1) showed that there was no significant difference in the number of germinated cysts of the three strains of *P. fragariae* ($P > 0.05$) at each temperature (Fig. 4.1). Therefore the cyst germination of each strain was similarly affected by temperature.

For each individual strain, a chi-square test on the number of germinated versus ungerminated cysts also showed that there was a significant difference in the number of germinated cysts at 10°C, 15°C and 20°C ($P < 0.001$ for 171, 293 and 372; Appendix I, section 10.2). Therefore the total number of germinated and ungerminated cysts could be combined for all three strains and a chi-square test showed a significant difference in the germination of cysts of *P. fragariae* at the different temperatures ($P < 0.001$). Thus cyst germination was dependent on temperature.

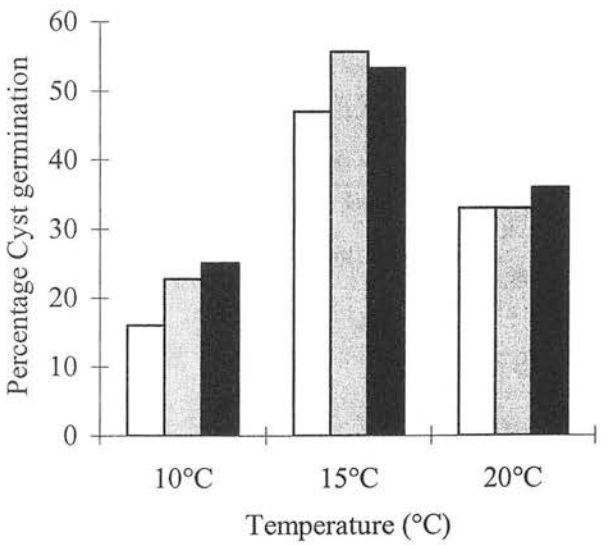

Fig. 4.1. Percentage germination of cysts of three strains of *P. fragariae* (171, white bars; 293, grey bars; 372, black bars) at three different temperatures: 10°C, 15°C and 20°C.

Figure 4.1 shows that the percentage germination was higher at 15°C than at 10°C or 20°C. Therefore the optimum temperature for cyst germination, 15°C, was used for further experiments involving cyst germination of *P. fragariae*.

4.1.3 Germination and orientation of the germ tube of cysts of three strains of *P. fragariae* in the presence of non-mycorrhizal roots and mycorrhizal roots of the strawberry cv Elsanta

4.1.3.i Experimental procedures

A suspension of 200 zoospores per millilitre of three strains of *P. fragariae* was sprayed onto a clean glass microscope slide onto which a root tip of an Elsanta strawberry plant had been placed. Each root was approximately 4 cm long and had been cut from either a non-mycorrhizal Elsanta plant or an Elsanta plant that was colonised with the mycorrhizal fungus *Glomus intraradices*. The slides were placed on a layer of moist tissue paper in a plastic box with a lid such that one slide for each pathogen strain was placed in each box. There were three replicates for each strain and root type. The slides were incubated at 15°C for 24 h in the dark. The slides were then removed from the boxes and a maximum of 100 cysts were examined per slide and the number of germinated and ungerminated cysts was counted. A cyst was considered to be germinated when the emerging germ tube was longer than the diameter of the cyst. The orientation of each germ tube was also assessed in relation to the position of the root on the microscope slide, and the number of germ tubes orientated in each of the four directions shown in Fig. 4.2 was counted.

4.1.3.ii Results

A chi-squared test indicated a significant difference in cyst germination for all three strains in the presence of mycorrhizal or non-mycorrhizal roots of Elsanta ($P < 0.001$). Therefore, the cyst germination was significantly reduced in the presence of the mycorrhizal root colonised by *G. intraradices* compared to that in the presence of the non-mycorrhizal root. The results shown in Fig. 4.4 illustrate that for each strain of *P. fragariae* there was a significantly greater number of germ tubes orientated towards the root (direction 1) than away from the root (direction 3) or laterally towards (direction 2) or away from the root tip (direction 4). This suggests that an exogenous factor was attracting the germ tubes to grow towards the root ($P < 0.001$ for strain 171, 293 and 372).

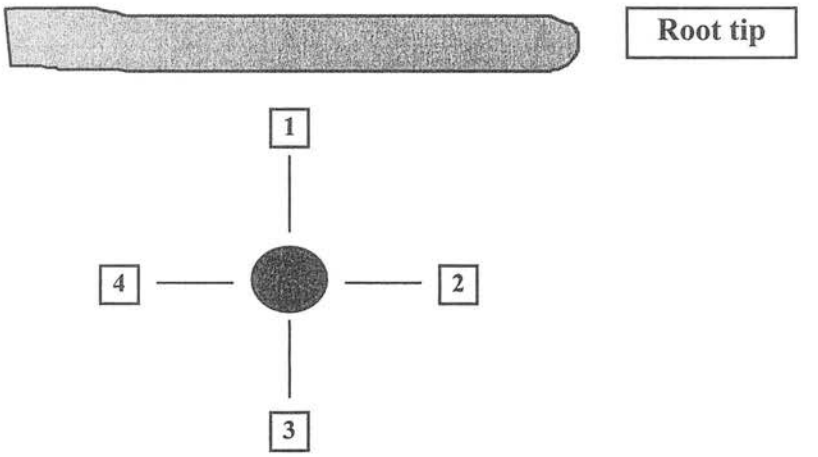

Fig. 4.2. The orientation of emerging germ tubes of *P. fragariae* in relation to the position of the root tip on the microscope slide.

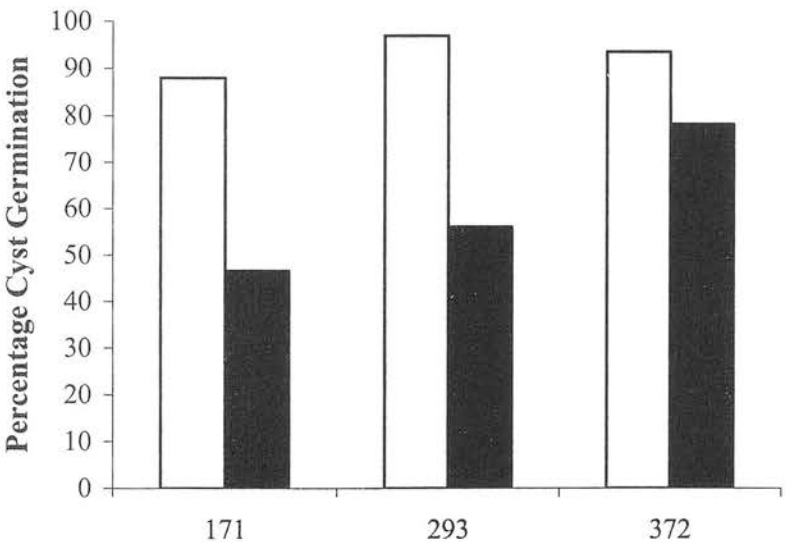

Fig. 4.3. Percentage germination of cysts of three strains (171, 293, and 372) of *P. fragariae* in the presence of non-mycorrhizal roots (white bars) and mycorrhizal roots (black bars) colonised by *G. intraradices*. The results represent the percentage germination of the total number of germinated cysts observed on three microscope slides for each strain of *P. fragariae* and each type of root.

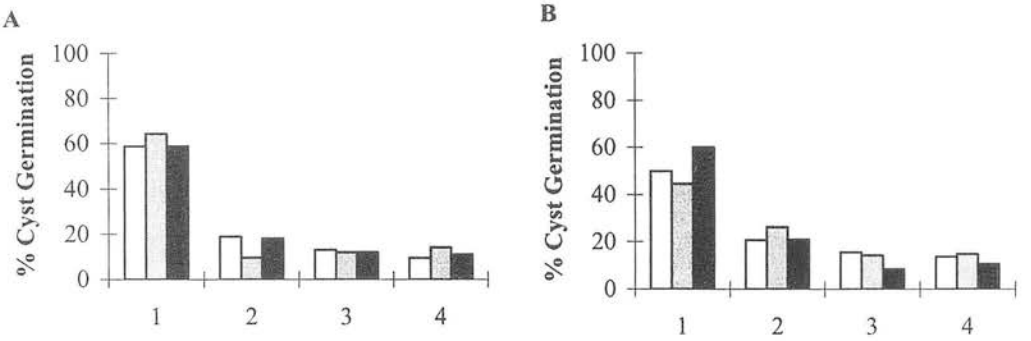

Fig. 4.4. Percentage germination of cysts in each of four different orientations (1, 2, 3 and 4, as described in Fig. 4.2) towards: (A) non-mycorrhizal roots; (B) mycorrhizal roots colonised by *G. intraradices*. The results represent the percentage germination of the total number of germinated cysts observed on three microscope slides for each strain of *P. fragariae* (strains: 171, white bars; 293, grey bars; 372, black bars).

4.1.4 Production of oospores of *P. fragariae* in roots in strawberry plants

4.1.4.i Experimental procedures

Twenty-eight Elsanta plants were grown by micropropagation until they formed roots, when they were planted into plastic pots containing fine-grade vermiculite and allowed to grow in a glasshouse for 2 weeks. Fourteen of the plants were then inoculated with 50 ml of a suspension of motile zoospores by pouring the zoospore suspension over the plants. Fourteen control plants received 50 ml of cold distilled water as a control. Plants were grown in a controlled environment chamber at 15°C until sampling occurred.

Eight root segments, each 2 cm in length, were removed from two inoculated plants 2 days after the inoculation with the *P. fragariae* and examined under a microscope for the presence of oospores in the root. This was repeated at 2 day intervals until roots had been examined at 2, 4, 6, 8, 10, 12 and 14 days after inoculation.

4.1.4.ii Results

Figure 4.5 shows the total number of oospores of *P. fragariae* observed in the plant roots. Oospores were not observed until 4 days after inoculation, and at 8 days after the inoculation, the greatest number of oospores was observed. The values for the number of oospores in the roots represent discrete, counted data and are not normally distributed. The oospores were also aggregated in roots, i.e. in a single plant, one root could contain no oospores while another root contained several hundred oospores. For

these reasons, parametric statistical analysis was not applicable to these results. Therefore the non-parametric Kruskal–Wallis analysis of variance using tied ranks was used to test for differences in the number of oospores produced at different days after inoculation of the plants. The values for each day after inoculation were first arranged in order of highest to lowest numbers of oospores. Ranks were then assigned to the data such that the highest number of oospores was ranked as 1, the next highest number of oospores was ranked 2, and so on. In the case of tied ranks where two values of the number of oospores were identical, each value was given the mean of the rank it would have been assigned if the values were not tied.

A Kruskal–Wallis analysis was then performed on the ranked values to obtain the test statistic, H. A corrected test statistic, H_c was then calculated to take into account the presence of the tied ranks. H_c was then compared to the chi-squared statistic at k–1 degrees of freedom where k equals the number of sets of data, i.e. seven sets in this case. The results showed that H_c (67.636) was much greater than the chi statistic ($\chi^2_{0.05,2} = 12.592$) (Appendix I, section 10.5). Therefore there was a significant difference in the number of oospores observed at different days after inoculation of the plants by *P. fragariae*.

In order to determine between which of the plants significant differences occurred, a non-parametric multiple comparison was performed (Zar, 1996). The rank sums were arranged in increasing order of magnitude: note that these represent the sum of the ranks and not the sum of the actual numbers of oospores in the root tips. The rank sums of the highest (2 days after inoculation) and the lowest (8 days after inoculation) orders of magnitude were then compared, and then the sums of the highest and next lowest (10 days after inoculation) orders of magnitude were compared. This was continued until a comparison of each rank sum with each of the lower rank sums had been made. Finally, the rank sums of the two lowest magnitudes were compared, i.e. 8 and 10 days after inoculation, respectively. The standard error (SE) was calculated for each comparison, based on the number of groups being compared (p), according to the following formula:

$$\text{S.E.} = \sqrt{[n(nk)(nk + 1)/12]}$$

where n is the number of observations in each treatment and k is the number of treatments. This test assumes that there is an equal number of observations in each treatment.

The test statistic, q, was then calculated and compared to the critical q value (equivalent to the chi distribution with ∞ degrees of freedom). The results in Appendix I, section 10.5.1, show that the number of oospores observed in root tips was significantly different at 2, 8, 10, 12 and 14 days after inoculation, but there was no significant difference between the number of oospores observed at 4 and 6 days after inoculation. Figure 4.5 shows that no oospores were observed at 2 days after inoculation. However, the number of oospores produced in the root tips then increased significantly, with the maximum number of oospores being observed at 8 and 10 days after inoculation, with no significant difference in the number of oospores observed on these days, suggesting that oospore production peaked at 8 to 10 days after inoculation. The number of oospores then decreased significantly at 12 and 14 days after inoculation.

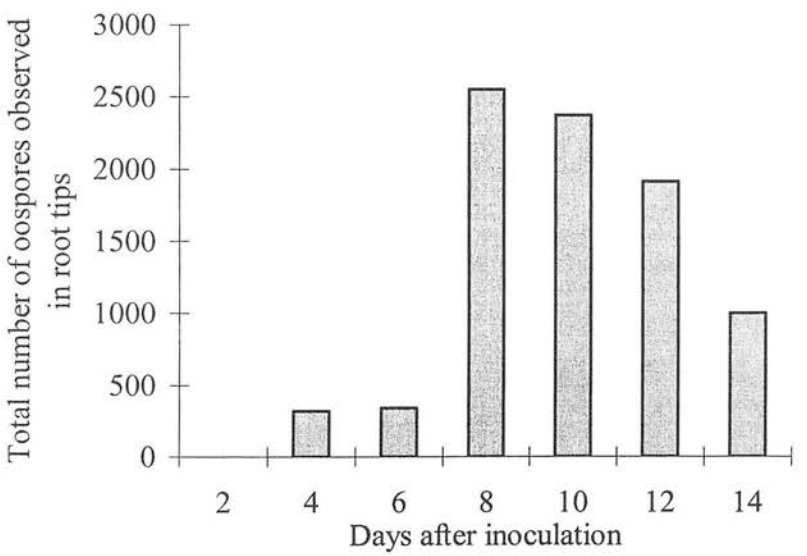

Fig. 4.5. Total number of oospores observed in root tips removed from strawberry plants inoculated with a motile, poured suspension of zoospores of *P. fragariae*. The root tips were removed and examined at 2, 4, 6, 8, 10, 12 and 14 days after the inoculation.

Therefore, at 15°C oospore production in infected strawberry roots reached a peak 8–10 days after inoculation by the pathogen *P. fragariae*, and then declined as oospores began to germinate to produce sporangia at the root surface. These sporangia, in turn, can germinate to release motile zoospores which can produce secondary infections by the pathogen, thus infecting adjacent plants in a field situation, or other roots on the same plant.

4.1.5 Comparison of a visual assessment of percentage infected root versus a physical measurement of the percentage of infected root in strawberry plants inoculated with zoospores of *P. fragariae*

4.1.5.i Experimental procedures

Two strains, 171 and 293, of *P. fragariae* were used to inoculate micropropagated strawberry plants of cultivar Elsanta with increasing amounts of zoospores using three different inoculation methods. Inoculation was performed by either pouring motile zoospores over the plant, pouring non-motile cysts over the plant, or by spraying the plant roots with a suspension of non-motile cysts, all as described in the Materials and Methods. Four plants were inoculated with each strain of *P. fragariae* for each inoculation method. The plants were randomised throughout a controlled environment chamber and grown at 15°C for 14 days when each plant was then removed from the pot and the root system washed free of debris under running tap water.

The percentage of red/brown rotting roots on each plant was assessed by two methods: a visual estimate of this percentage, and a physical measurement of the length of infected roots and the length of the whole root system, and calculation of the percentage of infected roots (length of infected root divided by the length of the whole root system and multiplied by 100). These two methods of assessing the percentage of infected root were compared to determine whether the rapid visual assessment was as accurate as the very time-consuming physical measurement of the root system.

4.1.5.ii Results

Figure 4.6 shows the mean percentage of infected root of the plants inoculated with either strain of the pathogen using the three different inoculation methods. Percentages have a binomial distribution rather than a normal distribution, and small or large percentages particularly differ greatly from a normal distribution. Therefore an arcsine transformation was applied to normalise the proportional data and the

resulting transformed values were used in the statistical analysis. The arcsine transformation involves transforming the square root of each proportion, not percentage, to its arcsine, i.e. the angle whose sine is $\sqrt{X}$ where X is the proportion.

The transformed value X' is calculated from:

$X' = \text{arcsine } \sqrt{X}$

A paired sample *t*-test was performed on the arcsine-transformed data to test for any differences between the means of the measured and estimated percentage infected roots for each plant inoculated with either strain 171 or 293 for each inoculation method (Appendix I, section 10.7).

The results shown in Table 4.1 indicate that, for plants inoculated with strain 171, there was no significant difference between the estimated and measured assessment of percentage infected root for each inoculation method. In plants inoculated with strain 293, there was no significant difference between the estimated or measured assessment of percentage infected root when plants were inoculated by non-motile poured cysts or non-motile sprayed cysts; however, a significant difference did exist when the inoculation method was that of motile poured zoospores.

This experiment was assessing the accuracy of the operator in assessing the percentage of infected root by a rapid visual examination of the whole root system. The method of inoculation of the plants will not affect this accuracy; the three inoculation methods simply provided three data sets on which to compare the assessment of percentage infected root. Therefore the significant difference observed between the estimated and measured assessment method in plants inoculated with a motile poured suspension of zoospores of strain 293 is not related to the inoculation method but indicates that the visual estimate of infected root may not always be entirely accurate.

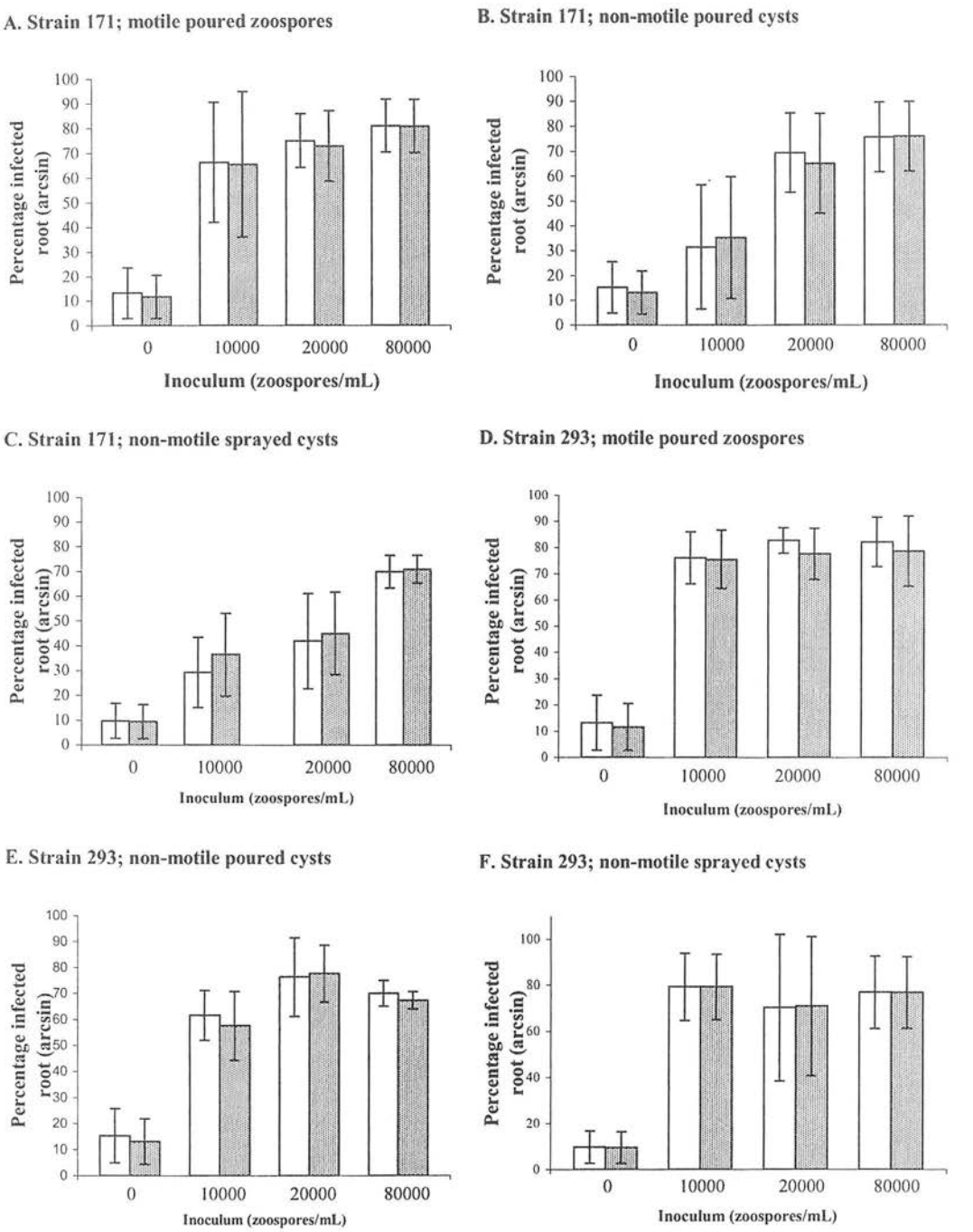

Fig. 4.6. Percentage infected root (arcsin transformed values) observed in strawberry plants inoculated with two strains of *P. fragariae*, 171 (A, B and C) and 293 (D, E and F) using three different inoculation methods: A and D, motile poured zoospores (MP); B and E, non-motile poured cysts (NMP); C and F, non-motile sprayed cysts (NMS). The percentage infected root was assessed by two methods: a physical measurement of the length of the infected root and the whole root system (white bars) and a visual estimate (shaded bars). The error bars represent the standard deviation.

Table 4.1. Results of a paired sample *t*-test to determine whether there was a significant difference between the estimated and measured percentage infected root in plants inoculated with strain 171 or 293 of *P. fragariae* with three inoculation methods: MP, motile poured zoospores; NMP, non-motile poured cysts; and NMS, non-motile sprayed cysts. *, significant at the 5% level; ns, not significant.

	171		293	
	t	Significance	*t*	Significance
MP	1.26	ns	2.62	*
NMP	0.47	ns	1.87	ns
NMS	–2.12	ns	–0.26	ns

However, the physical measurement of the length of infected root and the length of the whole root system is very laborious and time consuming. It is also subject to the error caused by pieces of root being broken off during removal of the plant from the vermiculite, which is inevitable regardless of the precautions taken to prevent this, the outcome of which is that the entire root system is not being measured. This error also applies to the visual assessment of the infected root as not all of the root system will be present then either.

A visual assessment of the infected root is a much quicker method to obtain this value than the actual measurement of the root system. Therefore, considering that both methods are subject to the error of the entire root system not being present, and that the *t*-test showed that there was a difference in the percentage infected root calculated by each method in only one out of a possible six sets of data, it was decided to use the visual estimate of percentage infected root in future experiments.

4.1.6 Comparison of inoculation method and strains of *P. fragariae* based on the visual assessment of percentage infected root

4.1.6.i Experimental procedures

Strawberry plants of the cultivar Elsanta were grown via micropropagation until they had been growing in rooting medium for 5 weeks, when sufficient roots had been formed. Plants were then removed from the micropropagation conditions and planted into pots containing medium-grade, watered vermiculite. Plants were allowed to grow in glasshouse conditions for 4 weeks before being inoculated with zoospores of two

strains of *P. fragariae* in one of three methods (as described in the Materials and Methods): (i) a suspension of motile zoospores was poured over the plants in the vermiculite-filled pots; (ii) a suspension of non-motile, encysted zoospores was poured over the plants in vermiculite-filled pots; and (iii) the roots were sprayed with a suspension of non-motile, encysted zoospores. Four plants were inoculated for each strain of *P. fragariae* and inoculation method and dose of zoospores. Plants were inoculated with either 10 000 zoospores, 20 000 zoospores or 80 000 zoospores, and control, non-inoculated plants were treated with cold distilled water. All plants were then randomised throughout a controlled temperature chamber and grown at 15°C for 14 days. A visual assessment of the percentage of infected root was then made for each plant.

4.1.6.ii Results

A three-factor analysis of variance was performed to test for any significant differences in the inoculation method, the two strains of *P. fragariae* and the level of zoospores used to inoculate the plants, and for any interaction between these factors. However, as percentage values are not normally distributed, they were first transformed using an arcsine transformation to normalise the data. The analysis of variance was then performed on the transformed data without the control plants, i.e. the plants that received no zoospore inoculum.

Table 4.2 shows the summary results of this analysis of variance without control, non-inoculated plants included (disease symptoms were not observed in these plants). This indicated a significant difference in the percentage of infected root observed with different inoculation methods ($P < 0.01$; see Appendix I, section 10.8), i.e. the inoculation method produced significantly different levels of disease as assessed by percentage of infected root.

There was also a significant difference in the level of disease resulting from inoculation of the plants with each strain of *P. fragariae* ($P < 0.01$). Figure 4.7 shows that the percentage of infected root resulting from inoculation of the plants by strain 293 was greater than that observed when plants were inoculated with strain 171. The analysis also showed a significant difference in the disease observed as the level of zoospore inoculum increased from 10 000 zoospores per plant to 80 000 zoospores per plant ($P < 0.01$).

However, there was no significant interaction between the inoculation method and the strains of *P. fragariae* ($P > 0.05$), i.e. the inoculation method did not significantly affect the disease caused by inoculation with either strain. The inoculation method also had no significant affect on the disease caused by inoculation with either 10 000, 20 000 or 80 000 zoospores per plant ($P > 0.05$).

There was a significant difference in disease caused by either of the two strains at different levels of zoospore inoculum ($P < 0.05$), and this was probably the result of the different behaviour of the strains at the lower level of zoospore inoculum, i.e. strain 171 showed a greater dose-related response whereas strain 293 produced the maximum disease response at an inoculation of 10 000 zoospores per plant.

However, the results showed that there was no significant interaction between the inoculation method, the strains of *P. fragariae* and the level of zoospore inoculum ($P > 0.05$), i.e. no one inoculation method favoured a strain and zoospore level.

Therefore differences caused by the inoculation level were consistent between strains and the zoospore dose.

4.1.7 Comparison of inoculation method and strains of *P. fragariae* based on the number of oospores observed in root tips

4.1.7.i Experimental procedures

The same plants that were grown and inoculated with the three different inoculation methods described above were assessed for disease by a microscopic examination of the number of oospores in the root tips of 8 roots removed from each plant. The numbers of oospores observed in the root tips were then subjected to a Kruskal–Wallis analysis of variance by ranks.

4.1.7.ii Results

Figure 4.8 shows the oospore numbers counted in the roots of the plants. The data analysis considered each strain of *P. fragariae* separately. In each analysis, the calculated Kruskal–Wallis statistic, H_c, was compared to the chi-square statistic.

Table 4.2. Summary of the three-factor analysis of variance to determine the effect of inoculation strawberry plants of cultivar Elsanta with different levels of zoospores of two strains (171 and 293) of *P. fragariae* using three different inoculation methods. ns, not significant; *, significant at the 5% level; **, significant at the 1% level. SS, sums of squares; df, degrees of freedom; MS, mean square; *F*, *F* distribution.

Variable	SS	df	MS	*F*	Significance
Total	32230.74	71	453.9541		
Inoculation Method (IM)	3487.01	2	1743.50	5.368	**
Strains of *P. fragariae* (SPf)	4151.73	1	4151.73	12.782	**
Zoospore level (Z)	4317.43	2	2158.71	6.646	**
IM v SPf	1636.55	2	818.27	2.519	ns
IM v Z	1713.05	4	428.26	1.318	ns
SPf v Z	2583.28	2	1291.64	3.976	*
IM v SPf v Z	700.43	16	43.78	0.135	ns
Error	15233.44	42	324.79		

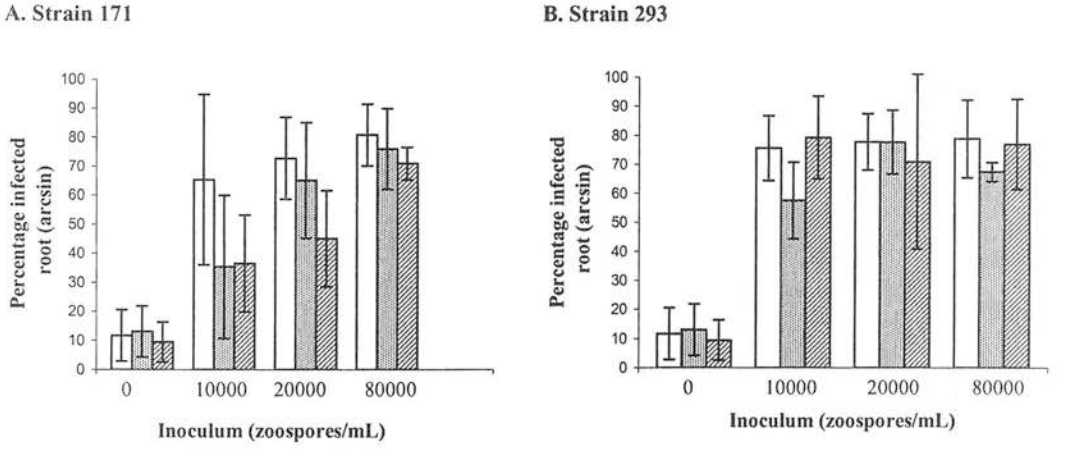

Fig. 4.7. A comparison of the disease resulting from inoculation of strawberry plants with zoospores of *P. fragariae* in one of three methods, with the resulting percentage infected root (arcsin transformed values) assessed by a visual estimate of strawberry plants inoculated with one of two strains of *P. fragariae*: 171 (A) and 293 (B). The inoculation methods used were: motile poured inoculation (white bars), non-motile poured cysts (dot shaded bars) and non-motile sprayed cysts (line shaded bars). The error bars represent the standard deviation.

The analysis showed no significant effect of increasing the level of zoospore inoculum from 10 000 to 80 000 zoospores per plant for plants inoculated with strain 293 of *P. fragariae* ($H_c = 0.107$; $\chi^2_{0.05,2} = 5.991$). The results in Table 4.3 (Appendix I, section 10.10) show that there was no significant effect of increasing the level of zoospore inoculum from 10 000 to 20 000 and 80 000 zoospores per plant for strain 293 of *P. fragariae* when plants were inoculated with any of the three different inoculation methods. However, there was a significant difference in the numbers of oospores observed in roots of plants inoculated using non-motile sprayed zoospores with strain 171 when the level of zoospore dose was increased from 10 000 to 80 000 ($H_c = 15.279$; $\chi^2_{0.05,2} = 5.991$).

When plants were inoculated with 10 000, 20 000 or 80 000 zoospores per plant using a motile poured suspension of zoospores, there was no significant difference in the number of oospores formed in the root tips of plants inoculated with either strain of the pathogen (Table 4.4; Appendix I, section 10.11). However, when plants received a non-motile poured suspension of cysts, a significant difference in disease due to the two strains of *P. fragariae* was observed when plants were inoculated with either 10 000 or 20 000 zoospores, but not at the highest level (80 000 zoospores per plant) of zoospore inoculum. Significant differences in the disease observed with the two strains of the pathogen were observed in plants receiving a non-motile sprayed inoculation of cysts at 10 000 zoospores per plant; higher levels of inoculum did not enable differences between the strains to be apparent. In those instances where a significant difference in oospore number was detected strain 293 gave higher numbers of oospores than strain 171.

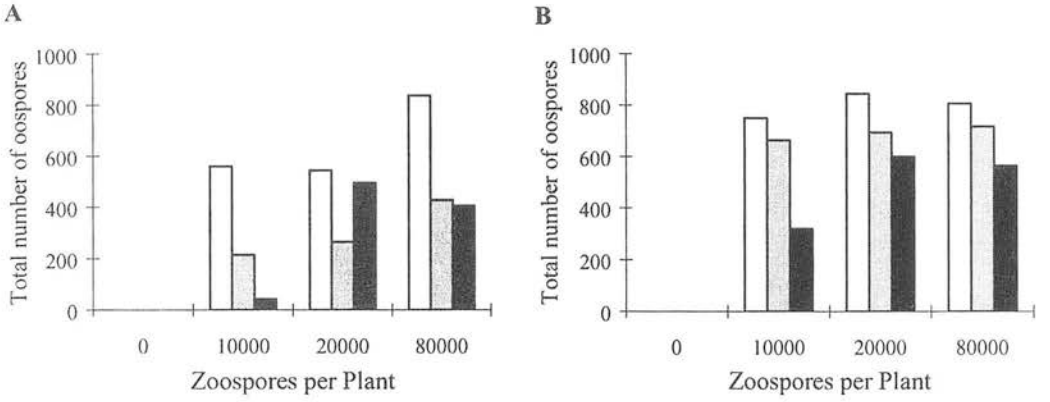

Fig. 4.8. The total number of oospores observed in root tips of strawberry plants inoculated with zoospores of *P. fragariae* in one of three methods with one of two strains of *P. fragariae*: 171 (A) and 293 (B). The inoculation methods used were: motile poured zoospores (white bars), non-motile poured cysts (grey bars) and non-motile sprayed cysts (black bars).

The results in Table 4.5 show that the inoculation method had a significant effect on the number of oospores formed in plant roots when plants were inoculated with 10 000 or 80 000 zoospores of strain 171, and with 10 000 zoospores of strain 293. A Kruskal–Wallis multiple comparison test using tied ranks was performed to determine which of the inoculation methods produced significantly different results.

Table 4.3. Results of the Kruskal–Wallis analysis of variance to determine whether increasing the level of zoospore inoculum (10 000, 20 000 and 80 000 zoospores per plant) had any significant effect on the number of oospores formed in root tips when plants were inoculated with three different inoculation methods (motile poured zoospores, non-motile poured cysts and non-motile sprayed cysts) and two strains of *P. fragariae* (171 and 293). The Kruskal–Wallis statistic, H_c was compared to the critical chi-square statistic ($\chi^2_{0.05,2} = 5.991$) at 2 degrees of freedom. ns, not significant; *, significant difference ($P < 0.05$).

	171		293	
	H_c	Significance	H_c	Significance
Motile Poured	5.670	ns	0.277	ns
Non-motile Poured	5.387	ns	0.145	ns
Non-motile Sprayed	12.279	*	0.107	*

Table 4.4. Results of the Kruskal–Wallis analysis of variance of the number of oospores formed in root tips to determine whether strains 171 and 293 of *P. fragariae* had any significant difference on the number of oospores formed in the root tips when plants received different levels of zoospore inoculum (10 000, 20 000 or 80 000 zoospores per plant) when inoculated with three different inoculation methods (motile poured zoospores, non-motile poured cysts and non-motile sprayed cysts). The Kruskal–Wallis statistic, H_c was compared to the critical chi-square statistic ($\chi^2_{0.05,2} = 3.841$) at 1 degree of freedom. ns, not significant; *, significant difference ($P < 0.05$).

	Motile Poured		Non-motile Poured		Non-motile Sprayed	
	H_c	Significance	H_c	Significance	H_c	Significance
10 000	2.510	ns	7.110	*	14.740	*
20 000	2.950	ns	8.567	*	0.995	ns
80 000	0.632	ns	3.495	ns	0.309	ns

The main conclusion of the results is that with the less virulent strain 171, the motile poured inoculation of 10 000 or 80 000 zoospores resulted in significantly greater numbers of oospores in root tips than either of the non-motile inoculation methods, and that there was no difference in the amount of disease, as measured by number of oospores, in plants inoculated with either the non-motile poured or non-motile sprayed inoculation methods. In contrast, with the more virulent strain 293, a significant difference between the numbers of oospores observed compared to the inoculation method used was only detected when the lowest level, i.e. 10 000 zoospores per plant, was inoculated onto a plant. At this low zoospore dose, the non-motile sprayed inoculation method resulted in significantly less disease as assessed by oospore numbers compared to the other inoculation methods.

Table 4.5. Results of the Kruskal–Wallis analysis of variance of the number of oospores formed in root tips to determine whether the inoculation method (motile poured zoospores, non-motile poured cysts or non-motile sprayed cysts) had any significant effect on the number of oospores formed in root tips of plants inoculated with one of the two strains of *P. fragariae* (171 and 293) at different levels of zoospore inoculum (10 000, 20 000 or 80 000 zoospores per plant) The Kruskal–Wallis statistic, H_c, was compared to the critical chi-square statistic ($\chi^2_{0.05,2} = 5.991$) at 2 degrees of freedom. ns, not significant; *, significant difference ($P < 0.05$).

	171		293	
	H_c	Significance	H_c	Significance
10 000	11.527	*	5.942	*
20 000	4.327	ns	5.385	ns
80 000	11.698	*	4.286	ns

4.1.8 Determination of the effect of inoculating two cultivars of strawberry, Elsanta and Rhapsody, with each of three strains of *P. fragariae*

4.1.8.i Experimental procedures

Strawberry plants of both cultivars were grown via micropropagation until they produced roots, at which point they were planted out into vermiculite-filled pots in the glasshouse and allowed to grow for 4 weeks. The plants were then inoculated with a motile poured inoculum of zoospores of one of the three strains of *P. fragariae* (strain 171, 293 and 372) such that a single plant received either cold distilled water (as a control), 5000 zoospores per plant or 80 000 zoospores per plant. Four plants were inoculated with each strain of *P. fragariae* and with distilled water for the controls, with four replicates for each treatment. The inoculated plants were then randomised throughout a controlled environment chamber and grown at 15°C for a period of 14 days, after which they were assessed for the level of disease due to the inoculation by counting the number of oospores in each of 10 infected root tips removed from each plant. The numbers of oospores were then subjected to a Kruskal–Wallis analysis of variance to determine any differences in the disease resulting from inoculation of each cultivar with each strain of the pathogen.

4.1.8.ii Results

Figure 4.9 shows the number of oospores detected in the root tips 14 days after the inoculation. The Kruskal–Wallis analysis of variance using tied ranks revealed that there was a significantly greater number of oospores in Elsanta plants compared to Rhapsody plants inoculated with strain 171 or 372 of *P. fragariae* using 5000 or 80 000 zoospores per plant (see Table 4.6 for results, and Appendix I, section 10.13 for analyses). Inoculation of the two strawberry cultivars with strain 293 resulted in no significant difference in the oospores observed in each cultivar inoculated with either 5000 or 80 000 zoospores per plant, as is suggested by the results shown in Fig. 9.

It was suggested by Kennedy (1992) that strain 293 is more virulent than strain 171, although this was not apparent from the results of the present experiments. It is possible that both strawberry cultivars were infected to a similar degree by zoospores of this strain, which was able to overcome the resistance of each cultivar. In contrast, strains 171 and 372 were unable to overcome the resistance of the Rhapsody cultivar

Table 4.6. Results of a Kruskal–Wallis analysis of variance to determine significant differences in the numbers of oospores observed in root tips removed from plants of two cultivars (Elsanta and Rhapsody) inoculated with a motile poured inoculation of either 5000 or 80 000 zoospores per plant of each of three strains of *P. fragariae* (171, 293 and 372). The Kruskal–Wallis statistic, H_c, was compared to the critical chi-square statistic ($\chi^2_{0.05,2} = 3.841$) at 1 degree of freedom. ns, not significant; *, significant difference ($P < 0.05$).

	171		293		372	
Zoospores/Plant	H_c	Significance	H_c	Significance	H_c	Significance
5000	7.730	*	0.730	ns	20.176	*
80 000	35.038	*	0.153	ns	29.489	*

as they infected the Rhapsody plants to a lesser degree than the disease, as assessed by the number of oospores in root tips, observed in Elsanta plants.

In Elsanta plants inoculated with 5000 zoospores per plant there was no significant difference between the level of disease caused by each strain of the pathogen ($H_c = 4.054$; $\chi^2_{0.05,2} = 5.991$). However, when each Elsanta plant was inoculated with 80 000 zoospores, the amount of disease resulting from each strain was significantly different ($H_c = 14.442$; $\chi^2_{0.05,2} = 5.991$) with strain 372 giving the greatest number of oospores. Inoculation of Rhapsody plants with either 5000 or 80 000 zoospores of the

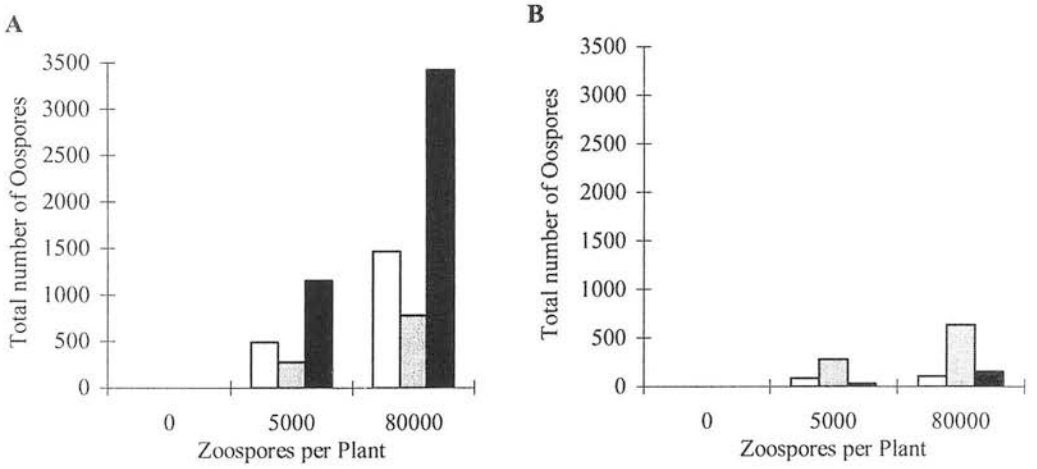

Fig. 4.9. The total number of oospores observed in root tips of strawberry plants of two cultivars inoculated with zoospores of each of three strains of *P. fragariae*: strain 171 (white bars); strain 293 (grey bars); and strain 372 (black bars). The strawberry cultivars were Elsanta (A) and Rhapsody (B).

pathogen resulted in significant differences in the level of disease caused by each strain (see Appendix I, section 10.14, for statistical analysis). A Kruskal–Wallis multiple comparison test using tied ranks was performed to determine which of the strains resulted in significantly different levels of disease.

The results indicated that at an inoculum of 80 000 zoospores per plant, disease due to strain 372 was significantly greater than that resulting from inoculation of Elsanta plants with strain 171 or 293, and that plants inoculated with strain 171 resulted in significantly more disease than those inoculated with strain 293.

However, for Rhapsody plants inoculated with 5000 zoospores per plant, strain 293 caused a significantly greater level of disease than either strain 171 or strain 372. In addition, strain 171 caused a significantly greater level of disease than strain 372. When the level of zoospores per plant was increased to 80 000 zoospores, strain 293 continued to result in a significantly greater amount of disease than strain 171 or strain 372, but there was no significant difference between the disease caused by either strain 171 or strain 372.

4.2. Discussion

The experiments described in this chapter investigated certain aspects of the biology of *P. fragariae*, and compared methods by which to inoculate strawberry plants with the pathogen and assess the resulting disease.

Germination of cysts of *P. fragariae* was shown to be affected by temperature, with 15°C being the more favourable temperature for cyst germination in all three strains of the pathogen (strain 171, 293 and 372) than either 10°C or 20°C. Therefore this optimum temperature was used for further experiments, e.g. when plants were inoculated with *P. fragariae* the inoculated plants were then grown at 15°C to promote maximum levels of cyst germination and therefore maximum levels of disease.

The results also indicated a significantly reduced level of cyst germination in the presence of a mycorrhizal root colonised by the mycorrhizal fungus *G. intraradices* compared to the germination observed in the presence of a non-mycorrhizal root. It has been suggested that the improved phosphorus nutrition which can result in a mycorrhizal plant can decrease root membrane permeability, causing a decrease in

root exudation from the host (Graham & Menge, 1982). It has been reported that mycorrhizal roots of sweet corn (*Zea mays* var *rogusa*) produced a reduced amount of rhizosphere leachate which was associated with a reduction in the production of sporangia and zoospores of the pathogen *Phytophthora cinnamomi* (Meyer & Linderman, 1986).

Specific attraction of zoospores of other zoosporic fungal pathogens of plants has been reported (Mitchell & Deacon, 1986; Chi & Sabo, 1978) suggesting that fungi are responding to a factor(s) exuded by the plant roots. Such factors may be flavonoids (Morris & Ward, 1992) or other components of the root exudates (Buxton, 1962) but the attraction has only been shown *in vitro* and not *in vivo* (Deacon, 1996).

The fact that the emerging germ tubes were significantly orientated towards the root indicates that some chemotropic affect was taking place. Although some germ tubes grew away from the root, the vast majority grew towards the root tip, which is the site of penetration from zoospore cysts of *P. fragariae*. In some instances, the emerging germ tube had changed direction in order to grow towards the root, suggesting an attraction of the root for the pathogen. This orientation of germ tubes towards the root was also observed with mycorrhizal roots in which the level of cyst germination had been significantly reduced. Therefore the presence of the mycorrhizal fungus had a significant effect on cyst germination but not on orientation of the emerging germ tube. This suggests that, once germinated, a cyst will grow towards the root regardless of the root type.

This may be a favourable strategy in terms of survival of the pathogen. Root type can influence cyst germination but, once germinated, the cyst will be attracted towards the root regardless of whether the root is a suitable host for the pathogen, thus the actual cyst germination is not wasted. In this way, fewer cysts will germinate in the presence of a root which is not attractive to the pathogen but of those cysts that do germinate the majority will be attracted towards the root; otherwise a germinated cyst will die if it does not locate a root within a finite time period.

Deacon (1996) suggested that two types of exogenous signals were required for root–zoospore attraction *in vivo*, although it was acknowledged that the range of signals to which cells respond *in vitro* is much greater. The *in vivo* signals are: (i) diffusible

attractants that encourage the zoospore to move towards the host surface; (ii) a host surface component that induces encystment.

Deacon and Donaldson (1993) described a homing sequence whereby the zoospore responds to various host and environmental cues as it moves towards the host root, encysts, produces a germ tube and penetrates the root. This sequence is preprogrammed so that progression through each stage occurs as a result of different stimuli acting on different receptors. Therefore the fact that the orientation of the germ tube towards the root was not significantly different for both mycorrhizal and non-mycorrhizal roots suggests that the germ tube responds to a signal which was not affected by the mycorrhizal colonisation. However, the germination of the cysts was reduced in the presence of the mycorrhizal root, indicating that the mycorrhizal colonisation did affect the signal which affects germ tube orientation.

It is known that zoospores of *Pythium* and *Phytophthora* have a fixed site of germination located very close to the flagellar insertion (Deacon, 1996), the consequences of which are that the zoospore docks onto a host root in such a position that the site of germ tube emergence is adjacent to the root. Therefore the *in vitro* conditions used in the experiments described here would not represent the ecological situation because it is assumed that, *in vivo*, the zoospore would encyst on the root surface rather than on the microscope slide used here.

Once a cyst of *P. fragariae* has germinated and infected a plant root a period of 4 days is required before oospores are produced in the root tips, with the maximum number of oospores produced at 8 and 10 days after inoculation and then declining significantly at 12 and 14 days. Oospores can germinate to produce sporangia at the root surface. Sporangia, in turn, germinate to release motile zoospores which can re-infect roots of the same plant or neighbouring plants in a field situation. Therefore, 8 to 10 days after the initial penetration of the plant by the pathogen, secondary infections in neighbouring roots or plants can occur as a result of zoospores released from the sporangia which are the products of oospore germination (Maas, 1984).

The visual measurement of the percentage of infected root resulted in a value not significantly different from that obtained by a physical measurement of the length of the infected root compared to the length of the whole root system on most occasions. Kennedy *et al.* (1986) and Milholland *et al.* (1989) reported that the visual estimate of

the percentage of infected root was in close agreement with actual measurements of infected roots. The percentage of infected root has been used by some workers as a reliable method of assessing disease due to *P. fragariae* (Kennedy *et al.*, 1986; Kennedy & Duncan, 1988). However, it has been recognised that such visual assessment procedures are subjective and frequently inconsistent (George & Milholland, 1986), largely because a red stele can be induced by other factors such as the presence of certain antibiotics or distilled water (Otterbacher *et al.*, 1969), and that production of oospores is a more reliable indicator of disease than the presence of rotting roots (Bain & Demaree, 1945).

The results of the present experiments showed that the level of disease varied with both the inoculation method used and the method of assessing the disease. The disease assessed as the percentage of infected root length suggested a significant difference between the two strains of the pathogen, whereas a significant difference based on the Kruskal–Wallis analysis of the number of oospores recorded in the roots was only apparent using a motile, poured suspension of zoospores when a low level (10 000 zoospores per plant) of zoospores of either strain was applied.

However, the different disease assessment methods could also produce a similar interpretation of the amount of disease. For example, the percentage infected root in plants inoculated with a motile, poured suspension of zoospores of strain 171 was much greater than when the inoculation was of non-motile cysts, and analysis of the number of oospores in the root tips also revealed this significant difference.

Any differences between strains or the inoculation method were most readily apparent when low levels of zoospore inoculum were applied to the plants, particularly if the inoculation used non-motile cysts. One must consider the behaviour of a zoospore being inoculated into a plant pot containing a strawberry plant. Motile zoospores are able to move through water in the growth medium in the plant pot and attach themselves directly onto a root surface by encysting. The germ tube then only has to penetrate the root in order to achieve infection of the root. In contrast, a non-motile zoospore has already encysted and is unable to move towards a plant root and must rely on the germinating cyst producing a germ tube which can locate and enter a root. Therefore it is possible that the use of motile zoospores can result either in higher

levels of disease, or the infection will be established more rapidly compared to the use of non-motile cysts.

The inoculation method also appeared to influence whether significant differences were observed between the disease due to strains with different virulence. For example, when disease was assessed by an analysis of the number of oospores observed in roots, with a motile, poured inoculation of zoospores, no significant difference between strains 171 and 293 was apparent as the level of zoospores increased from 10 000 to 80 000 zoospores per plant. However, using non-motile zoospores, a significant difference between strains 171 and 293 was observed when plants were inoculated with 10 000 and 20 000 cysts, while a significant difference was only apparent at the lowest level (10 000) of cysts per plant when the inoculum was applied as a non-motile sprayed inoculation of cysts. This may be a result of the motile, poured inoculation of zoospores being able to infect more roots and produce greater levels of disease and oospores than the non-motile cysts. Therefore, an inoculation of non-motile cysts may increase the probability of differences in the virulence of different strains of the pathogen being observed at low levels of zoospore inoculum. In addition, the range of levels of zoospore inoculum may need to be increased in order to compare differences in the virulence of strains in order to accommodate and observe these differences if the virulence of the strains is very different. If the categories of zoospore inoculum are few, strains with similar but different virulences may be unable to be distinguished, regardless of the inoculation method used or disease assessment method used.

Certainly, the results indicated that differences in the inoculation method, strain virulence or disease assessment were not apparent at the highest (80 000 zoospores per plant) level of inoculum, as all plants were then infected by *P. fragariae* to a similar high degree. Therefore, care must be taken to chose the most appropriate level and method of zoospore inoculum depending on the nature of the strains and host plants being used.

The results did indicate differences in the susceptibility of the two strawberry cultivars to different strains of the pathogen, such that when plants were inoculated with strain 171 or 372, the Elsanta plants exhibited a significantly greater number of oospores in the root tips than Rhapsody plants. This suggests that Elsanta plants were

more susceptible to disease resulting from strains 171 or 372 than Rhapsody plants. Indeed, it has been reported that Elsanta is more susceptible to this disease than Rhapsody (A.D.A.S., 1989).

However, the amount of disease resulting from inoculation of either Elsanta or Rhapsody plants with *P. fragariae* 293 was not significantly different, which was rather unexpected because of the differences in the susceptibility of the two cultivars to the pathogen. In addition, strain 293 had been reported previously to be more virulent than either strain 171 or 372 (Kennedy, 1992); therefore it had been expected that this strain would cause a greater level of disease in both cultivars than the other strains. However, the previous experiments described in this chapter suggested that a similar level of disease was caused in Elsanta plants inoculated with either strain 293 or strain 171. It is possible that the virulence of strain 293 had changed during the repeated culture of the pathogen. *P. fragariae* cultures have to be subcultured at intervals, and it has been suggested that the virulence of a particular isolate of the fungus could change during this process (Kennedy & Duncan, 1993).

Although strain 293 produced a significantly lower amount of disease in Elsanta than either of the other two strains, the level of disease in Rhapsody was significantly higher in plants inoculated with strain 293 compared to strain 171 or 372. Therefore the increased resistance of Rhapsody compared to Elsanta reduced the disease caused by strains 171 or 372.

Regardless of the method of disease assessment, considerable attention must be given to the statistical analysis of the results. For example, parametric analyses to obtain statistics such as mean, standard deviation and regression cannot be accurately applied to data that do not conform to a statistical probability distribution. The percentage infected root values do not approximate to a normal distribution and must therefore undergo an arcsine transformation before any statistical analysis can be performed, but parametric analyses are then appropriate. However, the values for the numbers of oospores observed in the root tips are counted, discrete data that are not normally distributed and, indeed, the number of oospores are aggregated and clustered within the root tips. Thus, these data must be analysed appropriately to take these factors into account and avoid introducing huge errors into the interpretation of the results. The Kruskal–Wallis analysis of variance enables these factors to be considered by ranking

the oospore values in order of the highest number of oospores to the lowest number of oospores. This overcomes the clustered effect of the oospore data and allows any significant differences to be observed and tested.

However, there have been several reports where the results of disease assessment have been analysed in an inappropriate manner which is subject to errors. For example, the disease score produced by Kennedy *et al.* (1986), although used in cluster analysis to suggest underlying patterns in the relationships between different isolates of *P. fragariae*, was also incorrectly used as mean disease scores and in regression analyses, thus applying parametric analyses to data that do not conform to a statistical probability distribution.

In addition, although Milholland *et al.* (1989) preferred to assess disease due to *P. fragariae* in terms of the number of oospores, the mean number of oospores per root segment was then used which would be subject to enormous variances and huge statistical errors. In any one plant, one root may contain no or a very small number of oospores while another root may contain several hundred oospores; any mean value will have a very large variance and will therefore be meaningless. Milholland *et al.* (1989) then used these values to calculate a disease severity index (DSI) where the DSI was equal to the mean number of oospores per root segment multiplied by the percentage of infected roots, all divided by 100. This had the effect of using oospore numbers, which do not conform to a probability distribution, in a parametric analysis which assumes that the data do conform to such a distribution. Although this DSI was considered to be a reliable and effective indicator of the differences in virulence of various isolates of *P. fragariae* when infecting different strawberry cultivars, its accuracy must be questioned because of the nature of the statistical analyses applied to the oospore data.

Because of the subjective nature of the disease assessment based on the percentage of infected root, and the distribution of the oospore data, further experiments will use the Kruskal–Wallis analysis of variance to assess disease in plants inoculated with motile, poured zoospores.

CHAPTER 5: Inoculation of strawberry plants with *Glomus mosseae*, *Glomus intraradices*, and *Glomus fistulosum*

5.1 Inoculation of micropropagated strawberry plants with the arbuscular mycorrhizal fungi *Glomus mosseae*, *Glomus intraradices*, and *Glomus fistulosum*

5.1.1 Introduction

The effect of colonisation of strawberry plants of two cultivars, Elsanta and Rhapsody, was assessed in terms of the growth and phosphorus nutrition of the plants. Disease resulting from subsequent inoculation of both mycorrhizal and non-mycorrhizal plants with the pathogen *Phytophthora fragariae* was assessed by counting the number of oospores in root tips of the plants and attempts made to determine whether there was a relationship between the mycorrhizal status of the host and infection by *P. fragariae*.

5.1.2 Experimental procedures

Plants of two strawberry cultivars, Elsanta and Rhapsody, were micropropagated to produce rooted plantlets, at which stage they were ready for transplantation into the glasshouse. A square of nylon was placed into the base of plastic plant pots of 0.5 L volume, which were then filled with sterilised sand to which lime (5 g L^{-1}) and bone meal (1.04 g L^{-1}) had been added. The pots were watered thoroughly with tap water and allowed to drain for 24 h. A planting hole was made in the centre of each pot, and approximately 30 g of mixed inoculum (consisting of colonised root fragments, fungal spores and hyphae) removed from a pot culture of the appropriate mycorrhizal fungus was placed into the hole. A rooted strawberry plant was removed from micropropagation and planted into this hole. Sand was gently pressed around the base of the plant to provide support. Care was taken to select plants of a similar size, so that any differences observed in plant growth during the experiment would not be a result of a particular plant being larger or smaller than the other replicates at the start of the experiment. Six plants of each strawberry cultivar were inoculated with each mycorrhizal fungus, and six control plants of each cultivar were inoculated with approximately 30 g of sterile sand. The sand around the base of the plant was watered and all plants placed in a randomized manner in one compartment of a glasshouse with average conditions of 20°C ± 2°C with mean daylight of 16 h per day.

After 8 weeks, the plants were removed from the pots and assessed for percentage mycorrhizal colonisation (%MC), root dry weight (RDW) and shoot dry weight (SDW), and the concentration [P] and total amount of phosphorus (P) in the shoots.

5.2 Results

5.2.1 Growth of plants during the experiment

Figure 5.1A illustrates the mean SDW, RDW, total P and [P] of mycorrhizal and non-mycorrhizal Elsanta and Rhapsody plants. An analysis of variance (see Appendix II, section 11.2) showed that there was no significant difference between SDW of mycorrhizal and non-mycorrhizal plants ($P > 0.05$) for each cultivar, i.e. the mycorrhizal colonisation had no significant effect on SDW. However, the mean SDW of Elsanta plants was significantly greater than that of Rhapsody plants ($P < 0.001$).

Mycorrhizal colonisation had no significant effect on the RDW of each cultivar (Fig. 5.1B; $P > 0.05$), although, once again, the RDW of Elsanta plants was significantly greater than that of Rhapsody plants ($P < 0.001$).

The amount of total P in the plant shoots was significantly greater in mycorrhizal compared to non-mycorrhizal plants (Fig. 5.1C; $P < 0.001$), with the mean amount of total P in plants colonised by *G. mosseae*, *G. intraradices* and *G. fistulosum* being significantly greater than that of the non-mycorrhizal plants. The total amount of P was significantly greater in Elsanta plants compared to Rhapsody plants ($P < 0.05$), which was not unexpected because the Elsanta plants had a significantly larger SDW than Rhapsody plants and larger plants may be assumed to contain more P than smaller plants.

Mycorrhizal colonisation also significantly increased the concentration of P in the shoots (Fig. 5.1D; $P < 0.001$). [P] is calculated as the total amount of P in milligrammes per gramme of SDW. As the total P in the shoots was significantly larger in mycorrhizal compared to non-mycorrhizal plants but there was no significant difference in the SDW of these plants, it appears that the significant difference in [P] is a result of the effect of the mycorrhizal colonisation on the amount of P in the shoots and not on the SDW.

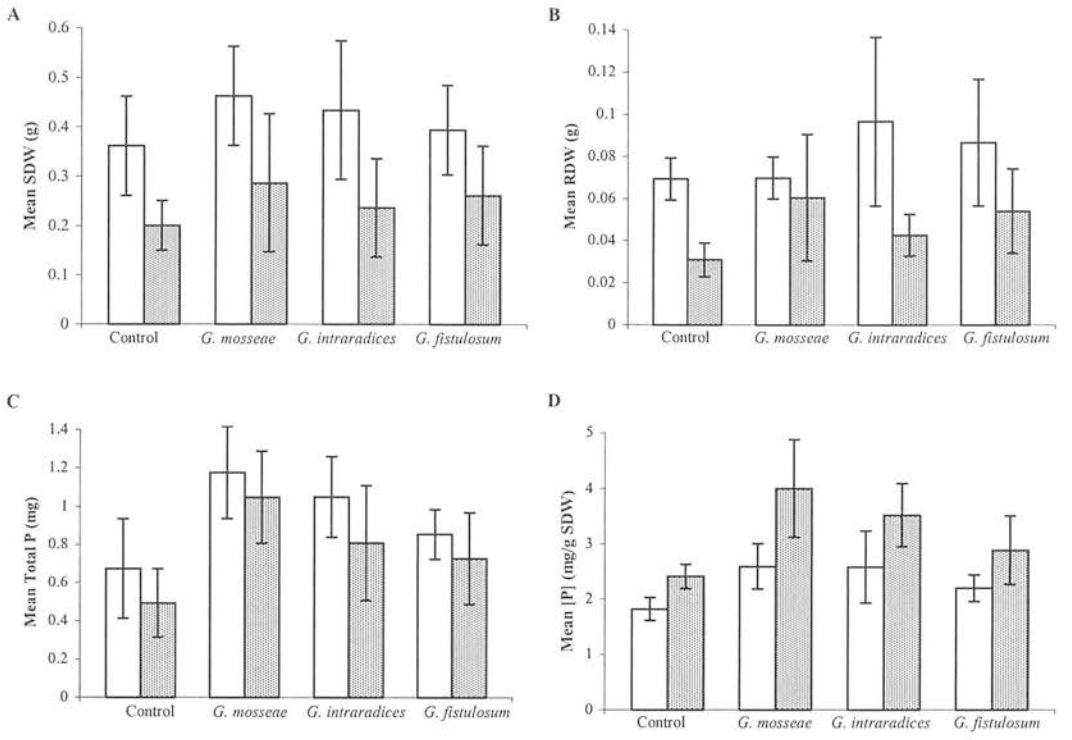

Fig. 5.1. Mean SDW (A), RDW (B), total P in the shoot (C) and P concentration (D) of Elsanta (white bars) and Rhapsody (shaded bars) plants for control, non-mycorrhizal strawberry plants, and plants colonised by *G. mosseae*, *G. intraradices* or *G. fistulosum*. Error bars represent the standard deviation.

The [P] value of Rhapsody plants was significantly greater than that of Elsanta plants (Fig. 5.1D; $P < 0.001$) even though the Elsanta plants were significantly larger and contained more phosphorus than Rhapsody plants. This indicates that the Rhapsody plants contained a greater amount of phosphorus relative to plant size than Elsanta plants.

Figure 5.2A shows the relationship between the root dry weight (RDW) and shoot dry weight (SDW) of the mycorrhizal and control, non-mycorrhizal Elsanta and Rhapsody plants. For both mycorrhizal and non-mycorrhizal Elsanta plants, there was a positive correlation between RDW and SDW, i.e. RDW increased as SDW increased (*G. mosseae*, $r = 0.533$; *G. intraradices*, $r = 0.820$; *G. fistulosum*, $r = 0.820$; control, $r = 0.903$). Correlation analysis (Table 5.1) indicated that this relationship was significant for non-mycorrhizal plants ($P < 0.05$) and those inoculated with *G. mosseae* ($P < 0.05$) and *G. fistulosum* ($P < 0.05$). In each of these three significant relationships, examination of the residuals suggests that the relationship can be assumed to be a straight line (see Appendix II, section 11.3.1). However, the results

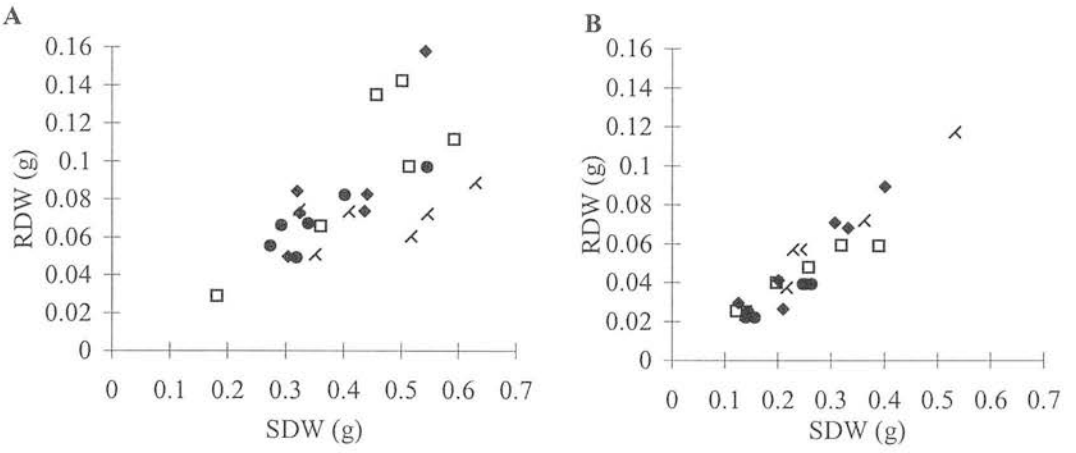

Fig. 5.2. The relationship between shoot dry weight (SDW) and root dry weight (RDW) of Elsanta (A) and Rhapsody (B) plants that were non-mycorrhizal (●) or colonised by *G. mosseae* (◆), *G. intraradices* (□) or *G. fistulosum* (×).

show that plants inoculated with *G. mosseae* and *G. intraradices* had a larger SDW than control plants; the RDW of plants inoculated with *G. intraradices* was also greater than in the non-mycorrhizal plants. However, there was no evidence to suggest that the relationship between RDW and SDW was causal. Therefore, although a significant positive correlation suggested that RDW increased as SDW increased, it was not necessarily the case that increasing SDW caused RDW to increase.

A more positive correlation was observed between RDW and SDW of all Rhapsody plants, with RDW increasing as SDW increased (non-mycorrhizal, $r = 0.985$; *G. mosseae*, $r = 0.978$; *G. intraradices*, $r = 0.965$; *G. fistulosum*, $r = 0.946$). Correlation analysis (Table 5.3) indicated that all four relationships were significant (non-mycorrhizal, *G. mosseae*, and *G. intraradices*, $P < 0.01$; *G. fistulosum*, $P < 0.05$). The results indicated that Rhapsody plants colonised by *G. mosseae* had a slightly larger SDW and RDW compared to control plants although this was not significant (Appendix II, section 11.3.4).

5.2.2 Relationship between plant growth and phosphorus (P)

The low-nutrient sand/peat growing medium contained only a low level of P that was available for uptake into the plants. The amount of P in the plant shoots was calculated in two different methods. The total P in the shoots represents the total

Table 5.1. Summary of correlation analysis of growth characteristics of Elsanta (RDW, Total P and [P]) varying with shoot dry weight of the plants. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as plant size (SDW) increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level; ***, significant at the 0.1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
Root dry weight	*G. mosseae*	0.277	ns	0.532	0.283
	G. intraradices	0.045	*	0.820	0.673
	G. fistulosum	0.045	*	0.819	0.671
	Control	0.014	*	0.903	0.816
Total P	*G. mosseae*	0.077	ns	0.764	0.583
	G. intraradices	0.018	*	0.887	0.788
	G. fistulosum	0.006	**	0.934	0.872
	Control	0.0001	***	0.991	0.982
[P]	*G. mosseae*	0.218	ns	–0.589	0.347
	G. intraradices	0.004	**	–0.946	0.895
	G. fistulosum	0.058	ns	–0.796	0.633
	Control	0.032	*	0.851	0.723

Table 5.2. Summary of linear regression analysis of growth characteristics of Elsanta (RDW, Total P and [P]) varying with %MC of the plant. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as %MC increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
Root dry weight	*G. mosseae*	0.806	ns	0.130	0.017
	G. intraradices	0.688	ns	–0.211	0.045
	G. fistulosum	0.459	ns	0.378	0.143
Shoot dry weight	*G. mosseae*	0.197	ns	–0.612	0.0.374
	G. intraradices	0.506	ns	–0.343	0.118
	G. fistulosum	0.906	ns	–0.063	0.004
Total P	*G. mosseae*	0.776	ns	–0.150	0.023
	G. intraradices	0.457	ns	–0.379	0.144
	G. fistulosum	0.811	ns	–0.127	0.016
[P]	*G. mosseae*	0.031	*	0.852	0.726
	G. intraradices	0.809	ns	0.128	0.016
	G. fistulosum	0.945	ns	–0.036	0.001

amount of P found in the whole shoot system of the plant and is measured in milligrammes. [P] is measured as the amount of P, in milligrammes, in the whole plant shoot per gramme of SDW. Therefore, larger plants may be expected to contain more total P than smaller plants and the total P will increase as the SDW increases; however, in order to determine whether this is a result of additional P being taken up by the plant, one must consider [P]. If there is no additional P uptake, as the SDW increases, the [P] will decrease. If there is only a slight uptake of additional P into the plant shoot, [P] will remain almost constant or fall only slightly as the plant size, i.e. SDW, increases. Therefore, these two measurements of P must be considered when interpreting the relationship between plant growth and P content of the plants and a comparison of total P and [P] provides information about the rate of accumulation of P in relation to the increase in SDW, RDW or %MC.

Figure 5.3A shows that for both mycorrhizal and non-mycorrhizal Elsanta plants, the amount of total P in the shoot increased as SDW increased, as illustrated by the positive correlation coefficients (non-mycorrhizal, $r = 0.991$; *G. mosseae*, $r = 0.764$; *G. intraradices*, $r = 0.887$; *G. fistulosum*, $r = 0.934$) (Table 5.1). However, correlation analysis (Table 5.1) indicated that this relationship was only significant in non-mycorrhizal plants ($P < 0.001$) and those inoculated with *G. intraradices* ($P < 0.01$) and *G. fistulosum* ($P < 0.01$). In each of these cases, a large proportion of the variation in total P in the shoots was accounted for by the variation in SDW. The total P content of plants inoculated with *G. mosseae* and *G. intraradices* was greater than that of control plants. This may be explained by the greater SDW of these plants compared to control plants, i.e. larger shoots contained more P than smaller shoots (Appendix II, section 11.3.3).

However, examination of the data relating to [P] of the shoots provides more information. Figure 5.3B and Table 5.1 show that in the non-mycorrhizal plants, as SDW increased, [P] increased significantly ($P < 0.001$; $r = 0.851$), representing an enhanced uptake of additional P into the plants in relation to shoot size. In contrast, [P] of the mycorrhizal plants decreased as SDW increased, indicating that additional P was not being taken up into the plants. This negative relationship was significant for plants colonised by *G. intraradices* ($P < 0.01$).

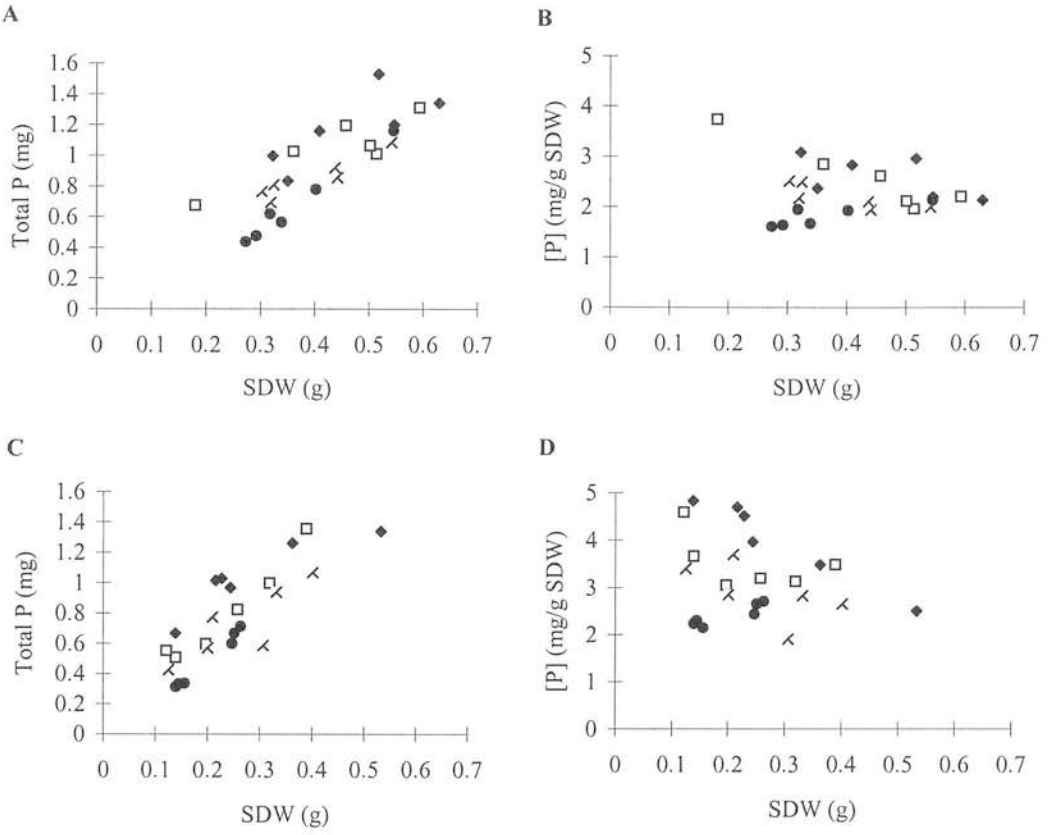

Fig. 5.3. The relationship between shoot dry weight (SDW) and total phosphorus (P) in the shoot and the concentration of phosphorus ([P]) in the shoot for Elsanta (A and B) and Rhapsody (C and D) plants that were non-mycorrhizal (●) or colonised by colonised by *G. mosseae* (◆), *G. intraradices* (□) or *G. fistulosum* (×).

In Rhapsody plants (Fig. 5.3C), there was a significant positive correlation between SDW and total P in all interactions (non-mycorrhizal and *G. intraradices*, $P < 0.001$; *G. mosseae* and *G. fistulosum*, $P < 0.05$) (Table 5.3). When considering the concentration of P in the plant shoots, that of the control plants increased as SDW increased significantly in a positive correlation ($P < 0.05$), indicating that larger shoots contained appreciably more P than the smaller shoots, whereas [P] in shoots of the mycorrhizal plants decreased as SDW increased in a negative relationship, which was only found to be significant for plants inoculated with *G. mosseae* ($P < 0.01$), indicating that the rate of uptake of additional P was smaller in the mycorrhizal plants compared to the control plants.

These results contrast with the findings of the analysis of variance described above, in which the mycorrhizal plants of both cultivars contained significantly more P in the shoots than non-mycorrhizal plants, although there was no difference in plant size.

This suggests that P must have accumulated in the mycorrhizal plants at a low level of mycorrhizal colonisation. Therefore a low threshold value of percentage of mycorrhizal colonisation (approximately 40%; see Fig. 5.4) was sufficient to cause an increase in additional P uptake into the plants. The increasing level of colonisation by the mycorrhizal fungi had no additional effect on the P uptake of the plants. The additional P uptake occurred at a low level of %MC when plants were probably smaller that at the sampling time. As plant size increased, there was no additional P uptake, resulting in an apparent negative relationship between SDW and P.

5.2.3 The effect of mycorrhizal colonisation on plant growth and P uptake

Figure 5.4 shows that the %MC of Elsanta plants colonised by *G. fistulosum* was lower than that of plants colonised by either of the two other mycorrhizal fungi, and that the level of colonisation by *G. mosseae* and *G. intraradices* was similar. The reduced level of colonisation by *G. fistulosum* may be a result of a lower number of infective propagules (i.e. spores, hyphae, infected root fragments) in the inoculum of the fungus. However, although the actual infectivity of the original mycorrhizal inoculum was not quantified, the 30 g that was applied to every strawberry plant was sufficient for root colonisation not to be limited by a lack of infective mycorrhizal propagules (Niemi & Vestberg, 1992). The root system of Elsanta plants may have been unfavourable for extensive colonisation by *G. fistulosum*. Also, the colonisation by *G. fistulosum* may have been slower than that by the other mycorrhizal fungi, causing a lower level of colonisation to be observed 8 weeks after the initial inoculation of the plants by the mycorrhizal fungi.

For each mycorrhizal fungus colonising Elsanta plants, there was a negative correlation between SDW and %MC (Fig. 5.4A; Table 5.2), indicating that an increase in the colonisation by the mycorrhizal fungi tended to result in a lower SDW of the host, i.e. the plant size decreased as the presence of the mycorrhizal colonisation increased. However, this relationship, which was most noticeable with *G. mosseae*, was not significant for any of the mycorrhizal fungi (Table 5.2).

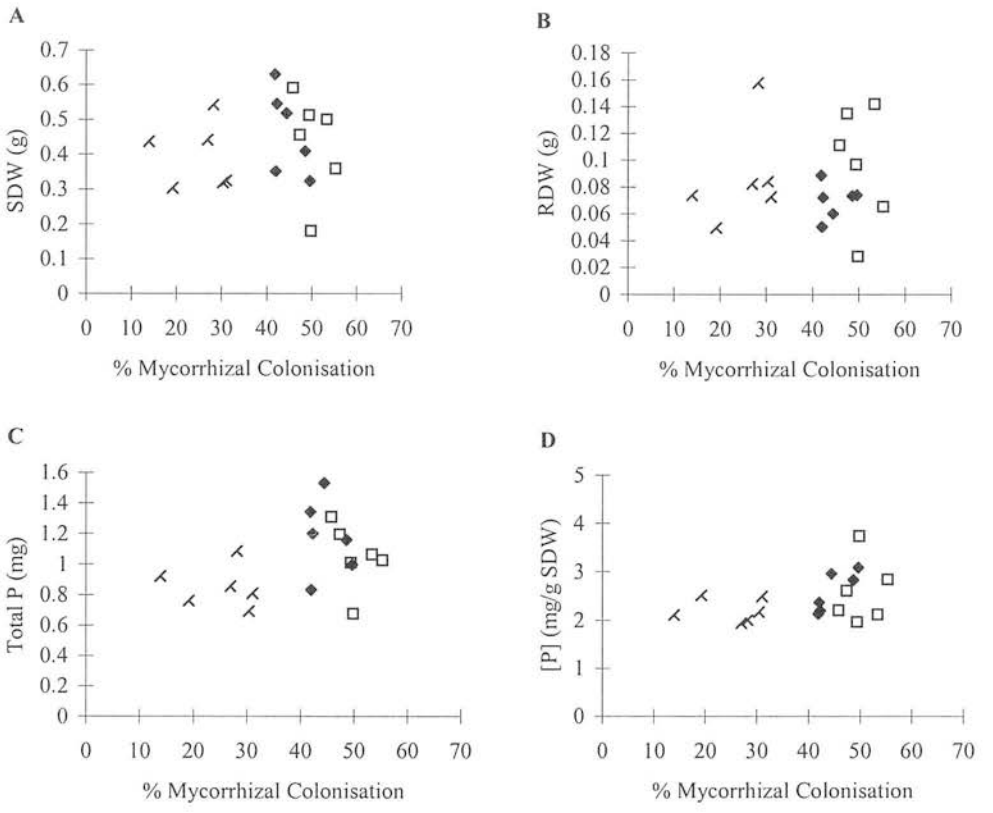

Fig. 5.4. The effect of increasing percentage mycorrhizal colonisation (%MC) on the shoot dry weight (SDW; A), root dry weight (RDW; B), total phosphorus in the shoot (P; C) and concentration of phosphorus in the shoot ([P]; D) of Elsanta plants colonised by *G. mosseae* (◆), *G. intraradices* (□) or *G. fistulosum* (×).

Figure 5.4B shows that increasing %MC had very little effect on the RDW of the Elsanta plants. For each mycorrhizal colonisation, the relationship between RDW and %MC was not significant (Table 5.2; see Appendix II, section 11.4.1). The results in Table 5.2 show that only a low proportion of the variation in RDW was a result of the effect of %MC (*G. mosseae*, 1.7%; *G. intraradices*, 4.5%; *G. fistulosum*, 14.3%). This can be compared to the relationship between RDW and SDW for Elsanta plants, in which a much larger proportion of the variation in RDW could be accounted for by the variation in SDW (*G. mosseae*, 28.3%; *G. intraradices*, 67.3%; *G. fistulosum*, 67.2%). Therefore, the presence of the mycorrhizal fungi had very little, if any, effect on the RDW of the host plant.

Increasing mycorrhizal colonisation appeared to have a negative effect on the total amount of P in the plant shoots, indicated by the negative correlation observed in Fig. 5.4C and Table 5.2 (*G. mosseae*, $r = -0.15$; *G. intraradices*, $r = -0.380$; *G. fistulosum*, $r = -0.127$). However, none of these relationships were significant, and

correlation analysis showed that a very low proportion of the variation in total amount of P in the shoots could be attributed to the effect of %MC (r^2 values in Table 5.2) compared to the amount of variation that could be accounted for by the increasing SDW (r^2 values in Table 5.1) (Appendix II, section 11.4.4). In fact, the SDW of the plants also decreased as the %MC increased. Smaller plants would be expected to contain less P than larger plants (Fig. 5.3A). Therefore the decreasing amount of total P was probably the result of the simultaneously decreasing SDW as the %MC increased.

Figure 5.4D shows that there was no effect of increasing %MC on [P] in plants colonised by *G. fistulosum*, and only a slight positive effect in plants colonised by *G. intraradices*. Although neither relationship was significant, ($P = 0.945$ and $P = 0.809$ for *G. fistulosum* and *G. intraradices*, respectively), it suggests that an increase in the mycorrhizal colonisation by these fungi had either no effect or a slight positive effect on [P] in the shoots (Table 5.2). Once again, only a very low proportion of the variability in [P] could be accounted for by the effect of %MC (r^2 values in Table 5.2) compared to the variation in [P] resulting from variation in the SDW of the plants (r^2 values in Table 5.1) (Appendix II, section 11.4.3). Indeed, both SDW and total P in the shoots tended to decrease as %MC increased. Therefore, as [P] is a function of the total amount of P divided by the SDW of the plants, [P] would be expected to remain approximately the same as both SDW and total P decreased, which was observed in Fig. 5.4D.

Examination of the results for Rhapsody plants shows a strong negative correlation between %MC and SDW in all mycorrhizal plants (Fig. 5.5A; *G. mosseae*, $r = -0.0611$; *G. intraradices*, $r = -0.953$; *G. fistulosum*, $r = -0.679$), indicating that the SDW decreased as the level of mycorrhizal colonisation increased. However, this relationship was only significant in plants colonised by *G. intraradices* (Table 5.4; $P = 0.003$). The relationship between RDW and %MC was similar (Fig. 5.5B) in that there was a negative correlation for all mycorrhizal fungi, although again this was only significant for plants colonised by *G. intraradices* (Table 5.4; $P < 0.05$).

Table 5.3. Summary of correlation analysis of growth characteristics of Rhapsody (RDW, Total P and [P]) varying with shoot dry weight of the plants. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as plant size (SDW) increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level; ***, significant at the 0.1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
Root dry weight	*G. mosseae*	0.0007	***	0.978	0.956
	G. intraradices	0.002	*	0.965	0.931
	G. fistulosum	0.004	*	0.945	0.894
	Control	0.0003	***	0.985	0.969
Total P	*G. mosseae*	0.013	*	0.905	0.818
	G. intraradices	0.001	***	0.972	0.944
	G. fistulosum	0.035	**	0.842	0.709
	Control	0.00009	***	0.992	0.984
[P]	*G. mosseae*	0.009	**	–0.975	0.951
	G. intraradices	0.265	ns	–0.543	0.295
	G. fistulosum	0.206	ns	–0.602	0.363
	Control	0.015	**	0.899	0.809

Table 5.4. Summary of linear regression analysis of growth characteristics of Rhapsody (RDW, Total P and [P]) varying with %MC of the plant. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as %MC increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level; ***, significant at the 0.1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
Root dry weight	*G. mosseae*	0.088	ns	–0.746	0.557
	G. intraradices	0.029	*	–0.858	0.736
	G. fistulosum	0.080	ns	–0.759	0.576
Shoot dry weight	*G. mosseae*	0.180	ns	–0.611	0.373
	G. intraradices	0.003	**	–0.953	0.908
	G. fistulosum	0.138	ns	–0.678	0.461
Total P	*G. mosseae*	0.132	ns	–0.687	0.472
	G. intraradices	0.005	**	–0.942	0.887
	G. fistulosum	0.696	ns	–0.205	0.042
[P]	*G. mosseae*	0.186	ns	0.623	0.389
	G. intraradices	0.280	ns	0.529	0.279
	G. fistulosum	0.001	***	0.972	0.946

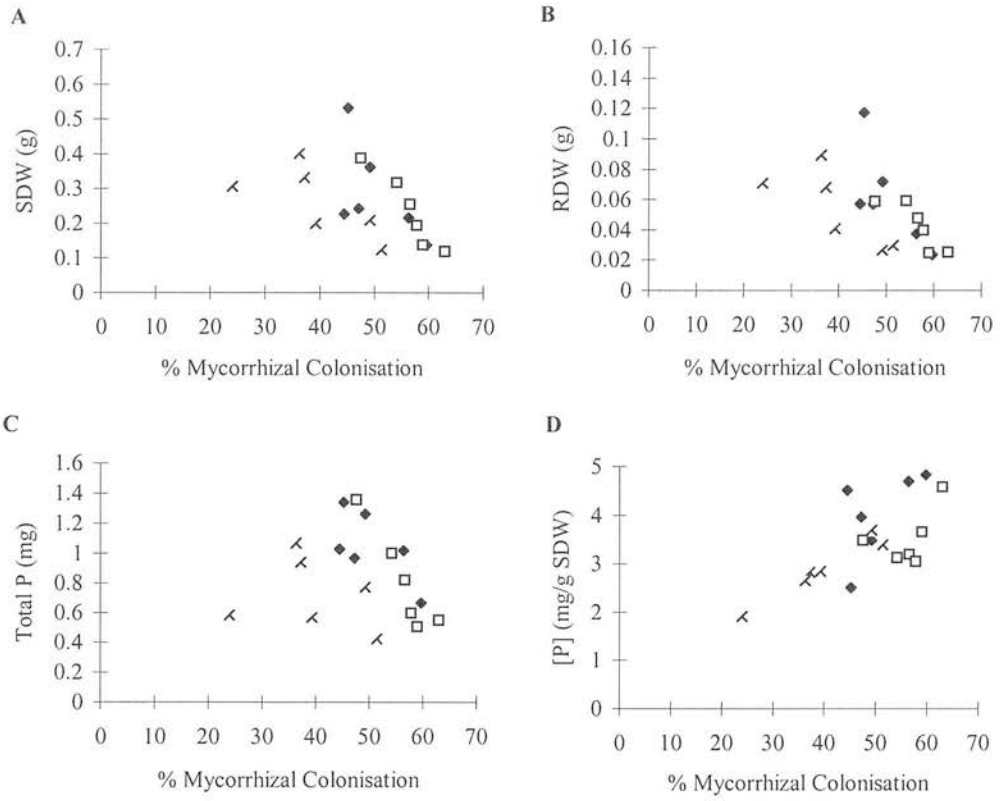

Fig. 5.5. The effect of increasing percentage mycorrhizal colonisation (%MC) on the shoot dry weight (SDW; A), root dry weight (RDW; B), total phosphorus in the shoot (P; C) and concentration of phosphorus in the shoot ([P]; D) of Rhapsody plants colonised by *G. mosseae* (◆), *G. intraradices* (□) or *G. fistulosum* (×).

Therefore, increasing colonisation of Rhapsody plants by either of the three mycorrhizal fungi resulted in a decrease in plant root and shoot size, strongly suggesting that the fungus had a negative effect on plant growth in this cultivar.

Figure 5.5C shows a negative relationship between %MC and total P in the shoot, although this was only significant for plants colonised by *G. intraradices* (Table 5.4; $P < 0.05$). Therefore, increasing the level of mycorrhizal colonisation resulted in a reduction in the total amount of P in the shoots. This may have been a result of the decreasing SDW as %MC increased, as smaller plants would be expected to contain less P than larger plants (see Fig. 5.3C).

Figure 5.4D shows that [P] in the shoots increased as the level of colonisation by either of the three mycorrhizal fungi increased. However, this was only significant for plants colonised by *G. fistulosum* (Table 5.4; $P < 0.01$; Appendix II, section 11.4.7).

The apparent effect of %MC on additional P uptake into these plants must be considered in relation to the effect of %MC on other aspects of plant growth. For example, it was shown that SDW, RDW and total P in the shoots decreased as the level of mycorrhizal colonisation increased. If SDW and total P in the shoots were decreasing at approximately the same degree, [P] would be expected to also decrease as %MC increased, as is the case in Elsanta plants. The fact that [P] (calculated as the amount of P (mg) per gramme of SDW) increased as %MC increased suggests that SDW was reduced to a greater degree than total P in the shoots as the %MC increased. Therefore, the apparent increase in [P] as %MC increased was a result of the concomitant decreasing SDW and total P as the %MC increased and probably not a result of increased P uptake into the plant.

These results must be considered in relation to the conclusions of the analysis of variance described earlier, in which the mycorrhizal plants were shown to contain significantly more P in their shoots than non-mycorrhizal plants, although there was no correlation between the increasing mycorrhizal colonisation and total P in the shoots. Therefore, it can be assumed that low levels of mycorrhizal colonisation caused additional P to be taken into the plant, because the increasing mycorrhizal colonisation shown in Fig. 5.4 had no effect on the P content of the shoots. The values shown in Fig. 5.4 indicate that approximately 15% mycorrhizal colonisation by *G. fistulosum* and approximately 40% mycorrhizal colonisation by *G. mosseae* or *G. intraradices* was sufficient to cause an increase in shoot P content, because values of colonisation above these had no effect on the total P in the plant.

5.2.4 Effect of inoculating mycorrhizal and non-mycorrhizal plants with *P. fragariae*

Disease resulting from subsequent inoculation of non-mycorrhizal and mycorrhizal plants with *P. fragariae* was assessed in terms of the number of oospores observed in each of 10 root tips removed from each plant. Oospores represent the sexual structures of *P. fragariae*; therefore their presence indicates that the pathogen has infected the root and sexual reproduction has occurred.

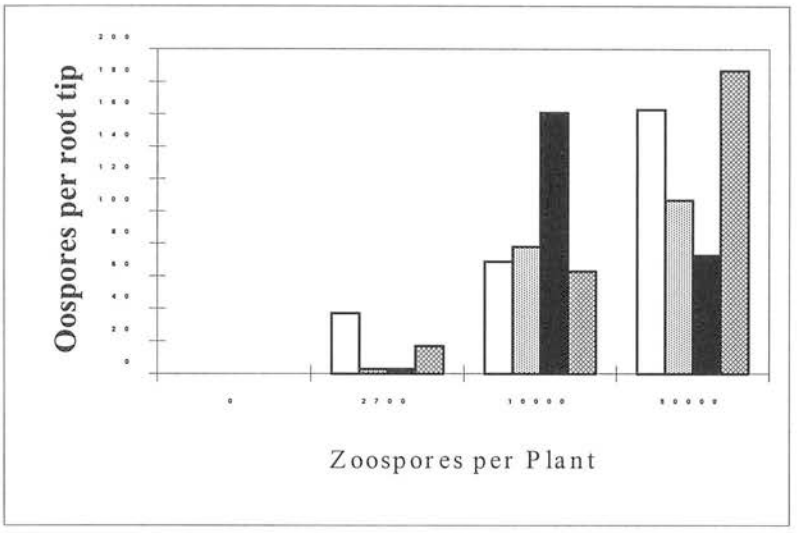

Fig. 5.6. Total number of oospores observed in root tips of non-mycorrhizal Elsanta plants (white bars) and those colonised by *G. mosseae* (grey-shaded bars), *G. intraradices* (black bars) and *G. fistulosum* (cross-hatched bars) inoculated with increasing levels of zoospores of *P. fragariae*.

Figure 5.6 shows that no oospores were observed in non-inoculated plants, and a small number of oospores were seen in plants inoculated with 2700 zoospores of *P. fragariae*, with a greater total number of oospores observed as the level of zoospores per plant of *P. fragariae* increased. A Kruskal–Wallis analysis of variance using tied ranks was performed to compare the numbers of oospores observed in the roots of mycorrhizal and non-mycorrhizal plants inoculated with 50 000 zoospores per plant.

Table 5.5 Inoculation of non-mycorrhizal Elsanta plants, plants colonised by *G. mosseae*, *G. intraradices* or *G. fistulosum* with the pathogen *P. fragariae*. The number of oospores was counted in 10 root tips taken from each plant.

	Non-mycorrhizal	*G. mosseae*	*G. intraradices*	*G. fistulosum*
Total number of oospores	163	107	73	187
Percentage of roots containing oospores	46	24	22	32
Mean number of oospores in roots containing oospores (standard deviation)	7.09 (4.02)	8.92 (9.39)	6.64 (4.34)	11.69 (11.68)

There was no significant difference between the number of oospores of *P. fragariae* observed in the root tips of either non-mycorrhizal plants or plants colonised by either *G. mosseae*, *G. intraradices* or *G. fistulosum* ($H_c = 7.399$; $\chi^2_{0.05,3} = 7.815$). Therefore, the presence of the mycorrhizal fungi appeared to have no effect on the level of disease due to *P. fragariae*.

However, Table 5.5 shows the actual number of oospores observed in the root tips and the mean number of oospores per root tip and the percentage of roots containing oospores. Analysis of the mean number of oospores per root tip is not a valid method to investigate these results because the data do not conform to a statistical probability distribution and therefore require a non-parametric statistical analysis. The proportion of root tips that contain oospores per treatment can be examined as this relies solely on the presence or absence of oospores in each root tip and does not take into account the distribution of oospores. A chi-squared test of the number of infected roots (Appendix II, section 11.6) showed that there was a significantly greater number of non-mycorrhizal infected roots compared to mycorrhizal infected roots ($P < 0.05$), as suggested by the value of percentage of infected roots in Table 5.5. However, the results in Table 5.5 also indicate that the greatest mean number of oospores was in plant roots colonised by *G. fistulosum*.

5.3 Discussion

Initially, it was difficult to discern the effect of the mycorrhizal colonisation on the plants in this experiment because many of the relationships, although apparent from the figures, were found to be not statistically significant. The initial analysis of variance showed that the mycorrhizal plants had significantly greater mean levels of total phosphorus in their shoots and an increased phosphorus concentration compared to non-mycorrhizal plants.

However, the results showed that the increase in mycorrhizal colonisation had a negative but not significant effect on SDW and no significant effect on RDW in both cultivars. In addition, a negative, but not significant, correlation was observed between increasing mycorrhizal colonisation and the total P or [P] in plant shoots for each mycorrhizal fungus, although this analysis also demonstrated that only a very low proportion of the variation in total P could be attributed to the effect of the

mycorrhizal colonisation. The observation of the effect of the increasing mycorrhizal colonisation had a minimum level of approximately 40% and 15% root colonisation for Elsanta and Rhapsody plants, respectively. Therefore, low levels of mycorrhizal colonisation caused the increased amount of total P in plant shoots observed as a result of the analysis of variance. Increasing the mycorrhizal colonisation above this level did not cause additional P to be taken up by the plant.

However, it must be remembered that the actual uptake of P into the shoots was not measured; the total amount of P in the shoots may not be representative of the P that had been taken into the plant during the 8-week period of colonisation by the mycorrhizal fungi. The values measured only show the situation at the exact time at which the plants were harvested for sampling. These values and those of the level of mycorrhizal colonisation in the roots could have changed over this time period. Sampling throughout the experiment may have detected the additional uptake of P at lower levels of mycorrhizal colonisation.

When the effect of increasing levels of mycorrhizal colonisation on the shoot size of the plant was investigated, it was clear that SDW decreased as mycorrhizal colonisation by each fungus increased, although this was not significant. Therefore, the presence of the mycorrhizal fungi was associated with a reduction in plant size, suggesting that the fungus had a negative effect on the plant. Again, this could be related to the threshold level of mycorrhizal colonisation, as the initial analysis of variance demonstrated no significant difference between the SDW of mycorrhizal and non-mycorrhizal plants. In a mycorrhizal symbiosis there is a passage of carbohydrates into the fungus across the arbuscular interface between the host and fungus (Smith & Gianinazzi-Pearson, 1988). This movement of carbohydrates into the arbuscles can be complemented by passage of P, which the mycorrhizal fungus has sequestered from the root zone and transported into the plant, from the fungus to the plant. However, it is possible that as the mycorrhizal colonisation increases above a particular threshold value, the fungus then becomes a drain on the plant's resources, causing the SDW to decrease as the mycorrhizal colonisation increased. The carbon drain from the plant to the fungus has been reported to cause growth depressions in some mycorrhizal plants, resulting in a loss of crop productivity (Cooper, 1984).

A similar negative effect of colonisation by *Glomus* spp. on strawberry plant health was reported by Vestberg (1994), in which mycorrhizal plants whose health was negatively affected by the mycorrhizal colonisation were found to be more susceptible to infection by *Phytophthora cactorum*. However, in the present experiments, the mycorrhizal colonisation had neither a positive nor a negative effect on disease due to the pathogen. Bååth & Hayman (1984) also reported that colonisation of strawberry roots by mycorrhizal fungi had no effect on subsequent disease caused by a root-invading pathogen. Therefore, it is possible that this exchange of carbohydrates and P at the arbuscles may have become unbalanced in favour of the fungus, which was then receiving carbohydrates from the plant without enhancing the P nutrition of the host. This could be considered to be a favourable arrangement for the fungus, allowing it to potentially colonise other plants in the vicinity while being provided with carbohydrates from the host plant.

Subsequent inoculation of the mycorrhizal and non-mycorrhizal plants by the pathogen *P. fragariae* showed that the mycorrhizal colonisation had no significant effect on the number of oospores of the pathogen observed in root tips compared to the non-mycorrhizal plants, i.e. the presence of the mycorrhizal fungi had no significant effect on the sexual reproduction of *P. fragariae*.

It has been suggested that a mycorrhizal colonisation provokes a continual low-level host defence response which is not sufficient to repel colonisation by the mycorrhizal fungi, but is enough to enable the plant to resist invasion by a pathogen (Gianinazzi, 1991). However, this did not appear to be the case in the interaction studied here. The presence of the mycorrhizal fungus had no effect on subsequent disease due to *P. fragariae*.

CHAPTER 6: Inoculation of strawberry plants with *Scutellospora nodosa* and *Acaulospora scrobiculata*

6.1 Inoculation of micropropagated strawberry plants with the arbuscular mycorrhizal fungi *Scutellospora nodosa* and *Acaulospora scrobiculata*

6.1.1 Introduction

The aim of these experiments was to determine the effect of the inoculation of micropropagated strawberry plants with the arbuscular mycorrhizal fungi *Scutellospora nodosa* and *Acaulospora scrobiculata*. Disease resulting from subsequent inoculation of mycorrhizal and non-mycorrhizal plants with *Phytophthora fragariae* was assessed and, by examination of the number of oospores of *P. fragariae* in the root tips, attempts were made to determine any effect of the mycorrhizal fungi on the resulting disease. A large proportion of research into the effect of arbuscular mycorrhizal fungi on host plants has concentrated on *Glomus* spp. It was therefore considered to be a novel approach to use alternative species to inoculate the strawberry plants. Also, other mycorrhizal species may possess useful properties and have different specific effects on a host plant compared to *Glomus* spp.

6.1.2 Experimental procedures

Plants of two strawberry cultivars, Elsanta and Rhapsody, were micropropagated to produce rooted plantlets, at which stage they were ready for transplantation into the glasshouse. A square of nylon was placed into the base of plastic plant pots of 0.5 L volume, which were then filled with sterilised sand to which lime (5 g L^{-1}) and bone meal (1.04 g L^{-1}) had been added. The pots were watered thoroughly with tap water and allowed to drain for 24 h. A planting hole was made in the centre of each pot, and approximately 30 g of mixed inoculum (consisting of colonised root fragments, fungal spores and hyphae) of the appropriate mycorrhizal fungus was placed into the hole. A rooted strawberry plant was removed from micropropagation and planted into this hole. Sand was gently pressed around the base of the plant to provide support. Care was taken to select plants of a similar size, so that any differences observed in plant growth during the experiment would not be a result of a particular plant being larger or smaller than the other replicates at the start of the experiment. There were six replicate plants of each strawberry cultivar inoculated with each mycorrhizal fungus, and six control plants of each cultivar were inoculated with approximately 30 g of

sterile sand. The sand around the base of the plant was watered and all plants placed in a randomized manner throughout one compartment of a glasshouse with average conditions of 20°C ± 2°C with mean daylight of 16 h per day.

After 8 weeks, some of the plants were removed from the pots and assessed for the percentage of mycorrhizal colonisation (%MC), root dry weight (RDW) shoot dry weight (SDW), and the concentration [P] and total amount of phosphorus (P) in the shoots (see Appendix III, section 12.1). The remaining plants were inoculated with a motile, poured inoculation of zoospores of strain 293 of *P. fragariae*, at varying doses, such that five plants of each strawberry cultivar were inoculated with each dose of zoospores. Control, non-inoculated plants were treated with 50 mL of distilled water instead of the motile zoospore suspension. The pots containing the plants were enclosed in loosely sealed plastic bags and randomised throughout a controlled environment chamber and grown at a temperature of 15°C for a period of 14 days. Ten root tips were then cut from each plant and examined under a stereoscopic light microscope for the presence of oospores of *P. fragariae*. The number of oospores per root tip was counted.

6.2 Results

6.2.1 Growth of plants during the experiment

Figure 6.1 shows the mean SDW, RDW, total P and P concentration of both Elsanta and Rhapsody plants determined at the end of the experiment when the plants were harvested. A two-factor analysis of variance (see Appendix III, section 12.2) showed that there was not a significant difference in the SDW of the two strawberry cultivars, Elsanta and Rhapsody ($P < 0.05$). However, there was a significant difference in the SDW of non-mycorrhizal plants and those colonised by either *A. scrobiculata* or *S. nodosa* (Fig. 6.1A; $P < 0.001$), indicating that for each cultivar, the SDW of the mycorrhizal plants was significantly different from the SDW of the non-mycorrhizal plants. Colonisation of both Elsanta and Rhapsody plants by *S. nodosa* significantly increased the SDW compared to the non-mycorrhizal plants, while colonisation of Elsanta plants by *A. scrobiculata* caused a significantly lower SDW than non-mycorrhizal plants and the effect of colonisation of Rhapsody by *A. scrobiculata* was

to significantly increase the SDW of the plants. This different effect of each mycorrhizal fungus on the SDW of each cultivar caused a significant interaction between the mycorrhizal status and cultivar to be observed ($P < 0.001$).

The RDW of each cultivar was significantly different, with that of Elsanta plants tending to be larger than the RDW of Rhapsody plants ($P < 0.01$). However, Fig. 6.1B shows that colonisation of plants of either strawberry cultivar by *A. scrobiculata* caused a significantly lower RDW ($P < 0.01$) than either non-mycorrhizal plants or plants colonised by *S. nodosa*, the RDW of which was similar to the non-mycorrhizal controls.

The total P in shoots of plants colonised by either *A. scrobiculata* or *S. nodosa* was significantly greater than that of non-mycorrhizal plants (Fig. 6.1C; $P < 0.001$), indicating that the mycorrhizal fungi increased the amount of total P in the shoots; the effect was more pronounced for plants colonised by *S. nodosa* than for plants colonised by *A. scrobiculata*. There was no significant difference in the response of the two cultivars ($P > 0.05$).

Colonisation of both cultivars by *A. scrobiculata* or *S. nodosa* resulted in a significant increase in [P] in the shoots (Fig. 6.1D; $P < 0.01$), as would be expected because the mycorrhizal plants had significantly greater SDW and total P than the non-mycorrhizal plants, and [P] is calculated as the amount of P in milligrammes per gramme of SDW. Therefore, as both the total P and SDW were increased in mycorrhizal plants, [P] would also increase.

Figure 6.2A shows the relationship between RDW and SDW of control, non-mycorrhizal plants and mycorrhizal plants of Elsanta. For Elsanta inoculated with *S. nodosa*, there was only a slight positive correlation ($r = 0.289$) between SDW and RDW, i.e. there was only a small increase in RDW as SDW increased, which was visible although the values were rather clustered. For the non-mycorrhizal Elsanta plants and those inoculated with *A. scrobiculata*, there was a more positive correlation

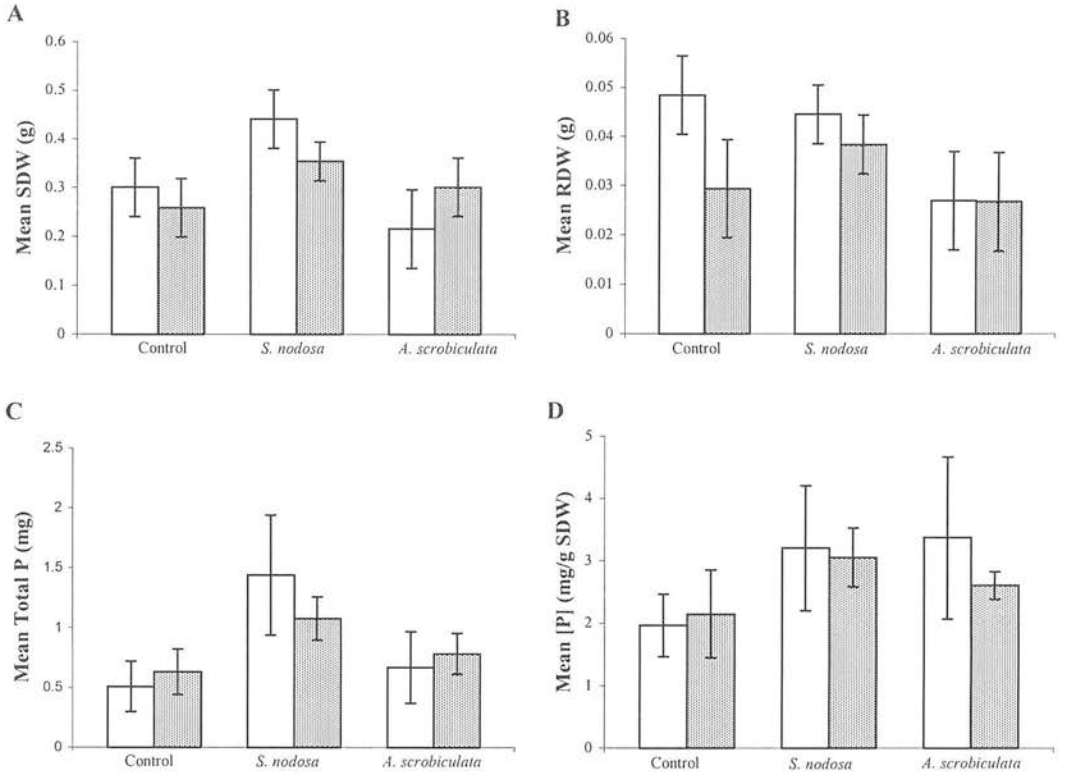

Fig. 6.1. Mean SDW (A), RDW (B), total P in the shoot (C) and P concentration (D) of Elsanta (white bars) and Rhapsody (shaded bars) plants of control, non-mycorrhizal plants and those colonised by *S. nodosa* or *A. scrobiculata*. The error bars represent the standard deviation.

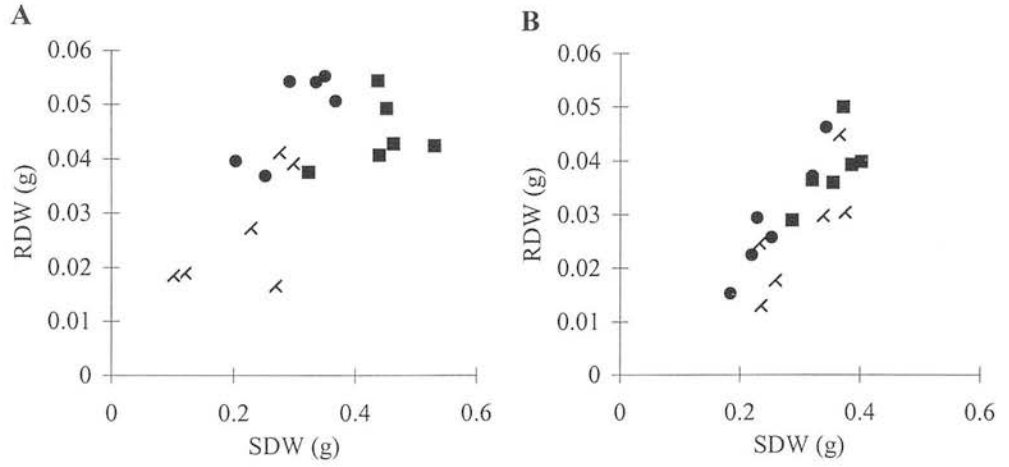

Fig. 6.2. The relationship between shoot dry weight (SDW) and root dry weight (RDW) of Elsanta (A) and Rhapsody (B) plants that were non-mycorrhizal (●) or colonised by *A. scrobiculata* (×) or *S. nodosa* (■).

($r = 0.798$ and $r = 0.661$, respectively) between SDW and RDW. Therefore, RDW increased as SDW increased.

Table 6.1 shows the summary of the results of correlation analysis of RDW and shoot phosphorus status against SDW, which provides more information about the plant growth and mineral status (see Appendix III, section 12.3.1). For non-mycorrhizal Elsanta plants, the relationship between RDW and SDW was not significant ($P = 0.057$), although a high proportion (64%) of the variation in RDW could be accounted for by the effect of the SDW of the plants. Therefore, Fig. 6.2A shows that RDW tended to increase as the shoot size increased, although this was not significant. In plants inoculated with *A. scrobiculata* and *S. nodosa* there was also not a significant correlation between RDW and SDW ($P > 0.05$), and a smaller proportion of the variation in RDW was a result of the SDW (44% and 8%, respectively). For these mycorrhizal plants, RDW did not appear to increase as shoot size increased; therefore other factors appear to have a greater effect on the RDW than the SDW of the plants. There is no evidence to suggest that the relationship between RDW and SDW was causal; indeed, this was unlikely to be the case. Therefore, although Fig. 6.2A indicated a correlation (not significant) between RDW and SDW, an increase in SDW did not cause an increase in RDW.

For both mycorrhizal and non-mycorrhizal Rhapsody plants, there was a strong positive correlation between RDW and SDW, indicating that the root growth increased with increasing shoot size. This relationship was significant for non-mycorrhizal plants ($P > 0.01$; Table 6.3; see Appendix III, section 12.3.4) with 91.9% of the variance in the RDW being accounted for by the increasing SDW. In contrast, with the mycorrhizal plants, although Fig. 6.2B showed that RDW appeared to increase as the shoot size increased, this relationship was not significant. In addition, much less of the variance in RDW was a result of the increasing SDW of plants inoculated with *S. nodosa* and *A. scrobiculata* (45.5% and 65.1%, respectively; Table 6.3). Therefore, the RDW of non-mycorrhizal Rhapsody plants was affected to a greater degree by the increasing shoot size, whereas factors other than shoot size had a greater effect on the RDW of the mycorrhizal plants. However, there was no evidence

Table 6.1. Summary of correlation analysis of growth characteristics of Elsanta (RDW, Total P and [P]) varying with shoot dry weight of the plants. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as plant size (SDW) increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
Root dry weight	*S. nodosa*	0.577	ns	0.289	0.084
	A. scrobiculata	0.152	ns	0.661	0.437
	Non-mycorrhizal	0.057	ns	0.798	0.636
Total P	*S. nodosa*	0.122	ns	0.699	0.489
	A. scrobiculata	0.422	ns	0.408	0.166
	Non-mycorrhizal	0.624	ns	0.256	0.065
[P]	*S. nodosa*	0.364	ns	0.455	0.207
	A. scrobiculata	0.189	ns	–0.619	0.384
	Non-mycorrhizal	0.079	ns	–0.761	0.579

Table 6.2. Summary of correlation analysis of growth characteristics of Elsanta (RDW, Total P and [P]) varying with %MC of the plant. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as %MC increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
RDW	*S. nodosa*	0.041	*	0.829	0.687
	A. scrobiculata	0.092	ns	0.741	0.549
SDW	*S. nodosa*	0.175	ns	0.635	0.403
	A. scrobiculata	0.386	ns	0.437	0.190
Total P	*S. nodosa*	0.00005	**	0.994	0.988
	A. scrobiculata	0.006	**	0.931	0.868
[P]	*S. nodosa*	0.0009	**	0.975	0.951
	A. scrobiculata	0.507	ns	0.341	0.116

to suggest a causal relationship between RDW and SDW, i.e. an increase in SDW did not cause an increase in RDW.

6.2.2 Relationship between plant growth and phosphorus in the plant

Considering the very low P status of the sand-based growing medium, very little P was available for uptake into the strawberry plants. A comparison of total P and [P] in the shoot provides information about the rate of accumulation of P in relation to the increase in SDW, RDW or %MC.

In non-mycorrhizal Elsanta plants (Fig. 6.3A), the total P in the shoot stayed almost constant ($r = 0.029$) as the SDW increased (Table 6.2; see Appendix III, section 12.3.3). This indicates that P was not being taken up into the plant, because the largest control plants contained almost the same amount of total P as the smallest control plants. The lack of P uptake was reflected in [P] in the shoots (Fig. 6.3B), where there was a strong negative correlation ($r = -0.761$) between SDW and [P]concentration, although, once again, this relationship was not significant. Therefore, as SDW and shoot size increased, very little, if any, additional P was taken up into the plant, causing [P] to decrease as the SDW increased.

A similar relationship was observed between SDW and total P of Elsanta plants inoculated with *A. scrobiculata*. There may have been a slight uptake of P into these plants as the SDW increases (Fig. 6.3A; $r = 0.408$), although the relationship was not significant. However, the negative correlation between [P] and SDW ($r = -0.620$) indicates that although this relationship was not significant, as the SDW increases, very little additional P was taken into the plant, causing a tendency for [P] to decrease.

The Elsanta plants inoculated with *S. nodosa* exhibited a very different relationship between SDW and P. Figure 6.3A shows that the total amount of P in the shoot actually increased as the SDW increased ($r = 0.700$). This was reflected in [P] in the shoots, which increased as SDW increased ($r = 0.455$). However, neither the relationship between total P in the shoots nor the relationship between [P]

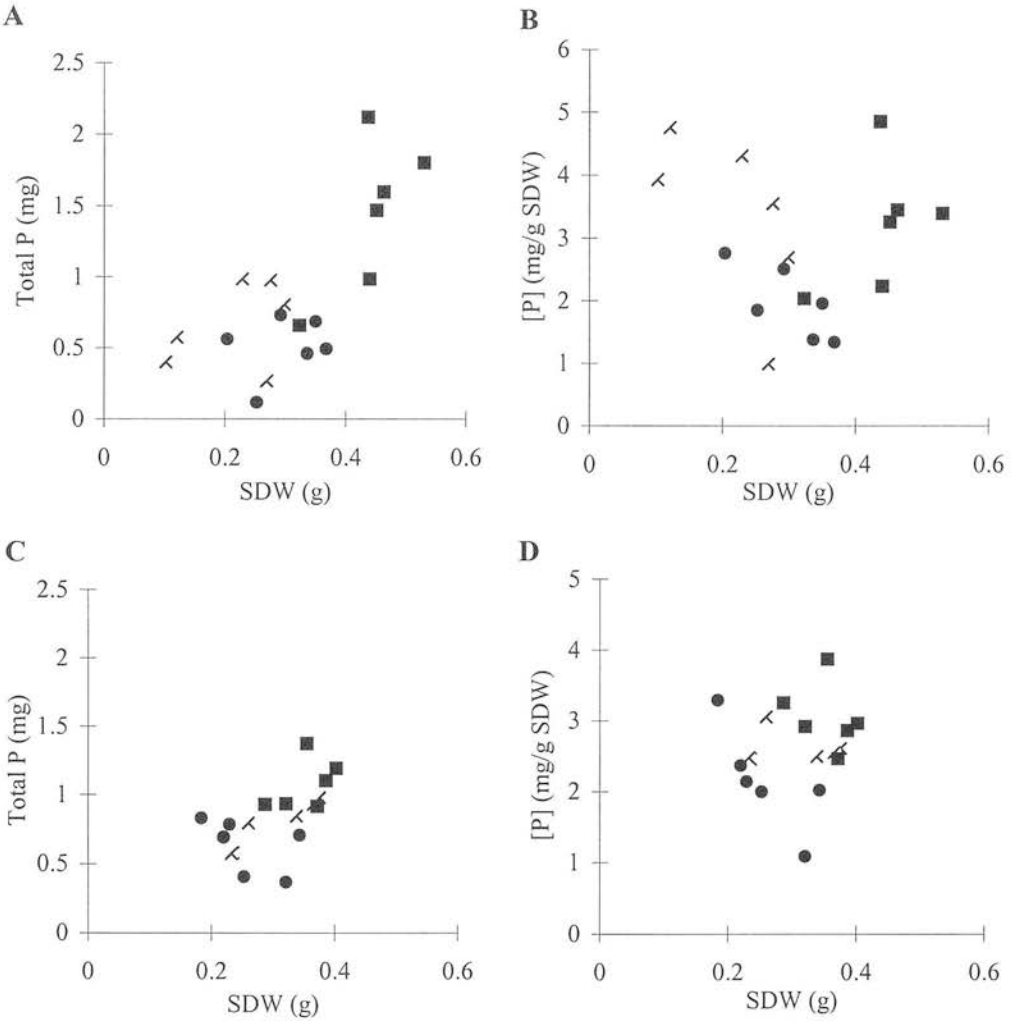

Fig. 6.3. The relationship between shoot dry weight (SDW) and total phosphorus (P) in the shoot and the concentration of phosphorus ([P]) in the shoot for Elsanta (A and B) and Rhapsody (C and D) plants that were non-mycorrhizal (●) or colonised by *A. scrobiculata* (×) or *S. nodosa* (■).

in the shoot was found to be significant (Table 6.2; see Appendix III, section 12.3.2). The positive relationship between [P] and SDW suggests that, as the shoot size increases, additional P is taken up into the plants.

For non-mycorrhizal Rhapsody plants, the relationship between SDW and P was similar to non-mycorrhizal Elsanta plants. As SDW increased the total P in the shoot remained at the same level or decreased marginally (Fig. 6.3C; $r = -0.039$) and consequently the concentration of P decreased (Fig. 6.3D; $r = -0.774$), indicating that

Table 6.3. Summary of correlation analysis of growth characteristics of Rhapsody (RDW, Total P and [P]) varying with shoot dry weight of the plants. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as plant size (SDW) increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
Root dry weight	*S. nodosa*	0.142	ns	0.675	0.455
	A. scrobiculata	0.053	ns	0.807	0.651
	Non-mycorrhizal	0.003	**	0.958	0.919
Total P	*S. nodosa*	0.354	ns	0.464	0.215
	A. scrobiculata	0.005	**	0.941	0.885
	Non-mycorrhizal	0.309	ns	–0.503	0.253
[P]	*S. nodosa*	0.587	ns	–0.282	0.079
	A. scrobiculata	0.848	ns	–0.102	0.010
	Non-mycorrhizal	0.071	ns	–0.774	0.598

Table 6.4. Summary of correlation analysis of growth characteristics of Rhapsody (RDW, Total P, [P]) varying with %MC of the plant. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as %MC increased were significant. *P*, probability; *r*, correlation coefficient; r^2, coefficient of determination. ns, not significant; *, significant at the 5% level; **, significant at the 1% level.

Growth characteristic	Inoculation of plant	*P*	Significance	*r*	r^2
RDW	*S. nodosa*	0.404	ns	0.422	0.178
	A. scrobiculata	0.265	ns	0.542	0.295
SDW	*S. nodosa*	0.125	ns	0.695	0.483
	A. scrobiculata	0.244	ns	0.563	0.318
Total P	*S. nodosa*	0.233	ns	0.574	0.330
	A. scrobiculata	0.474	ns	–0.367	0.134
[P]	*S. nodosa*	0.919	ns	0.054	0.003
	A. scrobiculata	0.265	ns	0.543	0.294

very little or no additional P is being taken up into the plant as the shoot size increases, i.e. the plants grow. However, neither of these relationships were statistically significant (Table 6.3; see Appendix III, section 12.3.6).

For Rhapsody plants colonised with *S. nodosa* or *A. scrobiculata*, there was a slight increase in total P in the shoots as SDW increased, as shown in Fig. 6.3C ($r = 0.464$ and $r = 0.941$, respectively). This relationship was significant for plants colonised with *A. scrobiculata* but not for plants colonised with *S. nodosa*.

The relationship between total P in the shoots and SDW was significant ($P < 0.01$) for plants inoculated with *A. scrobiculata*. This indicates that as SDW increased, there was some intake of additional P into the plant, because the [P] remained approximately the same; in the absence of any P uptake, [P] would decrease as shoot size increased, as was observed in the non-mycorrhizal plants.

In summary, non-mycorrhizal plants of both strawberry cultivars showed no uptake of additional P into the shoots as shoot size increased. In Elsanta, inoculation with *S. nodosa* or *A. scrobiculata* resulted in a significant uptake of P compared to the control plants. In Rhapsody plants, inoculation with either *S. nodosa* or *A. scrobiculata* caused some uptake of additional P into the shoot.

6.2.3 The effect of mycorrhizal colonisation on plant size and P uptake

Figure 6.4, B and D, shows that the percentage mycorrhizal colonisation (%MC) of both Elsanta and Rhapsody plants inoculated with *A. scrobiculata* was generally lower than those inoculated with *S. nodosa*. Therefore the root systems of both cultivars were colonised to a much smaller degree by *A. scrobiculata* compared to *S. nodosa*. This may have been the result of the mixed inoculum of *A. scrobiculata* containing fewer mycorrhizal propagules in the form of spores and infected root fragments which were available to colonise strawberry roots, or the strawberry plants were not a favourable host for the mycorrhizal fungus, resulting in the mycorrhizal colonisation being present but limited. In addition, the fact that the %MC is reduced could lessen the effect of the mycorrhizal fungus on the host plant, e.g. a lower level of

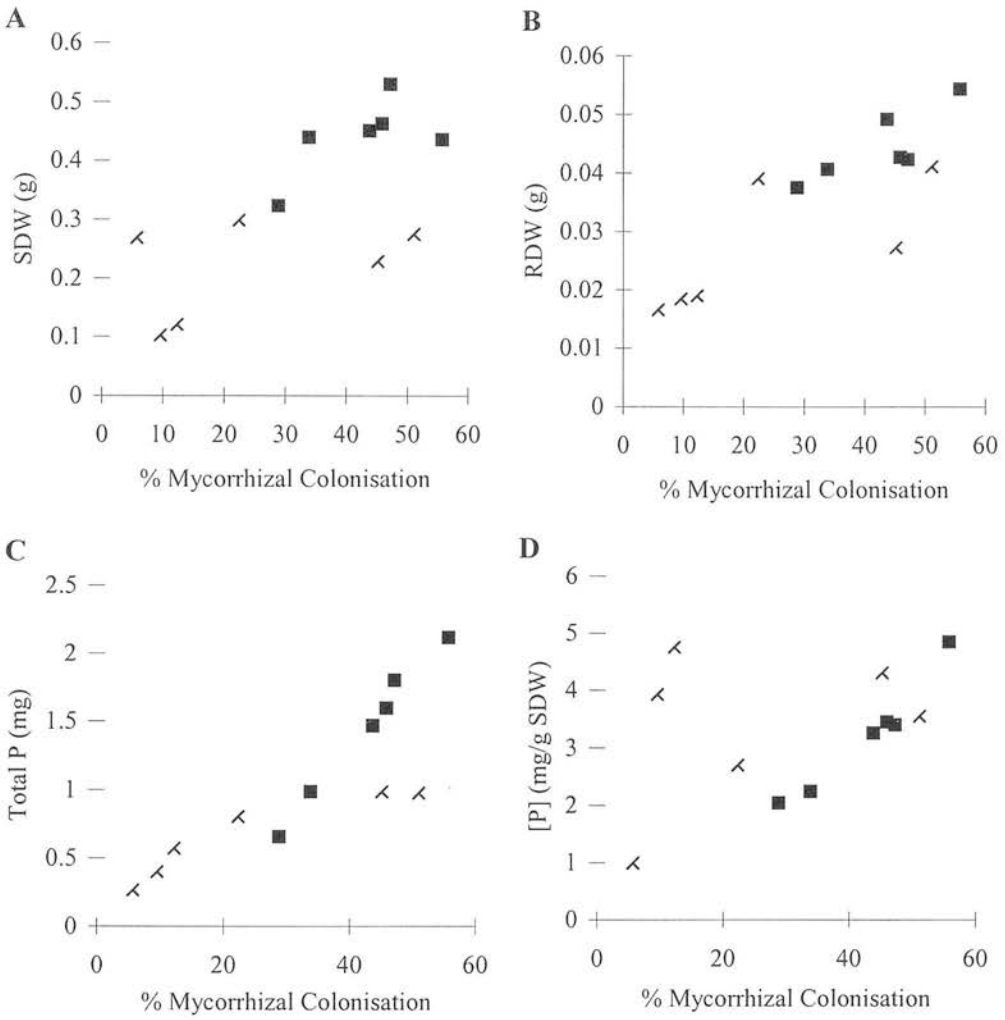

Fig. 6.4. The effect of increasing percent mycorrhizal colonisation (%MC) on the shoot dry weight (SDW; A), root dry weight (RDW; B), total phosphorus in the shoot (P; C) and concentration of phosphorus in the shoot ([P]; D) of Elsanta plants colonised by *A. scrobiculata* (×) or *S. nodosa* (■).

mycorrhizal colonisation may have a reduced capacity to uptake P into the host and affect host plant growth.

6.2.3.i Mycorrhizal colonisation of Elsanta plants

Regression analysis (Table 6.2) indicated that there was no significant effect of increasing %MC on SDW of plants inoculated with either *A. scrobiculata* ($P > 0.05$) or *S. nodosa* ($P > 0.05$) (see Appendix III, section 12.4.2. However, Fig. 6.4A shows a positive correlation between SDW and %MC in Elsanta plants inoculated with *S. nodosa* ($r = 0.635$), suggesting that plant size tended to increase as the colonisation of the plant roots by the mycorrhizal fungus increased. There was a significant

relationship between %MC and RDW of plants colonised by *S. nodosa* ($P < 0.05$) with 68% of the variation in RDW being able to be accounted for by the %MC. Examination of the residuals (Appendix III, section 12.4.1) indicates no obvious deviation from linearity and therefore the relationship can be assumed to be a straight line. This indicates that the size of the root system increased as the %MC of the root system increased, which could be a result either of more roots being produced or the additional biomass of the mycorrhizal fungus in the plant roots.

In contrast, in plants inoculated with *A. scrobiculata*, although the correlation between SDW and RDW and %MC was positive, Fig. 6.4, A and B, indicated that the SDW and RDW did not significantly increase as %MC increased. For example, the SDW and RDW remained at approximately the same level as the %MC increased from 20% to 50% of the root system. However, the lowest RDW and SDW values were associated with the lowest %MC and the highest RDW and SDW were associated with the highest %MC. Therefore, a relationship does appear to exist between these variables, with higher levels of %MC being associated with higher RDW, but it was found to be not significant ($P > 0.05$; Table 6.2).

The results shown in Fig. 6.4C indicate a significant relationship between %MC and the total P in the shoots for plants inoculated with both *S. nodosa* ($P < 0.001$) and *A. scrobiculata* ($P < 0.01$), with most of the variation in total P being accounted for by the %MC (86% and 98% for *A. scrobiculata* and *S. nodosa*, respectively; Table 6.2). This can be contrasted to the non-significant increase of total P observed as the SDW increases (Fig. 6.3A), in which only a low proportion of the variability in the total P (48.9% and 16.6% for *S. nodosa* and *A. scrobiculata*, respectively) was associated with increasing SDW. The results suggest that in the mycorrhizal plants the %MC had a greater effect on the amount and concentration of P in the shoots than did the shoot size.

With *S. nodosa* (Fig. 6.4C), examination of the residuals (Appendix III, section 12.4.4) shows that this relationship does not deviate considerably from a straight line, and that increasing the %MC will cause increases in the total P in the shoot. In contrast, the residuals for *A. scrobiculata* show a marked curve, indicating that the

relationship appears to plateau, i.e. increasing %MC raises the total P in the shoot until a value of %MC (approximately 25–30%) is reached beyond which further colonisation of the root by the fungus does not result in any additional increase in total P.

A *t*-test was performed to establish whether the two slopes observed in Fig. 6.4C were significantly different from each other (see Appendix III, section 12.5). The two slopes were found to be significantly different ($t_{\alpha=2\ (0.05),8} = 2.88$ vs critical value $t_{\alpha=2\ (0.05),8} = 2.306$), indicating that there was a difference in the effect of the mycorrhizal colonisation by each fungus on the total P in the plants, i.e. the effect of adding either mycorrhizal fungus was not the same, it depended on the interaction between the host plant and the fungus itself.

Examination of [P] in the shoots showed a significant relationship between %MC and [P] in plants inoculated with *S. nodosa* ($P < 0.001$; Table 6.2) in what appears, by examination of the residuals, to be a straight-line relationship (Appendix III, section 12.4.3). In contrast, the relationship was not significant for plants inoculated with *A. scrobiculata* ($P > 0.05$) with only 11% of the variation in [P] being accounted for by the effect of the %MC. This non-significant relationship is a result of the plateau effect of %MC on total P in the plant; the amount of total P in the shoot does not increase further as the %MC increases above a certain plateau level (Fig. 6.4C). Therefore, as the plants and SDW increase in size, [P] will fall.

6.2.3.ii Mycorrhizal colonisation of Rhapsody plants

The colonisation of Rhapsody plants by *A. scrobiculata* resulted in very variable levels of %MC; two plants had no visible mycorrhizal colonisation while another two plants showed 50–55%MC (Fig. 6.5A). Therefore the effects of the %MC are difficult to interpret accurately (see Appendix III, section 12.5–12.8). The highest levels of %MC were observed in the smallest Rhapsody plants, i.e. those with the smallest SDW and RDW, suggesting that the mycorrhizal colonisation had either no effect or a slightly deleterious effect on plant growth (Fig. 6.5, A and B).

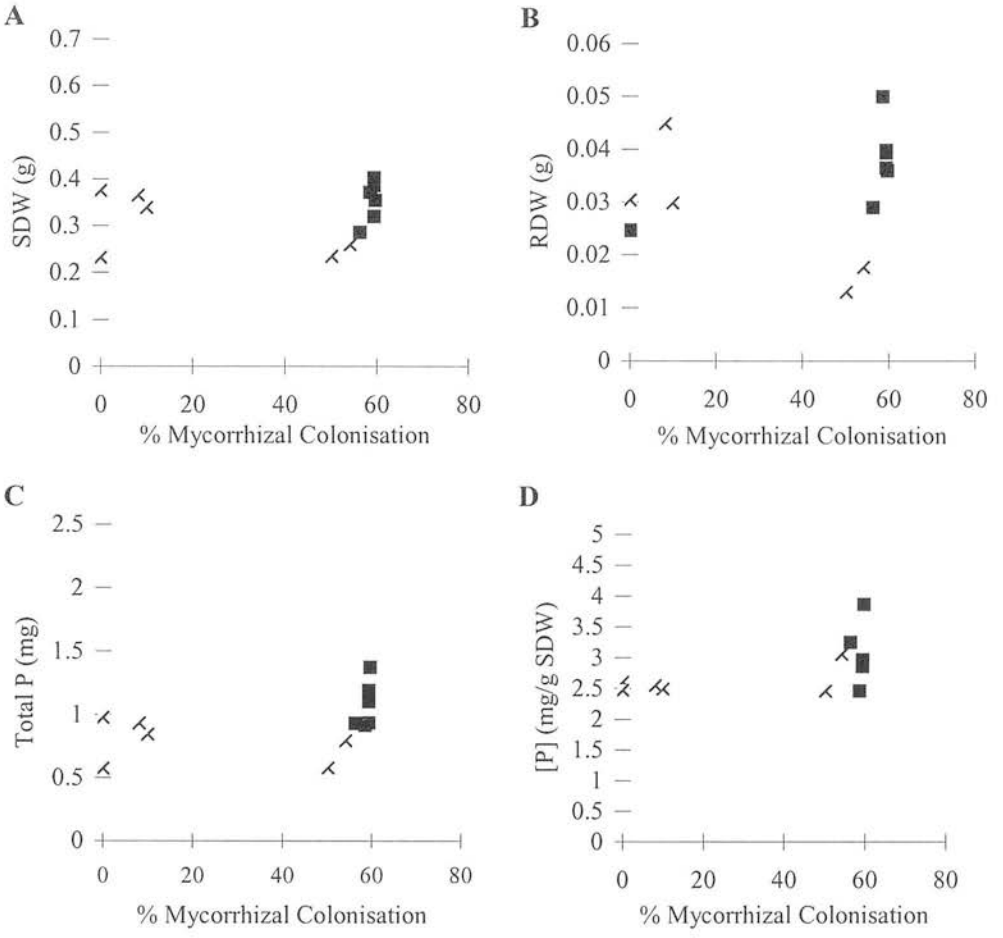

Fig. 6.5. The effect of increasing percent mycorrhizal colonisation (%MC) on the shoot dry weight (SDW; A), root dry weight (RDW; B), total phosphorus in the shoot (P; C) and concentration of phosphorus in the shoot ([P]; D) of Rhapsody plants colonised by *A. scrobiculata* (×) or *S. nodosa* (■).

As plant size appeared to be unaffected by the %MC for *A. scrobiculata*, it was not unexpected that the total P and [P] remained virtually unchanged as %MC increased. Therefore, the increased amount of mycorrhizal colonisation did not increase plant size or P uptake by Rhapsody plants. In contrast, all of the Rhapsody plants inoculated with *S. nodosa* had a high level of %MC, such that the values of %MC were clustered, making observation of any relationship with SDW, RDW or P

Table 6.5. Calculated *t* statistics for the one-tailed hypothesis that the mean SDW, RDW, total P and [P] of plants showing visible colonisation by *A. scrobiculata* was greater than the mean of each variable in plants showing no colonisation by the fungus. In each case, the calculated *t* statistic is smaller than the critical *t* statistic ($t_{c(0.05,\ 1,\ 3)}$) indicating that the presence of the mycorrhizal colonisation had no significant effect on either SWD, RDW, total P and [P].

	t statistic	Critical *t* statistic
SDW	–0.013	2.353
RDW	–0.034	2.353
Total P	0.013	2.353
[P]	0.036	2.353

content very difficult (Fig. 6.5). It is possible that a maximum level of mycorrhizal colonisation had been reached at approximately 60% of the root system. Therefore, it was impossible to observe whether %MC had an effect on plant size and P uptake of Rhapsody plants. The observation could be made that the values of RDW, SDW and P were also clustered, indicating that these plants with a very similar high level of %MC also had similar high values of plant size and P uptake.

A *t*-test with a one-tailed hypothesis was performed to determine whether there was any difference between the mean SDW, RDW, total P and [P] of the plants inoculated with *A. scrobiculata* but with no actual mycorrhizal colonisation (two data points located on the *y*-axis in Fig. 6.5) observed, and the inoculated plants for which mycorrhizal colonisation was visible (four plants). The results in Table 6.5 (see Appendix III, section 12.6) show that, in each case, the successful colonisation of Rhapsody plants by *A. scrobiculata* did not significantly increase the mean SDW, RDW, total P and [P] compared to plants in which no colonisation was observed.

6.3.1 Inoculation of mycorrhizal strawberry plants with *P. fragariae*

Subsequent inoculation of both mycorrhizal and non-mycorrhizal strawberry plants with different doses of the pathogen, *P. fragariae*, resulted in a number of plants showing symptoms of red stele disease. The amount of disease was assessed by counting the number of oospores of the pathogen in the root tips of the strawberry plants. None of the plants that were inoculated with a control, non-pathogenic solution of distilled water developed red stele disease. In addition, none of the Rhapsody plants

showed any disease symptoms; therefore the results concentrate on the effects of *P. fragariae* on Elsanta plants.

When a level of 2700 zoospores per plant was used to inoculate the plants, the non-mycorrhizal plants and those previously inoculated with *A. scrobiculata* showed no disease symptoms and no oospores were observed in the plant roots (Fig. 6.6). However, Elsanta plants colonised by the mycorrhizal fungus *S. nodosa* were found to contain oospores of *P. fragariae* in their root tips, i.e. these plants were infected by the pathogen *P. fragariae*. When the level of zoospore inoculum was increased to 50 000 zoospores per plant, non-mycorrhizal plants and those colonised by *S. nodosa* and *A. scrobiculata* showed disease symptoms and were found to contain oospores in their root tips (Fig. 6.6). A non-parametric analysis of variance was performed using the Kruskal–Wallis test.

The results showed that H_c (10.50) was greater than the chi-square statistic ($\chi^2_{0.05,2} = 5.991$), indicating a significant difference between the number of oospores observed in the root tips of non-mycorrhizal plants and mycorrhizal plants (see Appendix III, section 12.7).

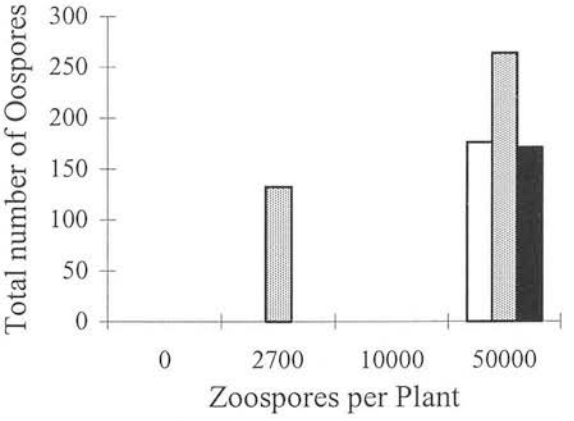

Fig. 6.6. Total number of oospores observed in root tips of Elsanta plants colonised by *A. scrobiculata* (white bar), colonised by *S. nodosa* (grey-shaded bars) and non-mycorrhizal Elsanta plants (black bar) inoculated with increasing levels of zoospores of *P. fragariae*.

Table 6.6. Results of the non-parametric multiple comparison of the Kruskal–Wallis data. The rank sums are first arranged in increasing order of magnitude. The differences in rank sums are then compared (Rx–Ry) and the q statistic calculated. *, significant at the 5% level.

Rank sums in increasing order of magnitude		
S. nodosa	*A. scrobiculata*	Non-mycorrhizal plants
3106	3819	4400

	Rx–Ry	SE	p	q	Critical q	Significance
Non-mycorrhizal vs *S. nodosa*	1294	307.205	3	4.212	3.314	*
Non-mycorrhizal vs *A. scrobiculata*	581	205.142	2	2.832	2.772	*
A. scrobiculata vs *S. nodosa*	713	205.142	2	3.476	2.772	*

In order to determine between which of the plants significant differences occurred, a non-parametric multiple comparison was performed.

The results indicated a significant difference in the distribution and number of oospores in non-mycorrhizal plants and plants inoculated with either *S. nodosa* or *A. scrobiculata*, and also a significant difference between plants colonised by the two mycorrhizal fungi (Table 6.6).

Because this analysis is based on the rank sums and not the actual values of oospore numbers, these results must be considered together with an examination of the actual data. Table 6.7 shows the mean numbers of oospores observed in infected roots, i.e. roots in which oospores were observed, the total number of oospores observed in the roots, and the percentage of roots in which oospores were observed. It can be seen that although the non-mycorrhizal plants contained a greater mean number of oospores per infected root, fewer roots were actually found to contain oospores, i.e. the percentage of roots in which oospores were observed was much lower (28%) compared to mycorrhizal plants (*S. nodosa*, 64%; *A. scrobiculata*, 46%), and the total number of

oospores observed is smaller in the non-mycorrhizal plants compared to plants inoculated with *S. nodosa*. Therefore, a greater number of roots of mycorrhizal plants actually contained oospores compared to non-mycorrhizal plants. However, Table 6.7 illustrates that this type of data must be examined carefully to avoid spurious conclusions being drawn; the actual distribution of oospores in the root tips must be considered. As an example, examination of the total number of oospores observed in non-mycorrhizal plants (171 oospores) and those inoculated with *A. scrobiculata* (176 oospores) could imply that there was little difference between the disease in these two groups of plants. However, when the percentage of infected roots is considered, it appears that a greater number of roots of plants inoculated with *A. scrobiculata* contained oospores compared to the non-mycorrhizal plants. The benefit of the Kruskal–Wallis test is that it takes into account both the number of oospores, in that the numbers are first ranked from highest to lowest, and the distribution of oospores, because the roots containing the lowest numbers of oospores or no oospores are given the highest ranks.

The conclusion of these analyses may be that the presence of the mycorrhizal fungi actually increased the number of oospores of *P. fragariae* in plants inoculated with *S. nodosa* or *A. scrobiculata*, and increased the number of roots in which oospores of *P. fragariae* were observed, in the case of both mycorrhizal fungi used, i.e. the sexual development of *P. fragariae* was enhanced when the mycorrhizal fungi were

Table 6.7. Inoculation of non-mycorrhizal plants, plants colonised by *S. nodosa* and plants colonised by *A. scrobiculata* with the pathogen *P. fragariae*. The number of oospores of *P. fragariae* was counted in 10 root tips from each inoculated plant.

	Non-mycorrhizal	*S. nodosa*	*A. scrobiculata*
Total number of oospores	171	264	176
Percentage of roots containing oospores	28	64	46
Mean number of oospores observed in roots containing oospores (standard deviation)	12.21 (11.41)	8.25 (7.03)	7.65 (6.37)

colonising the plant roots. Alternatively, the mycorrhizal root systems may be more readily infected by *P. fragariae*, hence a higher percentage of roots containing oospores, but sexual reproduction by *P. fragariae* in any particular infected root was less, as implied by the lower mean number of oospores.

A chi-square test was performed to determine whether the number of roots containing oospores was independent of the mycorrhizal status of the plants (see Appendix III, section 12.8). The results show that there was a significant difference in the number of roots containing oospores ($P < 0.01$). This indicates that the presence of the mycorrhizal colonisation increased the number of roots that contained oospores.

6.4 Discussion

The results show differences in the growth of each strawberry cultivar observed at the end of the experiment and in the effect of each treatment on each cultivar. First, the control, non-mycorrhizal Elsanta plants were larger than the Rhapsody plants with a greater SDW and RDW. This fundamental difference in growth indicated that each cultivar must be considered separately when assessing the plant growth and effect of mycorrhizal colonisation during the experiment.

With Elsanta plants, inoculation with *S. nodosa* increased plant growth, i.e. SDW, and increased P uptake compared to the control, non-mycorrhizal plants. Indeed, SDW and RDW were found to increase as the %MC of the plant roots increased, suggesting a direct, positive effect of the mycorrhizal inoculation on the plants. In Elsanta plants inoculated with *A. scrobiculata*, there was a slightly reduced RDW compared to the other treatments and the mycorrhizal colonisation of the roots was lower than that of the other mycorrhizal Elsanta plants. However, the results suggest that although increasing the mycorrhizal colonisation had very little effect on RDW and SDW of these plants, the presence of increasing mycorrhizal colonisation in the roots caused a slight increase in P uptake into the plants. Therefore, inoculation with *A. scrobiculata* increased P uptake into the plants compared to control plants, but this increased uptake was apparently not sufficient to affect plant growth and size.

Because the growing medium was nutrient deficient, it was assumed that increased plant growth was a result of an increase of nutrient uptake into the plant. Of course, the only nutrient component that was measured was P but as this has a major effect on plant health it is probable that any increase in P uptake into the plant from the growing medium would contribute to an increase in plant growth. It has been shown that other nutrients such as zinc and magnesium can be transported into a plant root as a result of a mycorrhizal infection. Therefore, although the P content of the plant was determined, it is possible that the increased uptake of other nutrients into the mycorrhizal plants could contribute to an increase in plant growth.

The Rhapsody plants were smaller than the Elsanta plants during the experiment. Also, although the level of mycorrhizal colonisation of roots was similar in both cultivars inoculated with *S. nodosa*, Rhapsody plants had a lower level of mycorrhizal colonisation with *A. scrobiculata* than Elsanta plants. This variation in the level of mycorrhizal colonisation may have contributed to the differences observed between the mycorrhizal plants of each cultivar.

The relationship between RDW and SDW was similar for non-mycorrhizal plants of both cultivars. However, the results indicated that the mycorrhizal colonisation of Rhapsody plants had very little, if any, effect on plant size, as the mycorrhizal plants had similar RDW and SDW to the control plants. It is possible that the low level of mycorrhizal colonisation was insufficient to have a significant effect on plant growth. However, it was shown that there was some uptake of P into Rhapsody plants inoculated with either *S. nodosa* or *A. scrobiculata*, but this uptake, although greater than that observed in the control plants, may have been insufficient to affect plant growth.

All the Rhapsody plants inoculated with *S. nodosa* had a very similar high level of mycorrhizal colonisation of their roots, making it impossible to determine the effect of an increasing mycorrhizal colonisation on the plants. However, it was observed that these mycorrhizal plants generally had a greater level of total P in the shoots compared to control plants. This indicated that the presence of the mycorrhizal colonisation caused an increase in P uptake compared to the control, non-mycorrhizal plants;

whether an increased mycorrhizal colonisation increased P uptake into the plants could not be determined.

Subsequent inoculation of these mycorrhizal and non-mycorrhizal plants with zoospores of the pathogen *P. fragariae* produced very interesting results. The Kruskal–Wallis analysis of the numbers of oospores in root tips showed that the non-mycorrhizal plants contained fewer oospores of the pathogen *P. fragariae* than plants colonised by either *S. nodosa* or *A. scrobiculata*. Plants colonised by *S. nodosa* also had a significantly higher number of oospores than those colonised by *A. scrobiculata*. This analysis clearly confirmed that the sexual development of *P. fragariae* was enhanced in plants colonised by these mycorrhizal fungi compared to non-mycorrhizal plants.

However, the effect of each mycorrhizal fungus did appear to differ. For example, *S. nodosa* greatly increased the percentage of roots infected and also the total reproductive output (number of oospores observed) of the pathogen. Colonisation by *A. scrobiculata* increased the percentage of infected roots but had little if any effect on the reproductive output of the pathogen.

It must be remembered that colonisation of plants by *S. nodosa* prior to introduction of the pathogen resulted in a significant increase in RDW, total P in the shoot and [P] in the shoot as the percentage of mycorrhizal colonisation increased, i.e. the mycorrhizal fungus had a beneficial effect on the plant. It is clear that this beneficial mycorrhizal colonisation did, however, increase the susceptibility of the plants to disease due to subsequent infection by the pathogen *P. fragariae*.

In plants colonised by *A. scrobiculata*, increased mycorrhizal colonisation had a significant effect on increasing the amount of total P in the shoots but this did not appear to affect plant size. However, the level of mycorrhizal colonisation, although not sufficient to greatly affect plant growth and lower than that in plants colonised by *S. nodosa*, was sufficient to increase the susceptibility of the plants to disease due to *P. fragariae*.

These results appear to be anomalous as it may have been expected that the plants that were beneficially affected by colonisation by the *S. nodosa* and *A. scrobiculata* would be 'fitter' and healthier and more able to resist disease due to the pathogen. It is possible that the most favourable host for the pathogen, *P. fragariae*, may be a healthy plant because there is no advantage to this pathogen to infect an unhealthy plant that may not survive long enough for the fungus to produce the resistant oospores which will ensure its survival after the host plant has died. These oospores can remain viable in the soil after an infected plant root has decayed, and can overwinter in preparation for emerging in the favourable climate of the spring when new host plants will be available for infection. Infection of a healthy plant which does not resist penetration by the pathogen will allow sexual development of *P. fragariae* to proceed, resulting in oospore production.

It must also be remembered that the presence of a mycorrhizal colonisation will occupy space within the plant root and the root will be penetrated from both inside and out by extramatrical hyphae penetrating the soil in the root zone, and by colonising hyphae trying to enter the root, respectively. Therefore the cortex of the root will have been breached many times; it is possible that this renders the root susceptible to entry by other potentially pathogenic fungi.

In a non-mycorrhizal plant, entry of a root by a germination tube emanating from a germinating zoospore of *P. fragariae* would alert the defence mechanisms of the host. However, in a mycorrhizal plant, the host's defence response is already aware of the presence of a foreign fungus in the plant roots; therefore perhaps the presence of another, pathogenic fungus will not alert the defence system further, allowing the pathogen to enter the roots without much resistance. By the same token, it has been suggested (Gianinazzi, 1991) that the defence system of a mycorrhizal plant is already primed by the presence of the mycorrhizal fungus which, although not sufficient to prevent the entry of the mycorrhiza, may be enough to prime the plant against any entry by another fungus such as a pathogen, thus rendering the plant more resistant to the pathogen than a non-mycorrhizal plant.

Therefore, the results clearly showed that although there were inherent differences in plant growth and the level of mycorrhizal colonisation of the two strawberry cultivars, the mycorrhizal plants exhibited different growth and P uptake compared to the control plants. In each case, the mycorrhizal inoculation had some effect on the host plants but this varied according to the particular combination of host plant and mycorrhizal fungus. This would be a result of the different characteristics of each cultivar and mycorrhizal fungus, indicating that different mycorrhizal fungi can have different effects on a variety of host plants. Differences in the response of strawberry cultivars to colonisation by different strains of mycorrhizal fungi have been reported (Cháves & Ferrera-Cerrato, 1990; Vestberg, 1992b). It is not, therefore, possible to predict the effect of colonisation by one mycorrhizal fungus on a variety of host plants, as each plant/fungus interaction can produce different results. For example, during this experiment, inoculation of Elsanta plants with *S. nodosa* resulted in significant increases in plant growth and P uptake into the plants, whereas a similar level of root colonisation of Rhapsody plants with this fungus produced slight but not significant increases.

In the same way, inoculation of a particular plant by different mycorrhizal fungi can result in different effects on the host plant. Therefore, the probable results of inoculation of a host plant with a mycorrhizal fungus cannot be predicted with accuracy; the inoculation may or may not affect plant growth or nutrient uptake, depending on the compatible nature of the interaction. In summary, each interaction of mycorrhizal fungus and host plant must be considered individually.

CHAPTER 7: Isozyme analysis of spore extracts from arbuscular mycorrhizal fungi

7.1 Isozyme analysis of spore extracts from arbuscular mycorrhizal fungi

7.1.1 Introduction

Morphological characteristics of the spores of arbuscular mycorrhizal fungi are frequently used to distinguish between species. However, accurate recognition of many of these characteristics requires much experience, and some of the spore features can vary depending on the spore's environment. In addition, the absence of a convenient method for axenic production of spores, because of the obligate nature of the fungi, exacerbates the problem of obtaining suitable spores for study. It has been noted that spores removed from field environments can show considerable variation in their morphological characteristics (Hepper *et al.*, 1988).

Problems also exist with defining the taxonomy of spores of arbuscular mycorrhizal fungi (Morton, 1988) and because of this, together with the difficulty in using morphological characteristics to identify spores, it is considered that a combination of morphological and biochemical or molecular analyses is preferable (Rosendahl *et al.*, 1989).

One such alternative method to provide information about the identity of arbuscular mycorrhizal spores is isozyme analysis. Banding patterns resulting from electrophoretic separation and subsequent staining of fungal isozymes are directly related to the gene products themselves and are therefore thought to reflect the fungal genotype more accurately than morphological characteristics (Rosendahl & Sen, 1992). Following electrophoretic separation of fungal isozymes, the gel can be stained to reveal the isozymes as bands of activity that have migrated certain distances in the gel. The migration distance in the gel is dependent on the size, shape and net charge of each isozyme, which are all directly related to the amino acid composition of the molecule. Therefore different mobilities reflect variations in the nucleotide sequence of the DNA, and variations in banding patterns can thus be used to distinguish between individuals (Rosendahl & Sen, 1992).

Attempts were made to extract isozymes from spores of a number of species of arbuscular mycorrhizal fungi, obtained from pot cultures of the fungi, and to stain the resulting electrophoretic gels for several isozymes.

7.1.2 Experimental procedures

Spores were extracted from individual pot cultures of a number of arbuscular mycorrhizal fungi by mixing an amount of approximately 30 g of pot culture substrate in water and then pouring the water through a 0.45 μm sieve, as described in the Materials and Methods (section 3.3). Spores were then placed in an Eppendorf tube with a volume of sucrose buffer (to which bromophenol blue had been added as a marker dye; see section 3.4.1.i in the Materials and Methods) and then base of the tube plunged into liquid nitrogen to freeze the spores. A sterile plastic rod was then used to crush the spores. A volume of 10 μm of the spore/buffer mixture was then loaded into each well of an electrophoresis gel. The gel was then subjected to electrophoresis at 150 V for 45 min. The resulting gel was then stained to reveal the presence of the isozyme banding pattern.

Spores of the following species were subjected to electrophoresis: *Glomus mosseae*, *Glomus fistulosum* V128, *G. fistulosum* 23A, and *Glomus intraradices*. The electrophoresis gels were stained for the presence of the following isozymes: α-esterase (EST), malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), and glutamate oxaloacetate transaminase (GOT).

7.1.3 Results

Sample preparation was generally time consuming in order to extract and prepare sufficient spores from a pot culture substrate for electrophoresis. Although a pot culture may appear to contain lots of spores, large numbers are frequently inviable as indicated by the lack of spore contents. The physical handling of spores requires much practice and manual dexterity in order to be accomplished at any speed.

7.1.3.i α-Esterase

A single well-defined α-esterase band was observed for *G. fistulosum* 23A, *G. mosseae* and *G. fistulosum* V128 as shown in Fig. 7.1 (lanes 3, 4 and 6, respectively). The esterase band observed for both *G. fistulosum* V128 and *G. fistulosum* 23A had

identical mobilities, indicating that the composition of the α-esterase of these geographical isolates was identical. Therefore, these geographical isolates of the same species, *G. fistulosum*, could not be distinguished on the basis of their α-esterase banding pattern.

However, the mobility of the α-esterase of the *G. mosseae* spores was different to that of the *G. fistulosum* isolates; therefore *G. mosseae* could be readily distinguished from the other two isolates in this instance. No isozyme band was observed for *G. intraradices*, probably because a sufficient number of these spores were not viable (Fig. 7.1, lanes 8 and 9).

It was apparent that the number of spores in the sample well affected the intensity of the resulting isozyme band. For example, 20 spores of either *G. mosseae*, *G. fistulosum* V128 or *G. fistulosum* 23A produced a much fainter band than resulted from electrophoresis of 40 spores of the same fungi, in the same gel (data not shown).

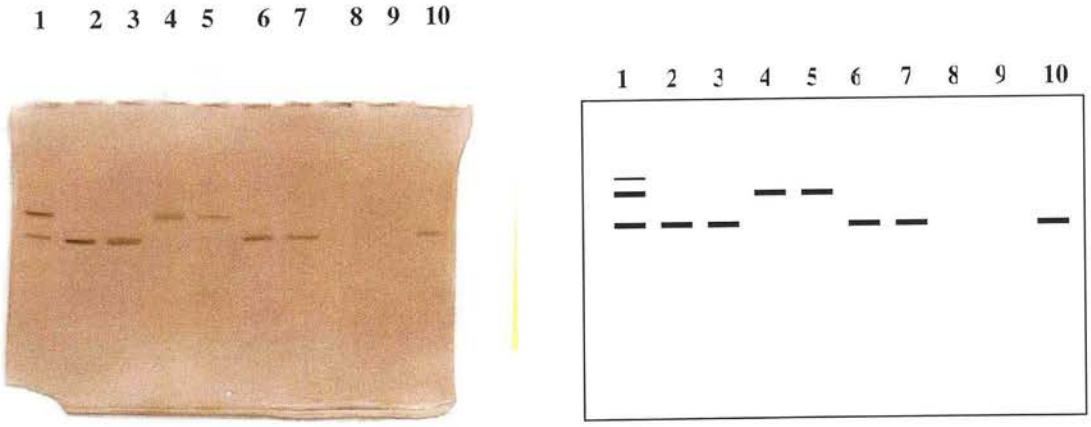

Fig. 7.1. Original image and representation of a Tris-HCl 4–20% gradient gel stained to show the presence of α-esterase isozyme bands. Lanes: 1, 40 spores of *G. fistulosum* 23A and *G. mosseae*; 2 and 3, 100 spores of *G. fistulosum* 23A; 4 and 5, 60 spores of *G. mosseae*; 6 and 7, 60 spores of *G. fistulosum* V128; 8 and 9, 60 spores of *G. intraradices*; 10, 40 spores of *G. fistulosum* V128 and *G. intraradices*.

7.1.3.ii Malate dehydrogenase

Spores of *G. mosseae* exhibited two isozyme bands when the electrophoresis gel was stained for malate dehydrogenase (Fig. 7.2, lane 5). These two bands were very close together. Isozyme staining of spores of *G. intraradices* indicated three bands for malate dehydrogenase (Fig. 7.2, lane 4). It must be noted that these particular spores had been freshly extracted from a pot culture substrate and a greater number of spores appeared viable than in the previous experiment staining for α-esterase.

The spores of *G. fistulosum* 23A and *G. fistulosum* V128 each had two isozyme bands for malate dehydrogenase; however the mobility of the bands differed for each isolate of *G. fistulosum* (Fig. 7.2, compare lanes 2 and 3). Therefore, staining for malate dehydrogenase enabled these geographical isolates to be distinguished.

7.1.3.ii Glutamate dehydrogenase and glutamate oxaloacetate transaminase

No isozyme bands were observed when electrophoresis gels were stained for the presence of these isozymes (data not shown). This may have been due to the lack of GDH and GOT in the fungal spores, or perhaps an insufficient number of viable spores being present to reveal the presence of these isozymes.

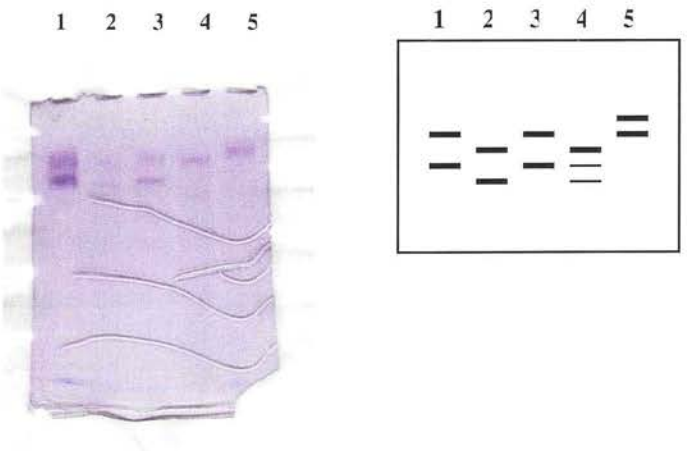

Fig. 7.2. Original and representation of a Tris-HCl 4–20% gradient gel stained to show the presence of malate dehydrogenase isozyme bands. Lanes: 1, 40 spores of *G. fistulosum* 23A, *G. fistulosum* V128, *G. intraradices* and *G. mosseae*; 2, 40 spores of *G. fistulosum* 23A; 3, 60 spores of *G. fistulosum* V128; 4, 60 spores of *G. intraradices*; 5, 60 spores of *G. mosseae*.

7.1.4 Discussion

The ability to distinguish between spores of arbuscular mycorrhizal fungi using isozyme analysis has the potential to provide a method to determine and confirm the identity of spores used for experimental purposes without having to rely on the observation of several morphological characteristics. However, this research showed that isozyme analysis relies on a considerable number of viable spores of each fungus being available. As arbuscular mycorrhizal fungi are difficult to produce in pure culture, it may be difficult to obtain sufficient viable spores for this technique. In addition, the actual method of extracting spores from pot culture and preparing them for electrophoresis requires considerable time and carries a potential risk of the technique itself failing due to a problem with the electrophoresis technique (e.g. an impurity in the gel which would distort the current passing through the gel). Therefore, although this method has been demonstrated to show differences between spores, it is considered that it is nt conclusive enough from which to draw definitive opinions over spore identity.

The success of using isozyme analysis to identify and compare mycorrhizal fungi can depend on the quality of the spores and the fungi used. Sen and Hepper (1986) showed that the mobility of four isozymes remained stable in *Glomus caledonium* spores removed from different host and soil combinations. In other studies, intraspecific variation in isozyme banding patterns has been observed (Hepper *et al.*, 1988), although subsequent analysis of the data showed that these isolates of *Glomus monosporum* formed a cluster which could be distinguished from clusters of isolates of other species. However, an examination of the spore morphology would not have indicated that the 19 isolates used in this study could be separated into the three distinct clusters that were suggested by the isozyme analysis.

The problems of accurately identifying species of arbuscular mycorrhizal spores using keys and descriptions of morphological characteristics remain. Considerable variation was observed in the isozyme pattern of isolates of *G. mosseae* (Hepper *et al.*, 1988), even though these isolates all fitted within species limits defined by published keys and descriptions (Morton, 1988; Hall, 1984).

Isozyme banding patterns are dependent on three features (Rosendahl & Sen, 1992). Multiple allelism at a single locus results in patterns of closely migrating bands. In this case, each allele codes for a structurally distinct polypeptide subunit and therefore variations in the subunit composition produces multiple isozyme bands. Second, multiple loci can code for single izosymes. For example, a putative monomorphic peptidase locus was expressed in internal and external mycelium of colonised roots but not spores of *G. caledonium* (Hepper *et al.*, 1988). Finally, secondary izosymes can result from non-genetic post-translational modifications or proteolytic activity during enzyme extraction. This is manifested as groups of several closely migrating bands with lower activities than the original unmodified band.

Further evidence for caution in a reliance on isozyme patterns for identifying arbuscular mycorrhizal fungi was suggested by Rosendahl (1989). Using a combination of a thorough study of morphological characteristics and isozyme analysis, it was apparent that there was great variation in isozyme pattern within morphologically similar isolates, i.e. some isolates that were morphologically alike had a correspondingly high similarity in isozyme pattern while others had very low similarity in the isozyme banding. Fossil evidence has shown that mycorrhizal arbuscles observed in an Early Devonian land plant (*Aglaophyton major*) are morphologically identical to those of extant mycorrhizas exaimed at the present time (Remy *et al.*, 1994). Rosendahl (1989) suggested that there may have been a faster divergence of isozymes whilst morphological divergence was very slow because of a lack of selection pressure. The fact that arbuscular mycorrhizal fungi were present in a similar form 4 million years ago (Remy *et al.*, 1994) suggets that the early symbioses were suitably efficient not to be put under any selection pressure to adapt to another environment.

It has been suggested that the availability of more accurate descriptions of both spores and morphological features other than spore walls, e.g. colour, shape, diameter, could provide further information about the taxonomy of *Glomus* spp. (Rosendahl, 1989).

Therefore, although isozyme analysis can provide information which may give an indication of the identity or taxonomy of spores of arbuscular mycorrhizal fungi, a

large proportion of the taxon must be studied, including isolates from a number of different habitats (Rosendahl, 1989). Considered opinion suggests that a combination of morphological analyses and molecular techniques such as isozyme analysis will provide more accurate information about spore taxonomy and identity than either of these techniques alone (Walker, 1992; Rosendahl *et al.*, 1989).

CHAPTER 8: General Discussion

8. General Discussion

This study represents an investigation into the interactions between micropropagated strawberry plants, the plant pathogen *Phytophthora fragariae*, and several species of arbuscular mycorrhizal fungi, and between the strawberry plants and each fungus individually. An attempt was made to elucidate the complex nature of each member of this interaction. In order to achieve this, the behaviour of the members of this interaction was studied individually before considering the effect of any interaction.

The experiments to study *P. fragariae* demonstrated that very precise, defined conditions are necessary for growth, sporulation and cyst germination of this fungus. For example, *P. fragariae* must be grown on agar containing sterols, e.g. agar containing beans or other appropriate vegetable extract before being immersed in a compost leachate for 3 days followed by immersion in cold distilled water in order to induce the agar-growing colonies to produce sporangia and release motile zoospores. The motile zoospores can then be induced to encyst by agitation and germination of the resulting cysts varies depending on the temperature, and it was shown that 15°C is the optimum temperature for germination. This is similar to oospore germination, which was also found to proceed at an optimum level at 15°C (Duncan, 1985).

Experiments showed that the germ tube produced by germinating cysts of *P. fragariae* was significantly orientated towards the root, in agreement with research by Deacon (1996), which suggested that zoospores and cysts respond to various host and environmental signals as a zoospore moves towards a root, encysts, germinates and enters the root. The specific *in vitro* attraction of zoospores towards plant roots has been reported, suggesting that fungi are responding to a factor(s) exuded by the roots (Mitchell & Deacon, 1986). In *in vitro* experiments, the attracting factor has been suggested to be flavonoids (Morris & Ward, 1992) or amino acid components of the root exudate (Buxton, 1962), although Deacon, studying zoospores of several *Pythium* and *Phytophthora* species, stressed that the range of signals to which zoospores respond *in vitro* is greater than that to which they respond *in vivo*, and that response *in vivo* has not been demonstrated because of the difficulties of studying a soil-borne interaction (Deacon, 1996).

Interestingly, the presence of a mycorrhizal colonisation in a strawberry root significantly reduced the level of cyst germination compared to cyst germination in the presence of a non-mycorrhizal root. Therefore the mycorrhizal colonisation appeared to suppress or alter production of a germination signal by the roots, thus reducing cyst germination. A reduced production of sporangia and zoospores of *Phytophthora cinnamomi* has been previously reported in the presence of mycorrhizal roots of *Zea mays* in which the amount of root leachate was reduced (Meyer & Linderman, 1986).

Observations showed that although cyst germination was reduced in the presence of the mycorrhizal root, the orientation of the resulting germ tubes was significantly towards the root for three strains of *P. fragariae* in the presence of mycorrhizal or non-mycorrhizal roots. Therefore, the mycorrhizal colonisation appeared to suppress the signal which encourages cyst germination, but had no effect on the signal which affects orientation of the germ tube towards the root. This observation correlates with Deacon's suggestion that the signals that act at each stage of the homing sequence of a zoosporic fungus infecting a root are different and are detected by different receptors (Deacon, 1996).

After a root is infected by *P. fragariae*, sexual reproduction can occur, resulting in the formation of oospores in the root. In the experiments performed here, the maximum production of oospores occurred approximately 8–10 days after infection of the root, and the number of oospores then decreased as oospores germinated to produce sporangia at the root surface. This is in some contrast to Law & Milholland (1991) who reported that oospore production peaked at 12–14 days after infection by the pathogen.

The importance of zoospores being produced from germinating oospores and released into drainage water has been emphasised in the literature (Duniway 1983), with the conclusion that the combination of low temperature and sufficient rainfall is favourable for infection and spread of the disease to occur in soils in Scotland from October to March (Duncan & Kennedy, 1995). A greater amount of secondary inoculum in the form of zoospores is produced from a more susceptible strawberry cultivar than from a resistant cultivar (Law & Milholland, 1991).

The results in the present study demonstrated that the choice of inoculation technique and disease assessment method have a considerable impact on the evaluation of the amount of disease in strawberry plants due to *P. fragariae*. It was established that a rapid visual estimate of the percentage of rotting, infected root was not significantly different from a physical measurement of the length of infected root, in agreement with Kennedy *et al.* (1986) and Milholland *et al.* (1989). However, the percentage of rotting root is considered to be too subjective (Milholland *et al.*, 1989) and to provide an inaccurate impression of the amount of disease, largely because the cause of the red stele of rotting roots has not been identified. Therefore, observation of oospores in the plant roots is considered to provide a positive identification of the fungal infection (Bain & Demaree, 1945). However, disease assessment will depend on the information required from the study. For example, classification of strawberry cultivars as either resistant or susceptible may be performed solely on the basis of percentage of infected root (Van de Weg *et al.*, 1993), although other researchers prefer to determine the number of oospores in the root tips (Milholland *et al.*, 1989).

An assessment of the number of oospores in root tips is relatively simple to perform but care must be taken regarding the statistical analysis of the resulting clustered, discrete data. In order to analyse the oospore data correctly, this thesis utilised the Kruskal–Wallis analysis of variance using tied ranks as a non-parametric statistical test. The results of these analyses provided a clear impression of any significant differences in the disease due to a particular strain or inoculation method.

The results showed that the inoculation method used to infect the strawberry plants must be considered, depending on the virulence of the strain of *P. fragariae* being used. For example, strain 293 was found to be particularly virulent and therefore infected strawberry plants to a high degree regardless of the inoculation method and zoospore dose used, whereas with the less virulent strain 171, a non-motile sprayed inoculation of zoospores enabled differences in disease as the zoospore dose increased to be observed. The results of this research showed that inoculation of strawberry plants with a low level of non-motile zoospores of the pathogen would allow any differences between strain virulence and plant resistance of the fungus to become

apparent. The virulence of the pathogen strains must be taken into account before the zoospore dose and inoculation method are selected.

Currently, resistance to strains of *P. fragariae* is tested by inoculating differential strawberry cultivars, which each have differing degrees of resistance to the pathogen, with different strains of the fungus. This approach has led to several putative observations of cultivar resistance, which have subsequently been observed to vary depending on the range of differential strawberry cultivars and pathogen strains used (Milholland *et al.*, 1989). It has been suggested that at least five race-specific resistance and corresponding virulence genes exist, and therefore strawberry differential host cultivars used to assess resistance should each have only one of the five resistance genes and be inoculated in conjunction with a universally susceptible cultivar (Van de Weg *et al.*, 1993). Therefore, it must be asked to what extent the results can be influenced by the experimental conditions and techniques employed. Results must be able to be compared across different studies, cultivars and pathogens and standardisation of criteria for inoculating host plants and assessing disease is the most effective method to ensure this.

Potential future work could involve attempting to quantify the level of disease due to inoculation of strawberry plants by *P. fragariae*. Mohan *et al.* (1989) used an enzyme-linked immunosorbent assay (ELISA) to determine the resistance or susceptibility of different host plant/pathogen strain interactions. Using this method, the ELISA was based on the reaction between mycelial antigens and antisera and a good correlation between this technique and a measurement of the percentage of infected root was observed. Therefore, this technique may enable very small amounts of the fungus to be detected and quantified, and crop protection schemes to be implemented prior to the devastating symptoms of the disease becoming apparent and causing crop losses.

The analysis of restriction fragment-length polymorphisms (RFLPs) has been used to differentiate between *Phytophthora* spp. associated with strawberry roots (Stammler *et al.*, 1993), suggesting that this technique could also be employed to detect and evaluate the amount of the fungus present in root samples.

In agreement with work by Hughes *et al.* (1978), Chaves & Ferrera-Cerrato (1990) and Niemi & Vestberg (1992), the results of this thesis demonstrated that each particular interaction between a plant host and arbuscular mycorrhizal fungus must be considered individually. The effect of a particular mycorrhizal fungus on a host plant may be different if the same fungus colonises another species or cultivar, and depends on the environmental conditions, particularly the nutrient status of the plant.

An arbuscular mycorrhizal colonisation can potentially affect the plant growth, in either a positive or negative manner. The results presented here suggest that colonisation of Elsanta strawberry plants by *Scutellospora nodosa* had a beneficial effect on plant growth, causing significant increases in the shoot and root dry weight and additional phosphorus to be taken up into the plant, positively correlated with increasing percentage mycorrhizal colonisation. In contrast, during the same experiment, increasing colonisation of Elsanta plants by the arbuscular mycorrhizal fungus *Ascrobiculata scrobiculata* had a positive effect on plant growth and phosphorus uptake only up to a threshold value of root colonisation by the fungus, beyond which further colonisation of the plant root system by the fungus had no additional effect on plant growth or nutrition. Therefore, there was a limit to the degree of benefit obtained by a plant from colonisation by *A. scrobiculata*.

When *Glomus* spp. were studied the results suggested that the threshold value of colonisation by the mycorrhizal fungus was reached at a relatively low level of percentage root colonisation. The mycorrhizal plants were larger and contained more phosphorus compared to the non-mycorrhizal, control plants, but any increases in percentage mycorrhizal colonisation did not cause a correlating increase in plant size or phosphorus uptake. This indicated that only a small degree of root colonisation by these mycorrhizal fungi was sufficient to cause an increase in plant growth and additional phosphorus uptake compared to non-mycorrhizal, control plants.

However, it must be remembered that the experiments described here only provide a measure of the plant size and phosphorus content at the end of the experiment; this does not provide information about the growth of the plants over the course of the experiment. An improvement on these experiments would be to sample plants at

various different time intervals after colonisation by the mycorrhizal fungus. This would provide more detailed information about the effect of the mycorrhizal colonization on the host plant as the colonisation progressed.

In these experiments, the percentage of mycorrhizal colonisation of Rhapsody plants was consistently lower than that of Elsanta plants. This is in agreement with work by Ravnskov & Jakobsen (1995) which indicated that the fungus involved, the mycorrhizal dependency of the host plant and the environmental conditions could affect the compatibility of a particular mycorrhizal association. Therefore, any commercial use of arbuscular mycorrhizal fungi to improve plant growth must first investigate the individual plant–mycorrhiza interaction. An arbuscular mycorrhizal fungus that encourages the plant growth of one cultivar cannot be assumed to similarly affect other cultivars of the same species (Dodd & Thomson, 1994).

It has been suggested that mycorrhizal colonisation will only result in a beneficial growth response of the host plant when growing in a low-phosphorus environment (Abbot & Fitter, 1982). However, it has been indicated that strawberry plants receiving commercial levels of phosphorus fertiliser will exhibit a growth response when colonised by mycorrhizal fungi (Werner *et al.*, 1990). It has been suggested that at certain times of a strawberry plant's life cycle, e.g. during fruiting, there is a high phosphorus demand, and strawberry plants will therefore benefit from the additional phosphorus provided by a compatible mycorrhizal colonisation (Dunne and Fitter, 1989; Fitter, 1991).

Therefore a useful extension to this work would be to investigate the effect of a mycorrhizal colonisation of micropropagated strawberry plants when planted into a nutrient-rich compost, such as has been described by Vestberg (1992c), where the inoculation persisted throughout the first year of the plants in the field.

More than one mycorrhizal fungus may colonise a host plant in the field (McGonigle & Fitter, 1990) and it is known that different mycorrhizal fungi affect plants differently in the field (Abbott & Robson, 1984; Sanders & Fitter, 1992). The interaction of mycorrhizal fungi with other soil microorganisms can also affect the

symbiosis, together with the fact that there are seasonal variations in the amount of micropropagated material available in the soil (Rosendahl *et al.*, 1989). In addition, mycorrhizal fungi may be in competition with other mycorrhizal fungi in the soil. These factors indicate that the natural colonisation of field plants is a more complex situation than laboratory experiments which frequently focus on only one factor, e.g. phosphorous nutrition. Abbott & Gazey (1994) emphasised that research into the diversity of mycorrhizal communities and the ecology of these fungi in the field is essential to manage soil conditions to promote the growth and activity of mycorrhizal fungi. Improved methods of identifying mycorrhizal fungi from field soils will aid in this research.

When mycorrhizal strawberry plants were subsequently inoculated with a low level of zoospores of the pathogen *P. fragariae*, only those plants colonised by *S. nodosa* exhibited disease symptoms. However, when 50 000 zoospores was used to inoculate each strawberry plant, oospores of *P. fragariae* were observed in the root tips of plants colonised by *S. nodosa* and *A. scrobiculata*. Analysis of the data showed a significantly greater number of oospores in plant roots colonised by *S. nodosa*, and in plants colonised by *A. scrobiculata* compared to control, non-mycorrhizal plants. Therefore the presence of either mycorrhizal fungus increased the disease due to *P. fragariae*.

However, the effect of each mycorrhizal fungus on subsequent infection by the pathogen did differ in that plants colonised by *S. nodosa* had a greater number of roots containing oospores and more oospores were observed in infected roots, indicating that the *S. nodosa* colonisation increased the total reproductive output of the pathogen (i.e. number of oospores observed) and the number of infected roots. In contrast, colonisation by *A. scrobiculata* increased the number of roots infected compared to non-mycorrhizal plants but had no effect on the number of oospores observed.

Previous experiments showed that colonisation of strawberry plants by *S. nodosa* increased the shoot and root dry weight and the uptake of additional phosphorus into the shoots compared to non-mycorrhizal plants and those colonised by *A. scrobiculata*. Therefore these 'fitter', apparently healthier plants were more

susceptible to infection by the pathogen *P. fragariae*. It has been suggested that it may be more favourable for a pathogen to invade a 'healthy' plant as it provides a greater opportunity for the pathogen to reproduce and survive to infect additional plants (C. Walker, personal communication). Also, colonisation by *S. nodosa* which increased the root dry weight may have allowed a greater number of potential entry points, i.e. root tips, for penetration by the germ tubes of *P. fragariae*.

There was no significant difference in the disease resulting from inoculation of strawberry plants colonised by *G. mosseae*, *G. intraradices* or *G. fistulosum* by *P. fragariae*, and no significant difference in the number of infected roots of either mycorrhizal or non-mycorrhizal plants. Therefore colonisation of Elsanta plants by either of these arbuscular mycorrhizal fungi had no effect on disease due to *P. fragariae*.

In a review of plant defences to colonisation by arbuscular mycorrhizal fungi, Gianinazzi-Pearson *et al.* (1996) concluded that colonisation by arbuscular mycorrhizal fungi results in an uncoordinated, weak activation of plant metabolism associated with defence. Most arbuscular mycorrhizal fungi show little or no host specificity; therefore a mycorrhizal fungus will colonise a variety of different host plants to varying degrees and have different effects on different host plants. However, in each case, the presence of the mycorrhizal fungus elicits either a low-grade or no defence response in the host plant. Small increases in the activities of chitinase (Spanu *et al.*, 1989), peroxidase (Spanu and Bonfante-Fasolo, 1988) and phenolic compounds (Gollotte *et al.*, 1993) have been reported during colonisation of leek roots by arbuscular mycorrhizal fungi, although this increased activity was found in the whole root and not necessarily in cells containing arbuscles.

Isoflavanoid phytoalexins are antimicrobial compounds related to a resistance response in plants; they often accumulate in plant tissues in response to infection by a plant pathogen. It has been reported that the isoflavanoids glyceollin and coumestrol accumulate in greater amounts in mycorrhizal soybean roots than in non-mycorrhizal roots (Morandi *et al.*, 1984). However, this accumulation is at a much lower and slower level than in pathogen interactions.

Most plant species are able to form arbuscular mycorrhizal symbioses (Newman & Reddel, 1987) with the exception of members of the families Cruciferae and Chenopodaceae and legumes of the genus *Lupinus*. However, Duc *et al.* (1989) first reported the genetic resistance to colonisation by arbuscular mycorrhizal fungi in mutants of two otherwise mycorrhizal legumes, *Pisum sativum* L. and *Vicia faba* L. A positive link was found between these myc$^-$ mutants and resistance to nodulation characterised as nod$^-$, and it was shown that three genes were involved in this resistance. Fungal appressoria were observed to form in contact with the root surface (Duc *et al.*, 1989) and further work by Gollotte *et al.* (1993) showed that phenolic compounds were deposited in the cell walls, a reaction usually associated with the activation of host defence responses to pathogen invasion. The fact that the fungal hyphae remained viable suggested that this defence response was responsible for the termination of the colonisation.

Kapulnik *et al.* (1996) suggested that mycorrhizal fungi are able to suppress the plant defence response either directly or indirectly by production of suppressors, and it has been suggested that the host defence factors may be mobile (Gianinazzi-Pearson & Gianinazzi, 1989). The majority of plant-mycorrhizal interactions are compatible, indicating that the fungus has suppressed the host defences and that the factor causing suppression is dominant.

Transgenic plant species have been genetically engineered to express high levels of defence-related genes, accompanied by an increase in their resistance to root fungal pathogens, and the question has been raised as to whether this affects the root colonisation by symbiotic fungi (Gianinazzi-Pearson *et al.*, 1996). However, studies have shown that transgenic species of *Nicotiana* (tobacco plants) that were more resistant to *Rhizoctonia solani* were colonised by *Glomus* species to a similar extent as non-transgenic tobacco plants (Vierheilig *et al.*, 1993; 1995). The reason why transgenic plants with constitutively expressed defence-related genes have no effect on a symbiotic colonisation of the plants is unknown, but represents a model system for understanding interactions between mycorrhizal fungi and plant defence systems (Gianinazzi-Pearson *et al.*, 1996). Recent work by Murphy *et al.* (1997) used a non-targeted cloning approach which showed that the expression of plant genes was

altered during mycorrhizal symbiosis and that the genes that were altered were probably not host-defence related.

Therefore, further study is required to elucidate the mechanisms behind and the effects of arbuscular mycorrhizal colonisation of plants.

There has been great interest in utilising the continuing advances in molecular biology to provide more information about the taxonomy of arbuscular mycorrhizal fungi, particularly because of the difficulties in obtaining sufficient accurate information about morphological characteristics, and interpreting this information with confidence. Isozyme banding patterns reflect variations in the nucleotide sequence of the spore's DNA (Rosendahl and Sen, 1992) and have therefore been used to attempt to distinguish between isolates of arbuscular mycorrhizal fungi. However, this approach is problematic because of the variation in morphology and isozyme banding patterns in morphologically similar isolates. Rosendahl (1989) reported that morphologically similar isolates of arbuscular mycorrhizal fungi can have a very low degree of similarity in their isozyme banding pattern. Isolates which fell within species limits defined by morphological descriptions (Morton, 1988) were found to exhibit considerable variation in their isozyme banding pattern (Hepper *et al.*, 1988). Therefore isozyme analysis may be useful for separating strains of arbuscular mycorrhizal fungi but other methods are required to separate fungi into distinct taxa (Rosendahl, 1989).

Several of the methods employed in this thesis were difficult to perform for various practical reasons. For example, large numbers of strawberry plants of two cultivars had to be continually maintained by micropropagation. Every experiment required large numbers of micropropagated plants of the same subculture and age, in addition to the stock of plants that had to be continually maintained to produce plants for future experiments. Therefore long-term planning and precise organisation was required. The *P. fragariae* strains were also continuously subcultured throughout the project, but on a laboratory scale that was much more manageable than a micropropagation environment. Zoospore production for inoculation experiments required exact timing,

and sufficient samples had to be produced to guarantee sufficient inoculum for the experiments.

A third task of continuous culturing was required throughout the project to maintain sufficient stocks of several species of arbuscular mycorrhizal fungi. Inoculum of specific mycorrhizal species was kindly provided by Dr C. Walker and Dr M. Vestberg. The initial amount of mycorrhizal inoculum was small, but this was multiplied by open pot culture to obtain sufficient amounts of inoculum for experimental purposes, and continuously maintained throughout the project.

However, successful timing of the fruition of all plant and fungal cultures and a major investment of time and labour did enable many relatively large-scale experiments to be performed. In combination with prolonged periods of recording measurements of many parameters and results, frequently over long periods of time because of the number of replicates and variables involved in the experiments, several very interesting and worthwhile sets of data were obtained.

The aim of the project was to investigate the effect of colonisation by mycorrhizal fungi on micropropagated strawberry plants, and whether this colonisation had any effect on subsequent inoculation of the plants by the pathogen *P. fragariae*. The results did show that colonisation of strawberry plants by certain mycorrhizal fungi was associated with enhanced growth (e.g. size, phosphorus nutrition) of the host plants, but this did not extend to all mycorrhizal species used. Indeed, one of the major findings of this project was that interactions between host plants and mycorrhizal fungi must be considered individually. In addition, the presence of a mycorrhizal fungal colonisation was sometimes observed to have no effect on disease resulting from subsequent inoculation of the plants with *P. fragariae* (e.g. *Glomus* spp.). In complete contrast, plants colonised by *S. nodosa* or *A. scrobiculata* showed a reduction in disease resulting from inoculation with the pathogen, even though plants colonised by *S. nodosa* were found to benefit from this colonisation in terms of growth and phosphorus nutrition.

Therefore, the aim of the project was achieved in that the interaction among host plants, mycorrhizal fungi and the pathogen *P. fragariae* was investigated, and the results highlight the fact that a better understanding of pathogen as well as mycorrhizal biology is required if the use of mycorrhizal fungi in crop protection is to be of any practical benefit.

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CHAPTER 10: Appendix I

10.1 Chi-square analysis to show the effect of temperature (10°C, 15°C or 20°C) on germination of cysts of three strains of *Phytophthora fragariae* (strain 171, 293 and 372)

Observed				
	171	293	372	
10	48	68	75	191
15	141	167	160	468
20	99	99	108	306
	288	334	343	965
Expected				
	171	293	372	
10	57.00311	66.10777	67.88912	
15	139.6725	161.9813	166.3461	
20	91.32435	105.9109	108.7648	
P	**0.443403**			

10.2 Chi-square analysis to show the effect of temperature (10°C, 15°C or 20°C) on germination of cysts of each of three strains of *Phytophthora fragariae* (strain 171, 293 and 372)

Strain 171			
Observed			
	Germ	Ungerm	
10	48	252	300
15	141	159	300
20	99	201	300
	288	612	900
Expected			
	Germ	Ungerm	
10	96	204	
15	96	204	
20	96	204	
P	**3.72E-15**		

Strain 372			
Observed			
	Germ	Ungerm	
10	75	225	300
15	160	140	300
20	108	192	300
	343	557	900
Expected			
	Germ	Ungerm	
10	114.3333	185.6667	
15	114.3333	185.6667	
20	114.3333	185.6667	
P	**5.36E-12**		

Strain 293			
Observed			
	Germ	Ungerm	
10	68	232	300
15	167	133	300
20	99	201	300
	334	566	900
Expected			
	Germ	Ungerm	
10	111.3333	188.6667	
15	111.3333	188.6667	
20	111.3333	188.6667	
P	**1.24E-16**		

10.3 Chi-square analysis to show cyst germination in the presence of mycorrhizal and non-mycorrhizal strawberry roots

Combine all three strains				
Observed				
	171	293	372	
Non-myc	264	291	281	836
Myc	140	168	235	543
	404	459	516	1379
Expected				
	171	293	372	
Non-myc	244.9195	278.2625	312.818	
Myc	159.0805	180.7375	203.182	
P	**0.001186**			

Strain 171			
Observed			
	Germ	Ungerm	
Non-myc	264	36	300
Myc	140	160	300
	404	196	600
Expected			
	Germ	Ungerm	
Non-myc	202	98	
Myc	202	98	
	P	**3.68E-27**	

Strain 293			
Observed			
	Germ	Ungerm	
Non-myc	291	9	300
Myc	168	132	300
	459	141	600
Expected			
	Germ	Ungerm	
Non-myc	229.5	70.5	
Myc	229.5	70.5	
P	**2.34E-32**		

Strain 372			
Observed			
	Germ	Ungerm	
Non-myc	281	19	300
Myc	235	65	300
	516	84	600
Expected			
	Germ	Ungerm	
Non-myc	258	42	
Myc	258	42	
P	**6.23E-08**		

10.4 Chi-square analysis to show germ tube orientation (positive, towards the root; negative, away from the root)of individual strains of *P. fragariae* (171, 293, 372) in the presence of mycorrhizal and non-myvorrhizal roots

171 Non-mycorrhizal					171 Mycorrhizal			
Observed	Positive	Negative			Observed	Positive	Negative	
1	155	109	264		1	70	70	140
2	50	214	264		2	29	111	140
3	34	230	264		3	22	118	140
4	25	239	264		4	19	121	140
	264	792	1056			140	420	560
Expected					Expected			
	66	198				35	105	
	66	198				35	105	
	66	198				35	105	
	66	198				35	105	
P	**2.18E-47**				*P*	**7.33E-14**		
293 Non-mycorrhizal					293 Mycorrhizal			

Observed	Positive	Negative		
1	187	104	291	
2	28	263	291	
3	35	256	291	
4	41	250	291	
	291	873	1164	
Expected				
	72.75	218.25		
	72.75	218.25		
	72.75	218.25		
	72.75	218.25		
P	**3.59E-69**			

Observed	Positive	Negative		
1	75	93	168	
2	44	124	168	
3	24	144	168	
4	25	143	168	
	168	504	672	
Expected				
	42	126		
	42	126		
	42	126		
	42	126		
P	**1.04E-11**			

372 Non-mycorrhizal				
Observed	Positive	Negative		
1	165	116	281	
2	51	230	281	
3	34	247	281	
4	31	250	281	
	281	843	1124	
Expected				
	70.25	210.75		
	70.25	210.75		
	70.25	210.75		
	70.25	210.75		
P	**6.22E-50**			

372 Mycorrhizal			
Observed	Positive	Negative	
1	141	94	235
2	49	186	235
3	20	215	235
4	25	210	235
	235	705	940
Expected			
	58.75	176.25	
	58.75	176.25	
	58.75	176.25	
	58.75	176.25	
P	**1.78E-46**		

10.5 Kruskal–Wallis analysis of variance using tied ranks to calculate any significant differences in the number of oospores produced at 2, 4, 6, 8, 10, 12 and 14 days after infection of a strawberry root by zoospores of *P. fragariae*

Days	Number of oospores arranged from highest to lowest						
	2	4	6	8	10	12	14
	0	81	98	269	290	318	211
	0	70	65	246	273	285	164
	0	56	64	238	235	285	129
	0	36	53	228	221	255	105
	0	35	39	219	198	230	93
	0	20	17	192	185	103	69
	0	14	6	179	183	95	58
	0	3	0	175	168	79	36
	0	2	0	169	124	78	35
	0	2	0	124	112	57	35
	0	0	0	109	79	42	31
	0	0	0	100	78	42	30
	0	0	0	86	64	38	5
	0	0	0	80	61	8	0
	0	0	0	70	58	0	0
	0	0	0	69	45	0	0

	Ranked						
	2	4	6	8	10	12	14
	94.5	37	33	6	2	1	15
	94.5	43.5	47	8	5	3.5	24
	94.5	54	48.5	9	10	3.5	25
	94.5	61.5	55	12	13	7	30
	94.5	64	59	14	16	11	35
	94.5	68	69	17	18	31	45.5
	94.5	70	72	20	19	34	51.5
	94.5	74	94.5	21	23	39.5	61.5
	94.5	75.5	94.5	22	26.5	41.5	64
	94.5	75.5	94.5	26.5	28	53	64
	94.5	94.5	94.5	29	39.5	57.5	66
	94.5	94.5	94.5	32	41.5	57.5	67
	94.5	94.5	94.5	36	48.5	60	73
	94.5	94.5	94.5	38	50	71	94.5
	94.5	94.5	94.5	43.5	51.5	94.5	94.5
	94.5	94.5	94.5	45.5	56	94.5	94.5
Rank Sum	1512	1190	1234	379.5	447.5	660	905

N		112
12/N(N+1)		0.000948
sum Ri		426493.8
3(n+1)		339
H		65.38734
m		12
sum T		46704
C		0.966754
Hc		67.63594
v(=k-1)		6
Chi		12.592

Hc>Chi: There is a significant difference between the no. of oospores at different days

10.5.1 Calculated and critical values of the q statistic for each comparison in the rank sums of the ranked number of oospores observed in roots removed from plants at 2, 4, 6, 8, 10, 12 and 14 days after inoculation with *P. fragariae*. SE, standard error; *p*, number of groups being compared; *q*, calculated *q* statistic. ns, not significant; *, significant difference

Arrange rank sums in increasing order of magnitude						
8	10	12	14	4	6	2
379.5	447.5	660	905	1190	1234	1512

	SE
p=7	129.9025
p=6	111.4271
p=5	92.9516
p=4	74.47595
p=3	56
p=2	37.52333

	Rx-Ry	SE	p	q	Table q
2 v 8	1132.5	129.9025	7	8.718075	4.17
2 v 10	1064.5	111.4271	6	9.553331	4.03
2 v 12	852	92.9516	5	9.166061	3.858
2 v 14	607	74.47595	4	8.150282	3.633
2 v 4	322	56	3	5.75	3.314
2 v 6	278	37.52333	2	7.408725	2.772
6 v 8	854.5	111.4271	6	7.668691	4.03
6 v 10	786.5	92.9516	5	8.461393	3.858
6 v 12	574	74.47595	4	7.707186	4.633
6 v 14	329	56	3	5.875	3.314
6 v 4	44	37.52333	2	1.172604	2.772
4 v 8	810.5	74.47595	5	10.88271	3.858
4 v 10	742.5	56	4	13.25893	3.633
4 v 12	530	37.52333	3	14.12455	3.314
4 v 14	285	37.52333	2	7.595276	2.772
14 v 8	525.5	74.47595	4	7.055969	3.633
14 v 10	457.5	56	3	8.169643	3.314
14 v 12	245	37.52333	2	6.529272	2.772
12 v 8	280.5	56	3	5.008929	3.314
12 v 10	212.5	37.52333	2	5.663144	2.772
10 v 8	68	37.52333	2	1.812206	2.772

10.6 Summary table to show estimated and measured percent infected root.

Key to Table

MP - motile, poured inoculation of zoospores
NMP - non-motile poured inculation of zoospores
NMS - non-motile sprayed inoculation of zoospores
M - measured percent infected root
Est - estimated percent infected root
Z/plant - zoospores per plant

Summary Table (% Infected Root)

Z/plant	MP 171M	MP 171Est	MP 293M	MP 293Est	NMP 171M	NMP 171Est	NMP 293M	NMP 293Est	NMS 171M	NMS 171Est	NMS 293M	NMS 293Est
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	8.040	8.000	8.040	8.000
0.000	17.960	12.000	17.960	12.000	13.800	9.000	13.800	9.000	4.670	4.000	4.670	4.000
0.000	4.210	3.000	4.210	3.000	13.750	10.000	13.750	10.000	0.000	0.000	0.000	0.000
0.000	7.720	8.000	7.720	8.000	8.990	8.000	8.990	8.000	2.990	3.000	2.990	3.000
10000	100.000	100.000	100.000	100.000	21.200	40.000	55.340	40.000	52.460	75.000	100.000	100.000
10000	91.530	95.000	93.320	90.000	75.210	70.000	88.810	85.000	5.750	15.000	73.870	75.000
10000	28.540	15.000	91.930	95.000	39.120	50.000	80.220	70.000	31.820	35.000	100.000	100.000
10000	87.600	90.000	83.750	80.000	0.000	0.000	81.340	85.000	14.820	20.000	95.400	95.000
20000	82.450	70.000	96.680	85.000	79.740	65.000	67.100	80.000	18.370	20.000	100.000	100.000
20000	100.000	100.000	100.000	100.000	62.680	50.000	100.000	100.000	28.190	35.000	100.000	100.000
20000	93.260	95.000	97.780	92.000	100.000	100.000	98.280	97.000	87.280	80.000	15.850	20.000
20000	87.780	85.000	97.050	97.000	90.340	90.000	95.650	95.000	43.250	65.000	95.490	95.000
80000	100.000	100.000	89.220	80.000	69.480	70.000	91.740	85.000	76.400	80.000	87.500	85.000
80000	92.850	90.000	100.000	100.000	100.000	100.000	79.070	80.000	93.780	95.000	72.600	75.000
80000	87.430	90.000	95.620	90.000	94.230	97.000	89.810	85.000	92.550	90.000	100.000	100.000
80000	100.000	100.000	100.000	100.000	96.800	95.000	91.320	90.000	87.230	90.000	100.000	100.000

Summary Table (Arcsin Values)

Z/plant	MP 171M	MP 171Est	MP 293M	MP 293Est	NMP 171M	NMP 171Est	NMP 293M	NMP 293Est	NMS 171M	NMS 171Est	NMS 293M	NMS 293Est
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	16.472	16.430	16.472	16.430
0	25.074	20.268	25.074	20.268	21.807	17.458	21.807	17.458	12.480	11.537	12.480	11.537
0	11.840	9.974	11.840	9.974	21.766	18.435	21.766	18.435	0.000	0.000	0.000	0.000
0	16.132	16.430	16.132	16.430	17.448	16.430	17.448	16.430	9.957	9.974	9.957	9.974
10000	90.000	90.000	90.000	90.000	27.415	39.232	48.065	39.232	46.410	60.000	90.000	90.000
10000	73.080	77.079	75.021	71.565	60.139	56.789	70.457	67.214	13.874	22.786	59.258	60.000
10000	32.292	22.786	73.496	77.079	38.716	45.000	63.593	56.789	34.339	36.271	90.000	90.000
10000	69.382	71.565	66.227	63.435	0.000	0.000	64.407	67.214	22.642	26.565	77.615	77.079
20000	65.233	56.789	79.502	67.214	63.249	53.729	54.999	63.435	25.379	26.565	90.000	90.000
20000	90.000	90.000	90.000	90.000	52.345	45.000	90.000	90.000	32.069	36.271	90.000	90.000
20000	74.953	77.079	81.431	73.570	90.000	90.000	82.464	80.026	69.105	63.435	23.461	26.565
20000	69.539	67.214	80.110	80.026	71.892	71.565	77.962	77.079	41.121	53.729	77.739	77.079
80000	90.000	90.000	70.833	63.435	56.465	56.789	73.298	67.214	60.935	63.435	69.295	67.214
80000	74.491	71.565	90.000	90.000	90.000	90.000	62.775	63.435	75.558	77.079	58.436	60.000
80000	69.235	71.565	77.920	71.565	76.101	80.026	71.384	67.214	74.160	71.565	90.000	90.000
80000	90.000	90.000	90.000	90.000	79.695	77.079	72.865	71.565	69.062	71.565	90.000	90.000

10.7 Paired sample *t*-tests to show any significant differences in the measured and estimated percent infected root using different inoculation methods and strains of *P. fragariae*

	MP 171M	MP 171Est
Mean	58.82813872	57.6446483
Variance	953.7136412	1034.84218
Observations	16	16
Pearson Correlation	0.993767478	
Hypothesized Mean Difference	0	
df	15	

	NMP 171M	NMP 171Est
Mean	47.93989991	47.34567848
Variance	919.2773581	909.4335087
Observations	16	16
Pearson Correlation	0.98605959	
Hypothesized Mean Difference	0	
df	15	

t Stat	1.26344505	
P(T<=t) one-tail	0.112857024	
t Critical one-tail	1.753051038	
P(T<=t) two-tail	0.225714047	
t Critical two-tail	2.131450856	

t Stat	0.47051729	
P(T<=t) one-tail	0.322377144	
t Critical one-tail	1.753051038	
P(T<=t) two-tail	0.644754287	
t Critical two-tail	2.131450856	

	NMS 171M	NMS 171Est
Mean	37.72281294	40.45049709
Variance	641.7828136	640.2356119
Observations	16	16
Pearson Correlation	0.979365014	
Hypothesized Mean Difference	0	
df	15	
t Stat	-2.121276809	
P(T<=t) one-tail	0.025485018	
t Critical one-tail	1.753051038	
P(T<=t) two-tail	0.050970037	
t Critical two-tail	2.131450856	

	NMS 293M	NMS 293Est
Mean	59.04460147	59.11735884
Variance	1179.462823	1166.029816
Observations	16	16
Pearson Correlation	0.999490652	
Hypothesized Mean Difference	0	
df	15	
t Stat	-0.262080271	
P(T<=t) one-tail	0.398412239	
t Critical one-tail	1.753051038	
P(T<=t) two-tail	0.796824478	
t Critical two-tail	2.131450856	

	MP 293M	MP 293Est
Mean	63.59913797	60.9100273
Variance	972.3585508	959.088185
Observations	16	16
Pearson Correlation	0.991344023	
Hypothesized Mean Difference	0	
df	15	
t Stat	2.627138943	
P(T<=t) one-tail	0.00952045	
t Critical one-tail	1.753051038	
P(T<=t) two-tail	0.019040901	
t Critical two-tail	2.131450856	

	NMP 293M	NMP 293Est
Mean	55.83059888	53.92105441
Variance	704.6097767	722.9635548
Observations	16	16
Pearson Correlation	0.988413832	
Hypothesized Mean Difference	0	
df	15	
t Stat	1.871522004	
P(T<=t) one-tail	0.040452812	
t Critical one-tail	1.753051038	
P(T<=t) two-tail	0.080905625	
t Critical two-tail	2.131450856	

10.8 Three-factor analysis of variance to illustrate any significant differences in the estimated percentage of infected root using three inoculation methods, two strains of *P. fragariae* and three levels of zoospore dose

MP					
10000		**20000**		**80000**	
171	**293**	**171**	**293**	**171**	**293**
90	90	65.23	79.5	90	70.83
73.08	75.02	90	90	74.49	90
32.29	73.5	74.95	81.43	69.23	77.92
69.38	66.23	69.53	80.11	90	90
264.75	**304.75**	**299.71**	**331.04**	**323.72**	**328.75**
70092.56	92872.56	89826.08	109587.5	104794.6	108076.6

NMP					
10000		**20000**		**80000**	
171	**293**	**171**	**293**	**171**	**293**
24.42	48.06	63.25	54.99	56.47	73.3
60.14	70.46	52.35	90	90	62.78
38.72	63.59	90	82.46	76.1	71.38
0	64.41	71.89	77.96	79.7	72.87
123.28	**246.52**	**277.49**	**305.41**	**302.27**	**280.33**
15197.96	60772.11	77000.7	93275.27	91367.15	78584.91

NMS					
10000		**20000**		**80000**	
171	**293**	**171**	**293**	**171**	**293**
46.41	90	25.38	90	60.94	69.3
13.87	59.26	32.07	90	75.56	58.44
34.34	90	69.11	23.46	74.16	90
22.64	77.62	41.12	77.74	69.06	90
117.26	**316.88**	**167.68**	**281.2**	**279.72**	**307.74**
13749.91	100412.9	28116.58	79073.44	78243.28	94703.91

	Summary Info.			
	Inoc.n	1852.72	1535.3	1470.48
	Strain	2155.88	2702.62	
	Z's	1373.44	1662.53	1822.53
	CF	327847.5		
	To SS	32230.74		
	Treat SS	18589.48		
	Error SS	13641.26		
	Inoc SS	3487.007		
	Strain SS	4151.731		
	Z's SS	4317.429		

	MP	NMP	NMS		
	888.18	703.04	564.66	**2155.88**	4647819
	964.54	832.26	905.82	**2702.62**	7304155
	1852.72	**1535.3**	**1470.48**		
	3432571	2357146	2162311		

	To SS	9275.291			
	Inoc SS	3487.007			
	Str SS	4151.731			
	Inoc*Str	1636.553			

	MP	NMP	NMS		
	569.5	369.8	434.14	**1373.44**	1886337
	630.75	582.9	448.88	**1662.53**	2764006
	652.47	582.6	587.46	**1822.53**	3321616
	1852.72	**1535.3**	**1470.48**		
	3432571	2357146	2162311		
	To SS	9517.49			
	Inoc SS	3487.007			
	Z's SS	4317.429			
	Inoc*Z's SS	1713.054			

	171	293		
	505.29	868.15	**1373.44**	1886337
	744.88	917.65	**1662.53**	2764006
	905.71	916.82	**1822.53**	3321616
	2155.88	**2702.62**		
	4647819	7304155		
	To SS	11052.44		
	Str SS	4151.731		
	Z's SS	4317.429		
	Str*Z's SS	2583.281		

% Infected Root: 3 Factor ANOVA without Controls							
Variable	SS	Df	MS	Calc F	F(0.05)	F(0.01)	
Total	32230.74	71	453.9541	1.397676			
Inoc	3487.007	2	1743.504	5.368062	3.11	4.88	S (0.01)
Strain	4151.731	1	4151.731	12.78274	3.96	6.96	S (0.01)
Zoospore	4317.429	2	2158.714	6.646452	2.72	4.04	S (0.01)
Inoc*str	1636.553	2	818.2763	2.519386	3.11	4.88	NS (0.05)
Inoc*Z's	1713.054	4	428.2636	1.318578	2.21	3.04	NS (0.05)
Str*Z's	2583.281	2	1291.64	3.976824	2.72	4.04	S (0.05)
In*Str*Z's	700.4247	16	43.77654	0.134783	1.72		NS (0.05)
Error	13641.26	42	324.792				

10.9 Oospores observed in root tips of Elsanta plants inoculated with strain 171 or strain 293 of *P. fragariae* by one of three methods: motile poured zoospores (MP), non-motile poured zoospores (NMP) or non-motile sprayed zoospores (NMS)

Zoospore	171MP	293 MP	171NMP	293 NMP	171 NMS	293NMS
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
10000	0	78	7	57	15	12
10000	58	52	0	24	9	3
10000	52	33	70	0	6	25
10000	142	80	29	0	7	18
10000	27	80	0	48	5	77
10000	40	49	0	0	0	22
10000	36	23	85	28	0	17
10000	70	62	0	13	0	0
10000	9	47	17	0	0	16
10000	18	56	7	45	0	5
10000	28	28	0	60	0	0
10000	11	34	0	5	0	30
10000	0	7	0	35	0	28
10000	17	72	0	12	0	14
10000	0	0	0	10	0	18
10000	0	21	0	48	0	0
10000	51	5	0	71	0	4
10000	0	4	0	0	0	16
10000	0	5	0	124	0	11
10000	0	13	0	83	0	4
20000	0	69	0	12	269	13
20000	92	119	0	33	34	97
20000	0	13	23	10	0	60
20000	0	73	41	30	102	21
20000	0	5	23	30	0	0
20000	0	68	8	93	0	32
20000	4	38	29	53	0	10
20000	15	50	16	41	0	40
20000	28	63	18	0	0	189
20000	67	39	26	6	0	27
20000	63	7	0	72	0	0
20000	27	62	0	46	0	0
20000	44	27	0	0	10	0
20000	30	63	0	0	8	0
20000	46	0	0	39	0	0

20000	14	23	0	59	22	0
20000	59	16	80	26	0	95
20000	25	40	0	72	18	0
20000	0	44	0	23	0	15
20000	29	25	0	48	31	0
80000	27	34	67	0	41	0
80000	46	17	113	0	52	131
80000	50	31	42	98	54	45
80000	36	4	23	0	38	38
80000	42	8	0	9	28	0
80000	32	41	0	64	0	0
80000	41	82	0	63	22	23
80000	23	62	67	30	0	11
80000	91	24	7	11	0	4
80000	18	56	9	32	5	0
80000	20	29	16	25	27	0
80000	59	31	15	30	5	93
80000	30	29	0	67	12	2
80000	68	26	13	11	25	33
80000	62	12	3	22	21	7
80000	0	0	2	80	5	0
80000	51	96	11	41	2	124
80000	36	67	10	39	36	27
80000	83	131	0	41	27	0
80000	20	25	29	54	5	26

10.10 Results of the Kruskal–Wallis analysis of variance to determine the effect of zoospore dose on oospore formation

10.10.1 *P. fragariae* strain 171

	171MP: Ranked Oospore Numbers		
	10000	20000	80000
	1	2	3
	5	7	4
	12	8	6
	13	10.5	9
	14.5	17.5	10.5
	22	19	14.5
	24	27.5	16
	30.5	29	17.5
	33	30.5	20
	39.5	33	21
	41	35	24
	44	42	24
	45	43	26
	53.5	46	27.5
	53.5	53.5	33

	171NMP: Ranked Oospore Numbers		
	10000	20000	80000
	2	3	1
	4	8	5.5
	10	10	5.5
	17	12	7
	27	14	10
	27	14	14
	45.5	16	18.5
	45.5	18.5	20
	45.5	25	21
	45.5	45.5	22
	45.5	45.5	23
	45.5	45.5	24
	45.5	45.5	27
	45.5	45.5	29
	45.5	45.5	30

	53.5	53.5	36
	53.5	53.5	37.5
	53.5	53.5	37.5
	53.5	53.5	39.5
	53.5	53.5	53.5
n	20	20	20
R	699	671	460
N	60		
12/N(N+1)	0.0032		
Sum Ri	57522.1		
3(N=1)	183		
H	5.597049		
m	10		
sum T	2796		
C	0.987052		
Hc	5.670471		
v (k-1)	2		
Chi	5.991		
	No Sig Diff		

	45.5	45.5	45.5
	45.5	45.5	45.5
	45.5	45.5	45.5
	45.5	45.5	45.5
	45.5	45.5	45.5
n	20	20	20
R	724	621	485
N	60		
12/N(N+1)	0.003279		
Sum Ri	57252.1		
3(N=1)	183		
H	4.71180		
m	6		
sum T	27054		
C	0.87471		
Hc	5.38667		
v (k-1)	2		
Chi	5.991		
No Sig Diff			

	171NMS: Ranked Oospore Numbers			
	10000	20000	80000	
	18	1	3	
	21	2	4	
	23	8	5	
	24	9	6	
	27	14.5	7	
	45.5	17	10	
	45.5	20	11.5	
	45.5	22	11.5	
	45.5	45.5	13	
	45.5	45.5	14.5	
	45.5	45.5	16	
	45.5	45.5	19	
	45.5	45.5	27	
	45.5	45.5	27	
	45.5	45.5	27	
	45.5	45.5	27	
	45.5	45.5	30	
	45.5	45.5	45.5	
	45.5	45.5	45.5	
	45.5	45.5	45.5	
n	20	20	20	
R	795.5	639.5	395	
N	60			
12/N(N+1)	0.00327			
Sum Ri	59890.2			

3(N=1)	183
H	13.3615
m	4
sum T	27102
C	0.87449
Hc	15.2792
v (k-1)	2
Chi	5.991
Hc>Chi	

	SE
p=3	78.1025
p=2	52.28129

Arrange rank sums in increasing order of magnitude

80000	20000	10000
395	639.5	795.5

	Rx-Ry	SE	p	q	Table q
10z v 80z	400.5	78.1025	3	5.12787	3.314
10z v 20 z	156	52.2812	2	2.98385	2.772
20z v 80z	244.5	52.2812	2	4.67662	2.772

10.10.2 *P. fragariae* strain 293

293MP: Ranked Oospore Numbers

	10000	20000	80000
	5.5	2	1
	5.5	8	3
	7	10	4
	9	11	12
	16	13.5	16
	18.5	13.5	18.5
	20	16	25
	22	21	29.5
	23	24	32.5
	29.5	26	32.5
	31	27	34.5
	36	28	34.5
	42.5	37	38
	44	39.5	39.5
	47.5	42.5	41
	51.5	46	45
	54	47.5	49
	54	51.5	50
	56.5	54	56.5
	59	59	59
n	20	20	20
R	632	577	621
N	60		
12/N(N+1)	0.003279		
Sum Ri	55899.7		

293NMP: Ranked Oospore Numbers

	10000	20000	80000
	1	3	2
	4	6.5	5
	8	6.5	9
	12	13	10
	14	16	11
	18	18	15
	18	20	23
	21	23	23
	27	25.5	25.5
	34	28	29
	37	31.5	31.5
	38	31.5	31.5
	41	35	36
	42.5	39	40
	46.5	42.5	44.5
	50	46.5	44.5
	55.5	49	48
	55.5	55.5	55.5
	55.5	55.5	55.5
	55.5	55.5	55.5
n	20	20	20
R	634	601	595
N	60		
12/N(N+1)	0.00327		
Sum Ri	55859.1		

3(N=1)	183		
H	0.277705		
m	14		
sum T	138		
C	0.999361		
Hc	0.277883		
v (k-1)	2		
Chi	5.991		
No Sig Diff			

3(N=1)	183		
H	0.14459		
m	9		
sum T	1128		
C	0.99477		
Hc	0.14534		
v (k-1)	2		
Chi	5.991		
No Sig Diff			

293NMS: Ranked Oospore Numbers			
	10000	20000	80000
	7	1	2
	14	4	3
	15	5	6
	19	8	9
	21	10	11
	23.5	13	12
	23.5	16.5	16.5
	25	22	18
	26.5	28	20
	26.5	30	32.5
	29	34	35
	31	51	38
	32.5	51	41
	36	51	51
	38	51	51
	38	51	51
	40	51	51
	51	51	51
	51	51	51
	51	51	51
n	20	20	20
R	598.5	630.5	601
N	60		
12/N(N+1)	0.003279		
Sum Ri	55846.68		
3(N=1)	183		
H	0.103852		
m	6		
sum T	6888		
C	0.968102		
Hc	0.107274		
v (k-1)	2		
Chi	5.991		
No Sig Diff			

10.11 Kruskal–Wallis analysis of variance to determine whether oospore formation was affected by the strain of *P. fragariae*

10000 Z/ MP Highest to lowest				20000 Z/ MP Highest to lowest		
	171	293			171	293
	1	2.5			2	1
	6	2.5			6	3
	8	4			8	4
	10.5	5			11	5
	12	7			13	8
	15	9			14.5	8
	16	10.5			19	10
	19.5	13			20	12
	21	14			21	14.5
	24	17			22.5	16
	25	18			24.5	17
	27	19.5			28	18
	28	22			29	22.5
	36.5	23			33	24.5
	36.5	26			37	26
	36.5	29			37	27
	36.5	30.5			37	30
	36.5	30.5			37	31
	36.5	32			37	32
	36.5	36.5			37	37
n	20	20		n	20	20
R	468.5	351.5		R	473.5	346.5
N	40			N	40	
12/N(N+1)	0.007317			12/N(N+1)	0.007317	
Sum Ri	17152.23			Sum Ri	17213.23	
3(N=1)	123			3(N=1)	123	
H	2.504085			H	2.950427	
m	5			m	5	
sum T	528			sum T	378	
C	0.997555			C	0.99825	
Hc	2.510223			Hc	2.955601	
v (k-1)	1			v (k-1)	1	
Chi	3.841			Chi	3.841	
No Sig Diff				No Sig Diff		

	80000 Z/ MP Highest to lowest				10000 Z/ NMP Highest to lowest		
	171	293			171	293	
	3	1			2	1	
	4	2			5	3	
	6	5			12	4	
	8.5	7			15	6	
	10	8.5			19.5	7	
	12	11			19.5	8.5	

	13	16.5			31	8.5	
	14	20			31	10	
	15	22.5			31	11	
	16.5	22.5			31	13	
	18.5	25.5			31	14	
	18.5	25.5			31	16	
	21	28			31	17	
	24	29			31	18	
	27	30			31	21	
	31	35			31	31	
	32.5	36			31	31	
	32.5	37			31	31	
	34	38			31	31	
	39.5	39.5			31	31	
n	20	20		n	20	20	
R	380.5	439.5		R	507	313	
N	40			N	40		
12/N(N+1)	0.00731			12/N(N+1)	0.007317		
Sum Ri	16897.0			Sum Ri	17750.9		
3(N=1)	123			3(N=1)	123		
H	0.63676			H	6.884634		
m	7			m	3		
sum T	42			sum T	6852		
C	0.99980			C	0.968269		
Hc	0.63689			Hc	7.11025		
v (k-1)	1			v (k-1)	1		
Chi	3.841			Chi	3.841		
No Sig Diff				Sig Diff			

20000 Z/ NMP Highest to lowest

	171	293			171	293	
	2	1			1	2	
	9.5	3.5			5	3	
	15	3.5			5	5	
	16.5	5			10	7	
	19	6			17	8	
	19	7			19	9	
	21	8			21	11.5	
	22	9.5			22	11.5	
	25	11			23	13	
	33.5	12			25	14	
	33.5	13.5			27	15.5	
	33.5	13.5			28.5	15.5	
	33.5	16.5			30	18	
	33.5	19			31	20	
	33.5	23			32	25	
	33.5	24			36.5	25	
	33.5	26			36.5	28.5	

80000 Z/ NMP Highest to lowest

	33.5	33.5	
	33.5	33.5	
	33.5	33.5	
n	20	20	
R	517.5	302.5	
N	40		
12/N(N+1)	0.007317		
Sum Ri	17965.63		
3(N=1)	123		
H	8.455793		
m	6		
sum T	2790		
C	0.98708		
Hc	8.566474		
v (k-1)	1		
Chi	3.841		
Sig Diff			

	36.5	36.5	
	36.5	36.5	
	36.5	36.5	
n	20	20	
R	479	341	
N	40		
12/N(N+1)	0.007317		
Sum Ri	17286.1		
3(N=1)	123		
H	3.483659		
m	6		
sum T	570		
C	0.99736		
Hc	3.492878		
v (k-1)	1		
Chi	3.841		
No Sig Diff!!!			

10000 Z/ NMS: Highest to lowest

	171	293
	11	1
	15	2
	16	3
	17	4
	18.5	5
	31.5	6.5
	31.5	6.5
	31.5	8
	31.5	9.5
	31.5	9.5
	31.5	12
	31.5	13
	31.5	14
	31.5	18.5
	31.5	20.5
	31.5	20.5
	31.5	22
	31.5	31.5
	31.5	31.5
	31.5	31.5
n	20	20
R	550	270
N	40	
12/N(N+1)	0.007317	
Sum Ri	18770	
3(N=1)	123	
H	14.34146	

20000 Z/ NMS: Highest to lowest

	171	293
	1	2
	3	4
	8	5
	10	6
	12	7
	14	9
	17.5	11
	19	13
	30	15
	30	16
	30	17.5
	30	30
	30	30
	30	30
	30	30
	30	30
	30	30
	30	30
	30	30
n	20	20
R	444.5	375.5
N	40	
12/N(N+1)	0.00731707	
Sum Ri	16929.025	
3(N=1)	123	
H	0.87091463	

m	5	
sum T	5838	
C	0.972965	
Hc	14.73996	
v (k-1)	1	
Chi	3.841	
Sig Diff		

m	2	
sum T	26976	
C	0.87507641	
Hc	0.9952441	
v (k-1)	1	
Chi	3.841	
No Sig Diff		

80000 Z/ NMS: Highest to lowest

	171	293
	4	1
	5	2
	7	3
	8.5	6
	10	8.5
	12	11
	14	14
	14	16
	17	18
	19	22
	20	23
	21	28
	25.5	29.5
	25.5	35.5
	25.5	35.5
	25.5	35.5
	29.5	35.5
	35.5	35.5
	35.5	35.5
	35.5	35.5
n	20	20
R	389.5	430.5
N	40	
12/N(N+1)	0.007317	
Sum Ri	16852.03	
3(N=1)	123	
H	0.3075	
m	5	
sum T	1086	
C	0.994971	
Hc	0.309054	
v (k-1)	1	
Chi	3.841	
No Sig Diff		

10.12 The number of oospores observed in root tips of strawberry plants of two cultivars (Elsanta and Rhapsody) inoculated with zoospores of each of three strains of *P. fragariae*: 171, 293 and 372

Elsanta					
171	171	293	293	372	372
5000	100000	5000	100000	5000	100000
0	24	25	13	5	66
0	11	0	76	0	13
4	20	18	24	4	0
6	31	5	55	38	0
51	14	0	26	1	147
10	6	15	22	0	63
156	165	5	59	0	27
12	116	44	29	0	0
47	50	11	12	0	0
10	13	5	0	0	0
2	16	2	30	41	19
0	0	17	1	49	44
4	1	7	108	12	44
15	20	35	38	1	13
0	36	42	0	0	88
0	28	0	6	0	44
0	125	0	14	0	87
0	100	0	0	0	24
0	26	0	0	0	192
0	30	0	0	0	14
0	12	2	7	0	389
4	8	0	0	56	141
5	25	0	9	33	0
0	0	0	43	11	67
0	6	0	57	44	411
17	78	0	11	44	125
0	160	0	104	120	301
0	54	0	4	210	261
0	0	0	1	4	114
0	65	0	13	11	99
39	53	11	0	129	22
7	12	6	0	45	54
52	17	8	0	0	54
51	47	4	7	0	127
0	0	19	7	11	113
0	0	0	4	0	118
0	27	0	0	250	143
0	16	0	0	37	0
0	54	0	0	0	0
0	0	0	0	0	0

Rhapsody					
171	171	293	293	372	372
5000	100000	5000	100000	5000	100000
27	7	33	65	0	4
4	11	11	0	0	19
0	13	7	2	11	17
1	29	2	0	0	4
0	3	5	4	0	3
0	0	5	68	0	22
0	0	24	39	0	31
0	0	20	17	0	0
0	0	2	0	0	0
0	0	1	12	2	0
8	0	0	8	0	17
5	11	4	25	0	14
0	0	0	18	0	11
0	1	10	45	0	2
12	3	19	9	0	0
27	1	0	0	0	7
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	4	0
0	0	1	0	0	0
0	0	6	5	0	0
0	0	4	39	15	0
0	0	12	45	0	0
0	0	0	92	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	6	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	7	0	13	0	5
0	11	0	37	0	0
0	10	0	7	0	0
0	0	0	14	0	0
0	0	0	28	0	0
0	0	0	22	0	0
0	0	0	2	0	0
0	0	0	6	0	0
0	0	0	7	0	0
0	0	0	0	0	0

10.13 Kruskal–Wallis analysis of variance to determine significant differences in the numbers of oospores observed in root tips removed from plants of two cultivars (Elsanta and Rhapsody) inoculated with a motile poured inoculation of either 5000 or 80 000 zoospores per plant of each of three strains of *P. fragariae* (171, 293 and 372)

10.13.1 *P. fragariae* strain 171

	171: 5000	
	Elsanta	Rhapsody
	1	7.5
	2	7.5
	3	12.5
	4	16
	5	18.5
	6	21.5
	9	25
	10	53
	11	53
	12.5	53
	14.5	53
	14.5	53
	17	53
	18.5	53
	21.5	53
	21.5	53
	21.5	53
	24	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
	53	53
n	40	40
R	1382.5	1857.5
N	80	
12/N(N+1)	0.001852	

	171: 100000	
	Elsanta	Rhapsody
	1	16
	2	28.5
	3	33.5
	4	33.5
	5	33.5
	6	36
	7	38.5
	8.5	38.5
	8.5	42.5
	10	42.5
	11	45
	12	45
	13	63.5
	14	63.5
	15	63.5
	17	63.5
	18	63.5
	19	63.5
	20	63.5
	21	63.5
	22.5	63.5
	22.5	63.5
	24	63.5
	25.5	63.5
	25.5	63.5
	27	63.5
	28.5	63.5
	30.5	63.5
	30.5	63.5
	33.5	63.5
	37	63.5
	40.5	63.5
	40.5	63.5
	45	63.5
	63.5	63.5
	63.5	63.5
	63.5	63.5
	63.5	63.5
	63.5	63.5
	63.5	63.5
n	40	40
R	1029	2211
N	80	
12/N(N+1)	0.001852	

Sum Ri	134040.3	
3(N=1)	243	
H	5.222801	
m	6	
sum T	166404	
C	0.674941	
Hc	7.738155	
v (k-1)	1	
Chi	3.841	
Sig Diff		

Sum Ri	148684.1	
3(N=1)	243	
H	32.34083	
m	11	
sum T	39402	
C	0.923031	
Hc	35.03765	
v (k-1)	1	
Chi	3.841	
Sig Diff		

10.13.2 *P. fragariae* strain 293

	293: 5000	
	Elsanta	Rhapsody
	1	4
	2	6
	3	7
	5	8.5
	8.5	13
	10	15
	11	17
	12	19.5
	15	21.5
	15	25
	18	25
	19.5	29
	21.5	29
	25	32.5
	25	32.5
	25	35.5
	29	35.5
	32.5	58.5
	32.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5
	58.5	58.5

	293: 100000	
	Elsanta	Rhapsody
	1	3
	2	5
	4	6
	7	10.5
	8	10.5
	9	13.5
	12	13.5
	15	16
	17	19
	18	21
	20	23.5
	22	25
	23.5	26
	27.5	27.5
	30	30
	30	32.5
	32.5	35.5
	34	37
	35.5	40
	40	40
	40	44
	40	44
	44	46
	48	48
	48	50.5
	52.5	50.5
	52.5	67
	67	67
	67	67
	67	67
	67	67
	67	67
	67	67
	67	67
	67	67
	67	67

	58.5	58.5
	58.5	58.5
	58.5	58.5
n	40	40
R	1539	1701
N	80	
12/N(N+1)	0.001852	
Sum Ri	131548.1	
3(N=1)	243	
H	0.6075	
m	9	
sum T	85392	
C	0.833193	
Hc	0.729123	
v (k-1)	1	
Chi	3.841	
No Sig Diff		

	67	67
	67	67
	67	67
n	40	40
R	1584	1656
N	80	
12/N(N+1)	0.001852	
Sum Ri	131284.8	
3(N=1)	243	
H	0.12	
m	13	
sum T	110784	
C	0.78359	
Hc	0.15314	
v (k-1)	1	
Chi	3.841	
No Sig Diff		

10.13.3 *P. fragariae* strain 372

	372: 5000	
	Elsanta	**Rhapsody**
	1	14
	2	17.5
	3	22
	4	24
	5	53.5
	6	53.5
	7	53.5
	8.5	53.5
	8.5	53.5
	10	53.5
	11	53.5
	12	53.5
	13	53.5
	15	53.5
	17.5	53.5
	17.5	53.5
	17.5	53.5
	20	53.5
	22	53.5
	22	53.5
	25.5	53.5
	25.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5

	372: 100000	
	Elsanta	**Rhapsody**
	1	25
	2	28.5
	3	30.5
	4	32.5
	5	32.5
	6	34.5
	7	38
	8	39
	9	40
	10	41.5
	11	41.5
	12	43
	13	44
	14	62.5
	15	62.5
	16	62.5
	17	62.5
	18	62.5
	19	62.5
	20.5	62.5
	20.5	62.5
	23	62.5
	23	62.5
	23	62.5
	26	62.5
	27	62.5
	28.5	62.5
	30.5	62.5

	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
	53.5	53.5
n	40	40
R	1236.5	2003.5
N	80	
12/N(N+1)	0.001852	
Sum Ri	138573.6	
3(N=1)	243	
H	13.6178	
m	5	
sum T	166416	
C	0.674918	
Hc	20.17697	
v (k-1)	1	
Chi	3.841	
Sig Diff		

	34.5	62.5
	36.5	62.5
	36.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
	62.5	62.5
n	40	40
R	1082	2158
N	80	
12/N(N+1)	0.001852	
Sum Ri	145692.2	
3(N=1)	243	
H	26.80037	
m	13	
sum T	46686	
C	0.908802	
Hc	29.48977	
v (k-1)	1	
Chi	3.841	
	Sig Diff	

10.14 Kruskal–Wallis analysis of variance to determine any significant differences between the disease due to the different strains of *P. fragariae*

10.14.1 Elsanta

	Elsanta: 5000		
	171	**293**	**372**
	3	6	1
	13	7	2
	14.5	8	4
	14.5	9	5
	17	10	11
	23	12	16
	33	21	18
	34	24.5	19.5
	39	28	19.5
	45.5	29	22
	45.5	30	24.5
	49.5	31	26
	52.5	32	27
	54.5	35	39
	59	36.5	42.5
	59	36.5	42.5

	Elsanta: 100000		
	171	**293**	**372**
	6	18	1
	7	19	2
	12.5	25	3
	15	30	4
	20	31	5
	24	32	8
	28	43	9
	34.5	44	10
	34.5	47.5	11
	37	49	12.5
	38	53.5	14
	39	57	16
	45	59.5	17
	46	68	21
	47.5	72	22
	50	72	23

	59	39	42.5
	63	42.5	54.5
	94	47	59
	94	49.5	59
	94	49.5	65.5
	94	49.5	65.5
	94	52.5	94
	94	59	94
	94	59	94
	94	65.5	94
	94	65.5	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
	94	94	94
n	40	40	40
R	2746.5	2156	2358
N	120		
12/N(N+1)	0.000826		
Sum Ri	443735.1		
3(N=1)	363		
H	3.723233		
m	13		
sum T	141138		
C	0.918317		
Hc	4.054409		
v (k-1)	2		
Chi	5.991		
No sig diff			

	51.5	76	26
	53.5	78.5	27
	55	80	29
	57	83	34.5
	61.5	83	34.5
	61.5	83	41
	64	86	41
	65.5	88.5	41
	65.5	88.5	51.5
	68	91	57
	72	91	59.5
	76	106.5	63
	76	106.5	68
	78.5	106.5	72
	81	106.5	72
	86	106.5	106.5
	86	106.5	106.5
	91	106.5	106.5
	106.5	106.5	106.5
	106.5	106.5	106.5
	106.5	106.5	106.5
	106.5	106.5	106.5
	106.5	106.5	106.5
	106.5	106.5	106.5
n	40	40	40
R	2372.5	3033.5	1854
N	120		
12/N(N+1)	0.000826446		
Sum Ri	456704.8625		
3(N=1)	363		
H	14.44203512		
m	13		
sum T	20058		
C	0.988391555		
Hc	14.6116537		
v (k-1)	2		
Chi	5.991		
Sig Diff			

	SE
p=3	78.1025
p=2	52.28129

	372	**171**	**293**		
	1854	2372.5	3033.5		
	Rx-Ry	SE	p	q	Table q
293 v 372	1179.5	78.10249676	3	15.1	3.314
293 v 171	661	52.28129047	2	12.64	2.772
171 v 372	518.5	52.28129047	2	9.918	2.772

10.14.2 Rhapsody

	Rhapsody: 5000		
	171	**293**	**372**
	2.5	1	7
	2.5	4	10.5
	8.5	5	20.5
	13	6	24
	17	8.5	74.5
	20.5	10.5	74.5
	27	12	74.5
	74.5	14	74.5
	74.5	15	74.5
	74.5	17	74.5
	74.5	17	74.5
	74.5	20.5	74.5
	74.5	20.5	74.5
	74.5	24	74.5
	74.5	24	74.5
	74.5	27	74.5
	74.5	27	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
	74.5	74.5	74.5
n	40	40	40
R	2549.5	1967	2744
N	120		
12/N(N+1)	0.000826		
Sum Ri	447415.2		
3(N=1)	363		
H	6.764638		
m	7		

		sum T	150			
		C	0.999913			
		Hc	6.765226			
		v (k-1)	2			
		Chi	5.991			
	SE	Sig Diff				
p=3	78.1025					
p=2	52.28129	**293**	**171**	**372**		
		1966.5	2549.5	2744		
	Arrange rank sums in increasing order of magnitude					
		Rx-Ry	SE	p	q	Table q
	372 v 293	777.5	78.1025	3	9.95	3.314
	372 v 171	194.5	52.28129	2	3.72	2.772
	171 v 293	583	52.28129	2	11.2	2.772

	Rhapsody: 100000		
	171	293	372
	11	1	10
	23.5	2	14.5
	27.5	3.5	16
	27.5	3.5	19
	27.5	5.5	19
	30	5.5	21.5
	35	7.5	27.5
	35	7.5	35
	46	9	40.5
	46	12	43
	51.5	13	43
	51.5	14.5	46
	86.5	17	49
	86.5	19	86.5
	86.5	21.5	86.5
	86.5	23.5	86.5
	86.5	25	86.5
	86.5	31	86.5
	86.5	32	86.5
	86.5	35	86.5
	86.5	35	86.5
	86.5	38.5	86.5
	86.5	38.5	86.5
	86.5	40.5	86.5
	86.5	43	86.5
	86.5	49	86.5
	86.5	49	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5
	86.5	86.5	86.5

			86.5	86.5	86.5	
			86.5	86.5	86.5	
			86.5	86.5	86.5	
			86.5	86.5	86.5	
			86.5	86.5	86.5	
		n	40	40	40	
		R	2834	1707	2720	
		N	120			
		12/N(N+1)	0.000826			
		Sum Ri	458484.5			
		3(N=1)	363			
		H	15.91278			
		m	16			
		sum T	314694			
		C	0.817873			
		Hc	19.4563			
		v (k-1)	2			
		Chi	5.991			
	SE	Sig Diff				
p=3	78.1025					
p=2	52.28129	**293**	**372**	**171**		
		1706.5	2719.5	2834		
Arrange rank sums in increasing order of magnitude						
		Rx-Ry	SE	p	q	Table q
	171 v 293	1127.5	78.1025	3	14.4	3.314
	171 v 372	114.5	52.28129	2	2.19	2.772
	372 v 293	1013	52.28129	2	19.4	2.772

CHAPTER 11: Appendix II

11.1 Shoot dry weight (SDW), root dry weight (RDW), total phosphorus in the shoot (P) and concentration of phosphorus in the shoot [P] of strawberry plants of cultivars Elsanta and Rhapsody colonised by *Glomus mosseae* (V57), *Glomus intraradices* (V98) and *Glomus fistulosum* (V128)

	Elsanta	Rhaps	Elsanta	Rhaps		Elsanta	Rhaps	Elsanta	Rhapsody		Total P (mg)	Total P (mg)	Arcsin %MC	Arcsin %MC
AMF	S. Dry Wt.	S. Dry Wt.	R. Dry Wt.	R. Dry Wt.	AMF	% Myc.C	% Myc C.	P Conc. mg/g SDWt.	P Conc. mg/g SDWt.	AMF	**Elsanta**	**Rhaps**	Elsanta	Rhapsody
V57	0.546	0.362	0.072	0.072	V57	45.250	57.250	2.200	3.484	V57	1.201	1.262	42.274	42.274
V57	0.351	0.216	0.051	0.038	V57	44.750	69.250	2.372	4.703	V57	0.832	1.016	41.986	41.986
V57	0.517	0.244	0.060	0.057	V57	49.000	53.750	2.960	3.969	V57	1.531	0.966	44.427	44.427
V57	0.630	0.138	0.089	0.024	V57	44.500	74.500	2.133	4.831	V57	1.343	0.667	41.842	41.842
V57	0.323	0.533	0.074	0.117	V57	58.000	50.250	3.088	2.514	V57	0.997	1.339	49.603	49.603
V57	0.409	0.228	0.074	0.057	V57	56.250	49.000	2.833	4.518	V57	1.158	1.028	48.590	48.590
V98	0.592	0.257	0.112	0.048	V98	51.250	69.500	2.215	3.206	V98	1.312	0.824	45.716	45.716
V98	0.513	0.389	0.097	0.059	V98	57.500	54.250	1.974	3.493	V98	1.013	1.358	49.313	49.313
V98	0.361	0.139	0.066	0.025	V98	67.500	73.250	2.851	3.671	V98	1.028	0.509	55.244	55.244
V98	0.181	0.319	0.029	0.059	V98	58.250	65.500	3.747	3.140	V98	0.678	1.001	49.749	49.749
V98	0.501	0.196	0.142	0.040	V98	64.250	71.500	2.128	3.062	V98	1.066	0.601	53.279	53.279
V98	0.456	0.121	0.135	0.025	V98	54.000	79.250	2.623	4.593	V98	1.197	0.555	47.294	47.294
V128	0.441	0.332	0.083	0.068	V128	20.500	36.500	1.938	2.831	V128	0.855	0.940	26.922	26.922
V128	0.437	0.209	0.074	0.027	V128	5.750	57.250	2.107	3.700	V128	0.920	0.774	13.874	13.874
V128	0.324	0.306	0.073	0.071	V128	26.500	16.500	2.495	1.913	V128	0.809	0.586	30.983	30.983
V128	0.303	0.402	0.050	0.089	V128	10.750	36.000	2.514	2.656	V128	0.763	1.066	19.140	19.140
V128	0.542	0.200	0.158	0.041	V128	22.250	40.000	2.003	2.849	V128	1.085	0.571	28.145	28.145
V128	0.319	0.125	0.084	0.030	V128	25.500	61.000	2.172	3.400	V128	0.694	0.425	30.330	30.330
C	0.545	0.247	0.097	0.039	C	0.000	0.000	2.133	2.438	C	1.162	0.601	0.000	0.000
C	0.339	0.156	0.067	0.022	C	0.000	0.000	1.674	2.152	C	0.567	0.336	0.000	0.000

C	0.273	0.145	0.055	0.025	C	0.000	0.000	1.610	2.301	C	0.440	0.332	0.000	0.000
C	0.318	0.263	0.049	0.039	C	0.000	0.000	1.951	2.706	C	0.621	0.713	0.000	0.000
C	0.402	0.251	0.082	0.039	C	0.000	0.000	1.935	2.653	C	0.778	0.667	0.000	0.000
C	0.292	0.140	0.066	0.022	C	0.000	0.000	1.637	2.246	C	0.478	0.313	0.000	0.000

11.2 Two-factor analysis of variance to test for significant differences in the shoot dry weight (SDW), root dry weight (RDW), total amount of phosphorus (P) in shoots or concentration of P in shoots between mycorrhizal and non-mycorrhizal plants of Elsanta and Rhapsody colonised by *Glomus mosseae* (V57), *Glomus intraradices* (V98) and *Glomus fistulosum* (V128)

SDW						
Anova: Two-Factor With Replication						
Summary						
	Elsanta	*Rhaps*	*Total*			
V57						
Count	6	6	12			
Sum	2.7754	1.72	4.4954			
Average	0.462567	0.286667	0.749233			
Variance	0.014535	0.01973	0.034265			
V98						
Count	6	6	12			
Sum	2.6043	1.4208	4.0251			
Average	0.43405	0.2368	0.67085			
Variance	0.021143	0.010992	0.032135			
V128						
Count	6	6	12			
Sum	2.3669	1.5742	3.9411			
Average	0.394483	0.262367	0.65685			

RDW						
Anova: Two-Factor With Replication						
Summary						
	Elsanta	*Rhaps*	*Total*			
V57						
Count	6	6	12			
Sum	0.42	0.3643	0.7843			
Average	0.07	0.060717	0.130717			
Variance	0.000171	0.001056	0.001226			
V98						
Count	6	6	12			
Sum	0.5811	0.2567	0.8378			
Average	0.09685	0.042783	0.139633			
Variance	0.001871	0.000237	0.002108			
V128						
Count	6	6	12			
Sum	0.5211	0.3254	0.8465			
Average	0.08685	0.054233	0.141083			

Variance	0.008916	0.010338	0.019253			
C						
Count	6	6	12			
Sum	2.1695	1.2017	3.3712			
Average	0.361583	0.200283	0.561867			
Variance	0.010056	0.003496	0.013552			
Total						
Count	24	24				
Sum	9.9161	5.9167				
Average	1.652683	0.986117				
Variance	0.054649	0.044556				
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.05316	3	0.01772	1.42896	0.248556	2.838746
Column	0.333233	1	0.333233	26.87226	6.58E-06	4.08474
Interaction	0.006729	3	0.002243	0.180885	0.908749	2.838746
Within	0.496026	40	0.012401			
Total	0.889148	47				

Variance	0.001368	0.000651	0.002018			
C						
Count	6	6	12			
Sum	0.4172	0.1862	0.6034			
Average	0.069533	0.031033	0.100567			
Variance	0.000309	8.08E-05	0.00039			
Total						
Count	24	24				
Sum	1.9394	1.1326				
Average	0.323233	0.188767				
Variance	0.003718	0.002024				
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	0.003199	3	0.001066	1.485869	0.232959	2.838746
Column	0.013561	1	0.013561	18.89383	9.23E-05	4.08474
Interaction	0.003105	3	0.001035	1.442239	0.244828	2.838746
Within	0.02871	40	0.000718			
Total	0.048576	47				

P Concentration						
Anova: Two-Factor With Replication						
Summary						
	Elsanta	*Rhaps*	*Total*			
V57						

Total P						
Anova: Two-Factor With Replication						
Summary						
	Elsanta	*Rhaps*	*Total*			
V57						

Count	6	6	12			
Sum	15.58488	24.01958	39.60446			
Average	2.59748	4.003263	6.600743			
Variance	0.17042	0.785791	0.956211			
V98						
Count	6	6	12			
Sum	15.53802	21.16526	36.70328			
Average	2.58967	3.527543	6.117213			
Variance	0.428475	0.325572	0.754047			
V128						
Count	6	6	12			
Sum	13.22872	17.34882	30.57754			
Average	2.204786	2.89147	5.096256			
Variance	0.060501	0.385926	0.446427			
C						
Count	6	6	12			
Sum	10.93968	14.49598	25.43566			
Average	1.82328	2.415997	4.239277			
Variance	0.045448	0.050591	0.096039			
Total						
Count	24	24				
Sum	55.2913	77.02964				
Average	9.215216	12.83827				
Variance	0.704844	1.547881				
ANOVA						

Count	6	6	12			
Sum	7.062379	6.278641	13.34101961			
Average	1.177063	1.04644	2.223503268			
Variance	0.061055	0.056804	0.117859394			
V98						
Count	6	6	12			
Sum	6.294008	4.84947	11.14347832			
Average	1.049001	0.808245	1.857246386			
Variance	0.046132	0.107218	0.153349952			
V128						
Count	6	6	12			
Sum	5.126084	4.361555	9.487638638			
Average	0.854347	0.726926	1.581273106			
Variance	0.018812	0.05964	0.078452062			
C						
Count	6	6	12			
Sum	4.046523	2.963119	7.009641904			
Average	0.67442	0.493853	1.168273651			
Variance	0.07132	0.034573	0.105893089			
Total						
Count	24	24				
Sum	22.52899	18.45278				
Average	3.754832	3.075464				
Variance	0.197319	0.258235				
ANOVA						

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	10.0329	3	3.344302	11.87647	1.06E-05	2.838746
Column	9.844907	1	9.844907	34.96178	6.27E-07	4.08474
Interaction	1.191135	3	0.397045	1.410008	0.253972	2.838746
Within	11.26362	40	0.281591			
Total	32.33257	47				

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.786145	3	0.595381615	10.45551	3.28414E-05	2.838746
Column	0.346156	1	0.346155758	6.078847	0.018068191	4.08474
Interaction	0.025444	3	0.008481483	0.148943	0.929763639	2.838746
Within	2.277772	40	0.056944312			
Total	4.435518	47				

11.3 Summary of linear regression analysis of growth characteristics of Elsanta and Rhapsody (RDW, Total P and P concentration) varying with shoot dry weight of the plants. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as plant size (SDW) increased were significant. Plants were colonised by *Glomus mosseae* (V57), *Glomus intraradices* (V98) and *Glomus fistulosum* (V128)

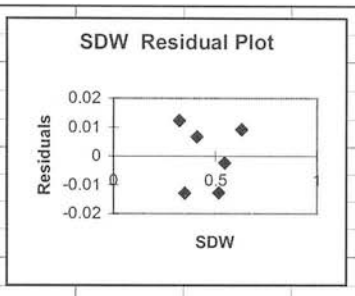
11.3.1 Linear regression analysis of root dry weight versus shoot dry weight in Elsanta

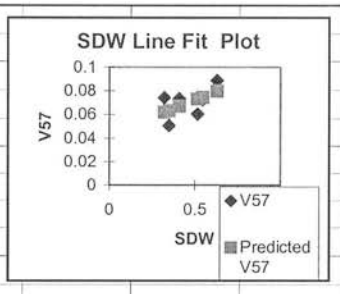
Elsanta: RDW versus SDW

G. mosseae

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.5326872
R Square	0.2837556
Adjusted R S	0.1046945
Standard Er	0.0123641
Observation	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.0002423	0.0002423	1.5846861	0.2765457
Residual	4	0.0006115	0.0001529		
Total	5	0.0008537			

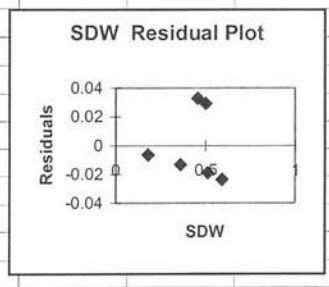
	Coefficients	*Standard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.0432935	0.0218073	1.9852709	0.1180874	-0.0172535	0.1038405	-0.0172535	0.1038405
SDW	0.0577355	0.0458639	1.2588432	0.2765457	-0.0696035	0.1850745	-0.0696035	0.1850745

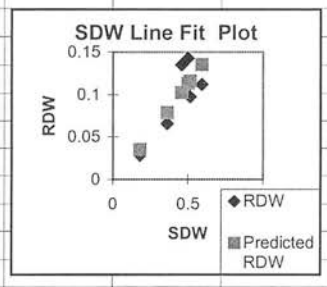
Elsanta: RDW versus SDW

G. intraradices

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.820419
R Square	0.673088
Adjusted R	0.59136
Standard E	0.027649
Observatio	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regressior	1	0.006296	0.006296	8.235695	0.045478
Residual	4	0.003058	0.000764		
Total	5	0.009353			

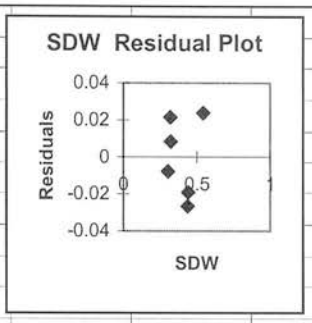
	Coefficients	*Standard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	-0.009074	0.038597	-0.235089	0.825684	-0.116237	0.09809	-0.116237	0.09809
SDW	0.244036	0.085036	2.86979	0.045478	0.007937	0.480135	0.007937	0.480135

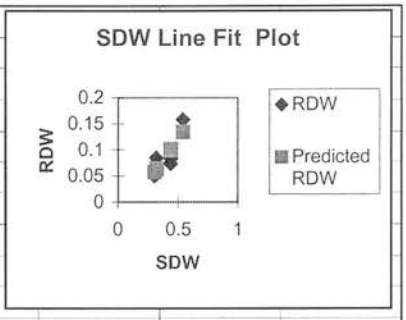
Elsanta: RDW versus SDW

G. fistulosum

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.819514
R Square	0.671604
Adjusted R	0.589505
Standard E	0.023693
Observatio	6





ANOVA

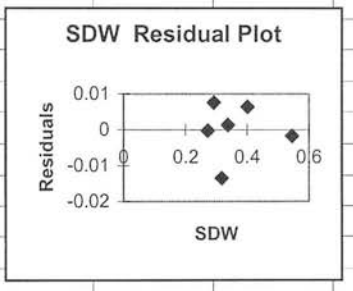
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.004592	0.004592	8.180409	0.045923
Residual	4	0.002245	0.000561		
Total	5	0.006838			

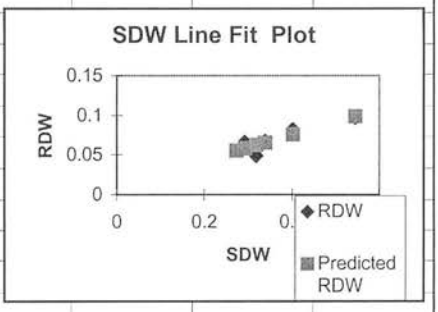
	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	-0.039762	0.045312	-0.877507	0.429757	-0.165568	0.086045	-0.165568	0.086045
SDW	0.320956	0.112217	2.860141	0.045923	0.009391	0.63252	0.009391	0.63252

Elsanta: Control: RDW&SDW

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.903232
R Square	0.815827
Adjusted R	0.769784
Standard E	0.008432
Observatio	6



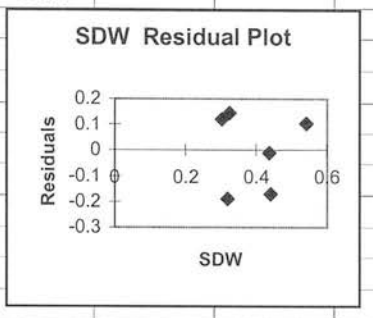


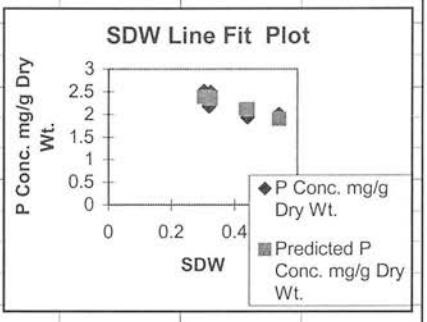
ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.00126	0.00126	17.71875	0.013593
Residual	4	0.000284	7.11E-05		
Total	5	0.001544			

	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.0123	0.014026	0.876997	0.430003	-0.026641	0.051242	-0.026641	0.051242
SDW	0.158284	0.037603	4.209364	0.013593	0.053882	0.262687	0.053882	0.262687

Elsanta: V128: SDW & P Conc





SUMMARY OUTPUT

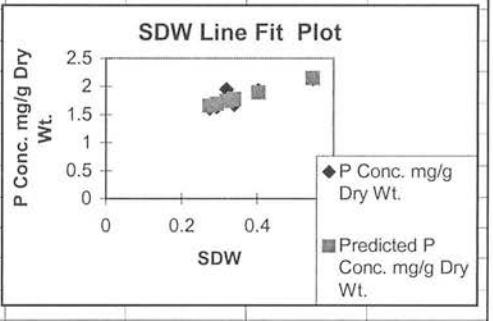
Regression Statistics	
Multiple R	0.795901
R Square	0.633458
Adjusted R	0.541823
Standard E	0.166494
Observatio	6

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.191625	0.191625	6.91281	0.058234
Residual	4	0.110881	0.02772		
Total	5	0.302506			

	Coefficients	*tandard Erı*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	3.022672	0.318415	9.492881	0.000687	2.13861	3.906735	2.13861	3.906735
SDW	-2.07331	0.788564	-2.629222	0.058234	-4.262718	0.116099	-4.262718	0.116099

Elsanta: Control: SDW & P Conc



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.850558
R Square	0.723448
Adjusted R	0.65431
Standard E	0.125343
Observatio	6

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.164396	0.164396	10.46384	0.031831
Residual	4	0.062844	0.015711		
Total	5	0.22724			

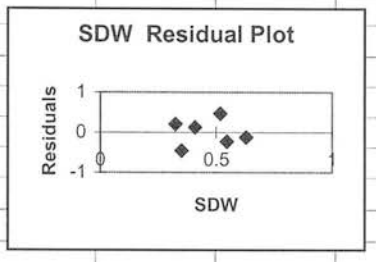
	Coefficients	*tandard Erı*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	1.169455	0.2085	5.608888	0.004964	0.590564	1.748345	0.590564	1.748345
SDW	1.80823	0.558995	3.234785	0.031831	0.256207	3.360253	0.256207	3.360253

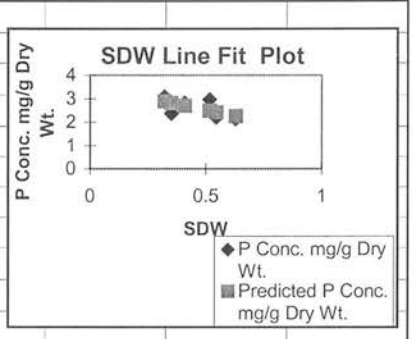
11.3.2 Linear regression analysis of P concentration in the shoot versus shoot dry weight in Elsanta

Elsanta: V57: SDW & P Conc

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.589377
R Square	0.347366
Adjusted R	0.184207
Standard E	0.372864
Observatio	6





ANOVA

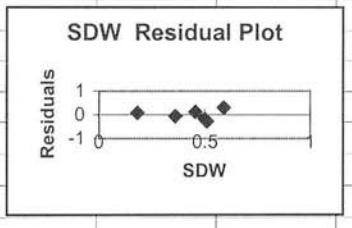
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.29599	0.29599	2.129006	0.218299
Residual	4	0.556109	0.139027		
Total	5	0.852099			

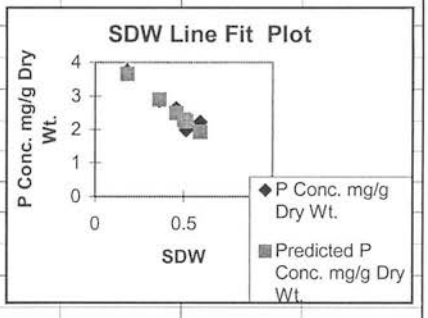
	Coefficients	*tandard Erı*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	3.530994	0.657642	5.369175	0.005811	1.705084	5.356904	1.705084	5.356904
SDW	-2.018117	1.383114	-1.459112	0.218299	-5.858265	1.82203	-5.858265	1.82203

Elsanta: V98: SDW & P Conc

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.94614
R Square	0.895182
Adjusted R	0.868977
Standard E	0.236939
Observatio	6





ANOVA

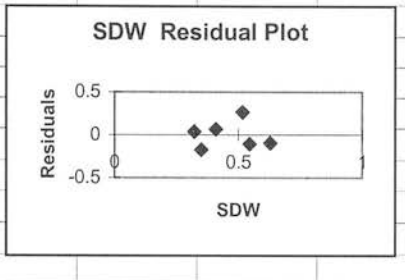
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	1.917813	1.917813	34.16128	0.004273
Residual	4	0.22456	0.05614		
Total	5	2.142373			

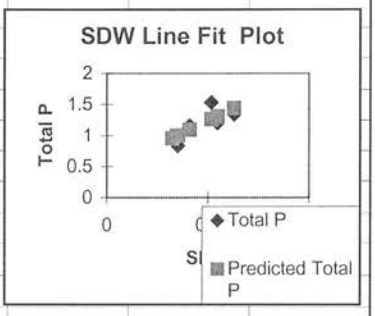
	Coefficients	*tandard Erı*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	4.438402	0.330766	13.41857	0.000178	3.520047	5.356757	3.520047	5.356757
SDW	-4.259261	0.728731	-5.844765	0.004273	-6.282547	-2.235975	-6.282547	-2.235975

11.3.3 Linear regression analysis of total P in the shoot versus shoot dry weight in Elsanta

Elsanta: V57: SDW & Total P

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.763597
R Square	0.583081
Adjusted R	0.478851
Standard E	0.178378
Observatio	6

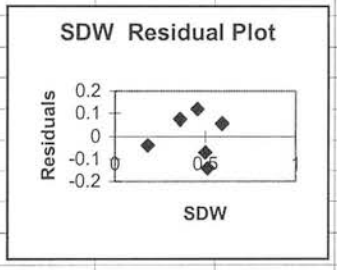
ANOVA

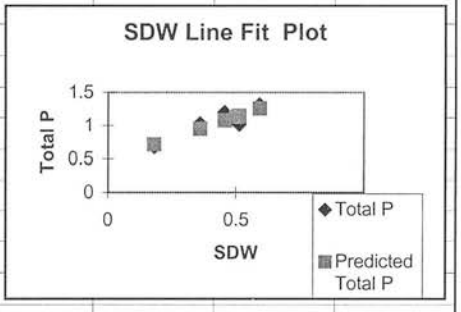
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.178	0.178	5.594192	0.077223
Residual	4	0.127275	0.031819		
Total	5	0.305276			

	Coefficients	*andard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.45314	0.314616	1.440293	0.22321	-0.420377	1.326656	-0.420377	1.326656
SDW	1.565015	0.661683	2.365204	0.077223	-0.272115	3.402144	-0.272115	3.402144

Elsanta: V98: SDW & Total P

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.887578
R Square	0.787795
Adjusted R	0.734743
Standard E	0.11062
Observatio	6

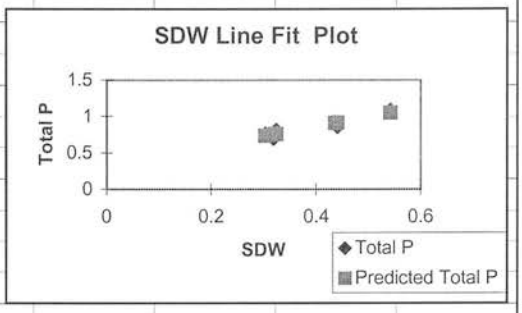
ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.181712	0.181712	14.84967	0.018248
Residual	4	0.048947	0.012237		
Total	5	0.23066			

	Coefficients	*andard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.479935	0.154425	3.107877	0.035946	0.051181	0.908689	0.051181	0.908689
SDW	1.311062	0.340224	3.853527	0.018248	0.366447	2.255678	0.366447	2.255678

Elsanta: V128: SDW & Total P

SUMMARY OUTPUT



Regression Statistics	
Multiple R	0.934282
R Square	0.872882
Adjusted R	0.841103
Standard E	0.054674
Observatio	6

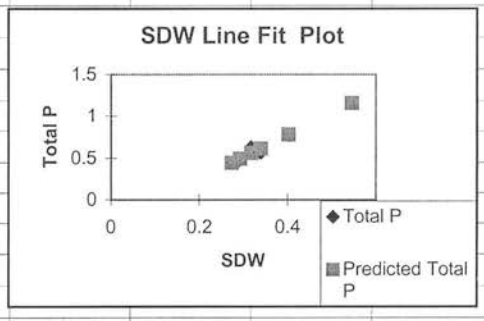
ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.082105	0.082105	27.46692	0.006336
Residual	4	0.011957	0.002989		
Total	5	0.094062			

	Coefficients	*tandard Erı*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.318979	0.104562	3.050618	0.038005	0.028668	0.609291	0.028668	0.609291
SDW	1.357137	0.258952	5.240889	0.006336	0.63817	2.076103	0.63817	2.076103

Elsanta: Control: SDW & Total P

SUMMARY OUTPUT



Regression Statistics	
Multiple R	0.991385
R Square	0.982844
Adjusted R	0.978555
Standard E	0.039108
Observatio	6

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.35048	0.35048	229.1598	0.000111
Residual	4	0.006118	0.001529		
Total	5	0.356598			

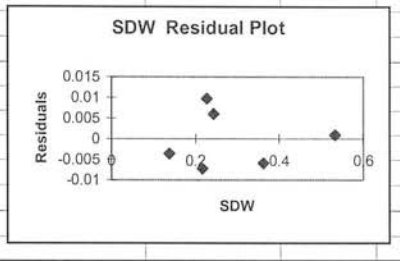
	Coefficients	*tandard Erı*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	-0.280238	0.065053	-4.307823	0.012568	-0.460855	-0.099621	-0.460855	-0.099621
SDW	2.640216	0.17441	15.13802	0.000111	2.155976	3.124456	2.155976	3.124456

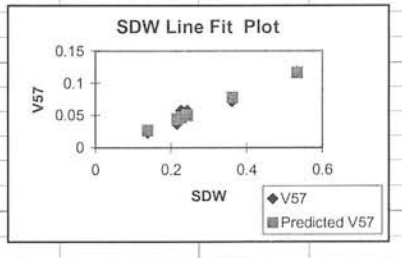
11.3.4 Linear regression analysis of root dry weight versus shoot dry weight in Rhapsody

Rhapsody: RDWversus SDW

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.97763034
R Square	0.95576108
Adjusted R	0.94470136
Standard E	0.00764004
Observatio	6

ANOVA

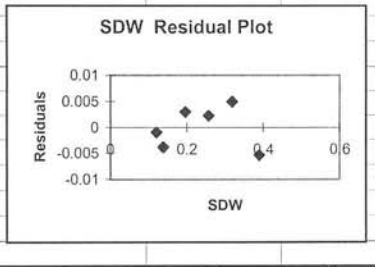
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.005044247	0.005044247	86.41813	0.000745
Residual	4	0.000233481	5.83702E-05		
Total	5	0.005277728			

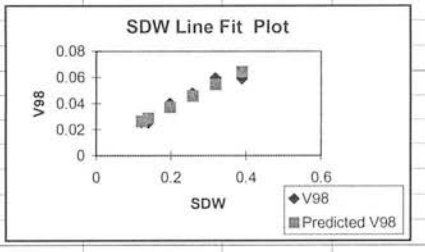
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	-0.0041063	0.007638888	-0.537550727	0.619399	-0.025315	0.017102708	-0.0253153	0.017102708
SDW	0.22612659	0.024324796	9.296135222	0.000745	0.15859	0.293663193	0.15859	0.293663193

Rhapsody: RDW versus SDW

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.96505039
R Square	0.93132225
Adjusted R	0.91415282
Standard E	0.00450985
Observatio	6

ANOVA

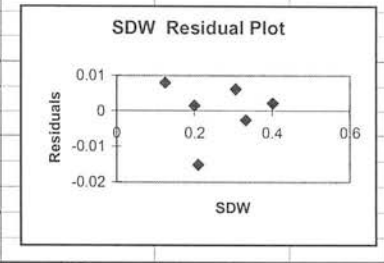
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.001103233	0.001103233	54.24303	0.001811
Residual	4	8.13549E-05	2.03387E-05		
Total	5	0.001184588			

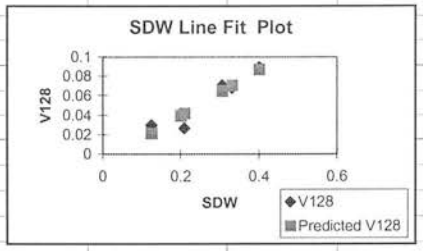
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	0.00923309	0.004913368	1.879177097	0.133408	-0.004409	0.022874814	-0.0044086	0.022874814
SDW	0.14168177	0.019237207	7.36498673	0.001811	0.088271	0.195092935	0.0882706	0.195092935

Rhapsody: V128: RDW & SDW

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.94558519
R Square	0.89413136
Adjusted R	0.86766419
Standard E	0.00928101
Observatio	6

ANOVA

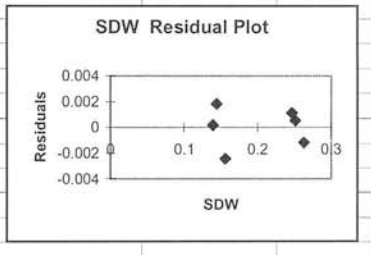
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.002909945	0.002909945	33.78267	0.004361
Residual	4	0.000344549	8.61372E-05		
Total	5	0.003254493			

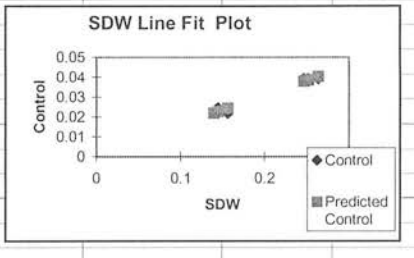
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	-0.0080188	0.011360877	-0.70582164	0.519237	-0.039562	0.023524163	-0.0395617	0.023524163
SDW	0.23727132	0.040822375	5.812286084	0.004361	0.12393	0.350612641	0.12393	0.350612641

Rhapsody: RDW & SDW

CONTROL

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.98472265
R Square	0.9696787
Adjusted R	0.96209838
Standard E	0.0017504
Observatio	6

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.000391938	0.000391938	127.9205	0.000348
Residual	4	1.22557E-05	3.06392E-06		
Total	5	0.000404193			

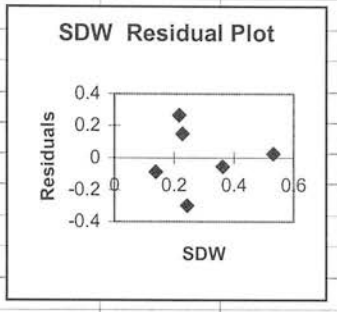
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	0.0010445	0.002746095	0.380356546	0.723016	-0.00658	0.008668894	-0.0065799	0.008668894
SDW	0.14973207	0.013238683	11.31019366	0.000348	0.112976	0.186488623	0.1129755	0.186488623

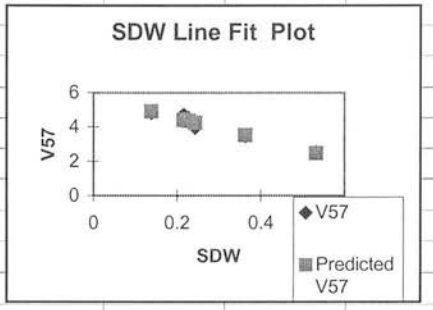
11.3.5 Linear regression analysis of P concentration versus shoot dry weight in Rhapsody

Rhapsody: V57: P Conc & SDW

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.974952
R Square	0.950532
Adjusted R	0.938165
Standard E	0.220429
Observatio	6

ANOVA

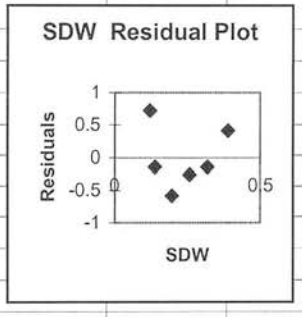
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	3.7346	3.7346	76.86089	0.000933
Residual	4	0.194356	0.048589		
Total	5	3.928956			

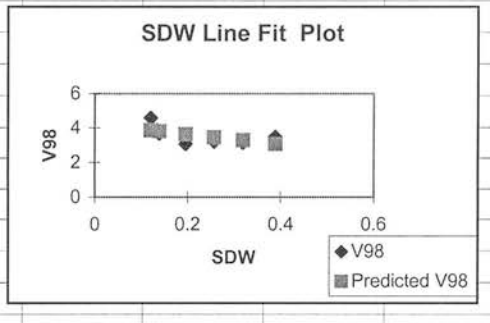
	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	5.767077	0.220396	26.16688	1.27E-05	5.155158	6.378995	5.155158	6.378995
SDW	-6.152838	0.701815	-8.767034	0.000933	-8.101393	-4.204282	-8.101393	-4.204282

Rhapsody: V98: P Conc & SDW

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.543158
R Square	0.29502
Adjusted R	0.118775
Standard E	0.535632
Observatio	6

ANOVA

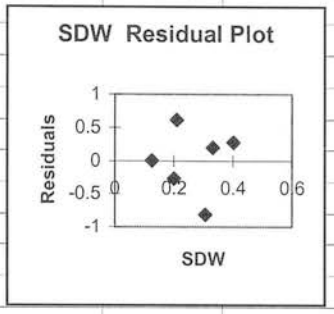
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.480252	0.480252	1.673923	0.265385
Residual	4	1.147608	0.286902		
Total	5	1.62786			

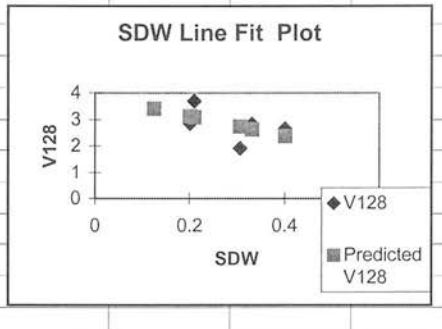
	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	4.227541	0.583559	7.244416	0.001927	2.607319	5.847762	2.607319	5.847762
SDW	-2.956071	2.284794	-1.293802	0.265385	-9.29969	3.387549	-9.29969	3.387549

Rhapsody: V128: P Conc & SDW

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.602241
R Square	0.362695
Adjusted R	0.203368
Standard E	0.554474
Observatio	6

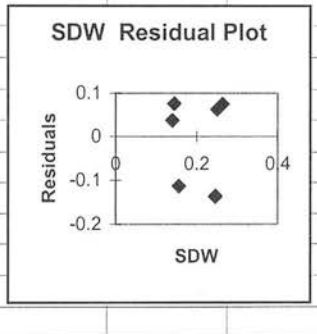
ANOVA

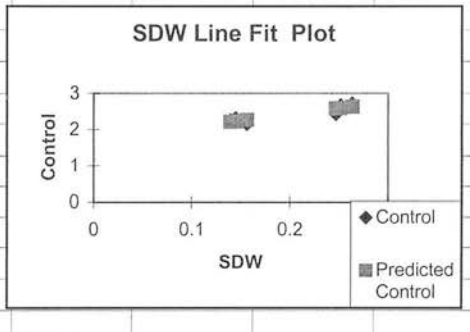
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.699867	0.699867	2.276426	0.205853
Residual	4	1.229764	0.307441		
Total	5	1.929631			

	Coefficients	*andard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	3.856897	0.678731	5.682515	0.004734	1.972435	5.741359	1.972435	5.741359
SDW	-3.679685	2.438843	-1.508783	0.205853	-10.45101	3.091643	-10.45101	3.091643

Rhapsody: Control: P Conc & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.899495
R Square	0.809091
Adjusted R	0.761363
Standard E	0.109877
Observatio	6

ANOVA

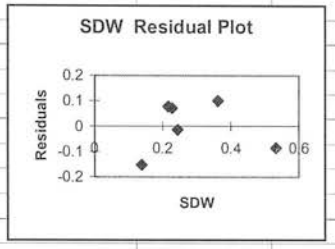
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.204665	0.204665	16.95236	0.014644
Residual	4	0.048292	0.012073		
Total	5	0.252957			

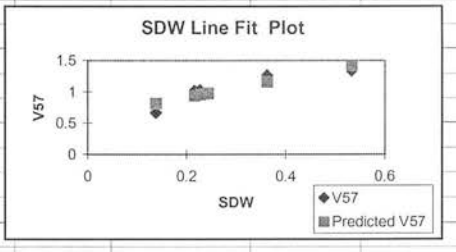
	Coefficients	*andard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	1.73071	0.172379	10.04016	0.000553	1.252108	2.209311	1.252108	2.209311
SDW	3.42159	0.831023	4.117324	0.014644	1.114296	5.728884	1.114296	5.728884

11.3.6 Linear regression analysis of total P in the shoot versus shoot dry weight in Rhapsody

Rhapsody: V57: Total P & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.90456407
R Square	0.81823616
Adjusted R	0.7727952
Standard E	0.11360548
Observatio	6

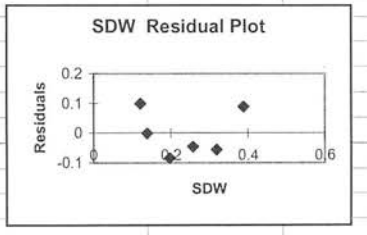
ANOVA

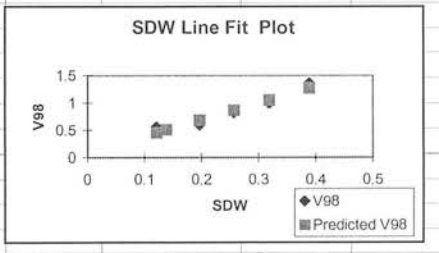
	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.232396572	0.232396572	18.00658	0.013227
Residual	4	0.051624818	0.012906204		
Total	5	0.28402139			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	0.60644736	0.113588317	5.338994135	0.005929	0.291075	0.921819735	0.291075	0.921819735
SDW	1.53485838	0.361703507	4.243415809	0.013227	0.530606	2.539110394	0.5306064	2.539110394

Rhapsody: V98: Total P & SDW

SUMMARY OUTPUT





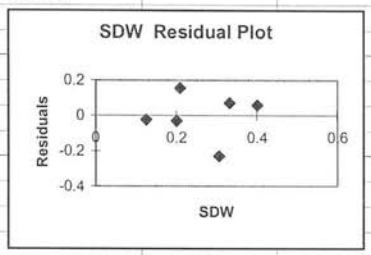
Regression Statistics	
Multiple R	0.97170665
R Square	0.94421381
Adjusted R	0.93026726
Standard E	0.08646738
Observatio	6

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.50618373	0.50618373	67.70233	0.001189
Residual	4	0.029906428	0.007476607		
Total	5	0.536090158			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	0.08959732	0.094204108	0.951097808	0.395412	-0.171956	0.351150398	-0.1719558	0.351150398
SDW	3.03482997	0.36883536	8.228142677	0.001189	2.010777	4.058883218	2.0107767	4.058883218

Rhapsody: V128: Total P & SDW



SUMMARY OUTPUT

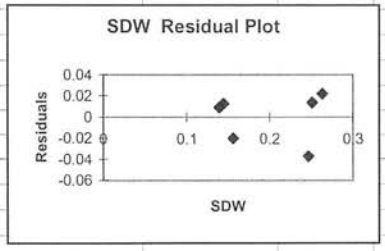
Regression Statistics	
Multiple R	0.8420255
R Square	0.70900694
Adjusted R	0.63625867
Standard E	0.14728681
Observatio	6

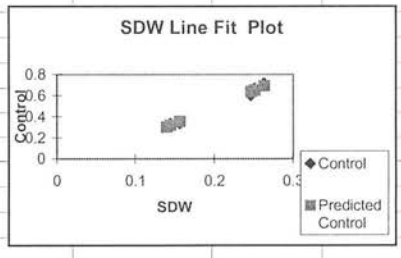
ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.211424613	0.211424613	9.746032	0.035463
Residual	4	0.086773615	0.021693404		
Total	5	0.298198229			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	0.19629881	0.180293606	1.088772983	0.337458	-0.304278	0.696875146	-0.3042775	0.696875146
SDW	2.02246338	0.647838488	3.121863585	0.035463	0.223772	3.821155109	0.2237717	3.821155109

Rhapsody: Control: Total P & SDW





SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.99211004
R Square	0.98428233
Adjusted R	0.98035291
Standard E	0.02606277
Observatio	6

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	0.170150324	0.170150324	250.4906	9.31E-05
Residual	4	0.002717073	0.000679268		
Total	5	0.172867397			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.000%*	*Upper 95.000%*
Intercept	-0.1309849	0.040888181	-3.203490803	0.032792	-0.244509	-0.017460887	-0.2445089	-0.017460887
SDW	3.119771	0.197118316	15.82689559	9.31E-05	2.572482	3.667060316	2.5724817	3.667060316

11.4 Summary of linear regression analysis of growth characteristics of Elsanta and Rhapsody (RDW, Total P, P concentration) varying with percentage of mycorrhizal colonisation (%MC) of the plant. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as %MC increased were significant

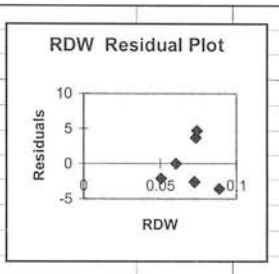
11.4.1 Linear regression of root dry weight and %MC in Elsanta

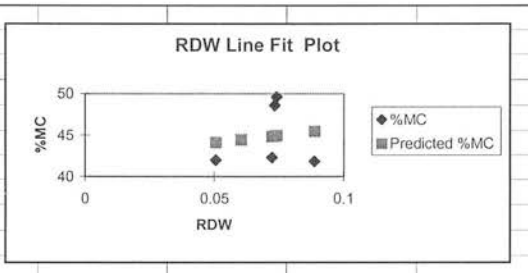
Elsanta: RDW versus %MC

G. mosseae

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.13036751
R Square	0.01699569
Adjusted R Sc	-0.2287554
Standard Erro	3.85975678
Observations	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.030298747	1.030299	0.069158	0.805556581
Residual	4	59.59088952	14.89772		
Total	5	60.62118826			

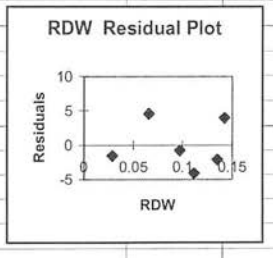
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	42.3555833	9.380177002	4.515435	0.010696	16.31198287	68.3991838	16.31198287	68.3991838
RDW	34.7391167	132.098268	0.262979	0.805557	-332.0252327	401.503466	-332.0252327	401.503466

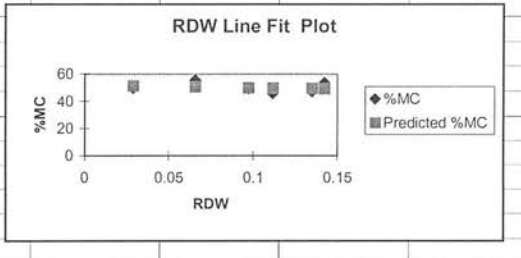
Elsanta: RDW versus %MC

G. intraradices

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.2109859
R Square	0.04451505
Adjusted R Sc	-0.1943562
Standard Erro	3.92207011
Observations	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.866643783	2.866644	0.186356	0.688217168
Residual	4	61.53053588	15.38263		
Total	5	64.39717966			

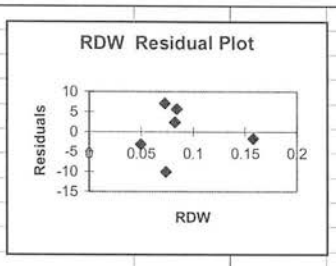
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	51.794793	4.241446524	12.21159	0.000258	40.01862513	63.5709608	40.01862513	63.57096079
RDW	-17.506523	40.55350482	-0.43169	0.688217	-130.1013364	95.0882899	-130.1013364	95.08828987

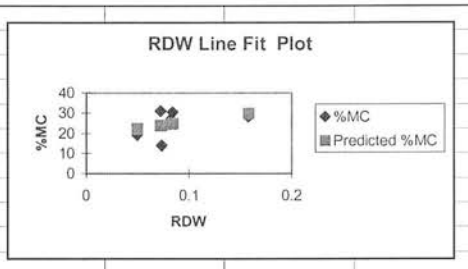
Elsanta: RDW versus %MC

G. fistulosum

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.37816822
R Square	0.1430112
Adjusted R Sc	-0.071236
Standard Erro	7.10839772
Observations	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	33.72860195	33.7286	0.667506	0.459788817
Residual	4	202.1172724	50.52932		
Total	5	235.8458743			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	18.7989158	8.010223341	2.346865	0.078785	-3.441075628	41.0389073	-3.441075628	41.03890728
RDW	70.2343211	85.96505344	0.81701	0.459789	-168.4434251	308.912067	-168.4434251	308.9120673

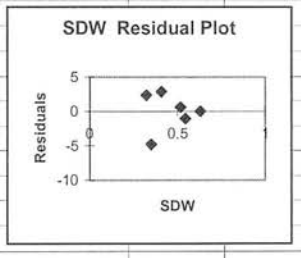
11.4.2 Linear regression of shoot dry weight and %MC in Elsanta

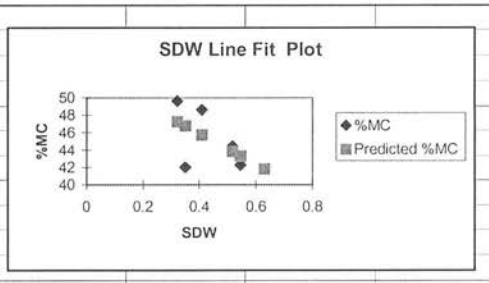
Elsanta: SDW versus %MC

G. mosseae

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.61176617
R Square	0.37425785
Adjusted R Sc	0.21782231
Standard Erro	3.07949805
Observations	6





ANOVA

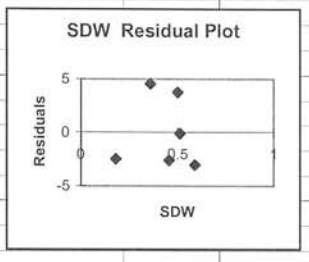
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	22.68795537	22.68796	2.392409	0.19682989
Residual	4	37.9332329	9.483308		
Total	5	60.62118826			

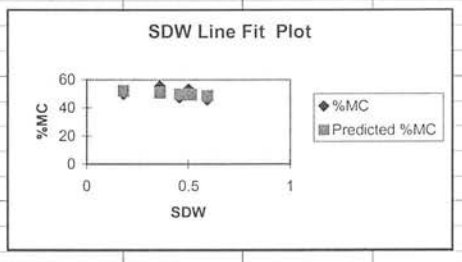
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	52.9602909	5.431493295	9.750595	0.00062	37.88001667	68.0405651	37.88001667	68.04056508
SDW	-17.668738	11.42320028	-1.546742	0.19683	-49.38469248	14.0472159	-49.38469248	14.04721592

Elsanta: SDW versus %MC

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.34292442
R Square	0.11759716
Adjusted R Sc	-0.1030036
Standard Erro	3.7690932
Observations	6

ANOVA

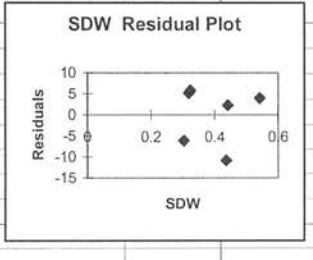
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	7.572925422	7.572925	0.533077	0.505776835
Residual	4	56.82425424	14.20606		
Total	5	64.39717966			

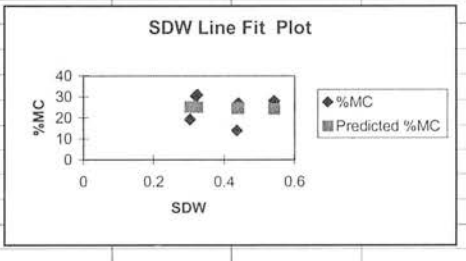
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	53.7729777	5.261640828	10.21981	0.000517	39.16429054	68.3816649	39.16429054	68.3816649
SDW	-8.463752	11.59225601	-0.730121	0.505777	-40.64908107	23.7215772	-40.64908107	23.72157716

Elsanta: SDW versus %MC

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.06302434
R Square	0.00397207
Adjusted R Sc	-0.2450349
Standard Erro	7.66337195
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.936795577	0.936796	0.015952	0.905588665
Residual	4	234.9090788	58.72727		
Total	5	235.8458743			

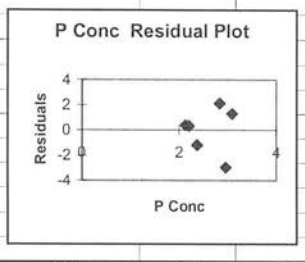
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	26.7071461	14.65596963	1.822271	0.142501	-13.98443332	67.3987255	-13.98443332	67.39872555
SDW	-4.5841721	36.29596986	-0.1263	0.905589	-105.3581487	96.1898044	-105.3581487	96.18980445

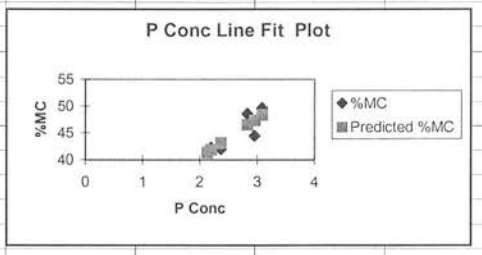
11.4.3 Linear regression of P concentration in the shoot and %MC in Elsanta

Elsanta: P Concentration versus %MC

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.85231447
R Square	0.72643996
Adjusted R Sc	0.65804995
Standard Erro	2.03614432
Observations	6

ANOVA

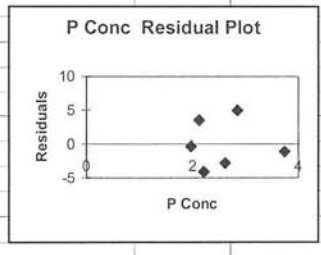
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	44.03765357	44.03765	10.62202	0.031105937
Residual	4	16.5835347	4.145884		
Total	5	60.62118826			

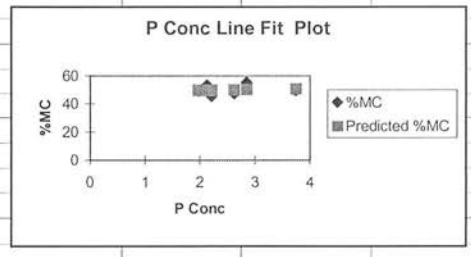
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	26.1141043	5.789471812	4.510619	0.010735	10.03992036	42.1882883	10.03992036	42.1882883
P Conc	7.18897489	2.205786313	3.259144	0.031106	1.064717593	13.3132322	1.064717593	13.31323219

Elsanta: P Concentration versus %MC

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.12801393
R Square	0.01638757
Adjusted R Sc	-0.2295155
Standard Erro	3.97938018
Observations	6

ANOVA

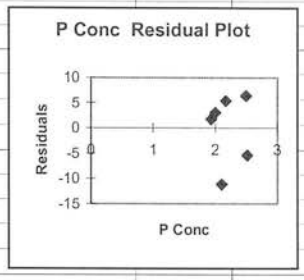
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.055313129	1.055313	0.066642	0.809028016
Residual	4	63.34186653	15.83547		
Total	5	64.39717966			

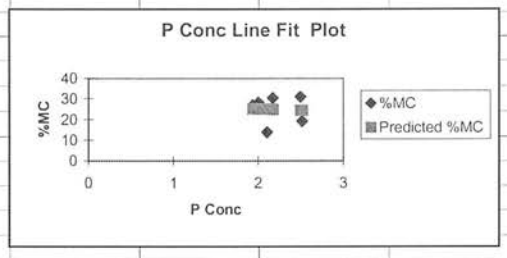
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	48.2817314	7.225640741	6.682	0.002608	28.22009502	68.3433679	28.22009502	68.34336786
P Conc	0.70184807	2.718741231	0.258152	0.809028	-6.846603345	8.25029949	-6.846603345	8.250299489

Elsanta: P Concentration versus %MC

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.03681546
R Square	0.00135538
Adjusted R Sc	-0.2483058
Standard Erro	7.67343166
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.319660278	0.31966	0.005429	0.944801764
Residual	4	235.5262141	58.88155		
Total	5	235.8458743			

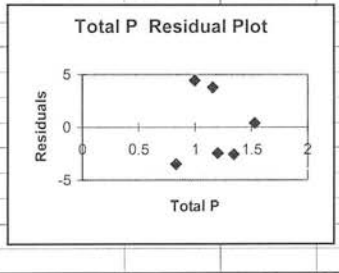
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	27.1652063	30.91932764	0.878583	0.429237	-58.68078739	113.0112	-58.68078739	113.0112
P Conc	-1.0279635	13.95156665	-0.073681	0.944802	-39.76380265	37.7078757	-39.76380265	37.70787569

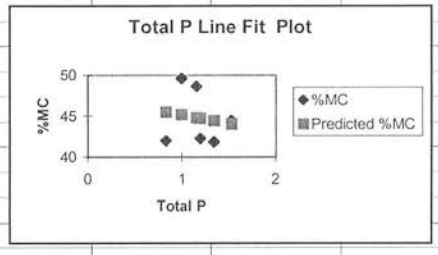
11.4.4 Linear regression of total P in the shoot and %MC in Elsanta

Elsanta: Total P versus %MC

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.15017146
R Square	0.02255147
Adjusted R Sc	-0.2218107
Standard Erro	3.84883396
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.367096777	1.367097	0.092287	0.776436101
Residual	4	59.25409149	14.81352		
Total	5	60.62118826			

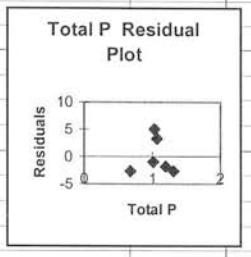
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	47.2782048	8.348613197	5.663001	0.004794	24.0986905	70.457719	24.0986905	70.45771901
Total P	-2.1161848	6.965994786	-0.303788	0.776436	-21.45692703	17.2245573	-21.45692703	17.22455735

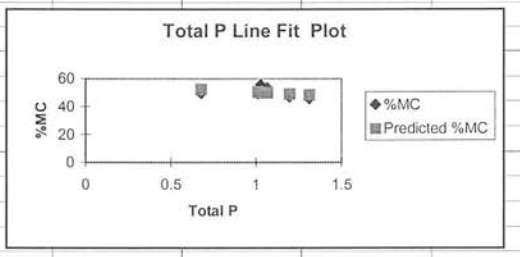
Elsanta: Total P versus %MC

G. intraradices

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.37970081
R Square	0.14417271
Adjusted R Sc	-0.0697841
Standard Erro	3.71190194
Observations	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	9.284315724	9.284316	0.67384	0.457820028
Residual	4	55.11286394	13.77822		
Total	5	64.39717966			

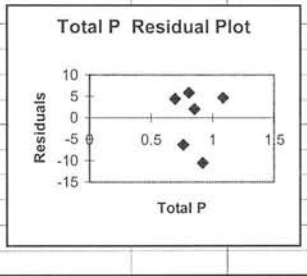
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	56.7545491	8.247900763	6.88109	0.002337	33.85465801	79.6544403	33.85465801	79.65444028
Total P	-6.3443799	7.728776088	-0.820878	0.45782	-27.8029469	15.1141871	-27.8029469	15.11418707

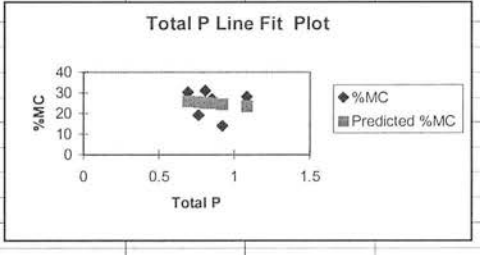
Elsanta: Total P versus %MC

G. fistulosum

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.12688261
R Square	0.0160992
Adjusted R Sc	-0.229876
Standard Erro	7.61657642
Observations	6





ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.796928913	3.796929	0.06545	0.810697444
Residual	4	232.0489454	58.01224		
Total	5	235.8458743			

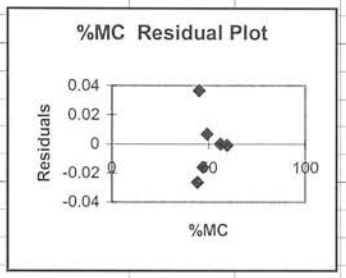
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	30.3268111	21.44378705	1.414247	0.230191	-29.21080979	89.864432	-29.21080979	89.86443204
Total P	-6.3534401	24.83433348	-0.255833	0.810697	-75.30474652	62.5978664	-75.30474652	62.59786637

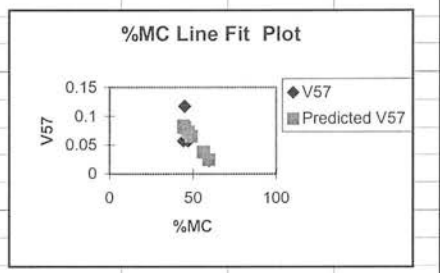
11.4.5 Linear regression of root dry weight and %MC in Rhapsody

Rhapsody: RDW versus %MC

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.746613986
R Square	0.557432443
Adjusted R Sq	0.446790554
Standard Erro	0.024164806
Observations	6

ANOVA

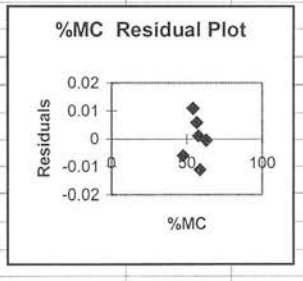
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.002941977	0.002942	5.038168	0.088172451
Residual	4	0.002335751	0.000584		
Total	5	0.005277728			

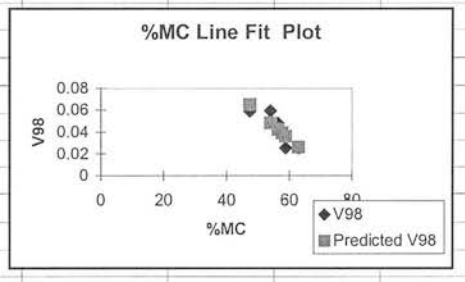
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.255473601	0.087326429	2.925502	0.043006	0.013016062	0.497931	0.013016	0.497931
%MC	-0.00387086	0.001724531	-2.244586	0.088172	-0.008658937	0.000917	-0.008659	0.000917

Rhapsody: RDW versus %MC

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.858063535
R Square	0.736273029
Adjusted R Sq	0.670341287
Standard Erro	0.008837532
Observations	6

ANOVA

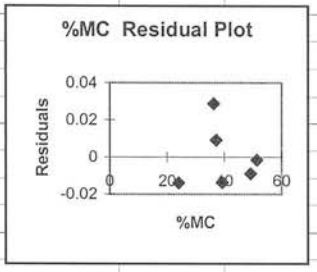
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.00087218	0.000872	11.1672	0.028789217
Residual	4	0.000312408	7.81E-05		
Total	5	0.001184588			

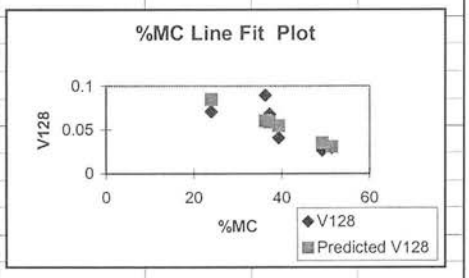
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.185275882	0.042792639	4.32962	0.012353	0.066464223	0.304088	0.066464	0.304088
%MC	-0.00253369	0.000758195	-3.341736	0.028789	-0.004638777	-0.000429	-0.004639	-0.000429

Rhapsody: RDW versus %MC

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.759116764
R Square	0.576258261
Adjusted R Sq	0.470322826
Standard Erro	0.01856788
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001875429	0.001875	5.439712	0.080048507
Residual	4	0.001379065	0.000345		
Total	5	0.003254493			

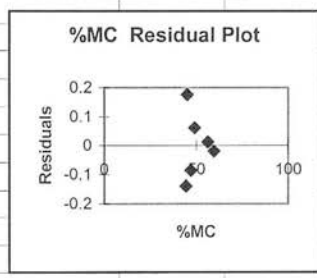
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.131465173	0.033970307	3.870002	0.017993	0.037148285	0.225782	0.037148	0.225782
%MC	-0.00195392	0.000837757	-2.332319	0.080049	-0.004279906	0.000372	-0.00428	0.000372

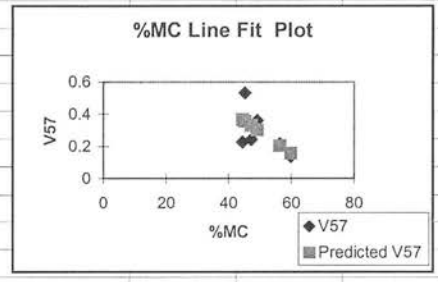
11.4.6 Linear regression of shoot dry weight and %MC in Rhapsody

Rhapsody: SDW versus %MC

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.610838708
R Square	0.373123927
Adjusted R Sq	0.216404908
Standard Erro	0.124339009
Observations	6

ANOVA

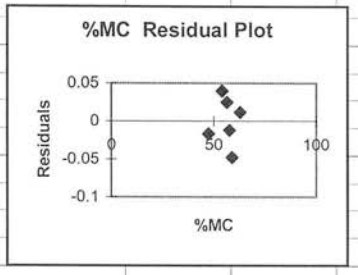
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.036808337	0.036808	2.380847	0.197701207
Residual	4	0.061840756	0.01546		
Total	5	0.098649093			

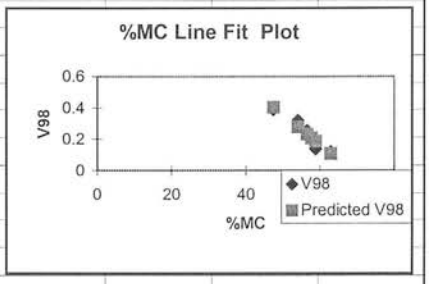
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.975551134	0.449334532	2.171102	0.095692	-0.272004113	2.223106	-0.272004	2.223106
%MC	-0.01369181	0.008873505	-1.542999	0.197701	-0.038328664	0.010945	-0.038329	0.010945

Rhapsody: SDW versus %MC

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.953016422
R Square	0.9082403
Adjusted R Sq	0.885300375
Standard Erro	0.035507143
Observations	6

ANOVA

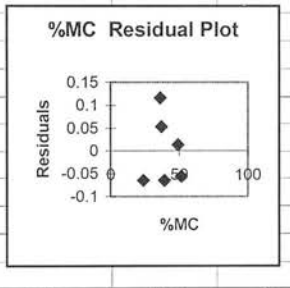
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.049916051	0.049916	39.59212	0.003259328
Residual	4	0.005043029	0.001261		
Total	5	0.05495908			

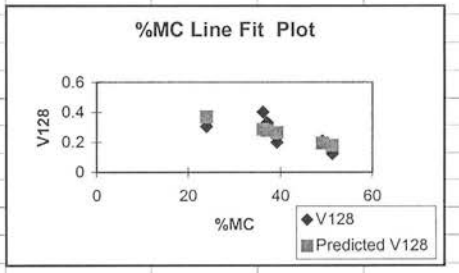
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	1.314776008	0.171930843	7.647121	0.001571	0.837418472	1.792134	0.837418	1.792134
%MC	-0.01916769	0.00304625	-6.292227	0.003259	-0.027625457	-0.01071	-0.027625	-0.01071

Rhapsody: SDW versus %MC

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.678681568
R Square	0.460608671
Adjusted R Sq	0.325760839
Standard Erro	0.083487045
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.023808187	0.023808	3.415766	0.138280955
Residual	4	0.027880347	0.00697		
Total	5	0.051688533			

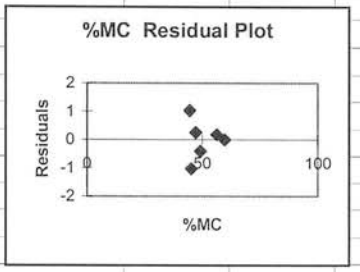
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.537541849	0.152741215	3.519298	0.024466	0.113463372	0.96162	0.113463	0.96162
%MC	-0.00696175	0.003766818	-1.848179	0.138281	-0.017420139	0.003497	-0.01742	0.003497

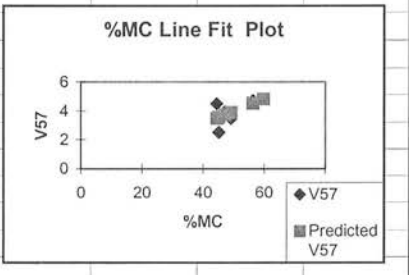
11.4.7 Linear regression of P concentration in the shoot and %MC in Rhapsody

Rhapsody: P Concentration versus %MC

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.623794467
R Square	0.389119537
Adjusted R Sq	0.236399422
Standard Erro	0.774616426
Observations	6

ANOVA

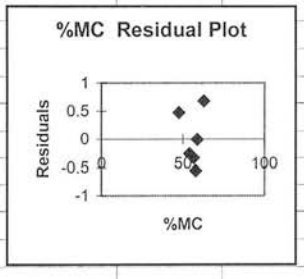
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.528833522	1.528834	2.547926	0.185673603
Residual	4	2.400122428	0.600031		
Total	5	3.92895595			

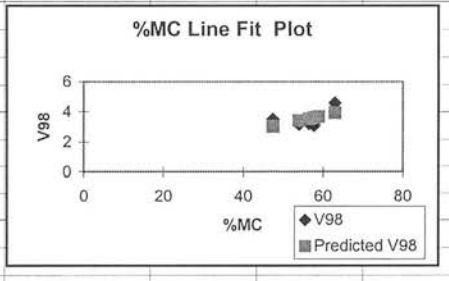
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	-0.43643458	2.799297768	-0.155909	0.883657	-8.208547261	7.335678	-8.208547	7.335678
%MC	0.088240492	0.055280826	1.596222	0.185674	-0.065244005	0.241725	-0.065244	0.241725

Rhapsody: P Concentration versus %MC

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.529097563
R Square	0.279944231
Adjusted R Sq	0.099930289
Standard Erro	0.541329443
Observations	6

ANOVA

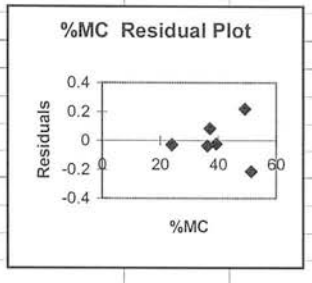
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.455710124	0.45571	1.555125	0.280412561
Residual	4	1.172150262	0.293038		
Total	5	1.627860386			

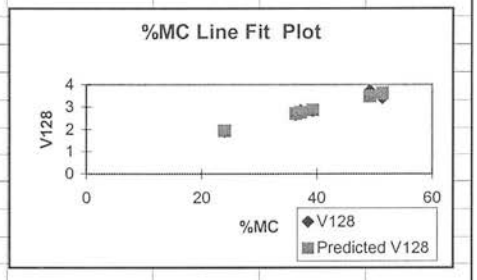
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.270426408	2.621197331	0.103169	0.922794	-7.007199165	7.548052	-7.007199	7.548052
%MC	0.057915406	0.046442054	1.247047	0.280413	-0.071028674	0.186859	-0.071029	0.186859

Rhapsody: P Concentration versus %MC

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.972869491
R Square	0.946475046
Adjusted R Sq	0.933093807
Standard Erro	0.160688699
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.826347951	1.826348	70.7315	0.001094112
Residual	4	0.103283432	0.025821		
Total	5	1.929631383			

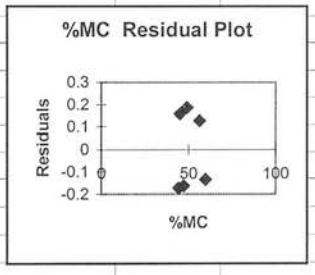
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.481354553	0.293983182	1.637354	0.176898	-0.334875305	1.297584	-0.334875	1.297584
%MC	0.060974366	0.007250048	8.410202	0.001094	0.040844964	0.081104	0.040845	0.081104

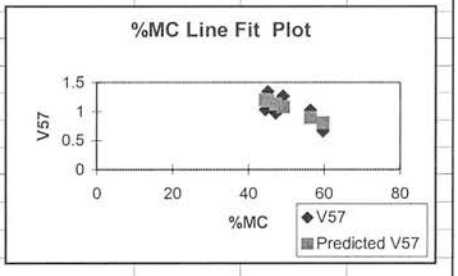
11.4.8 Linear regression of total P in the shoot and %MC in Rhapsody

Rhapsody: Total P versus %MC

G. mosseae

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.687109255
R Square	0.472119129
Adjusted R Sq	0.340148911
Standard Erro	0.193603628
Observations	6

ANOVA

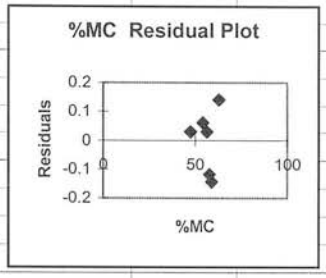
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.134091931	0.134092	3.577467	0.131534828
Residual	4	0.149929459	0.037482		
Total	5	0.28402139			

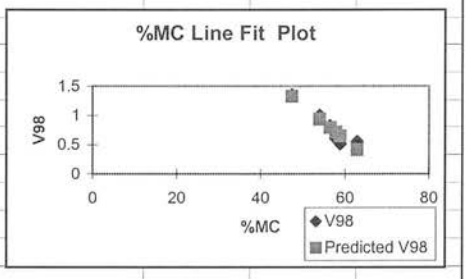
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	2.361285265	0.699642023	3.374991	0.027914	0.418763572	4.303807	0.418764	4.303807
%MC	-0.02613299	0.013816604	-1.891419	0.131535	-0.064494115	0.012228	-0.064494	0.012228

Rhapsody: Total P versus %MC

G. intraradices

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.941996092
R Square	0.887356637
Adjusted R Sq	0.859195797
Standard Erro	0.12286883
Observations	6

ANOVA

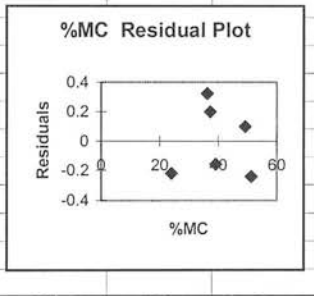
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.47570316	0.475703	31.5103	0.004949104
Residual	4	0.060386998	0.015097		
Total	5	0.536090158			

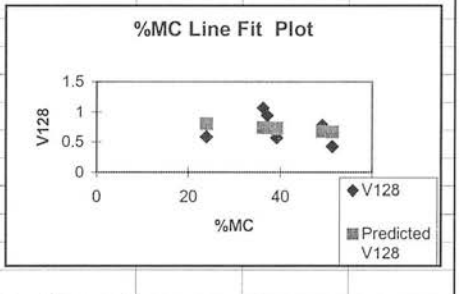
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	4.136043406	0.594949074	6.951928	0.002249	2.484196541	5.78789	2.484197	5.78789
%MC	-0.05917221	0.010541235	-5.613404	0.004949	-0.08843943	-0.029905	-0.088439	-0.029905

Rhapsody: Total P versus %MC

G. fistulosum

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.205401763
R Square	0.042189884
Adjusted R Sq	-0.19726264
Standard Erro	0.267215868
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.012580949	0.012581	0.176193	0.696230293
Residual	4	0.28561728	0.071404		
Total	5	0.298198229			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*wer 95.000*	*per 95.000*
Intercept	0.926959315	0.488876764	1.8961	0.130826	-0.430382994	2.284302	-0.430383	2.284302
%MC	-0.00506072	0.012056403	-0.419754	0.69623	-0.038534731	0.028413	-0.038535	0.028413

11.5 Numbers of oospores observed in root tips of non-mycorrhizal (0) and mycorrhizal Elsanta plants inoculated with 50 000 zoospores per plant of *Phytophthora fragariae*. Mycorrhizal plants were colonised by either *Glomus mosseae* (V57), *Glomus intraradices* (V98) or *Glomus fistulosum* (V128)

Highest - Lowest				Rank			
50000/0	50000/V57	50000/V98	50000/V128	50000/0	50000/V57	50000/V98	50000/V128
20	33	14	48	4	2	9.5	1
13	17	14	26	12	5.5	9.5	3
12	14	10	17	14	9.5	17	5.5
10	10	7	15	17	17	28	7

9	9	6	14	20.5	20.5	32.5	9.5
9	6	4	12	20.5	32.5	45	14
8	6	4	12	24.5	32.5	45	14
8	3	4	9	24.5	51.5	45	20.5
8	3	3	8	24.5	51.5	51.5	24.5
7	2	3	6	28	58.5	51.5	32.5
7	2	2	5	28	58.5	58.5	38.5
6	1	2	4	32.5	63.5	58.5	45
6	1		4	32.5	63.5	132.5	45
5			3	38.5	132.5	132.5	51.5
5			2	38.5	132.5	132.5	58.5
5			2	38.5	132.5	132.5	58.5
5				38.5	132.5	132.5	132.5
5				38.5	132.5	132.5	132.5
4				45	132.5	132.5	132.5
4				45	132.5	132.5	132.5
3				51.5	132.5	132.5	132.5
2				58.5	132.5	132.5	132.5
2				58.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
				132.5	132.5	132.5	132.5
			n	50	50	50	50
Sum R	20100		R	4311	5369	5486.5	4933.5

N(N+1)/2	20100		N	200			
			12/N(N+1)	0.000299			
			sum Ri	20100			
			3(N+1)	603			
			H	5.071561			
			m	13			
			sum T	2517112			
			C	0.685353			
			Hc	7.399924			
			v (=k-1)	2			
			Chi	7.815			
			Hc<Chi				
			Do not reject null hypothesis				
			i.e. no difference between myc and controls				

11.6 Chi-square test to determine any significant differences in the number of root tips containing oospores of *P. fragariae* in non-mycorrhizal Elsanta plants and those colonised by *G. mosseae*, *G. intraradices* or *G. fistulosum*

Observed			
	Inf Roots	Non-inf	
Control	23	27	50
Gm	12	38	50
Gi	11	39	50
Gf	16	34	50
	62	138	200
Expected			
	15.5	34.5	
	15.5	34.5	
	15.5	34.5	
	15.5	34.5	
Chi Prob	0.03981		

CHAPTER 12: Appendix III

12.1 Shoot dry weight (SDW), root dry weight (RDW), total phosphorus in the shoot (P) and concentration of phophorus in the shoot [P] of strawberry plants of cultivars Elsanta and Rhapsody colonised by *Acaulospora scrobiculata* (AS) and *Scutellospora nodosa* (SN)

	Arcsine	Arcsine	RDW	RDW		SDW	SDW	Absorb	Absorb			P mg/ml	P mg/ml	Pmg/gSDW	Pmg/gSDW
AMF	Elsanta	Rhapsody	Elsanta	Rhapsody	AMF	Elsanta	Rhapsody	Elsanta	Rhapsody	c	m	Elsanta	Rhapsody	Elsanta	Rhapsody
AS1	22.38	0.00	0.0391	0.0304	AS1	0.2986	0.3752	0.2	0.245	-0.00955	52.05818	0.0040	0.0049	2.6961	2.6064
AS2	5.74	9.97	0.0166	0.0298	AS2	0.269	0.3382	0.06	0.21	-0.00955	52.05818	0.0013	0.0042	0.9932	2.4940
AS3	51.06	8.13	0.0412	0.0448	AS3	0.2756	0.3655	0.245	0.233	-0.00955	52.05818	0.0049	0.0047	3.5484	2.5495
AS4	12.25	50.18	0.019	0.013	AS4	0.1209	0.2352	0.14	0.141	-0.00955	52.05818	0.0029	0.0029	4.7521	2.4591
AS5	45.14	54.18	0.0273	0.0177	AS5	0.2288	0.2596	0.247	0.197	-0.00955	52.05818	0.0049	0.0040	4.3077	3.0567
AS6	9.55	0.00	0.0185	0.0246	AS6	0.1022	0.2323	0.095	0.14	-0.00955	52.05818	0.0020	0.0029	3.9300	2.4732
SN1	43.71	59.67	0.0493	0.0359	SN1	0.4511	0.3549	0.373	0.348	-0.00955	52.05818	0.0073	0.0069	3.2580	3.8705
SN2	47.15	59.34	0.0424	0.0398	SN2	0.5302	0.4023	0.459	0.301	-0.00955	52.05818	0.0090	0.0060	3.3951	2.9656
SN3	33.83	56.32	0.0407	0.029	SN3	0.4398	0.2867	0.247	0.233	-0.00955	52.05818	0.0049	0.0047	2.2410	3.2502
SN4	28.83	58.53	0.0376	0.05	SN4	0.323	0.3718	0.162	0.229	-0.00955	52.05818	0.0033	0.0046	2.0404	2.4649
SN5	45.86	59.34	0.0428	0.0393	SN5	0.4628	0.3858	0.406	0.278	-0.00955	52.05818	0.0080	0.0055	3.4496	2.8634
SN6	55.70	59.34	0.0544	0.0364	SN6	0.4366	0.3204	0.542	0.234	-0.00955	52.05818	0.0106	0.0047	4.8533	2.9203
C1	0	0	0.0507	0.0294	C1	0.368	0.2293	0.119	0.118	-0.00955	52.05818	0.0025	0.0025	1.3420	2.1370
C2	0	0	0.0543	0.0225	C2	0.292	0.2201	0.181	0.126	-0.00955	52.05818	0.0037	0.0026	2.5070	2.3660
C3	0	0	0.0541	0.0371	C3	0.3358	0.3206	0.111	0.081	-0.00955	52.05818	0.0023	0.0017	1.3791	1.0850
C4	0	0	0.0552	0.0462	C4	0.3501	0.3432	0.169	0.171	-0.00955	52.05818	0.0034	0.0035	1.9593	2.0211
C5	0	0	0.0396	0.0257	C5	0.2037	0.2531	0.137	0.122	-0.00955	52.05818	0.0028	0.0025	2.7639	1.9968
C6	0	0	0.0369	0.0153	C6	0.2527	0.1841	0.112	0.148	-0.00955	52.05818	0.0023	0.0030	1.8479	3.2877

12.2 Two-factor analysis of variance to test for significant differences in the shoot dry weight, root dry weight, total amount of P in shoots or concentration of P in shoots between mycorrhizal and non-mycorrhizal plants of cultivars Elsanta and Rhapsody

RDW								SDW							
SUMMARY	Elsanta	Rhaps	Total					SUMMARY	Elsanta	Rhaps	Total				
AS1								*AS1*							
Count	6.0000	6.0000	12.0000					Count	6.000	6.000	12.000				
Sum	0.1617	0.1603	0.3220					Sum	1.295	1.806	3.101				
Average	0.0270	0.0267	0.0268					Average	0.216	0.301	0.258				
Variance	0.0001	0.0001	0.0001					Variance	0.007	0.004	0.007				
SN1								*SN1*							
Count	6.0000	6.0000	12.0000					Count	6.000	6.000	12.000				
Sum	0.2672	0.2304	0.4976					Sum	2.644	2.122	4.765				
Average	0.0445	0.0384	0.0415					Average	0.441	0.354	0.397				
Variance	0.0000	0.0000	0.0000					Variance	0.004	0.002	0.005				
C1								*C1*							
Count	6.0000	6.0000	12.0000					Count	6.000	6.000	12.000				
Sum	0.2908	0.1762	0.4670					Sum	1.802	1.550	3.353				
Average	0.0485	0.0294	0.0389					Average	0.300	0.258	0.279				
Variance	0.0001	0.0001	0.0002					Variance	0.004	0.004	0.004				
Total								*Total*							
Count	18.0000	18.0000						Count	18.000	18.000					
Sum	0.7197	0.5669						Sum	5.741	5.478					
Average	0.0400	0.0315						Average	0.319	0.304					
Variance	0.0002	0.0001						Variance	0.014	0.005					

ANOVA								ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*		*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	
Sample	0.0015	2.0000	0.0007	8.5498	0.0012	3.3158		Sample	0.134	2.000	0.067	15.736	$2.12x10^{-5}$	3.316	
Columns	0.0006	1.0000	0.0006	7.5618	0.0100	4.1709		Columns	0.002	1.000	0.002	0.449	0.508	4.171	
Interaction	0.0006	2.0000	0.0003	3.2582	0.0524	3.3158		Interaction	0.048	2.000	0.024	5.607	0.009	3.316	
Within	0.0026	30.0000	0.0001					Within	0.128	30.000	0.004				
Total	0.0052	35.0000						Total	0.312	35.000					

P Conc. (mg/g SDW)									**Total P (mg)**							
SUMMARY	Elsanta	Rhaps	Total						SUMMARY	Elsanta	Rhapsody	Total				
AS1									*AS1*							
Count	6.000	6.000	12.000						Count	6.000	6.000	12.000				
Sum	20.228	15.639	35.866						Sum	4.012	4.700	8.712				
Average	3.371	2.606	2.989						Average	0.669	0.783	1.452				
Variance	1.848	0.052	1.023						Variance	0.091	0.030	0.121				
SN1									*SN1*							
Count	6.000	6.000	12.000						Count	6.000	6.000	12.000				
Sum	19.237	18.335	37.572						Sum	8.630	6.455	15.085				
Average	3.206	3.056	3.131						Average	1.438	1.076	2.514				
Variance	1.020	0.223	0.571						Variance	0.286	0.034	0.320				
C1									*C1*							
Count	6.000	6.000	12.000						Count	6.000	6.000	12.000				
Sum	11.799	12.894	24.693						Sum	3.405	3.163	6.568				
Average	1.967	2.149	2.058						Average	0.567	0.527	1.095				

Variance	0.335	0.503	0.390					Variance	0.014	0.014	0.027			
Total								*Total*						
Count	18.000	18.000						Count	18.000	18.000				
Sum	51.264	46.867						Sum	16.047	14.318				
Average	2.848	2.604						Average	2.674	2.386				
Variance	1.358	0.374						Variance	0.391	0.077				
ANOVA								ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*		*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	8.157	2.000	4.078	6.147	0.006	3.316		Sample	3.271	2.000	1.636	20.951	0.000	3.316
Columns	0.537	1.000	0.537	0.809	0.375	4.171		Columns	0.083	1.000	0.083	1.063	0.311	4.171
Interaction	1.385	2.000	0.693	1.044	0.364	3.316		Interaction	0.355	2.000	0.178	2.276	0.120	3.316
Within	19.904	30.000	0.663					Within	2.342	30.000	0.078			
Total	29.983	35.000						Total	6.052	35.000				

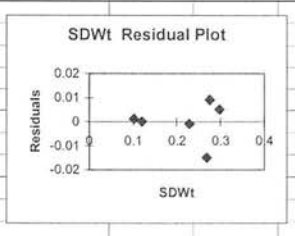
12.3 Summary of linear regression analysis of growth characteristics of Elsanta and Rhapsody (RDW, Total P and P concentration) varying with shoot dry weight of the plants. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics as plant size (SDW) increased were significant

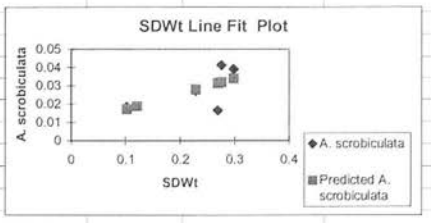
12.3.1 Linear regression analysis of root dry weight versus shoot dry weight in Elsanta

Elsanta: A. scrobiculata: RDW & SDW

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.661354122
R Square	0.437389274
Adjusted R Square	0.296736593
Standard Error	0.009129164
Observations	6





ANOVA

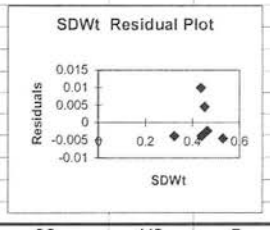
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000259168	0.000259	3.109712	0.152603417
Residual	4	0.000333367	8.33E-05		
Total	5	0.000592535			

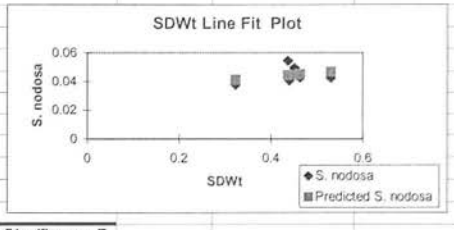
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.008465951	0.011124702	0.761005	0.489046	-0.022421237	0.0393531	-0.022421237	0.03935314
SDWt	0.085633767	0.048560706	1.763437	0.152603	-0.049192647	0.2204602	-0.049192647	0.22046018

Elsanta: S. nodosa: RDW & SDW

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.289736599
R Square	0.083947297
Adjusted R Square	-0.145065879
Standard Error	0.006603225
Observations	6





ANOVA

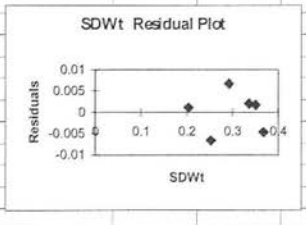
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.5983E-05	1.6E-05	0.366561	0.577556404
Residual	4	0.00017441	4.36E-05		
Total	5	0.000190393			

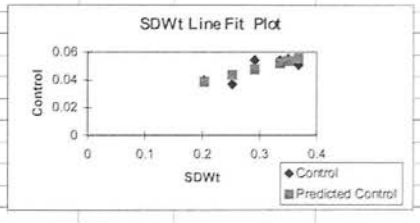
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.032784849	0.019591136	1.673453	0.169553	-0.021608977	0.0871787	-0.021608977	0.087178674
SDWt	0.026665749	0.044043383	0.605443	0.577556	-0.095618538	0.14895	-0.095618538	0.148950037

Elsanta: Control: RDW & SDW

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.798112083
R Square	0.636982897
Adjusted R Square	0.546228622
Standard Error	0.005460053
Observations	6





ANOVA

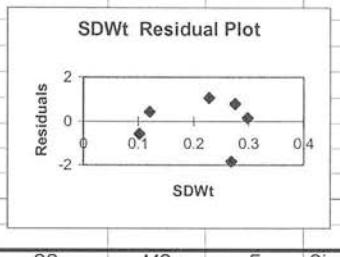
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000209245	0.000209	7.018765	0.057023749
Residual	4	0.000119249	2.98E-05		
Total	5	0.000328493			

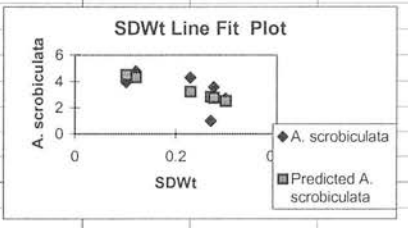
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.017734064	0.011812515	1.501295	0.207682	-0.015062804	0.0505309	-0.015062804	0.050530933
SDWt	0.102311276	0.038618301	2.649295	0.057024	-0.004910539	0.2095331	-0.004910539	0.209533092

12.3.2 Linear regression analysis of P concentration in the shoot versus shoot dry weight in Elsanta

Elsanta: A. scrobiculata: P Conc & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.61983319
R Square	0.38419319
Adjusted R Square	0.23024148
Standard Error	1.19257601
Observations	6

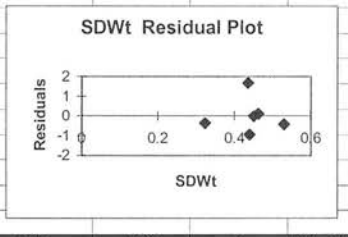
ANOVA

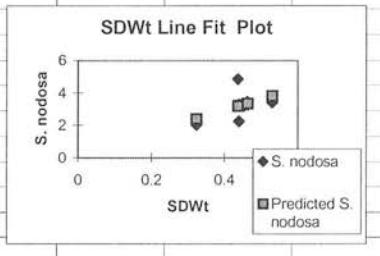
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.549255768	3.549256	2.495544	0.189318056
Residual	4	5.688950155	1.422238		
Total	5	9.238205923			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	5.53434999	1.453260431	3.80823	0.018969	1.499443818	9.5692562	1.499443818	9.569256
SDWt	-10.0212668	6.343662168	-1.579729	0.189318	-27.63413309	7.5915994	-27.6341331	7.591599

Elsanta: S. nodosa: P Conc & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.45524089
R Square	0.20724427
Adjusted R Square	0.00905534
Standard Error	1.00548422
Observations	6

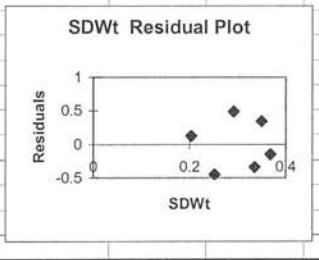
ANOVA

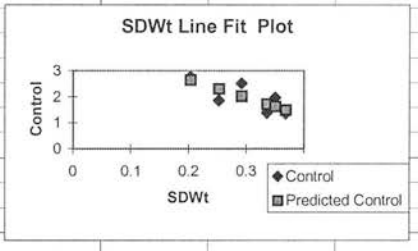
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.057191467	1.057191	1.04569	0.364311694
Residual	4	4.043994027	1.010999		
Total	5	5.101185494			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	0.18469516	2.983175245	0.061912	0.953603	-8.097944306	8.4673346	-8.09794431	8.467335
SDWt	6.85806171	6.706560101	1.02259	0.364312	-11.76237283	25.478496	-11.7623728	25.4785

Elsanta: Control: P Conc & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.7611111
R Square	0.5792901
Adjusted R Square	0.47411263
Standard Error	0.4199939
Observations	6

ANOVA

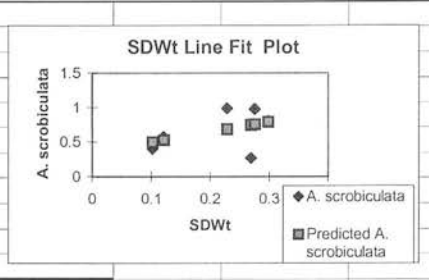
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.971536973	0.971537	5.507739	0.078785416
Residual	4	0.705579492	0.176395		
Total	5	1.677116466			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	4.06065916	0.90863306	4.468976	0.011083	1.53788412	6.5834342	1.53788412	6.583434
SDWt	-6.97149633	2.970566752	-2.346857	0.078785	-15.21912893	1.2761363	-15.2191289	1.276136

12.3.3 Linear regression analysis of total P in the shoot versus shoot dry weight in Elsanta

Elsanta: A. scrobiculata: Total P & SDW

SUMMARY OUTPUT



Regression Statistics	
Multiple R	0.408128
R Square	0.16656847
Adjusted R Square	-0.04178942
Standard Error	0.30825324
Observations	6

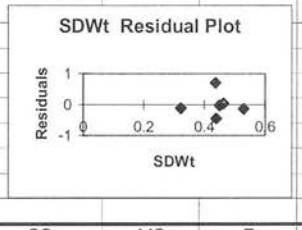
ANOVA

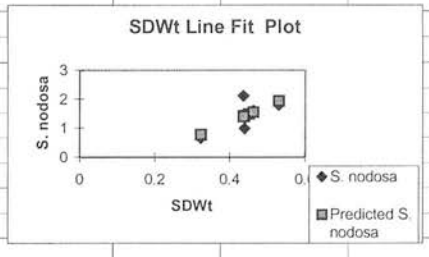
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.075962309	0.075962	0.799434	0.421798623
Residual	4	0.380080246	0.09502		
Total	5	0.456042556			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	0.35220764	0.37563412	0.937635	0.401517	-0.690722038	1.3951373	-0.69072204	1.395137
SDWt	1.46606443	1.639689561	0.894111	0.421799	-3.086453051	6.0185819	-3.08645305	6.018582

Elsanta: S. nodosa: Total P & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.69963067
R Square	0.48948307
Adjusted R Square	0.36185384
Standard Error	0.42756075
Observations	6

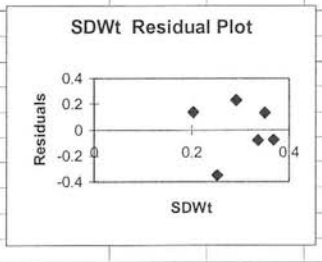
ANOVA

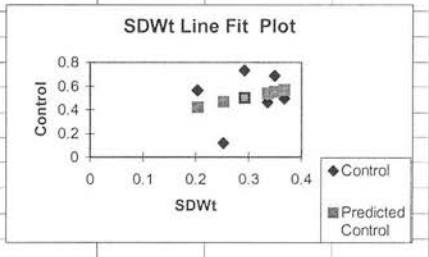
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.701105175	0.701105	3.835196	0.121782683
Residual	4	0.731232772	0.182808		
Total	5	1.432337947			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	-1.02230876	1.268531738	-0.805899	0.465482	-4.544324788	2.4997073	-4.54432479	2.499707
SDWt	5.58490963	2.851821847	1.958366	0.121783	-2.333033576	13.502853	-2.33303358	13.50285

Elsanta: Control: Total P & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.25598488
R Square	0.06552826
Adjusted R Square	-0.16808968
Standard Error	0.23638826
Observations	6

ANOVA

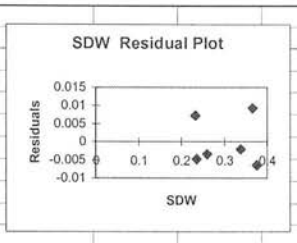
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.015673798	0.015674	0.280493	0.624409808
Residual	4	0.223517643	0.055879		
Total	5	0.239191441			

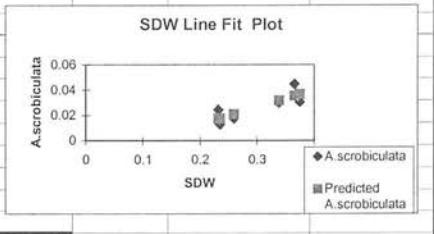
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	0.2433425	0.511412647	0.475824	0.659022	-1.17656958	1.6632546	-1.17656958	1.663255
SDWt	0.88548959	1.671945995	0.529616	0.62441	-3.756586299	5.5275655	-3.7565863	5.527565

12.3.4 Linear regression analysis of root dry weight versus shoot dry weight in Rhapsody

Rhapsody: A. scrobiculata: RDW & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.80654257
R Square	0.650510917
Adjusted R Square	0.563138646
Standard Error	0.007383835
Observations	6

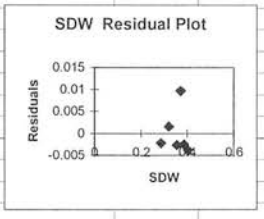
ANOVA

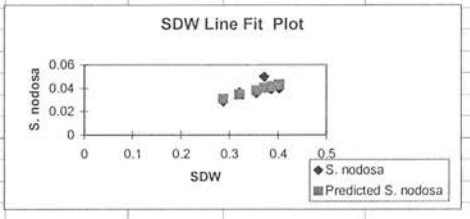
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000405924	0.000406	7.445279	0.052518519
Residual	4	0.000218084	5.45E-05		
Total	5	0.000624008			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	-0.014344481	0.01534736	-0.934655	0.402879	-0.056955673	0.0282667	-0.056955673	0.02826671
SDW	0.136415774	0.049994716	2.728604	0.052519	-0.002392099	0.2752236	-0.002392099	0.275223646

Rhapsody: S. nodosa: RDW & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.674580231
R Square	0.455058488
Adjusted R Square	0.31882311
Standard Error	0.005669513
Observations	6

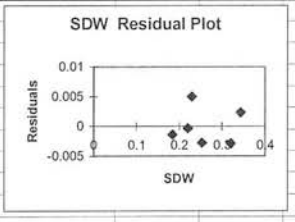
ANOVA

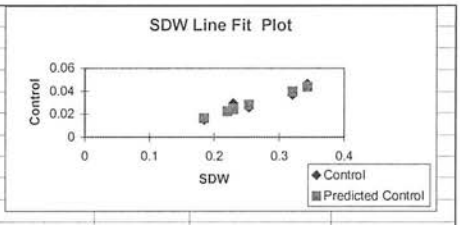
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000107366	0.000107	3.340237	0.141616383
Residual	4	0.000128574	3.21E-05		
Total	5	0.00023594			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.000440846	0.020898158	0.021095	0.98418	-0.057581862	0.0584636	-0.057581862	0.058463553
SDW	0.107335372	0.058729217	1.827632	0.141616	-0.055723411	0.2703942	-0.055723411	0.270394156

Rhapsody: Controls: RDW & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.958815546
R Square	0.919327251
Adjusted R Square	0.899159064
Standard Error	0.003483368
Observations	6

ANOVA

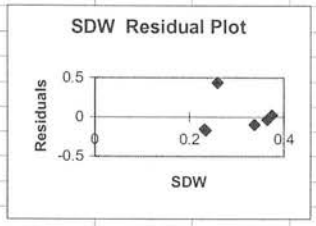
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000553098	0.000553	45.58304	0.002509311
Residual	4	4.85354E-05	1.21E-05		
Total	5	0.000601633			

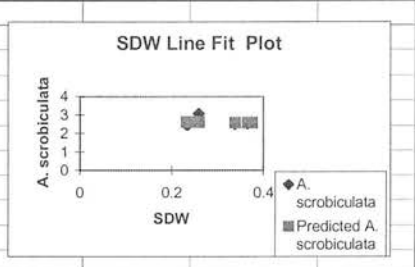
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	-0.014811549	0.006696193	-2.211936	0.091429	-0.033403198	0.0037801	-0.033403198	0.003780101
SDW	0.170968325	0.025322934	6.751521	0.002509	0.100660443	0.2412762	0.100660443	0.241276207

12.3.5 Linear regression analysis of P concentration in the shoot versus shoot dry weight in Rhapsody

Rhapsody: A. scrobiculata: P Conc & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.10200895
R Square	0.01040583
Adjusted R Square	-0.23699272
Standard Error	0.25268594
Observations	6

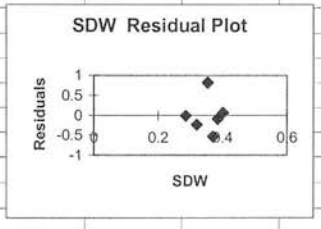
ANOVA

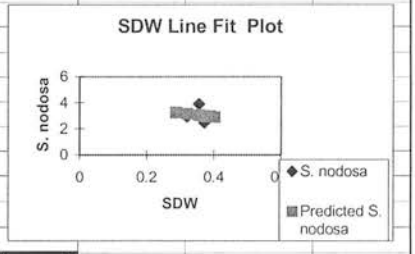
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.002685602	0.002686	0.042061	0.847517312
Residual	4	0.255400735	0.06385		
Total	5	0.258086337			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	2.71208979	0.525209724	5.163823	0.00668	1.253870805	4.1703088	1.253870805	4.170309
SDW	-0.35088347	1.710894291	-0.205088	0.847517	-5.101097385	4.3993305	-5.10109738	4.39933

Rhapsody: S. nodosa: P Conc & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.28250292
R Square	0.0798079
Adjusted R Square	-0.15024012
Standard Error	0.50626743
Observations	6

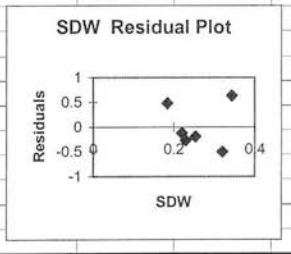
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.088917521	0.088918	0.346918	0.587518602
Residual	4	1.025226828	0.256307		
Total	5	1.114144348			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	4.14820882	1.86613152	2.222892	0.090321	-1.033013633	9.3294313	-1.03301363	9.329431
SDW	-3.08888787	5.244311208	-0.588998	0.587519	-17.64946021	11.471684	-17.6494602	11.47168

Rhapsody: Control: P Conc & SDW

SUMMARY OUTPUT



Regression Statistics	
Multiple R	0.77365136
R Square	0.59853643
Adjusted R Square	0.49817054
Standard Error	0.50245758
Observations	6

ANOVA

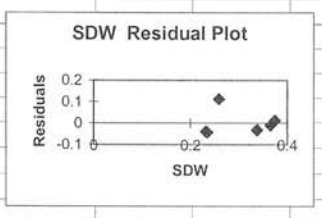
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.505577914	1.505578	5.963544	0.071052219
Residual	4	1.009854461	0.252464		
Total	5	2.515432374			

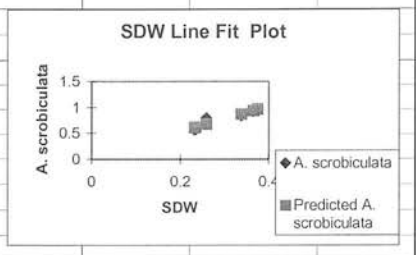
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	4.45385319	0.96589065	4.611136	0.009947	1.772105273	7.1356011	1.772105273	7.135601
SDW	-8.92002892	3.652700325	-2.442037	0.071052	-19.06157186	1.221514	-19.0615719	1.221514

12.3.6 Linear regression analysis of total P in the shoot versus shoot dry weight in Rhapsody

Rhapsody: A. scrobiculata: Total P & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.94052206
R Square	0.88458175
Adjusted R Square	0.85572719
Standard Error	0.06562249
Observations	6

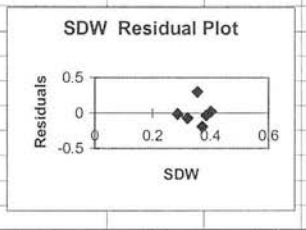
ANOVA

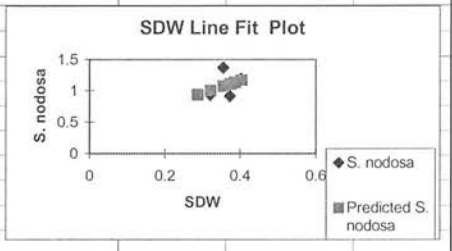
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.132016712	0.132017	30.65656	0.005201232
Residual	4	0.017225246	0.004306		
Total	5	0.149241957			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	0.04277643	0.136396869	0.313617	0.769485	-0.335922776	0.4214756	-0.33592278	0.421476
SDW	2.4601215	0.444318933	5.536837	0.005201	1.226491818	3.6937512	1.226491818	3.693751

Rhapsody: S. nodosa: Total P & SDW

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.46390862
R Square	0.21521121
Adjusted R Square	0.01901401
Standard Error	0.18211945
Observations	6

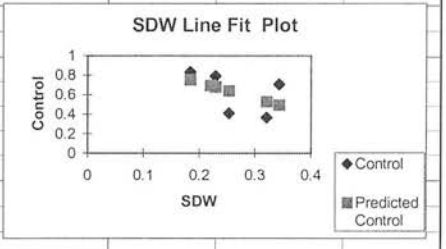
ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.036381849	0.036382	1.096913	0.354056233
Residual	4	0.132669982	0.033167		
Total	5	0.169051831			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	0.37713999	0.671303021	0.561803	0.604237	-1.486699853	2.2409798	-1.48669985	2.24098
SDW	1.97583582	1.886534748	1.047336	0.354056	-3.262035194	7.2137068	-3.26203519	7.213707

Rhapsody: Control: Total P & SDW

SUMMARY OUTPUT



Regression Statistics	
Multiple R	0.50326728
R Square	0.25327795
Adjusted R Square	0.06659744
Standard Error	0.19075252
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.049367255	0.049367	1.356746	0.308832334
Residual	4	0.145546098	0.036387		
Total	5	0.194913353			

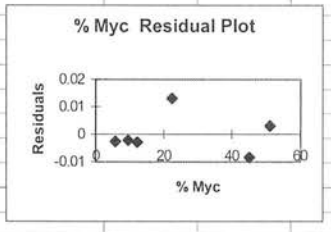
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000*
Intercept	1.04849904	0.366689818	2.859362	0.045959	0.030402785	2.0665953	0.030402785	2.066595
SDW	-1.61522912	1.386707716	-1.164794	0.308832	-5.465354948	2.2348967	-5.46535495	2.234897

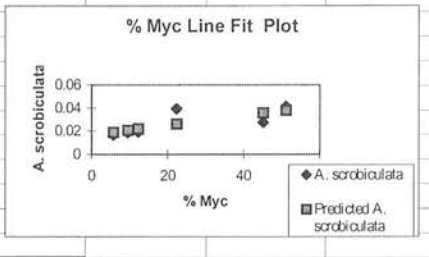
12.4 Summary of linear regression analysis of growth characteristics of Elsanta and Rhapsody (RDW, Total P, P concentration) varying with percentage of mycorrhizal colonisation (%MC) of the plant. A *t*-test was applied to each correlation relationship to determine whether the changes in the growth characteristics that were studied as %MC increased were significant

12.4.1 Linear regression analysis of root dry weight versus %MC in Elsanta

Elsanta: A. scrobiculata: RDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.7406841
R Square	0.548613
Adjusted R Square	0.4357663
Standard Error	0.0081771
Observations	6

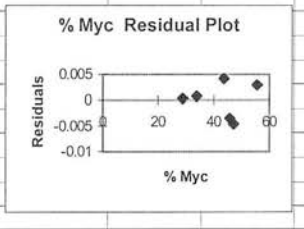
ANOVA

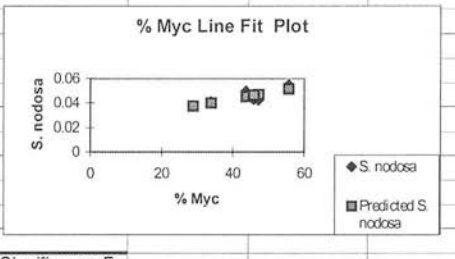
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000325072	0.000325	4.861576	0.092148257
Residual	4	0.000267463	6.69E-05		
Total	5	0.000592535			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.0167738	0.005696066	2.944797	0.042187	0.000958909	0.032588605	0.000958909	0.03258861
% Myc	0.0004179	0.000189515	2.204898	0.092148	-0.000108318	0.00094404	-0.00010832	0.00094404

Elsanta: S. nodosa: RDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.8294381
R Square	0.6879676
Adjusted R Square	0.6099595
Standard Error	0.0038539
Observations	6

ANOVA

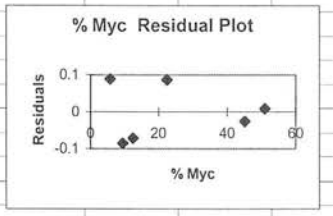
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000130984	0.000131	8.819181	0.041156097
Residual	4	5.94089E-05	1.49E-05		
Total	5	0.000190393			

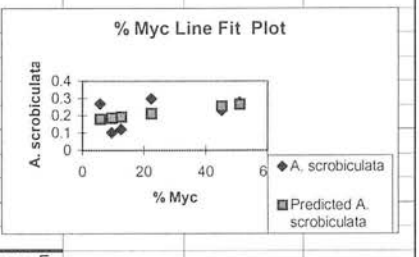
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.0221076	0.00771365	2.866035	0.045651	0.000691018	0.043524159	0.000691018	0.04352416
% Myc	0.0005275	0.000177623	2.969711	0.041156	3.43273E-05	0.001020648	3.43273E-05	0.00102065

12.4.2 Linear regression analysis of shoot dry weight versus %MC in Elsanta

Elsanta: A. scrobiculata: SDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.4370273
R Square	0.1909929
Adjusted R Square	-0.0112589
Standard Error	0.0845458
Observations	6

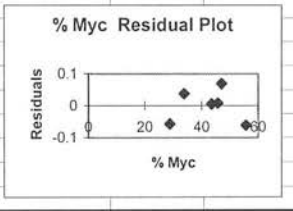
ANOVA

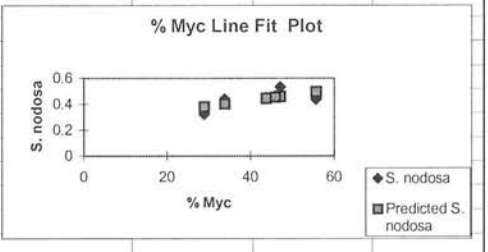
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.006750084	0.00675	0.944332	0.386193589
Residual	4	0.028591991	0.007148		
Total	5	0.035342075			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.1694784	0.058893275	2.877721	0.045116	0.005964125	0.332992692	0.005964125	0.33299269
% Myc	0.0019041	0.00195945	0.971768	0.386194	-0.003536187	0.007344447	-0.00353619	0.00734445

Elsanta: S. nodosa: SDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.6351
R Square	0.4033521
Adjusted R Square	0.2541901
Standard Error	0.0579035
Observations	6

ANOVA

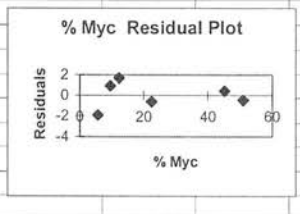
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.009066406	0.009066	2.704121	0.175434391
Residual	4	0.013411242	0.003353		
Total	5	0.022477648			

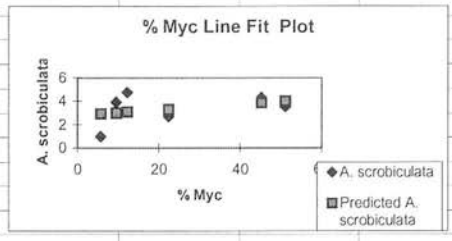
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.2540078	0.115896073	2.191686	0.093516	-0.067771913	0.575787589	-0.06777191	0.57578759
% Myc	0.0043885	0.002668744	1.644421	0.175434	-0.003021097	0.011798175	-0.0030211	0.01179817

12.4.3 Linear regression analysis of P concentration in the shoot versus %MC in Elsanta

Elsanta: A. scrobiculata: P Conc & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.3416345
R Square	0.1167141
Adjusted R Square	-0.1041074
Standard Error	1.4282837
Observations	6

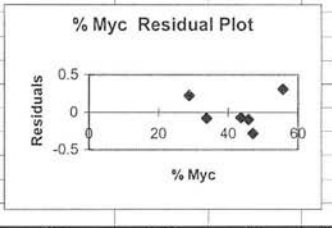
ANOVA

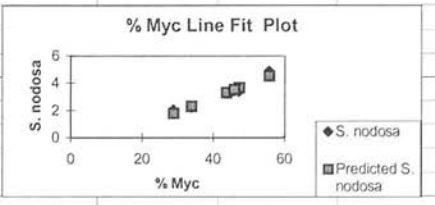
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.078228912	1.078229	0.528545	0.507485094
Residual	4	8.159977011	2.039994		
Total	5	9.238205923			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*.ower 95.000%*	*Jpper 95.000%*
Intercept	2.7851849	0.994919574	2.799407	0.048841	0.022839643	5.547530249	0.022839643	5.54753025
% Myc	0.0240656	0.033102173	0.727011	0.507485	-0.06784091	0.115972201	-0.06784091	0.1159722

Elsanta: S. nodosa: P Conc & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.9756415
R Square	0.9518764
Adjusted R Square	0.9398455
Standard Error	0.2477335
Observations	6

ANOVA

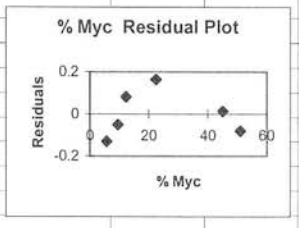
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.855697945	4.855698	79.11925	0.000882777
Residual	4	0.245487549	0.061372		
Total	5	5.101185494			

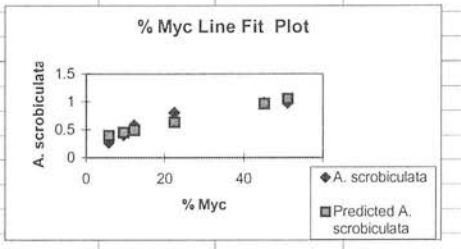
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*.ower 95.000%*	*Jpper 95.000%*
Intercept	-1.1115605	0.495848436	-2.241734	0.088452	-2.488259302	0.265138329	-2.4882593	0.26513833
% Myc	0.1015613	0.011417923	8.8949	0.000883	0.069859986	0.133262589	0.069859986	0.13326259

12.4.4 Linear regression analysis of total P in the shoot versus %MC in Elsanta

Elsanta: A. scrobiculata: Total P & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.931666477
R Square	0.868002424
Adjusted R Squar	0.83500303
Standard Error	0.122674887
Observations	6

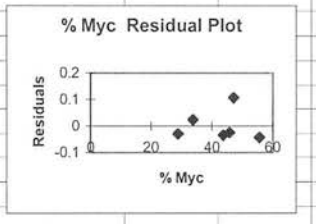
ANOVA

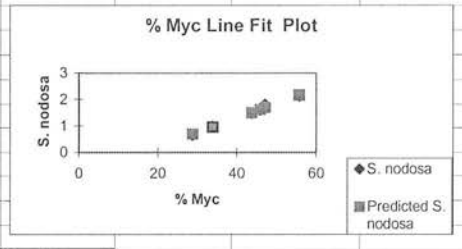
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.395846044	0.395846	26.30359	0.00684466
Residual	4	0.060196512	0.015049		
Total	5	0.456042556			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	0.313549557	0.085453365	3.669248	0.021405	0.07629249	0.55080663	0.076292488	0.550806627
% Myc	0.0145816	0.002843136	5.128702	0.006845	0.00668777	0.02247543	0.006687772	0.022475429

Elsanta: S. nodosa: Total P & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.99420183
R Square	0.988437279
Adjusted R Squar	0.985546599
Standard Error	0.064346181
Observations	6

ANOVA

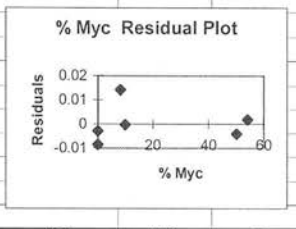
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.415776223	1.415776	341.9393	5.0331E-05
Residual	4	0.016561724	0.00414		
Total	5	1.432337947			

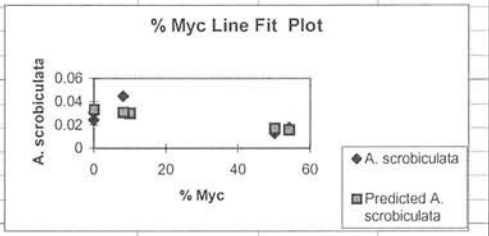
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Jpper 95.000%*
Intercept	-0.893184821	0.128791436	-6.935126	0.00227	-1.25076791	-0.53560173	-1.250767913	-0.53560173
% Myc	0.054840281	0.002965686	18.4916	5.03E-05	0.0466062	0.06307436	0.0466062	0.063074362

12.4.5 Linear regression analysis of root dry weight versus %MC in Rhapsody

Rhapsody: A. scrobiculata: RDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.7160754
R Square	0.512764
Adjusted R Square	0.3909551
Standard Error	0.0087184
Observations	6

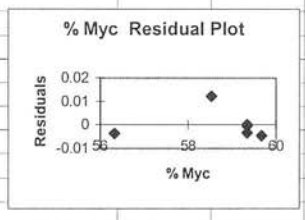
ANOVA

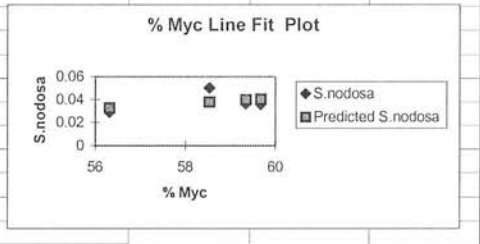
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000319969	0.00032	4.209575	0.109475703
Residual	4	0.000304039	7.6E-05		
Total	5	0.000624008			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.0332535	0.00477692	6.961281	0.002238	0.019990599	0.046516364	0.019990599	0.04651636
% Myc	-0.0003202	0.000156088	-2.051725	0.109476	-0.000753621	0.000113121	-0.00075362	0.00011312

Rhapsody: S. nodosa: RDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.422565
R Square	0.1785612
Adjusted R Square	-0.0267985
Standard Error	0.0069608
Observations	6

ANOVA

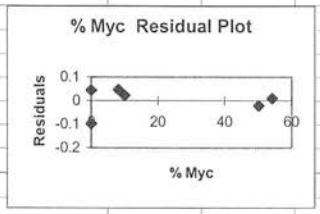
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.21297E-05	4.21E-05	0.869505	0.403879311
Residual	4	0.00019381	4.85E-05		
Total	5	0.00023594			

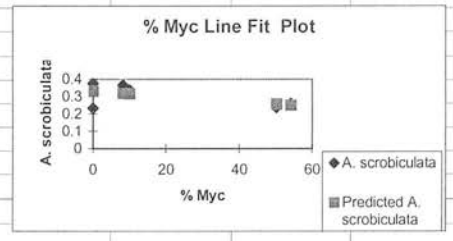
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	-0.0977797	0.146069233	-0.669407	0.539892	-0.503333784	0.307774308	-0.50333378	0.30777431
% Myc	0.0023176	0.002485442	0.932472	0.403879	-0.004583103	0.009218315	-0.0045831	0.00921832

12.4.6 Linear regression analysis of shoot dry weight versus %MC in Rhapsody

Rhapsody: A. scrobiculata: SDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.5636527
R Square	0.3177044
Adjusted R Square	0.1471305
Standard Error	0.0609978
Observations	6

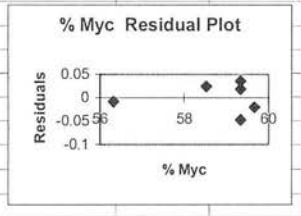
ANOVA

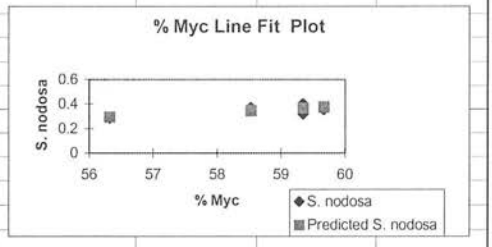
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.006930093	0.00693	1.862562	0.244058362
Residual	4	0.014882927	0.003721		
Total	5	0.02181302			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*.ower 95.000%*	*Jpper 95.000%*
Intercept	0.3314216	0.033421601	9.916388	0.000581	0.238628145	0.424215013	0.238628145	0.42421501
% Myc	-0.0014904	0.001092067	-1.364757	0.244058	-0.004522477	0.001541664	-0.00452248	0.00154166

Rhapsody: S. nodosa: SDW & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.6951721
R Square	0.4832643
Adjusted R Square	0.3540804
Standard Error	0.0346973
Observations	6

ANOVA

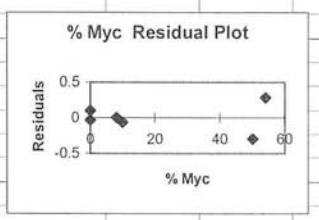
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.004503682	0.004504	3.740901	0.125217743
Residual	4	0.004815613	0.001204		
Total	5	0.009319295			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*.ower 95.000%*	*Jpper 95.000%*
Intercept	-1.054348	0.728108544	-1.448064	0.221168	-3.075905554	0.967209625	-3.07590555	0.96720963
% Myc	0.0239623	0.012389138	1.934141	0.125218	-0.010435495	0.058360171	-0.01043549	0.05836017

12.4.7 Linear regression analysis of P concentration in the shoot versus %MC in Rhapsody

Rhapsody: A. scrobiculata: P conc & %MC

SUMMARY OUTPUT



Regression Statistics	
Multiple R	0.5430756
R Square	0.2949311
Adjusted R Square	0.1186639
Standard Error	0.2132889
Observations	6

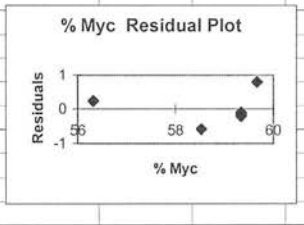
ANOVA

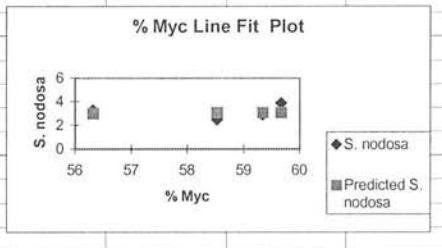
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.076117692	0.076118	1.673205	0.265471535
Residual	4	0.181968645	0.045492		
Total	5	0.258086337			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	2.5056519	0.116864165	21.44072	2.8E-05	2.181184326	2.830119546	2.181184326	2.83011955
% Myc	0.0049394	0.003818593	1.293524	0.265472	-0.005662694	0.01554158	-0.00566269	0.01554158

Rhapsody: S. nodosa: P Conc & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.0536339
R Square	0.0028766
Adjusted R Square	-0.2464043
Standard Error	0.5270056
Observations	6

ANOVA

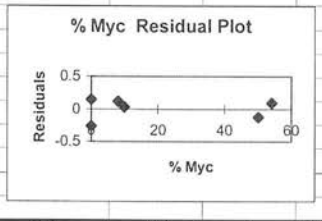
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.003204942	0.003205	0.01154	0.919626302
Residual	4	1.110939407	0.277735		
Total	5	1.114144348			

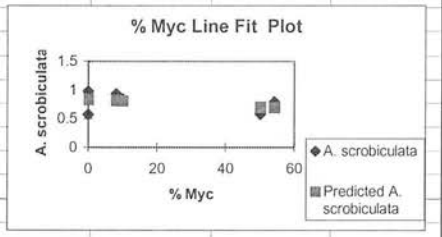
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	1.868065	11.05899195	0.168918	0.874059	-28.83668268	32.57281263	-28.8366827	32.5728126
% Myc	0.0202141	0.188174386	0.107422	0.919626	-0.502242789	0.542671078	-0.50224279	0.54267108

12.4.8 Linear regression analysis of total P in the shoot versus %MC in Rhapsody

Rhapsody: A. scrobiculata: Total P & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.367026
R Square	0.1347081
Adjusted R Square	-0.0816149
Standard Error	0.1796788
Observations	6

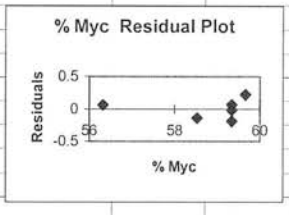
ANOVA

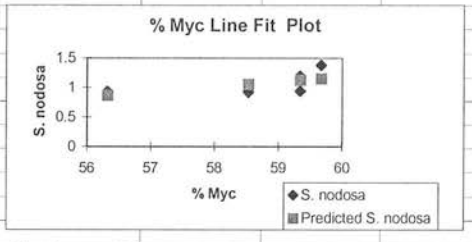
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.020104101	0.020104	0.622718	0.474181649
Residual	4	0.129137856	0.032284		
Total	5	0.149241957			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	0.8350879	0.098448672	8.48247	0.001059	0.561749986	1.108425788	0.561749986	1.10842579
% Myc	-0.0025385	0.003216858	-0.789125	0.474182	-0.011469951	0.006392947	-0.01146995	0.00639295

Rhapsody: S. nodosa: Total P & %MC

SUMMARY OUTPUT





Regression Statistics	
Multiple R	0.5748392
R Square	0.3304401
Adjusted R Square	0.1630502
Standard Error	0.1682188
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.05586151	0.055862	1.974074	0.232716139
Residual	4	0.113190321	0.028298		
Total	5	0.169051831			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.000%*	*Upper 95.000%*
Intercept	-3.8828816	3.53000241	-1.099966	0.333097	-13.68375979	5.917996639	-13.6837598	5.91799664
% Myc	0.0843921	0.06006479	1.405017	0.232716	-0.082374867	0.251159011	-0.08237487	0.25115901

12.5 The results of a *t*-test to determine whether there was a significant difference between the two slopes of the variation in total P in shoots of Elsanta plants colonised by *S. nodosa* or *A. scrobiculata*

	Elsanta	Total P			100		
AMF	% Myc	A. scrobiculata			1	2	
AS1	22.38254	0.805044		Sum x2	5420.186	11315.53	
AS2	5.73917	0.267184		Sum xy	124.8505	392.7083	
AS3	51.06118	0.977927		Sum y2	3.138661	13.84474	
AS4	12.24732	0.574532		n	6	6	
AS5	45.14324	0.985611		b	0.023034	0.034705	
AS6	9.545526	0.401649		Res SS	0.262809	0.2157	
	% Myc	S. nodosa		Res df	4	4	
SN1	43.71041	1.469684					
SN2	47.15061	1.800084					
SN3	33.83316	0.985611		Res MS	0.059814		
SN4	28.82804	0.659053					
SN5	45.85957	1.596466		s(b1-b2)	0.00404		

SN6	55.70379	2.118958		t	-2.88886		
				v	8		
				Table t	2.306		
				Calc t>Table t			
				Therefore reject null hypothesis			
				, i.e. the two slopes are not equal			

12.6 Calculated *t* statistics for the one-tailed hypothesis that the mean SDW, RDW, total P and P concentration of plants showing visible colonisation by *A. scrobiculata* was greater than the mean of each variable in plants showing no colonisation by the fungus. In each case, the calculated *t* statistic is smaller than the critical *t* statistic indicating that the presence of the mycorrhizal colonisation had no significant effect on either SWD, RDW, total P and P concentration.

Rhapsody: A.scrobiculata			
SDW: Any difference between plants with			
no %MC and those with MC			
	Is x1 > x2		
	x1	x2	
	0.3382	0.3752	
	0.3655	0.2323	
	0.2352		
	0.2596		
n	4	2	
v	3	1	
mean	0.299625	0.30375	
SS	0.370681	0.194738	
sp(2)	0.141355		
s(x1 - x2)	0.325601		
t	-0.01267		
Crit t	2.132		
t < Crit t			

Rhapsody: A.scrobiculata			
RDW: Any difference between plants with			
no %MC and those with MC			
	Is x1 > x2		
	x1	x2	
	0.0298	0.0304	
	0.0448	0.0246	
	0.013		
	0.0177		
n	4	2	
v	3	1	
mean	0.026325	0.0275	
SS	0.003377	0.001529	
sp(2)	0.001621		
s(x1 - x2)	0.034864		
t	-0.0337		
Crit t	2.132		
t < Crit t			

Rhapsody: A.scrobiculata			
Total P: Any difference between plants with			
no %MC and those with MC			
	Is x1 > x2		
	x1	x2	
	0.843462	0.977927	
	0.931825	0.574532	
	0.578374		
	0.793518		
n	4	2	
v	3	1	
mean	0.786795	0.77623	
SS	2.543913	1.286428	
sp(2)	0.957585		

Rhapsody: A.scrobiculata			
P Conc.: Any difference between plants with			
no %MC and those with MC			
	Is x1 > x2		
	x1	x2	
	2.493974	2.606415	
	2.549452	2.473234	
	2.459073		
	3.056695		
n	4	2	
v	3	1	
mean	2.639799	2.539824	
SS	28.11004	12.91029	
sp(2)	10.25508		

s(x1 - x2)	0.84746		
t	0.012467		
Crit t	2.132		
t < Crit t			

s(x1 - x2)	2.773321		
t	0.036049		
Crit t	2.132		
t < Crit t			

12.7 Number of oospores observed in root tips of non-mycorrhizal Elsanta plants and those colonised by *A. scrobiculata* and *S. nodosa* inoculated with increasing levels of zoospores of *P. fragariae*

Highest-Lowest			Ranks		
50000/0	50000/SN	50000/AS	50000/0	50000/SN	50000/AS
33	30	23	1	2	6.5
28	26	21	3	4	9
25	16	15	5	10.5	13.5
23	16	15	6.5	10.5	13.5
22	15	14	8	13.5	16
10	15	12	23	13.5	19
9	13	11	26.5	17	21
7	12	9	33	19	26.5
4	12	8	43.5	19	30
3	10	7	50	23	33
3	10	6	50	23	35.5
2	9	5	58.5	26.5	39
1	9	5	66	26.5	39
1	8	5	66	30	39
0	8	4	110	30	43.5
0	7	3	110	33	50
0	6	3	110	35.5	50
0	5	3	110	39	50
0	5	2	110	39	58.5
0	4	2	110	43.5	58.5
0	4	1	110	43.5	66
0	3	1	110	50	66
0	3	1	110	50	66
0	3	0	110	50	110
0	3	0	110	50	110
0	2	0	110	58.5	110
0	2	0	110	58.5	110
0	2	0	110	58.5	110
0	2	0	110	58.5	110
0	2	0	110	58.5	110
0	1	0	110	66	110
0	1	0	110	66	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110

0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
0	0	0	110	110	110
		n	50	50	50
		R	4400	3106	3819
		N	150		
		12/N(N+1)	0.00053		
		sumRi	871839.9		
		3(N+1)	453		
		H	8.901955		
		m	15		
		sum T	513894		
		C	0.847728		
		Hc	10.50095		
		v(=k-1)	2		
		Chi	5.991		
		Reject the null hypothesis			
		There is a difference in Oospore no.			

	SE		Arrange rank sums in increasing order of magnitude				
p=3	307.2051		Sn	As	Control		
p=2	205.1422		3106	3819	4400		
			Rx-Ry	SE	p	q	Table q
		Con v Sn	1294	307.2051	3	4.212169	3.314
		Con v As	581	205.1422	2	2.832181	2.772
		As v Sn	713	205.1422	2	3.475637	2.772

12.8 Chi-square test to determine any significant differences in the number of root tips containing oospores of *P. fragariae* in non-mycorrhizal Elsanta plants and those colonised by *S. nodosa* or *A. scrobiculata*.

Observed	Inf Roots	Non-inf R	
Control	14	36	**50**
SN	32	18	**50**
AS	23	27	**50**
	69	**81**	**150**
Expected	Inf Roots	Non-inf R	
Control	23	27	
SN	23	27	
AS	23	27	
	Chi Prob	**0.001471**	