# SOME ASPECTS OF THE COILED SPROUT CONDITION OF POTATOES CAUSED BY *VERTICILLIUM NUBILUM*

By

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C O N T E N T S

SECTION 1.a.

ABSTRACT OF THESIS

The incidence of coiled sprout in potatoes has been associated with several factors. The main aims of the investigations described in this thesis were to study further the effects of inoculation treatment with V. nubilum and seed storage treatment in relation to the occurrence of the disorder.

The results of inoculation tests with V. nubilum confirmed that the fungus is pathogenic to potatoes, giving rise to symptoms in the form of light brown lesions on the stem bases. The fungus was found to be confined to the cortical tissues of the host. The browning was either superficial or accompanied by longitudinal and transverse crackings, with or without russetting, and was readily distinguished from the dark brown well-defined lesions caused by R. solani but not from those caused by O. pustulans in the absence of russetting. The extent of surface browning with V. nubilum was more with soil-borne than with seed-borne inoculum. The fungus was observed to persist in a sterilized soil culture for at least 3 years, but in a field survey of its incidence in east Scotland it was not isolated from any of the 35 crops examined.

Infection by V. nubilum was associated with an increased incidence of coiled sprout. This relationship was less evident in glasshouse studies where seed tubers were disinfected with an organo-mercurial solution before inoculation. This effect may be related to the persistence of mercury in the tubers. Low traces of mercury were noted to retard growth of the pathogen in Czapek Dox agar and the growth was completely inhibited with higher values of mercury.

There was generally more coiling in stem bases of plants from

seed tubers sprouted in light than from desprouted or unsprouted seed tubers. Different varieties of potatoes showed great differences in susceptibility to coiling caused by sprouting, but tended to show very little differences in response to inoculation with V. nubilum. The increased coiling associated with sprouting had no apparent effect on tuber number or final weight yield but inoculation with V. nubilum appeared to cause reduction in both.

Observations on the various forms of coiling suggested that coiling induced by sprouting tubers in light before planting and that due to infection by V. nubilum occur independently. Coiling accompanied by fasciation and splitting or, more rarely, by fasciation only were found to be associated with sprouting seed tubers in light before planting whereas coiling accompanied by swelling and splitting but without fasciation was characteristic in the case of infection by V. nubilum. Coiling with swelling only was found in all treatments but mainly occurred in association with V. nubilum: this type of coiling also occurred occasionally in absence of V. nubilum in unsprouted and desprouted tubers, but the causal factor in this instance is not known.

# SECTION 1.

## INTRODUCTION.

## 1.1. General Review.

Detailed descriptions of the sprout abnormality in potatoes referred to as "coiled sprout" have appeared only recently in published records. Pitt, Hardie, Hall and Graham (1964) described the coiled or twisted sprout condition as one where sprouts coil below soil level, the area of curvature often being swollen. The sprouts bear light brown lesions, usually on the inside of the coil. It was also noted that, in severe cases, the lesions were accompanied by transverse and longitudinal cracks which may contain white spore-bearing mycelium. In a later paper (Pitt, Hardie, Hall and Graham, 1965), the same workers reported that, where sprouts have been badly affected, side shoots develop below the soil level and these can continue to grow normally. Further accounts of coiled sprout were given by Moorby (1965) and Moorby and McGee (1966). According to Moorby and McGee (1966), the coiled sprout disorder first becomes evident in the field as a very uneven emergence of the crop and, when affected plants do emerge, they produce many more stems than would be expected from the number of growing sprouts on the tubers planted. The sprouts lose their negatively geotropic habit and produce tight coils; they are often fasciated and, in extreme examples, the stems are split. The coiling was associated with the loss of normal apical dominance relationships of the sprout and was considered to allow stolons to become negatively geotropic and emerge, so producing multi-stemmed plants.

Pitt et al. (1965) referred to coiled sprout as having been observed sporadically throughout Britain on a number of varieties but they made no assessment of its economic significance. According to Milthorpe and Moorby (1966), coiled sprout is found mainly in the United Kingdom but has also been reported from the Channel Islands and the north-west mainland of Europe. Moorby and McGee (1966) indicated that the condition has been reported with increasing frequency in recent years from the main areas of early potato production in Britain and that the disorder may cause a financial loss of the order of £30 per acre, based on estimates by an Eastern Region N.A.A.S. survey (N.A.A.S. Eastern Region Crops Group, 1964). From the observations of Moorby and McGee, coiling delays emergence and, in turn, growth of new tubers, while the large number of stems increases the number of tubers formed with no equivalent increase in yield; hence the mean tuber size is reduced and the time to lifting of marketable tubers lengthened. Since early varieties are most often affected, the lower economic returns may be associated with this delay in tuber bulking. Lapwood, Hide and Hirst (1967) have also noted that the disorder has become more common and that, by delaying the emergence of sprouts and tuberisation, coiled sprout can cause a considerable financial loss, especially to growers of first early varieties. Among the varieties found to be most affected may be included Arran Pilot, Duke of York and Ulster Premier, while locally grown seed in England shows a great tendency to coil than does seed from Scotland (Moorby and McGee, 1966).

With respect to the causal nature of coiled sprout, several factors have been associated with the condition. In studies carried out by Pitt et al. (1964, 1965), Verticillium nubilum Pethybr. was isolated from coiled sprouts and the fungus was shown to cause the disorder. Field observations were reported which confirmed that the condition is commonly associated with this fungus (Pitt et al., 1965). However, V. nubilum was considered unlikely to be the sole cause, but these workers were unable to produce coiling in tests with herbicides or with compacted soil in pots (Pitt et al., 1965). On the other hand, Moorby and McGee (1966) failed to produce coiling by infection of sprouts with spores of V. nubilum and their attempts to isolate the fungus from coiled sprouts were successful in only a small percentage of cases and then no more than in uncoiled sprouts. From studies on the effects of different sprouting treatments, they concluded that the major factor controlling the appearance of coiling and fasciation was the degree of development of the sprouts at planting, the larger and more developed the sprouts the greater the coiling. It was also suggested that another factor contributing to the production of coils is low soil temperature after planting, but this only appeared to have an effect when sprouts were quite small. Despite their failure to find any relationship between V. nubilum and coiling, Moorby and McGee (1966) did not rule out possible later effects of the fungus after physiological changes had occurred in the sprout apex, or the alternative possibility that coiling may be induced independently by either some

physiological change in well developed sprouts or by V. nubilum. Lapwood et al. (1967) confirmed that sprouting of seed tubers in light increases coiling, that not all coiled sprouts bear lesions and that V. nubilum can be isolated from many lesions on coils. They also suggested that soil compaction and deep planting increase the incidence of the abnormality.

In considering these different factors which have been associated with coiled sprout, no single theory of a causal mechanism emerges which would seem to satisfy all the findings to date, and as recently as 1967 the view was expressed by Lapwood et al. that the etiology of coiled sprout is not yet determined. The various findings concerning the causal nature of the disorder may be broadly grouped into those which indicate a non-pathogenic origin, with a physiological basis associated with certain methods of seed treatment before planting or certain soil conditions after planting, or that a fungal pathogen, V. nubilum, is involved.

## 1.2. Seed treatment and soil factors in relation to coiled sprout.

The relationship between coiled sprout and planting seed tubers sprouted in light has been observed in several experiments (Moorby and McGee, 1966; Lapwood et al., 1967) and Moorby and McGee found that, over a wide range of sprout sizes, the amount of coiling was a function of sprout size at planting. Thus, where tubers were stored at different temperatures in light, there was in

general a tendency for higher temperatures to give larger and more developed sprouts at planting and, in turn, a greater incidence and intensity of coiling after planting. Moreover, from general observations, the varieties found to be most affected are those which tend to produce large sprouts at planting, and locally grown seed in England, which produces larger sprouts, was reported to show a greater tendency to coil than seed from Scotland. The relationship between coiling and sprout length, however, was not considered a direct one as the parts which coil are those in the apical bud at the time of planting and hence contribute only a small amount to the total sprout size. It appeared more likely that the correlation between sprout size and coiling reflected the changing metabolism of the elongating regions of sprouts with their increase in length, these regions developing in such a way as to produce a greater tendency to coiling.

The relationship between coiling and the attainment of a certain sprout size or degree of sprout development may thus be based on associated physiological changes at the sprout apex. Where tubers were sprouted in darkness and a large amount of tip death occurred less coiling was observed than after storage in light, where the original sprout apices continued to grow (Moorby and McGee, 1966). It was reported that sprouts and branches which start to grow after tip death coil only infrequently: this suggested that the physiological changes leading to coiling do not take place in inhibited buds, and that the buds remain, therefore, in an earlier phase of development which proceeds only when the buds are released

from inhibition. The view that coiled sprout arises from a form of physiological ageing is also attributed to Burton (Cherry, 1965). The internal reactions concerned in the changes, however, are not known (Moorby and McGee, 1966). In summarizing the position, Moorby and McGee stated that the development predisposing the sprouts to coil and fasciate appears to proceed throughout storage and that the size of the sprout no doubt reflects differences in metabolic patterns in both tuber tissue and growing regions of the sprout and in the supply of substrates and growth factors to these regions; just what reactions are involved, however, remain unknown.

In their account of coiled sprout Pitt et al. (1964, 1965), did not report any incidence of fasciation either alone or in association with coiling although they observed swelling of the coiled part of the stem. Moorby (1965) and Moorby and McGee (1966), however, reported that sprouts that produce coils are often fasciated and that the relation between fasciation and sprout size and development followed patterns similar to those between coiling and sprout size and development. Although these results might suggest that coiling and fasciation were 2 different expressions of the same controlling processes, Moorby and McGee (1966) found little or no correlation between the intensity of coiling and the intensity of fasciation and suggested that the extents to which sprouts become coiled or fasciated are controlled by different processes. According to White (1948), fasciation is a morphological term used to describe a series of abnormal growth phenomena resulting from many different causes and leading to a flattening of the main axis

of the plant. Although the expansion of the stem is often the most striking feature of this condition, all parts of the plant may be affected. He reported that the apical growing region may become linear and comb-like or develop numerous growing points, producing a witches'-broom effect. In other instances the growing point may coil or be distorted into a tangle of coils. White (1948) classified fasciations into the following 5 groups according to their causal factors:

1) Genetically induced fasciations which breed true, as in Pisum sativum umbellatum (Mummy pea) and Nicotiana tabacum fasciata.

2) Fasciations that arise due to various natural environmental causes, the selfed seed of which does not reproduce this character, i.e. fasciations in Erigeron canadensis ascribed to injuries caused by a Dipteran fly Cecidomyia erigeroni and effects related to time of planting and spacing of plants, as in flax stems in Formosa.

3) Fasciations that occur spontaneously, the initial cause of which is unknown, but which have been propagated vegetatively. Propagation through seed is unknown or imperfectly investigated, as in the cristate form of Opuntia serpentina (Boxing Glove Cactus).

4) Fasciations that are induced artificially by known procedures and which possibly, in some cases, have been asexually propagated, e.g. medium X-ray dosage of soaked seed of Helianthus annuus, crushing the young stems of Viola tricolor and cutting off the root tips of Vicia faba have resulted in fasciation.

5) Fasciations that have not been investigated experimentally and which remain unclassified as to their transmissibility, i.e. in

the common dandelion (Taraxacum officinale), sweet potato (Ipomoea batatas), pumpkin (Cucurbita pepo). Hard winters, severe pruning, insect mutilation, fungal and bacterial infections, unfavourable growth conditions abruptly succeeded by favourable conditions, soil friction, nematodes, chromosome aberration, and highly-manured or very rich soil have been suggested or regarded as causes. Horsfall and Dimond (1959) reported that, aside from gene mutation, fasciation may result from environmental effects such as frost, pressure, alteration of food and water relationships, pruning, mutilation as well as from more specific etiological agencies such as Corynebacterium fasciens and X-irradiation. Nutritional changes due to correlative disturbances in growth substance relationships appear to play a role in the incidence of fasciation (Horsfall and Dimond, 1959).

Based on unpublished observations of Morris, Moorby and McGee (1966) noted that another factor which may contribute to the production of coiled sprout in potato is low soil temperature after planting, but in their own experiments planting temperature only had an effect when the sprouts were quite small. The mechanism controlling this effect was suggested to be possibly related in some way to the slower growth rate of emerging sprouts at the lower temperatures and hence the increased time to emergence.

Lapwood et al. (1967) showed that incidence of coiled sprout was increased by chitting the seed, planting deeply and compacting the soil. From unchitted seed, coiled stems were produced only on rolled soil, while coiling was most abundant with plants from chitted

seed in rolled soil. Rolling the soil increased the incidence of coiling and fasciation more than did chitting but very few coils were produced in chitted seed planted 1 cm. deep, even in rolled soil.

## 1.3. Verticillium nubilum Pethybr. and its association with coiled sprout

V. nubilum is a species of the genus Verticillium (Nees) which belongs to the family Moniliaceae of the order Moniliales of the group Fungi Imperfecti. The genus is distinguished by its creeping, septate and sterile hyaline or slightly coloured hyphae bearing erect and branched conidiophores. Branches of the first order are whorled, opposite or alternate; branches of the second order are whorled, dichotomous or trichotomous on branches of the first order. Further branches are similar to those of the second order with the terminal ones usually flask-shaped and distinctly pointed at the apex. Conidia are always borne singly on the branchlets and soon fall away. They are round, elliptical, ovate, inverted egg-shaped or short spindle-shaped, hyaline or slightly coloured (Gilman, 1957).

The species V. nubilum was first recorded by Pethybridge (1919) who accidentally discovered it in Ireland on surface of an injured blighted potato tuber in 1916. Pethybridge (1919) reported that it has aerial growth and conidiophores like V. albo-atrum Reinke and Berthold, but with larger conidia. It forms a large

number of chlamydospores which are spherical and hyaline at first but soon develop thickish, dark brown or black walls and which may be borne singly or in groups of up to seven or so. They may be terminal but are frequently intercalary in rows of 3 or 4 or more. The hyphae bearing these chlamydospores remain visible for a long time. The chlamyospores are usually absent in media containing gelatine. Isaac (1953a) confirmed the classification of V.nubilum by Pethybridge and stated that it is characterized by producing a large number of chlamydospores which are distinguishable from those produced by other species of the genus, V. nigrescens Pethybr. and V. tricorpus Isaac, by their large size. V. nubilum produces no other type of resting body, whereas V. tricorpus forms microsclerotia and resting mycelium and V. dahliae Kleb. and V. albo-atrum, which do not form chlamydospores, produce microsclerotia and resting mycelium respectively. It is further distinguished from V. nigrescens by the presence of hyphae among the chlamydospores in old cultures (Isaac, 1953a). He summarised the characteristics of the species of the genus Verticillium as shown in Table 1.

When he first isolated the fungus, Pethybridge (1919) made no reference to the extent of its incidence in stocks of potato. Isaac (1953a), isolated the fungus from wilted potato stalks from Kent and reported working with a culture of the fungus obtained from wilting tomato plants, said to have been inoculated from infected soil of a strawberry field in Kent, as well as a culture from Central Bureau Voor Schimmelculture, Baarn. Pitt et al. (1965)

reported the presence of the fungus in association with coiled sprout in potatoes in the field but did not indicate its general incidence. In a survey of the incidence of Verticillium species in seed potato stocks in Britain in 1965, MacGarvie and Hide (1966) found V. nubilum, V. nigrescens and V. tricorpus present in 10·2, 8·5 and 72·5 per cent of the stocks respectively, but V. albo-atrum and V. dahliae were not found. The 3 species found were isolated more often from sprouts and the rose end of the tuber than from the heel end. MacGarvie (1965) has also isolated V. nubilum from a potato sample from the Netherlands.

A considerable number of inoculation experiments were carried out with V. nubilum on living potato stalks and tubers by Pethybridge (1919). In no case was infection found to occur and there was no indication of any ability of the fungus to invade or grow in the vessels of the wood such as characterizes the parasite V. albo-atrum. Isaac (1953a), however, reported the development of wilting from inoculation of potatoes and tomatoes with the fungus, thus establishing the first record of its role as a plant pathogen. He reported that the introduction of the fungus into the plant through a wound gave a more rapid development of a vascular wilt than through growing plants in contaminated soil. The vascular wilt in potato and tomato plants caused by V. nubilum showed a standard sequence of events which were summarized by Isaac (1953a) as follows:

1) The lower leaves of infected plants lost pigmentation, became dessicated and dropped.

2) The next immediately higher leaves followed the same pattern

Table 1.

Comparison of species of Verticillium

| | V.dahliae | V.albo-atrum | V.nigrescens | V.nubilum | V.tricorpus |
|---|---|---|---|---|---|
| Resting bodies | Micro-sclerotia | Resting mycelium | Chlamydo-spores (diam.) (7-10μ) | Chlamydo-spores (diam. ) (8·5-15·5μ) | Microsclerotia resting mycelium and chlamydospores (diam.7·5-11·0μ) |
| Colour of young Prostrate hyphae | White | White | White | White | Yellow |
| Hyaline sectors | Very frequent | Frequent | Never recorded | Very frequent | Very frequent |
| Optimum temperature for growth (°C) | 22·5 | 20·0-22·5 | 22·5-25·0 | 20·0-22·5 | 20·0-22·5 |
| Growth in culture at 30°C | Fairly good | Nil | Fairly good | Nil | Only fair |
| Optimum pH for growth | 5·3-7·2 | 8·0-8·6 | 5·3-7·2 | 7·2-8·6 | 7·2-8·0 |
| Growth at pH 3·6 | Fairly good | Fair | Good | Very poor | Yeast-like mass |
| Growth in ammonium nitrate medium | Nil | Nil | Nil | Fairly good | Nil |
| Best source of carbon | Sucrose and dextrose | Glycerine | Sucrose and dextrose | Sucrose and dextrose | Sucrose, dextrose, maltose and Glycerine, |
| Increase in concen-tration of peptone | Stimulates growth | Stimulates growth | Stimulates growth | Slows down growth | Slows down growth |
| Host range | Wide | Wide | Fairly wide | Tomato and potato only | Tomato only |
| Host reaction | Rapid | Usually very rapid | Fairly rapid | Slow | Slow |

(After Isaac, I. (1953a); Trans. Brit. mycol. Soc., 36, 180-195)

of symptoms and fell away. This process continued till the whole plant was defoliated.

3) The wood of the host plant was discoloured by the fungal mycelium together with gum.

4) The presence of fungal hyphae in the xylem tissue of the host stimulated the production of tylosis.

According to Isaac (1953a), the pathogen was entirely confined to the internal tissues of the host xylem and he made no reference to external symptoms of necrosis. To conclude, he reported that the 4 preceding symptoms of wilt are typical reactions by many hosts to parasitism by species of Verticillium and added that since V. nubilum and V. tricorpus are pathogenic to a narrow range of host plants, and since the external symptoms of disease are induced only after a protracted time, it appears evident that these isolates are weaker pathogens than V. albo-atrum, V. dahliae and V. nigrescens.

Pitt et al. (1964, 1965) confirmed that V. nubilum was pathogenic to potatoes but did not reproduce the pattern of disease development described by Isaac (1953a). They reported that infected plants from tubers, inoculated by dipping in a spore suspension of the fungus, did not show wilting but, instead, the coiled sprout disorder in potatoes and that the organism also caused external symptoms in the form of necrotic lesions usually to the inside of the coil. Affected sprouts showed typical symptoms including swelling, coiling, suberisation and necrosis. The apices of many of the young sprouts were killed and others showed a reticulate russet-coloured roughening of the entire surface. Lesions

on larger sprouts varied in size from 1 or more millimetres to several centimetres in length and again had a roughened, reticulate appearance. Sprouts also bore transverse and longitudinal lens-shaped cracks, a number of which contained the sporing fungus (Pitt et al., 1965). The mycelium was found to penetrate within and between the first 4 or 5 layers of the cortex. They reported the re-isolation of V. nubilum from the necrotic lesions in the sprouts. Pitt et al.(1965) observed that coiling was obtained from a stock of undisinfected tubers planted in sterilized as well as unsterilized soil, while tubers from the same stock disinfected in a solution of methoxyethyl mercuric chloride (100 ppm. Hg) for 15 minutes showed no coiling when planted in both types of soil. They concluded that, since the disease control was obtained by disinfecting naturally contaminated tubers with an organo-mercurial solution immediately before planting, it appears that the pathogen is carried in either the soil adhering to tubers or as a superficial infection or both. They also noted that J.E.E. Jenkins from England found that mercurial dipping at lifting reduced the disorder in Craigs Royal variety by 98 per cent. However, a more recent report by Jenkins showed that dipping at lifting may not always be successful in controlling the coiled sprout (Pitt et al., 1965).

MacGarvie and Hide (1966) found potato shoots inoculated with V. nubilum showed severe browning and cracking but they did not report any production of coiling. Lapwood et al. (1967) confirmed that V. nubilum can be isolated from many lesions on coils, but

their observations showed that not all coiled sprouts bear lesions. Moorby and McGee (1966) failed in their attempts to induce coiling in plants from previously inoculated mother tubers, although they reported a very slight contamination of the fungus in coiled and uncoiled potato sprouts.

Other fungi which have been found associated with browning of potato sprouts include Oospora pustulans Owen and Wakefield, Rhizoctonia solani Kühn and Colletotrichum atramentarium (Berk. & Br.) Taubenh (Hirst and Salt, 1959, and Salt, 1964). Hirst and Salt (1959) reported that lesions caused by O. pustulans are at first small discrete light brown spots. These later coalesce to form brown patches which sometimes occupy the whole cortex but do not penetrate deeper. Later, the colour darkens and small transverse cracks appear in the epidermis and eventually deep and wide longitudinal cracks may develop in the cortex, which can then be detached easily to expose the clean vascular tissue. Lesions caused by R. solani were described as having a deeper penetration with more discrete and dark coloured margins. Infection by C. atramentarium of the cortical tissue of the potato stem may occur from the soil level down to the mother tuber and cause the outer tissue to peel off easily (McKay, 1955), but Hirst and Salt (1959) rarely found the fungus in actively growing sprouts. Although these fungi have been associated with browning of the stem bases none of them is reported to cause coiling in potato sprouts.

The experimental work in the present study was designed to investigate further the possible association of V. nubilum with the

coiled sprout disorder and also the effect of seed tuber treatment on the incidence of coiling. The work may be broadly divided into the following sections:

1) Glasshouse studies on the effects of seed tuber inoculation with V. nubilum, seed storage treatments and seed disinfection on the incidence of coiled sprout (Section 3).

2 ) A survey of the incidence of coiled sprout and of fungi associated with brown lesions on potato stem bases in crops grown in east Scotland (Section 4).

3) Field studies on the effects of seed tuber inoculation with V. nubilum, seed storage treatments and seed disinfection on the incidence of coiled sprout and yield in different varieties (Section 5).

# SECTION 2.

# GENERAL MATERIALS AND METHODS.

## 2.1. Methods of seed tuber inoculation with V. nubilum

The cultures of V. nubilum used in the first inoculation tests were derived from isolates provided by Dr. Q.D. MacGarvie, Department of Agriculture and Fisheries for Scotland, East Craigs, Edinburgh. These isolates had been made from lesions on potato sprouts of a stock grown near Arbroath, in the county of Angus, in 1965 (MacGarvie and Hide, 1966) and were maintained on Czapek Dox agar medium. For later work, cultures of the fungus were used which had been re-isolated from lesions on stems of inoculated potato tubers.

In preparing a suspension of the fungus for inoculation, 3-4 weeks old cultures grown on Czapek Dox agar were used. The growth was transferred with a sterile scalpel into tap water and agitated in an auto-mixer to give a uniform suspension. The concentration of the suspension was assessed on the basis of the number of chlamydospores per ml. and then diluted to give about $6 \times 10^6$ spores per ml. Tubers for the glasshouse experiments (Section 3.) were inoculated by immersing in the suspension for 5 minutes immediately before planting. In the field experiments (Section 5.) sterilized John Innes field soil No.1 was added to the suspension to make up a slurry in which the tubers were immersed for 1 minute.

## 2.2. Methods of disinfection of seed tubers

In most cases, the seed tubers planted in the experiments were surface disinfected either before inoculation with V. nubilum

or before other experimental treatments were applied. Disinfection was usually carried out by dipping the tubers in a solution of methoxyethyl mercuric chloride (150 ppm. Hg) for 1 minute, in the case of glasshouse experiments (Section 3.), and 30 seconds, in case of field experiments (Section 5.). With the glasshouse experiments the tubers were washed in water before disinfection. After dipping, the tubers were allowed to dry for at least 2 days and then washed in an attempt to remove residual traces of mercury, except in Sections 3.5. and 5.2. where no washing was carried out after disinfection.

In some of the glasshouse work (Sections 3.6. and 3.7.), 2 per cent formalin and 70 per cent alcohol were used as disinfectants in place of the organo-mercurial solution. The period of dipping in the disinfectant was 5 minutes with alcohol and 2 minutes with formalin and the tubers were allowed to dry for at least 1 hour before further treatments were applied.

## 2.3. Methods of isolating V. nubilum from stem lesions

For the recovery of V. nubilum from plants from inoculated seed tubers or for detecting its presence in field work, the following 2 methods of isolation were used:

Method A

Small pieces of stem bases, about 1 cm. long, which showed brown lesions were washed in tap water, dipped in a 1/20 solution of Chloros (Sodium hypochlorite solution) for 30-60 seconds and

finally washed in 2 changes of sterilized distilled water. These pieces were then placed in Petri dishes with Czapek Dox agar and incubated for 5-10 days at 20-23°C.

Method B

Pieces of stem bases, about 2 cm. long, which showed surface lesions were washed and sterilized in Chloros for 2 minutes. After re-washing in sterilized distilled water, the pieces were placed on damp paper towelling in sterilized plastic boxes, each containing layers of damp cotton wool under its lid and incubated for 2-4 days at 23°C. The fungal hyphae usually started to emerge after 48 hours and the sporulating hyphae could be seen later under the microscope. Spores could then be picked off with fine forceps and streaked on Czapek Dox agar plates. Pure cultures were more readily obtained by this method than from method A, which often gave bacterial contamination.

These 2 methods were also used for isolating other species of fungi from the different experimental treatments or in the field survey (Section 4).

## 2.4. Method of assessment of coiling

From preliminary observations, varying degrees of deviation from a straight vertical axis may be observed in the direction of the underground stem growth of potatoes. In the early experiments (Section 3.1.), the scale 0-4 for the varying degrees of coiling was used, as illustrated in Fig.1. In all other experiments,

however, only categories 3 and 4 were classified as showing coiling, as it was felt that only marked deviations of more than $90^{o}$ from the vertical axis could validly be described as coiled.

In 1967, records were also made of the incidence of fasciation and splitting and the occurrence of these abnormalities in association with coiling are illustrated in Fig. 2, where the following forms of coiling are shown:

A. Coiling associated with fasciation, which may extend for a short distance above the soil level. The fasciated part is also split and such a splitting may occur throughout the whole of the fasciated part or be confined to the region of the coil. In extreme cases, after coiling, the growing tip of the sprout may pass through this split and may emerge or coil again.

B. This type of coiling is similar to A, except that the fasciated part is intact but flattened and swollen with a clean line marking the apparent fusion of 2 stem axes.

C. Coiling in this category shows no fasciation but is seen to be associated with swelling accompanied by splitting on either side of the coil, although most often on the concave side.

D. D is similar to C, except that the coiling is not accompanied by any splitting.

The incidence of fasciation and splitting without coiling were also recorded in 1967.

Where the incidence of coiling or any other abnormality in plants or stem bases was calculated on a percentage basis the original percentages were transformed into the corresponding angles

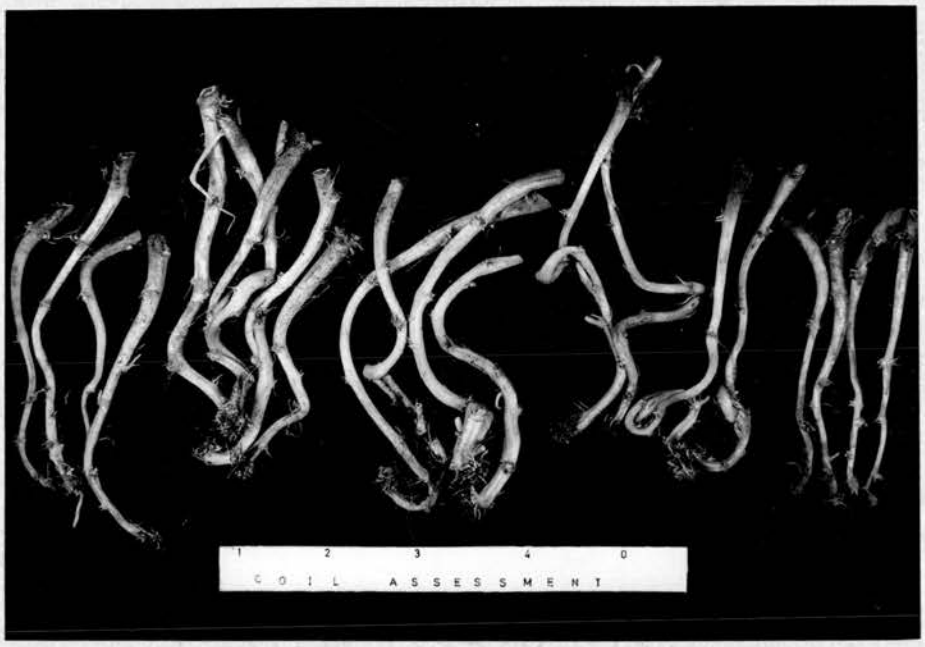


Fig. 1. Intensity of coiling (Scale 0-4)

1. Very slight )
2. Slight ) Less than 90° from vertical
3. Moderate )
4. Severe ) Over 90° from vertical
0. Normal

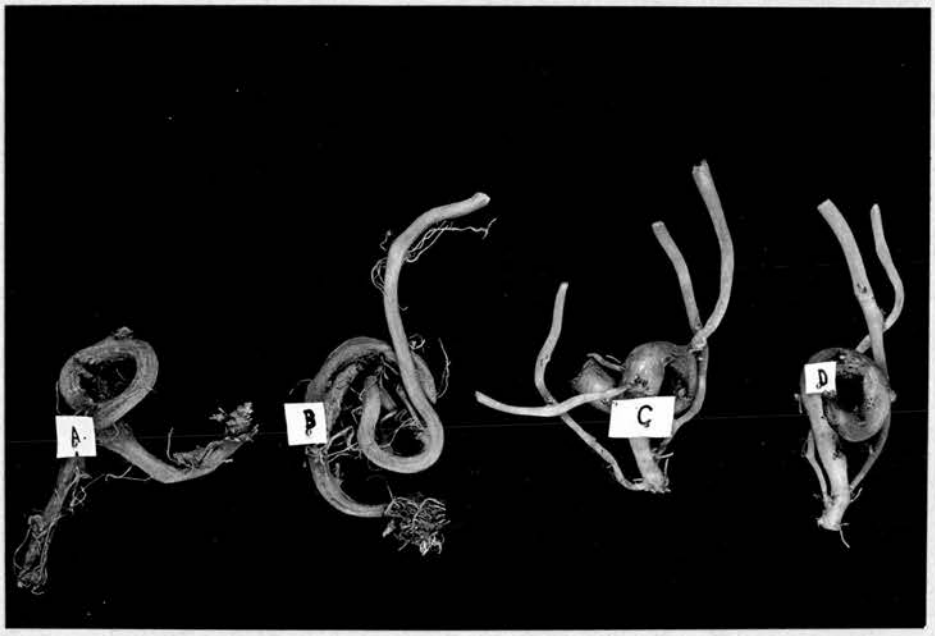


Fig. 2. Forms of coiling

A. Coiling, fasciation and splitting

B. Coiling and fasciation

C. Coiling and splitting

D. Coiling only

for purposes of statistical analysis. In presenting the results the transformed values and their equivalent de-transformed percentages are given in Tables in the text and the original percentages given in tables in the Appendix. This procedure is also adopted in the case of the percentage of stem bases showing brown lesions.

## 2.5. Assessment of incidence of brown lesions

In the field investigations, there was a general incidence of brown lesions on stem bases which may be mainly attributed to infection by various fungal contaminants and it was not possible to relate their occurrence to experimental treatments. In glass-house experiments, where greater control of fungal contamination was possible, counts were made of the numbers of stem bases showing brown lesions in relation to the various experimental treatments and, in some cases, assessments of the surface area affected by browning were made by recording the number of stem bases in the following categories:

a) Clean sprouts.

b) Up to 10 per cent of the surface area affected.

c) 10-25 per cent of the surface area affected.

d) Over 25 per cent of the surface area affected.

The various forms of lesions observed in this study are illustrated in Fig. 3. as follows:

0. No brown lesions.

1. Superficial browning.

2. Superficial browning with splitting.

3. Superficial browning with splitting and russetting.

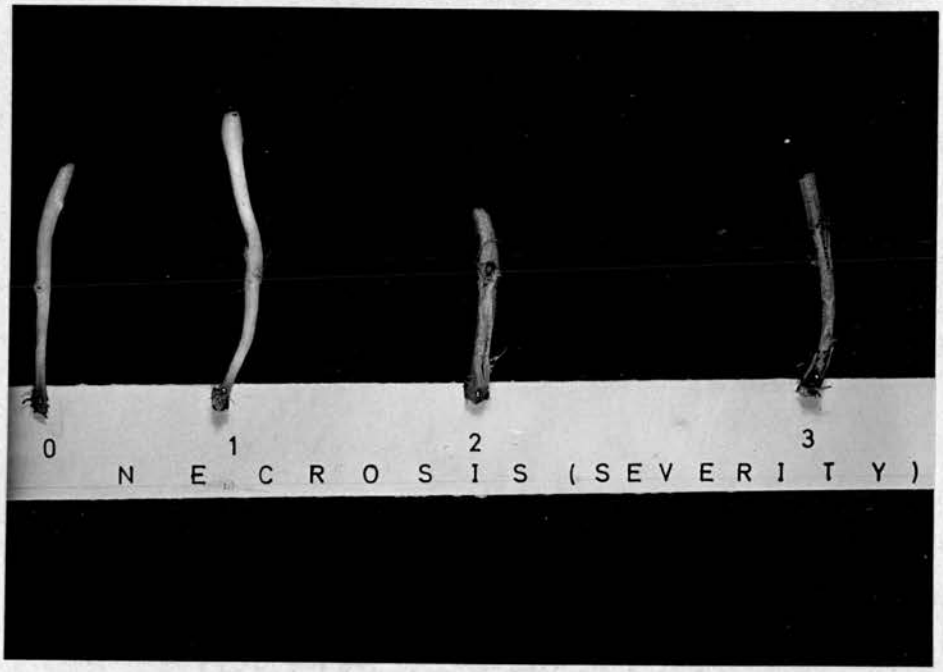


Fig. 3. Forms of brown lesions on stem bases

0. No brown lesions

1. Superficial browning

2. Browning and splitting

3. Browning, splitting and russetting

# SECTION 3.

# GLASSHOUSE AND LABORATORY STUDIES ON THE EFFECTS OF SEED TUBER INOCULATION WITH *VERTICILLIUM NUBILUM*, SEED STORAGE TREATMENT AND SEED DISINFECTION ON THE INCIDENCE OF COILED SPROUT

## 3.1. Inoculation tests with *V. nubilum*

### 3.1.1. Introduction

Over the period October, 1965 - March, 1966, a series of pot experiments was carried out to study the effect of artificial inoculation of seed tubers with *V. nubilum* on subsequent sprout growth. The main object of the investigation was to examine the findings of previous work (Pitt *et al.*, 1964 and 1965), which indicated a causal relationship between this fungus and incidence of coiled sprout.

### 3.1.2. Materials and methods

Cultures of *V. nubilum* derived from isolates made from potato plants in 1965 (Section 2.1.) were used in 2 seed tuber inoculation tests planted on 29 October (Experiment 1a) and 21 January (Experiment 1b). In a third test (Experiment 1c), tuber inoculation was carried out with cultures of the fungus isolated from lesions developed on the sprouts of inoculated tubers of experiment 1a. In a fourth investigation tubers were planted in the same soil as had been used in experiment 1a, in order to test for the presence of any residual soil-borne inoculum (Experiment 1d).

The tubers used in each experiment were from the same stock of the variety Arran Pilot, harvested in early October 1965 and held in commercial bulk storage prior to sampling. Following the sampling, the tubers were washed and surface disinfected by dipping in an organo-mercurial solution (Section 2.2.). The inoculation of seed tubers in experiments

1a, 1b and 1c was carried out by dipping them in a spore suspension of V. nubilum containing $6{\cdot}0 \times 10^6$ chlamydospores per ml. The tubers were then immediately planted in sterilized John Innes field soil No.1 in 7 in. sterilized pots, with a 2 to 3 in. soil covering, and the soil kept damp by regular watering. Other tubers were dipped in an autoclaved chlamydospore suspension of the fungus with the same concentration and planted as controls (Section 2.1.).

In all experiments the date of first leaf emergence above the soil level was recorded for each pot, and each plant was examined 25 days after its emergence. The incidence of coiling was recorded on the scale 0-4 (Section 2.4., Fig. 1.) and a count was made of the number of stems showing brown lesions (Section 2.5., Fig. 3.). The isolation of fungi from brown lesions, with particular reference to V. nubilum, was carried out by the 2 methods described in Section 2.3.

The details of each experiment were as follows:

Experiment 1a:

A sample of tubers was obtained from bulk storage on 12 October, 1965, disinfected and allowed to dry. In order to reduce any possible delay in sprout growth, at such an early date after harvest, the tubers were held in a container and subjected to a dormancy breaking treatment with Rindite (7:3:1 parts by volume of ethylene chlorohydrin, ethylene dichloride and carbon tetrachloride) for 1 week (Whitehead, McIntosh and Findlay, 1953). The tubers were then washed and

stored at a temperature of 36-40°F (2·2-4·4°C) until 29 October, when 20 tubers were inoculated with V. nubilum and planted, while a further 18 tubers were planted as controls. Half the inoculated and half the control tubers were placed in a random manner in a heated glasshouse at 51-67°F (10·6-19·4°C) and the remainder placed in an unheated glasshouse at 32-54°F (0-12·2°C) until 29 November, when they were transferred to the heated glasshouse.

Experiment 1b:

Forty tubers, obtained from bulk storage on 12 October and stored at 36-40°F (2·2 - 4·4°C), were washed and disinfected on 18 January. On 21 January, half the tubers were inoculated with V. nubilum and planted while the remaining tubers were planted as controls. Ten inoculated and 10 control pots were placed at random in a glasshouse at 50-70°F (10·0-21·1°C) and the remainder in a second glasshouse at 42-60°F (5·6-15·6°C).

Experiment 1c:

A further 20 tubers, obtained on 12 October and stored at 36-40°F (2·2-4·4°C), were washed and surface disinfected on 2 February. On 4 February, 10 tubers were inoculated with V. nubilum spore suspension made up from cultures of the fungus isolated from infected sprouts of experiment 1a and planted. The remaining 10 tubers were planted as controls and all pots were kept in a glasshhuse at 50-70°F (10·0-21·1°C).

Experiment 1d:

Twenty tubers were obtained from bulk storage on 12 October and stored at 36-40°F (2·2-4·4°C) until 18 December when they were washed and disinfected. They were then held at a temperature of 50-60°F (10·0-15·6°C) in sterilized plastic boxes with transparent covers before planting in soil of 10 of the pots of inoculated and 10 of the pots of the control tubers from experiment 1a, 21 days after the old plants had been removed. Due to differences in time of removal of plants in experiment 1a, planting of tubers in this experiment extended over a period of 18 days (4-21 January). Owing to this delay in planting, sprout growth of 1-1·5 cm. was evident on some of the later planted tubers. In the 3 previous experiments, tubers were unsprouted at the time of planting.

3.1.3. Results

From Table 2, it may be seen that a proportion of tubers in most of the experiments failed to show emergence and, on examination at the end of the experiments, were found to be decayed with symptoms of soft rot. The fairly high incidence of rotting may have been due to the washing or wetting of tubers before planting encouraging bacterial infection, but there was no evidence to show that the incidence of soft rot was increased by the inoculation treatment. In all experiments, the dates of emergence were variable with no consistent trend in relation to the experimental treatments. The late emergence

in experiment 1a (ii) may be related to low soil temperatures following planting, while the rapid emergence in experiment 1d may be attributed to the tubers showing some degree of sprouting at planting time.

Table 2.

Experimental treatments and plant emergence.

| Experimental details | | | Inoculation treatment | Mean sprout length at planting cm. | No. of tubers planted | No. of emerged plants | Average no. of days to first leaf emergence |
|---|---|---|---|---|---|---|---|
| No. | Date of planting | Glasshouse temperature °F | | | | | |
| 1a (i) | 29 Oct. | 51-67 | Seed inoculated | 0·0 | 10 | 4 | 36·5 |
| | | | Control | 0·0 | 9 | 6 | 25·3 |
| 1a (ii) | 29 Oct. | 32-54 / 51-67 | Seed inoculated | 0·0 | 10 | 7 | 61·1 |
| | | | Control | 0·0 | 9 | 6 | 53·8 |
| 1b (i) | 21 Jan. | 50-70 | Seed inoculated | 0·0 | 10 | 10 | 38·2 |
| | | | Control | 0·0 | 10 | 7 | 52·0 |
| 1b (ii) | 21 Jan. | 42-60 | Seed inoculated | 0·0 | 10 | 10 | 31·0 |
| | | | Control | 0·0 | 10 | 10 | 34·7 |
| 1c | 4 Feb. | 50-70 | Seed inoculated | 0·0 | 10 | 9 | 40·3 |
| | | | Control | 0·0 | 10 | 6 | 42·5 |
| 1d | 4-21 Jan. | 46-70 | Soil inoculated | 1·5 | 10 | 10 | 14·8 |
| | | | Control | 1·0 | 10 | 9 | 16·2 |

The results in Table 3 show that no coiling was formed in experiments 1a and 1c but 6 of each of the inoculated and control treatments in 1b showed coils and, in experiment 1d,

coiling occurred in 7 of the 10 inoculated and 3 of the 9 control plants. The Table also shows the actual number of stems found to be coiled, indicating that affected plants may show more than 1 coiled stem.

Table 3.

Incidence of coiling in relation to inoculation with V. nubilum.

| Experiment | | Inoculation treatment | No. of plants showing coiling as a fraction of the total no. of plants | No. of stem bases showing coiling as a fraction of the total no. of stem bases. |
|---|---|---|---|---|
| 1a | (i) | Inoculated | 0/4 | 0/45 |
| | | Control | 0/6 | 0/58 |
| | (ii) | Inoculated | 0/7 | 0/67 |
| | | Control | 0/6 | 0/60 |
| 1b | (i) | Inoculated | 2/10 | 2/53 |
| | | Control | 2/7 | 4/36 |
| | (ii) | Inoculated | 4/10 | 5/85 |
| | | Control | 4/10 | 7/66 |
| 1c | | Inoculated | 0/9 | 0/44 |
| | | Control | 0/6 | 0/27 |
| 1d | | Inoculated | 7/10 | 26/170 |
| | | Control | 3/9 | 10/115 |

The incidence of slight coiling in relation to the inoculation treatment is shown in Appendix 1, but the results showed no consistent differences between treatments.

Brown lesions were observed on the stem bases of most of the plants in the inoculated treatments and occasionally in the controls (Table 4). V. nubilum was isolated from majority of

plants in the inoculated treatments, together with R. solani in some cases, whereas only R. solani was isolated from the control treatments. In a few instances, the isolation methods failed to show the presence of any fungal species associated with lesions.

Table 4.

Incidence of brown lesions on stem bases and associated fungal isolates in relation to inoculation treatment with V. nubilum.

| Experiment | | Inoculation treatment | No. of plants showing brown lesions on underground stem bases as a fraction of the total no. of plants | No. of plants from which fungi were isolated from lesions | |
|---|---|---|---|---|---|
| | | | | V. nubilum | R. solani |
| 1a | (i) | Inoculated | 4/4 | 4 | 2 |
| | | Control | 3/6 | 0 | 2 |
| | (ii) | Inoculated | 5/7 | 5 | 1 |
| | | Control | 2/6 | 0 | 2 |
| 1b | (i) | Inoculated | 10/10 | 8 | 1 |
| | | Control | 2/7 | 0 | 2 |
| | (ii) | Inoculated | 9/10 | 9 | 0 |
| | | Control | 2/10 | 0 | 1 |
| 1c | | Inoculated | 8/9 | 8 | 4 |
| | | Control | 3/6 | 0 | 3 |
| 1d | | Inoculated | 9/10 | 9 | 2 |
| | | Control | 4/9 | 0 | 3 |

### 3.1.4. Conclusions

The results of this series of experiments failed to provide any evidence of a causal relationship between coiled sprout and *V. nubilum* as reported by Pitt *et al.* (1964, 1965). In experiment 1b, a number of plants from both the inoculated and control tubers showed coiling. The only other experiment where coiling occurred was 1d, where the higher incidence of coiled sprout from the inoculated treatment may be associated with the sprout length of the inoculated tubers at planting, which was generally greater than that of the control tubers (Table 2). This result would be in keeping with the findings of Moorby and McGee (1966), who considered that the degree of sprout development at planting was a major factor controlling the appearance of coiling.

The occurrence of brown lesions in association with *V. nubilum* confirmed previous observations on the pathogenicity of the fungus (Pitt *et al.*, 1964, 1965; MacGarvie and Hide, 1966). Browning was found where seed tubers were inoculated with a spore suspension of the fungus and also developed on stems growing in soil from which affected plants had been removed 3 weeks prior to planting surface disinfected tubers. With respect to the persistence of *V. nubilum* in soil, it was observed in incidental studies that the fungus could be re-isolated from inoculated soil previously sterilized and kept in a closed tube at room temperature for 3½ years. It appeared from observations

in these experiments that the lesions were more extensive from soil-borne inoculum than from seed-borne, although no actual assessments of the extent of browning were carried out.

## 3.2. Effects of soil and seed eye-core inoculation with *V. nubilum* on the incidence of coiling and brown lesions on stem bases.

### 3.2.1. Introduction

It was observed in the previous experiments (Section 3.1.), that browning appeared to develop more extensively on stem bases of plants from soil contaminated with *V. nubilum* than on plants where the inoculum was seed-borne. An experiment was therefore carried out over the period 18 February - 15 April, 1966, to study the effect of different methods of *V. nubilum* inoculation on the extent of symptom development on potato sprouts.

### 3.2.2. Materials and methods

In order to economize in space, eye-cores were used in place of whole seed tubers as planting material. Single eye-cores, 1 in. in length were taken on 20 February by means of a 5/8 in. sterilized cork borer from Arran Pilot tubers. The tubers had been obtained from a commercial bulk store on 12 October, 1965, and held at 36-40°F (2·2-4·4°C) until 18 February, when they were surface disinfected in an organo-mercurial solution. The cores were then stored at room temperature 50-60°F (10·0-15·6°C)

in clean plastic boxes with transparent covers for 3 weeks, when they were re-sterilized with the organo-mercurial solution. Two weeks later, on 31 March, the cores were washed and planted in 6 in. pots containing sterilized John Innes field soil No.1 with the following 4 treatments, using a V. nubilum suspension with a concentration of $6{\cdot}3 \times 10^6$ chlamydospores per ml. in all cases.

a) Soil drenched with a V. nubilum suspension at the rate of 50 ml. per pot 1 week before planting.

b) Soil drenched with a V. nubilum suspension at the rate of 50 ml. per pot immediately ~~before~~ after planting.

c) Eye-cores dipped in a V. nubilum suspension for 5 minutes immediately before planting.

d) Eye-cores dipped in an autoclaved V. nubilum suspension for 5 minutes and planted as controls.

Three cores were planted in each pot and 7 replicated pots were used for each treatment. The pots were arranged in a random manner in a glasshouse at a temperature of 50-70°F (10·0-21·1°C). At the time of planting the eye-cores, sprout growth up to 3 cm. in length was evident. It was observed that most of the main sprout tips were dead and that several secondary branches had grown up to about 2 cm. long. Assessments of coiling and of the incidence and extent of browning on the stem bases (Sections 2.4. and 2.5. respectively) were carried out after plant emergence.

3.2.3. Results

All the eye-cores showed plant emergence 14 days after planting, with the exception of 2 which had been dipped in a viable spore suspension and were found to have broken down completely with soft rot at the completion of the experiment. This, again, may be attributed to the wetting of the planting material having encouraged bacterial infection.

Coiling was observed only in plants of 2 of the treatments, on sprouts from 3 eye-cores where V. nubilum had been added to the soil immediately after planting and on sprouts from 2 eye-cores from the control treatment (Table 5).

Table 5.

Incidence of coiling in stem bases in relation to inoculation with V. nubilum.

| Inoculation treatment | No. of cores showing coiling as a fraction of the total no. of cores | No. of stem bases showing coiling as a fraction of the total no. of stem bases |
|---|---|---|
| a) Sterilized soil + V. nubilum suspension, added 1 week before planting | 0/21 | 0/83 |
| b) Sterilized soil + V. nubilum suspension, added immediately after planting | 3/21 | 5/102 |
| c) Eye-cores dipped in a V. nubilum suspension immediately before planting in sterilized soil | 0/19 | 0/102 |
| d) Eye-cores dipped in an autoclaved V. nubilum suspension immediately before planting in sterilized soil | 2/21 | 3/113 |

Brown lesions were found only once on sprouts from the control treatment, on 60 per cent of the sprouts from seed inoculation and on at least 90 per cent of sprouts from the soil to which V. nubilum had been added either 1 week before or immediately after planting (Table 6). The results also indicate that the surface area of stem bases affected by browning was negligible in the case of the control treatment and greater where the fungus had been added to the soil than where the eye-cores were inoculated with V. nubilum. With all 3 inoculation treatments the symptoms included surface necrosis in the form of superficial browning, splitting and deeper cracking and a roughened reticulate appearance of the surface. The increased extent of browning with soil inoculation compared with eye-cores inoculation was associated with a greater incidence of general surface necrosis rather than any increase in the incidence of splitting or cracking.

Using method B (Section 2.3.) for making isolations from the lesions on the stem bases, no isolate was obtained from the control, while 70 per cent of the lesions from soil inoculation 1 week before planting, 80 per cent from soil inoculation immediately after planting and 85 per cent from cores dipped in the viable spore suspension gave V. nubilum. No fungal isolates were obtained from clean sprouts in any treatment or brown lesions in the control.

Table 6,

Incidence of browning on stem bases in relation to inoculation with *V. nubilum*

| Inoculation treatment | Percentage of stem bases in each infection category in Section 2.5. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Area affected by browning | | | | Types of browning | | | |
| | Clean | up to 10 per cent | 10-25 per cent | Over 25 per cent | Clean | Surface browning only | Surface browning + splitting | Surface browning + splitting + russetting |
| a) Sterilized soil + *V.nubilum* suspension, added 1 week before planting | 2·1 | 25·9 | 44·0 | 28·0 | 2·1 | 58·0 | 25·9 | 14·0 |
| b) Sterilized soil + *V.nubilum* suspension added immediately after planting | 4·9 | 2·16 | 28·5 | 45·0 | 4·9 | 68·6 | 2·0 | 24·5 |
| c) Eye-cores dipped in a *V.nubilum* suspension immediately before planting in sterilized soil | 40·2 | 22·6 | 12·7 | 24·5 | 40·2 | 36·2 | 3·9 | 19·7 |
| d) Eye-cores dipped in an autoclaved *V.nubilum* suspension immediately before planting in sterilized soil | 99·1 | 0·9 | 0·0 | 0·0 | 99·1 | 0·9 | 0·0 | 0·0 |

### 3.2.4. Conclusions

As in Section 3.1., the results provided no evidence of causal relationship between *V. nubilum* and coiling. The incidence of the disorder was again low and occurred in only a small number of plants from 1 of the inoculation treatments and from the control. At the time of planting there was an appreciable amount of sprout growth on the eye-cores but this was associated with a high incidence of tip death, and Moorby and McGee (1966), in relating the incidence of coiling to sprout development at planting, have observed that sprouts which start to grow after tip death coil only infrequently.

The extent of surface necrosis on stem bases associated with *V. nubilum* was found to be greater where the inoculum was present in the soil than when it was carried on the eye-core, thus confirming the observations made in Section 3.1. One possible explanation for this is that the shoots are exposed to more frequent infection when they grow up through the inoculum than when the inoculum is carried from the seed tuber on the growing shoot.

## 3.3. Effect of sprouting seed tubers in light on the incidence of coiled sprout.

### 3.3.1. Introduction

Moorby and McGee (1966) indicated that the incidence of coiled sprout is associated with the planting of seed tubers sprouted in light and that the amount of coiling was a function of sprout size at planting. In an attempt to confirm this finding, an experiment was carried out over the period 3 January - 22 March, 1966, to study the effect of sprouting seed tubers in light on the incidence of coiling.

### 3.3.2. Materials and methods

On 3 January, 20 tubers of the variety Arran Pilot, which had been stored at 36-40°F (2·2-4·4°C) since 12 October 1965, were surface disinfected with organo-mercurial solution (Section 2.2.). Ten tubers were then stored at 36-40°F (2·2-4·4°C) and the remaining 10 were put in a chitting tray and placed in a glasshouse at 55-65°F (12·8-18·3°C). The tubers were planted on 31 January, when the mean length of the longest sprouts of the light-sprouted tubers was 1·4 cm., and the tubers stored at the low temperature were still unsprouted. Three of the light-sprouted tubers showed symptoms of sprout tip death and 2 of the unsprouted were discarded because of dry rot. Each tuber was planted in a 7 in. sterilized plastic pot, containing sterilized John Innes field soil No.1, at a depth of 2-3 in. below the soil surface and the pots

arranged randomly in a glasshouse at a temperature of 55-65°F (12·8-18·3°C). The incidence of coiling and brown lesions was recorded after plant emergence.

3.3.3. Results

Both treatments showed complete emergence and the average numbers of days to emergence were 24·7 and 39·3 for sprouted and unsprouted tubers respectively.

Coiling of the stem bases was observed on 5 of the 10 plants from tubers sprouted in light and on 2 of the 8 plants from unsprouted tubers (Table 7). The area of coiling was swollen in the coiled sprouts from both treatments, but there was no evidence of fasciation or splitting.

Table 7.

Incidence of coiling in stem bases in relation to sprouting treatments.

| Sprouting treatment | No. of tubers showing coiling as a fraction of the total no. of plants | No. of stem bases showing coiling as a fraction of the total no. of stem bases |
|---|---|---|
| Seed tubers sprouted in light at 55-65°F | 5/10 | 6/73 |
| Seed tubers stored at 36-40°F (unsprouted at planting) | 2/8 | 3/53 |

Brown lesions were observed in both treatments at the tips and on the underground part of the stems of all except 1 plant from the unsprouted treatment (Table 8). Attempts

were made to isolate fungi from the necrotic areas but the results were negative.

Table 8.

Incidence of brown lesions on stem bases in relation to sprouting treatments.

| Sprouting treatment | No. of plants with brown lesions as a fraction of the total no. of plants | No. of stem bases with brown lesions as a fraction of the total no. of stem bases |
|---|---|---|
| Seed tubers sprouted in light at 55-65°F | 10/10 | 64/73 |
| Seed tubers stored at 36-40°F (unsprouted at planting) | 7/8 | 43/53 |

3.3.4. Conclusions

The results of this experiment provided limited evidence that the incidence of coiling was increased where tubers were sprouted in light. The increase may have been greater, had there not been some tip death at planting. There was an extensive occurrence of brown lesions on the stem bases and sprout tips of both treatments, but these failed to show the presence of any fungal isolates. The lesions may possibly be associated with damage to the buds of the seed tuber eyes due to treatment with the organo-mercurial solution, which also appeared as the possible cause of the occurrence of sprout tip death in the sprouted tubers prior to planting. However, it

is difficult to explain why the incidence had been especially high in this experiment and not in others where similar treatments were applied.

## 3.4. Effect of storage treatment and seed tuber inoculation with *V. nubilum* on the incidence of coiled sprout.

### 3.4.1. Introduction

The results from Sections 3.1., and 3.3. provided some evidence of a relationship between the occurrence of coiled sprout and sprouting of seed tubers in light before planting, but failed to show any association between *V. nubilum* and coiling, although the fungus was found to cause brown lesions on the stem bases (Sections 3.1. and 3.2.). In the present investigation, a comprehensive study was made over the period 29 December 1965 - 1 July 1966, of the effects of storage treatment and inoculation of seed tubers with *V. nubilum* on the incidence of coiled sprout.

### 3.4.2. Materials and methods

One hundred and sixty tubers of the variety Arran Pilot, lifted in early October, 1965, and stored from 12 October at 36-40°F (2·2-4·4°C), were washed and disinfected with an organo-mercurial solution on 18 December. They were then divided into 4 lots of 40 tubers each for storage in clean and sterilized covered boxes at 4 different temperatures, 53-60°F (11·7-15·6°C), 46-50°F (7·8-10·0°C), 40-45°F (4·4-7·2°C) and 36-40°F (2·2-4·4°C). Nine weeks later, 20 of the tubers from each temperature range were selected at random and the length of sprout growth was recorded (Table 9). Following

the removal of sprout growth, the 4 lots of tubers were washed and placed in chitting trays in a glasshouse at 55-65°F (12·8-18·3°C) for sprouting in light for 13 weeks. On 27 May, the length of sprout growth in the remaining tubers from each of the 4 storage temperature treatments was recorded (Table 9) and the tubers washed after desprouting. On the same day, the lengths of the longest sprouts of the tubers sprouted in light were measured and counts made of the number of sprouts over 1 cm. in length per tuber (Table 9). One half of the tubers from each of these 8 storage treatments were then inoculated with V. nubilum by dipping in a spore suspension ($6{\cdot}0 \times 10^6$ chlamydospores per ml.) immediately before planting on 27 May. The remaining tubers were dipped in an autoclaved spore suspension of the fungus of the same concentration, before planting as controls. Each tuber was planted in a 7 in. sterilized plastic pot containing sterilized John Innes field soil No.1, 2-3 in. below the soil level. Half the pots from each of these 16 treatments (5 replicates per treatment) were placed in a glasshouse at 50-70°F (10·0-21·1°C) and the second half were placed in a second glasshouse at 45-55°F (7·2-12·8°C) which eventually rose to 50-70°F (10·0-21·1°C) after the first 2 weeks. The experiment thus comprised 8 storage treatments, 2 inoculation and 2 after-planting temperature treatments in an 8 x 2 x 2 factorial design. The plants were examined over the period 15 June - 1 July, following emergence, assessments were made of the incidence of coiling, fasciation and

splitting and of lesions on stem bases.

3.4.3. Results

When stored in the dark, the longest sprouts were produced by tubers stored at the 2 higher temperatures, which also gave a greater number of sprouts over 1 cm. (Table 9). All sprouts grown in darkness were removed before either sprouting in light or planting. With tubers sprouted in light for 13 weeks before planting, the mean lengths of the longest sprouts ranged from 2·8 to 5·1 cm., somewhat longer sprouts tending to be produced where tubers had been previously stored at higher temperatures (Table 9).

A complete emergence occurred in all treatments and the average number of days from planting to first leaf emergence for the various treatments is shown in Table 10. It was found, in general, that tubers sprouted in light before planting showed emergence about 1 week earlier than those desprouted just before planting, but there was no evidence that the inoculation treatment or the slight difference in glasshouse temperatures during the first 2 weeks after planting had any effect on emergence rate.

From the results of the assessments of the incidence of coiling in relation to the experimental treatments (Table 11), it may be seen that coiling was found in treatments where tubers had been sprouted in light before planting, whereas no coiling was recorded in any of the treatments where tubers had

Table 9.

Sprout length and number of sprouts per tuber in relation to storage treatment.

| Storage treatment | Average Length of of longest sprout at time of desprouting. cm. | Average No. of sprouts more than 1 cm. per tuber at desprouting time | After resprouting in light | |
|---|---|---|---|---|
| | | | Mean length of longest sprout at planting cm. | Average No. of sprouts per tuber at planting |
| Stored at 53-60°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 82·7 | 7·3 | 4·0 | 6·2 |
| Stored at 46-50°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 90·8 | 4·7 | 5·1 | 6·2 |
| Stored at 40-45°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 8·0 | 0·6 | 2·8 | 7·1 |
| Stored at 36-40°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 0·1 | 0·2 | 3·4 | 6·5 |
| Stored at 53-60°F for 22 weeks in dark, desprouted and planted | 131·1 | 11·2 | - | - |
| Stored at 46-50°F for 22 weeks in dark, desprouted and planted | 146·1 | 8·4 | - | - |
| Stored at 40-45°F for 22 weeks in dark, desprouted and planted | 39·8 | 7·1 | - | - |
| Stored at 36-40°F for 22 weeks in dark, desprouted and planted | 2·3 | 2·1 | - | - |

Table 10.

Average number of days to plant emergence in relation to storage treatment, inoculation with V. nubilum and glasshouse temperature during first 2 weeks after planting.

| Storage treatment | Glasshouse temperature during first 2 weeks after planting and inoculation treatment | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| Stored at 53-60°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 9·8 | 10·0 | 11·4 | 9·8 |
| Stored at 46-50°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 9·8 | 9·6 | 11·0 | 9·2 |
| Stored at 40-45°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 10·0 | 11·4 | 10·8 | 12·0 |
| Stored at 36-40°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 13·2 | 12·0 | 11·6 | 10·6 |
| Stored at 53-60°F for 22 weeks in dark, desprouted and planted | 18·0 | 17·6 | 18·0 | 17·8 |
| Stored at 46-50°F for 22 weeks in dark, desprouted and planted | 17·2 | 17·0 | 17·6 | 17·2 |
| Stored at 40-45°F for 22 weeks in dark, desprouted and planted | 18·4 | 17·8 | 18·4 | 18·8 |
| Stored at 36-40°F for 22 weeks in dark, desprouted and planted | 18·0 | 18·9 | 19·0 | 18·8 |

Table 11.

Number of plants showing coiling in relation to storage treatment, inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting (From 5 plants per treatment)

| Storage treatment | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| Stored at 53-60°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 2 | 2 | 3 | 3 |
| Stored at 46-50°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 2 | 2 | 3 | 0 |
| Stored at 40-45°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 3 | 4 | 3 | 2 |
| Stored at 36-40°F for 9 weeks in dark, desprouted and resprouted in light at 55-65°F for 13 weeks | 3 | 3 | 4 | 3 |
| Stored at 53-60°F for 22 weeks in dark, desprouted and planted | 0 | 0 | 0 | 0 |
| Stored at 46-50°F for 22 weeks in dark, desprouted and planted | 0 | 0 | 0 | 0 |
| Stored at 40-45°F for 22 weeks in dark, desprouted and planted | 0 | 0 | 0 | 0 |
| Stored at 36-40°F for 22 weeks in dark, desprouted and planted | 0 | 0 | 0 | 0 |

been desprouted before planting (Figs. 4a-5b). Only 1 sprouted treatment failed to show any coiling, namely where tubers were held at 46-50°F before being resprouted in light and planted as controls in the glasshouse at the lower temperature during the first 2 weeks after planting.

Since coiling was only obtained in tubers sprouted in light the results for sprouted tubers were considered separately from those of desprouted tubers for purposes of statistical analysis. Thus, in each part the analysis was carried out for a 4 x 2 x 2 factorial experiment (Cochran and Cox 1960).

The analysis of variance for the data within the sprouted treatments indicated that pre-sprouting storage temperatures, inoculation with V. nubilum and glasshouse temperature for first 2 weeks after planting had no significant effects on the incidence of coiling, based on the number of plants showing coiling (Appendix 3 ) or expressed as a percentage of the total number of stem bases showing coiling per treatment (Appendix 4).

In considering other abnormalities associated with coiling (Appendix 2), it was found that, of about 15 per cent coiled sprouts, 50 per cent showed coiling associated with fasciation and splitting, 7 per cent showed coiling associated with splitting only, 6 per cent showed coiling associated with fasciation only and 38 per cent showed coiling without an association with any other abnormality. In addition, 7 per cent of the total number

of stem bases showed fasciation and splitting without coiling (Appendix 2). From the analysis of variance of the data for the incidence of each of these abnormalities in relation to the experimental treatments, it was found that none of the treatments showed significant effects (Appendices 5, 7, 8 and 9) except in the case of coiling associated with splitting (Appendix 6). This was found only in the glasshouse kept at the higher temperature during the first 2 weeks after planting ($P = 0{\cdot}05$) although its incidence was very low (Table 12).

Table 12.

Effect of temperature after planting on the incidence of coiling associated with splitting in stem bases from sprouted tubers. (Transformed data given in brackets)

| Glasshouse temperature during first 2 weeks after planting | Percentage of stem bases showing coiling and splitting |
|---|---|
| 50-70°F | 0·21 (2·66) |
| 45-55°F | 0·00 (0·00) |
| Standard error | (± 0·85) |

The incidence of brown lesions on stem bases from sprouted tubers was significantly greater in treatments inoculated with *V. nubilum* than in the controls ($P = 0{\cdot}01$) as shown in Table 13 (Appendix 10).

Fig. 4a.

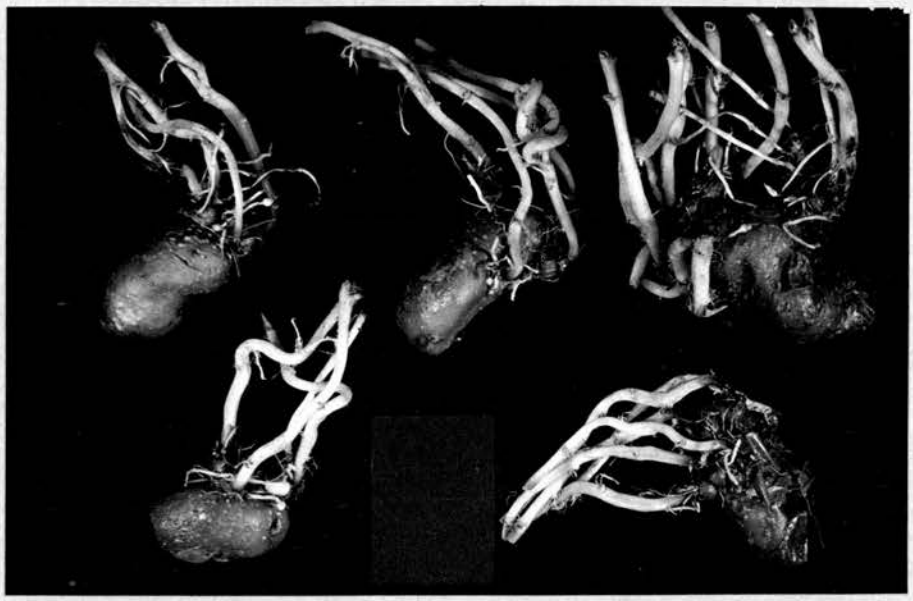

Fig. 4b.

Fig. 4a. Stem bases from tubers sprouted in light and inoculated with V. nubilum at planting.

Fig. 4b. Stem bases from tubers sprouted in light but not inoculated at planting (Control).

Symptoms of coiling present in both treatments; brown lesions present on stem bases from inoculated tubers but generally absent from control tubers.

Fig. 5a.

Fig. 5b.

Fig. 5a. Stem bases from desprouted tubers inoculated with V. nubilum at planting.

Fig. 5b. Stem bases from desprouted tubers not inoculated at planting (Control).

Coiling absent in both treatments; brown lesions present on stem bases from inoculated tubers but generally absent from control tubers.

Table 13.

Effect of inoculation with *V. nubilum* on the incidence of brown lesions on stem bases from sprouted tubers. (Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing brown lesions | |
|---|---|---|
| Inoculated | 99·75 | (87·12) |
| Control | 2·90 | (9·76) |
| Standard error | | (± 2·56) |

There was also a more frequent incidence of coiled stem bases showing brown lesions in inoculated treatments than in the control ($P = 0·001$) as shown in Table 14 (Appendix 11).

Table 14.

Effect of inoculation with *V. nubilum* on the incidence of coiled stem bases with brown lesions from sprouted tubers. (Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing brown lesions | |
|---|---|---|
| Inoculated | 8·70 | (17·13) |
| Control | 0·11 | (1·87) |
| Standard error | | (± 2·31) |

The analysis of variance for the data on the incidence of both brown lesions and brown lesions associated with splitting on stem bases from the desprouted tubers also showed that the inoculation treatment had a significant effect (Appendices 12 and 13). Tubers inoculated with *V. nubilum* gave a significantly

higher incidence of stem bases with brown lesions (P = 0·001) and with brown lesions and splitting (P = 0·01) than control tubers (Tables 15 and 16).

Table 15.

Effect of inoculation with V. nubilum on the general incidence of brown lesions on stem bases from desprouted tubers.
(Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing brown lesions |
|---|---|
| Inoculated | 77·0 (61·85) |
| Control | 0·70 (4·80) |
| Standard error | (± 2·65) |

Table 16.

Effect of inoculation with V. nubilum on the incidence of brown lesions associated with splitting on stem bases from desprouted tubers.
(Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing brown lesions associated with splitting |
|---|---|
| Inoculated | 0·85 (5·37) |
| Control | 0·00 (0·00) |
| Standard error | (± 1·36) |

From 180 isolation tests, 85 per cent of brown lesions on stem bases from inoculated treatments gave V. nubilum and 4 per cent gave R. solani, whereas 20 per cent of the control treatments gave R. solani and no fungal isolates were obtained from the remaining brown lesions.

### 3.4.4. Conclusions

In confirmation of the findings of Moorby and McGee (1966), the results of this experiment indicated a definite relationship between the incidence of coiling and the sprouting condition of the tuber at planting. Where tubers had been sprouted in light before planting, about half showed coiling in their sprouts whereas with tubers which had been desprouted at the time of planting no coiling occurred. This effect was shown over a wide range of temperatures either before sprouting in light or before desprouting and planting. Although the storage temperature differences before sprouting gave some variation in sprout length at planting, they had no significant effect on the incidence of coiling. The mean sprout lengths at planting for the various sprouting treatments, however, were all above 2 cm. and, according to Moorby and McGee (1966), the increase in percentage of sprouts coiling with increasing sprout size was found up to a range of 2 cm.

Moorby and McGee suggested that the development predisposing the sprouts to coil appears to proceed throughout storage and that the size of the sprout reflects differences in both tuber tissue and growing regions of the sprout and in the supply of substrate and growth factors to these regions. In view of the absence of any effect of marked temperature differences in the storage of tubers prior to sprouting in light on the incidence of coiling, it would appear the developmental changes leading

to coiling proceed in the growing regions of the sprout rather than in the tuber tissue. In all sprouted treatments, the slight temperature difference during the first 2 weeks after planting had no marked effect on the incidence of the abnormality. There was no definite evidence of an effect of V. nubilum on the incidence of coiling although the fungus was again associated with a high incidence of brown lesions on the stem bases.

## 3.5. Effects of organo-mercury disinfection of tubers naturally contaminated with V. nubilum on the incidence of coiled sprout and stem lesions

### 3.5.1. Introduction

In the previous tests on the pathogenicity of V. nubilum (Sections 3.1., 3.2. and 3.4.), artificial methods of inoculation with a spore suspension of the fungus were applied. In this experiment, carried out in the period 20 February - 1 May, 1966, stocks of seed tubers suspected of being naturally contaminated with V. nubilum were used and an attempt was made to investigate further the findings of Pitt et al. (1965), that control of the coiled sprout disorder in naturally contaminated tubers may be obtained by disinfecting them with an organo-mercurial solution immediately before planting.

### 3.5.2. Materials and methods

Samples of seed tubers of the variety Arran Pilot from 2 stocks, both of which were reported to have given symptoms of coiled sprout in the previous year, were obtained on 16 February, 1966. Tubers in 1 stock (A) were unsprouted, while those of the other (B) possessed etiolated sprouts averaging 3 cm. in length and were desprouted before applying the experimental treatments. One half of the tubers from each stock were surface disinfected in a solution of methoxyethyl mercuric chloride (150 ppm. Hg) for 1 minute, air dried at room temperature for 2 days and then planted in sterile 7 in. pots containing sterilized John Innes

field soil No.1. The remaining half of each stock was planted at the same time, but without washing or surface disinfection. Planting was carried out on 21 February for stock A and on 28 February for stock B, 28 tubers being used at the first date and 24 at the second. The pots were placed in a random manner in a glasshouse at 50-70°F (10·0-21·1°C). Following first leaf emergence, each plant was lifted and examined for the presence of coiling and brown lesions on the stem bases and attempts were made to isolate fungi associated with these lesions, using methods described in Section 2.3.

3.5.3. Results

A number of seed tubers failed to produce plants and were found to be rotted at the end of the period of plant examination (Table 17). The incidence of rotting was greater in the case of the disinfected treatment. The results in Table 17 show also that the average number of days from planting to first leaf emergence was about 5 days more for the disinfected tubers than for the untreated tubers.

Table 17.

Plant emergence in relation to seed tuber disinfection treatment.

| Stock | No. of emerged plants as a fraction of the total no. of tubers planted | | Average no. of days to first leaf emergence | |
|---|---|---|---|---|
| | Disinfected | Untreated | Disinfected | Untreated |
| A | 9/14 | 13/14 | 30 | 25 |
| B | 10/12 | 12/12 | 34 | 30 |

Fig. 6a.

Fig. 6b.

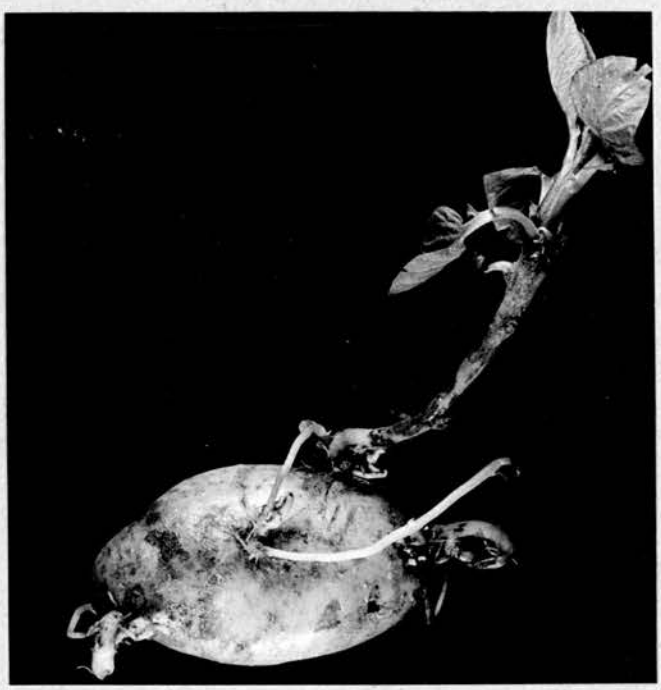

Fig. 6a. Symptoms of coiling on a stem base from a seed tuber disinfected in an organo-mercurial solution. Brown lesions absent on stem bases.

Fig. 6b. Symptoms of coiling on a stem base from untreated seed tuber.
Browning present on most stem bases with dark well defined lesions caused by R. solani.

No coiling was observed on plants from stock A, but 3 plants from the disinfected and 1 from the untreated tubers from stock B showed the coiled sprout condition (Table 18, Figs. 6a, 6b).

Table 18.

Incidence of coiled sprout in relation to seed tuber disinfection treatment.

| Stock | No. of plants showing coiled sprout as a fraction of the total no. of plants. | | No. of stem bases showing coiling as a fraction of the total no. of stem bases. | |
|---|---|---|---|---|
| | Disinfected | Untreated | Disinfected | Untreated |
| A | 0/9 | 0/13 | 0/39 | 0/46 |
| B | 3/10 | 1/12 | 4/56 | 2/55 |

Brown lesions were found on the underground stem bases of most plants from the untreated tubers but on only 2 of the 9 plants of 1 stock in the case of disinfected tubers (Table 19). In stock A, fungi associated with the brown lesions on stem bases from untreated tubers included V. nubilum, O. pustulans and R. solani. Only R. solani was found associated with lesions on stem bases from disinfected tubers of this stock (Table 19). In stock B, lesions were only found on stems from untreated plants and from these lesions V. nubilum, O. pustulans, R. solani and a species of Fusarium was isolated (Table 19).

Table 19.

Incidence of brown lesions on stem bases and associated fungal isolates in relation to seed tuber disinfection treatment.

| Stock | Disinfection treatment | No. of plants showing brown lesions on stem bases as a fraction of the total no of plants | No. of plants from which isolates obtained from brown lesions | | | |
|---|---|---|---|---|---|---|
| | | | V.nubilum | O.pustulans | R.solani | Fusarium sp. |
| A | (Disinfected | 2/9 | 0 | 0 | 2 | 0 |
| | (Untreated | 11/13 | 4 | 9 | 2 | 0 |
| B | (Disinfected | 0/10 | 0 | 0 | 0 | 0 |
| | (Untreated | 8/12 | 6 | 4 | 3 | 3 |

3.5.4. Conclusions

Both seed stocks A and B were found to be naturally infected with V. nubilum, but the results did not show any relationship between the presence of the fungus and the incidence of coiling. No coiling was found in 1 of the stocks and, in the other, the very slight incidence of coiling was more in evidence in plants from disinfected tubers than in those from untreated tubers. Disinfection of the seed tubers with an organo-mercurial solution immediately before planting checked the development of brown lesions associated with

V. nubilum, O. pustulans and a Fusarium species on stem bases, but some lesions associated with R. solani still occurred on sprout bases from disinfected tubers.

## 3.6. Effects of seed tuber inoculation with V. nubilum in relation to the method of disinfection before inoculation.

### 3.6.1. Introduction

In a consideration of the results of inoculation with V. nubilum (Sections 3.1., 3.2. and 3.4.), a possible reason which may be suggested for their failure to establish a causal relationship between the fungus and the coiled sprout, as reported by Pitt et al. (1964, 1965), was the use of organo-mercurial solution as a disinfectant prior to inoculation. In part of the work carried out by Pitt et al. (1965), 70 per cent ethyl alcohol had been used to disinfect seed tubers before inoculation (Pitt, 1967). This difference in disinfectants used in the respective experiments might account for the divergence in results, although it was established that the use of an organo-mercurial disinfectant allowed a successful carry-over of the fungus from inoculated tubers on to the stem bases which gave rise to the production of brown lesions. It was possible, however, that the use of mercury had exerted a residual effect which influenced the activity of the fungus on the sprouts or the reaction of the host tissues. Two experiments were therefore carried out in 1967 to study the effect of V. nubilum inoculation of seed tubers on subsequent sprout growth, using different disinfectants prior to inoculation. In addition studies were carried

out to assess the amount of residual mercury on tubers after disinfection with methoxyethyl mercuric chloride and its effect on growth of V. nubilum in artificial media. The experiments in this section may therefore be considered in 2 parts as follows:

Part i: The effects of different seed tuber disinfectants and V. nubilum inoculation on the incidence of coiled sprout and lesions on stem bases.

Part ii: The assessment of mercury residues in tubers disinfected with an organo-mercurial solution and their effects on growth of V. nubilum.

3.6.2. Materials and methods

Part i:

Experiment 1.

Sixty tubers of the variety Arran Pilot, lifted in early October 1966 and immediately stored at 36-40°F (2·2-4·4°C), were divided into 3 groups of 20 on 10 January, 1967, and subjected to the following 3 disinfection treatments before inoculation with V. nubilum:

a) Disinfected in methoxyethyl mercuric chloride solution (Section 2.2.).

b) Disinfected in 70 per cent ethyl alcohol (Section 2.2.).

c) Untreated.

Each of these 3 groups was then divided into 2 lots of 10, on 13 January, and 1 lot from each disinfection treatment was inoculated with a spore suspension of V. nubilum

($6 \cdot 3 \times 10^6$ chlamydospores per ml.) and planted in 7 in. sterilized pots containing sterile Vermiculite. The remaining tubers of the 3 groups were planted as untreated controls. All inoculated and control tubers were planted 2-3 in. below the Vermiculite level and the pots were arranged randomly in a glasshouse at a temperature of 43-65°F (6·1-18·3°C).

Experiment 2.

Seventy tubers from a different stock of the variety Arran Pilot, lifted in early October, 1966, and stored at 36-40°F (2·2-4·4°C) were divided into 5 groups of 14 on 11 March, 1967, for the following 5 disinfection treatments before inoculation with V. nubilum:

a) Disinfected in methoxyethyl mercuric chloride solution (Section 2.2.).
b) Disinfected in 70 per cent ethyl alcohol (Section 2.2.).
c) Untreated.
d) Disinfected in 2 per cent formalin (Section 2.2.).
e) Washed with water only.

On 14 March, 7 tubers from each of the 5 disinfection groups were dipped in a V. nubilum suspension ($5 \cdot 9 \times 10^6$ chlamydospores per ml.) and planted. The remaining tubers from each group were planted as uninoculated controls. The method of planting and glasshouse conditions were similar to those in experiment 1.

After emergence plants in each experiment were removed and examined and the incidence of coiling and brown lesions recorded. In addition, fungal isolates associated with the lesions were identified.

Part ii:

Forty tubers of the variety Arran Pilot, harvested on 10 October, 1967, and held at 36-40°F (2·2-4·4°C), were divided into 2 lots of 20 on 18 October. One lot was disinfected with methoxyethyl mercuric chloride solution (150 ppm. Hg) for 1 minute, dried at room temperature for 2 days and then washed with tap water. The second lot was only washed with tap water and then both lots of tubers were stored at 40-53°F (4·4-11·7°C). On 15 December, determinations of the amount of residual mercury present on 2 samples of tubers from each lot were carried out by the Chemistry Section, Department of Agriculture and Fisheries for Scotland, East Craigs, Edinburgh.

In a second study, a methoxyethyl mercuric chloride solution (150 ppm. Hg) was prepared on 18 October by dissolving 5·0 gms. of the proprietary compound Aretan in 1 litre of sterilized distilled water. From this solution, further solutions containing 15, 1·5 and 0·15 ppm. mercury were prepared using sterilized distilled water for dilution. One ml. of each of these 4 solutions was pipetted into each of 10 sterile Petri dishes and 1 ml. of sterilized distilled water was added to each of a further series of 10 dishes as controls. To each plate, 9 mls. of autoclaved Czapek Dox agar medium were then added when

the temperature of the medium was about 40°C. After the agar had set, each plate was inoculated in the centre with an 8 mm. disc of a 3 weeks old culture of V. nubilum in Czapek Dox agar medium. The inoculated plates were then incubated at 55-70°F (12·8-21·1°C) for 22 days, during which period the diameter of growth in each plate was measured daily.

3.6.3. Results

Part i:

Some tubers in the inoculated and several of the control treatments failed to show emergence and the majority of these were found to be rotted. This might in most cases be related to wetting the tubers before planting (Table 20). The emergence rate was irregular, tending to be slower in experiment 1 than in experiment 2 (Table 20). It also tended to be earlier where tubers were disinfected with the organo-mercurial compound. In experiment 2, there was a very slight tendency for inoculated tubers to show a slower rate of emergence and, in experiment 1, it was observed that 3 of the 5 tubers untreated before inoculation and 1 of the 3 alcohol disinfected and inoculated tubers which failed to show plant emergence above soil level produced sprouts which were coiled (Table 20).

Coiling did not occur on any of the plants grown from the uninoculated control tubers but was found to a varying extent in all except 1 of the treatments where tubers had been inoculated with V. nubilum before planting (Table 21). In both experiments, the disinfection with the organo-mercurial solution

Table 20.

Plant emergence in relation to different disinfection treatments and inoculation with V. nubilum.

| Disinfection treatment before inoculation | No. of emerged plants (no. of plants which failed to emerge but gave coiled sprout given in brackets) | | Average no. of days to first leaf emergence | |
|---|---|---|---|---|
| | Inoculated | Control | Inoculated | Control |
| Experiment 1. (10 tubers planted per treatment) | | | | |
| a) Methoxyethyl mercuric chloride | 9 | 10 | 39·1 | 39·8 |
| b) 70 per cent ethyl alcohol | 7(+ 1) | 7 | 45·0 | 45·1 |
| c) Untreated | 5(+ 3) | 10 | 46·6 | 44·3 |
| Experiment 2. (7 tubers planted per treatment) | | | | |
| a) Methoxyethyl mercuric chloride | 6 | 7 | 26·3 | 23·2 |
| b) 70 per cent ethyl alcohol | 5 | 5 | 28·0 | 26·0 |
| c) Untreated | 4 | 7 | 28·3 | 28·3 |
| d) 2 per cent formalin | 6 | 7 | 30·7 | 28·3 |
| e) Washed only | 5 | 6 | 29·2 | 23·2 |

tended to give a low incidence of coiling from inoculated tubers. In experiment 1, tubers disinfected with alcohol prior to inoculation gave the most frequent incidence of coiling. In experiment 2, the incidence of coiling was higher where tubers were washed only before inoculation than with the other treatments although the numbers of tubers involved in all cases were small (Table 21).

Table 21.

Incidence of coiled sprout in relation to different disinfection treatments and inoculation with *V. nubilum*.

| Disinfection treatment before inoculation | No. of plants showing coils as a fraction of the total no. of plants. | | No. of stem bases with coiling as a fraction of the total no. of stem bases. | |
|---|---|---|---|---|
| | Inoculated | Control | Inoculated | Control |
| Experiment 1. | | | | |
| a) Methoxyethyl mercuric chloride | 1/9 | 0/10 | 1/40 | 0/52 |
| b) 70 per cent ethyl alcohol | 7/8 | 0/7 | 11/38 | 0/29 |
| c) Untreated | 3/8 | 0/10 | 7/37 | 0/41 |
| Experiment 2. | | | | |
| a) Methoxyethyl mercuric chloride | 2/6 | 0/7 | 2/41 | 0/50 |
| b) 70 per cent alcohol | 3/5 | 0/5 | 3/16 | 0/17 |
| c) Untreated | 0/4 | 0/7 | 0/17 | 0/49 |
| d) 2 per cent formalin | 2/6 | 0/7 | 2/18 | 0/29 |
| e) Washed only | 5/5 | 0/6 | 12/39 | 0/32 |

Brown lesions were found on the stem bases of most plants from the inoculated treatments of both experiments and from a few plants of the control treatments, where tubers had been washed only or were untreated (Table 22). With inoculated treatments the proportion of stem bases showing brown lesions tended to be less for tubers disinfected with the organo-mercurial solution than for the other disinfection treatments (Table 22), although it may be seen that the total number of stem bases tended to be higher with this treatment. Most of

the browning was of a superficial type with a few plants showing splitting of the necrotic areas (Table 23). In the isolation of fungi from brown lesions, plants from all inoculated treatments of both experiments gave V. nubilum, with 2 from the washed only and 3 from the tubers untreated before inoculation also giving O. pustulans. From the 8 plants showing brown lesions in the control treatments, 6 gave O. pustulans and the remaining 2 gave no isolates. No splitting of stem bases was observed in the control treatments (Table 23).

Table 22.

Incidence of brown lesions on stem bases in relation to different seed tuber disinfection treatments and inoculation with V. nubilum.

| Disinfection treatment before inoculation | No. of plants showing brown lesions on stem bases as a fraction of the total no. of plants. | | No. of stem bases showing brown lesions as a fraction of the total no. of stem bases. | |
|---|---|---|---|---|
| | Inoculated | Control | Inoculated | Control |
| Experiment 1. | | | | |
| a) Methoxyethyl mercuric chloride | 9/9 | 0/10 | 30/40 | 0/52 |
| b) 70 per cent alcohol | 8/8 | 0/7 | 38/38 | 0/29 |
| c) Untreated | 8/8 | 4/10 | 35/37 | 14/41 |
| Experiment 2. | | | | |
| a) Methoxyethyl mercuric chloride | 6/6 | 0/7 | 27/41 | 0/50 |
| b) 70 per cent alcohol | 5/5 | 0/5 | 14/16 | 0/17 |
| c) Untreated | 4/4 | 2/7 | 17/17 | 10/49 |
| d) 2 per cent formalin | 5/6 | 0/7 | 15/18 | 0/29 |
| e) Washed only | 5/5 | 2/6 | 39/39 | 7/32 |

Table 23.

Number of stem bases showing browning and splitting in relation to different disinfection treatments and inoculation with *V. nubilum*.

| Disinfection treatment before inoculation | Inoculation treatment | | | | | |
|---|---|---|---|---|---|---|
| | Inoculated | | | Control | | |
| | No. of clean stem bases | No. of stem bases showing surface browning only | No. of stem bases showing surface browning + splitting | No. of clean stem bases | No. of stem bases showing surface browning only | No. of stem bases showing surface browning + splitting |
| Experiment 1. | | | | | | |
| a) Methoxyethyl mercuric chloride | 10 | 29 | 1 | 52 | 0 | 0 |
| b) 70 per cent alcohol | 0 | 33 | 5 | 29 | 0 | 0 |
| c) Untreated | 2 | 32 | 3 | 27 | 14 | 0 |
| Experiment 2. | | | | | | |
| a) Methoxyethyl mercuric chloride | 14 | 25 | 2 | 50 | 0 | 0 |
| b) 70 per cent alcohol | 2 | 10 | 4 | 17 | 0 | 0 |
| c) Untreated | 0 | 16 | 1 | 39 | 10 | 0 |
| d) 2 per cent formalin | 3 | 9 | 6 | 29 | 0 | 0 |
| e) Washed only | 0 | 30 | 9 | 25 | 7 | 0 |

Microscopic examination of stem lesions:

Cross sections made from stem bases obtained from tubers disinfected with the organo-mercurial solution in experiment 2 and showing brown lesions indicated that some fungal hyphae were present in the cortex (Fig. 7) using the stain mixture Pianese IIIb stain (Gatenby and Painter, 1937). These hyphae could be seen to occupy inter- and intra-cellular positions in the cortical tissue but did not penetrate into the xylem. There were no conidiophores to confirm the pathogen was a Verticillium species but, since only V. nubilum was isolated from the remainder of the stem, it was assumed that the fungus observed was V. nubilum.

Part ii:

From Table 24 it may be seen that tubers disinfected with an organo-mercurial solution had an average residual mercury value of 1·15 ppm. after 2 months storage at 40-53°F, while the control tubers had none.

Table 24.

Residual mercury in Arran Pilot tubers in relation to disinfection with methoxyethyl mercuric chloride solution (150 ppm. Hg).

| Disinfection treatment | Residual Hg in ppm. Replicate 1 | Replicate 2 | Mean |
|---|---|---|---|
| Tubers dipped in the methoxy-ethyl mercuric solution for 1 minute, dried and washed | 1·00 | 1·30 | 1·15 |
| Control tubers (Washed only) | 0·00 | 0·00 | 0·00 |

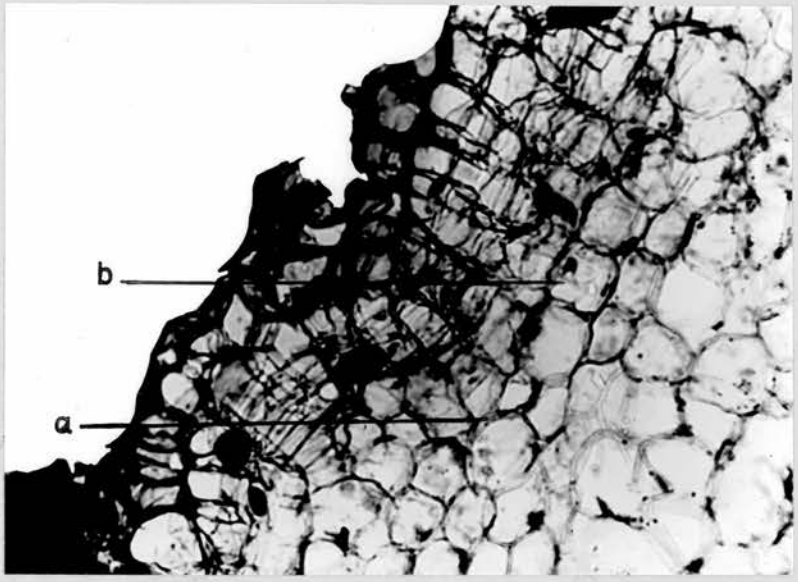


Fig. 7. Cross section of a stem base from a seed tuber inoculated with V. nubilum.

a) Mycelial hyphae in intercellular spaces of the cortex.

b) Mycelial hyphae within a cell in the cortex.

In considering the effect of varying concentrations of mercury in the growth medium on the growth of V. nubilum, the results illustrated in Fig. 8, (Appendix 14) show that measurable growth in the control plates started on the third day after inoculation. The earliest growth for treatments containing traces of mercury was on the fifth day in the media containing 0·015 and 0·15 ppm. mercury. Plates containing 1·5 ppm. showed a very slow growth rate and there was not a visible increase in the diameter of colonies until the fourteenth day after inoculation. From the time when growth became visible, the control medium and media with the 2 lowest concentrations of mercury gave progressive increases in colony sizes until, after 3 weeks, the fungus covered the area of the plates containing the control medium and extended to about two-thirds of the diameter of plates containing 0·015 or 0·15 ppm. mercury (Fig. 9a-c). In contrast, with 1·5 ppm. mercury growth was very slow and tended to be erect and fluffy rather than spreading (Fig. 9d). Where the mercury concentration was 15 ppm. no growth occurred and the inoculum eventually dried out and turned brown (Fig. 9e).

### 3.6.4. Conclusions

The results of the 2 seed tuber inoculation experiments, in Part i, provided evidence that V. nubilum is associated with the coiled sprout disorder in potatoes in confirmation of the findings of Pitt et al. (1964, 1965). However, the results

FIG. 8.

GROWTH OF V. NUBILUM IN RELATION TO CONCENTERATION OF MERCURY( PPM. ) IN CZAPEK DOX AGAR .

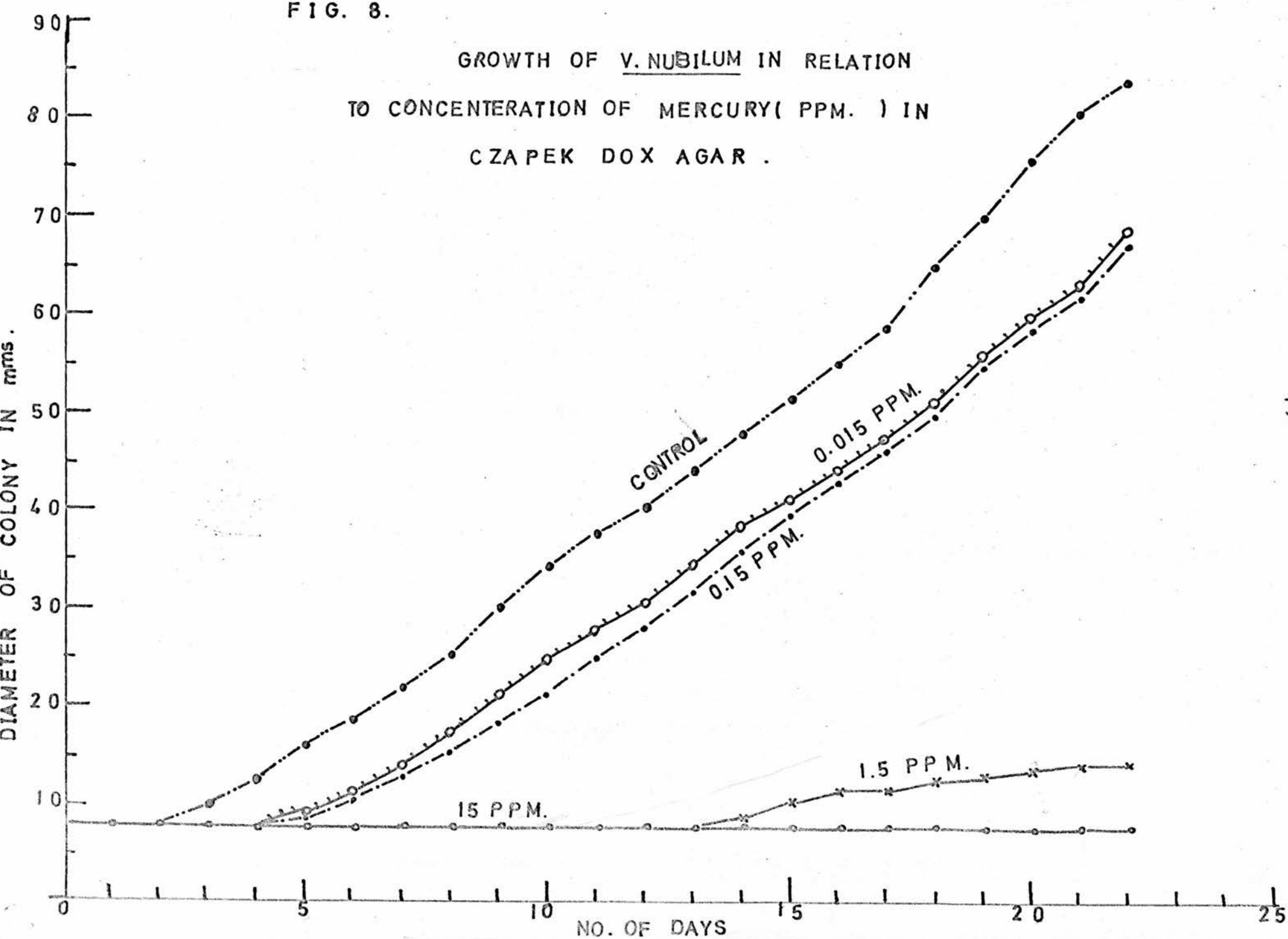

Fig. 9a.

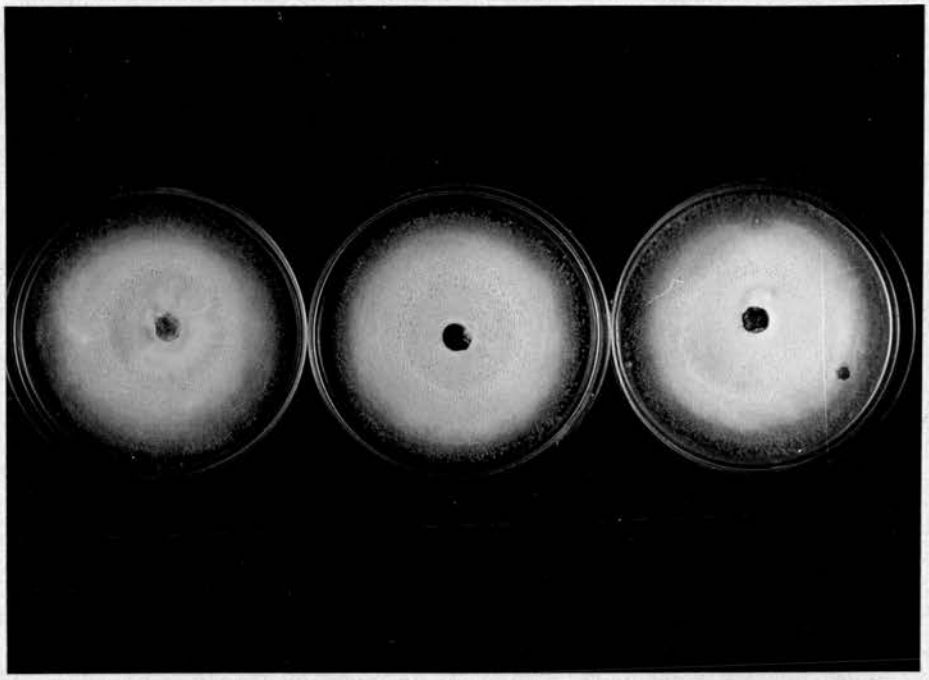

Fig. 9b.

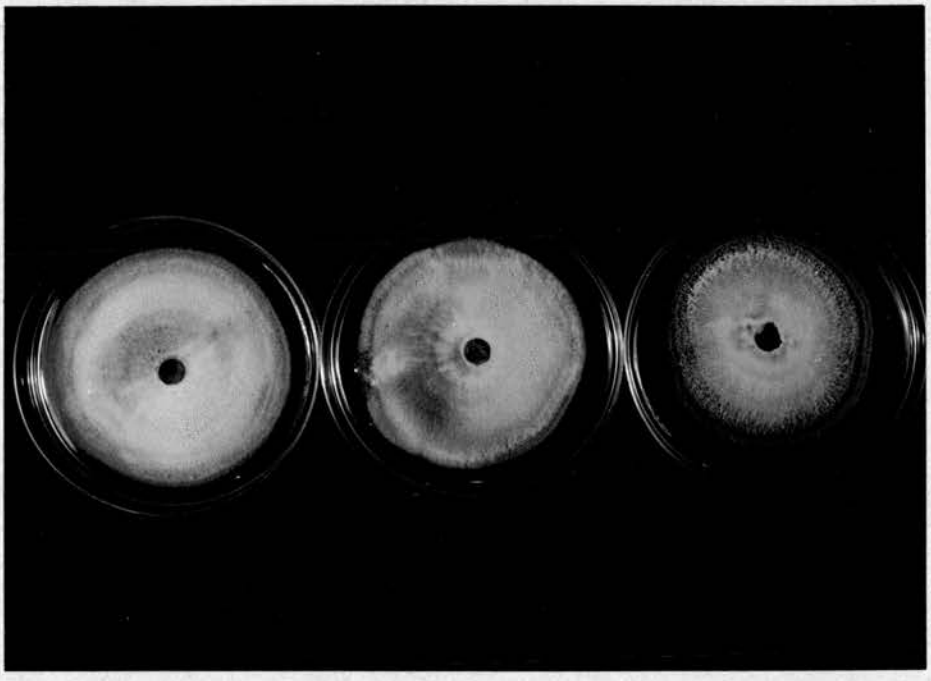

Fig. 9a. Growth of V. nubilum on Czapek Dox agar containing no mercury (Control) 22 days after inoculation.

Fig. 9b. Growth of V. nubilum on Czapek Dox agar containing 0·015 ppm. mercury 22 days after inoculation.

Fig. 9c.

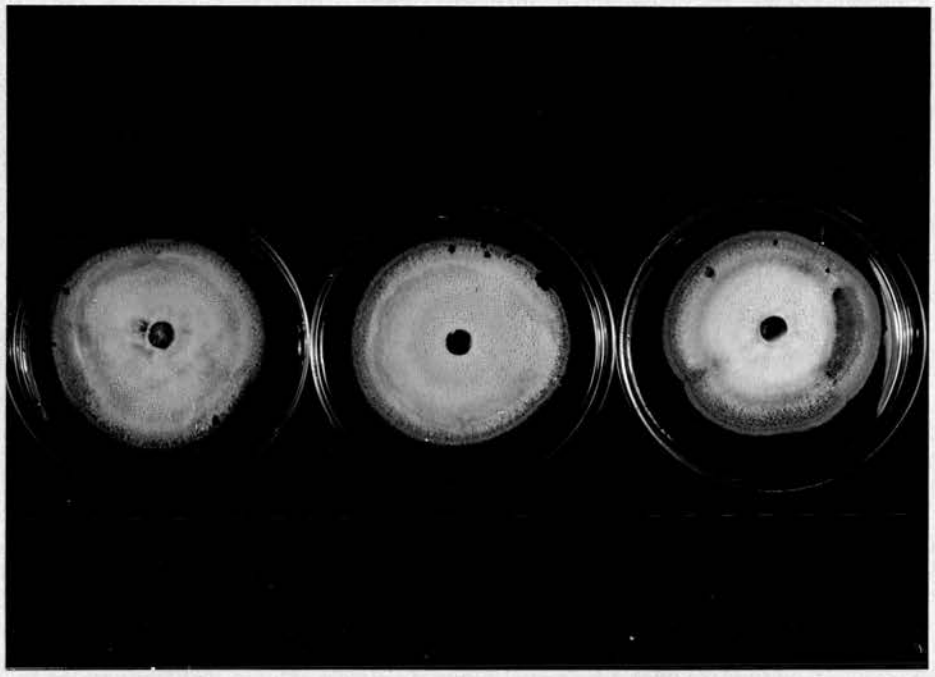

Fig. 9d.

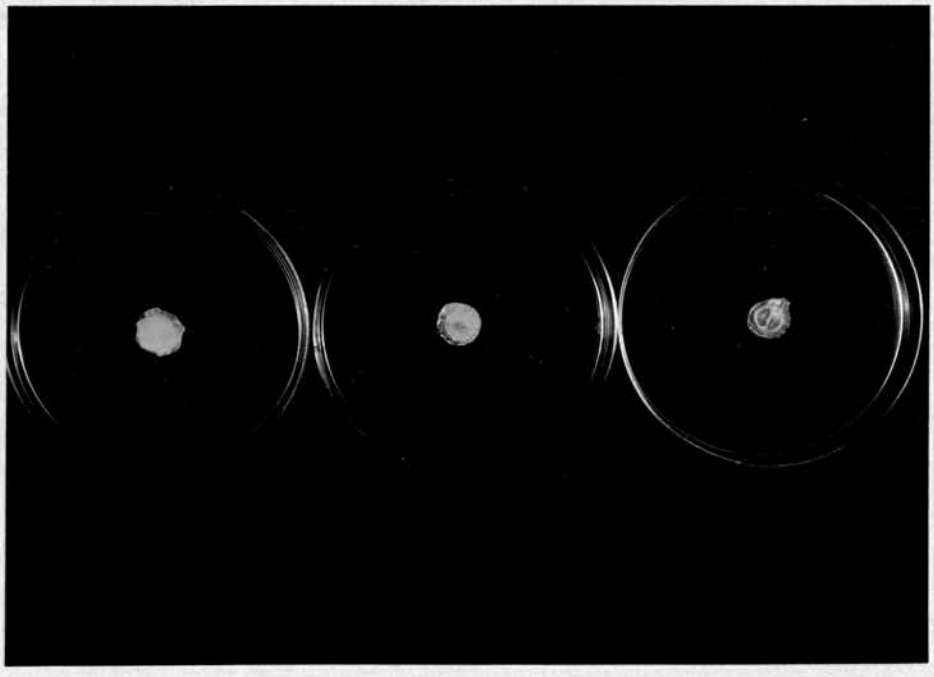

Fig. 9c. Growth of V. nubilum on Czapek Dox agar containing 0·15 ppm. mercury 22 days after inoculation.

Fig. 9d. Growth of V. nubilum on Czapek Dox agar containing 1·5 ppm. mercury 22 days after inoculation.

Fig. 9e. Growth of V. nubilum in Cazpek Dox agar containing 15 ppm. mercury 22 days after inoculation.

also suggest that the relationship of the fungus with this disorder may be influenced by the type of disinfectant used on the seed tuber prior to inoculation, and it would appear that the use of mercury may have an inhibitory effect on the subsequent activity of the fungus. The results of analysis of organo-mercury disinfected tubers showed that the concentration left in the tuber was much higher than the concentrations which slightly retarded growth in Czapek Dox agar medium and was only slightly less than that which led to a marked suppression in fungal growth.

It was observed in experiment 2 (Part i) that there was no coiling in plants from inoculated tubers which had not been washed or disinfected before inoculation, although some coiling was observed with the same treatment in experiment 1. While the numbers of tubers involved was small, the results are of interest in that they might suggest a possible antagonistic effect from the soil in 1 stock of tubers. Schippers and Schermer (1966) noted that V. albo-atrum developed readily on seedlings of Senecio vulgaris when internally-infected achenes of the plant were placed on agar or on an autoclaved soil. The fungus, however, did not grow from these infected achenes in natural soil or autoclaved soil exposed to unfiltered air, nor could it be isolated on cherry agar from internally infected achenes that had been incubated in natural soil for 55 hours or more at 24°C. These workers concluded that the growth of V. albo-atrum was inhibited or the mycelium was lysed in the

achenes in natural soil and did not contribute to the dissemination of the fungus. Autoclaved soil exposed to unfiltered air for 5 minutes or more and then incubated for 24 hours at 24°C inhibited or completely prevented V. albo-atrum from developing from infected achenes. Several fungi trapped from the air inhibited growth of V. albo-atrum from infected achenes (Schippers and Schermer, 1966).

## 3.7. Effect of seed tuber inoculation with V. nubilum, O.pustulans, and R. solani on the incidence of coiled sprout and stem lesions.

### 3.7.1. Introduction

In previous experiments (Sections 3.1., 3.4., 3.5. and 3.6.), it was observed that in some cases O. pustulans and R. solani were isolated from brown lesions on stem bases. O. pustulans was found only on stems from tubers which had not been disinfected before planting, whereas R. solani could occur on plant growth from both disinfected and undisinfected tubers. Symptoms produced by R. solani in the form of well defined dark brown lesions (Fig. 6b) were generally readily distinguished from the light brown lesions associated with V. nubilum, but lesions from which O. pustulans alone were isolated were often confused with those from infection with V. nubilum. Moreover, in some instances, 2 or all 3 fungal species were isolated from the same sprout. In an attempt to define the symptoms produced by each fungus and to study any possible interaction between any 2 or all 3 fungi combined, in their effects on sprout growth, 2 experimental studies were carried out in 1967 on the effects of inoculation with the 3 fungi either alone or in combination. In experiment 1 the inoculum for each treatment was added to soil, while in experiment 2 the tubers were inoculated before planting.

### 3.7.2. Materials and methods

Experiment 1.

Small sized (1·5 in. diameter) unsprouted seed tubers of Arran Pilot, harvested in early October, 1966, and stored at 36-40°F (2·2-4·4°C), were disinfected with 70 per cent alcohol (Section 2.2.) on 26 May, 1967. After drying the tubers were planted to a depth of 2-3 in. in sterilized 7 in. pots containing sterilized John Innes field soil No.1. Three tubers were planted to each pot. The pots were divided into 8 groups of 5 and each group allocated to 1 of the following soil inoculation treatments:

a) V. nubilum, b) O. pustulans, c) R. solani, d) V. nubilum + O. pustulans, e) V. nubilum + R. solani, f) O. pustulans + R. solani, g) V. nubilum + O. pustulans + R. solani, and h) Control.

For the inoculations, separate suspensions in 1 litre of water were made up from 8 slopes of 4 weeks old cultures of each of the 3 fungi grown on Czapek Dox agar. Fifty ml. of each fungal suspension was added to the soil of each pot of the appropriate treatments - a pot treated with 1 fungus receiving 50 ml. of the suspension, and a pot treated with 2 or the 3 fungi receiving 50 ml. of suspension of each fungus. The inoculation was carried out immediately after planting and the pots placed in a random manner in a glasshouse at 43-65°F (5·1-18·3°C).

Experiment 2.

Eighty tubers of the variety King Edward, lifted on 17 August, 1967 and kept at 50-67°F (10·0-19·4°C) until 3 October, were disinfected with 70 per cent alcohol. The tubers were divided into 8 lots of 10 and 1 group allotted to each of the following seed tuber inoculation treatments: a) V. nubilum, b) O. pustulans, c) R. solani, d) V. nubilum + O. pustulans, e) V. nubilum + R. solani, f) O. pustulans + R. solani, g) V. nubilum + O. pustulans + R. solani, and h) Control.

The 3 fungal suspensions were prepared, as in experiment 1, and the mixed suspensions prepared by adding equal volumes of the respective suspensions to the same container and agitating. The 10 tubers for each treatment were immersed in the appropriate suspension for 5 minutes and immediately planted and the 10 control tubers were planted without immersion. The tubers were planted separately in clean 7 in. pots containing sterilized John Innes field soil No.1, 2-3 in. below the soil surface, and the pots were then arranged randomly in a glasshouse at 43-65°C (5·1-18·3°C).

In both experiments, following emergence, the plants were examined for coiling and the presence of brown lesions on the stem bases, from a proportion of which fungal isolates were made.

### 3.7.3. Results

Experiment 1.

Most treatments in experiment 1 did not show complete emergence (Table 25) and on examination at the end of the experiment the tubers which failed to show emergence were found to be rotted. Although *R. solani* alone and in combination with *V. nubilum* showed the highest rate of rotting there was no evidence to suggest this was associated with the experimental treatments. There were only small differences in the average number of days to emergence between treatments (Table 25).

Table 25.

Plant emergence in relation to soil inoculation with *V. nubilum*, *O. pustulans* and *R. solani*.

| Soil inoculation treatment | No. of plants emerged as a fraction of the total no. of tubers planted | Average no. of days to first leaf emergence. |
|---|---|---|
| a) *V. nubilum* | 15/15 | 27·4 |
| b) *O. pustulans* | 13/15 | 27·4 |
| c) *R. solani* | 11/15 | 28·0 |
| d) *V. nubilum* + *O. pustulans* | 12/15 | 31·8 |
| e) *V. nubilum* + *R. solani* | 9/15 | 31·8 |
| f) *O. pustulans* + *R. solani* | 13 /15 | 28·6 |
| g) *V. nubilum* + *O. pustulans* + *R. solani* | 15/15 | 30·2 |
| h) Control | 13/15 | 29·8 |

No coiling was found in any of the 8 treatments. However, there was a frequent incidence of brown lesions on the stem bases (Table 26). All the 7 inoculated treatments showed some splitting combined with surface browning, but with V. nubilum and R. solani there were instances of deeper cracking and russetting (Table 26). Lesions caused by R. solani were usually distinguished by their dark brown colour with cracks and russetting. V. nubilum and O. pustulans were more difficult to differentiate by the type of lesions formed. The lesions caused by V. nubilum tended to be light brown in colour, varying from a few millimetres to several centimetres in length and possibly changing on exposure to air to a darker brown or pale orange colour. The lesions were either entirely smooth or split and occasionally formed deeper transverse and longitudinal russetted cracks. Basing on observations from this experiment, lesions caused by O. pustulans were light brown in colour and of a similar size to those caused by V. nubilum, but mostly smooth. However, occasionally slight splitting was associated with O. pustulans infection but there was an absence of russetting as found with V. nubilum. Without the presence of the deep russetted cracks, it was not possible to distinguish between lesions caused by V. nubilum and O. pustulans and the only reliable method of identification was an isolation test. However, on isolation, each fungus was obtained from stems of its respective inoculation treatments. R. solani was also found

Table 26.

Incidence of brown lesions in relation to soil inoculation with *V. nubilum*, *O.pustulans* and *R. solani*.

| Soil inoculation treatment | No. of plants showing brown lesions as a fraction of the total no. of plants | No. of stem bases affected | | | |
|---|---|---|---|---|---|
| | | Type of lesion | | | |
| | | Clean | Surface browning | Surface browning + splitting | Surface browning + deep cracks + russetting |
| a) *V. nubilum* | 13/15 | 10 | 23 | 12 | 6 |
| b) *O. pustulans* | 12/13 | 4 | 43 | 10 | 0 |
| c) *R. solani* | 11/11 | 0 | 11 | 10 | 18 |
| d) *V. nubilum* + *O. pustulans* | 10/12 | 13 | 25 | 13 | 5 |
| e) *V. nubilum* + *R. solani* | 9/9 | 0 | 9 | 17 | 17 |
| f) *O. pustulans* + *R. solani* | 13/13 | 0 | 17 | 20 | 13 |
| g) *V. nubilum* + *O. pustulans* + *R. solani* | 15/15 | 2 | 30 | 18 | 23 |
| h) Control | 4/13 | 38 | 8 | 8 | 4 |

in some plants from pots which had not been treated with this fungus, including the controls.

Experiment 2.

None of the 8 treatments showed complete emergence (Table 27) and on examination of the tubers which did not produce plants, at the end of the experiment, they were found to be rotted. The average number of days to emergence was earlier for the control than for any of the inoculated tubers (Table 27). Within the inoculation treatments, emergence seemed to be slightly later with *V. nubilum* and *R. solani* inoculation.

Table 27.

Plant emergence in relation to seed tuber inoculation with *V. nubilum*, *O. pustulans* and *R. solani*.

| Seed tuber inoculation treatment | No. of emerged plants as a fraction of the total no. of tubers per treatment | Average no. of days to first leaf emergence |
|---|---|---|
| a) *V. nubilum* | 7/10 | 44·7 |
| b) *O. pustulans* | 7/10 | 34·0 |
| c) *R. solani* | 6/10 | 43·5 |
| d) *V. nubilum* + *O. pustulans* | 8/10 | 39·0 |
| e) *V. nubilum* + *R. solani* | 8/10 | 42·5 |
| f) *O. pustulans* + *R. solani* | 8/10 | 37·8 |
| g) *V. nubilum* + *O. pustulans* + *R. solani* | 7/10 | 40·9 |
| h) Control | 9/10 | 29·0 |

The results in Table 28 show that coiling occurred only in treatments receiving *V. nubilum* alone or in combination with 1 or both other fungi.

Table 28.

Incidence of coiled sprout in relation to seed tuber inoculation with *V. nubilum*, *O. pustulans* and *R. solani*.

| Seed tuber inoculation treatment | No. of plants showing coiling as a fraction of the total no. of plants. | No. of stem bases showing coiling as a fraction of the total no. of stem bases. |
|---|---|---|
| a) *V. nubilum* | 4/7 | 4/15 |
| b) *O. pustulans* | 0/7 | 0/10 |
| c) *R. solani* | 0/6 | 0/9 |
| d) *V. nubilum* + *O. pustulans* | 3/8 | 3/13 |
| e) *V. nubilum* + *R. solani* | 2/8 | 2/10 |
| f) *O. pustulans* + *R. solani* | 0/8 | 0/16 |
| g) *V. nubilum* + *O. pustulans* + *R. solani* | 3/7 | 4/16 |
| h) Control | 0/9 | 0/20 |

Brown lesions on the stem bases, were found frequently in all inoculated treatments, but none were found on any of the plants from control tubers (Table 29). The lesions were mainly of the surface browning type with a few stem bases showing splitting or russetting and cracking from *V. nubilum* or *R. solani* inoculation treatments (Table 29).

The result of isolation tests on stem bases with brown lesions is shown in Table 30.

Table 29.

Incidence of brown lesions on stem bases in relation to seed tuber inoculation with *V. nubilum*, *O. pustulans* and *R. solani*.

| Seed tuber inoculation treatment | No. of plants with brown lesions as a fraction of the total no. of plants | No. of stem bases affected | | | |
|---|---|---|---|---|---|
| | | Type of lesion | | | |
| | | Clean | Surface browning only | Surface browning + splitting | Surface browning + splitting + russetting |
| a) *V. nubilum* | 7/7 | 0 | 10 | 3 | 2 |
| b) *O. pustulans* | 7/7 | 2 | 8 | 0 | 0 |
| c) *R. solani* | 6/6 | 0 | 3 | 3 | 3 |
| d) *V. nubilum* + *O. pustulans* | 8/8 | 0 | 8 | 5 | 0 |
| e) *V. nubilum* + *R. solani* | 8/8 | 0 | 6 | 3 | 1 |
| f) *O. pustulans* + *R. solani* | 6/8 | 4 | 8 | 0 | 4 |
| g) *V. nubilum* + *O. pustulans* + *R. solani* | 6/7 | 2 | 9 | 2 | 3 |
| h) Control | 0/9 | 20 | 0 | 0 | 0 |

Table 30.

Isolation of fungal species from brown lesions on stem bases in relation to inoculation with *V. nubilum*, *O. pustulans* and *R. solani*.

| Seed tuber inoculation treatment | Total no. of stem bases | No. of stem bases from which isolates obtained | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *V. nubilum* | *O. pustulans* | *R. solani* | *V. nubilum* + *O. pustulans* | *V. nubilum* + *R. solani* | *O. pustulans* + *R. solani* | *V. nubilum* + *O. pustulans* + *R. solani* |
| a) *V. nubilum* | 15 | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| b) *O. pustulans* | 10 | 0 | 8 | 0 | 0 | 0 | 0 | 0 |
| c) *R. solani* | 9 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| d) *V. nubilum* + *O. pustulans* | 13 | 1 | 3 | 0 | 9 | 0 | 0 | 0 |
| e) *V. nubilum* + *R. solani* | 10 | 0 | 0 | 0 | 0 | 10 | 0 | 0 |
| f) *O. pustulans* + *R. solani* | 16 | 0 | 1 | 4 | 0 | 0 | 7 | 0 |
| g) *V. nubilum* + *O. pustulans* + *R. solani* | 16 | 0 | 0 | 3 | 0 | 4 | 2 | 5 |
| h) Control | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

### 3.7.4. Conclusions

Coiling was only found in 1 of the 2 experiments, where seed tubers inoculation rather than soil inoculation was carried out. It was, however, only those treatments where infection with V. nubilum was involved which gave rise to plants showing the disorder. This result therefore, provided further evidence to that of the previous experiments (Section 3.6.) of a relationship between V. nubilum and coiled sprout. There was no evidence of an association between O. pustulans or R. solani and coiling but both of these fungi and V. nubilum gave rise to brown lesions on the stem bases. The lesions caused by R. solani were clearly distinguished by their darker brown colour and clearly defined margins and the frequent occurrence of splitting and russetting. The lesions caused by O. pustulans and V. nubilum tended to be similar in appearance, i.e. the form of a light superficial browning, but splitting occurred more often with V. nubilum and only this fungus appeared to give rise to cracking and russetting.

# SECTION 4.

## A SURVEY OF THE INCIDENCE OF COILED SPROUT AND OF FUNGI ASSOCIATED WITH NECROTIC LESIONS ON POTATO STEM BASES IN CROPS GROWN IN EAST SCOTLAND

## 4. A survey of the incidence of the coiled sprout in potato stocks grown in east Scotland, 1966.

### 4.1. Introduction

In June, 1966, a survey was carried out to assess the incidence of coiled sprout in commercial potato crops in the counties of East Lothian, Mid Lothian and Fife. At the same time, counts were made of the frequency of stem bases showing brown lesions and attempts were made to isolate and identify fungi associated with sprout growth abnormalities.

### 4.2. Materials and methods

Samples of 10 plants were collected from the potato stocks grown on 12 farms and examined for coiling and the presence of brown lesions on stem bases. From each sample, segments of a proportion of any underground stems which showed discoloration or distortion were taken, washed, surface disinfected in Chloros and subjected to the isolation tests described in Section 2.3. for the identification of the fungal species present.

A total of 35 stocks was sampled and details relating to each crop with respect to variety, storage treatment, planting date and after-planting cultivation are given in Appendix 15. The survey included 15 crops of the variety King Edward, 6 of Epicure, 5 of Craigs Royal, 4 of Pentland Dell, 2 of Majestic and of Redskin and 1 crop of Golden Wonder (Table 31). About two-thirds of the stocks had been chitted before planting and, for slightly more than a half of the crops, chemical methods of

weed control had been applied. The planting dates extended over the period early March to early May.

## 4.3. Results

Table 31.

Number of crops surveyed and number in which coiled sprout was observed in relation to variety, planting date, chitting and post-planting cultivation.

| Variety and time of planting | Seed treatment and cultivation treatment after planting | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Chitted | | | | Unchitted | | | |
| | Normal cultivation | | Chemical weed control | | Normal cultivation | | Chemical weed control | |
| | Total no. of crops | No. of crops showing coiling | Total no. of crops | No. of crops showing coiling | Total no. of crops | No. of crops showing coiling | Total no. of crops | No. of crops showing coiling |
| King Edward (late March to early April) | 4 | 0 | 2 | 0 | 6 | 0 | 3 | 0 |
| Epicure (early March to early April) | 2 | 0 | 4 | 3 | 0 | 0 | 0 | 0 |
| Craigs Royal (early March) | 1 | 0 | 4 | 3 | 0 | 0 | 0 | 0 |
| Pentland Dell (late March to early April) | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Majestic (mid April to early May) | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Redskin (early April to early May) | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Golden Wonder (early April) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

Of the 35 crops examined, only 7 showed any coiling (Table 31). Three of the crops that showed the disorder were of the variety Epicure, 3 of Craigs Royal and 1 of Majestic. In all cases where coiling was observed, the seed had been chitted before planting and, in all except that of Majestic, chemical weed control had been carried out (Table 31). The percentage of the total number of stem bases showing coiling, within crops in which the disorder was found, ranged from 2·9 to 13·0 (Table 32). Brown lesions were found in association with coils in all cases but were also very frequent in stems not showing coiling. Both R. solani and O. pustulans were isolated from lesions in plants in 6 of the crops where coiling was found and, in 1 crop, only O. pustulans was found (Table 32). All except 1 of the crops of Epicure and all the crops of Craigs Royal involved were from the same farm (Appendix 15) and had been planted early in light sandy or sandy loam soils. Growth had been delayed by a prolonged cold period in March and, at the time of the survey, the emergence was poor with many of the sprouts attacked by R. solani.

In the total number of crops surveyed, there was only a very occasional incidence of splitting of stem bases and in only 1 instance was fasciation observed (Appendix 15).

Brown lesions were found on the stem bases in all the crops sampled but there was no obvious trend in the pattern of incidence in relation to chitting, post-planting cultivation or date of planting (Table 33). However, as previously noted, the most

Table 32

Incidence of coiling and brown lesions on underground stem bases in crops showing coiled sprout and of fungal species isolated from lesions.

| Crop and farm | Percentage of stem bases showing coils | Percentage of stem bases showing brown lesions | Fungal species isolated from lesions | | |
|---|---|---|---|---|---|
| | | | *R. solani* | *O. pustulans* | *Verticillium* spp. |
| Epicure (Scoughall) | 13 | 82·6 | + | + | - |
| Epicure (Scoughall) | 4·3 | 100·0 | + | + | - |
| Epicure (Archerfield) | 2·9 | 58·8 | - | + | - |
| Craigs Royal (Scoughall) | 11·4 | 88·7 | + | + | - |
| Craigs Royal (Scoughall) | 6·5 | 90·3 | + | + | - |
| Craigs Royal (Scoughall) | 4·0 | 80·0 | + | + | - |
| Majestic (Luffness Mains) | 2·9 | 85·4 | + | + | - |

severe necrosis occurred in association with heavy attacks by *R. solani* in light soils.

Table 33.

Incidence of brown lesions on underground stem bases in relation to variety chitting and post-planting cultivation.

| Crop | Mean percentage of stem bases showing brown lesions | | | |
|---|---|---|---|---|
| | Chitted | | Unchitted | |
| | Normal cultivation | Chemical weed control | Normal cultivation | Chemical weed control |
| King Edward | 64·9 | 46·6 | 68·5 | 77·3 |
| Epicure | 61·7 | 74·2 | - | - |
| Craigs Royal | 42·2 | 79·5 | - | - |
| Pentland Dell | 83·5 | - | - | 91·2 |
| Majestic | 85·4 | - | 100·0 | - |
| Redskin | 75·8 | - | - | - |
| Golden Wonder | - | 78·5 | - | - |

Of the fungi associated with brown lesions, *R. solani* only was isolated from 1 and *O. pustulans* only from 7 of the crops and in the remaining 27 crops both fungi were present. In no case was *V. nubilum* or any other *Verticillium* species recorded (Table 34).

Table 34

Fungi isolated from brown lesions on stem bases in relation to variety chitting and post-planting cultivation.

| Crop | Post-planting cultivation | No. of crops from which fungi isolated | | | | | |
|---|---|---|---|---|---|---|---|
| | | Chitted | | | Unchitted | | |
| | | R. solani | O. pustulans | R. solani + O. pustulans | R. solani | O. pustulans | R. solani + O. pustulans |
| King Edward | Normal cultivation | - | 1 | 3 | - | 2 | 4 |
| | Chemical weed control | - | 1 | 1 | - | 1 | 2 |
| Epicure | Normal cultivation | - | 1 | 2 | - | - | - |
| | Chemical weed control | - | - | 3 | - | - | - |
| Craigs Royal | Normal cultivation | - | - | 1 | - | - | - |
| | Chemical weed control | - | - | 4 | - | - | - |
| Pentland Dell | Normal cultivation | - | - | 2 | - | - | - |
| | Chemical weed control | - | - | - | - | 1 | 1 |
| Majestic | Normal cultivation | - | - | 2 | - | - | - |
| | Chemical weed control | - | - | - | - | - | - |
| Redskin | Normal cultivation | - | - | 2 | - | - | - |
| | Chemical weed control | - | - | - | - | - | - |
| Golden Wonder | Normal cultivation | - | - | - | - | - | - |
| | Chemical weed control | 1 | - | - | - | - | - |

## 4.3. Conclusions

Coiling was found in only one fifth of the stocks examined and then only to a slight extent. In all cases where coiling did occur, the seed tubers had been sprouted in light before planting and, in 6 of the 7 cases of coiling, chemical weed control had been used in place of harrowing down and ridging up of drills. R. solani and O. pustulans were found frequently in all stocks but in no case was V. nubilum isolated. Although the survey was of a limited nature, the observations tend to be in agreement with the findings of Moorby and McGee (1966) and Lapwood et al. (1967) that chitting increases the prevalence of coiling in sprouts and that an increased depth of soil over the seed tuber might aggravate its incidence (Lapwood et al, 1967). Moreover, low soil temperature also might have been associated with outbreaks of coiling, as suggested by Moorby and McGee (1966).

## SECTION 5.

# FIELD STUDIES ON THE EFFECTS OF SEED TUBER INOCULATION WITH *VERTICILLIUM NUBILUM*, SEED STORAGE TREATMENT AND SEED DISINFECTION ON THE INCIDENCE OF COILED SPROUT AND YIELD IN DIFFERENT VARIETIES

## 5.1. Effects of storage treatment and seed tuber inoculation with V. nubilum on the incidence of coiled sprout and tuber yield in the varieties Arran Pilot and Majestic.

### 5.1.1. Introduction

Field experiments were carried out in 1966 and 1967 to study the effects of different sprouting treatments of seed tubers and seed tuber inoculation with V. nubilum on the incidence of coiled sprout. The studies aimed to investigate further the relationship between the disorder and sprout development at planting, as reported by Moorby and McGee (1966), and the findings of Pitt et al. (1964, 1965), that V. nubilum can cause coiling. Two varieties were used in the experiments, Arran Pilot, which shows vigorous sprout growth, and Majestic, which has a slow rate of sprout growth. In addition to assessing the incidence of coiling, yield of tubers in relation to the various factors was measured in duplicated trials.

### 5.1.2. Materials and methods

For the experiment in 1966, seed tubers of the varieties Arran Pilot and Majestic were obtained from a commercial store in early December 1965 and stored at 38°F (3·3°C) until 4 February when they were disinfected with an organo-mercurial solution. On 6 February, the tubers of each variety were divided into 3 groups of 80 and allotted to the following 3 storage treatments:

(a) Storage at about 50°F (10·0°C) in light (Sprouted).

(b) Storage at about 50°F (10·0°C) in darkness (Desprouted).

(c) Storage at about 38°F (3·3°C) in darkness (Unsprouted).

On 9 April, the sprout lengths were measured from samples of 40 tubers from each treatment and, after all the tubers had been washed, any sprout growth on tubers stored in darkness was removed. The tubers from each storage treatment and variety were then subdivided into 2 groups of 40. On 11 April, tubers of 1 group were inoculated with a spore suspension of V. nubilum by dipping for 1 minute in a slurry of the fungus ($6{\cdot}0 \times 10^6$ chlamydospores per ml.), as described in Section 2.1., and immediately planted at a spacing of 12 in. in 28 in. drills. The tubers of the second group were planted at the same time without further treatment, as uninoculated controls. A basically similar experiment was carried out in the following year with Arran Pilot and Majestic tubers lifted in October 1966 and stored at 38°F (3·3°C) from lifting until 4 February 1967 when, following tuber disinfection, the storage treatments were applied. Sprout measurements were recorded on 7 April and the tubers planted at a spacing of 24 in. in 28 in. drills on 9 April, immediately after the inoculation treatment ($5{\cdot}8 \times 10^6$ chlamydospores per ml.) was carried out. Following the ridging up of drills, chemical methods of weed control were applied in both years, using paraquat and linuron in 1966, and linuron only in 1967.

The experiments thus comprised 2 variety, 2 inoculation and

3 storage treatments which were arranged factorially and laid out in the field in 4 replicates of a split-plot, randomized block design with each sub-plot consisting of 10 seed tubers. In 1966, the 2 varieties formed main plots and the 2 inoculation and 3 storage treatments were arranged as sub-plots but, in 1967, the 2 inoculation treatments formed main plots and the sub-plots comprised the 2 varieties and 3 storage treatments.

All tubers gave plant emergence. Following emergence, plants were lifted and the stem bases of sprouts examined for coiling and brown lesions, and samples of sprouts being taken from each treatment and segments incubated in an attempt to isolate V. nubilum and other fungal species present (Section 2.3.). In 1967, the numbers of stem bases showing fasciation and splitting were also recorded.

In both years, an exact duplicate of each experiment was laid out in an adjacent area of land and the plants allowed to grow to full maturity. In October, the tubers were lifted for assessments of tuber weights and numbers in relation to the various treatments. In grading the yield, tubers more than $2\frac{1}{4}$ in. in diameter were classified as ware, those between $2\frac{1}{4}$ and $1\frac{1}{4}$ in. as seed and those less than $1\frac{1}{4}$ in. in diameter as chats.

Analyses of variance for data obtained for all observations were carried out for a split-plot 2/2 x 3 factorial design (Cochran and Cox, 1960).

5.1.3. Results

Sprout growth in storage:

The length of sprout growth just before planting varied with variety and storage treatment, as shown in Table 35. Tubers which had been stored at 50°F (10·0°C) in darkness showed long sprouts and all sprout growth was removed before planting. In the case of tubers stored at 38°F (3·3°C) in darkness, a slight amount of sprout growth had occurred in tubers of Arran Pilot and this was removed, although this treatment was classified as unsprouted. The tubers which had been sprouted in light showed sturdy green sprouts which were kept intact at planting and were longer in Arran Pilot than in Majestic, and for both varieties the sprout length was greater in the second year (Table 35).

Table 35.

Sprout length in relation to storage treatment and variety, 1966 and 1967.

| Storage treatment | Mean length of longest sprout in cm. | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | 1966 | 1967 | 1966 | 1967 |
| Sprouted | 4·0 | 6·4 | 1·8 | 3·0 |
| Desprouted | 95·0 | 120·0 | 40·0 | 60·0 |
| Unsprouted | 3·0 | 4·0 | 0·0 | <1·0 |

Incidence of coiling:

The percentage of plants which showed coiling in 1 or more stem bases in relation to variety and storage treatment and in relation to variety and inoculation treatment for both years are shown in Tables 36 and 37 respectively.

The incidence of coiled sprouts was, in general, lower in 1966 than in 1967. In 1966, none of the treatments investigated had any significant effect on the percentage of plants which showed coiling (Appendix 16). There was however, a trend for coiling to be more frequent in Arran Pilot than in Majestic and the difference between varieties was significant ($P = 0{\cdot}05$) from an analysis of variance of the percentage of the total number of stem bases which coiled, as shown in Table 38 (Appendix 18).

The results for 1967 showed that variety, storage and inoculation treatments all had significant effects on the incidence of coiling (Appendices 17 and 19). Thus Arran Pilot gave a higher incidence of coiling than Majestic ($P = 0{\cdot}001$), expressed as percentage of plants (Table 36) or of stem bases showing coiling (Table 38); the sprouted storage treatment increased the percentage of plants ($P = 0{\cdot}01$) and of stem bases ($P = 0{\cdot}001$) showing coiling compared with desprouted and unsprouted treatments, as shown in Tables 36 and 39. Tubers inoculated with V. nubilum produced more coiling than uninoculated tubers whether expressed as percentage of plants ($P = 0{\cdot}01$) or of stem bases ($P = 0{\cdot}05$) showing coiling, as

Table 36.

Effects of variety and storage treatment on the percentage of plants showing coiling, 1966 and 1967. (Transformed data given in brackets)

| Storage treatment | Percentage of plants showing coiling | | | | | |
|---|---|---|---|---|---|---|
| | 1966 | | | 1967 | | |
| | Arran Pilot | Majestic | Mean | Arran Pilot | Majestic | Mean |
| | | | (± 3·75) | | (± 2·17) | (± 1·53) |
| Sprouted | 22·1(28·08) | 3·2(10·37) | 10·8(19·23) | 84·0(66·43) | 29·4(32·80) | 58·0(49·62) |
| Desprouted | 21·9(27·87) | 10·7(19·06) | 15·9(23·47) | 62·5(52·24) | 25·7(30·49) | 43·7(41·37) |
| Unsprouted | 26·2(30·79) | 2·5(9·08) | 11·6(19·94) | 63·5(52·80) | 32·3(34·65) | 47·8(43·72) |
| Mean | 23·7(28·91) | 4·9(12·84) | | 70·6(57·16) | 29·1(32·65) | |
| | (± 3·75) | | | (± 1·25) | | |

Standard errors for means in body of the Table for 1966.

1) for comparisons between variety means at any 1 storage treatment (± 5·77)

2) for comparisons between storage treatment means of any variety (± 5·31)

Table 37.

Effects of variety and seed tuber inoculation with *V. nubilum* on the percentage of plants showing coiling, 1966 and 1967. (Transformed data given in brackets)

| Inoculation treatment | 1966 | | | 1967 | | |
|---|---|---|---|---|---|---|
| | Arran Pilot | Majestic | Mean | Arran Pilot | Majestic | Mean |
| | | | (± 3·06) | | | (± 1·58) |
| Inoculated | 25·4(30·26) | 7·1(15·45) | 15·1(22·85) | 86·9(68·79) | 37·8(37·91) | 64·4(53·35) |
| Control | 21·4(27·57) | 3·2(10·22)) | 10·5(18·90) | 50·9(45·52) | 21·2(27·39) | 35·3(36·45) |
| Mean | 23·4(28·92) | 4·9(12·84) | | 70·6(57·16) | 29·1(32·65) | |
| | (± 3·75) | | | (± 1·25) | | |

Standard errors for means in body of the Table:-

| | 1966 | 1967 |
|---|---|---|
| 1) for comparisons between variety means for 1 inoculation treatment | (± 4·89) | (± 1·77) |
| 2) for comparisons between inoculation treatment means of 1 variety | (± 4·33) | (± 2·02) |

indicated in Tables 37 and 40 respectively.

In the analysis of variance of the percentage of plants showing coils in 1967 (Appendix 17), significant interactions were found between variety and storage treatments and variety and inoculation treatments. Table 36 shows that only Arran Pilot gave a significant increase in the percentage of plants which produced coils from sprouting in light ($P = 0{\cdot}01$) compared with other storage treatments and, as may be seen from Table 37, Arran Pilot gave a more marked response to the inoculation treatment than Majestic ($P = 0{\cdot}001$).

Table 38.

Effect of variety on the percentage of stem bases showing coiling, 1966 and 1967.
(Transformed data given in brackets)

| Variety | Percentage of stem bases showing coiling | |
|---|---|---|
| | 1966 | 1967 |
| Arran Pilot | 4·1 (11·69) | 17·8 (25·20) |
| Majestic | 1·1 (5·92) | 9·5 (17·76) |
| | (± 1·25) | (± 0·90) |

Table 39.

Effect of storage treatment on the percentage of stem bases showing coiling, 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing coiling |
|---|---|
| Sprouted | 17·4 (24·64) |
| Desprouted | 11·0 (19·40) |
| Unsprouted | 12·1 (20·39) |
| | (± 1·11) |

Table 40.

Effect of seed tuber inoculation with V. nubilum on the percentage of stem bases showing coiling, 1967.
(Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing coiling |
|---|---|
| Inoculated | 17·8 (24·98) |
| Control | 9·5 (17·97) |
| | (± 1·18) |

Incidence of fasciation and splitting in association with coiling:

In 1967, the numbers of stem bases which showed coiling were sub-divided into those which showed coiling only and those which showed coiling with splitting or fasciation or both fasciation and splitting.

From the analysis of variance of the data for incidence of stem bases showing coiling without fasciation or splitting,

variety and inoculation treatments were found to have significant effects, but the effect of storage treatment was non-significant (Appendix 20). The percentage of stem bases that showed coiling only was greater in Arran Pilot than in Majestic (P = 0·001) and with tubers inoculated with V.nubilum than in uninoculated tubers (P = 0·01), as is shown in Tables 41 and 42 respectively.

Table 41.

Effect of variety on the percentage of stem bases showing coiling only, 1967.
(Transformed data given in brackets)

| Variety | Percentage of stem bases showing coiling only |
|---|---|
| Arran Pilot | 11·8 (20·10) |
| Majestic | 6·7 (15·01) |
| | (± 0·84) |

Table 42.

Effect of seed tuber inoculation with V. nubilum on the percentage of stem bases showing coiling only, 1967.
(Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing coiling |
|---|---|
| Inoculated | 12·40 (20·61) |
| Control | 6·30 (14·51) |
| | (± 0·69 ) |

The incidence of coiling and splitting was generally low but was also significantly increased (P = 0·001) by seed tuber inoculation, as shown in Table 43 (Appendix 21). There was, however, a significant interaction between variety and inoculation treatments, and it may be seen from Table 43 that the increase with inoculation was significant in Arran Pilot (P = 0·01) but not in Majestic. Moreover, with inoculation, Arran Pilot produced a significantly higher proportion of stem bases showing coiling and splitting than Majestic (P = 0·01) but gave no sprouts in this category in the control treatment.

Table 43.

Effect of variety and seed tuber inoculation with V. nubilum on the percentage of stem bases showing coiling and splitting, 1967.
(Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing coiling and splitting | | |
|---|---|---|---|
| | Arran Pilot | Majestic | Mean |
| | | | (± 0·33) |
| Inoculated | 3·00(9·94) | 0·43(3·78) | 1·40(6·86) |
| Control | 0·00(0·00) | 0·09(1·76) | 0·02(0·88) |
| Mean | 0·80(4·97) | 0·2(2·77) | |
| | (± 1·05) | | |

Standard errors for means in body of the Table:-

1) for comparisons between variety means at 1 inoculation treatment (± 1·49)

2) for comparisons between inoculation treatment means for 1 variety (± 1·10)

No coiling associated with fasciation only occurred in any treatment but, as shown in Appendix 22, the incidence of coiling with both fasciation and splitting was significantly influenced by variety and storage treatment and there was, also a significant interaction between those 2 factors. Coiling associated with fasciation and splitting was only found in tubers sprouted in light (P = 0·001) and was more frequent with this treatment in Arran Pilot than in Majestic (P = 0·01), as shown in Table 44. The inoculation treatment had no significant effect on the incidence of sprouts in this category (Appendix 22).

Table 44.

Effects of variety and storage treatments on the percentage of stem bases showing coiling, fasciation and splitting, 1967. (Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing coiling, fasciation and splitting | | |
|---|---|---|---|
| | Arran Pilot | Majestic | Mean |
| | (± 1·49) | | (± 1·04) |
| Sprouted | 12·6(20·80) | 3·1(10·16) | 7·1(15·48) |
| Desprouted | 0·0(0·00) | 0·0(0·00) | 0·0(0·00) |
| Unsprouted | 0·0(0·00) | 0·0(0·00) | 0·0(0·00) |
| Mean | 1·5(6·93) (± 0·85) | 0·35(3·39) | |

Incidence of fasciation and splitting without coiling:

Fasciation and splitting and splitting alone were observed to occur without coiling but no fasciation without coiling or

splitting was found on stem bases. The proportion of stem bases with splitting alone was not significantly affected by any of the experimental treatments (Appendix 23). The incidence of fasciation with splitting was found to be significantly affected by storage treatment and the interactions of storage treatment with variety and storage and inoculation treatments (Appendix 24). Table 45 shows that seed tubers sprouted in light gave more fasciation and splitting on stem bases than desprouted or unsprouted tubers ($P = 0{\cdot}001$) and that this effect was greater in Majestic than in Arran Pilot ($P = 0{\cdot}01$). As may be seen in Table 46, inoculation with V. nubilum gave a reduced percentage of stem bases in this category for tubers sprouted in light when compared with uninoculated sprouted tubers ($P = 0{\cdot}01$).

Table 45.

Effects of variety and storage treatments on the percentage of stem bases showing fasciation and splitting without coiling, 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing fasciation and splitting without coiling | | |
|---|---|---|---|
| | Arran Pilot | Majestic | Mean |
| | | (± 1·35) | (± 0·96) |
| Sprouted | 1·20(6·22) | 4·90(12·82) | 2·70(9·52) |
| Desprouted | 0·00(0·00) | 0·00(0·00) | 0·00(0·00) |
| Unsprouted | 0·14(2·13) | 0·00(0·00) | 0·04(1·07) |
| Mean | 0·2 (2·78) | 0·6 (4·27) | |
| | | (± 0·78) | |

Table 46.

Effects of seed tuber inoculation with V. nubilum and storage treatment on the percentage of stem bases showing fasciation and splitting without coiling, 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing fasciation and splitting without coiling | | |
|---|---|---|---|
| | Inoculated | Control | Mean |
| | | | (± 0·96) |
| Sprouted | 0·91(5·47) | 5·50(13·57) | 2·70(9·52) |
| Desprouted | 0·00(0·00) | 0·00(0·00) | 0·00(0·00) |
| Unsprouted | 0·14(2·13) | 0·00(0·00) | 0·04(1·07) |
| Mean | 0·20(2·53) | 0·60(4·52) | |
| | (± 0·54) | | |

Standard errors for means in body of the Table:

1) for comparisons between inoculation treatment means at 1 storage treatment (± 1·23)

2) for comparisons between storage treatment means of 1 inoculation treatment (± 1·35)

Incidence of brown lesions and associated fungal isolates:

Brown lesions were formed in all sprouts from the experimental treatments for 1966 and 1967 and, using the humid chamber method of isolation (Section 2.3.), several fungi were isolated from these lesions as shown in Table 47. R. solani occurred frequently on stem bases from all treatments for both years. O. pustulans was found fairly frequently in 1966 but was absent in 1967. There was a very occasional incidence of a Fusarium species in both years and of Cylindrocarpon radicicola

Table 47.

Incidence of fungal species isolated from brown lesions on coiled and normal stem bases in relation to seed tuber inoculation with V. nubilum, 1966 and 1967.

| Year, inoculation treatment and growth form of stem bases | | V.nubilum | R.solani | O.pustulans | Fusarium sp. | C.atramentarium | Cylindrocarpon radicicola Wollenw. |
|---|---|---|---|---|---|---|---|
| 1966 Inoculated | Coiled | + | + | + | traces | - | - |
| | Normal | + | + | + | traces | - | - |
| 1966 Control | Coiled | - | + | + | traces | - | - |
| | Normal | - | + | + | traces | - | - |
| 1967 Inoculated | Coiled | + | + | - | traces | traces | traces |
| | Normal | + | + | - | traces | traces | traces |
| 1967 Control | Coiled | - | + | - | traces | traces | traces |
| | Normal | - | + | - | traces | traces | traces |

Wollenw. and C. atramentarium in 1967. V. nubilum was found only on stem bases from inoculated tubers but was present in both coiled and normal stem bases.

Tuber yield and tuber number per acre and size grading of produce:

The total tuber yield per acre was significantly affected by variety and storage treatment in both years, and also by inoculation treatment in 1966 (Appendices 25 and 26). In 1967, the main effect of inoculation with V. nubilum was not significant, but this factor showed a significant interaction with storage treatment and with storage treatment and variety. Also, in 1967, the interaction between variety and storage treatment was significant (Appendix 26). Majestic gave a higher yield than Arran Pilot variety in both 1966 ($P = 0{\cdot}05$) and 1967 ($P = 0{\cdot}001$), and tubers sprouted in light gave a higher yield than desprouted and unsprouted tubers ($P = 0{\cdot}001$) in both years (Table 48 ). In 1967, yield from desprouted tubers was significantly higher than that from unsprouted tubers ($P = 0{\cdot}01$) in Arran Pilot but not in Majestic (Table 48).

In 1966, tubers inoculated with V. nubilum gave generally a significantly lower yield than uninoculated tubers ($P = 0{\cdot}05$). In 1967, this reduction in yield with V. nubilum inoculation was found in the sprouted tubers ($P = 0{\cdot}01$) but not in desprouted or unsprouted treatments (Table 49).

Table 48.

Effects of variety and storage treatment on total yield, 1966 and 1967.

| Storage treatment | Yield in tons per acre | | | | | |
|---|---|---|---|---|---|---|
| | 1966 | | | 1967 | | |
| | Arran Pilot | Majestic | Mean | Arran Pilot | Majestic | Mean |
| | | | ± 0·31 | ± 0·58 | | ± 0·41 |
| Sprouted | 8·18 | 12·34 | 10·26 | 15·98 | 17·70 | 16·84 |
| Desprouted | 7·74 | 11·41 | 9·57 | 13·88 | 14·35 | 14·12 |
| Unsprouted | 6·54 | 10·08 | 8·31 | 11·27 | 14·98 | 13·13 |
| Mean | 7·49 | 11·28 | | 13·71 | 15·68 | |
| | ± 0·60 | | | ± 0·34 | | |

Standard errors for means in body of Table for 1966:

1) for comparisons between variety means at 1 storage treatment ± 0·70
2) for comparisons between storage treatment means of 1 variety ± 0·44

Table 49.

Effects of storage treatment and seed tuber inoculation with V. nubilum on total yield, 1966 and 1967.

| Storage treatment | Yield in tons per acre | | | | | |
|---|---|---|---|---|---|---|
| | 1966 | | | 1967 | | |
| | Inoculated | Control | Mean | Inoculated | Control | Mean |
| | ± 0·44 | | ± 0·31 | | | ± 0·41 |
| Sprouted | 9·58 | 11·94 | 10·26 | 15·18 | 18·50 | 16·84 |
| Desprouted | 8·96 | 10·19 | 9·57 | 14·32 | 13·91 | 14·12 |
| Unsprouted | 7·90 | 8·72 | 8·31 | 12·83 | 13·42 | 13·13 |
| Mean | 8·81 | 9·95 | | 14·11 | 15·28 | |
| | ± 0·25 | | | ± 0·54 | | |

Standard errors for body of the Table for 1967;

1) for comparison between inoculation treatment means at 1 storage treatment ± 0·72
2) for comparison between storage treatment means of 1 inoculation treatment ± 0·58

The total number of tubers per acre was significantly affected by the inoculation treatment in 1966, while the effects of variety and storage treatment were not significant (Appendix 27). Table 50 shows that the total number of tubers per acre in this year was less for treatments inoculated with V. nubilum than for the uninoculated treatments (P = 0·05). In 1967, none of the 3 factors investigated showed significant main effect on tuber number. There was, however, a significant interaction between variety, inoculation and storage treatments and except in the case of desprouted tubers of Arran Pilot, inoculated seed again tended to give lower tuber number per acre than uninoculated controls (Appendix 28).

Table 50.

Effect of seed tuber inoculation with V. nubilum on the total number of tubers per acre, 1966.

| Inoculation treatment | Total no. of tubers per acre |
|---|---|
| Inoculated | 175494·4 |
| Control | 202189·6 |
| | ± 6824·1 |

With respect to the grading of produce, the proportion of ware was generally less in 1966 than in 1967, when the spacing of plants was wider. The percentage contribution of each of the 3 size categories of tubers, ware, seed and chats, to the total yield per acre was significantly affected by variety in both years (Appendices 29 to 34). Table 51 shows that Arran Pilot produced a lower percentage of ware in 1966 (P = 0·05)

and 1967 (P = 0·001) and a correspondingly higher percentage of seed tubers in 1967 (P = 0·001) and of chats in 1966 (P = 0·01) the Majestic. The main effects of the storage and inoculation treatments on the grading of the crop were not significant in 1966, but the 2 factors showed a significant interaction on their effects on the proportion of ware in the total yield (Appendix 29). The percentage of ware was found to be significantly less in unsprouted uninoculated tubers than in the inoculated tubers subjected to the same storage treatment and sprouted control tubers gave a higher percentage of ware than unsprouted control tubers. In 1967, storage treatment had a significant effect on the proportion of the different size categories (Appendices 30, 32 and 34) and, as shown in Table 53, sprouted tubers gave a significantly higher percentage of ware and correspondingly lower percentages of seed and chats than desprouted or unsprouted tubers. In 1967, the inoculation treatment was found to have no significant effect on crop grading.

Table 51.

Effect of variety on the percentage of ware, seed and chat tubers in the total yield, 1966 and 1967.
(Transformed data given in brackets)

| Variety | Percentage of tubers by weight in total yield | | | | | |
|---|---|---|---|---|---|---|
| | 1966 | | | 1967 | | |
| | Ware | Seed | Chat | Ware | Seed | Chat |
| Arran Pilot | 0·2(2·55) | 80·9(64·08) | 17·9(25·02) | 59·8(50·64) | 37·8(37·89) | 2·4(8·81) |
| Majestic | 3·8(11·26) | 85·3(67·43) | 9·2(17·67) | 70·4(57·02) | 27·6(31·73) | 1·9(7·92) |
| | (± 1·53) | (± 1·23) | (± 0·46) | (± 0·76) | (± 0·78) | (± 0·33) |

Table 52.

Effects of seed tuber inoculation with V. nubilum and storage treatment on the percentage of ware in the total yield, 1966.
(Transformed data given in brackets)

| Storage treatment | Percentage of ware by weight in total yield | | |
|---|---|---|---|
| | Inoculation treatment | | Mean |
| | Inoculated | Control | |
| | (± 2·16) | | (± 1·53) |
| Sprouted | 1·90(7·97) | 3·30(10·44) | 2·40(9·20) |
| Desprouted | 0·33(3·27) | 1·40(7·68) | 0·91(5·48) |
| Unsprouted | 2·50(9·09) | 0·27(2·99) | 1·10(6·04) |
| Mean | 1·40(6·78) | 1·50(7·03) | |
| | (± 1·25) | | |

Table 53.

Effect of storage treatment on the percentage of ware, seed and chat tubers in the total yield, 1966 and 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of tubers by weight in the total yield | | | | | |
|---|---|---|---|---|---|---|
| | 1966 | | | 1967 | | |
| | Ware | Seed | Chat | Ware | Seed | Chat |
| Sprouted | 2·40(9·20) | 81·40(64·45) | 13·40(21·49) | 74·30(59·52) | 24·1(29·31) | 1·70(7·48) |
| Desprouted | 0·91(5·48) | 85·10(67·29) | 12·60(20·81) | 63·20(52·66) | 34·5(35·99) | 2·10(8·39) |
| Unsprouted | 1·10(6·04) | 82·80(65·51) | 13·70(21·73) | 57·50(49·31) | 39·80(39·12) | 2·60(9·23) |
| | (± 1·53) | (± 1·08) | (± 0·83) | (± 0·94) | (± 0·95) | (± 0·41) |

5.1.4. Conclusions

The incidence of coiling on stem bases appeared, in general, to be higher in 1967 than in 1966 but it is difficult to account for this difference between years. The sprout lengths on tubers sprouted in light before planting were greater in 1967 and the higher incidence of coiling in sprouted tubers in this year may be related to an effect of sprout size, although in both years the sprout lengths were above the range within which Moorby and McGee (1966) found differences in incidence relating to sprout size. However, this size effect would not explain the higher incidence of coiling in desprouted or unsprouted tubers, also in the second year. Moorby and McGee (1966) indicated that lower soil temperatures may give a greater incidence of coiling but only where sprouts are short. Only slight differences in soil temperatures for the month after planting were found between the 2 years, the average at 8 in. soil depth being 46·3°F in 1966 and 47·1°F in 1967.

In 1966, there was a slight tendency for the incidence of coiling to be higher in Arran Pilot than in Majestic, but storage and inoculation treatments did not exert any significant effects. However, in 1967, all the factors investigated significantly influenced the incidence of coiling expressed as a percentage of plants or stem bases showing coiling. Arran Pilot produced more coiling than Majestic, sprouted tubers gave more coiling than unsprouted or desprouted tubers and seed tuber inoculation with *V. nubilum* also increased the incidence

of the disorder. In the case of the percentages of plants showing coiling, there were also significant interactions between variety and inoculation treatment and between storage treatment and variety: only Arran Pilot showed a significant increase with sprouting and this variety also showed a more marked response to inoculation with V. nubilum. These results confirm previous findings of Moorby and McGee (1966) that sprouting of seed tubers and varieties with more vigorous sprout growth increase the incidence of coiling. At the same time, they also confirm findings by Pitt et al. (1964, 1965) that V. nubilum is associated with the incidence of the coiled sprout condition in potatoes. In addition, the results indicate that the occurrence of fasciation with or without coiling is related to sprouting seed tubers in light rather than the presence of V. nubilum. From results of both years it was observed that coiling could occur in unsprouted and desprouted uninoculated tubers, suggesting that coiling may also be caused by factors other than those investigated.

In relating the tuber yield results to results obtained from their duplicate experiments where records of the incidence of coiling were made, it would appear that the greater incidence of coiling associated with sprouted tubers was not accompanied by any reductions in final yield. The storage treatments tended to have no significant effect on tuber number per acre and higher weight yields were obtained from sprouted than from

desprouted or unsprouted tubers which showed less coiling.

Seed tuber inoculation with V. nubilum appeared, in general, to reduce the number of tubers produced per acre in the subsequent crop. This was associated with significant weight yield reduction from inoculation of seed tubers of all storage treatments in 1966 and in tubers sprouted in light in 1967.

## 5.2. Effects of seed tuber disinfection, storage treatment and seed tuber inoculation with V. nubilum on the incidence of coiled sprout.

### 5.2.1. Introduction

The results of previous work (Pitt et al., 1965) have indicated that disinfection of seed tubers with an organo-mercurial solution may control the coiled sprout disorder from tubers naturally contaminated with V. nubilum. The results from the glasshouse experiments, in this present work, suggest that, where tubers are disinfected with an organo-mercurial solution prior to inoculation with V. nubilum, there is a tendency for less coiling to develop than where alcohol or formalin is used before inoculation (Section 3.6.). In 1966 and 1967, field experiments were carried out to study the effects on growth of organo-mercurial disinfection of seed tubers of the variety Arran Pilot which were subsequently inoculated with V. nubilum or planted without inoculation. The experiments also included various storage treatments, which were applied following the time of carrying out the disinfection treatment and before the tubers were inoculated.

### 5.2.2. Materials and methods

For the experiment in 1966, tubers of the variety Arran Pilot were obtained from a commercial bulk store in early December, 1965, and stored at 38°F (3·3°C) until 4 February of

the following year, when they were divided into 2 groups of 240. One group was disinfected in an organo-mercurial solution (Section 2.2.) and the second group was left untreated. On 6 February, the tubers from each group were subdivided into 3 lots of 80 which were subjected to the following 3 storage treatments:

a) Storage at about 50°F (10·0°C) in light (Sprouted)

b) Storage at about 50°F (10·0°C) in darkness (Desprouted)

c) Storage at about 38°F (3·3°C) in darkness (Unsprouted)

On 9 April, samples of 40 disinfected and 40 untreated tubers from each storage treatment were taken and their sprout lengths measured. Any sprout growth on disinfected or untreated tubers stored in the dark was removed and, on 11 April, each group of 80 tubers from the respective disinfection and storage treatments was divided into 2 groups of 40. One group was dipped in a spore suspension of V. nubilum ($6{\cdot}0 \times 10^6$ chlamydospores per ml.) as described in Section 2.1. and immediately planted, while the second group was planted directly as uninoculated controls. The tubers were planted in 28 in. drills at a spacing of 12 in. In a similar experiment in 1967, tubers were lifted in early October, 1966, and stored at 38°F (3·3°C) until 4 February, when the disinfection treatment was carried out and the tubers allotted to the 3 storage treatments. Following the measurements of sprout length on 7 April, the inoculation treatment with V. nubilum ($5{\cdot}8 \times 10^6$ chlamydospores per ml.) and planting at a spacing of 24 in. in

28 in. drills were carried out on 9 April. After the ridging up of drills and before plant emergence chemical methods of weed control were applied in both years, using paraquat and linuron in 1966, and linuron only in 1967.

Each experiment comprised 2 disinfection, 3 storage and 2 inoculation treatments which were arranged factorially and laid out in the field in 4 replicates of a split-plot randomized block design with each sub-plot consisting of 10 tubers. In 1966, the 2 varieties formed main plots and the 2 inoculation and 3 storage treatments were arranged as sub-plots but, in 1967, the 2 inoculation treatments formed main plots and the sub-plots comprised the 2 varieties and 3 storage treatments.

Following emergence, plants were lifted and the stem bases of sprouts examined for coiling and brown lesions. Samples of stem bases were also taken from each treatment and segments incubated in an attempt to isolate V. nubilum and other fungal species present. In 1967, the numbers of stem bases showing fasciation and splitting were also recorded.

Analyses of variance of the collected data were carried out for a split-plot randomized block factorial design (Cochran and Cox 1960).

### 5.2.3. Results

Sprout growth in storage:

From Table 54, it may be seen that storage in the dark at about 50°F (10·0°C) was associated with the production of long

etiolated sprouts which were removed before planting. Tubers stored at about 38°F (3·3°C) showed a slight amount of sprout growth which was also removed before planting. The tubers sprouted in light at about 50°F (10·0°C) produced sturdy green sprouts which were kept intact at planting.

Table 54.

Sprout length in relation to seed tuber disinfection and storage treatment, 1966 and 1967.

| Storage treatment | Mean length of longest sprout in cm. | | | |
|---|---|---|---|---|
| | Disinfection treatment | | | |
| | 1966 | | 1967 | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 4·0 | 4·0 | 6·4 | 7·0 |
| Desprouted | 95·2 | 150·0 | 120·0 | 135·0 |
| Unsprouted | 3·0 | 4·3 | 4·0 | 3·5 |

Plant emergence:

In 1967, all the tubers planted showed plant emergence, but, in 1966, there was some blanking in sub-plots (Appendix 35) and, although the differences in emergence failure were not marked between treatments, significantly higher numbers of plants were obtained from tubers which had been disinfected before planting ($P = 0·05$) than from untreated tubers (Table 55). This may relate to disinfection affording some measure of control of dry rot, symptoms of which were seen in some tubers which failed to show emergence.

Table 55.

Plant emergence in relation to seed tuber disinfection, 1966.

| Disinfection treatment | No. of plants recovered from 10 tubers planted per treatment |
|---|---|
| Disinfected | 7·3 |
| Untreated | 5·9 |
| | ± 0·19 |

The average number of days to emergence was only recorded in 1966 (Table 56). Emergence was generally several days earlier in sprouted tubers compared with that from unsprouted or desprouted tubers, but there appeared to be no consistent differences in rates of emergence in relation to the other factors (Table 56).

Table 56.

Number of days to plant emergence in relation to seed tuber disinfection treatment, seed tuber inoculation with V. nubilum and storage treatment, 1966.

| Storage treatment | Average no. of days to first leaf emergence | | | |
|---|---|---|---|---|
| | Disinfection and inoculation treatments | | | |
| | Disinfected | | Untreated | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 35·0 | 32·2 | 35·6 | 32·0 |
| Desprouted | 40·8 | 45·1 | 50·0 | 48·7 |
| Unsprouted | 40·3 | 43·8 | 39·0 | 42·0 |

Incidence of coiling:

The general incidence of coiling appeared to be higher in 1967 than in 1966. In 1966, only the storage treatment had a significant effect on the percentage of stem bases showing coiling (Appendix 36) but, in 1967, the analysis of variance showed that the main effects of both storage and inoculation treatments were significant and that there was a significant interaction between the 2 factors (Appendix 37). The effects of the disinfection treatment were non-significant. In 1966, the unsprouted tubers were found to give significantly less coiling than sprouted or desprouted tubers ($P = 0{\cdot}01$ and $0{\cdot}05$ respectively) but the differences between the sprouted and desprouted treatments were non-significant (Table 57). In 1967, however, the sprouted tubers gave more coiling than the other storage treatments ($P = 0{\cdot}001$), both of which showed a similar average incidence (Table 57). Inoculation with V. nubilum increased the amount of coiling compared with that from the control tubers in 1967 ($P = 0{\cdot}01$), as may also be seen from Table 57. The increase in incidence of coiling with inoculation was significant for each of the 3 storage treatments ($P = 0{\cdot}05$) but was most marked in unsprouted tubers. In the control treatment, sprouted tubers gave significantly more coiling than desprouted ($P = 0{\cdot}05$) and unsprouted tubers ($P = 0{\cdot}01$) and the difference between desprouted and unsprouted tubers was non-significant. However, in treatments inoculated with V. nubilum the increase in frequency of coiling from sprouting

## Table 57.

Effects of storage treatment and inoculation with *V. nubilum* on the percentage of total stem bases showing coiling, 1966 and 1967. (Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing coiling | | | | | |
|---|---|---|---|---|---|---|
| | 1966 | | | 1967 | | |
| | Inoculated | Control | Mean | Inoculated | Control | Mean |
| | | (± 2·84) | (± 2·01) | | | (± 0·84) |
| Sprouted | 11·2(19·57) | 9·7(18·18) | 10·5(18·87) | 37·0(37·48) | 24·0(29·36) | 30·3(33·42) |
| Desprouted | 7·8(16·21) | 8·7(17·12) | 8·2(16·67) | 28·8(32·45) | 18·2(25·25) | 23·3(28·85) |
| Unsprouted | 2·8(9·65) | 1·4(6·67) | 2·0(8·16) | 35·1(36·34) | 14·6(22·44) | 24·1(29·39) |
| Mean | 6·8(15·14) | 5·8(13·99) | | 33·6(35·42) | 18·8(25·68) | |
| | | (± 1·64) | | | (± 0·96) | |

Standard errors for means in body of the Table for 1967:

1) for comparisons between inoculation treatment means for 1 storage treatment (± 1·36)

2) for comparisons between storage treatment means of 1 inoculation treatment (± 1·91)

tubers in light was only significant when compared with desprouted tubers (P = 0·05).

Incidence of fasciation and splitting in association with coiling:

Records of the incidence of fasciation and splitting were made in 1967 only. The number of sprouts which showed coiling without fasciation or splitting was not affected by the storage or disinfection treatments but the inoculation treatment was found to have significant effect (Appendix 38), there being an increased incidence from inoculation with V. nubilum (P = 0·01) as shown in Table 58).

Table 58.

Effect of seed tuber inoculation with V. nubilum on the percentage of stem bases showing coiling only, 1967. (Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing coiling only |
|---|---|
| Inoculated | 23·9(29·28) |
| Control | 15·3(23·00) |
| | (± 0·67) |

The incidence of coiling and splitting (Appendix 39) was also significantly increased by inoculation with V. nubilum (P = 0·01), as shown in Table 59. The analysis of variance also showed that the incidence was affected by a significant interaction between the disinfection and storage treatments (Appendix 39). With untreated tubers storage treatment had

no significant effect on the incidence of coiling and splitting. However, disinfection decreased the incidence of stem bases in this category where tubers were sprouted (P = 0·05) and with disinfection unsprouted tubers gave more coiling and splitting than sprouted tubers (P = 0·01), as shown in Table 60.

Table 59.

Effect of seed tuber inoculation with V. nubilum on the percentage of stem bases showing coiling and splitting, 1967. (Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing coiling and splitting |
|---|---|
| Inoculated | 6·00(14·23) |
| Control | 0·07 (1·48) |
| | (± 0·90) |

Table 60.

Effects of seed tuber disinfection and storage treatments on the percentage of stem bases showing coiling and splitting, 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing coiling and splitting | | |
|---|---|---|---|
| | Disinfected | Untreated | Mean |
| | (± 1·28) | | (±0·91) |
| Sprouted | 0·61(4·50) | 2·40(8·83) | 1·40(6·67) |
| Desprouted | 1·60(7·25) | 1·90(7·95) | 1·70(7·60) |
| Unsprouted | 3·70(10·98) | 1·80(7·62) | 2·60(9·30) |
| Mean | 1·70(7·58) | 2·00(8·13) | |
| | (±0·74) | | |

No stem bases showing coiling and fasciation only were found. With respect to the incidence of coiling associated with fasciation and splitting, only storage treatment had a significant effect (Appendix 40): the results in Table 61 show that the sprouted tubers gave an increased incidence of stem bases in this category (P = 0·001) compared with desprouted, which produced very few and unsprouted tubers, which produced none.

Table 61.

Effect of storage treatment on the percentage of stem bases showing coiling, fasciation and splitting, 1967. (Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing coiling, fasciation and splitting |
|---|---|
| Sprouted | 8·40(16·83) |
| Desprouted | 0·01(0·49) |
| Unsprouted | 0·00(0·00) |
| | (±0·87) |

Incidence of fasciation and splitting without coiling:

The incidence of splitting alone was not affected by the experimental treatments (Appendix 41) and fasciation alone did not occur. However, storage treatment was found to have a significant effect on the incidence of stem bases showing both fasciation and splitting without coiling (Appendix 42). Tubers sprouted in light produced a small proportion of stem

bases showing fasciation and splitting, whereas neither desprouted nor unsprouted tubers produced any in this category (P = 0·001), as shown in Table 62.

Table 62.

Effect of storage treatment on the percentage of stem bases showing fasciation and splitting without coiling, 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of stem bases showing fasciation and splitting without coiling |
|---|---|
| Sprouted | 2·0(8·17) |
| Desprouted | 0·0(0·00) |
| Unsprouted | 0·0(0·00) |
| | (± 0·90) |

Incidence of brown lesions and associated fungal isolates:

Brown lesions were noted in all stem bases from the experimental treatments in both years and, using the humid chamber method of isolation (Section 2.3.), several fungi were found associated with the lesions, as shown in Table 63. *R. solani* occurred frequently on stem bases from all treatments for both years. *O. pustulans* was found fairly frequently in 1966 but was absent in 1967. There was a very occasional incidence of a *Fusarium species* in both years and of *Cylindrocarpon radicicola* Wollenw. and *C. atramentarium* in 1967. *V. nubilum* was found only on stems bases from inoculated tubers but was present in both coiled and normal stem bases.

Table 63.

Fungal species isolated from brown lesions in relation to seed tuber inoculation with *V. nubilum* and disinfection treatment, 1966 and 1967.

| Disinfection and inoculation treatments | | Fungal species isolated from brown lesions | | | | | |
|---|---|---|---|---|---|---|---|
| | | *V.nubilum* | *R.solani* | *O.pustulans* | *Fusarium* sp. | *C.atramentarium* | *C.radicicola* |
| 1966 Disinfected | Inoculated | + | + | + | traces | - | - |
| | Control | - | + | + | traces | - | - |
| 1966 Untreated | Inoculated | + | + | + | traces | - | - |
| | Control | - | + | + | traces | - | - |
| 1967 Disinfected | Inoculated | + | + | - | traces | traces | traces |
| | Control | - | + | - | traces | traces | traces |
| 1967 Untreated | Inoculated | + | + | - | traces | traces | traces |
| | Control | - | + | - | traces | traces | traces |

5.2.4. Conclusions

As in the previous field experiments (Section 5.1.) the general incidence of coiling was higher in 1967 than in 1966 but, again no reasons appeared to explain this difference between years. In 1966, only storage treatment had a significant effect on the number of stem bases showing coils and tubers stored at low temperature in the dark gave less coiling than those which had been sprouted in light or had been stored at a relatively higher temperature in the dark and desprouted before planting. In 1967, however, inoculation with V. nubilum as well as storage treatment was found to affect the amount of coiling. Without inoculation tubers sprouted in light gave more coiling than desprouted and unsprouted tubers, while inoculation with V. nubilum increased the incidence of coiling for all storage treatments, the effect tending to be most marked in unsprouted tubers. Disinfection of seed tubers before applying the storage or inoculation treatments had no effect on the general incidence of coiling in 1966 and only exerted a minor effect on the incidence of coiling and splitting in sprouted tubers in 1967.

The results of assessments of the incidence of the different types of coiling again indicated that the occurrence of coiling in association with fasciation and splitting or fasciation and splitting without coiling was related to sprouting tubers in light but not to inoculation with V. nubilum which gave rise to more coiling either alone or with splitting only.

## 5.3. Incidence of coiled sprout in different varieties in relation to seed tuber inoculation with V. nubilum and storage treatment.

### 5.3.1. Introduction

In inoculation tests with V. nubilum on 10 varieties, Pitt et al. (1965) found that all the varieties examined were susceptible to infection with V. nubilum and all exhibited a full range of coiled sprout symptoms. However, in associating the appearance of coiling with the degree of sprout development at planting, Moorby and McGee (1966) indicated that varieties such as Arran Pilot, Duke of York and Ulster Premier, which tend to produce large sprouts during storage, are most affected by the disorder. Field trials were thus carried out in 1966 and 1967 to investigate the incidence of coiled sprout in relation to seed tuber inoculation with V. nubilum and sprouting treatment in a wide range of commercial varieties.

### 5.3.2. Materials and methods

Thirty six tubers of each of 23 varieties, from stocks grown at East Craigs (Department of Agriculture and Fisheries for Scotland), Edinburgh, and held after lifting in a cold room, were obtained on 14 November, 1965, and stored at 38°F (3·3°C) until 17 February, 1966, when they were disinfected in an organo-mercurial solution (Section 2.2.). After drying, 18 tubers from each variety were placed in paper bags and stored

at 38°F (3·3°C) to prevent sprouting. The remaining 18 tubers of each variety were sprouted in chitting trays and kept in a glasshouse at 50-70°F (10·0-21·1°C). On 10 April, each group of 18 from the 2 storage treatments for each variety was divided into 2 lots of 9. The tubers of 1 lot were dipped in a slurry of a spore suspension of V. nubilum ($5·7 \times 10^6$ chlamydospores per ml.) for 1 minute (Section 2.1.) and immediately planted, while those of the second lot were planted as uninoculated controls. In the following year, the experiment was repeated using 80 tubers of 24 varieties obtained on 17 November, 1966, from East Craigs. The same storage and inoculation treatments were applied and the tubers planted on 7 April, 1967. In both years the seed spacing was 12 in. in 28 in. drills. Following the ridging up of drills, chemical methods of weed control were applied using linuron and paraquat in 1966 and linuron only in 1967.

The treatments were arranged factorially and laid out in the field in a split-plot randomized block design with the 2 storage and 2 inoculation treatments forming main plots and the varieties forming sub-plots. In 1966, 3 replicates were used with each sub-plot consisting of 3 tubers and, in 1967, there were 4 replicates with each sub-plot consisting of 5 tubers.

No records were made of rate of emergence and, in both years, plants were lifted 14 weeks after planting and examined for coiling and brown lesions. Samples of stem bases were also taken from each treatment for fungal isolation tests

(Section 2.3.). In addition, in 1967, records were made of incidence of fasciation and splitting.

Analyses of variance of the data were carried out for a split-plot randomized block design (Cochran and Cox 1960).

5.2.3. Results

Incidence of coiling:

From the results in both years, the percentage of plants with 1 or more stem bases showing coiling was found to vary with variety and storage treatment and there was also a significant interaction between these 2 factors (Appendices 43 and 44). In general, sprouted tubers gave more coiling than unsprouted tubers and there were marked differences between varieties (Tables 64 and 65). With certain exceptions, varieties tended to occupy the same relative position in order of susceptibility to coiling for the 2 storage treatments, but the differences were much greater when the seed tubers had been sprouted. Highly susceptible varieties such as Arran Pilot and Duke of York tended to give a marked reaction to the sprouting treatment, whereas less susceptible varieties such as Arran Consul and Golden Wonder gave only a slight response. The results in Tables 64 and 65 also show that there were some changes in the relative positions of varieties in the 2 years, but Arran Pilot, Duke of York, Pentland Dell, Red Skin and Majestic showed a high incidence of coiling whereas King Edward, Pentland Crown, Sharpe's Express, Golden Wonder and Arran Consul showed a

Table 64.

Effect of variety and storage treatment on the percentage of plants showing coils, 1966.
(Transformed data given in brackets)

| Variety | Percentage of plants showing coils | | |
|---|---|---|---|
| | Storage treatment | | Mean |
| | Unsprouted | Sprouted | |
| Arran Pilot | 37·1(37·50) | 93·3(75·00) | 69·1(56·25) |
| Duke of York | 20·3(26·76) | 98·3(82·50) | 66·5(54·63) |
| Majestic | 44·4(41·76) | 39·8(39·12) | 42·1(40·44) |
| Paracrinkle-free King Edward | 14·1(22·50) | 52·8(46·62) | 32·2(34·56) |
| Home Guard | 1·1( 5·88) | 65·7(54·12) | 25·0(30·00) |
| Pentland Dell | 6·7(15·00) | 47·4(43·52) | 24·0(29·26) |
| Dr. McIntosh | 4·2(11·76) | 52·8(46·62) | 23·8(29·19) |
| Great Scot | 5·4(13·38) | 44·3(41·76) | 21·4(27·57) |
| Redskin | 9·2(17·63) | 22·6(28·38) | 15·3(23·01) |
| Record | 25·0(30·00) | 6·7(15·00) | 14·6(22·50) |
| Ulster Chieftain | 6·1(14·35) | 25·0(30·00) | 14·3(22·18) |
| Red Pentland Beauty | 14·6(22·50) | 12·7(20·88) | 13·7(21·69) |
| King Edward | 16·7(24·12) | 6·7(15·00) | 11·2(19·56) |
| Pentland Beauty | 1·7( 7·50) | 22·6(28·38) | 9·5(17·94) |
| Arran Banner | 1·1( 5·88) | 23·7(29·12) | 9·1(17·50) |
| Golden Wonder | 1·7( 7·50) | 18·3(25·32) | 7·9(16·32) |
| Kerr's Pink | 20·3(26·76) | 0·0( 0·00) | 5·3(13·38) |
| Pentland Crown | 4·2(11·76) | 1·1( 5·88) | 2·4( 8·82) |
| Up-to-Date | 1·1( 5·88) | 4·1(11·76) | 2·4( 8·82) |
| Epicure | 0·0( 0·00) | 6·7(15·00) | 1·7( 7·50) |
| Arran Consul | 0·0( 0·00) | 5·4(13·38) | 1·4( 6·69) |
| Craigs Royal | 1·1( 5·88) | 0·0( 0·00) | 0·3( 2·94) |
| Sharpe's Express | 0·0( 0·00) | 0·0( 0·00) | 0·0( 0·00) |
| | | | (± 7·78) |
| Mean | 7·1(15·40) | 24·2(29·44) | |
| | | (± 2·52) | |

Standard errors for means in body of the Table:

1) for comparisons between variety means for 1 storage treatment (± 11·00)

2) for comparisons between storage treatment means of 1 variety (± 11·06)

Table 65.

Effect of variety and storage treatment on the percentage of plants showing coils, 1967.
(Transformed data given in brackets)

| Variety | Percentage of plants showing coils | | |
|---|---|---|---|
| | Storage treatment | | Mean |
| | Unsprouted | Sprouted | |
| Arran Pilot | 55·0(47·89) | 88·2(69·94) | 73·3(58·92) |
| Duke of York | 33·9(35·63) | 84·1(66·48) | 60·5(51·06) |
| Ulster Premier | 28·4(32·23) | 76·1(60·72) | 52·6(46·48) |
| Pentland Dell | 31·4(34·05) | 72·9(58·61) | 52·3(46·33) |
| Redskin | 21·0(27·27) | 76·3(60·86) | 48·4(44·06) |
| Dr. McIntosh | 31·0(33·85) | 53·3(46·88) | 41·9(40·36) |
| Majestic | 23·7(29·14) | 48·9(44·34) | 35·8(36·74) |
| Up-to-Date | 33·2(35·20) | 38·3(38·23) | 35·7(36·71) |
| Paracrinkle-free King Edward | 24·4(29·57) | 45·7(42·55) | 34·6(36·06) |
| Red Pentland Beauty | 27·2(31·45) | 41·5(40·10) | 34·2(35·77) |
| Home Guard | 20·1(26·61) | 48·8(44·28) | 33·6(35·44) |
| Pentland Beauty | 21·2(27·40) | 42·7(40·82) | 31·5(34·11) |
| Craigs Royal | 21·4(27·56) | 37·7(37·86) | 29·2(32·71) |
| Arran Banner | 27·2(31·45) | 30·8(33·69) | 29·0(32·57) |
| Great Scot | 24·4(29·57) | 32·5(34·77) | 28·3(32·17) |
| Ulster Chieftain | 9·1(17·60) | 52·8(46·58) | 28·2(32·09) |
| Pentland Crown | 17·4(24·67) | 39·3(38·80) | 27·2(31·73) |
| Sharpe's Express | 21·4(27·56) | 33·7(35·49) | 27·3(31·52) |
| Golden Wonder | 23·7(29·14) | 30·6(33·61) | 27·1(31·38) |
| Kerr's Pink | 11·9(20·20) | 45·7(42·54) | 27·1(31·37) |
| King Edward | 23·9(29·28) | 28·4(32·17) | 26·1(30·73) |
| Epicure | 23·3(28·85) | 26·8(31·15) | 25·0(30·00) |
| Arran Consul | 11·4(19·70) | 25·4(30·23) | 17·8(24·97) |
| Record | 7·3(15·73) | 23·1(28·71) | 14·3(22·22) |
| | | | (± 3·71) |
| Mean | 23·3(28·82) | 47·1(43·31) | |
| | (± 3·22) | | |

Standard errors for means in body of the Table:

1) for comparisons between variety means for 1 storage treatment (± 5·25)

2) for comparisons between storage treatment means of 1 variety (± 6·06)

relatively low incidence in both years. The variety Ulster Premier, which was tested only in 1967, appeared to be one of the more susceptible varieties.

Based on the averaged results for the 2 years, the varieties studied are tentatively grouped according to their susceptibilities to coiling in Table 66.

In 1966, inoculation with V. nubilum had no significant effect on the incidence of coiling (Appendix 43) but, in 1967, there was a significant interaction between the storage and inoculation treatments (Appendix 44): as shown in Table 67, inoculation with V. nubilum had no significant effect on sprouted tubers but significantly increased the frequency of plants showing coils in unsprouted tubers ($P = 0{\cdot}05$).

In considering the percentage of the total number of stem bases which coiled in relation to the various treatments, the general pattern of the results was found to be more or less similar to that based on the percentage of plants showing coils (Appendix 45 and 46). It may be seen from Tables 68 and 69, however, that the relative positions of varieties tended to vary and this may be attributed to differences in the actual stem bases produced per variety and whether the plant from a seed tuber produced 1 or more coiled stem bases.

Table 66.

Relative susceptibility of different varieties to coiled sprout, based on the mean percentage of plants showing coils, 1966 and 1967.

| Susceptibility group | Variety | Mean Percentage of plants showing coils |
|---|---|---|
| Very susceptible | Arran Pilot | 64·7 |
| | Duke of York | 58·3 |
| | * Ulster Premier | 52·6 |
| Susceptible | Pentland Dell | 42·4 |
| | Majestic | 41·9 |
| | Paracrinkle-free King Edward | 38·2 |
| | Dr. McIntosh | 36·4 |
| | Home Guard | 36·1 |
| | Redskin | 36·0 |
| Fairly susceptible | Red Pentland Beauty | 31·5 |
| | Great Scot | 31·4 |
| | Ulster Chieftain | 30·5 |
| | Arran Banner | 29·5 |
| | Pentland Beauty | 28·8 |
| | King Edward | 27·4 |
| Fairly resistant | Record | 24·1 |
| | Up-to-Date | 23·5 |
| | Golden Wonder | 23·3 |
| | Pentland Crown | 21·1 |
| | Epicure | 20·8 |
| | Craigs Royal | 19·3 |
| | Kerr's Pink | 18·7 |
| | Sharpe's Express | 16·3 |
| | Arran Consul | 15·7 |

* Results for 1967 only.

Table 67

Effect of storage treatment and seed tuber inoculation with V. nubilum on the percentage of plants showing coils, 1967.
(Transformed data given in brackets)

| Storage treatment | Percentage of plants showing coils | | |
|---|---|---|---|
| | Inoculation treatment | | Mean |
| | Inoculated | Control | |
| | | (± 4·55) | (± 3·22) |
| Unsprouted | 37·5(37·76) | 11·6(19·87) | 23·2(28·82) |
| Sprouted | 44·2(41·65) | 49·9(44·97) | 47·1)43·31) |
| Mean | 40·8(39·71) | 28·7(32·42) | |
| | | (± 3·22) | |

Table 68.

Effect of variety on the percentage of stem bases showing coiling, 1966.
(Transformed data given in brackets)

| Variety | Percentage of stem baaes showing coiling |
|---|---|
| Arran Pilot | 22·00(27·96) |
| Duke of York | 13·10(21·17) |
| Home Guard | 6·10(14·25) |
| Paracrinkle-free King Edward | 5·90(14·09) |
| Majestic | 4·40(12·10) |
| Dr. McIntosh | 4·10(11·62) |
| Great Scot | 3·90(11·39) |
| Pentland Dell | 3·50(10·84) |
| Arran Banner | 3·30(10·48) |
| Red Pentland Beauty | 2·70( 9·47) |
| Ulster Chieftain | 2·30( 8·71) |
| Redskin | 2·20( 8·59) |
| King Edward | 1·70( 7·51) |
| Pentland Beauty | 1·50( 7·05) |
| Record | 1·40( 6·77) |
| Golden Wonder | 0·78( 5·06) |
| Kerr's Pink | 0·78( 5·05) |
| Up-to-Date | 0·70( 4·80) |
| Pentland Crown | 0·56( 4·27) |
| Epicure | 0·54( 4·22) |
| Arran Consul | 0·21( 2·63) |
| Craigs Royal | 0·05( 1·29) |
| Sharpe's Express | 0·03(1·05) |
| | (± 7·78) |

Table 69.

Effect of variety on the percentage of stem bases showing coiling, 1967.
(Transformed data given in brackets)

| Variety | Percentage of stem bases showing coiling |
|---|---|
| Pentland Dell | 26·8(31·18) |
| Arran Pilot | 21·5(27·65) |
| Dr. McIntosh | 15·5(23·21) |
| Duke of York | 14·8(22·66) |
| Ulster Premier | 14·7(22·59) |
| Majestic | 13·7(21·70) |
| Redskin | 12·6(20·83) |
| Paracrinkle-free King Edward | 10·7(18·98) |
| Up-to-Date | 10·5(18·91) |
| Red Pentland Beauty | 10·4(18·78) |
| Sharpe's Express | 9·3(17·79) |
| Pentland Crown | 9·2(17·63) |
| Pentland Beauty | 8·8(17·22) |
| Home Guard | 8·6(17·03) |
| Kerr's Pink | 8·5(16·98) |
| King Edward | 8·5(16·78) |
| Arran Banner | 8·0(16·44) |
| Ulster Chieftain | 7·9(16·28) |
| Golden Wonder | 7·8(16·25) |
| Great Scot | 7·8(16·24) |
| Craigs Royal | 7·2(15·55) |
| Epicure | 6·6(14·86) |
| Arran Consul | 5·1(13·10) |
| Record | 3·9(11·32) |
| | (± 1·74) |

Incidence of fasciation and splitting with coiling:

Records of the incidence of fasciation and splitting were made in 1967 only. The percentage of stem bases which showed coiling without fasciation or splitting was affected by variety only (Appendix 47): those varieties which showed a low incidence of all forms of coiling such as Arran Consul, Golden Wonder and Epicure, showed a low incidence of stem bases in this category (Table 70).

Table 70.

Effect of variety on the percentage of stem bases showing coiling without fasciation or splitting, 1967.
(Transformed data given in brackets)

| Variety | Percentage of stem bases showing coiling without fasciation or splitting |
|---|---|
| Pentland Dell | 20·4(26·86) |
| Arran Pilot | 14·6(22·43) |
| Dr. McIntosh | 11·4(19·73) |
| Majestic | 11·2(19·57) |
| Up-to-Date | 8·0(16·48) |
| Red Pentland Beauty | 7·9(16·28) |
| King Edward | 6·8(15·12) |
| Ulster Premier | 6·8(15·09) |
| Paracrinkle-free King Edward | 6·6(14·93) |
| Duke of York | 6·5(14·77) |
| Kerr's Pink | 6·3(14·55) |
| Redskin | 6·3(14·55) |
| Home Guard | 5·7(13·87) |
| Great Scot | 5·3(13·38) |
| Pentland Crown | 5·3(13·33) |
| Arran Banner | 5·1(13·07) |
| Ulster Chieftain | 5·1(13·03) |
| Craigs Royal | 5·0(12·98) |
| Sharpe's Express | 4·6(12·50) |
| Pentland Beauty | 4·0(11·52) |
| Epicure | 3·9(11·42) |
| Golden Wonder | 3·3(10·46) |
| Record | 3·3(10·41) |
| Arran Consul | 2·6( 9·26) |
| | (± 1·82) |

The incidence of coiling and splitting (Appendix 48) was only affected significantly by the inoculation treatment and was increased where seed tubers were inoculated with V. nubilum ($P = 0{\cdot}01$), although the incidence was very low (Table 71).

Table 71.

Effect of seed tuber inoculation with V. nubilum on the percentage of stem bases showing coiling and splitting, 1967.
(Transformed data given in brackets)

| Inoculation treatment | Percentage of stem bases showing coiling and splitting. |
|---|---|
| Inoculated | 0·12(1·98) |
| Control | 0·00(0·37) |
| | (±0·34) |

None of the experimental treatments had any significant effect on the incidence of coiling and fasciation, which rarely occurred (Appendix 49). The incidence of coiling associated with both fasciation and splitting varied with storage treatment and variety and there was a significant interaction between these 2 factors (Appendix 50). The results in Table 72 show that sprouting tubers in light increased the percentage of stem bases in this category ($P = 0{\cdot}01$): with unsprouted tubers the incidence was very low and differences between varieties were only made evident where tubers were sprouted.

Table 72.

Effect of variety and storage treatment on the percentage of stem bases showing coiling associated with fasciation and splitting, 1967.
(Transformed data given in brackets)

| Variety | Percentage of stem bases showing coiling associated with fasciation and splitting | | |
|---|---|---|---|
| | Storage treatment | | Mean |
| | Unsprouted | Sprouted | |
| Ulster Premier | 0·09(1·68) | 13·70(21·75) | 4·10(11·71) |
| Duke of York | 0·08(1·66) | 12·50(20·72) | 3·80(11·19) |
| Redskin | 0·00(0·00) | 11·50(19·82) | 3·00( 9·91) |
| Arran Pilot | 0·00(0·00) | 9·60(18·03) | 2·40( 9·02) |
| Sharpe's Express | 0·00(0·00) | 7·00(15·36) | 1·80(7·68) |
| Pentland Beauty | 0·00(0·00) | 5·50(13·52) | 1·40(6·76) |
| Golden Wonder | 0·00(0·00) | 5·20(13·16) | 1·30(6·58) |
| Pentland Dell | 0·00(0·00) | 4·40(12·04) | 1·10(6·02) |
| Pentland Crown | 0·00(0·00) | 4·30(12·01) | 1·10(6·00) |
| Up-to-Date | 0·14(2·14) | 2·70(9·48) | 1·00(5·81) |
| Ulster Chieftain | 0·00(0·00) | 3·50(10·82) | 0·89(5·41) |
| Dr. McIntosh | 0·00(0·00) | 3·30(10·46) | 0·83(5·23) |
| Arran Consul | 0·09(1·76) | 1·70(7·57) | 0·66(4·66) |
| Epicure | 0·00(0·00) | 2·50(9·10) | 0·62(4·55) |
| Craigs Royal | 0·00(0·00) | 2·50(8·98) | 0·61(4·50) |
| Kerr's Pink | 0·00(0·00) | 2·00(8·13) | 0·52(4·11) |
| Arran Banner | 0·00(0·00) | 1·80(7·69) | 0·45(3·84) |
| Great Scot | 0·00(0·00) | 1·60(7·17) | 0·39(3·59) |
| Paracrinkle-free King Edward | 0·00(0·00) | 1·50(6·89) | 0·36(3·44) |
| Home Guard | 0·00(0·00) | 1·20(6·38) | 0·31(3·19) |
| Majestic | 0·00(0·00) | 0·59(4·37) | 0·14(2·18) |
| Red Pantland Beauty | 0·00(0·00) | 0·57(4·30) | 0·14(2·15) |
| Record | 0·00(0·00) | 0·20(2·53) | 0·06(1·26) |
| King Edward | 0·00(0·00) | 0·04(1·29) | 0·01(0·65) |
| | | | (± 1·54) |
| Mean | 0·00(0·30) | 3·3(10·49) | |
| | | (± 0·92) | |

Standard errors for means in body of the Table:

1) for comparisons between variety means for 1 storage treatment (± 2·18)

2) for comparisons between storage treatment means of 1 variety (± 2·32)

Incidence of fasciation and splitting without coiling:

The analyses of variance showed that none of the treatments had any significant effect on the incidence of fasciation only or splitting only (Appendices 51 and 52) but that the incidence of both fasciation and splitting varied significantly with variety and storage treatment (Appendix 53). The results in Table 73 show that a very slight amount of fasciation and splitting occurred in only 2 varieties when tubers were not sprouted before planting, but that the abnormality occurred in all except the variety Pentland Beauty with sprouted tubers. When tubers were sprouted in light marked differences in incidence between varieties were evident. However, a second order interaction between variety, inoculation and storage treatment (Appendix 53) indicated that less fasciation and splitting occurred in sprouted tubers of certain varieties with inoculation with V. nubilum than with comparable uninoculated tubers ($P = 0{\cdot}05$).

Incidence of brown lesions and associated fungal isolates:

Brown lesions were found in all stem bases in both years and the fungal species isolated from these lesions are shown in Table 74. R. solani occurred frequently on stem bases from all treatments for both years. O. pustulans was found fairly frequently in 1966 but was absent in 1967. There was a very occasional incidence of a Fusarium species in both years and of C. radicicola and C. atramentarium in 1967.

Table 73.

Effect of variety and storage treatment on the percentage of stem bases showing fasciation and splitting without coiling, 1967.
(Transformed data given in brackets)

| Variety | Percentage of stem bases showing fasciation and splitting without coiling, 1967. | | |
|---|---|---|---|
| | Storage treatment | | Mean |
| | Unsprouted | Sprouted | |
| Golden Wonder | 0·00(0·00) | 12·60(20·76) | 3·30(10·38) |
| Sharpe's Express | 0·00(0·00) | 10·50(18·90) | 2·70(9·45) |
| Duke of York | 0·08(1·66) | 8·60(17·07) | 2·60(9·36) |
| Epicure | 0·00(0·00) | 4·10(11·64) | 1·00(5·82) |
| Redskin | 0·00(0·00) | 3·40(10·61) | 0·86(5·30) |
| Ulster Chieftain | 0·00(0·00) | 3·20(10·29) | 0·80(5·15) |
| Ulster Premier | 0·00(0·00) | 1·60(7·22) | 0·39(3·61) |
| Craigs Royal | 0·00(0·00) | 1·50(7·15) | 0·39(3·58) |
| Kerr's Pink | 0·00(0·00) | 1·20(6·04) | 0·28(3·02) |
| Up-to-Date | 0·00(0·00) | 1·00(5·74) | 0·25(2·87) |
| Home Guard | 0·00(0·00) | 0·72(4·86) | 0·18(2·43) |
| Paracrinkle-free King Edward | 0·00(0·00) | 0·62(4·52) | 0·16(2·26) |
| Pentland Dell | 0·16(2·30) | 0·12(1·87) | 0·13(2·09) |
| Arran Banner | 0·00(0·00) | 0·48(3·96) | 0·12(1·98) |
| King Edward | 0·00(0·00) | 0·39(3·59) | 0·10(1·80) |
| Arran Consul | 0·00(0·00) | 0·39(3·58) | 0·10(1·79) |
| Record | 0·00(0·00) | 0·24(2·78) | 0·07(1·39) |
| Great Scot | 0·00(0·00) | 0·13(2·05) | 0·04(1·03) |
| Dr. McIntosh | 0·00(0·00) | 0·12(2·01) | 0·03(1·01) |
| Arran Pilot | 0·00(0·00) | 0·10(1·81) | 0·03(0·91) |
| Pentland Crown | 0·00(0·00) | 0·07(1·54) | 0·02(0·77) |
| Majestic | 0·00(0·00) | 0·06(1·44) | 0·01(0·72) |
| Red Pentland Beauty | 0·00(0·00) | 0·06(1·39) | 0·01(0·69) |
| Pentland Beauty | 0·00(0·00) | 0·00(0·00) | 0·00(0·00) |
| | | | (± 1·48) |
| Mean | 0·00(0·17) | 1·2 (6·28) | |
| | | (± 0·87) | |

Standard errors for means in body of the Table:-

1) for comparisons between variety means for 1 storage treatment (± 2·09)

2) for comparisons between storage treatment means of 1 variety (± 2·23)

Table 74.

Fungal species isolated from brown lesions on stem bases in relation to inoculation treatment, 1966 and 1967.

| Year, inoculation treatment and growth form of stem bases | | Fungal species isolated from brown lesions on stem bases | | | | | |
|---|---|---|---|---|---|---|---|
| | | V.nubilum | R.solani | O.pustulans | Fusarium sp. | C.atramentarium | C.radicicola |
| 1966 Inoculated | Coiled | + | + | + | traces | - | - |
| | Uncoiled | + | + | + | traces | - | - |
| 1966 Control | Coiled | - | + | + | traces | - | - |
| | Uncoiled | - | + | + | traces | - | - |
| 1967 Inoculated | Coiled | + | + | - | traces | traces | traces |
| | Uncoiled | + | + | - | traces | traces | traces |
| 1967 Control | Coiled | traces | + | - | traces | traces | traces |
| | Uncoiled | traces | + | - | traces | traces | traces |

V. nubilum was isolated from most of the stem bases from inoculated tubers but, in 1967 only, it was also observed very occasionally on both coiled and uncoiled stem bases from control tubers. This may be traced to some contamination having taken place at the time of planting, although the number of stem bases involved appeared to be very small.

5.3.4. Conclusions

The results for both years showed that sprouting tubers in light gave an increased amount of coiling over unsprouted tubers and that marked differences in the incidence of coiling between different varieties were evident, more especially when tubers were sprouted before planting, Tables 64 and 65. Among the varieties which tended to show a high incidence of coiling were Duke of York, Arran Pilot and Ulster Premier, varieties which were observed by Moorby and McGee (1966) to be most affected by the disorder and which were noted to produce large sprouts during storage. Moreover, Arran Consul and Golden Wonder which characteristically show a slow rate of sprout growth (Burton, 1957, 1966) tended to show a low incidence of coiling. However, the relationship between the relative incidence of coiling and vigour of sprout growth for different varieties did not appear to hold in all cases e.g. Majestic gave a higher incidence of coiling than the more rapid sprouting Kerr's Pink in both years. Inoculation with V.nubilum

affected the incidence of coiling in only 1 year, 1967, when it gave an increase in coiling in unsprouted tubers (Table 67). No interaction between the inoculation treatment and varieties was found, which would suggest that varieties respond similarly to infection by V. nubilum as reported by Pitt et al. (1965).

As in the previous experiments, the increase in coiling with sprouting was associated with an increased incidence of fasciation, whereas V. nubilum inoculation was not associated with any increase in fasciation.

# SECTION 6

# GENERAL DISCUSSION AND SUMMARY

## 6.1. General discussion

The incidence of coiled sprout in potatoes has been associated with several different factors: Pitt et al. (1964, 1965) indicated a causal relationship between the disorder and the fungus V. nubilum; according to Moorby and McGee (1966), the major factor controlling the appearance of coiling was the degree of development of sprouts at planting; low soil temperature after planting (Moorby and McGee, 1966), soil compaction and deep planting (Lapwood et al., 1967) may also increase the incidence of distorted stem bases. In the present studies, the main aims were to study further the effects of V. nubilum and seed storage treatment on underground stem growth.

The results show that, in general, tubers sprouted in light to form well developed sprouts at planting give a higher incidence of coiling than unsprouted or desprouted tubers. In glasshouse studies, where sterilized soil was used and the tubers disinfected, the coils associated with sprouted tubers did not usually bear brown lesions, but were often accompanied by fasciation and splitting. In field studies, where there was less control over fungal contamination, browning of the stem bases occurred generally but, again, the occurrence of fasciation and splitting was linked with coiling associated with sprouting seed tubers before planting. With sprouted tubers, varieties were found to vary appreciably in their tendency to give coiling. In general, varieties found to be most affected were those which characteristically produce large

sprouts, confirming the observations of Moorby and McGee (1966). Thus, Arran Pilot gave more coiling than Majestic (Section 5.1.) and, in studies on 24 varieties (Section 5.3.), Arran Pilot, Duke of York and Ulster Premier, which all show vigorous sprout growth, gave a high incidence of the disorder, while varieties giving a relatively low incidence included Arran Consul and Golden Wonder, which show a slow rate of sprout growth (Burton, 1957, 1966). Differences in the incidence of coiling between varieties tended to be less marked when tubers were unsprouted or desprouted before planting.

Inoculation tests with V. nubilum (Sections 3.1., 3.2., 3.4., 3.6. and 3.7.) established that the fungus is pathogenic to potato sprouts, giving rise to the production of lesions in the form of a superficial browning (Fig. 3(1)) or, in more severe cases, browning accompanied by splitting (Fig. 3(2)) or splitting and russetting (Fig. 3(3)). The extent of browning appeared to be greater from soil than from seed tuber inoculation (Section 3.2.). This may possibly be attributed to the stem growing up through the inoculum in the soil and being exposed to more frequent infection than where the inoculum was carried from the seed tuber on to the growing sprout: an alternative possibility was that the disinfection of the seed tuber with an organo-mercurial solution before inoculation partially inhibited the activity of the seed-borne inoculum due to mercury residues persisting on the tuber (Section 3.6.). Evidence of a causal relationship between V. nubilum and the coiled sprout condition was established in the

results from certain experiments (Sections 3.6., 3.7., 5.1., 5.2. and 5.3.), thus confirming the findings of Pitt et al. (1964, 1965). In tests where a mercury compound was used as a disinfectant before seed tuber inoculation, coiling associated with V. nubilum was rarely found in glasshouse experiments and in only 1 year of the 2 years when field experiments were carried out. However, in glasshouse tests using alcohol or formalin as a disinfectant in place of an organo-mercurial solution, it was found that infection with V. nubilum produced coiling.

Residues of mercury were found on tubers disinfected with a solution of an organo-mercurial compound, even after washing. These were shown to be sufficient to suppress the activity of V. nubilum (Section 3.6.) although, in all cases where organo-mercury seed disinfection was carried out before inoculation, the fungus was still carried over on to the stems and gave rise to brown lesions. It is possible, however, that these residues may have interfered with the activity of the fungus or sprout tissues in such a way as to inhibit the development of coiling symptoms. Hamilton and Ruthven (1967) have shown that the mercury residues in dipped tubers are mainly found in the peel, especially with tubers dipped after a period of storage as was the case in the present studies. This might suggest that the effect of the mercury was mainly on the activity of the fungus at the surface of the tuber. On the other hand, it may be assumed that the level of penetration of mercury in the unsuberized regions of the eye tissues would be relatively high. This could possibly

give rise to correspondingly high values for mercury at the growing bud regions of the tuber which might affect the sprout tissues, although Hamilton and Ruthven concluded that potatoes grown from treated seed did not show increased residues compared with those of untreated seed. A further possible explanation to account for mercury reducing the incidence of coiling in some instances may relate to chemical injury to the bud tissues: Moorby and McGee (1966) have observed that sprouts that begin to grow after tip death rarely coil in the case of sprouted tubers, but it is not known if this would necessarily apply where coiling was associated with V. nubilum.

The coiled sprout condition associated with V. nubilum tended to be in the form of a swollen coil alone or in association with splitting, but without fasciation when the mother tubers were not sprouted before planting. In the light of observations on the incidence of coiled sprout in relation to storage treatment and inoculation with V. nubilum, the various forms of coiling may be distinguished as follows:

a) Coiling associated with fasciation and splitting in the underground part of the stem: in extreme cases, the growing point of the sprout may pass through the split after coiling and grow normally or coil again. This type of coiling is common where tubers are sprouted in light before planting (Fig. 2A.).

b) Coiling associated with fasciation without splitting: this type is rarely found and its occurrence is limited to instances where tubers have been sprouted in light (Fig. 2B.).

c) Coiling and splitting, where the stem may be swollen but does not show fasciation: the split is more often on the concave side of the coil but may also occur on the convex side and may sometimes also develop deep transverse cracks with or without russetting. This type is common with V. nubilum infection of stems from both sprouted and unsprouted tubers, although mainly from the latter (Fig. 2C.).

d) Coiling alone, giving symptoms similar to those described for the previous category (c) but with no cracking or splitting: when V. nubilum is present the coiling is accompanied by smooth brown spots from which the fungus can be isolated. This type is found to occur in V. nubilum inoculated tubers but may also occur in both sprouted and unsprouted uninoculated tubers. It was also the most common form of coiling which occurred occasionally in branches arising after the death of the main growing point of a sprout.

In discussing possible effects of V. nubilum on the incidence of coiling, Moorby and McGee (1966) suggested that once physiological changes occur in the developing regions of sprouts which prevent normal negatively geotropic growth, V. nubilum may have an effect in causing or increasing coiling or, alternatively, they suggest that coiling may be induced independently by either some physiological changes in well developed sprouts or by V. nubilum. From the previous observations on the forms of coiling associated with either sprouting treatment or with

V. nubilum, it would appear that the second possibility, i.e. that coiling induced by sprouting and that induced by V. nubilum are independent phenomena, is the more likely. Thus, not only do the forms of coiling associated with the 3 factors tend to be different, but coiling may occur in plants from sprouted tubers where all attempts to isolate V. nubilum have failed. Moreover, V. nubilum gives more marked increased in the incidence of coiling in unsprouted or desprouted tubers than in sprouted tubers (Sections 5.1., 5.2. and 5.3.), sprouted tubers giving a high incidence of the disorder without inoculation with the pathogen. Further possible evidence to suggest that coiling due to V. nubilum and that due to sprouting seed tubers are independent phenomena is that the different varieties tested showed marked differences in incidence of coiled sprout in relation to sprouting treatment but, with the exception of 1 case (Section 5.1.), there were no apparent differences in varietal response to inoculation with V. nubilum (Sections 5.1. and 5.3.).

In considering the incidence and economic importance of coiled sprout, the evidence would suggest that the occurrence of the disorder as a practical problem is more closely linked with sprouting treatment and possibly other cultural factors than with V. nubilum. Thus, its main economic effect is associated with delayed bulking rates in early varieties in early ware growing districts, where seed tubers are invariably sprouted (Moorby and McGee, 1966). The results of yield studies (Section 5.1.), however, indicated that final yields do not appear to be adversely

affected by an increased incidence of coiling associated with sprouting tubers. It was established that V. nubilum can remain viable in soil sterilized before inoculation for over 3 years at room temperature (Section 3.1.), which might suggest its capacity to persist in field soils. MacGarvie and Hide (1966) found the fungus present in 10·2 per cent of the potato stocks examined in an extensive survey carried out in Great Britain in 1965 and Pitt et al. (1965) reported that seed tubers may be naturally contaminated by the fungus and that it was commonly associated with coiled sprout in the field. From yield studies (Section 5.1.), infection by the fungus was found to be associated with a reduction in tuber numbers produced and a possible reduction in yield. However, the evidence from a field survey (Section 4.), although limited, indicated that V. nubilum was not of general occurrence in field crops in east Scotland, as it was not isolated from plants from any of the 35 crops examined.

The form of brown lesions associated with V. nubilum is clearly distinguished from that of R. solani but not from that caused by O. pustulans (Section 3.7.). However, neither of these 2 species is associated with coiling, either in reports of other workers or from the present investigation (Section 3.7.). It may, therefore, be assumed that coiling of potato stem bases due to V. nubilum infection is not a simple, possibly unilateral effect of injury in the growing regions. A distortion of growth due to fungal infection in the pre-emergence stage has been described in certain cereal diseases i.e. pre-emergence blight in oats due

to infection by Helminthosporium avenae Eidam or Fusarium species (Anon, 1952). The symptoms of infection by H. avenae include a twisted or contorted appearance of seedlings below soil level (Anon, 1933), but neither in this early extensive work on the disease nor in later accounts (Coffman, 1961) is any mechanism suggested to explain the development of coiling.

Correlative disturbances in growth-substance relationships have been implicated in the incidence of certain types of growth distortion (Horsfall and Dimond, 1959). In an account of the mechanism by which growth is altered in an infected plant, Wood (1967) reported that a higher plant, at any stage of its development, is regarded as a culmination of a series of reactions between the growth regulating-substances and the metabolic processes. In a healthy plant in a suitable external environment, these reactions follow a pattern delineated by its genome and result in a normal growth. Infection with a parasite can alter this pattern of growth in 1 of the following 3 ways:

1) The parasite itself produces into the infected tissue 1 or more substances with growth-regulating activity. These may be identical with, or very similar to, growth-regulating substances which are normal products of the metabolism of healthy plants and which, when present in appropriate concentrations, regulate normal growth and development. It is also possible that the parasite produces substances with growth-regulating activity, but which do not have an equivalent in healthy plants.

2) The parasite produces substances which themselves have no growth-regulating activity, but which, in one way or another, alter the amount or activity of growth substances of host origin in the tissues. This may happen because the parasite causes such growth-regulating substances to be inactivated, or because the metabolism of the host is altered so that different quantities of the active substances are produced.

3) The parasite produces substances which alter the capacity of host tissues to respond to growth-regulating substances originating in the host or in the parasite.

Based on this account by Wood (1967) of the possible effects of infection by a parasite, it might be suggested that the mechanism underlying the relationship between V. nubilum and the coiled sprout condition of potatoes is a temporary ill-balance in growth-regulating substances in the sprout tip caused by the fungal invasion of the host tissues. The growth-regulating substances involved may be gibberellins or gibberellin-like substances which are known to occur naturally in varying amounts throughout the potato plant (Booth, 1963; Brian 1966). The resumption of normal growth of the sprout tip after coiling is possibly due to the ability of gibberellins to diffuse freely in all directions (Wood, 1967), thus restoring the balance of distribution in different parts of the tip. Aube and Sackston (1965), while working with different genera and species of fungi, reported that many species of Verticillium produced gibberellin-

like substances that increased the growth rate of a dwarf mutant variety of Zea mays, while other species of the genus were noted to produce substances which inhibited growth of the dwarf mutant of maize. However, further work on V. nubilum is necessary to establish its growth-regulating activity in potato stems.

## 6.2. General summary

1) Glasshouse, laboratory and field studies were carried out on factors associated with the incidence of coiled sprout of potato, with particular reference to the effects of seed tuber inoculation with V. nubilum and storage treatment.

2) Inoculation of seed tubers with V. nubilum produced symptoms of infection on the underground stem bases of subsequent growth in the form of light brown lesions which were either superficial or accompanied by splitting or longitudinal and transverse deep cracking and, in some cases, russetting. Browning on the stem bases caused by this fungus was readily distinguished from the dark brown, well defined lesions caused by R. solani but was difficult to differentiate from that caused by O. pustulans in the absence of russetting symptoms. Microscopic examination of V. nubilum-infected stem bases indicated that the fungus was confined to the cortical tissues.

3) The extent of browning caused by V. nubilum was observed to be more with soil-borne than with seed-borne inoculum. In incidental studies, it was found that V. nubilum could persist in sterilized soil held at room temperature for over 3 years. However, in a field survey of potato crops grown in east Scotland in 1966, V. nubilum was not isolated from any of 35 stocks examined.

4) Results of laboratory tests showed that traces of mercury as

low as 0·015 ppm slightly retarded growth of V. nubilum in Czapek Dox agar and at 15 ppm the growth was completely inhibited. Tubers disinfected with an organo-mercurial solution and subsequently washed gave residual mercury values of an order sufficient to retard growth of the fungus.

5) V. nubilum was shown to cause coiling of stem bases. In glasshouse studies, the incidence of coiling in response to seed tuber inoculation with V. nubilum was greater when 2 per cent formalin or 70 per cent alcohol was used as a disinfectant before inoculation in place of an organo-mercurial solution. In 1 of the 2 years when field studies were carried out, seed tubers inoculated with V. nubilum gave more coiling than uninoculated tubers, despite the use of organo-mercurial solution for seed tuber disinfection. The response to V. nubilum appeared to be more marked in unsprouted or desprouted tubers than with tubers sprouted in light before planting.

6) Coiling associated with V. nubilum was characteristically associated with a swelling of the coiled part which may or may not be accompanied by splitting. The splitting, if present occurred most often on the inside of the coil.

7) In considering the development of coiling due to infection by V. nubilum, it is suggested that the disorder is not a simple effect of injury to tissues and a possible mechanism involving a disturbance in the balance of growth-regulating substances is briefly discussed.

8) Seed tubers sprouted in light before planting gave an increased incidence of coiled sprout compared with unsprouted or desprouted tubers. This effect of sprouting occurred in the absence of V. nubilum and the observations indicated that coiling induced by sprouting treatment and that induced by V. nubilum occur independently. The increased coiling of stem bases from sprouted tubers was often accompanied by fasciation and splitting, whereas fasciation was not associated with the increased amount of coiling caused by seed tuber inoculation with V. nubilum.

9) Different varieties of potatoes showed marked differences in the incidence of coiling, more especially in response to sprouting treatment: with few exceptions, it was found that varieties which show vigorous sprout growth gave more coiling than those which have a characteristically slow rate of sprout growth. There were generally no apparent differences in varietal response to infection with V. nubilum.

10) The increased coiling associated with sprouting seed tubers in light before planting did not affect the final weight yield or total number of tubers produced but seed tuber inoculation with V. nubilum tended to decrease tuber numbers and final weight yield.

11) When the main tip of a sprout was killed or damaged the branches given off from the sprout rarely showed coiling. A small incidence of coiling was observed in plants from unsprouted or desprouted tubers in the absence of V. nubilum and the factors involved in those instances are not known.

# SECTION 7

# ACKNOWLEDGEMENTS, REFERENCES AND APPENDICES

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## 7.3. APPENDICES

### Appendix 1

Percentage of stem bases showing coiling on the scale 0-4 in relation to inoculation with *V. nubilum* (October, 1965 - March, 1966).

| Experiment | | Treatment | Percentage of stem bases in each coiling category of Section 2.4. Fig. 1. | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 |
| 1a | i | inoculated | 49·0 | 22·2 | 28·8 | 0·0 | 0·0 |
| | | control | 66·8 | 26·0 | 7·2 | 0·0 | 0·0 |
| | ii | inoculated | 62·6 | 26·9 | 10·5 | 0·0 | 0·0 |
| | | control | 61·6 | 21·7 | 16·7 | 0·0 | 0·0 |
| 1b | i | inoculated | 58·6 | 18·8 | 18·8 | 2·6 | 1·2 |
| | | control | 55·6 | 24·2 | 9·1 | 11·1 | 0·0 |
| | ii | inoculated | 58·4 | 13·1 | 22·6 | 5·9 | 0·0 |
| | | control | 38·8 | 22·7 | 27·9 | 10·6 | 0·0 |
| 1c | | inoculated | 72·8 | 13·6 | 13·6 | 0·0 | 0·0 |
| | | control | 81·5 | 11·1 | 7·4 | 0·0 | 0·0 |
| 1d | | inoculated | 43·4 | 16·7 | 24·6 | 9·4 | 5·9 |
| | | control | 63·5 | 8·7 | 19·1 | 5·2 | 3·5 |

## Appendix 2.

Incidence of coiling and other abnormalities in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature after planting.

| Storage temperature before sprouting in light and inoculation with *V. nubilum* | Inoculation treatment | Total no. of sprouts | Stem bases showing coiling and other abnormalities | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Coiling + fasciation + splitting | Coiling + splitting | Coiling + fasciation | Coiling only | Total Coiled | Fasciation + splitting without coiling |
| **a) Glasshouse temperature during first 2 weeks after planting 50-70°F** | | | | | | | | |
| 53-60°F | Control | 23 | 1 | 1 | 0 | 0 | 2 | 3 |
| 53-60°F | Inoculated | 30 | 2 | 0 | 0 | 1 | 3 | 3 |
| 46-50°F | Control | 29 | 0 | 1 | 0 | 1 | 2 | 6 |
| 46-50°F | Inoculated | 31 | 4 | 1 | 0 | 0 | 5 | 0 |
| 40-45°F | Control | 34 | 0 | 1 | 1 | 4 | 6 | 0 |
| 40-45°F | Inoculated | 27 | 2 | 0 | 0 | 2 | 4 | 3 |
| 36-40°F | Control | 37 | 5 | 1 | 0 | 0 | 6 | 3 |
| 36-40°F | Inoculated | 42 | 3 | 0 | 0 | 1 | 4 | 0 |
| **b) Glasshouse temperature during first 2 weeks after planting 45-55°F** | | | | | | | | |
| 53-60°F | Control | 31 | 0 | 0 | 0 | 5 | 5 | 3 |
| 53-60°F | Inoculated | 28 | 5 | 0 | 0 | 4 | 9 | 2 |
| 46-50°F | Control | 23 | 0 | 0 | 0 | 0 | 0 | 2 |
| 46-50°F | Inoculated | 32 | 2 | 0 | 1 | 3 | 6 | 2 |
| 40-45°F | Control | 32 | 3 | 0 | 0 | 0 | 3 | 1 |
| 40-45°F | Inoculated | 30 | 1 | 0 | 2 | 3 | 6 | 1 |
| 36-40°F | Control | 30 | 5 | 0 | 0 | 1 | 6 | 2 |
| 36-40°F | Inoculated | 26 | 3 | 0 | 0 | 2 | 5 | 4 |
| Total | | 485 | 36 | 5 | 4 | 27 | 72 | 35 |

## Appendix 3

Number of plants showing coils in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

(From 5 plants per treatment)

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 2 | 2 | 3 | 3 |
| 46-50°F | 2 | 2 | 3 | 0 |
| 40-45°F | 3 | 4 | 3 | 2 |
| 36-40°F | 3 | 3 | 4 | 3 |

Analysis of variance

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 0·00 | NS |
| Storage temperature (T) | 3 | 0·35 | NS |
| Inoculation treatment (I) | 1 | 0·20 | NS |
| T x G | 3 | 0·17 | NS |
| I x G | 1 | 0·45 | NS |
| I x T | 3 | 0·10 | NS |
| I x T x G | 3 | 0·08 | NS |
| Error | 64 | 0·27 | |

NS Not significant

## Appendix 4

Percentage of stem bases showing coiling in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 11·7 | 6·9 | 29·0 | 14·5 |
| 46-50°F | 16·6 | 6·5 | 17·3 | 0·0 |
| 40-45°F | 13·0 | 15·8 | 21·5 | 7·3 |
| 36-40°F | 12·0 | 17·2 | 19·0 | 27·8 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 172·155 | NS |
| Storage temperature (T) | 3 | 315·682 | NS |
| Inoculation treatment (I) | 1 | 494·188 | NS |
| T x G | 3 | 240·630 | NS |
| I x G | 1 | 386·284 | NS |
| I x T | 3 | 213·656 | NS |
| I x T x G | 3 | 57·970 | NS |
| Error | 64 | 309·052 | |

NS Not significant

## Appendix 5

Percentage of stem bases showing coiling, fasciation and splitting in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 6·7 | 2·9 | 16·7 | 0·0 |
| 46-50°F | 13·7 | 0·0 | 6·7 | 0·0 |
| 40-45°F | 6·9 | 0·0 | 4·0 | 7·3 |
| 36-40°F | 8·5 | 15·2 | 10·7 | 25·6 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 36·639 | NS |
| Storage temperature (T) | 3 | 505·056 | NS |
| Inoculation treatment (I) | 1 | 308·082 | NS |
| T x G | 3 | 57·450 | NS |
| I x G | 1 | 98·708 | NS |
| I x T | 3 | 319·437 | NS |
| I x T x G | 3 | 189·507 | NS |
| Error | 64 | 220·218 | |

NS Not significant

## Appendix 6

Percentage of stem bases showing coiling and splitting in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 0·0 | 4·0 | 0·0 | 0·0 |
| 46-50°F | 2·9 | 2·5 | 0·0 | 0·0 |
| 40-45°F | 0·0 | 2·0 | 0·0 | 0·0 |
| 36-40°F | 0·0 | 2·0 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 141·372* | |
| Storage temperature (T) | 3 | 6·657 | NS |
| Inoculation treatment (I) | 1 | 47·945 | NS |
| T x G | 3 | 6·657 | NS |
| I x G | 1 | 47·945 | NS |
| I x T | 3 | 7·146 | NS |
| I x T x G | 3 | 7·146 | NS |
| Error | 64 | 28·841 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 1% level
NS Not significant

## Appendix 7

Percentage of stem bases showing coiling and fasciation in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 0·0 | 0·0 | 0·0 | 0·0 |
| 46-50°F | 0·0 | 0·0 | 4·0 | 0·0 |
| 40-45°F | 0·0 | 4·0 | 6·7 | 0·0 |
| 36-40°F | 0·0 | 0·0 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 15·548 | NS |
| Storage temperature (T) | 3 | 42·920 | NS |
| Inoculation treatment (I) | 1 | 15·548 | NS |
| T x G | 3 | 7·841 | NS |
| I x G | 1 | 97·670 | NS |
| I x T | 3 | 7·841 | NS |
| I x T x G | 3 | 42·920 | NS |
| Error | 64 | 33·187 | |

NS Not significant

## Appendix 8

Percentage of stem bases showing coiling only in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 5·0 | 0·0 | 12·4 | 14·5 |
| 46-50°F | 0·0 | 4·0 | 6·7 | 9·0 |
| 40-45°F | 6·2 | 9·8 | 10·9 | 0·0 |
| 36-40°F | 4·0 | 0·0 | 18·3 | 2·2 |

**Analysis of variance on transformed data**

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 193·816 | NS |
| Storage temperature (T) | 3 | 182·862 | NS |
| Inoculation treatment (I) | 1 | 169·271 | NS |
| T x G | 3 | 286·784 | NS |
| I x G | 1 | 141·760 | NS |
| I x T | 3 | 30·995 | NS |
| I x T x G | 3 | 183·745 | NS |
| Error | 64 | 142·845 | |

NS Not significant

## Appendix 9

Percentage of stem bases showing fasciation and splitting in relation to storage temperature before sprouting in light, seed tuber inoculation with V. nubilum and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 10·3 | 9·7 | 5·7 | 9·8 |
| 46-50°F | 0·0 | 23·0 | 6·9 | 10·0 |
| 40-45°F | 10·7 | 0·0 | 3·3 | 4·0 |
| 36-40°F | 0·0 | 9·7 | 15·3 | 7·2 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 6·556 | NS |
| Storage temperature (T) | 3 | 120·920 | NS |
| Inoculation treatment (I) | 1 | 116·957 | NS |
| T x G | 3 | 128·623 | NS |
| I x G | 1 | 67·253 | NS |
| I x T | 3 | 323·372 | NS |
| I x T x G | 3 | 305·773 | NS |
| Error | 64 | 186·195 | |

NS Not significant

## Appendix 10

Percentage of stem bases showing brown lesions in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 91·0 | 0·0 | 100·0 | 14·0 |
| 46-50°F | 100·0 | 19·2 | 100·0 | 12·0 |
| 40-45°F | 92·0 | 12·0 | 100·0 | 16·0 |
| 36-40°F | 97·8 | 2·9 | 100·0 | 10·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 434·546 | NS |
| Storage temperature (T) | 3 | 134·653 | NS |
| Inoculation treatment (I) | 1 | 119689·661*** | |
| T x G | 3 | 224·844 | NS |
| I x G | 1 | 24·294 | NS |
| I x T | 3 | 40·536 | NS |
| I x T x G | 3 | 29·008 | NS |
| Error | 64 | 263·068 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 11

Percentage of stem bases showing coiling and brown lesions in relation to storage temperature before sprouting in light, seed tuber inoculation with *V. nubilum* and glasshouse temperature for first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F | 6·7 | 0·0 | 29·0 | 3·3 |
| 46-50°F | 16·6 | 2·5 | 17·3 | 0·0 |
| 40-45°F | 13·0 | 0·0 | 21·5 | 0·0 |
| 36-40°F | 10·5 | 0·0 | 15·0 | 5·0 |

**Analysis of variance on transformed data**

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 509·211 | NS |
| Storage temperature (T) | 3 | 3·013 | NS |
| Inoculation treatment (I) | 1 | 4654·882*** | |
| T x G | 3 | 143·512 | NS |
| I x G | 1 | 227·993 | NS |
| I x T | 3 | 45·057 | NS |
| I x T x G | 3 | 46·364 | NS |
| Error | 64 | 213·058 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 12

Percentage of stem bases from desprouted tubers, showing brown lesions in relation to storage temperature, seed tuber inoculation with *V. nubilum* and glasshouse temperature during first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F (22 weeks) | 75·7 | 2·2 | 55·2 | 9·0 |
| 46-50°F (22 weeks) | 42·8 | 5·4 | 95·0 | 6·5 |
| 40-45°F (22 weeks) | 74·3 | 4·0 | 78·0 | 0·0 |
| 36-40°F (22 weeks) | 59·8 | 0·0 | 76·3 | 0·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS |
|---|---|---|
| Glasshouse temperature (G) | 1 | 800·269 NS |
| Storage temperature (T) | 3 | 141·439 NS |
| Inoculation treatment (I) | 1 | 65097·407*** |
| T x G | 3 | 731·822 NS |
| I x G | 1 | 613·612 NS |
| I x T | 3 | 233·898 NS |
| I x T x G | 3 | 851·902 NS |
| Error | 64 | 280·643 |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 13

Percentage of stem bases from desprouted tubers, showing brown lesions associated with splitting in relation to storage temperature, inoculation with *V. nubilum* and glasshouse temperature for first 2 weeks after planting.

| Storage temperature before sprouting in light | Glasshouse temperature during first 2 weeks after planting and inoculation treatments | | | |
|---|---|---|---|---|
| | 50-70°F | | 45-55°F | |
| | Inoculated | Control | Inoculated | Control |
| 53-60°F (22 weeks) | 4·0 | 0·0 | 15·7 | 0·0 |
| 46-50°F (22 weeks) | 6·9 | 0·0 | 0·0 | 0·0 |
| 40-45°F (22 weeks) | 5·7 | 0·0 | 5·0 | 0·0 |
| 36-40°F (22 weeks) | 0·0 | 0·0 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Glasshouse temperature (G) | 1 | 0·001 | NS |
| Storage temperature (T) | 3 | 91·664 | NS |
| Inoculation treatment (I) | 1 | 577·602** | |
| T x G | 3 | 82·655 | NS |
| I x G | 1 | 0·001 | NS |
| I x T | 3 | 91·664 | NS |
| I x T x G | 3 | 82·655 | NS |
| Error | 64 | 73·696 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 14

**Diameters of *V. nubilum* colonies at 1 day intervals after inoculation in relation to concentration of mercury in Czapek-Dox agar growth medium.**

| Days after inoculation | Diameter of colony in mm. (mean of 10 plates) | | | | |
|---|---|---|---|---|---|
| | Mercury concentration in ppm | | | | |
| | 0 | 0·015 | 0·15 | 1·5 | 15 |
| 1 | 8 | 8 | 8 | 8 | 8 |
| 2 | 8 | 8 | 8 | 8 | 8 |
| 3 | 10 | 8 | 8 | 8 | 8 |
| 4 | 12·5 | 8 | 8 | 8 | 8 |
| 5 | 16·0 | 9 | 8·6 | 8 | 8 |
| 6 | 19·0 | 11 | 10·7 | 8 | 8 |
| 7 | 22·1 | 14 | 13·0 | 8 | 8 |
| 8 | 25·3 | 17·5 | 15·5 | 8 | 8 |
| 9 | 30·6 | 21·5 | 18·7 | 8 | 8 |
| 10 | 34·5 | 25·0 | 21·3 | 8 | 8 |
| 11 | 37·6 | 28·0 | 25·0 | 8 | 8 |
| 12 | 40·3 | 30·2 | 28·4 | 8 | 8 |
| 13 | 43·9 | 34·8 | 31·9 | 8 | 8 |
| 14 | 47·7 | 38·6 | 36·2 | 9·0 | 8 |
| 15 | 51·5 | 41·2 | 39·8 | 10·4 | 8 |
| 16 | 54·9 | 44·9 | 42·8 | 11·8 | 8 |
| 17 | 58·7 | 47·5 | 46·0 | 11·8 | 8 |
| 18 | 65·0 | 50·8 | 50·0 | 12·6 | 8 |
| 19 | 70·3 | 55·6 | 55·0 | 13·3 | 8 |
| 20 | 76·0 | 59·6 | 58·7 | 13·8 | 8 |
| 21 | 80·7 | 63·0 | 62·1 | 14·1 | 8 |
| 22 | 84·1 | 68·8 | 66·8 | 14·5 | 8 |

## Appendix 15

### Details of field survey on the coiled sprout of potatoes (June 1966)

| Farm | Variety | Date of sampling | Date of planting | Seed tuber treatment | Post-planting treatment | Observations on stem bases | | | | | | | Fungal isolates from brown lesions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Total No. | No. showing browning | No. showing coiling & browning | No. clean and coiled | % showing coiling | % showing browning | No. showing splitting, fasciation or both without coiling | R.solani | O.pustulans | R.solani + O.pustulans | Verticillium spp. |
| Highfield | King Edward | 10/6 | 22-30 March | Not chitted | Chemical weed control | 28 | 25 | 0 | 0 | 0 | 87·8 | 1 | - | - | + | - |
| " | " " | " | " | " " | Normal cultivation | 31 | 21 | 0 | 0 | 0 | 67·7 | 1 | - | - | + | - |
| " | " " | " | " | " " | " " | 43 | 29 | 0 | 0 | 0 | 67·4 | 0 | - | - | + | - |
| " | " " | " | " | Chitted | Chemical weed control | 45 | 24 | 0 | 0 | 0 | 53·3 | 1 | - | + | - | - |
| " | " " | " | " | " | Normal cultivation | 65 | 29 | 0 | 0 | 0 | 44·6 | 0 | - | - | + | - |
| " | " " | " | " | " | " " | 41 | 18 | 0 | 0 | 0 | 43·7 | 0 | - | - | + | - |
| " | Pentland Dell | " | " | Not chitted | Chemical weed control | 34 | 28 | 0 | 0 | 0 | 82·4 | 0 | - | - | + | - |
| " | " " | " | " | Chitted | Normal cultivation | 28 | 25 | 0 | 0 | 0 | 89·2 | 0 | - | - | + | - |
| " | " " | " | " | Not chitted | Chemical weed control | 29 | 29 | 0 | 0 | 0 | 100·0 | 0 | - | + | - | - |
| Luffness Mains | Majestic | 20/6 | 7 May | Chitted | Normal cultivation | 41 | 35 | 1 | 0 | 2·9 | 85·4 | 0 | - | - | + | - |
| " " | Pentland Dell | " | 5 April | " | " " | 27 | 21 | 0 | 0 | 0 | 77·8 | 0 | - | - | + | - |
| " " | Redskin | " | 7 " | " | " " | 31 | 24 | 0 | 0 | 0 | 77·4 | 0 | - | - | + | - |
| " " | " | " | 2 May | " | " " | 43 | 31 | 0 | 0 | 0 | 74·2 | 0 | - | - | + | - |
| " " | Epicure | " | 4 April | " | " " | 17 | 11 | 0 | 0 | 0 | 64·7 | 0 | - | - | + | - |
| Archerfield | Craigs Royal | " | Early March | " | Chemical weed control | 34 | 20 | 0 | 0 | 0 | 58·8 | 0 | - | - | + | - |
| " | Epicure | " | " | " | " " " | 34 | 20 | 1 | 0 | 2·9 | 58·8 | 0 | - | + | - | - |
| Shiells | King Edward | 21/6 | " | " | Normal cultivation | 42 | 30 | 0 | 0 | 0 | 71·4 | 0 | - | + | - | - |
| Brownrigg | Majestic | 15/6 | Early April | Not chitted | " " | 24 | 24 | 0 | 0 | 0 | 100·0 | 0 | - | - | + | - |
| " | King Edward | " | " | " " | " " | 32 | 22 | 0 | 0 | 0 | 68·8 | 0 | - | + | - | - |
| Peaston Bank | King Edward | 17/6 | " | " " | " " | 46 | 25 | 0 | 0 | 0 | 54·4 | 0 | - | + | - | - |
| East Saltoun | Golden Wonder | 20/6 | " | Chitted | Chemical weed control | 33 | 26 | 0 | 0 | 0 | 78·5 | 0 | + | - | - | - |
| " " | King Edward | " | " | " | " " " | 36 | 14 | 0 | 0 | 0 | 38·9 | 0 | - | - | + | - |
| " " | " " | " | " | Not chitted | " " " | 34 | 22 | 0 | 0 | 0 | 64·7 | 0 | - | - | + | - |
| " " | " " | " | " | " " | " " " | 21 | 17 | 0 | 0 | 0 | 80·9 | 0 | - | + | - | - |
| Scoughall | Epicure | 10/6 | 8 March | Chitted | " " " | 23 | 19 | 3 | 0 | 13·0 | 82·6 | 1 | - | - | + | - |
| " | " | " | " | " | " " " | 47 | 47 | 2 | 0 | 4·3 | 100·0 | 0 | - | - | + | - |
| " | Craigs Royal | " | Early March | " | " " " | 44 | 39 | 5 | 0 | 11·4 | 88·7 | 2 | - | - | + | - |
| " | " " | " | " | " | " " " | 25 | 20 | 1 | 0 | 4·0 | 80·0 | 0 | - | - | + | - |
| " | " " | " | " | " | " " " | 31 | 28 | 2 | 0 | 6·5 | 90·3 | 0 | - | - | + | - |
| Saltcoats | Epicure | 20/6 | 8 March | " | Normal cultivation | 29 | 17 | 0 | 0 | 0 | 58·6 | 0 | - | - | + | - |
| Ferrygate | " | " | 2 " | " | Chemical weed control | 18 | 10 | 0 | 0 | 0 | 55·5 | 0 | - | - | + | - |
| Kingston | King Edward | 15/6 | 20-27 March | Not chitted | Normal cultivation | 45 | 37 | 0 | 0 | 0 | 82·2 | 1 | - | - | + | - |
| " | " " | " | " | " | " " | 41 | 29 | 0 | 0 | 0 | 70·7 | 0 | - | - | + | - |
| East Fenton | Craigs Royal | 20/6 | 1 March | Chitted | " " | 45 | 19 | 0 | 0 | 0 | 42·2 | 0 | - | - | + | - |
| " " | King Edward | " | 1 April | " | " " | 33 | 33 | 0 | 0 | 0 | 100·0 | 0 | - | - | + | - |

## Appendix 16

Percentage of plants showing coils in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 31·5 | 25·0 | 11·3 | 5·0 |
| Desprouted | 19·4 | 30·6 | 16·3 | 11·7 |
| Unsprouted | 33·1 | 22·2 | 7·8 | 5·0 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 294·539 | NS |
| Variety | (V) | 1 | 3103·143 | NS |
| Error a | | 3 | 347·793 | |
| Inoculation treatment | (I) | 1 | 188·052 | NS |
| Storage treatment | (S) | 2 | 82·523 | NS |
| V x I | | 1 | 19·514 | NS |
| V x S | | 2 | 174·369 | NS |
| I x S | | 2 | 170·194 | NS |
| V x I x S | | 2 | 88·388 | NS |
| Error b | | 30 | 225·143 | |
| Total | | 47 | 277·084 | |

NS Not significant

## Appendix 17

Percentage of plants showing coiling in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 94·7 | 39·7 | 62·5 | 20·6 |
| Desprouted | 80·0 | 32·5 | 42·5 | 20·6 |
| Unsprouted | 77·2 | 42·5 | 47·5 | 23·1 |

Analysis of variance on transformed data

| Source of variation | | DF | MS |
|---|---|---|---|
| Blocks | | 3 | 39·130 NS |
| Inoculation treatment | (I) | 1 | 3425·222** |
| Error a | | 3 | 60·098 |
| Variety | (V) | 1 | 7209·054*** |
| Storage treatment | (S) | 2 | 288·810** |
| I x V | | 1 | 487·361*** |
| I x S | | 2 | 35·002 NS |
| V x S | | 2 | 262·412** |
| I x V x S | | 2 | 29·968 NS |
| Error b | | 30 | 37·672 |
| Total | | 47 | 293·231 |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 18

Percentage of stem bases showing coiling in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 5·9 | 6·7 | 3·2 | 1·0 |
| Desprouted | 4·7 | 5·7 | 4·5 | 2·2 |
| Unsprouted | 5·5 | 3·1 | 1·6 | 0·8 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 13·997 | NS |
| Variety | (V) | 1 | 399·986* | |
| Error a | | 3 | 37·320 | |
| Inoculation treatment | (I) | 1 | 40·586 | NS |
| Storage treatment | (S) | 2 | 43·452 | NS |
| V x I | | 1 | 23·834 | NS |
| V x S | | 2 | 17·918 | NS |
| I x S | | 2 | 12·393 | NS |
| V x I x S | | 2 | 7·151 | NS |
| Error b | | 30 | 52·061 | |
| Total | | 47 | 49·830 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 19

Percentage of stem bases showing coiling in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 29·9 | 16·0 | 17·6 | 10·2 |
| Desprouted | 19·7 | 10·5 | 12·0 | 5·3 |
| Unsprouted | 23·6 | 11·9 | 10·3 | 5·8 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 12·726 | NS |
| Inoculation treatment | (I) | 1 | 588·545* | |
| Error a | | 3 | 33·235 | |
| Variety | (V) | 1 | 664·135*** | |
| Storage treatment | (S) | 2 | 124·215*** | |
| I x V | | 1 | 17·077 | NS |
| I x S | | 2 | 6·981 | NS |
| V x S | | 2 | 2·353 | NS |
| I x V x S | | 2 | 2·520 | NS |
| Error b | | 30 | 19·579 | |
| Total | | 47 | 48·237 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 20

Percentage of stem bases showing coiling not associated with fasciation or splitting in relation to variety, storage treatment and seed tuber inoculation with V. nubilum, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 14·4 | 12·2 | 5·4 | 3·5 |
| Desprouted | 13·2 | 9·7 | 12·0 | 4·5 |
| Unsprouted | 19·1 | 8·7 | 10·3 | 5·8 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 10·787 | NS |
| Inoculation treatment | (I) | 1 | 446·514** | |
| Error a | | 3 | 11·342 | |
| Variety | (V) | 1 | 311·701*** | |
| Storage treatment | (S) | 2 | 26·302 | NS |
| I x V | | 1 | 2·793 | NS |
| I x S | | 2 | 47·320 | NS |
| V x S | | 2 | 16·944 | NS |
| I x V x S | | 2 | 19·906 | NS |
| Error b | | 30 | 17·051 | |
| Total | | 47 | 33·189 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 21

Percentage of stem bases showing coiling associated with splitting in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 1·8 | 0·0 | 0·0 | 0·9 |
| Desprouted | 6·6 | 0·8 | 0·0 | 0·8 |
| Unsprouted | 4·4 | 3·2 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | | DF | MS |
|---|---|---|---|
| Blocks | | 3 | 7·893 NS |
| Inoculation treatment | (I) | 1 | 428·651*** |
| Error a | | 3 | 2·593 |
| Variety | (V) | 1 | 58·047 NS |
| Storage treatment | (S) | 2 | 28·449 NS |
| I x V | | 1 | 188·126** |
| I x S | | 2 | 59·870 NS |
| V x S | | 2 | 9·492 NS |
| I x V x S | | 2 | 32·338 NS |
| Error b | | 30 | 26·501 |
| Total | | 47 | 37·481 |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 22

Percentage of stem bases showing coiling associated with fasciation and splitting in relation to variety, storage treatment and seed tuber inoculation with V. nubilum, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 13·7 | 3·8 | 12·2 | 5·8 |
| Desprouted | 0·0 | 0·0 | 0·0 | 0·0 |
| Unsprouted | 0·0 | 0·0 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 19·418 | NS |
| Inoculation treatment | (I) | 1 | 0·001 | NS |
| Error a | | 3 | 7·508 | |
| Variety | (V) | 1 | 150·993** | |
| Storage treatment | (S) | 2 | 1278·062*** | |
| I x V | | 1 | 1·833 | NS |
| I x S | | 2 | 0·001 | NS |
| V x S | | 2 | 150·993** | |
| I x V x S | | 2 | 1·833 | NS |
| Error b | | 30 | 17·216 | |
| Total | | 47 | 76·848 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 23

Percentage of stem bases showing splitting without coiling or fasciation in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 0·0 | 0·0 | 0·0 | 0·0 |
| Desprouted | 2·2 | 0·0 | 0·0 | 0·0 |
| Unsprouted | 0·5 | 1·3 | 0·0 | 0·6 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 15·511 | NS |
| Inoculation treatment | (I) | 1 | 17·756 | NS |
| Error a | | 3 | 8·120 | |
| Variety | (V) | 1 | 0·234 | NS |
| Storage treatment | (S) | 2 | 13·854 | NS |
| I x V | | 1 | 9·097 | NS |
| I x S | | 2 | 4·823 | NS |
| V x S | | 2 | 14·877 | NS |
| I x V x S | | 2 | 5·018 | NS |
| Error b | | 30 | 12·611 | |
| Total | | 47 | 11·776 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 24

Percentage of stem bases showing fasciation and splitting in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 0·0 | 4·9 | 4·8 | 6·7 |
| Desprouted | 0·0 | 0·0 | 0·0 | 0·0 |
| Unsprouted | 2·2 | 0·0 | 0·0 | 0·0 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 2·090 | NS |
| Inoculation treatment | (I) | 1 | 47·439 | NS |
| Error a | | 3 | 6·900 | |
| Variety | (V) | 1 | 26·458 | NS |
| Storage treatment | (S) | 2 | 434·990 | *** |
| I x V | | 1 | 6·525 | NS |
| I x S | | 2 | 116·582 | ** |
| V x S | | 2 | 82·709 | ** |
| I x V x S | | 2 | 43·622 | NS |
| Error b | | 30 | 14·671 | |
| Total | | 47 | 40·496 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 25

Total yield (tons per acre) in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 7·442 | 8·913 | 11·721 | 12·966 |
| Desprouted | 7·158 | 8·317 | 10·758 | 12·065 |
| Unsprouted | 6·438 | 6·642 | 9·364 | 10·801 |

Analysis of variance

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 12·320 | NS |
| Variety | (V) | 1 | 172·764* | |
| Error a | | 3 | 8·773 | |
| Inoculation treatment | (I) | 1 | 15·516*** | |
| Storage treatment | (S) | 2 | 15·639*** | |
| V x I | | 1 | 0·444 | NS |
| V x S | | 2 | 0·431 | NS |
| I x S | | 2 | 0·316 | NS |
| V x I x S | | 2 | 0·574 | NS |
| Error b | | 30 | 1·521 | |
| Total | | 47 | 7·054 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 26

Total yield (tons per acre) in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 14·373 | 15·996 | 17·586 | 19·411 |
| Desprouted | 15·075 | 13·569 | 12·688 | 15·127 |
| Unsprouted | 10·262 | 15·401 | 12·281 | 14·561 |

Analysis of variance

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 0·133 | NS |
| Inoculation treatment | (I) | 1 | 16·240 | NS |
| Error a | | 3 | 7·025 | |
| Variety | (V) | 1 | 46·414 | *** |
| Storage treatment | (S) | 2 | 59·236 | *** |
| I x V | | 1 | 0·553 | NS |
| I x S | | 2 | 14·890 | ** |
| V x S | | 2 | 10·692 | * |
| I x V x S | | 2 | 11·613 | * |
| Error b | | 30 | 2·752 | |
| Total | | 47 | 7·662 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 27

Total number of tubers per acre in relation to variety, storage treatment and seed tuber inoculation with V. nubilum, 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 201786·3 | 216787·2 | 191140·5 | 206625·3 |
| Desprouted | 159687·0 | 193560·0 | 175655·7 | 205657·5 |
| Unsprouted | 176623·5 | 191624·4 | 148073·4 | 198882·9 |

### Analysis of variance

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 1743471424·594 | NS |
| Variety | (V) | 1 | 65642631·878 | NS |
| Error a | | 3 | 3523913986·711 | |
| Inoculation treatment | (I) | 1 | 8551572402·328* | |
| Storage treatment | (S) | 2 | 2881797397·477 | NS |
| V x I | | 1 | 350380231·203 | NS |
| V x S | | 2 | 804180780·219 | NS |
| I x S | | 2 | 394402162·688 | NS |
| V x I x S | | 2 | 473547975·727 | NS |
| Error b | | 30 | 1117652316·402 | |
| Total | | 47 | 1434195268·586 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 28

Total number of tubers per acre in relation to variety, storage treatment, and seed tuber inoculation with V. nubilum, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 46777·0 | 44438·2 | 58148·7 | 50406·3 |
| Desprouted | 54116·2 | 45889·9 | 51212·8 | 55890·5 |
| Unsprouted | 47099·6 | 50325·6 | 58068·0 | 55809·8 |

### Analysis of variance

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 23198384·204 | NS |
| Inoculation treatment | (I) | 1 | 557318433·068 | NS |
| Error a | | 3 | 153026657·303 | |
| Variety | (V) | 1 | 53442503·400 | NS |
| Storage treatment | (S) | 2 | 34076669·477 | NS |
| I x V | | 1 | 1355088·020 | NS |
| I x S | | 2 | 32203395·797 | NS |
| V x S | | 2 | 30859148·481 | NS |
| I x V x S | | 2 | 112216465·113* | |
| Error b | | 30 | 27361214·603 | |
| Total | | 47 | 50645509·464 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 29

Percentage of ware in total weight yield in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 2·6 | 2·0 | 5·5 | 9·8 |
| Desprouted | 0·0 | 2·6 | 2·8 | 3·2 |
| Unsprouted | 0·0 | 0·0 | 11·1 | 2·2 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 183·525 | NS |
| Variety | (V) | 1 | 910·377* | |
| Error a | | 3 | 56·395 | |
| Inoculation treatment | (I) | 1 | 0·814 | NS |
| Storage treatment | (S) | 2 | 64·733 | NS |
| V x I | | 1 | 34·841 | NS |
| V x S | | 2 | 61·134 | NS |
| I x S | | 2 | 125·180* | |
| V x I x S | | 2 | 85·164 | NS |
| Error b | | 30 | 37·361 | |
| Total | | 47 | 73·597 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 30

Percentage of ware in total weight yield in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 69·7 | 78·2 | 69·4 | 78·9 |
| Desprouted | 65·4 | 68·8 | 55·1 | 62·8 |
| Unsprouted | 47·6 | 67·7 | 50·3 | 63·8 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 46·764 | NS |
| Inoculation treatment | (I) | 1 | 32·588 | NS |
| Error a | | 3 | 7·071 | |
| Variety | (V) | 1 | 488·913*** | |
| Storage treatment | (S) | 2 | 433·233*** | |
| I x V | | 1 | 0·103 | NS |
| I x S | | 2 | 30·454 | NS |
| V x S | | 2 | 43·292 | NS |
| I x V x S | | 2 | 11·361 | NS |
| Error b | | 30 | 14·009 | |
| Total | | 47 | 45·533 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 31

Percentage of seed in total weight yield in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum* 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 78·0 | 78·2 | 86·2 | 81·4 |
| Desprouted | 85·8 | 78·2 | 87·8 | 86·8 |
| Unsprouted | 81·2 | 81·7 | 80·1 | 87·1 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 2·064 | NS |
| Variety | (V) | 1 | 134·825 | NS |
| Error a | | 3 | 36·368 | |
| Inoculation treatment | (I) | 1 | 9·127 | NS |
| Storage treatment | (S) | 2 | 32·774 | NS |
| V x I | | 1 | 13·637 | NS |
| V x S | | 2 | 7·597 | NS |
| I x S | | 2 | 42·381 | NS |
| V x I x S | | 2 | 25·817 | NS |
| Error b | | 30 | 18·586 | |
| Total | | 47 | 22·290 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 32

Percentage of seed in total weight yield in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 28·6 | 20·3 | 28·4 | 19·5 |
| Desprouted | 32·0 | 29·3 | 42·6 | 35·1 |
| Unsprouted | 49·2 | 30·0 | 46·9 | 33·8 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 52·407 | NS |
| Inoculation treatment | (I) | 1 | 32·056 | NS |
| Error a | | 3 | 9·540 | |
| Variety | (V) | 1 | 456·127 | *** |
| Storage treatment | (S) | 2 | 401·191 | *** |
| I x V | | 1 | 0·005 | NS |
| I x S | | 2 | 31·615 | NS |
| V x S | | 2 | 41·429 | NS |
| I x V x S | | 2 | 11·433 | NS |
| Error b | | 30 | 14·511 | |
| Total | | 47 | 44·270 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 33

Percentage of chats in total weight yield in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Storage treatment | Variety and inoculation treatment | | | |
|---|---|---|---|---|
| | Arran Pilot | | Majestic | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 19·4 | 19·8 | 8·3 | 8·7 |
| Desprouted | 14·2 | 19·2 | 9·5 | 10·1 |
| Unsprouted | 18·8 | 18·3 | 8·8 | 10·7 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 58·319* | |
| Variety | (V) | 1 | 647·651** | |
| Error a | | 3 | 4·976 | |
| Inoculation treatment | (I) | 1 | 15·974 | NS |
| Storage treatment | (S) | 2 | 3·693 | NS |
| V x I | | 1 | 0·285 | NS |
| V x S | | 2 | 15·918 | NS |
| I x S | | 2 | 4·711 | NS |
| V x I x S | | 2 | 8·064 | NS |
| Error b | | 30 | 11·034 | |
| Total | | 47 | 26·587 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 34

Percentage of chats in total weight yield in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation treatment and variety | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Arran Pilot | Majestic | Arran Pilot | Majestic |
| Sprouted | 1·8 | 1·5 | 2·1 | 1·6 |
| Desprouted | 2·5 | 1·9 | 2·3 | 2·1 |
| Unsprouted | 3·1 | 2·3 | 2·8 | 2·4 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 4·451 | NS |
| Inoculation treatment | (I) | 1 | 0·055 | NS |
| Error a | | 3 | 3·741 | |
| Variety | (V) | 1 | 9·557 | NS |
| Storage treatment | (S) | 2 | 12·250* | |
| I x V | | 1 | 0·538 | NS |
| I x S | | 2 | 0·174 | NS |
| V x S | | 2 | 0·081 | NS |
| I x V x S | | 2 | 0·492 | NS |
| Error b | | 30 | 2·684 | |
| Total | | 47 | 3·005 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 35

Mean numbers of tubers showing plant emergence per sub-plot in relation to surface disinfection, storage treatments and seed tuber inoculation with *V. nubilum*, 1966.

| Storage treatment | No. of emerged plants (10 tubers planted per sub-plot) | | | |
|---|---|---|---|---|
| | Disinfected | | Untreated | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 7·8 | 7·3 | 5·8 | 6·3 |
| Desprouted | 7·3 | 6·3 | 6·0 | 5·8 |
| Unsprouted | 8·0 | 7·5 | 5·8 | 5·8 |

**Analysis of variance**

| Source of variation | DF | MS | |
|---|---|---|---|
| Blocks | 3 | 0·34 | NS |
| Disinfection treatment (D) | 1 | 25·52* | |
| Error a | 3 | 0·91 | |
| Inoculation treatment (I) | 1 | 1·02 | NS |
| Storage treatment (S) | 2 | 1·02 | NS |
| D x I | 1 | 1·69 | NS |
| D x S | 2 | 1·27 | NS |
| I x S | 2 | 0·40 | NS |
| D x I x S | 2 | 0·06 | NS |
| Error b | 30 | 1·27 | |
| Total | 47 | 1·61 | |

* Significant at 5% level

NS Not significant

Appendix 36.

Percentage of stem bases showing coiling in relation to surface disinfection, and storage treatment and seed tuber inoculation with V. nubilum, 1966.

| Storage treatment | Disinfection and inoculation treatments | | | |
|---|---|---|---|---|
| | Disinfected | | Untreated | |
| | Inoculated | Control | Inoculated | Control |
| Sprouted | 4·4 | 8·0 | 22·0 | 14·2 |
| Desprouted | 7·2 | 9·5 | 11·1 | 9·6 |
| Unsprouted | 4·7 | 1·6 | 3·4 | 4·5 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Blocks | 3 | 30·044 | NS |
| Disinfection treatment (D) | 1 | 261·897 | NS |
| Error a | 3 | 32·629 | |
| Inoculation treatment (I) | 1 | 15·992 | NS |
| Storage treatment (S) | 2 | 511·962** | |
| D x I | 1 | 37·697 | NS |
| D x S | 2 | 103·566 | NS |
| I x S | 2 | 15·369 | NS |
| D x I x S | 2 | 52·571 | NS |
| Error b | 30 | 64·684 | |
| Total | 47 | 81·087 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 37

Percentage of stem bases showing coiling in relation to surface disinfection, and storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation and disinfection treatments | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 36·7 | 37·7 | 22·7 | 25·4 |
| Desprouted | 28·5 | 29·5 | 19·7 | 16·8 |
| Unsprouted | 33·8 | 36·6 | 16·5 | 13·2 |

Analysis of variance on transformed data

| Source of variation | DF | MS |
|---|---|---|
| Blocks | 3 | 15·132 NS |
| Inoculation treatment (I) | 1 | 1138·036** |
| Error a | 3 | 21·942 |
| Disinfection treatment (D) | 1 | 0·005 NS |
| Storage treatment (S) | 2 | 100·024*** |
| I x D | 1 | 12·725 NS |
| I x S | 2 | 52·820* |
| D x S | 2 | 4·543 NS |
| D x I x S | 2 | 8·276 NS |
| Error b | 30 | 11·340 |
| Total | 47 | 41·138 |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 38

Percentage of stem bases showing coiling without fasciation and splitting in relation to surface disinfection, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation and disinfection treatments | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 25·6 | 21·0 | 14·7 | 13·9 |
| Desprouted | 21·9 | 23·0 | 19·7 | 15·8 |
| Unsprouted | 24·0 | 29·6 | 15·4 | 13·2 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Blocks | 3 | 24·349 | NS |
| Inoculation treatment (I) | 1 | 473·391** | |
| Error a | 3 | 10·893 | |
| Disinfection treatment (D) | 1 | 3·544 | NS |
| Storage treatment (S) | 2 | 8·142 | NS |
| I x D | 1 | 20·507 | NS |
| I x S | 2 | 32·001 | NS |
| D x S | 2 | 6·547 | NS |
| D x I x S | 2 | 16·228 | NS |
| Error b | 30 | 15·253 | |
| Total | 47 | 25·247 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

Appendix 39

Percentage of stem bases showing coiling associated with splitting in relation to surface disinfection, storage treatment and seed tuber inoculation with V. nubilum, 1967.

| Storage treatment | Inoculation and disinfection treatments | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 3·3 | 6·9 | 0·0 | 0·8 |
| Desprouted | 6·6 | 6·0 | 0·0 | 0·5 |
| Unsprouted | 9·8 | 7·0 | 1·1 | 0·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Blocks | 3 | 24·241 | NS |
| Inoculation treatment (I) | 1 | 1951·488** | |
| Error a | 3 | 19·226 | |
| Disinfection treatment (D) | 1 | 3·793 | NS |
| Storage treatment (S) | 2 | 28·652 | NS |
| I x D | 1 | 1·633 | NS |
| I x S | 2 | 14·512 | NS |
| D x S | 2 | 59·280* | |
| D x I x S | 2 | 10·132 | NS |
| Error b | 30 | 13·166 | |
| Total | 47 | 57·605 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 40

Percentage of stem bases showing coiling associated with fasciation and splitting in relation to surface disinfection, and storage treatments and seed tuber inoculation with *V. nubilum*, 1967.

| Storage treatment | Inoculation and disinfection treatments | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 7·8 | 9·8 | 8·1 | 10·7 |
| Desprouted | 0·0 | 0·0 | 0·0 | 0·5 |
| Unsprouted | 0·0 | 0·0 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 2·866 | NS |
| Inoculation treatment | (I) | 1 | 9·305 | NS |
| Error a | | 3 | 15·115 | |
| Disinfection treatment | (D) | 1 | 23·523 | NS |
| Storage treatment | (S) | 2 | 1468·116 | *** |
| I x D | | 1 | 0·065 | NS |
| I x S | | 2 | 2·772 | NS |
| D x S | | 2 | 10·838 | NS |
| D x I x S | | 2 | 3·086 | NS |
| Error b | | 30 | 12·054 | |
| Total | | 47 | 72·725 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

Appendix 41

Percentage of stem bases showing splitting without fasciation or coiling in relation to surface disinfection, and storage treatment and seed tuber inoculation with V. nubilum, 1967.

| Storage treatment | Inoculation and disinfection treatments | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 0·9 | 0·0 | 0·0 | 1·7 |
| Desprouted | 0·0 | 0·0 | 0·0 | 0·0 |
| Unsprouted | 1·0 | 2·0 | 0·0 | 0·0 |

Analysis of variance on transformed data

| Source of variation | DF | MS | |
|---|---|---|---|
| Blocks | 3 | 7·547 | NS |
| Inoculation treatment (I) | 1 | 11·712 | NS |
| Error a | 3 | 23·880 | |
| Disinfection treatment (D) | 1 | 1·832 | NS |
| Storage treatment (S) | 2 | 15·225 | NS |
| I x D | 1 | 9·096 | NS |
| I x S | 2 | 18·925 | NS |
| D x S | 2 | 0·477 | NS |
| D x I x S | 2 | 17·644 | NS |
| Error b | 30 | 15·647 | |
| Total | 47 | 14·699 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 42

Percentage of stem bases showing fasciation and splitting without coiling in relation to surface disinfection, and storage treatment and inoculation with *V. mubilum*, 1967.

| Storage treatment | Inoculation and surface disinfection treatments | | | |
|---|---|---|---|---|
| | Inoculated | | Control | |
| | Disinfected | Untreated | Disinfected | Untreated |
| Sprouted | 2·2 | 4·0 | 3·3 | 2·7 |
| Desprouted | 0·0 | 0·0 | 0·0 | 0·0 |
| Unsprouted | 0·0 | 0·0 | 0·0 | 0·0 |

**Analysis of variance on transformed data**

| Source of variation | DF | MS | |
|---|---|---|---|
| Blocks | 3 | 14·757 | NS |
| Inoculation treatment (I) | 1 | 6·443 | NS |
| Error a | 3 | 14·064 | |
| Disinfection treatment (D) | 1 | 3·122 | NS |
| Storage treatment (S) | 2 | 356·122 | *** |
| I x D | 1 | 20·363 | NS |
| I x S | 2 | 6·443 | NS |
| D x S | 2 | 3·125 | NS |
| D x I x S | 2 | 20·363 | NS |
| Error b | 30 | 12·910 | |
| Total | 47 | 27·144 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 43.

Percentage of plants showing coils in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 11·1 | 33·3 | 55·6 |
| Arran Consul | 0·0 | 0·0 | 16·7 | 11·1 |
| Arran Pilot | 16·7 | 66·7 | 100·0 | 66·7 |
| Craigs Royal | 11·1 | 0·0 | 0·0 | 0·0 |
| Dr. McIntosh | 11·1 | 11·1 | 55·6 | 50·0 |
| Duke of York | 11·1 | 44·4 | 83·3 | 100·0 |
| Epicure | 0·0 | 0·0 | 16·7 | 16·7 |
| Golden Wonder | 16·7 | 0·0 | 38·9 | 11·1 |
| Great Scot | 16·7 | 11·1 | 50·0 | 38·9 |
| Home Guard | 11·1 | 0·0 | 55·6 | 66·7 |
| Kerr's Pink | 11·1 | 44·4 | 0·0 | 0·0 |
| King Edward | 0·0 | 55·6 | 16·7 | 16·7 |
| Majestic | 44·4 | 44·4 | 66·7 | 22·2 |
| Paracrinkle-free King Edward | 33·3 | 16·7 | 55·6 | 50·0 |
| Pentland Beauty | 16·7 | 0·0 | 33·3 | 27·8 |
| Pentland Crown | 11·1 | 11·1 | 11·1 | 0·0 |
| Pentland Dell | 0·0 | 33·3 | 1·6 | 98·0 |
| Record | 33·3 | 33·3 | 33·3 | 0·0 |
| Red Pentland Beauty | 33·3 | 16·7 | 27·8 | 16·7 |
| Redskin | 22·2 | 11·1 | 11·1 | 50·0 |
| Sharpe's Express | 0·0 | 0·0 | 0·0 | 0·0 |
| Up-to-Date | 11·1 | 0·0 | 11·1 | 11·1 |
| Ulster Chieftain | 1·6 | 33·3 | 38·9 | 27·8 |
| | 14·5 | 19·3 | 32·9 | 32·0 |

Analysis of variance

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 2 | 562·309 | NS |
| Storage treatment | (S) | 1 | 13599·219** | |
| Inoculation treatment | (I) | 1 | 365·858 | NS |
| S x I | | 1 | 518·804 | NS |
| Error a | | 5·9 | 873·993 | |
| Variety | (V) | 22 | 2564·204*** | |
| S x V | | 22 | 1292·481* | |
| I x V | | 22 | 940·123 | NS |
| S x I x V | | 22 | 664·896 | NS |
| Error b m | | 173·1 | 726·962 | |
| Total | | 272 | 970·035 | |

* Significant at 5% level  *** Significant at 0·1% level
** Significant at 1% level  NS Not significant

## Appendix 44

Percentage of plants showing coils in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 38·8 | 28·8 | 35·0 | 33·3 |
| Arran Consul | 21·3 | 13·3 | 32·0 | 30·8 |
| Arran Pilot | 65·0 | 38·8 | 80·0 | 83·0 |
| Craigs Royal | 25·0 | 30·0 | 18·3 | 70·0 |
| Dr. McIntosh | 38·3 | 25·0 | 55·0 | 45·0 |
| Duke of York | 60·0 | 15·0 | 80·0 | 72·5 |
| Epicure | 43·8 | 15·0 | 35·0 | 31·3 |
| Golden Wonder | 45·0 | 15·0 | 35·0 | 40·0 |
| Great Scot | 50·0 | 11·3 | 30·0 | 42·3 |
| Home Guard | 37·9 | 20·0 | 47·5 | 50·0 |
| Kerr's Pink | 25·0 | 11·3 | 52·5 | 45·0 |
| King Edward | 35·0 | 25·0 | 35·0 | 35·0 |
| Majestic | 50·0 | 10·0 | 46·7 | 51·3 |
| Paracrinkle-free King Edward | 41·3 | 20·0 | 45·5 | 47·5 |
| Pentland Beauty | 45·0 | 15·0 | 43·8 | 48·8 |
| Pentland Crown | 45·0 | 6·3 | 35·0 | 50·0 |
| Pentland Dell | 55·0 | 15·0 | 76·7 | 62·5 |
| Record | 23·8 | 10·0 | 25·0 | 33·8 |
| Red Pentland Beauty | 47·5 | 20·0 | 37·5 | 52·5 |
| Redskin | 33·8 | 20·0 | 65·0 | 75·0 |
| Sharpe's Express | 40·0 | 15·0 | 40·0 | 35·0 |
| Up-to-Date | 48·8 | 25·0 | 35·0 | 48·8 |
| Ulster Chieftain | 30·0 | 10·0 | 55·0 | 50·0 |
| Ulster Premier | 36·7 | 33·8 | 70·0 | 70·0 |
| | 40·9 | 18·7 | 46·3 | 50·1 |

Analysis of variance

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 24180·505** | |
| Storage treatment | (S) | 1 | 20164·874* | |
| Inoculation treatment | (I) | 1 | 5100·716 | NS |
| S x I | | 1 | 10789·938* | |
| Error a | | 9 | 1991·621 | |
| Variety | (V) | 23 | 1089·639*** | |
| S x V | | 23 | 372·100* | |
| I x V | | 23 | 317·346 | NS |
| S x I x V | | 23 | 169·927 | NS |
| Error b | | 276 | 220·353 | |
| Total | | 383 | 606·179 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 45

Percentage of stem bases showing coiling in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1966.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 6·1 | 9·3 | 15·6 |
| Arran Consul | 0·0 | 0·0 | 2·4 | 2·6 |
| Arran Pilot | 2·6 | 41·5 | 38·0 | 25·6 |
| Craigs Royal | 2·4 | 0·0 | 0·0 | 0·0 |
| Dr. McIntosh | 2·1 | 4·8 | 6·4 | 21·9 |
| Duke of York | 1·5 | 5·6 | 29·6 | 31·4 |
| Epicure | 0·0 | 0·0 | 5·1 | 7·1 |
| Golden Wonder | 3·3 | 0·0 | 4·2 | 1·8 |
| Great Scot | 4·2 | 1·3 | 11·5 | 7·0 |
| Home Guard | 4·7 | 0·0 | 16·2 | 28·3 |
| Kerr's Pink | 1·8 | 11·7 | 0·0 | 0·0 |
| King Edward | 0·0 | 12·0 | 3·3 | 4·8 |
| Majestic | 8·7 | 9·7 | 10·4 | 6·7 |
| Paracrinkle-free King Edward | 8·6 | 4·8 | 20·2 | 7·4 |
| Pentland Beauty | 2·8 | 0·0 | 6·7 | 4·8 |
| Pentland Crown | 2·1 | 1·8 | 5·1 | 0·0 |
| Pentland Dell | 0·0 | 4·2 | 0·5 | 36·8 |
| Record | 4·8 | 6·7 | 4·8 | 0·0 |
| Red Pentland Beauty | 7·7 | 2·2 | 5·3 | 6·7 |
| Redskin | 4·2 | 2·1 | 1·9 | 10·5 |
| Sharpe's Express | 1·6 | 0·0 | 0·0 | 0·0 |
| Up-to-Date | 6·7 | 0·0 | 2·6 | 2·2 |
| Ulster Chieftain | 0·5 | 13·3 | 3·6 | 7·8 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS |
|---|---|---|---|
| Blocks | | 2 | 91·998 NS |
| Storage treatment | (S) | 1 | 1793·263*** |
| Inoculation treatment | (I) | 1 | 166·073 NS |
| S x I | | 1 | 25·909 NS |
| Error a | | 5·9 | 75·070 |
| Variety | (V) | 22 | 471·225*** |
| S x V | | 22 | 198·921 NS |
| I x V | | 22 | 152·224 NS |
| S x I x V | | 22 | 171·454 NS |
| Error b | | 173·1 | 134·456 |
| Total | | 272 | 173·638 |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 46

Percentage of stem bases showing coiling in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 10·8 | 7·2 | 14·1 | 10·1 |
| Arran Consul | 8·7 | 1·3 | 11·6 | 12·6 |
| Arran Pilot | 22·6 | 10·0 | 31·2 | 32·9 |
| Craigs Royal | 6·0 | 6·6 | 5·2 | 22·1 |
| Dr. McIntosh | 20·6 | 11·8 | 19·3 | 19·3 |
| Duke of York | 17·6 | 4·7 | 25·0 | 23·6 |
| Epicure | 12·5 | 3·1 | 9·4 | 11·1 |
| Golden Wonder | 16·8 | 5·1 | 9·3 | 16·3 |
| Great Scot | 17·0 | 2·5 | 7·7 | 15·4 |
| Home Guard | 10·5 | 4·0 | 14·1 | 14·7 |
| Kerr's Pink | 9·7 | 5·3 | 16·4 | 15·3 |
| King Edward | 15·1 | 6·1 | 12·0 | 13·2 |
| Majestic | 18·5 | 4·1 | 27·9 | 20·9 |
| Paracrinkle-free King Edward | 18·4 | 7·1 | 12·7 | 16·5 |
| Pentland Beauty | 16·7 | 2·9 | 13·3 | 15·5 |
| Pentland Crown | 14·6 | 6·2 | 12·3 | 19·9 |
| Pentland Dell | 35·6 | 9·2 | 41·5 | 36·7 |
| Record | 11·8 | 1·1 | 6·4 | 9·9 |
| Red Pentland Beauty | 14·6 | 4·6 | 13·2 | 20·9 |
| Redskin | 9·9 | 5·2 | 21·1 | 25·1 |
| Sharpe's Express | 11·3 | 5·5 | 14·3 | 17·2 |
| Up-to-Date | 13·4 | 6·4 | 14·5 | 19·9 |
| Ulster Chieftian | 7·9 | 4·7 | 19·5 | 14·7 |
| Ulster Premier | 16·1 | 8·5 | 22·9 | 23·8 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 6046·941** | |
| Storage treatment | (S) | 1 | 5492·227* | |
| Inoculation treatment | (I) | 1 | 633·495 | NS |
| S x I | | 1 | 4437·229* | |
| Error a | | 9 | 547·039 | |
| Variety | (V) | 23 | 311·516*** | |
| S x V | | 23 | 63·707 | NS |
| I x V | | 23 | 72·722 | NS |
| S x I x V | | 23 | 43·924 | NS |
| Error b | | 276 | 48·285 | |
| Total | | 383 | 152·193 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 47

Percentage of stem bases showing coiling without fasciation or splitting in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 10·8 | 7·2 | 9·1 | 7·3 |
| Arran Consul | 7·2 | 1·3 | 1·7 | 10·9 |
| Arran Pilot | 22·0 | 10·0 | 18·0 | 17·9 |
| Craigs Royal | 6·0 | 6·6 | 5·9 | 9·3 |
| Dr. McIntosh | 19·3 | 11·8 | 14·6 | 13·8 |
| Duke of York | 15·3 | 4·7 | 11·8 | 6·4 |
| Epicure | 10·9 | 3·1 | 8·4 | 3·5 |
| Golden Wonder | 14·2 | 5·1 | 6·3 | 2·1 |
| Great Scot | 13·3 | 2·5 | 5·1 | 11·5 |
| Home Guard | 9·4 | 4·0 | 13·2 | 7·8 |
| Kerr's Pink | 8·7 | 5·3 | 12·9 | 10·1 |
| King Edward | 13·9 | 6·1 | 10·3 | 10·7 |
| Majestic | 14·6 | 4·1 | 24·5 | 19·6 |
| Paracrinkle-free King Edward | 16·8 | 5·8 | 9·4 | 9·0 |
| Pentland Beauty | 16·7 | 2·9 | 6·7 | 4·1 |
| Pentland Crown | 14·6 | 6·2 | 8·3 | 9·5 |
| Pentland Dell | 35·6 | 9·2 | 24·8 | 21·2 |
| Record | 11·8 | 1·1 | 6·4 | 5·7 |
| Red Pentland Beauty | 13·6 | 4·6 | 13·2 | 15·3 |
| Redskin | 8·9 | 5·2 | 11·5 | 10·1 |
| Sharpe's Express | 11·3 | 5·5 | 3·9 | 8·6 |
| Up-to-Date | 11·2 | 6·4 | 12·0 | 12·4 |
| Ulster Chieftain | 7·9 | 4·7 | 15·6 | 5·7 |
| Ulster Premier | 16·1 | 7·2 | 10·7 | 7·1 |

### Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 7503·743** | |
| Storage treatment | (S) | 1 | 111·787 | NS |
| Inoculation treatment | (I) | 1 | 1608·557 | NS |
| S x I | | 1 | 1627·299 | NS |
| Error a | | 9 | 487·810 | |
| Variety | (V) | 23 | 255·131*** | |
| S x V | | 23 | 73·920 | NS |
| I x V | | 23 | 80·585 | NS |
| S x I x V | | 23 | 54·410 | NS |
| Error b | | 276 | 53·074 | |
| Total | | 383 | 145·093 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 48

Percentage of stem bases showing coiling and splitting in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Consul | 0·0 | 0·0 | 1·7 | 0·0 |
| Arran Pilot | 0·6 | 0·0 | 4·0 | 0·0 |
| Craigs Royal | 0·0 | 0·0 | 0·0 | 0·0 |
| Dr. McIntosh | 1·3 | 0·0 | 0·9 | 0·0 |
| Duke of York | 1·0 | 0·0 | 0·0 | 0·0 |
| Epicure | 1·6 | 0·0 | 0·0 | 0·0 |
| Golden Wonder | 2·6 | 0·0 | 0·0 | 0·0 |
| Great Scot | 3·7 | 0·0 | 0·0 | 0·0 |
| Home Guard | 1·2 | 0·0 | 0·0 | 1·0 |
| Kerr's Pink | 0·9 | 0·0 | 1·7 | 0·0 |
| King Edward | 1·2 | 0·0 | 0·9 | 0·0 |
| Majestic | 3·9 | 0·0 | 0·0 | 0·0 |
| Paracrinkle-free King Edward | 1·6 | 1·3 | 2·4 | 0·0 |
| Pentland Beauty | 0·0 | 0·0 | 2·1 | 1·0 |
| Pentland Crown | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Dell | 0·0 | 0·0 | 2·8 | 0·0 |
| Record | 0·0 | 0·0 | 0·0 | 1·2 |
| Red Pentland Beauty | 1·0 | 0·0 | 0·0 | 1·0 |
| Redskin | 1·0 | 0·0 | 0·8 | 0·0 |
| Sharpe's Express | 0·0 | 0·0 | 0·0 | 0·0 |
| Up-to-Date | 0·0 | 0·0 | 0·0 | 0·9 |
| Ulster Chieftain | 0·0 | 0·0 | 1·4 | 0·0 |
| Ulster Premier | 0·0 | 0·0 | 0·0 | 0·0 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 34·195 | NS |
| Storage treatment | (S) | 1 | 0·645 | NS |
| Inoculation treatment | (I) | 1 | 248·829** | |
| S x I | | 1 | 14·222 | |
| Error a | | 9 | 21·946 | NS |
| Variety | (V) | 23 | 11·807 | NS |
| S x V | | 23 | 14·151 | NS |
| I x V | | 23 | 13·300 | NS |
| S x I x V | | 23 | 16·063 | NS |
| Error b | | 276 | 14·170 | |
| Total | | 383 | 15·006 | |

* Significant at 5% level  *** Significant at 0·1% level
** Significant at 1% level  NS Not significant

## Appendix 49

Percentage of stem bases showing coiling and fasciation in relation to storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Consul | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Pilot | 0·0 | 0·0 | 1·0 | 0·0 |
| Craigs Royal | 0·0 | 0·0 | 0·0 | 0·0 |
| Dr. McIntosh | 0·0 | 0·0 | 0·0 | 0·0 |
| Duke of York | 0·0 | 0·0 | 0·0 | 0·0 |
| Epicure | 0·0 | 0·0 | 0·0 | 0·0 |
| Golden Wonder | 0·0 | 0·0 | 0·0 | 0·0 |
| Great Scot | 0·0 | 0·0 | 0·0 | 0·0 |
| Home Guard | 0·0 | 0·0 | 0·0 | 0·0 |
| Kerr's Pink | 0·0 | 0·0 | 0·0 | 0·0 |
| King Edward | 0·0 | 0·0 | 0·0 | 2·5 |
| Majestic | 0·0 | 0·0 | 0·0 | 0·0 |
| Paracrinkle-free King Edward | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Crown | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Dell | 0·0 | 0·0 | 0·0 | 0·0 |
| Record | 0·0 | 0·0 | 0·0 | 0·0 |
| Red Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Redskin | 0·0 | 0·0 | 0·0 | 0·0 |
| Sharpe's Express | 0·0 | 0·0 | 0·0 | 0·0 |
| Up-to-Date | 0·0 | 0·0 | 0·0 | 0·0 |
| Ulster Chieftain | 0·0 | 0·0 | 0·0 | 0·0 |
| Ulster Premier | 0·0 | 0·0 | 0·0 | 0·0 |

### Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 0·862 | NS |
| Storage treatment | (S) | 1 | 2·339 | NS |
| Inoculation treatment | (I) | 1 | 0·124 | NS |
| S x I | | 1 | 0·124 | NS |
| Error a | | 9 | 1·355 | |
| Variety | (V) | 23 | 1·183 | NS |
| S x V | | 23 | 1·183 | NS |
| I x V | | 23 | 1·280 | NS |
| S x I x V | | 23 | 1·280 | NS |
| Error b | | 276 | 1·232 | |
| Total | | 383 | 1·229 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 50

Percentage of stem bases showing coiling with fasciation and splitting in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 0·0 | 5·0 | 2·7 |
| Arran Consul | 1·5 | 0·0 | 8·2 | 1·7 |
| Arran Pilot | 0·0 | 0·0 | 8·2 | 15·0 |
| Craigs Royal | 0·0 | 0·0 | 0·0 | 12·7 |
| Dr. McIntosh | 0·0 | 0·0 | 3·8 | 5·5 |
| Duke of York | 1·3 | 0·0 | 13·2 | 17·2 |
| Epicure | 0·0 | 0·0 | 1·0 | 7·5 |
| Golden Wonder | 0·0 | 0·0 | 3·0 | 14·2 |
| Great Scot | 0·0 | 0·0 | 2·6 | 3·9 |
| Home Guard | 0·0 | 0·0 | 0·9 | 5·9 |
| Kerr's Pink | 0·0 | 0·0 | 1·7 | 5·2 |
| King Edward | 0·0 | 0·0 | 0·8 | 0·0 |
| Majestic | 0·0 | 0·0 | 3·4 | 1·3 |
| Paracrinkle-free King Edward | 0·0 | 0·0 | 0·9 | 7·6 |
| Pentland Beauty | 0·0 | 0·0 | 4·4 | 10·4 |
| Pentland Crown | 0·0 | 0·0 | 4·0 | 10·4 |
| Pentland Dell | 0·0 | 0·0 | 3·8 | 15·5 |
| Record | 0·0 | 0·0 | 0·0 | 3·0 |
| Red Pentland Beauty | 0·0 | 0·0 | 0·0 | 4·6 |
| Redskin | 0·0 | 0·0 | 8·9 | 15·0 |
| Sharpe's Express | 0·0 | 0·0 | 10·5 | 8·6 |
| Up-to-Date | 2·2 | 0·0 | 2·5 | 6·5 |
| Ulster Chieftain | 0·0 | 0·0 | 2·5 | 9·0 |
| Ulster Premier | 0·0 | 1·4 | 12·3 | 16·7 |

### Analysis of variance on transformed data

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 281·269 | NS |
| Storage treatment | (S) | 1 | 9958·249 | *** |
| Inoculation treatment | (I) | 1 | 626·282 | NS |
| S x I | | 1 | 794·682 | NS |
| Error a | | 9 | 160·733 | |
| Variety | (V) | 23 | 135·639 | *** |
| S x V | | 23 | 115·727 | ** |
| I x V | | 23 | 44·930 | NS |
| S x I x V | | 23 | 35·648 | NS |
| Error b | | 276 | 38·016 | |
| Total | | 383 | 83·020 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 51

Percentage of stem bases showing fasciation only in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Consul | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Pilot | 0·0 | 0·0 | 0·0 | 0·0 |
| Craigs Royal | 0·0 | 0·0 | 0·0 | 4·2 |
| Dr. McIntosh | 0·0 | 0·0 | 0·0 | 0·0 |
| Duke of York | 0·0 | 0·0 | 0·0 | 0·0 |
| Epicure | 0·0 | 0·0 | 0·0 | 1·6 |
| Golden Wonder | 0·0 | 0·0 | 0·0 | 0·0 |
| Great Scot | 0·0 | 0·0 | 0·0 | 2·2 |
| Home Guard | 0·0 | 0·0 | 0·0 | 1·0 |
| Kerr's Pink | 0·0 | 0·0 | 0·0 | 0·0 |
| King Edward | 0·0 | 0·0 | 0·0 | 0·0 |
| Majestic | 2·4 | 0·0 | 0·0 | 0·0 |
| Paracrinkle-free King Edward | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Crown | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Dell | 0·0 | 0·0 | 0·0 | 0·0 |
| Record | 0·0 | 0·0 | 0·0 | 0·0 |
| Red Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Redskin | 0·0 | 0·0 | 0·0 | 0·0 |
| Sharpe's Express | 0·0 | 0·0 | 0·0 | 0·0 |
| Up-to-Date | 0·0 | 0·0 | 0·0 | 0·0 |
| Ulster Chieftain | 0·0 | 0·0 | 0·0 | 2·5 |
| Ulster Premier | 0·0 | 0·0 | 0·0 | 1·0 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 22·319 | NS |
| Storage treatment | (S) | 1 | 16·265 | NS |
| Inoculation treatment | (I) | 1 | 16·265 | NS |
| S x I | | 1 | 34·428 | NS |
| Error a | | 9 | 26·356 | |
| Variety | (V) | 23 | 3·960 | NS |
| S x V | | 23 | 4·750 | NS |
| I x V | | 23 | 4·750 | NS |
| S x I x V | | 23 | 3·960 | NS |
| Error b | | 276 | 4·355 | |
| Total | | 383 | 5·154 | |

* Significant at 5% level
** Significant at 1% level
*** Significant at 0·1% level
NS Not significant

## Appendix 52

Percentage of stem bases showing splitting only in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Consul | 0·0 | 0·0 | 0·0 | 0·0 |
| Arran Pilot | 0·0 | 0·0 | 0·0 | 0·0 |
| Craigs Royal | 0·0 | 0·0 | 0·0 | 0·0 |
| Dr. McIntosh | 0·0 | 0·0 | 0·0 | 0·0 |
| Duke of York | 0·0 | 0·0 | 0·0 | 0·0 |
| Epicure | 0·0 | 0·0 | 1·1 | 0·0 |
| Golden Wonder | 0·0 | 0·0 | 0·0 | 0·0 |
| Great Scot | 0·0 | 0·0 | 0·0 | 0·0 |
| Home Guard | 0·0 | 0·0 | 0·0 | 0·0 |
| Kerr's Pink | 0·0 | 0·0 | 0·0 | 0·0 |
| King Edward | 0·0 | 0·0 | 1·9 | 0·0 |
| Majestic | 0·0 | 0·0 | 0·0 | 0·0 |
| Paracrinkle-free King Edward | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Crown | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Dell | 0·0 | 0·0 | 0·0 | 0·0 |
| Record | 0·0 | 0·0 | 0·0 | 0·0 |
| Red Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Redskin | 0·0 | 0·0 | 0·0 | 0·0 |
| Sharpe's Express | 0·0 | 0·0 | 0·0 | 0·0 |
| Up-to-Date | 0·0 | 0·0 | 0·0 | 0·0 |
| Ulster Chieftain | 0·0 | 0·0 | 0·0 | 0·0 |
| Ulster Premier | 0·0 | 0·0 | 0·0 | 0·0 |

**Analysis of variance on transformed data**

| Source of variation | | DF | MS | |
|---|---|---|---|---|
| Blocks | | 3 | 0·707 | NS |
| Storage treatment | (S) | 1 | 2·057 | NS |
| Inoculation treatment | (I) | 1 | 2·057 | NS |
| S x I | | 1 | 2·057 | NS |
| Error a | | 9 | 0·707 | |
| Variety | (V) | 23 | 1·000 | NS |
| S x V | | 23 | 1·000 | NS |
| I x V | | 23 | 1·000 | NS |
| S x I x V | | 23 | 1·000 | NS |
| Error b | | 276 | 1·059 | |
| Total | | 383 | 1·042 | |

* Significant at 5% level  *** Significant at 0·1% level
** Significant at 1% level  NS Not significant

## Appendix 53

Percentage of stem bases showing fasciation and splitting in relation to variety, storage treatment and seed tuber inoculation with *V. nubilum*, 1967.

| Variety | Storage and inoculation treatments | | | |
|---|---|---|---|---|
| | Unsprouted | | Sprouted | |
| | Inoculated | Control | Inoculated | Control |
| Arran Banner | 0·0 | 0·0 | 0·0 | 3·8 |
| Arran Consul | 0·0 | 0·0 | 1·7 | 1·4 |
| Arran Pilot | 0·0 | 0·0 | 0·0 | 1·6 |
| Craigs Royal | 0·0 | 0·0 | 0·0 | 6·2 |
| Dr. McIntosh | 0·0 | 0·0 | 0·0 | 1·9 |
| Duke of York | 1·3 | 0·0 | 1·1 | 28·9 |
| Epicure | 0·0 | 0·0 | 2·3 | 15·7 |
| Golden Wonder | 0·0 | 0·0 | 11·9 | 14·8 |
| Great Scot | 0·0 | 0·0 | 2·0 | 0·0 |
| Home Guard | 0·0 | 0·0 | 0·0 | 3·9 |
| Kerr's Pink | 0·0 | 0·0 | 1·3 | 4·6 |
| King Edward | 0·0 | 0·0 | 1·9 | 1·3 |
| Majestic | 0·0 | 0·0 | 0·0 | 1·0 |
| Paracrinkle-free King Edward | 0·0 | 0·0 | 2·9 | 1·9 |
| Pentland Beauty | 0·0 | 0·0 | 0·0 | 0·0 |
| Pentland Crown | 0·0 | 0·0 | 0·0 | 1·1 |
| Pentland Dell | 0·0 | 2·5 | 0·0 | 1·7 |
| Record | 0·0 | 0·0 | 0·0 | 3·6 |
| Red Pentland Beauty | 0·0 | 0·0 | 0·9 | 0·0 |
| Redskin | 0·0 | 0·0 | 0·8 | 15·0 |
| Sharpe's Express | 0·0 | 0·0 | 14·4 | 19·7 |
| Up-to-Date | 0·0 | 0·0 | 1·4 | 3·9 |
| Ulster Chieftain | 0·0 | 0·0 | 2·4 | 11·0 |
| Ulster Premier | 0·0 | 0·0 | 2·9 | 5·9 |

### Analysis of variance on transformed data

| Source of variation | | DF | MS |
|---|---|---|---|
| Blocks | | 3 | 190·047 NS |
| Storage treatment | (S) | 1 | 3594·558*** |
| Inoculation treatment | (I) | 1 | 713·706 NS |
| S x I | | 1 | 685·779 NS |
| Error a | | 9 | 146·884 |
| Variety | (V) | 23 | 138·420*** |
| S x V | | 23 | 133·055*** |
| I x V | | 23 | 44·735 NS |
| S x I x V | | 23 | 59·169* |
| Error b | | 276 | 35·025 |
| Total | | 383 | 65·738 |

* Significant at 5% level  *** Significant at 0·1% level
** Significant at 1% level  NS Not significant