RESISTANCE TO THE PEA WEEVIL IN *PISUM* SPECIES

by

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"A plant breeder's opinion on insect control is generally as limited as the public opinion: insects are nasty and noxious and must be controlled by insecticides. This entomophobia might be caused by a lack of training of plant breeders in entomology, often being limited to instruction in pesticide usage. Entomologists, on the other hand, are mostly not trained in plant breeding. This educational gap, which is less prominent between plant breeders and phytopathologists, might be one of the main reasons why plant breeding for resistance to insects and mites is lagging behind breeding for resistance to diseases."

O.M.B. de Ponti (1981)

Pea weevils visiting a Dun pea flower.

Frontispiece photograph courtesy of G. Baker

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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CONTENTS

SUMMARY

1. The pea weevil, *Bruchus pisorum* (L.) is a serious pest of field peas in southern Australia. It can be controlled by insecticide sprays but their use adds to the cost of production. The development of resistant cultivars may overcome or reduce the need for chemical control measures. My study was concerned with identifying useful sources of genetic resistance to the pea weevil and the means by which resistance may be bred into cultivars.

2. Mass screening was undertaken to obtain sources of pea germplasm that were resistant to the pea weevil. The material screened included local cultivars, landraces and wild pea types. Some were lines reputed to be resistant to the pea weevil. After three seasons of field trials, 1650 of the original 1825 Pisum accessions had been discarded and 106 of the 175 remaining accessions could not be categorised because they did not flower or set sufficient seed. This left 69 accessions that were potentially resistant, including all of the *P. fulvum* accessions.

3. To investigate possible mechanisms of resistance, no-choice testing of whole plants was undertaken in a growth room and a glasshouse. These tests demonstrated the presence of a high level of resistance to the pea weevil in the *P. fulvum* accessions. The results obtained for oviposition antixenosis were variable and dependant on the test environment. The no-choice tests also indicated the presence of a feeding antixenosis and / or an antibiosis which was in the pod wall, the testa or the cotyledons. It was later found to be in the testa or cotyledons.

4. The inheritance of oviposition antixenosis and cotyledon antibiosis were investigated. The pea cultivar Pennant was crossed to the resistant *P. fulvum* accessions.

Pennant was the female parent as the reciprocal cross is not successful. The presence of pink flowers in the F_1, when Pennant has a white flower, confirmed that hybrids of Pennant by *P. fulvum* were obtained. The F_1 and F_2 progeny and their parents from four of these crosses were evaluated. Pods were sampled for the number of eggs laid on them and the infested seed harvested from each plot was assessed for the emergence of adult weevils. No clear genetic segregation ratios were detected. In most instances the F_1 and F_2 plants resembled the Pennant parent. This lack of evidence for genetic segregation for resistance is difficult to explain. In the Discussion reference is made to possible cytoplasmic factors.

5. The presence of antibiosis in the cotyledons was confirmed by the non-emergence and death of some of the larvae and the longer period of development of larvae that did survive to emerge as adults. However the free-choice offered to weevils in the field trial reduced infestation rates on many of the less preferred lines and meant only small numbers of seed were available for study. As many of the larvae entering the cotyledons did emerge as adults, this suggests variation in the seed antibiosis is influenced by the environment and is inherited as a quantitative trait.

6. Results obtained in a field trial suggested pea weevils preferred to lay their eggs on some genotypes and at certain stages of development. A bio-assay was developed to assess this preference and obtain information on oviposition antixenosis and cotyledon antibiosis. It was confirmed that the stage of pod development and length influence the number of eggs laid. It was also established the first hatching of eggs occurred over a wide range of pod development from all stages, swollen to green-wrinkled . A seed antibiosis assay was developed, timing the hatching of eggs to occur in the middle of this pod development range.

7. Pod preference bio-assays in both choice and no-choice situations demonstrated that there were differences between accessions even when pod length and stage of

development were standardised. The number of eggs laid on pods of several *P. fulvum* accessions were significantly lower than on the control cultivar.

8. The development of the pea weevil from laying of the egg on the pod to adult emergence from the seed, was monitored in several *P. fulvum* accessions and in a control cultivar. The results indicated that there was no resistance mechanism in the pod wall in the *P. fulvum* accessions. However significantly fewer larvae penetrated the seed coat in the *P. fulvum* accessions than in the control cultivar . The proportion of larvae that entered the cotyledons and emerged as adults in these *P. fulvum* accessions was also significantly lower than in the control cultivar. These results demonstrated the presence of resistance mechanisms in both the seed coat and the cotyledons of *P. fulvum.*

9. Various resistance mechanisms were found to affect the development of the pea weevil. Bio-assays were established to detect the presence of antixenosis and antibiosis. Results from these bio-assays were used to construct a model for resistance to the pea weevil. The model will allow breeders to assess the merits of including the various *P. fulvum* accessions in their breeding programs.

CHAPTER 1

INTRODUCTION

The field pea (*Pisum sativum* L.) is Australia's second most important grain legume after lupins. It is the most important grain legume in New South Wales (50,000 ha), Victoria (160,000 ha) and South Australia (115,000 ha) and the area sown in Western Australia has increased to 50,000 hectares this decade (Mahoney 1991). The value of the crop has risen from $21 million in 1982/83 to $100 million in 1989/90 (Australian Agriculture 1992).

The reasons for the increase in field pea production over the last decade include lower prices for some other broadacre crops and an increased demand for protein meal in the intensive livestock industries, both in Australia and overseas. Farmers are also aware of the benefits of using field peas in rotations. They reduce the level of disease in following cereal crops, provide an opportunity to control grassy weeds and result in an increase in available soil nitrogen. Markets have also emerged for premium grade peas, as substitutes for chickpeas and lentils for human consumption.

Further increases in pea production in Australia are restricted by several factors, but most importantly yield. Yield is affected by pests, diseases and weeds in the crop. The pea weevil, *Bruchus pisorum* (L.) is one of the most damaging insect pests of peas. Pea weevils reduce the yield by consuming a large part of the seeds they infest. Infested seed has to be sold as stockfeed because emerging adults contaminate grain. Infested seed also has an increased likelihood of cracking while being harvested.

The damage caused to a crop can be reduced by monitoring for the weevils' invasion and adopting a spraying strategy. This will minimise losses and ensure that most seed is acceptable as feed or milling, though in some instances seed cleaning is necessary. A high

standard is only achieved when a farmer is proficient and has time to monitor the crop, and sprays and harvests at the appropriate time. This can be a problem as the weevil and eggs are not easily observed and the damage caused by the larvae is not seen at harvest. The size and duration of weevil invasion determines the number and types of sprays needed. Most chemicals registered in Australia provide protection for a maximum of seven days. Invasions can continue for many weeks in some seasons, thus requiring several applications (Michael *et al.* 1990; Baker & Phillips 1992). Some crops require a border spray only, while others require a spray of the whole crop. The cost of chemicals and their application severely reduces the profitability of growing peas in Australia. There are also concerns about the impact of spray drift on the environment and the marketing of grain with insecticide residues.

Cultural methods have been used to reduce the damage caused by the pea weevil. Baker (1990c) found early harvesting reduced grain-shatter at harvest, and grain fumigation stopped adult emergence and weight losses associated with larval feeding. However at the earliest possible harvest date the majority of larvae are third and fourth instars and have damaged enough of the seed to prevent the material being sold as premium seed.

Biological control agents have been tried in Australia. The larval parasite, *Triaspis thoracius* was imported from France to Western Australia in the late 1930s but failed to establish (Wilson 1960; Clausen 1978). Baker (1990b) suggested importing another parasitic wasp, the egg parasite *Uscana senex* (Trichogrammatidae) which has recorded rates of parasitism of 85% in Eastern Europe (Karpova 1950). However the use of insecticides against the pea weevil would affect the survival of *U. senex*. Also, in parts of Southern Europe with climates similar to the pea growing areas of Australia, the rates for parasitism for weevil eggs are always less than five per cent (Baker 1990b).

The limited success of cultural methods, the failure of biological control and the reliance on expensive insecticides demonstrates the need for alternative control measures. One

attractive option would be to develop pea weevil-resistant cultivars. None of the field pea cultivars currently grown in Australia have any known resistance to pea weevil (pers. comm. Dr Ali, S.A. Dept Agric), although some resistance has been reported in other regions of the world such as the USA and Russia (Pesho *et al.* 1977; Posylaeva 1988).

Before a program for breeding resistance to pea weevil can be started, several questions need to be answered. They are:

1. Will the germplasm identified in the literature as being resistant provide resistance under Australian conditions and are there other sources of resistant germplasm ?
2. Can mechanisms of resistance be identified and efficient screening procedures be developed to evaluate germplasm ?
3. How are resistance mechanisms inherited ?
4. Will any of the resistance genes be of value in a breeding program ?

My study was concerned with these questions. Emphasis was placed on determining sources of resistance and the identification of mechanisms involved.

CHAPTER 2

LITERATURE REVIEW

2.1. The pest species - *Bruchus pisorum (L.)*

2.1.1. Taxonomy

Bruchus pisorum (L.), commonly known as the pea weevil, was described by Linnaeus (1758) and given the name *Dermestes pisorum.* Linnaeus (1767) later created the genus *Bruchus* for the seed-beetles and designated *B. pisorum* as the type species. The genus *Bruchus* was originally placed within the family Curculionidae (weevils) because of the elongated facial parts and four-segmental tarsi. This is probably why it remains universally, though incorrectly, known as the pea weevil. The seed-beetles were later separated into their own family Bruchidae by Spinola (1843) and Lacordaire (1845) reinforced the position of Bruchidae within the superfamily Chrysomeloidea.

2.1.2. Species description

B. pisorum is a short stout beetle with an oval body about 5mm in length. It ranges in colour from a soft grey brown to black with patches of white scales that form white spots on the elytra. The elytra are shorter than the abdomen, exposing large white patches at its base. The head is short and strongly constricted behind the eyes. Antennae extend to less than one third of the body length. The pea weevil also has a well-defined denticle on the lateral margin of the pronotum.

2.1.3. Host specificity

The majority of seed-beetle species are classed as either oligophagous or monophagous. Members of the genus *Bruchus* are known to associate only with plants of the tribe Vicieae (Borowiec 1987a). The pea weevil is known to infest several species from this tribe including *Pisum sativum*, *Lathyrus sativus*, *L. odoratus*, *Vicia faba* and *V. leucantha* (Johnson 1981a). However a study by Burov (1980; cited by Annis & O'Keeffe 1984a)

described the pea weevil as monophagous. Tahhan and van Emden (1989) believed the pea weevil has been confused with *Bruchus dentipes* Baudi, and that it is wrongly classed as a pest of *V. faba*. Thus the pea weevil appears to be monophagous and confusion between species led to earlier reports that it is polyphagous.

2.1.4. Distribution

The natural range of the pea weevil is Asia Minor, wherever its host species is present (Borowiec 1987a). Its ability to survive in the dry pea seed for an extended time has led to it being transported to other regions rather than through migrating naturally, such that it is now considered cosmopolitan. Harris (1841) reported that, while on a collecting trip for Linnaeus in 1748, Peter Kalm found the pea weevil in the USA and infestations were of such a high level that the pea could no longer be grown successfully as a crop in several States. The weevil has since spread and become a pest in all pea growing areas of the USA (Whitehead 1930; Brindley 1933). In 1918 Skaife reported the establishment of the pea weevil in South Africa and infestations were over 50% of seed in the south-western districts of the Cape Province.

The pea weevil is a serious pest of peas in most of Southern Russia (Vasil'ev 1939). Also a survey found the pea weevil in south-eastern Europe and the Middle East including, Bulgaria, Yugoslavia, Albania, Greece, Turkey, Syria, Lebanon, Israel, Iran and Afghanistan (Borowiec 1987b). Its presence has also been reported in Japan (Yoshida 1959) and parts of China (Anon 1966). It is also found in South America and has been described as the principal pest of peas in Chile where up to 85% of the harvested seed from the Southern provinces can be infested (Olalquiaga 1953).

By the early 1930s, the pea weevil was established in several areas of south Western Australia (Newman 1932). By 1936 it had become a serious pest and, in 1937, a July-sown trial recorded a seed infestation level of 92.5% (Newman & Elliot 1938). The pea weevil spread to South Australia in the late 1950s (Birks 1965).

2.1.5. Life cycle

The pea weevil is a univoltine species. In South Australia adult weevils leave their over-wintering sites and arrive in pea crops in early Spring. They may arrive as early as mid-August, but most years they arrive in early September (Baker 1990a). Estimates of fecundity range from three (Panji & Sood 1976) to 735 (Brindley 1939) eggs per female. The bright yellow-orange eggs are laid singly on the surface of pods and the eggs usually hatch in three to five weeks, depending on the temperature (Skaife 1918). Young larvae chew directly through the pod wall from the underside of the egg. Once inside the pod they search for a soft developing seed. The pea weevil has four distinct larval instars (Brindley 1933). Larval development ranges from seven to 11 weeks and pupation from two to three weeks in Victoria (Smith 1990). Adults either emerge over summer from the seed of unharvested crops and fly to over-wintering sites, or remain in harvested seed until the following Spring, or until they are disturbed.

2.1.6. Behaviour of the pea weevil

The behaviour of the pea weevil is poorly understood. When pea weevils arrive in a pea crop, they congregate along its edge. Just how they find the crop and why they stay close to the edge for some time is not known. However it appears that the range of species acceptable for oviposition is narrower than the range suitable for larval development (Jermy & Szentesi 1978). This was confirmed by Annis and O'Keeffe (1984a) who found no difference in the survival of larvae placed in green pods of *P. sativum* and *L. sativus.*

The arrival of pea weevils in a crop often coincides with the commencement of flowering, but if there are no flowers they shelter in the vegetative parts of the crop. Panji and Sood (1975) found that feeding on pea pollen by both sexes was a prerequisite for copulation, whereas Pesho and Van Houton (1982) found the ingestion of pea pollen did not initiate the development of ovaries. Ovaries of the weevil have been shown to mature when the adult feeds on the pollen of species other than that of the cultivated pea (Annis & O'Keeffe

1984b). This suggests the weevil may feed on other pollen sources in the field if it enters a pea crop before flowering.

The females fly through the crop searching for pea pods on which to oviposit. It is not known if this is a random process or if they select pods of a particular length and age. It is also unknown whether the presence of eggs on a pod influences subsequent oviposition on it. Longer pods have more eggs and few eggs are laid on pods once seeds have filled (Brindley 1933; Smith 1990).

The seed-beetle *Callosobruchus maculatus* (F) also prefers to oviposit on green rather than mature pods (Messina 1984). The age of a pod affects its acceptability for oviposition and small pods are unacceptable because of their size. The attractiveness of pods could be related to pod diameter, because experiments using glass rods as a substrate for oviposition have shown the diameters of glass rods to be more important than their length (Avidov *et al.* 1965). This may account for the rapid increase in the attractiveness of pods in the early stages of development. Pods retain their attractiveness until they begin to mature and this is related to changes in surface texture. Egg dispersal on seeds for *C. maculatus* was found to range from random to completely uniform, depending on the weevil population used and legume host (Messina & Mitchell 1989). Other experiments using *C. maculatus* found they were more likely to oviposit on seeds free of eggs; once all had eggs, those with fewer eggs were chosen for oviposition (Mitchell 1975). Egg-spacing behaviour may be an adaptation to optimise larval survival by limiting competition. Variation in selection pressures among different environments could account for the observed differences in egg laying behaviour. Factors such as larval aggressiveness in the seed may also be affected by competition (Dick & Credland 1984). Larval competition may be an important selective agent in the egg laying behaviour of the pea weevil as more than one larva may enter a seed, but only one adult will emerge.

The larvae of *C. maculatus* feeding in a single seed appear to respond to vibrations from each other's chewing. One larva will continue to feed normally while others are inhibited as a result of the vibrations (Thanthianga & Mitchell 1987). This could explain why only one adult pea weevil emerges from a seed when intra-specific competition occurs for the limited food source provided by a seed.

2.2. The host species - *Pisum sativum* L.

2.2.1. Classification

Part of my study of the pea weevil was concerned with the resistance of species other than *Pisum sativum* to infestation and reference is made here to the botanical relationship of peas to these other species. Tournhefort (1700; cited by Makasheva 1984) placed peas along with several species of *Lathyrus* and *Vicia* into the genus *Pisum*. Linnaeus (1753) reviewed *Pisum* and identified four species in the genus, namely *P. sativum*, *P. arvense*, *P. ochrus* and *P. maritimum*. The number of *Pisum* species has changed many times since. De Candolle (1886) mentioned eight species of European or Asiatic pea, while at the other extreme Lamprecht (1966) concluded peas were a single species because differences within the genus could be accounted for by simple chromosome rearrangements. Davis (1970) described *Pisum* as a ditypic genus consisting of *P. sativum* and *P. fulvum*, with *P. sativum* divided into several subspecies and varieties. This view of *Pisum* has since been widely supported by other taxonomists (Ben-Ze'ev & Zohary 1973; Kupicha 1981).

A perennial form of pea originally described by Steven in 1812 as *Orobus formosum* (Makasheva 1984) has been included in several genera including *Pisum*. This monotypic species has been recognised as a separate member of the tribe Vicieae and is known as *Vavilovia formosum* A. Fed. (Davis 1970; Ben-Ze'ev & Zohary 1973; Kupicha 1981). Hybrids of *P. sativum* and *V. formosum* were made by Arkady Goluberb at the Vavilov Institute in Russia (pers comm. R. Reid) and so *Vavilovia* can be considered as part of the

Pisum gene pool. It is not known whether the hybrids were tested for pea weevil resistance.

2.2.2. *Distribution of the Pisum gene pool*

The pea originated in West Asia (Makasheva 1984). The centres of origin for the various subspecies of *P. sativum*, according to Zeven and de Wet (1982) are Ethiopia and Yemen for *P. sativum* ssp. *abyssinicum*; northern Iraq, Jordan, Syria, Iran, Israel, Turkey and Cyprus for *P. sativum* ssp. *syriacum* (syn *humile*); Syria, northern Israel, Lebanon, southern coastal Turkey, Greece, Cyprus, the Adriatic coast of Yugoslavia, Italy, Morocco, Algeria, Tunisia, southern Spain, Southern France, Crimea and Caucasia for *P. sativum* ssp. *elatius*. The distribution of the non-cultivated species *P. fulvum* is restricted to Israel, Jordan, Lebanon, western Syria and southern Turkey (Ben-Ze'ev & Zohary 1973).

Vavilovia formosum is found in alpine areas of Lebanon, northern Iraq, north west Iran and Caucasia (Davis 1970).

2.2.3. *Domestication and spread of the pea*

Zohary and Hopf (1988) cited evidence for the presence of the pea in early Neolithic farming villages of the Near East around 7,500 BC, but whether it was cultivated is unknown. They considered the most reliable trait indicating domestication of the pea was the development of a smooth seed coat and it is not until the late Neolithic period (5400-5050 BC) that the remains of smooth seed-coated types are found. Archaeological evidence suggests that cultivation of peas began in the Near East about the time wheat and barley were being domesticated. Zohary and Hopf (1973) also believed domesticated peas were associated with the spread of wheat and barley into Neolithic Europe. Neolithic sites in Greece (van Zeist & Bottema 1971), Bulgaria (Hopf 1973) and Yugoslavia (Hopf 1974) contained evidence of the cultivation of peas. Peas had been spread into central Europe, Russia, Egypt and India by the late Neolithic or Bronze Age (Zohary & Hopf 1988). Makasheva (1984) suggested the domesticated pea did not reach China from Afghanistan

much earlier than 100 BC, Japan from India by 300 AD. They did not reach England until as late as 400 AD. The pea was taken to the new world by Colombus in 1493 (Makasheva 1984). It was introduced with white settlement into Australia and substantial areas were grown in Tasmania as early as 1829 (Wood & Russell 1979).

2.3. Plant resistance - theoretical aspects

2.3.1. Co-evolution

Natural populations of plants can be severely damaged by a plethora of living organisms including fungi, bacteria, viruses, insects, mites and nematodes, but this does not generally occur unless a new pest species is introduced into a population. The reason for this is best demonstrated by the theory of co-evolution which attempts to explain the interaction over time between host and a pest species. Any individuals within a pest population that obtain an increase in fitness (reproductive success) from a new adaptation to better utilise their host species will have a competitive advantage over other individuals in the population. With each generation their genes will comprise a higher proportion of the gene pool and this will exert selection pressure on the host species. Over time individuals in the host population which are able to resist the attack of the pest species will gain a fitness advantage over susceptible members in their population and will increase in abundance. Co-evolution develops into a dynamic process of adaptation vs. counter-adaptation between the host and pest species where a quasi-equilibrium is eventually reached between the two species (MacKenzie 1980).

2.3.2. Gene for gene concept

Natural populations of plants possess many forms of pest resistance, controlled by one or more genes. Genes giving rise to a pronounced phenotypic effect are major genes, in contrast to minor genes or polygenes which provide small additive effects on the phenotype (Mather (1941). A gene for gene interaction was demonstrated between flax and flax rust (Flor 1942). For every major gene for rust resistance in flax there appeared to be a matching gene for virulence in the parasitic species. A host plant will show a

resistant reaction if it has a gene for resistance and the parasite has an avirulent allele at the corresponding gene locus. However if the parasite possesses an allele for virulence at the locus the plant is susceptible. Many investigations into the gene for gene relationship with other hosts and parasites have followed. It has been described for the relationship between the Hessian fly and wheat (Hatchett & Gallun 1970) and yellow rust and wheat (Johnson *et al.* 1972). In contrast there are numerous reports of stable resistance occurring with major genes without a gene for gene interaction, including eyespot disease of wheat (Scott & Hollins 1977) and the berry disease of coffee (Van der Graaff 1981). There are other examples where race or biotype-specific genes occur in gene for gene relationships but do not account fully for the observed resistance (Driver 1962; Hooker 1967). While the gene for gene model does not appear to be appropriate for all resistances involving major genes, Johnson (1984) believed that it helps explain much of the variation observed between races or biotypes of pests in relation to host resistance. Johnson and Lupton (1987) reasoned that successful breeding with major genes for resistance can be attributed to certain aspects of the population biology and epidemiology of the pest along with the types and combinations of genes used.

2.3.3. Specificity of resistance

The terms vertical and horizontal resistance have been used to explain race or biotype specific resistance and non-specific resistance respectively (Vanderplank 1963). These terms are frequently used in other contexts, which has led to misunderstandings of their original meanings (Vanderplank 1978). An example of misinterpretation was made by Gallun and Khush (1980) who described a major gene as one which conveys vertical resistance, while horizontal resistance was controlled by many genes. Vanderplank (1978) has previously stated that there is no evidence to suggest that more genes are involved in horizontal resistance than in vertical resistance. Another interpretation of Vanderplank's theory was provided by MacKenzie (1980). He suggested major genes or vertical genes act as the first line of defence of the host species and will remove individuals from newly arrived pest populations that do not possess the appropriate

virulence genes. The second line of defence in the host is provided by horizontal genes and they restrict the pest population from increasing once it is established.

2.3.4. *Durable resistance*

The term durable resistance proposed by Johnson and Law (1975) avoids the complications associated with other terminology. It simply describes a resistance that has remained effective for many years over a wide area, in environments that are favourable to the disease or pest species. It does not imply a cause for the resistance or its genetic basis and it does not have to convey complete resistance to the disease or pest (Johnson 1984). When breeding for durable resistance, Johnson (1984) believed no single method was applicable in all situations and listed two methods. The first involves testing a newly developed cultivar at many locations and the second testing it against as many races of the pathogen as are available in existing collections. He believed neither method was as powerful as growing a resistant cultivar over many seasons in an environment favouring disease.

2.3.5. *Insect resistance in plants*

Painter (1951) defined resistance to insects as "the relative amount of heritable qualities possessed by the plant which influences the ultimate degree of damage done by an insect". In agriculture it is the ability of one cultivar to produce a higher yield of quality produce than another under the same level of insect pressure. Painter also recognised several levels of resistance, ranging from immunity to high susceptibility.

He divided resistance into three categories.

> *Non-preference* (*antixenosis*) is an insect's response to those plants which are unsuitable as hosts, resulting in avoidance of this plant during the search for food, oviposition sites or shelter.

Antibiosis is where the insect's biology is adversely affected by its interaction with the plant, ie. its survival, development or reproduction is altered.

Tolerance is the ability of a plant to withstand infestation and support populations that would severely damage susceptible plants.

These categories are not mutually exclusive, they may interact and complement each other. For example, a non-preferred host can display antibiotic effects and a plant with a low level of antibiosis may be less affected if it is highly tolerant under high insect pressure (Horber 1980). Also, selection pressure on a pest population can be affected by non-preference and antibiosis but not by a tolerant plant (Horber 1980).

Antixenosis was proposed by Kogan and Ortman (1978) to replace non-preference because it projects the avoidance reaction as an aspect of insect behaviour rather than a property of the plant. Lowe (1987) found that Painter's definition of mechanisms of resistance were valuable to the plant breeder though none indicated the possible causes of the resistance.

2.4. Breeding for insect resistance in crop plants

Breeding for resistance requires an understanding of the interactions between the pest and the crop (Bellotti & Kawano 1980; Lowe 1987) including a knowledge of the pest's population dynamics, life cycle, feeding and oviposition habits. Insect resistance has been known for many years but breeding for resistance to insects has been less common than breeding for disease resistance. The was due in part to the advent of powerful insecticides in the 1940s and the cheap control of pests for nearly three decades (Lowe 1987). Plant breeding for resistance has been attempted where insecticides have failed to be effective.

2.4.1. Germplasm

The chances of identifying sources of resistance are related to the diversity of germplasm screened. Most breeders search first among adapted cultivars, then screen older cultivars

and landraces, and finally assess wild relatives of the species. If resistance occurs in an existing cultivar and is inherited simply, the cultivar can be used in crosses and backcrosses while selecting for resistance. The progeny are screened for undesirable traits that may be inherited from the less improved material and wild relatives. Resistance is often found in landraces or wild relatives of a crop species and transfer of the resistance to the crop may require intensive selection procedures or special techniques (Ortman & Peters 1980). For example bridging species can be used, or embryo rescue may be required to place the resistance gene in a suitable background.

2.4.2. *Sources of insects*

Insects used in resistance studies may be laboratory-reared or field-collected. Laboratory cultures produce a continuous supply of insects free from parasites and diseases, though at a considerable cost (Tingey 1986). A disadvantage with them is that they may be different from natural populations in their behaviour, physiology and genetic characteristics and be of little value in testing germplasm (Schoonhoven 1967). Conversely, field populations of insects are usually easy and inexpensive to collect and represent the pest species from an agricultural environment, but they are seasonal and unpredictable (Tingey 1986). Infection of field populations with pathogens and parasites can alter insect behaviour and development.

In the case of the pea weevil, it would be extremely difficult to culture large numbers of this species in the laboratory and unnecessary when good quality insects can be obtained from harvested seed. Another problem with this univoltine species is its facultative diapause which has to be broken before the weevil can be used out of its period of seasonal activity.

2.4.3. *Mass Screening methods*

Germplasm can be mass screened for resistance in the field or laboratory (glasshouse). However, it is difficult to duplicate field conditions in the glasshouse or grow plants representative of those in the field, while field screening can utilise cropping procedures

(Tingey 1986). Laboratory screening allows testing with different biotypes of the pest and testing when the plant is at its most susceptible stage. Laboratory screening can usually be undertaken most of the year and not just when the insects are active in the field. However laboratory screening is constrained by time and suitable facilities. This has resulted in many researchers using field trials for the initial mass screening purposes, followed by more intensive studies in the field or laboratory on a small number of accessions to eliminate escapes and determine the mechanisms of resistance.

The mass screening of germplasm in the field allows the rapid processing of many accessions, because large differences are all that are required (Ortman & Peters 1980). The screening indicates susceptibility rather than resistance in germplasm and should be undertaken for at least two seasons (Bellotti & Kawano 1980). Selection should be minimised to accessions from the most resistant material. Accessions do not need to be replicated, though controls should be randomly spaced through the trial to assess the damage and evenness of attack. Results can be presented as a ratio to the standard deviation or standard error of the mean for the control genotypes (Tingey 1986). This design allows assessment even when there is a limited supply of seed of an accession.

Field trials should be evaluated at the time of insect attack to identify those with temporal escape mechanisms. Painter (1951) refers to this as host evasion, a pseudo-resistance that occurs if a test plant passes through a susceptible growth stage when the pest species is not active. He notes that early maturing cultivars may avoid attack. Most breeders would only consider using pseudo-resistance characters like early maturity if it is not associated with a yield penalty.

2.4.4. *Release of insects in field trials*

When germplasm is screened in the field trials, the natural population needs to exert sufficient selection pressure to damage the majority of accessions. Trials should be sown in areas prone to attack. In some years populations may be inadequate or not invade the

trial when the majority of accessions are at a susceptible stage (Bellotti & Kawano 1980). Releases of laboratory-reared or field-collected insects will increase the density and ensure an early peak in the pest population (Tingey 1986). The releases should coincide with climatic conditions that are favourable to infestation (Bellotti & Kawano 1980).

2.4.5. Free-choice, no-choice and sequential trials

The response of an insect to various host genotypes may differ depending on the type of test used.

In *Free-choice tests*, insects have access to two or more plant genotypes at a time. They can be undertaken in the field or laboratory and provide information on the insects preference for a genotype. Mass screening is a form of free-choice test though usually without replication. A genotype which appears to be "resistant" when the pest is given a choice may not maintain its resistance when grown as a pure crop (Wiseman *et al.* 1961).

In *No-choice tests* a population of insects has access to only one host genotype. The tests may be conducted in the laboratory or field. A no-choice test can provide supplementary information to a free-choice test. In the field, confinement cages or spatially isolated field sites of a single genotype have been used to minimise the biases arising from a free-choice (Tingey 1986). Confinement techniques are also used in the laboratory with entire plants, plant parts or plant extracts. In such a no-choice test, the density of the pest is regulated, immigration and emigration are eliminated (Tingey 1986). However in some cases insects are less discriminating when they are kept in cages (Singer 1986).

Sequential tests allow insects access to a sequence of plant genotypes, one at a time. This shows the investigator how experience gained by an insect on one genotype affects its behaviour on other genotypes. Different effects have been recorded for different species. For example, *C. maculatus* beetles are unlikely to accept less preferred plants once they have encountered a more preferred genotype (Mark 1982). Other insect species have shown the opposite behaviour and some will not accept a plant for oviposition if they

detect that other members of the species have attempted and failed. This form of test may not be relevant for crops grown as large scale monocultures, but it could clarify results obtained in a free choice test.

2.5. Bruchid resistance in legume crops

Legume species contain many secondary plant chemicals, which by definition are compounds that are not involved in primary metabolism. Some are known to act as anti-metabolites (Gatehouse *et al.* 1990), while others are storage compounds or regulators of plant metabolism and growth (Rhoades 1979). The substances belong to many chemical groups and some have exhibited deterrent or toxic effects on bruchids (Stamopoulos 1987). The groups implicated are tannins, lectins (phytohaemagglutinins), alkaloids, cyanogenic glycosides, saponins, enzyme inhibitors, non-protein amino acids and heteropolysaccarides (Stamopoulos 1987). While many of these compounds are known to be toxic to vertebrates (Levin 1976; Bell 1978, 1980), bruchid resistance has been developed in two agriculturally important legume species, *Phaseolus vulgaris* and *Vigna unguiculata* without associated toxicity problems.

High levels of antibiosis have been found to the bruchids, *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) in some wild forms of *P. vulgaris*, a major legume crop native to South America (Schoohoven *et al.* 1983). In the most resistant accession the emergence of *Z. subfasciatus* adults was depressed to 12% of the mean of susceptibles, adult weight was reduced by 50% and the developmental period was increased significantly. Similar results were obtained for *A. obtectus*. Two groups of compounds in the cotyledons are associated with this resistance. A hetero-polysaccharide was identified as the compound responsible for resistance to *A. obtectus* (Gatehouse *et al.* 1987). Subsequent research, however, suggested that arcelin, a protein present in resistant wild accessions, but absent in susceptible wild and cultivated accessions, was responsible for the resistance to both *A. obtectus* and *Z. subfasciatus* (Harsmen *et al.* 1987). Four distinct electrophoretic variants have been isolated and larval feeding trials have shown

arcelin-1 to be responsible for the resistance to *Z. subfasciatus* and arcelin-4 to *A. obtectus*. Resistance to *A. obtectus* has also been associated with a seed factor other than arcelin (Kornegay & Cardona 1991).

The arcelin variant responsible for resistance to *Z. subfasciatus* is inherited as a single dominant gene (Osborn *et al.* 1986) and resistance to *A. obtectus* appears to be inherited by two recessive but complementary genes (Kornegay & Cardona 1991).

In several *V. unguiculata* accessions, resistance in pods to *C. maculatus* was reported to be dominant and maternally inherited (Fatunla & Badaru 1983). The antibiosis to larvae in seeds was inherited as a complex recessive character, consisting of both major and minor genes (Redden *et al.* 1984). Another study indicated that seed resistance was controlled by two recessive genes (Adjadi *et al.* 1985). The genes are inherited independently and cytoplasmic factors are involved (Rusoke and Fatunla 1987), which accounts for the complex inheritance (Redden *et al.* 1984). Other evidence suggests that resistance in the pod is not linked to resistance in the seed (Rusoke & Fatunla 1987). It appears that two genes at most are involved in the various forms of bruchid resistance reported in the literature.

The durability of bruchid resistance has been questioned by Dick & Credland (1986a). They cite a publication by Redden *et al.* (1983) in which a Brazilian population of *C. maculatus* responded differently to other populations of *C. maculatus* than to the resistant accession of *V unguiculata*. This may limit the success of resistant varieties produced for widespread distribution. Other evidence suggests that resistance to bruchids reliant on a few genes may be short-lived. The survival and developmental rates of a population of weevils reared on a resistant variety were found to increase after only three generations in the laboratory (Dick & Credland 1986b). Similar results have been obtained with other insect pests (Claridge & Den Hollander 1982). Changes observed following intensive selection in the laboratory should only be used as an indicator only for

what may happen in the field given that no alternative host genotypes are present (Credland 1990). It must also be remembered there are many examples of resistant cultivars which have been grown for many years without a breakdown in resistance (Adkisson & Dyke 1980).

2.6. Pea weevil resistance in peas

2.6.1. Background

There have been several attempts to identify pea germplasm resistant to pea weevil over the last 80 years. In 1918 Skaife reported the establishment of pea weevil in South Africa with infestations of over 50% in parts of the Cape Province and the failure of 12 cultivars to resist attack in field trials. He cited a report by Carville in the American Entomologist, of the cultivar Prussian Blue, which was immune to the pea weevil.

Newman and Elliot commenced trials in Western Australia in 1938 to identify resistant cultivars of peas and other legume fodder species immune to the weevil. They found that *Lathyrus* species were resistant to the weevil (Anon 1947). At the same time in Europe, selection trials showed that some green-seeded pea varieties were providing a degree of resistance (Ufer 1949). Attempts were made in Western Australia to cross peas with several species of *Lathyrus* to incorporate its resistance (Fisher 1953).

Breeding programs in the USSR (Vilkova & Kalesnichenko 1973; Aleksandrova 1977; Sokolov 1977; Verbitskii & Pokazeeva 1980) led to the identification of seven sources of combined resistance to the pea weevil and the pea aphid *Acyrthosiphon pisum* (Harris) (Posylaeva 1988). Whether or not the release of resistant cultivars has occurred in the USSR is uncertain, but a putative resistant line (WIR 4739) from the Vavilov Institute has been introduced for screening in Australia.

In the 1970s American researchers showed a renewed interest in the development of pea cultivars resistant to the pest and screened 1571 *Pisum* introductions (Pesho *et al.* 1977).

After two years of field trials, six lines were classified as non-preferred. Peduncles of the resistant material were significantly shorter than those of susceptible cultivars. Advanced breeding lines were obtained from the American program but they did not show resistance under South Australian field conditions (Ali 1984). Pesho's original resistant introductions were also field tested in Chile with little success (pers. comm. M. Gerding, Instituto de investigaciones Agropecurias, Chile) and there have been no reports on them in recent literature.

Neither Russian nor American literature mentions the Prussian Blue cultivar or whether *P. fulvum* accessions were screened.

2.7. Conclusions

It is probable that resistance to the pea weevil may yet be found in *Pisum* germplasm because the wild relatives do not appear to have been screened. The evidence suggests the weevil is a monophagous species which could indicate the presence of resistance in closely related species. Screening of other legume crop species and their close relatives has identified germplasm resistant to other bruchids. Results from these screening programs show that relatively few accessions possess genes for resistance to bruchids and most of these are wild types or relatives of the crop species. This indicates that a large number of *Pisum* accessions should be screened, including the wild subspecies and as many accessions of *P. fulvum* as can be obtained.

It appears that all of the pea relatives and subspecies described in my review can be crossed with commercially grown cultivars and it should be feasible to transfer pea weevil resistance. Bruchid resistance has been shown to be simply inherited in the instances where it has been investigated. Two genes have been implicated with a significant mechanism of resistance in *V. unguiculata* and both of these were recessive which the homozygotes had to be selected.

Field trials using advanced *P. vulgaris* and *V. unguiculata* lines and cultivars resistant to species of bruchids have been reported. If the chemical compounds conveying resistance could be identified then simple tests could be developed to screen the progeny of crosses. Fortunately there have been no reports suggesting that resistant weevil biotypes have developed, that resistant compounds in host-plants cause death in rats or higher animals, or that bruchid resistance is associated with large yield penalties in the crop. It can be inferred that bruchid-resistant cultivars could be developed which possess durability and high yields and are not toxic to humans or stock.

Mass screening procedures to evaluate germplasm for resistance and to select progeny in segregating populations have been developed in several legume crops against various bruchids. Similar procedures were used in my research to mass-screen *Pisum* germplasm and select resistant progeny.

CHAPTER 3

MASS SCREENING OF *PISUM* GERMPLASM FOR RESISTANCE TO THE PEA WEEVIL

3.1. Introduction

There have been reports in the literature from the USSR and the USA over the last 20 years of resistance to the pea weevil in the genus *Pisum* (Aleksandrova 1977 & Pesho *et al.* 1977). Accessions identified as being resistant have been incorporated into breeding programs in both countries and advanced lines were introduced into Australia from the American program for testing. Results have shown these lines are not resistant under Australian field conditions (pers. comm. Dr Ali, South Australian Department of Agriculture). It was not known why the American lines failed and it was decided to investigate more fully the absence of resistance under local conditions by mass screening a wide range of pea germplasm over several years at numerous sites.

3.2. Materials and Methods

Pea germplasm was obtained from local and overseas sources, including the Australian Temperate Field Crops Collection, Commonwealth Scientific and Industrial Research Organisation of Australia (CSIRO), South Australian Department of Agriculture, Western Australian Department of Agriculture, Waite Agricultural Research Institute (South Australia), United States Department of Agriculture (USDA) and the John Innes Institute (United Kingdom). All the material available in the Australian collections was requested, including breeding lines as well as wild and landrace accessions. Many of the lines received were later found to be duplicates, but were kept as separate lines for field screening. The lines imported from the John Innes Institute and the USDA were those not available in the Australian collections. The material from the USDA had previously been implicated in US trials to be resistant to the pea weevil (Pesho *et al* 1977). Germplasm

obtained from the John Innes collection consisted of wild pea types, landrace accessions and primitive cultivars. This material did not arrive in Adelaide until mid 1990, where it was grown in quarantine for one generation. The wild pea types and the primitive populations were processed first so they could be used in the 1991 screening trials. Priority was given to these lines because they were considered more likely to be resistant to the pea weevil than the primitive cultivars .

A total of 1,825 *Pisum* accessions were evaluated for resistance in 15 field trials over three seasons and nine sites beginning in 1989. An unreplicated trial design was used at all sites. This was considered sufficient to eliminate any obviously susceptible material. When sufficient seed was available, an accession was sown at more than one site a year. In the 14 South Australian trials all lines were hand sown in one metre rows (20 seeds per accession), with half a metre space between accessions and a one metre row spacing, or into nine litre plastic pots at up to ten seeds per pot (Plate 3.1). The one Western Australian trial was machine sown, with five metres of row per accession and a row spacing of one metre.

Pot trials were confined to the Waite because of the special requirements of this form of trial. Five of these trials were sown over a three year period. Pot trials were useful because Australian collections usually provided fewer than ten seeds per accession and germplasm could be screened without the need for seed multiplication. Sterilised soil was used in all pots. The potting soil was a mixture of 40% German peat and 60% washed river sand, with a pH of 6.5, adjusted with hydrated and normal lime. Black polythene sheeting or woven mat was placed under the pots to suppress weed growth. Water was supplied to the pots by overhead sprinklers or drippers. A slow release fertiliser (18% N, 4.8% P, 9.1% K and 3.7% S) was added to all potted material once it had germinated. Pots were spaced at about 75cm centres to reduce intertwining of plants of different accessions.

Plate 3.1. Examples of the unreplicated germplasm evaluation trials showing (a) a field trial and (b) a pot trial.

All seed, sown directly into field plots or pots, received a fungicidal seed dressing of P-Pickle® (480 g/kg thiram and 266 g/kg thiabendazole). Seed from the CSIRO collection, because of its age, was sown in pots at the Waite bird cage site in 1989. The seed was surface sterilised with a 10% sodium hypochlorite solution for five minutes, then rinsed in distilled water. Seed was germinated on moist filter paper in petri dishes in an incubator at 25°C before being transplanted into the pots. This procedure was also used with accessions which did not emerge in plots. All accessions suspected of being hard seeded were scarified before being sown. Some hard-seeded lines were also germinated in petri dishes before being sown into pots. Accessions which did not germinate on filter paper were surface sterilised and grown under sterile conditions on a standard PDA nutrient agar containing streptomycin sulphate (100 ppm).

Observations made on the accessions included flowering date and flower colour. Australian field pea cultivars were used as controls in each trial to compare weevil damage rates with the test material. Various other species from the tribe Vicieae were included in the 1989 trials because of a report that pea weevil may attack the faba bean (*Vicia faba* L.) in Iraq (Al-rawy and Kaddou 1971). A review by Johnson (1981b) also lists *V. faba* as a host species along with *Lathyrus sativus* L., *L. odoratus* and *Vicia leucantha* L.

All trial sites except Charlick and Turretfield in 1989 and Northam in 1990 were sown some distance away from known pea weevil populations. A source of pea weevils was provided to these sites provided by scattering weevil infested seed on several occasions beginning in early September and continuing until flowering ceased. This procedure provided weevils for the entire flowering and podding period.

All trials were hand harvested to conserve the limited quantities of seed, to maximise the collection of dehiscing lines and to decrease splitting of infested seed. In 1989 the harvested seed was stored at room temperature in seed envelopes so that an assessment could be made of the number of weevils that would complete their life cycle and emerge.

When this was repeated in 1990, the straw itch mite (*Pyemotes herfsi* Oudemans) had infested many of the samples by early January , so the seed was placed in a 15°C cool room. This slowed the activity of the mite sufficiently to allow the remaining weevil larvae to develop into adults and emerge.

Seed harvested in 1991 was placed in the cool room by the middle of December to negate the mite problem. Seed stored at room temperature was sampled for weevil emergence after three months, while seed stored in the cool room was left for four months. The seed was evaluated on the percentage emergence of adult weevils. A minimum of 20 seeds were usually sampled, with lines being considered susceptible and discarded when two seeds in 20 or ten per cent of a larger sample was infested with adult weevils. However some lines which yielded less than the required 20 seeds per plot were discarded if they were heavily damaged. A seed was classed as having been successfully attacked, if at the time of sampling a live larva, an exit window or an exit hole was found.

3.3. Results

After three seasons of field trials 1650 of the original 1825 *Pisum* accessions had been discarded, 106 could not be categorised because they did not flower or set sufficient seed and 69 that were potentially resistant. The level of successful weevil attack measured across all sites was less than five per cent for 30 accessions (Table 3.1a), five to ten per cent for 16 accessions (Table 3.1b) and ten per cent for 23 accessions (Table 3.1c). This compares to mean successful attack rates of 7.5-100% in the control pea cultivars (Table 3.2). Sixteen accessions with less than five per cent successful weevil attack are of the wild pea species *P. fulvum* and the majority of them were tested at more sites than other accessions with this level of adult emergence (Table 3.1a). It is also important to note that of the 18 *P. fulvum* accessions obtained for screening purposes adult emergence did not exceed five per cent for any of them (Tables 3.1a &3.1b). Many of the lines with ten per cent adult emergence were tested at very few sites and may have been discarded if testing had continued (Table 3.1c).

Table 3.1a. *Pisum* accessions with emergence from seed of adult pea weevil at less than 5% in field trials between 1989 and 1991.

Accession[1]	Other number	Species	% emergence at 1989 field sites					% emergence at 1990 field sites					% emergence at 1991 field sites		
			BC	UR	CL	TF	GP	BC	WP	GP	GG	TF	BC	WP	NF
	Mean for control cultivars		50.0	36.7	26.0	13.3	7.5	54.0	32.5	72.5	10.0	10.7	22.5	27.5	25.7
PIG 49	PI 343955	*P. fulvum*	0.0					0.0					0.0	0.0	0.0
PIG 111		*P. fulvum*	0.0					0.0					0.1	0.0	0.0
PIG 112		*P. fulvum*	0.0		0.0			0.0	3.3	0.0		0.0	0.0	0.0	0.0
NGB 1256	JI 1392	*P. fulvum*	0.0					0.0/0.0					0.2/1.4	0.0	0.0
ATC 114		*P. fulvum*						0.0					2.5	0.0	0.0
JI 2204	WIR 3397	*P. fulvum*											1.0	1.0	0.0
ATC 113		*P. fulvum*								0.0			0.0		0.0
IC 63466		*P. fulvum*											0.0	0.0	
JI 849		*P. fulvum*											0.0		0.0
JI 1006		*P. fulvum*											0.0		0.0
JI 1010		*P. fulvum*											0.0	4.8	
JI 1011		*P. fulvum*											0.0	0.0	
JI 1012		*P. fulvum*											0.0		
JI 1796		*P. fulvum*											0.0	0.0	
JI 2205	WIR 6070	*P. fulvum*											2.5		
JI 2206	WIR 6071	*P. fulvum*												2.5	0.0
JI 1794		*P. sativum* ssp. *humile*													
NGB 1437		*P. sativum*		0.0											
NGB 1490		*P. sativum*					0.0							2.5	
ATC 31		*P. sativum*					0.0								
ATC 309		*P. sativum*					0.0							3.0	
ATC 1		*P. sativum*								0.0					
ATC 278		*P. sativum*												2.5	
ATC 445		*P. sativum*													0.0
WA 401		*P. sativum*								0.0					
NGB 154	JI 430	*P. sativum* convar *speciosum*										0.0			
NGB 5147		*Pisum* sp								0.0					
NGB 1424	JI 101	*Pisum* sp								0.0					
SA 1354		*Pisum* sp								0.0					
SA 1358		*Pisum* sp													

[1]Accession abbreviations explained in Table 3.1b.

Table 3.1b. *Pisum* accessions with emergence from seed of adult pea weevil at more than 5%, but less than 10% in field trials between 1989 and 1991.

Accession[1]	Other number	Species	% emergence at 1989 field sites					% emergence at 1990 field sites					% emergence at 1991 field sites		
			BC	UR	CL	TF	GP	BC	WP	GP	GG	TF	BC	WP	NF
	Mean for control cultivars		50.0	36.7	26.0	13.3	7.5	54.0	32.5	72.5	10.0	10.7	22.5	27.5	25.7
JI 1458	NGB 1571	*P. abyssinicum*											5.0		
PIG 277	CPI 53306	*P. fulvum*	5.0	0.0		0.0			0.0				2.3	0.0	0.0
JI 2203	WIR 2523	*P. fulvum*						0.0		5.0			0.0	0.0	0.0
NGB 1429	PI 222071	*P. sativum*				0.0						7.8			
NGB 1352		*P. sativum*								5.0					
ATC 12		*P. sativum*							5.0						
ATC 239	MU 57	*P. sativum*					0.0		5.0			0.0			
ATC 308		*P. sativum*					0.0		0.0					5.0	
JI 197		*P. sativum*													6.0
NGB 1411		*Pisum* sp								5.0					
PI 180868	JI 750	*Pisum* sp										4.1			
PI 164837	JI 1367	*Pisum* sp						5.0							
SA 959		*Pisum* sp								5.0					
SA 1353		*Pisum* sp								5.0					
SA 1374		*Pisum* sp								5.0					
WA 120	N 87012	*Pisum* sp													9.1

[1]PIG = Plant Industries Genetics (C.S.I.R.O..- Canberra, Australia), NGB = Nordic Gene Bank (Sweden), ATC = Australian Temperate Field Crops Collection (Horsham, Australia), JI = John Innes Institute (Norwich, England), IC = ICARDA (Aleppo, Syria), WA = Western Australian Department of Agriculture (Perth, Australia), SA = South Australian Department of Agriculture (Adelaide, Australia), PI = Plant Introductions (USDA, United States of America), WIR = Vavilov Institute of Plant Industry (Leningrad, Russia), CPI = Commonwealth Plant Introductions (C.S.I.R.O.- Canberra, Australia), MU = Melbourne University (Melbourne, Australia), N = origin of these accession unknown, P = origin of these accession unknown.

Table 3.1c. *Pisum* accessions with emergence from seed of adult pea weevil at 10% in field trials between 1989 and 1991.

Accession[1]	Other number	Species	% emergence at 1989 field sites					% emergence at 1990 field sites					% emergence at 1991 field sites		
			BC	UR	CL	TF	GP	BC	WP	GP	GG	TF	BC	WP	NF
	Mean for control cultivars		50.0	36.7	26.0	13.3	7.5	54.0	32.5	72.5	10.0	10.7	22.5	27.5	25.7
ATC 178	M U679	*P. sativum*								10.0					
ATC 315		*P. sativum*								10.0					
NGB 1972		*P. sativum*						10.0							
ATC 221	MU 20	*P. sativum*								10.0					
ATC 225	MU 647	*P. sativum*		10.0					10.0						
NGB 1586	JI 1473	*P. sativum*		10.0											
NGB 1588	JI 1475	*P. sativum*		10.0											
NGB 1602		*P. sativum*		10.0					5.0						
NGB 1632		*P. sativum*		10.0					0.0			0.0			
WA 8		*P. sativum*				0.0			10.0						
NGB 862		*P. sativum*								0.0				10.0	7.5
ATC 315		*P. sativum*												10.0	9.8
JI 213		*P. sativum*						10.0							
NGB 1951		*P. transcaucasicum*						10.0						5.0	9.3
PI 164758		*Pisum* sp								10.0			0.0		0.0
SA 1408		*Pisum* sp								10.0					
SA 1369		*Pisum* sp								10.0					
SA 1355		*Pisum* sp								10.0					
SA 1356		*Pisum* sp								10.0					
JI 962	PI 343960	*Pisum* sp						10.0					0.0		
SA 516	P 86-76	*Pisum* sp								10.0					
NGB 1421	PI 207508	*Pisum* sp						10.0					5.0		
PI 166082		*Pisum* sp													

[1]Accession abbreviations explained in Table 3.1b.

Table 3.2. Sites used in screening for resistance to the pea weevil showing the type of trial used, weevil releases at each site, number of accessions sown and harvested, mean adult emergence per site and for cultivars, and the number of accessions discarded from each trial between 1989 and 1991.

Year	Site	Type of trial	Weevils released	Number of plots sown	Number of plots harvested	Mean weevil development per trial (% seed)	Mean weevil development for cultivars (% seed)	Control cultivars sampled	Accessions discarded (>10% infestation)
1989	Waite bird cage	pot	yes	300	286	38.6	50.0	5	253
	Urrbrae	field	yes	210	205	31.8	36.7	3	172
	Charlick	field	no	253	202	19.8	26.0	5	118
	Turretfield	field	no	215	192	11.3	13.3	3	59
	Waite glasshouse plots	pot	yes	126	126	2.1	7.5	2	7
	Waite grass garden	field	yes	48	30	12.0	22.5	4	10
1990	Waite bird cage	pot	yes	251	220	21.7	54.0	2	180
	Waite plots	field	yes	252	238	29.7	32.5	6	189
	Waite glasshouse plots	pot	yes	463	351	28.6	72.5	2	234
	Waite grass garden	field	yes	47	31	44.1	10.0	1	24
	Turretfield	field	yes	650	388	7.9	10.7	4	110
	Northam	field	no	134	118	85.6	100.0	7	108
1991	Waite bird cage	pot	yes	160	149	38.2	22.5	1	111
	Waite plots	field	yes	202	124	20.1	27.5	1	71
	Northfield	field	yes	315	277	26.7	25.7	1	230

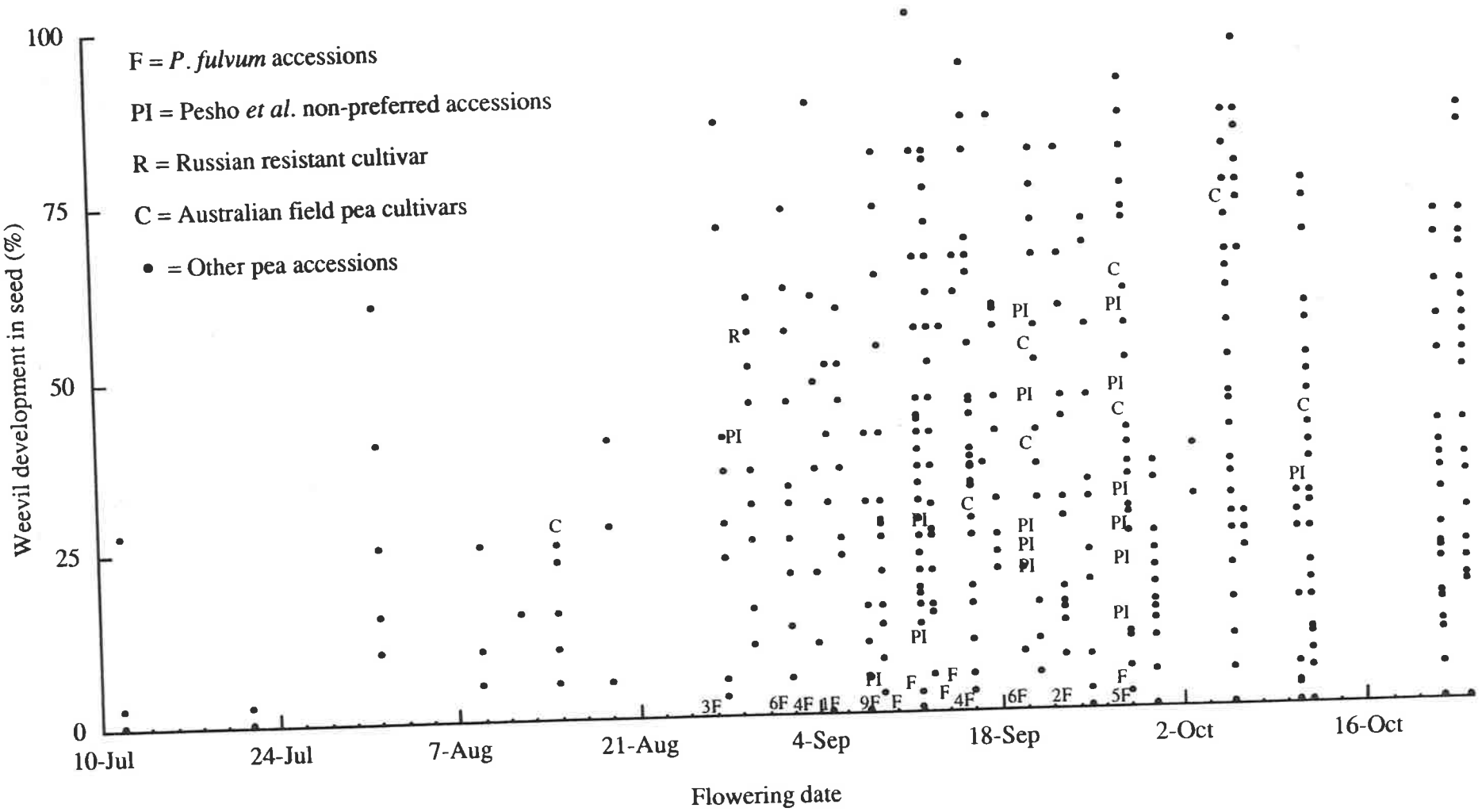


Figure 3.1. The flowering dates and emergence results of adult pea weevil for *Pisum* accessions harvested from the six screening trials where the flowering dates of accessions were recorded between 1989 and 1991. Local cultivars, resistant accessions imported from overseas and *P. fulvum* accessions are individually labelled. The sites used were Waite bird cage 1989, 1990 and 1991, Waite plots 1990 and 1991 and the Waite glasshouse site 1990.

The *P. fulvum* accessions grew taller in pot culture. It was also observed that pod size increased in these accessions, but higher levels of seed attack were not noticed (Table 3.1a).

The lower level of weevil damage in *P. fulvum* could not be attributed to a different flowering and podding period as the *P. fulvum* accessions flowered at the same time as many of the heavily damaged *P. sativum* accessions (Figure 3.1). This combined result for the six trials where flowering date was recorded allowed for a better comparison of the *P. fulvum* accessions with the local cultivars of field peas. It also shows how the *P. fulvum* germplasm compares with the resistant accessions identified by Pesho *et al* (1977) and the cultivar WIR 4739 identified by the Russians. The individual adult emergence results for putatively (Pesho *et al* 1977) non-preferred accessions (Table 3.3) showed that all except PI 164758 were discarded from the screening program and even the results for this accession place it in a marginal category (Table 3.1c). A similar result was obtained for these accessions when they were screened for pea weevil resistance in Chile in 1981 (Table 3.3).

Seed from non *Pisum* genera in the tribe Vicieae was harvested from 29 plots over three sites in 1989 (Table 3.4). The majority of the harvested accessions were from Turretfield which was a lightly damaged site, however some accessions were harvested from the Waite bird cage and the Charlick sites which were more heavily damaged (Table 3.2). All seed examined from the non *Pisum* lines was free of weevil damage and the evidence suggests all these genera are resistant to the weevil.

A total of 1,300 pots were used over the three years to speed up the screening procedure. The high rate of seed recovery for the potted accessions can be assessed if the ratio of plots harvested to plots sown is considered (Table 3.2). Only at the 1990 Waite glasshouse plots, where many of the accessions were sown late in the season, was seed harvested from less than 85% of the plots sown in the trial, whereas the percentage of accessions

Table 3.3. Development by pea weevil from putatively antixenotic accessions (Pesho's *et al.* 1977) in screening trials for pea weevil resistance in the United States, Chile and Australia.

Accession	Seed supporting weevil development (%)							
	US[1] 1972	US[1] 1974	CH[2] 1981	BC 1990	GP 1990	TF 1990	WP 1991	NF 1991
PI 164304	4.5					41.7		
PI 164758	1.3		42.2	10.0			5.0	9.3
PI 165949	4.0		42.0	15.0/65.0[3]		0.0		
PI 166051	0.0						32.5	
PI 174917	2.9			30.0/60.0[3]		5.9		
PI 174919	1.8			45.0/20.0[3]		10.5		
PI 198027	2.7	3.0	64.5		37.5			
PI 244149	7.0	1.5	83.5					
PI 244241	2.3	2.3	70.5		30.0			
PI 244254	4.0	0.6	77.1		20.0			6.0
PI 244263	1.0	1.0	92.3		20.0			
PI 263026	1.3	1.5	91.6		45.0			
PI 269768	0.7		66.8	40.0				
PI 280612	4.0	1.0	70.8		25.0			
PI 285726	4.9	0.7	80.4					
PI 297082	5.0	1.7	72.1					
PI 343286	0.3			30.0		11.6		

[1]Results published by Pesho *et al.* (1977).
[2]Results obtained by pers. comm. M. Gerding, Instituto de investigaciones Agropecurias, Chile.
[3]Adult emergence result for a second plot at the same site.

US=United States of America. CH=Chile. Australian sites (BC=Waite bird cage GP=Waite glasshouse plots TF=Turretfield WP=Waite plots NF=Northfield).

Table 3.4. Species from the tribe Vicieae used in the 1989 screening trials for pea weevil resistance showing the number of accessions and where they were harvested.

Species	Number of accessions	Plots harvested		
		Waite bird cage	Charlick	Turretfield
Lathyrus cicera	4	1	1	4
Lathyrus ochrus	1			1
Lathyrus sativus	1			1
Lathyrus tingitanus	1			1
Lathyrus unconspicus	1		1	1
Lens esculenta	4		3	3
Vicia cordata	1		1	1
Vicia cracca	1		1	1
Vicia ervilea	2			2
Vicia faba	3			3
Vicia lathyroides	1		1	1
Vicia lutea	1		1	
Vicia narbonensis	5		1	4
Vicia sativa	1			1
Vicia sp1	1	1		
Vicia sp2	1	1	1	1

recovered from ground sown plots was lower than this at four sites. The irrigation of the field pots aided in the survival of material poorly adapted to the local environment such as the late flowering accessions and certainly increased the number of accessions that were successfully screened. The screening procedure was greatly enhanced by the weevil releases which provided a steady supply of fresh weevils which could utilise these late-flowering accessions. The weevil releases ensured that field sites that did not have a

history of cropping with peas were subject to a high level of infestation which greatly reduced the number of field escapes and therefore the time associated with repeat screening of accessions.

The mean adult emergence per site and the mean adult emergence of cultivars sown at each site provided a measure of activity by adult weevils or weevil pressure at each site (Table 3.2). The level of seed damage for both these criteria was generally higher at sites where weevils were released. In 1990 Northam was the only site where adult emergence was higher than at any of the sites where weevils were released and this demonstrated the size of the pea weevil population in that area of Western Australia. The number of lines rejected by the screening process at each site indicates that both field pots and field plots are more than adequate for initial screening of germplasm for resistance (Table 3.2).

3.4. Discussion

It is clear from the field screening trials that none of the pea cultivars used have any resistance to the pea weevil and that material imported into Australia from the USA and the USSR does not have the required level of resistance either. The failure of *P. sativum* material classified by Pesho *et al.* (1977) in the US as highly resistant in Australian and Chilean trials is puzzling, although Pesho and his colleagues admit the trials were undertaken in a free-choice situation. Results obtained in Australia and Chile were also from choice trials, but they were apparently subjected to much higher pea weevil populations. Pesho *et al.* (1977) correlated the resistance they observed to shorter peduncles and this suggests that the only real defence their material had to the pea weevil was the concealment of pods in the foliage. This appears to break down when more pea weevils are searching for oviposition sites.

It is also clear from the results that *P. fulvum* shows considerable resistance to the pea weevil in a choice situation and it is possible that the weevil may fail to recognise *P. fulvum* as a host species. The information on the flowering dates (Figure 3.1) for the

P. fulvum accessions and the other *Pisum* germplasm removes the possibility that the *P. fulvum* material avoided the pea weevil. It is quite evident the *P. fulvum* accessions flowered and set pods when sexually mature adult pea weevils were active in the field. Although many accessions, especially those of *P. fulvum*, perform extremely well when a choice is available, there is a need to determine how they will perform when a choice is not available (Tables 3.1). This would provide information on how the pea weevil might respond to cultivars derived from this material.

Results from the screening of non-*Pisum* germplasm for pea weevil resistance in 1989 are encouraging, even though only 16 other species of the tribe Vicieae were tested and it was in a choice situation. The results indicate that the pea weevil will not readily infest other legume species (Table 3.4), unlike the reports of Al-rawy and Kaddou (1971) and Johnson (1981b) who list the pea weevil as a pest of faba beans. This result was disputed by Tahhan and van Emden (1989), who believed the pea weevil had been confused with *Bruchus dentipes* Baudi on faba beans. Such confusion could explain why the weevil in the past has been referred to as a pest of faba beans. If the pea weevil is host specific, it can be argued that any resistance genes that are identified and used against the pea weevil could form the basis of a stable resistance, because other legume species do not appear to be hosts for the pea weevil.

Taxonomists generally regard *P. fulvum* to be a separate species. Ben-Ze'ev and Zohary (1973) have found *P. fulvum* growing along side *P. elatius* and *P. humile*, but have found no evidence of spontaneous hybridisation between *P. fulvum* and them or introgression of distinctly *P. fulvum* genes into other *Pisum* species. They believe that this is because of the highly cleistogamous nature of the genus. This could explain the difference in the field response by the pea weevil for *P. fulvum* compared to the other members of the genus *Pisum*. The pods of *P. fulvum* do, however, have a strong tendency to shatter. It is not known if pods that contain weevil larvae entrance holes, or which are infested, dehisce earlier than usual and so avoid being harvested in the screening trials.

Although the emphasis in these trials was placed on screening wild relatives, landraces and primitive cultivars for resistance to the pea weevil, many accessions were found to be duplicates and others were cultivars or breeding lines which have already been screened by pea breeders. It is conceivable that fewer than 1,000 accessions of the 1,825 screened were distinct accessions. This is probably a small proportion of the total pea gene pool and could mean that any resistance genotypes identified are a fraction of those that do exist. It is also possible that resistant genotypes could have been overlooked if they were included in a mixture, as often occurs in landraces.

Screening of germplasm in small unreplicated plots was an effective way of eliminating the greater proportion of accessions. Unfortunately it did not allow the landrace material to be properly evaluated. Within a self-pollinating species, such as peas, there may be wide genetic variability in a landrace or in the wild material, so seed stocks in germplasm collections are maintained from a large number of plants. Nevertheless a multiplication from many plants could not be undertaken with the germplasm imported from the USDA and John Innes because of quarantine limitations at the Waite Institute, so seed was harvested from a maximum of five plants per accession. Furthermore seed was harvested from a maximum of ten plants per accession in field and from a maximum of 20 plants per accession in the field plots if all plants survived to produce seed. Seed from accessions grown through quarantine and plots using 20 seed or fewer would probably not represent all the genotypes in a wild or landrace accession. The method of harvesting plots also led to a bias against wild and landrace material because seed from a plot was pooled and the presence of a resistant genotype among other genotypes could be overlooked when the sample was evaluated. The use of unreplicated plots also rules out a rigorous statistical separation of accessions and only provided results of the weevil's preference because it is a highly mobile species. The ten per cent cut off was intended to remove the obviously susceptible accessions. It must be also remembered that the design of the trial was to enable the assessment of a large number of accessions in the time available.

CHAPTER 4

NO-CHOICE TESTING OF *PISUM* ACCESSIONS FOR RESISTANCE TO PEA WEEVIL IN A PLANT GROWTH ROOM

4.1. Introduction

Sixty-nine accessions of *Pisum* were identified as potential sources of resistance to the pea weevil following three years of screening germplasm in the field. These accessions were those in which adult weevils emerged from ten per cent of the seed or less at all sites (Chapter 3). The weevils had a free-choice during the field screening, but the lines identified are only of value if their resistance is maintained in a no-choice situation, as exists in a crop. This is particularly important if the mechanism involved is a form of antixenosis. Insects like the pea weevil, though capable of flying several kilometres to a host crop, effectively have a limited range over which they can exercise a choice. A no-choice situation occurs when an area is sown to a single genotype. It is difficult to test a genotype for antixenosis before it has been developed as a cultivar and sown as a broadacre crop. No-choice testing has been simulated in the field using enclosures to obtain a measure of oviposition (Smith *et al.* 1980). However there are technical problems associated with this type of test. The pea weevil is only active for about six weeks of the year which limits the time available for experiments. To make a valid comparison across genotypes in the field they should flower at approximately the same time and this may require several sowings. Many enclosures are required when the trial is replicated. These factors severely limit the number of accessions that can be screened.

Annis and O'Keeffe (1984a) used choice and no-choice situations in a glasshouse to screen for resistance to pea weevil. As their results were encouraging, it was decided to undertake no-choice testing in a controlled environment. The aim was to separate the highly resistant accessions from those that were less resistant or non-resistant.

4.2. Materials and Methods

The selection of accessions for no-choice testing was based on the 1989 field results. The Waite bird-cage site recorded the highest incidence of pea weevil damage with only 15 accessions recording less than ten per cent adult emergence. All five *P. fulvum* accessions tested in 1989 were among the 15 with PIG 277 being the only one in which any emergence was recorded. Two more *P. fulvum* accessions (WIR 2523 & WIR 3397) were included because of the promise shown by *P. fulvum*, together with a pea cultivar (WIR 4739) imported from the USSR that is reputed to be resistant (pers. comm. Dr Ali, S.A. Dept Agric). Other accessions of Vicieae were tested in single replicates, but were not included in the statistical analysis. The Australian pea cultivar Pennant was chosen as the control because of its susceptibility to the pea weevil and its compact habit when grown in a glasshouse.

Beginning in April, sequential sowings were made, to enable testing of accessions at similar stages of growth. Seeds were surface sterilised as described in Chapter 3 and germinated on moist filter paper in petri dishes in an incubator at 25°C . All the *P. fulvum* and *P sativum* ssp. *humile* accessions were hard-seeded and so they were scarified. The seedlings were transplanted into nine litre pots. Wire trellises provided support. Incandescent lights were used during winter to meet the day-length requirement of accessions dependant on photo-period to induce flowering. Insects and diseases attacking the plants were only controlled with non-persistent contact pesticides. Sprays were used only when necessary and never less than two weeks prior to testing. Eight cages were constructed and placed in a growth room (Plate 4.1) for the no-choice test.

In a preliminary experiment weevil-infested seed, harvested from crops in 1989, was stored at 4, 10 and 15°C to determine if storage temperature influenced the break in diapause. Weevils which had been held at 10°C were the first to lay fertile eggs. They

Plate 4.1. No-choice trial in a growth room.

were transferred to 25°C as required from the end of May, and fed pollen. These mated and began to lay fertile eggs after one week. This meant that testing of accessions could be undertaken from early June. All weevils stored at other temperatures were then placed at 10°C, as was all subsequent material.

Pea weevils, removed from storage one week prior to a test, were sexed and 20 pairs placed in each mating container (Disposable Products # 22520, 250ml plant tissue culture container) with a water supply and pollen (Plate 4.2). The no-choice testing was carried out in a plant growth cabinet because the temperature in unheated glasshouses at that time of the year was below the threshold for weevil activity. The growth room was maintained at 25±2°C with a photo-period of 16L:8D, the artificial lighting being provided by sodium vapour lamps. Pots containing three to five plants with green pods of different lengths and maturity were selected for testing. This maximised the oviposition choice of weevils. Most tests used eight cages, containing one of the six accessions or the two cv. Pennant controls. All plants were at a similar stage of development. Two rows of four cages with a cv. Pennant control in each row were placed in the growth cabinet, with the accessions randomised within rows.

Containers with mating weevils were selected at random and placed in each cage and the lid of the container removed. The container with its supply of pollen was left in the cage as a prior test had shown that plants aborted many of their flowers in the growth cabinet and would not provide pollen. A water bottle with a filter paper wick was placed on top of each cage to provide water for the weevils. The plants were watered daily. After a week the plants were removed and scored.

The total number of pods per cage for each accession was recorded and the eggs counted on a sub-sample of 50 pods if this number of pods was available. The pods were not removed from the plants. Pods of various lengths and developmental stages from different parts of the pea vine were assessed. After several tests the sub-sample was

Plate 4.2. Pea weevil mating container

increased to 100 pods, if available, because of the variation within accessions and the very low incidence of eggs on other accessions. After assessment, the plants were placed in the glasshouse to allow the hatching larvae to infest the seed. When the plants were mature, the seed was harvested and kept at 25°C to enable adult pea weevils to develop and emerge. The estimated number of eggs laid on each accession was used as a measure for the presence of antixenosis and the number of adult weevils to emerge was used as a measure of pod and seed resistance.

The results were analysed as a completely randomised block design. It was assumed that there was no influence of pea plants in one cage on weevils in another cage. An analysis of variance was carried out for the estimated number of eggs per accession, the adult emergence from seed and the relationship between these two variables. The data were transformed using the square root transformations ($\sqrt{x+0.5}$) or ($\sqrt{y+0.5}$) where necessary for the analysis. A pair-wise comparison of the cv. Pennant and the test accessions was made for adult emergence and estimated number of eggs using Scheffe's test (Scheffe 1959). Since the number of replicates varied for each accessions, it was calculated as:

$$\hat{\sigma}_{w}S = \sqrt{\hat{\sigma}^2\left(\frac{1}{n1}+\frac{1}{n2}\right)(k-1)F}$$

where $\hat{\sigma}_{w}S$ = Scheffe's statistic, $\hat{\sigma}^2$ = Residual MS, n_i = number of replicates, k = number of means to be compared. The F value in the equation is for the degrees of freedom of k-1 and v, the df associated with the Residual MS for the level of significance (1-α). The relation between the number of eggs per cage and the number of adult weevils to emerge suggested a curvilinear response for most accessions. The exact shape of the response was not determined as there were only three to five values for each test accession. A pair-wise comparison of accessions was made with each test and the control to determine if the lines were parallel, distinct, or coincident and if the slope of the line was independent of

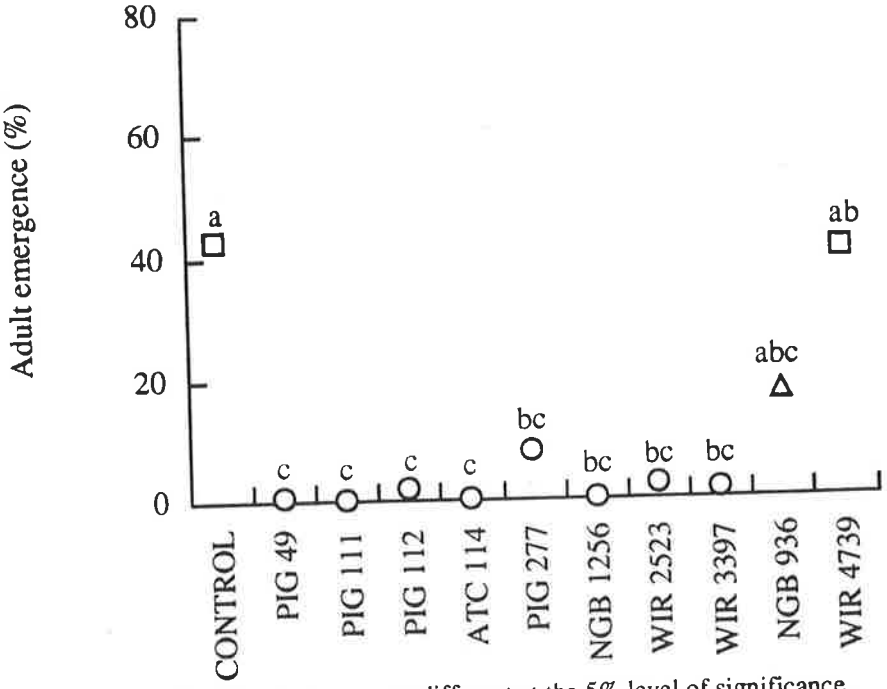
the estimate of eggs per cage. The hypotheses for these comparisons is given in Appendix 1.

4.3. Results

The percentage of weevils which emerge from seed has been considered first as this character is the ultimate indicator of resistance. The percentage emergence of weevils from seed was higher in *P. sativum* than in *P. fulvum* (Figure 4.1). Only the *P. fulvum* accessions showed any overall resistance with emergence ranging from 0.1% for the most resistant to 8.0% in the least resistant. All of the *P. fulvum* accessions were significantly different from both the cv. Pennant control and the Russian *P. sativum* cultivar. No accession was statistically different from the *P. sativum* ssp *humile* line. These results indicate the presence of resistance mechanisms in the *P. fulvum* accessions and the need for further analysis.

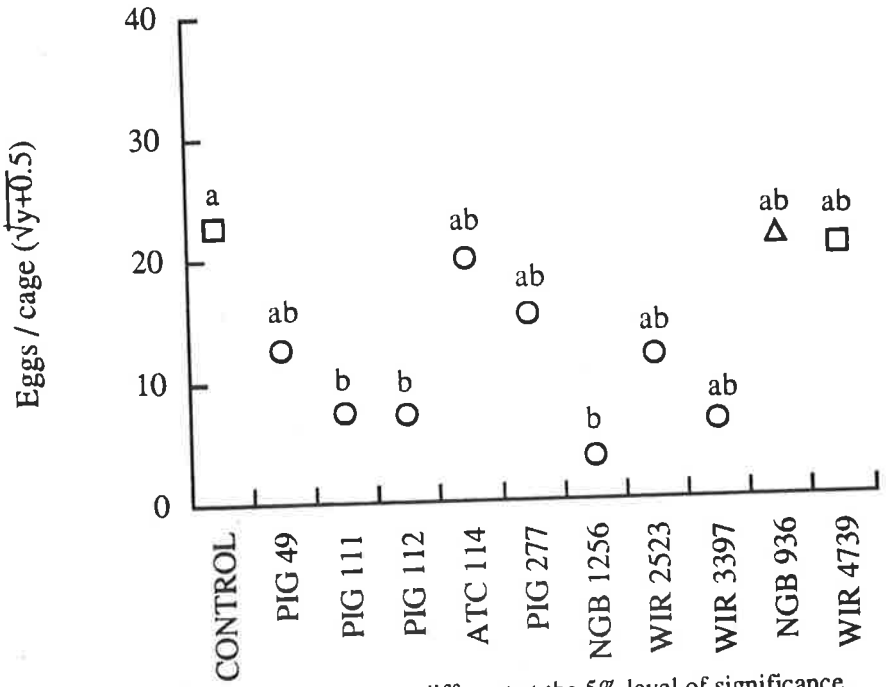
The oviposition results show that significantly fewer eggs were laid on the three *P. fulvum* accessions PIG 111, PIG 112 and NGB 1256 than on the cv. Pennant. All other accessions were intermediate and differences among then were not significant (Figure 4.2). Although WIR 3397 has a lower value than PIG 111 and 112 in Figure 4.2 it was evaluated in a lesser number of replicates and was not significantly different from the control. The results demonstrate that when weevils are given no choice of pea genotype on which to lay their eggs they still lay more eggs on some accessions.

With many of the accessions, the number of adults to emerge was positively associated with the number of eggs laid. However a regression analysis indicates that for some accessions many eggs were laid but adults did not emerge. Figure 4.3a shows the lines of best fit for accessions which were significantly different ($p<0.05$) from the control and Figure 4.3b those that were not significantly different. The data for PIG 111, ATC 114 and PIG 49 clearly indicate that many eggs may be laid that do not result in adult weevils emerging from seed.



Symbols with the same letter are not different at the 5% level of significance.

Figure 4.1. The mean percentage emergence of adult weevils from the harvested seed for *P. sativum* accessions (□), *P. fulvum* accessions (○) and a *P. sativum* ssp. *humile* accession (△).



Symbols with the same letter are not different at the 5% level of significance.

Figure 4.2. The effect of antixenosis on oviposition by female weevils in no-choice conditions on *P. sativum* accessions (□), *P. fulvum* accessions (○) and a *P. sativum* ssp. *humile* accession (△).

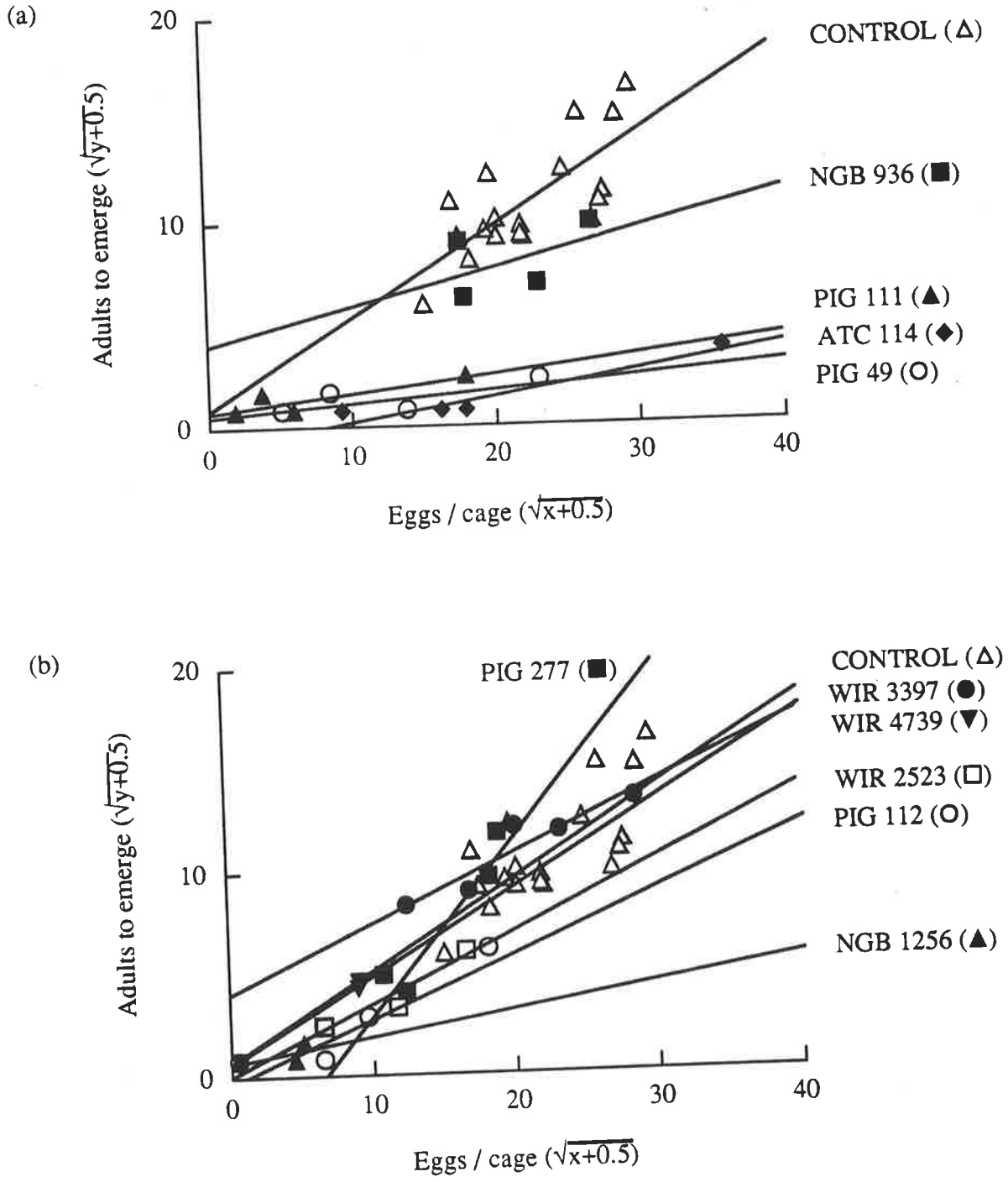


Figure 4.3. The relation between the number of adults (y) on eggs per cage (x) for (a) regressions significantly different from the control cv. Pennant and (b) regressions not significantly different from the control cv. Pennant.

4.4. Discussion

The results demonstrate that there is a high level of resistance to the pea weevil in the eight *P. fulvum* accessions, as indicated by the low level of adult emergence from seed (Figure 4.1). The differences between accessions for the percentage emergence of weevils from seed harvested provide a measure of plant resistance and demonstrated the need for other analyses to determine the underlying mechanisms of resistance. There appeared to be at least two mechanisms of resistance, a lack of oviposition (Figure 4.2) and a reduction in the emergence of adults (Figure 4.3a). Whether the same mechanisms are common to the various resistant accessions of *P. fulvum* is not known. NGB 936 developed pod callus which may be a form of resistance in certain circumstances. There was no evidence of resistance in the Russian cultivar WIR 4739.

The number of seeds produced by an accession can influence the percentage emergence of adults. This could occur without the influence of a seed mechanism. The number of larvae to enter pods in different accessions could be the same, however different accessions produce different numbers of pods and seeds per pod. Therefore the total number of seeds produced is a component in the calculation of adult emergence even if there is no competition between larvae and each infests a separate seed. The *P. fulvum* accessions and NGB 936 produced many more seeds than the cv. Pennant, and though not useful to a plant breeder it can be considered a mechanism of escape for the plant which must be accounted for in future experiments.

There were lower rates of oviposition on all *P. fulvum* accessions than on the controls (Figure 4.2). Numerous eggs were laid on the aluminium frames of the cages rather than the pods of the *P. fulvum* accessions indicating the extent of the antixenosis. The pea weevil will sometimes lay eggs on species outside its normal host range when it has no choice (Annis and O'Keeffe 1984a), as indicated by the example of a *Lathyrus tingitanus* accession which received an estimated 351 eggs compared to 835 eggs in the control.

An antixenosis and or an antibiosis mechanism(s) may be located in the pod wall, the testa or the cotyledons. The plant tissues responsible for resistance, and the chemistry and genetics of the observed oviposition antixenosis in the *P. fulvum* accessions are unknown. The entire plant, or a part such as the pod wall may be responsible for changes in the behavioural response of the pea weevil. This may depend on the presence of one or a group of volatile compounds or the number and morphology of pods. *P. fulvum* accessions produced more, but smaller pods than the control cultivar. However, the *P. sativum* ssp. *humile* accession (NGB 936) also produces small pods which have similar a morphology to the *P. fulvum* accessions. The estimated number of eggs per cage for NGB 936 did not differ from the control (Figure 4.2), so pod size alone is unlikely to be an important resistance factor.

The environment in the growth room was not ideal for growth and it appears the low light intensity was the reason the plants aborted their flowers. It was also observed that young pods stopped growing, but continued to mature under these conditions and in several instances the entire plant senesced. Any plants which senesced during the test period were excluded from the analysis. The remaining eight *Pisum* accessions of the original 15 accessions selected for the no-choice testing were not replicated because of high adult emergence in a single test, plant senescence in the growth room or because suitable plants were not available for screening. These accessions have since been rejected in the 1990 or 1991 mass screening field trials (Chapter 3).

The slope of the regression for NGB 936 was different from that of the control (Figure 3a) suggesting that it was more resistant to the pea weevil in the presence of high numbers of eggs. However this seems unlikely and a more plausible explanation is the sporadic development of pod callus in this accession which could reduce the number of larvae that penetrate the pod wall. The callus developed beneath the eggs and prevented larvae boring directly into the pod so that they had to move to a callus free area of pod to penetrate the pod wall (Plate 4.3a). This could have affected the survival of larvae as was

Plate 4.3. Callus development under pea weevil eggs (a) *P. sativum* ssp. *humile* and (b) *L. tingitanus*

reported by Annis and O'Keeffe (1984a) for *L. tingitanus* (Plate 4.3b). Dodds and Matthews (1966) described the spontaneous development of callus on pea pods in some glasshouse grown accessions as neoplastic pods and associated this development of callus as a plant response to low levels of light. The growth room and the glasshouse were both low light areas, but pod callus was only induced on NGB 936 in the glasshouse in response to a wound. This would suggest that the callus response in NGB 936 is different to the response described by Dodds and Matthews. Callus growth was not found in association with weevil eggs in the field on NGB 936, nor did it develop as a wounding response in the field. This is unfortunate because, apart from deterring the entry of larvae into pods, larvae would be exposed to predators, parasites and any pesticides as they crawl across pods in the presence of callus.

CHAPTER 5

TESTING FOR OVIPOSITION ANTIXENOSIS IN A GLASSHOUSE

5.1. Introduction

In Chapter 4, the no-choice testing of accessions demonstrated lower rates of oviposition on *P. fulvum* accessions than on the control cv. Pennant. However the differences were significant for only three accessions. The *P. fulvum* and control plants were different morphologically and were grown in an environment which did not allow normal development. The behaviour of weevils also was not normal; they laid eggs on the cages and on pods of species outside the normal host range. In this test oviposition was estimated as eggs laid per cage, using a sample of pods. The experiment may not have adequately distinguished between accessions as it did not take account of morphological differences between pods. Pennant produced fewer pods than the test lines and this could increase competition between weevils for places to lay eggs. However the pods were nearly twice as long and provided a much larger area for oviposition.

The seed-beetle *Callosobruchus maculatus* is known to assess egg load on the host (Messina & Renwick 1985; Wilson 1988) and will lay eggs according to the size of host seeds (Messina & Mitchell 1989). Egg spacing behaviour in *C. maculatus* has also been shown to regulate the level of competition and the fitness of larvae (Mitchell 1975). Although many pea weevil larvae can enter the same seed, on most occasions only one will reach maturity. This suggests that survival would be optimised by the weevil adjusting egg load for different sizes of pods.

In Chapter 4 light intensity in the growth room was monitored, but it is not known whether light affected the growth of plants or the behaviour of the weevil. Apart from reducing light intensity, a cage can alter the environment in terms of temperature, relative humidity,

and air movement (Tingey 1986). This can result in changes in the behaviour of captive insects which may cause them to be less discriminating, such as in the choice of oviposition sites (Singer 1986). Some aspects of oviposition in the growth room conflicted with what was expected and this problem needs to be resolved. Thus the aim of this experiment was to investigate the effect of pod morphology on oviposition in an environment more conducive to normal plant growth.

5.2. Materials and Methods

The preparation of plants and weevils used in each no-choice test was the same as in Chapter 4. The number of lines was reduced to five of *P. fulvum*, and the control *P. sativum* cv. Pennant. The test accessions were PIG 111, PIG 112 and NGB 1256, categorised as antixenotic, and PIG 49 and ATC 114 which did not differ significantly from the control (Chapter 4). Nine cages were placed in a heated glasshouse on 17 September for this experiment, which continued for eight weeks. Three cages of Pennant and three cages of two test accessions were set up each week. Mated weevils were added to each cage, but supplementary water bottles were not used. Temperatures in the glasshouse were between 21 and 31°C. None of the plants died during the test period, though some flowers aborted. After a week plants were removed from the cages, the pods were harvested, and assessed for length, development stage and the number of weevil eggs. The pod development categories used were based on those described by Knott (1987).

The results were analysed as a completely randomised block as in Chapter 4. An analysis of variance was carried out for the number of eggs per accession, number of eggs per pod, pod surface area and number of eggs per mm^2 of pod surface. The data were adjusted where necessary using the square root transformation ($\sqrt{y+0.5}$). Scheffe's test (Scheffe 1959) was used to make pair-wise comparisons between accessions because of the variation in numbers of replicates.

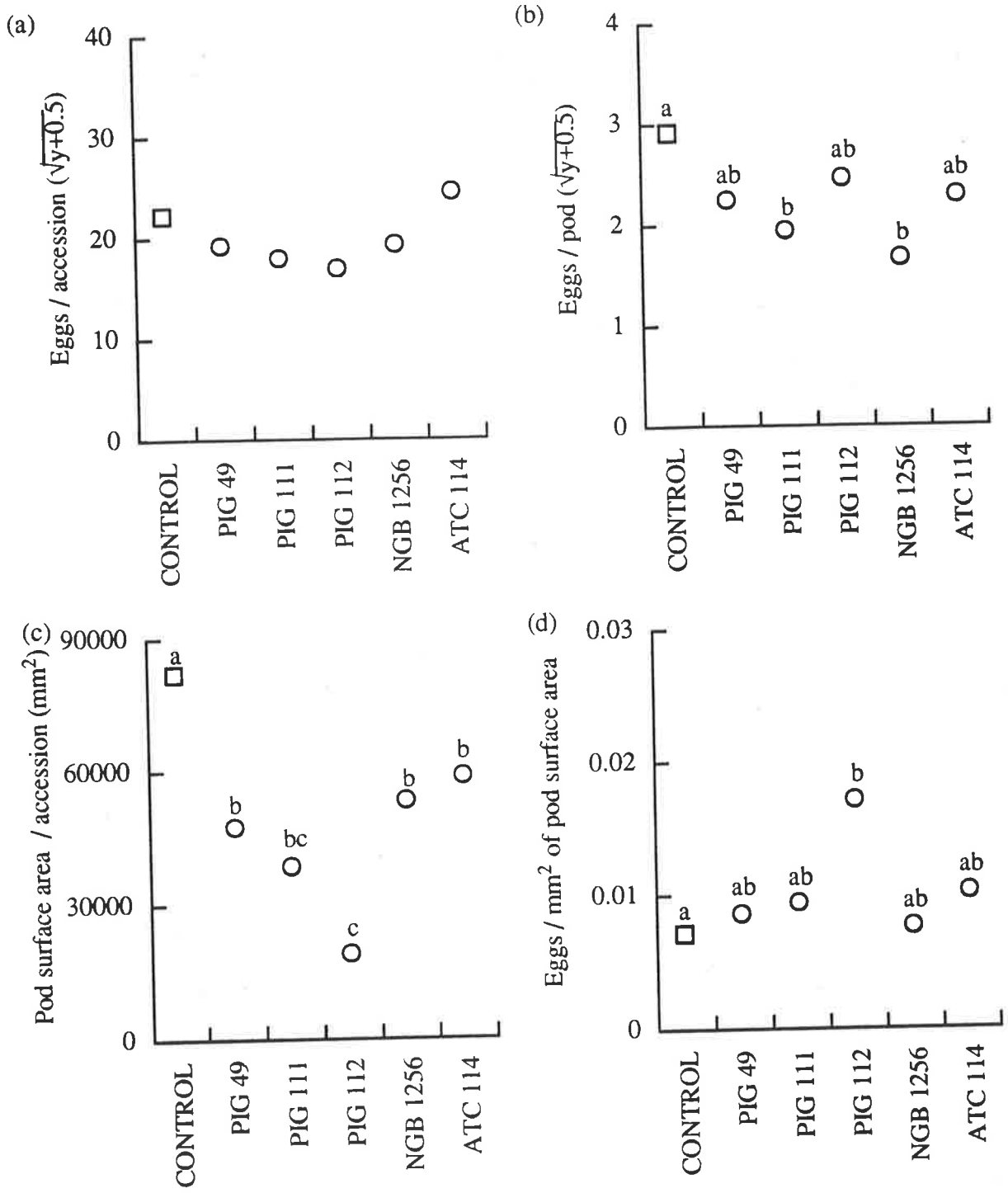
The pod surface area and number of eggs per mm^2 of surface were estimated from pod length for each accession separately. An area was estimated from a sample of 20 pods of

varying lengths and development. The areas were estimated from the regression of area on pod length. The regression values were obtained for pods of known lengths that were passed beneath a leaf area scanner. Area varied little with stage of development but this was not taken into account. These data were fitted to a series of quadratic polynomial models (Appendix 2). The regression analysis for the six genotypes indicated that it was better to fit a quadratic polynomial than a linear model to the data. The resulting curves were parallel with the same linear and quadratic regression coefficients, but with different intercepts.

5.3. Results

The number of eggs laid per accession were not significantly different from the control as was found as in Chapter 4 (Figure 5.1a) although the number of eggs laid on the *P. fulvum* accessions tended to be lower. There was considerable variation around the mean number of eggs per accession suggesting factors like the size and number of pods may influence the result.

Differences in the number of eggs laid per pod were found among accessions, however only PIG 111 and NGB 1256 differed significantly from the control (Figure 5.1b). These produce smaller pods (Figure 5.1c) than the other accessions and this could explain the differences in the number of eggs laid per pod. The control pods had twice the area of many of the *P. fulvum* accessions. The area for PIG 49 was significantly larger than in PIG 112 and similar for that in PIG 111, ATC 114 and NGB 1256. Because of the large area of the control pods, they were excluded from further analysis which was only repeated on the *P. fulvum* accessions. This analysis indicated that the differences in the areas available for oviposition evident in Figure 5.1c were very highly significant. The differences in area between the control and test accessions could account for the perceived preference for the control pods.



Symbols with the same letter are not different at the 5% level of significance.

Figure 5.1. Oviposition under no-choice conditions on the control *P. sativum* cv. Pennant (□) and *P. fulvum* accessions (○). (a) Eggs per accession. (b) Eggs per pod of each accession. (c) Pod surface area available for oviposition. (d) Egg density observed for each accession.

Evidence with a bearing on this matter is given in Figure 5.1d where a significant difference between accessions in egg density (eggs laid per mm^2 of pod surface area) was observed. This was due to the single difference between the control pods which had the lowest density and PIG 112 which had the highest density. None of the other differences was significant.

5.4. Discussion

The no-choice testing in the glasshouse demonstrated that there were no differences in egg density on control and test pods. This was is in contrast to the results obtained in the field screening trial (Chapter 3) and the growth room experiment (Chapter 4) and demonstrates how the environment and experimental design (free-choice or no-choice) can possibly influence the outcome of an experiment.

The type of measurements taken may also influence conclusions gained from an experiment. This analysis of weevil oviposition in a no-choice situation suggests that the number of eggs laid on a pod is determined by its size. It indicates that, for a given size of pod, the number of eggs laid would be the same and the control would be no more susceptible than the *P. fulvum* accessions. Therefore the use of eggs per pod would be misleading because it is not a good measurement of resistance if pod area differs between accessions.

The glasshouse environment used in this experiment sustained all the plants, whereas in the growth room (Chapter 4) the control plants appeared to tolerate the light conditions better than many of the *P. fulvum* accessions. In Chapter 4 the number of eggs per cage was 526 for the control and here it was 519, a similar result. However the number of eggs per cage had at least doubled on those test lines which were common to both experiments except for ATC 114. The environment in the growth room evidently decreased the attractiveness of the *P. fulvum* accessions to the pea weevil and could be the reason why

more eggs were laid on them in the glasshouse. However a different response may have occurred in this experiment than that experienced in the field.

The use of numbers of eggs per accession and eggs per pod provided consistent and sometimes significant differences between the control and the *P. fulvum* accessions. However they do not allow for differences between the accessions in the number and size of pods. The use of the pod area available to the ovipositing weevils removes many of the affects associated with a difference in pod morphology while maintaining the weevils in an environment where they can interact with an entire plant. Results from an analysis of egg density per accession suggested that pod size is the only factor involved in determining the number of eggs laid. This includes the effect that different pod lengths might have on competition between ovipositing females or from eggs already laid on a pod. A simple test comparing pods with different lengths would provide information on competitive interactions between ovipositing weevils.

Testing with a no-choice procedure removed the preferences associated with different genotypes in a free-choice situation (Chapter 3). It also allowed the density of the pest species to be regulated. However it did not give individual weevils the choice of whether to alight or leave the test plant even if the weevil perceived it to be unsuitable for oviposition. In this experiment each gravid weevil either laid or did not lay eggs on the pods of the available genotype. The results indicated that either the oviposition antixenosis found against the *P. fulvum* accessions in free-choice situations is a weak form of resistance, or that gravid females are less discriminating when confined. The results also suggest that confined weevils are less discriminating, but the use of isolated *P. fulvum* field plots could put this hypothesis to a more rigorous test.

CHAPTER 6

INHERITANCE OF RESISTANCE TO THE PEA WEEVIL IN THE GENUS *PISUM*

6.1. Introduction

Evidence was presented in Chapter 4, and reinforced by the results to be presented in Chapters 8 and 9, for at least two mechanisms of plant resistance to the pea weevil. With one the weevil prefers not to oviposit on certain accessions (antixenosis), and with the second there is a seed resistance (antibiosis). These mechanisms were restricted to several weedy accessions of *P. fulvum* which are poorly adapted to the local farming environment. A breeding program will be required to introduce the desired genes into a useful background. Before this can be done the inheritance of the mechanisms needs to be understood. The aim of the experiment presented here was to investigate the inheritance of antixenosis and antibiosis mechanisms from crosses made between *P. sativum* and *P. fulvum* accessions.

6.2. Materials and Methods

The five *P. fulvum* accessions identified as resistant to the pea weevil were PIG 112 and NGB 1256 for antixenosis, PIG 49 and ATC 114 for seed antibiosis and PIG 111 for both (Chapter 4). Plants of each accession and the susceptible cultivar Pennant were grown in a glasshouse. Crosses were made with Pennant as the female and the five resistant accessions as males. A comprehensive investigation by Ben-Ze'ev and Zohary (1973) showed that *P. sativum* had to be the female parent to obtain viable F_1 progeny from crosses between these two species. Fifty crosses were made for each *P. fulvum* accession. With the exception of ATC 114 enough F_1 and F_2 seed was obtained for the trial. Pods developed normally from the cross of Pennant with ATC 114, but few seeds developed and

plants derived from the F_1 seeds also failed to set many seeds. Anther squashes of these F_1 plants, stained with 2% acetocarmine, showed the plants to be male sterile as the anthers had failed to dehisce.

It was not feasible to screen plants using the no-choice procedure of Chapter 4, though this would have removed the influence of choice in determining the inheritance of antixenosis. Instead the material was sown in pots placed in a field trial. The trial design used was a randomised block design with five replicates, each of 108 pots. Each replicate contained four sowings of the susceptible cultivar Pennant, with two pots of five seeds sown at four weekly intervals. The aim was to overlap the flowering and podding of the resistant parents (PIG 49, PIG 111, PIG 112 & NGB 1256) and their F_1 and F_2 progeny which were sown only once. The F_2 seed from each cross were sown individually with 21 pots per replicate. The four resistant parents and the F_1 plants from each cross were sown with two pots per replicate and five seeds per pot. The use of five seeds in each pot increased the probability that some plants would reach maturity and thereby ensured a supply of flowers and pods for the pea weevil. Enough material was included to determine if resistance was a simply inherited trait in the F_2 populations .

These crosses have a low seedling vigour (Ben-Ze'ev and Zohary 1973). The seed of the parental material and the F_1 plants were scarified, surface sterilised and germinated on moist filter paper in petri dishes in an incubator at 25°C before sowing into pots. All other material was treated with P-Pickle® and sown directly into pots. The trial material was sown together with the first sowing of Pennant between 31 May and 3 June 1991. The other sowings of Pennant were on 28 June, 26 July and 23 August. The trial was drip irrigated as described in Chapter 3 and conditions optimised to enhance seedling establishment. A slow release fertiliser (18% N, 4.8% P, 9.1% K and 3.7% S) was added to the pots after germination.

The trial was monitored for the presence of the pea weevil and once individuals from the natural population were observed their numbers were supplemented by an additional 2,000 weevils released during the flowering and podding period. The weevils were released throughout the trial and because of the high mobility of this species it was assumed they would have occupied the whole area. They were collected during the previous season from seed harvested at the edge of an infested pea crop. The first eggs were found on pods on 13 September.

The trial was sampled on two occasions, 14-18 October and 28 October-1 November. Twenty pods per pot were selected at random and examined. The length, developmental stage and the number of eggs laid on each pod were recorded to provide information on the inheritance of pod antixenosis. If 20 pods per pot were not found then the number of eggs was extrapolated to a 20 pod sample. To obtain the mean values for each genotype, the number of eggs on the 20 pods sampled from each plant were averaged for all plants respectively of the parents, the F_1 and F_2 plants. Pods which were drying off, were not selected and care was taken not to dislodge any eggs. During sampling it was noticed that many pods had been chewed by the larvae of the native budworm (*Heliothis punctigera* Wallengren) but, in the absence of a selective insecticide, they were not controlled. Other characters assessed were flowering date and flower colour.

Seed harvested from each pot was bulked and kept at 25°C to enable adult weevils to develop and emerge. Once weevils began emerging, each seed was individually inspected for penetration of the seed testa by larvae (seed infestation) and for adult emergence from cotyledons which had been entered (adult emergence per seed entered). These data were collected to obtain information on the inheritance of the possible antibiosis mechanism in the seed. The means are based in some instances on a fewer number of plants than for the antixenosis analyses as some plants died, some had pods but no seeds and pods on others had shattered. Many of the pods on the F_1 and F_2 plants contained none or very few seeds, and the pods shattered as they dried off as with *P. fulvum* parents. These factors

contributed to the small amount of seed harvested from some plants and is the reason why most plants were harvested while partially green. The cotyledons represent the next generation which means the F_1 plants carry pods containing F_2 seeds with embryos and cotyledons which will have segregated. This was considered in the analysis of inheritance of seed antibiosis.

6.3. Results

All of the F_1 plants were variegated and many of the F_2 plants grew slowly compared to the parental material. 47% of the F_2 plants were variegated (Plate 6.1) as described by Ben-Ze'ev and Zohary (1973). 7% of the trial material grew so poorly the plants did not reach flowering. Plants in the trial started flowering on the 31 July. All F_1 plants had pale pink flowers indicating they were crosses and not selfs. If selfing had occurred the plants would have produced white flowers. A hail storm on the 23 August destroyed 10% of the trial and reduced the yield from many of the early-flowering plants.

The data presented were for the first sampling period, as many of the plants, including the four *P. fulvum* parents had matured and were harvested before the second sampling. As results for the four crosses and their progeny were similar, only the results for the Pennant by PIG 49 cross are presented for illustrative purposes. This cross was chosen because the morphology of PIG 49 was closer to that of Pennant than the other *P. fulvum* parents. The fourth sowing of Pennant had not flowered by the time sampling of the trial commenced and data relating to it were not included in the results. The combined data for the first three sowings of Pennant will be referred to as that for *all sowings*.

Pennant flowered three weeks earlier (on average 11 August) than the cross material from their common sowing on the 31 May (Figure 6.1). From this sowing the susceptible parent had been flowering for up to five weeks before weevils began laying eggs in mid-September, while the resistant parent and the F_1 plants had been flowering for only two and three weeks respectively.

Plate 6.1. Variegated hybrids showing (a) stunted seedling and (b) close-up of an affected leaflet.

The mean number of eggs laid declined for each of the three sowings of the susceptible parent (Figure 6.1 & Table 6.1). Because of the differences in flowering time and the effect this has on the potential for oviposition it is necessary to compare data on the F_1 and F_2 progeny to values for oviposition on the control from flowerings that occurred at the same time. This was the second sowing for the F_1 progeny. The F_2 progeny began flowering over an extended period which encompassed the three sowings of the control (Figure 6.1 overlay), so oviposition on their pods needs to be compared to the combined results from all sowings.

Table 6.1 Mean, standard error and range of eggs per twenty pod sample from the susceptible parent (1st, 2nd, 3rd & all sowings), resistant parent, F_1 and F_2 progeny.

Generation	Mean ± SE (eggs per 20 pods)	Range
susceptible parent - all sowings	18.1 ± 4.1[1]	0 to 79.0
susceptible parent - 1st sowing	30.0 ± 8.1	9.0 to 79.0
susceptible parent - 2nd sowing	12.9 ± 5.3	0 to 42.9
susceptible parent - 3rd sowing	8.5 ± 4.5	0 to 33.3
resistant parent	1.6 ± 0.6	0 to 7.0
F_1 progeny	10.1 ± 1.4	0 to 25.0
F_2 progeny	4.1 ± 0.8	0 to 35.0

[1] mean calculated on raw data not the weighted average from the 1st, 2nd and 3rd sowings of Pennant

From the comparison of the parents to the F_1 progeny, it is evident there was a wide range for the number of eggs laid per 20 pods on the susceptible parent and over half the pods bore eggs. On the resistant plants there was a narrow range of egg numbers, with no eggs on most of the pods (Table 6.1 & Figure 6.2a). The range of the number of eggs on pods of the F_1 progeny was closer to the susceptible than the resistant parent, and the mean number of eggs per pod was also close to the susceptible parent.

The number of eggs laid on 20 pod samples from the three sowings of the susceptible parent and the F_2 progeny were also compared (Table 6.1 & Figure 6.2b). The distributions for the resistant parent and F_1 progeny are included for comparison. The range in egg distribution for all sowings of the susceptible parent was wider, with a higher mean than for the second sowing of the susceptible parent. The distribution of eggs laid on pods of the F_2 plants was more discrete, with a much lower mean. It was heavily skewed toward a small number of eggs per sample. This skew suggests that antixenosis is dominant, however such an interpretation is not clearly supported by the data from F_1 progeny.

The mean number of infested seed per plant for the second flowering of the susceptible parent was higher and the range was greater than for the resistant parent (Table 6.2 & Figure 6.3a). The F_1 progeny were more similar to the susceptible than to the resistant parent. The mean level of seed infestation for all sowings of the susceptible parent was lower than for the second sowing of the susceptible parent (Table 6.2 & Figure 6.3). The distributions representing the level of seed infestation (Figure 6.3) are similar to those for oviposition on pods (Figure 6.2) and again the mean value of the F_1 progeny was closer to that of the second sowing of the susceptible parent than to the resistant parent. The distribution of infested seed for the F_2 progeny (Figure 6.3b) did not show the pronounced skewness observed for oviposition on the same F_2 progeny (Figure 6.2b) and suggests that a factor additional to the number of eggs laid has influenced the level of seed infestation. These diagrams provide little evidence on the nature of inheritance of this resistance mechanism as the distributions for infested seed produced from the F_1 and F_2 plants resemble the susceptible parent.

When the lengths and stage of development of pods for the parents, F_1 and F_2 plants are considered, it is evident that more eggs were found on longer pods (Figure 6.4). This is most clear for the susceptible parent where pods up to 70mm and pods that had finished had more eggs on them. No eggs were found on yellow-wrinkled pods. Pods of the

resistant parent rarely exceeded a length of 50mm and given the small sample no trend was apparent. There was also no strong evidence for a trend in the F_1 or F_2 progeny.

Table 6.2 Mean, standard error and range of infested seed per plant harvested from the susceptible parent (1st, 2nd, 3rd sowing & all sowings), resistant parent, F_1 and F_2 progeny.

Generation	Mean ± SE (% of infested seed/plant	Range
susceptible parent - all sowings	25.4 ± 8.7[1]	0 to 100
susceptible parent - 1st sowing	21.8 ± 4.4	0 to 35.7
susceptible parent - 2nd sowing	35.2 ± 10.0	0 to 100
susceptible parent - 3rd sowing	25.4 ± 8.7	0 to 58.3
resistant parent	0.5 ± 0.4	0 to 3.6
F_1 progeny	23.7 ± 6.9	0 to 25.0
F_2 progeny	18.9 ± 3.9	0 to 100

[1]mean calculated on raw data not the weighted average from the 1st, 2nd and 3rd sowings of Pennant

Some of the eggs laid on the pods of the *P. fulvum* plants hatched and the larvae penetrated the pod wall. This suggested that the pod wall was not a factor in resistance and that a seed mechanism was involved.

Evidence for an antibiosis mechanism based in the cotyledons is apparent from the large difference in weevil emergence between the susceptible and resistant parents, but the F_2 and F_3 seed showed little evidence for segregation of this character, with all plants in the samples producing mostly susceptible seeds (Table 6.3 & Figure 6.5) (Note that F_1 and F_2 plants bear F_2 and F_3 seed, respectively). However it was observed that adults did not develop as quickly in the F_2 and F_3 seed. In five out of 107 F_2 seeds, and five F_3 out of 202 seeds an adult did not form. In contrast adults emerged from all but four of the 666 infested seeds from the susceptible parent. This result for the susceptible parent indicates that some weevil larvae may die in the cotyledons as the result of factors other than the

antibiosis mechanism. Two of the 17 infested seeds of the resistant parent produced an adult weevil. The survival of a few weevils in the cotyledons of the resistant parent indicates that the antibiosis mechanism is not always completely effective and may be influenced by the environment.

Table 6.3 Mean, standard error and range of emergence per seed entered for the susceptible parent (1st, 2nd, 3rd sowing & all sowings), resistant parent, F_2 and F_3 cotyledons.

Generation	Mean ± SE (% emergence from seed entered)	Range
susceptible parent - all sowings	98.6 ± 1.4[1]	92.9 to 100
susceptible parent - 1st sowing	99.8 ± 0.3	97.8 to 100
susceptible parent - 2nd sowing	99.8 ± 0.2	98.4 to 100
susceptible parent - 3rd sowing	98.6 ± 1.4	92.9 to 100
resistant parent	3.3 ± 3.3	0 to 6.7
F_2 progeny	97.0 ± 1.3	93.8 to 100
F_3 progeny	98.1 ± 1.5	60.0 to 100

[1] mean calculated on raw data not the weighted average from the 1st, 2nd and 3rd sowings of Pennant

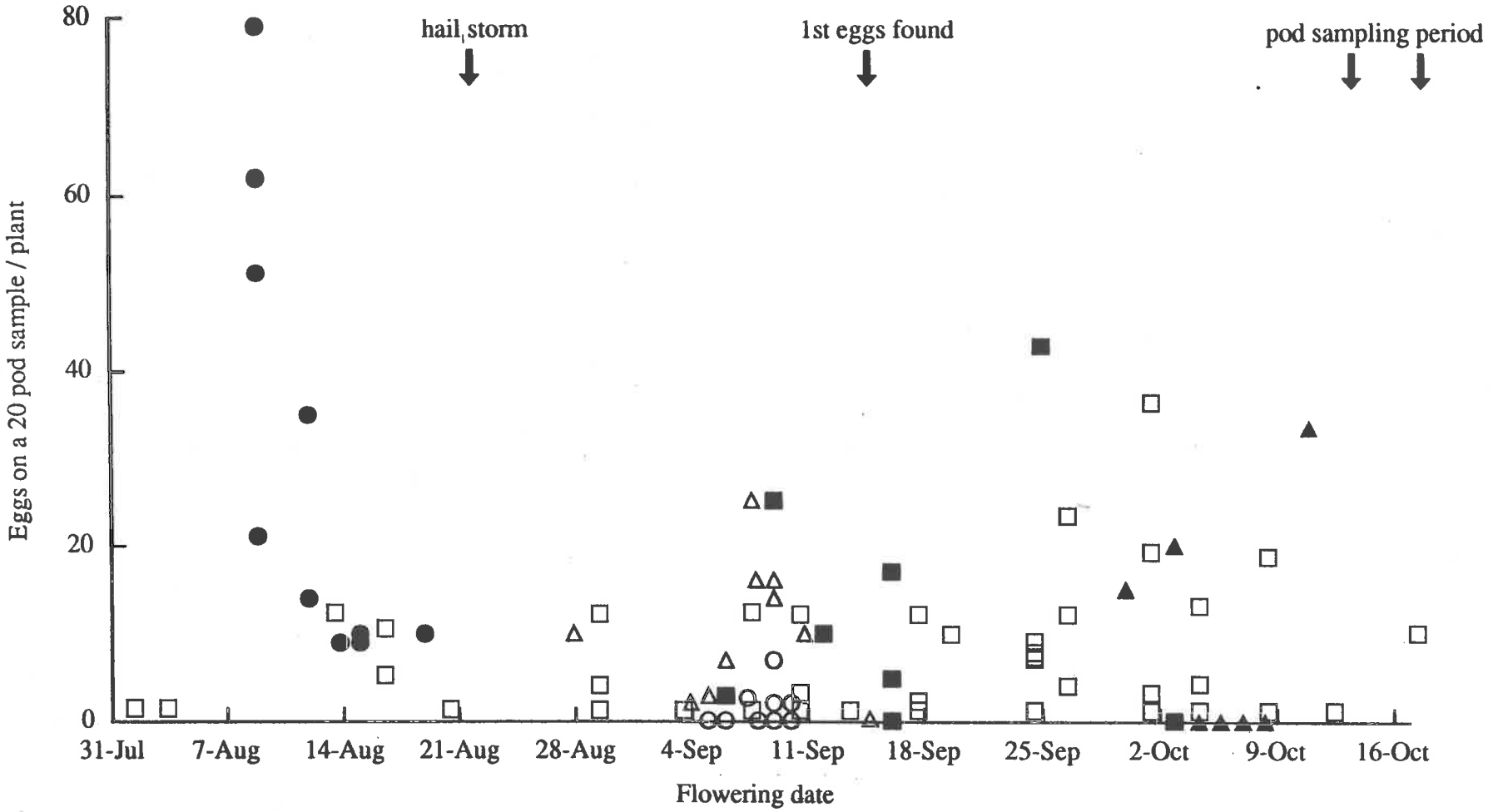


Figure 6.1. The relationship between flowering date and the number of eggs laid on a 20 pod sample per plant at three times of sowing for Susceptible parent; 1st sowing (●), 2nd sowing (■), 3rd sowing (▲), Resistant parent (O), F_1 (Δ)and F_2 plants (□) sampled from 14-18/10/91. There was a four week period between each sowing of the susceptible parent and the first pea weevil eggs were found at the trial site on the 13/9/91.

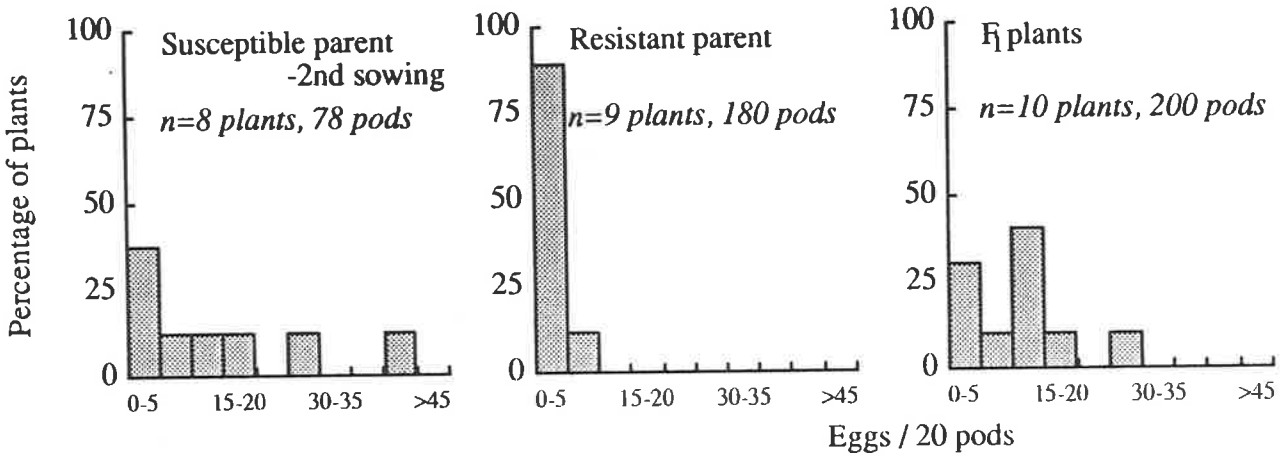


Figure 6.2a. The percentage of plants falling in various categories according to the number of eggs laid on 20 pods of each plant of the Susceptible parent (2nd sowing), the Resistant parent and the F_1 plants.

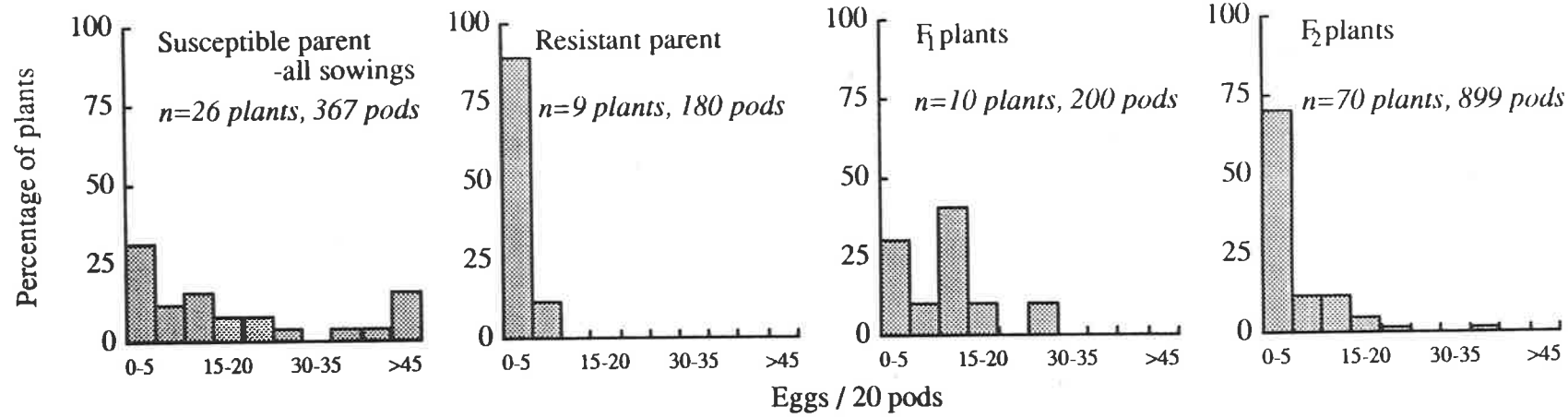


Figure 6.2b. The percentage of plants falling in various categories according to the number of eggs laid on 20 pods of each plant of the Susceptible parent (all sowings), the Resistant parent and F_1 and F_2 plants.

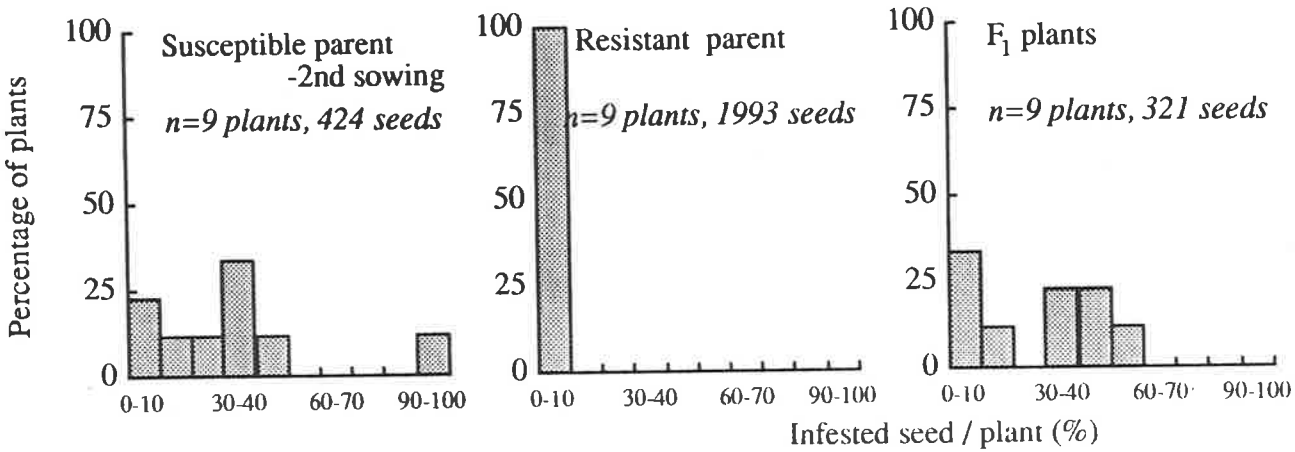


Figure 6.3a. The percentage of plants falling in various categories according to the number of infested seed from each plant of the Susceptible parent (2nd sowing), the Resistant parent and the F_1 plants.

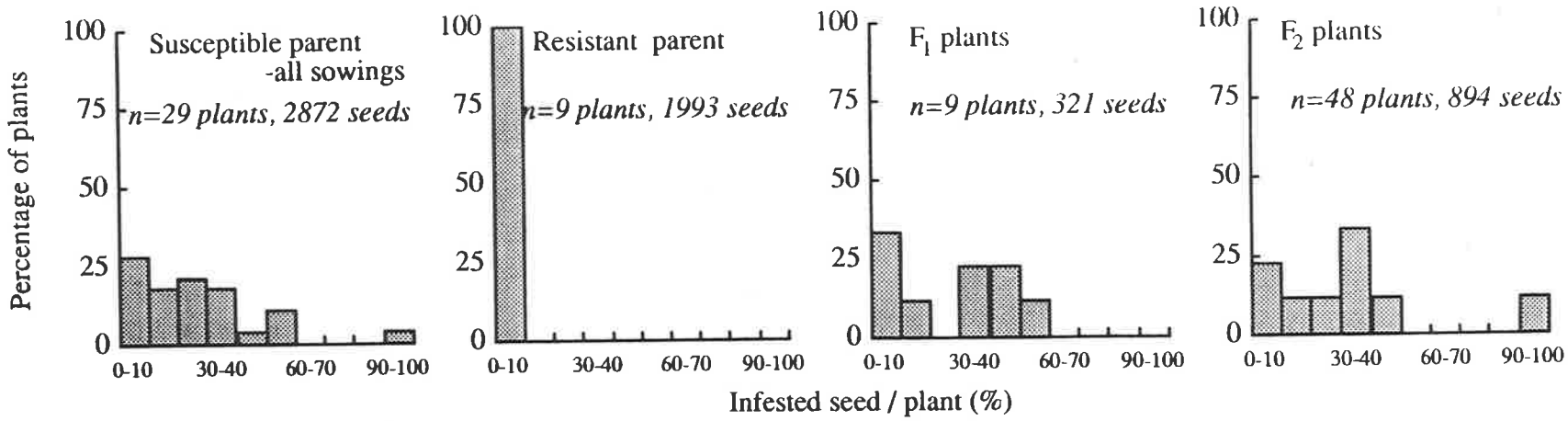


Figure 6.3b. The percentage of plants falling in various categories according to the number of infested seed from each plant of the Susceptible parent (all sowings), the Resistant parent and F_1 and F_2 plants.

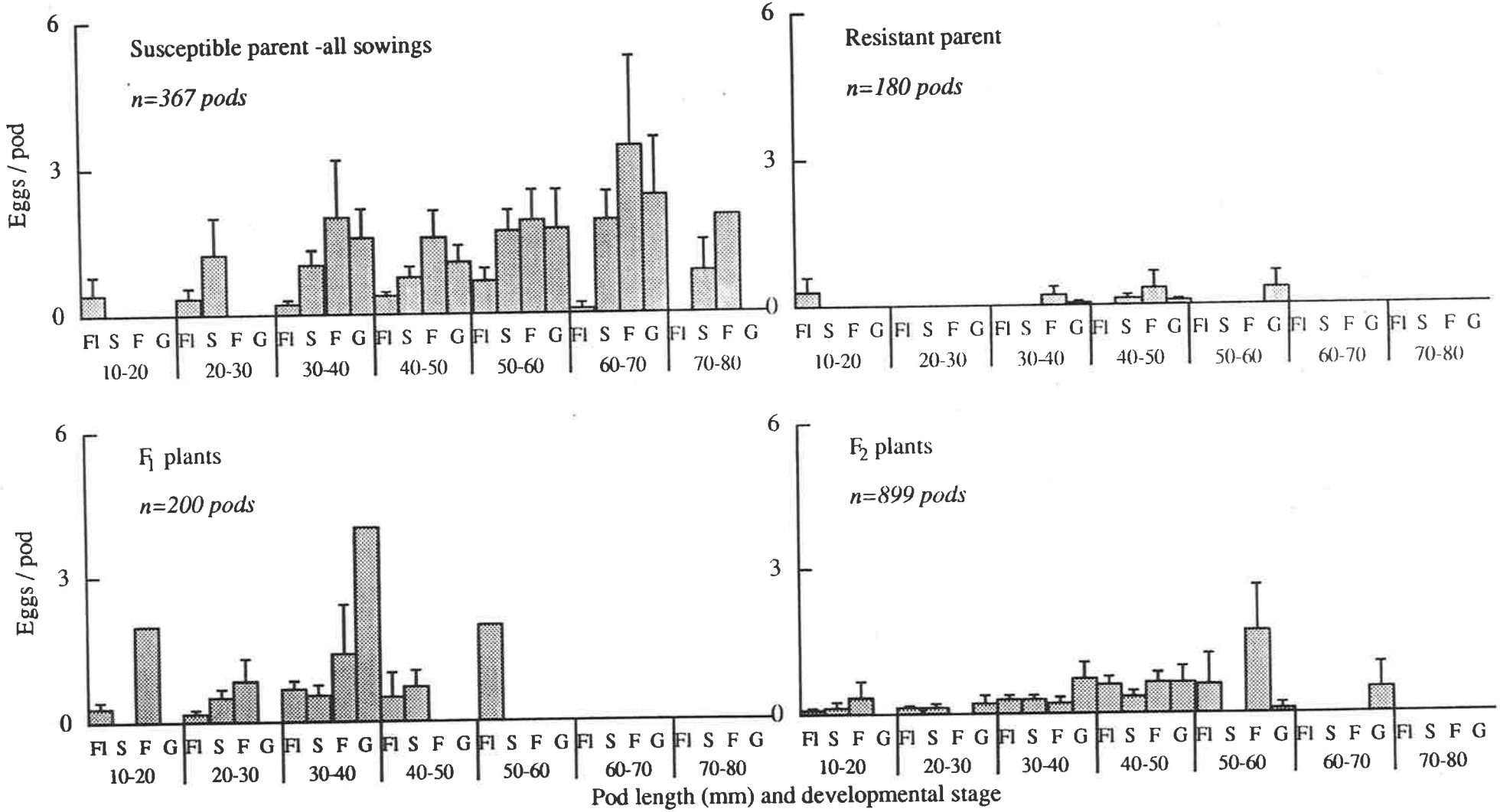


Figure 6.4. The number of eggs laid per pod of different lengths and stages of development for the susceptible parent (all sowings), the resistant parent, the F_1 and F_2 plants. The pod development categories used were Fl=flat, S=swollen, F=filled, and G=green wrinkled from Knott (1987). Yellow wrinkled pods were not included because no eggs were found on them. Absence of a standard error bar denotes a single data point.

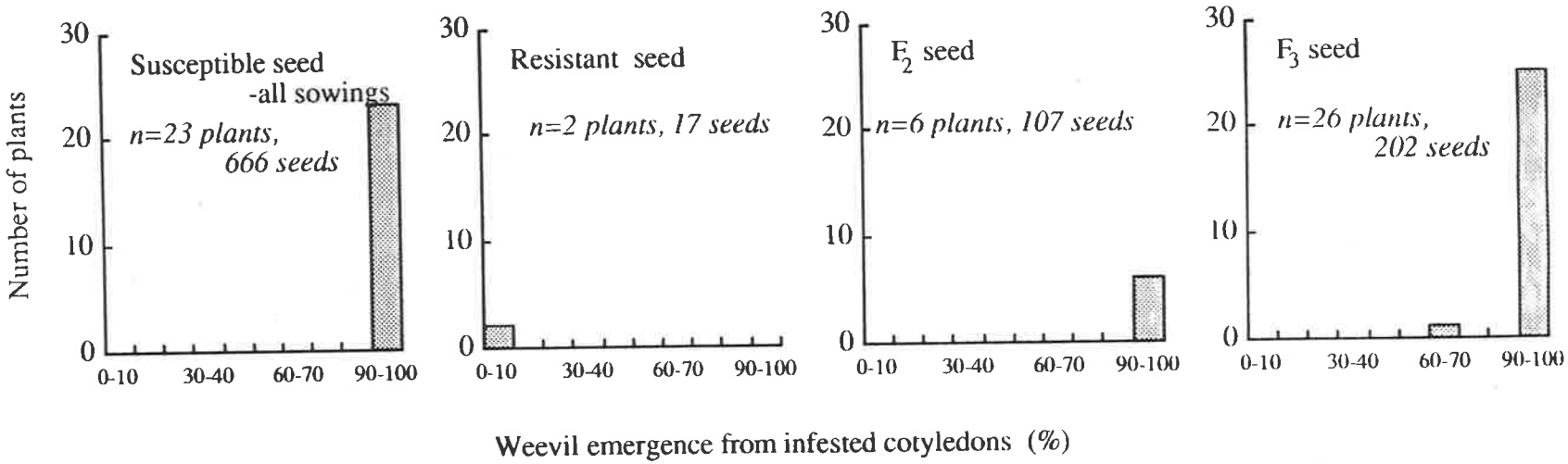


Figure 6.5. The number of plants falling in various categories according to the number of weevils to emerge from seed where the cotyledons had been entered.

6.4. Discussion

To obtain a realistic interpretation of the field trial it was essential to use flowering date and to consider the length and development of pods as associated variables. Even when these were considered, the inheritance of antixenosis was not clearly expressed in the F_1 and F_2 progeny. The level of oviposition on F_2 progeny suggests antixenosis is dominant, but this interpretation was not reinforced by the results for F_1 progeny.

The results also indicate that many susceptible pods escaped infestation. For all sowings of the susceptible parent, 39% of pods escaped. The escapes were consistent across length classes which suggests a significant number of the pods of F_2 progeny were also escapes. Escapes make the detection of resistant progeny in the field difficult. The effect of pod length and development needs to be investigated further and a simple test is required which allows the selection of resistant progeny without the influence of pod morphology.

The results suggest that one mechanism of resistance to the pea weevil is located in the cotyledons. Both the death of some larvae in the seed and the longer development period of larvae that did survive in the seed indicate that it is a form of antibiosis. The free-choice available in the field led to a low number of eggs being laid on many progeny and therefore the rate of infestation and sample size was at such a low level that meaningful interpretation of the F_2 and F_3 seed data was difficult. Although relatively few larvae did penetrate the cotyledons of the F_2 and F_3 seeds, most of them emerged as adults. This could indicate that seed antibiosis is inherited as a quantitative trait because none of the F_3 seed from the 26 F_2 plants were as resistant as the *P. fulvum* parent. This would explain the observed absence of resistance in the small number of seeds from the F_1 and F_2 plants.

The inheritance of the two characters, antixenosis and the antibiotic factor in the cotyledons, has been studied in this chapter and although there were clear differences

between the susceptible and resistant parents, it has not been possible to obtain clear pictures of segregation in the F_2 or F_3 progeny. For antixenosis, the factors confounding the results included differences in flowering time, which meant that pods of the different genotypes were not at a similar stage of development when the weevils were laying eggs. The presence of a natural infestation and the release of weevils did not overcome this problem. There was also an effect of differences in pod lengths which influenced oviposition and could confound genotypic differences between the crosses. Finally there was a possible misclassification of material when susceptible genotypes escaped infestation. For a study to reveal genetic differences among the plants, it is necessary to overcome these confounding factors, probably by working under more controlled environmental conditions.

Only one set of crosses has been presented here but the others gave similar results. It may be necessary to concentrate on just one cross and work with very large numbers of plants of the parents, F_1s and F_2s. And to further resolve the mode of inheritance, attempts should be made to provide the reciprocal cross to determine if cytoplasmic factors are involved. If resistance is inherited cytoplasmically then it would not be present in the cross progeny considered in this study, as the resistant parent provided pollen only.

Another less likely explanation for the apparent absence of resistance in the cross material is that any possible genetic ratio is being influenced by the mortality that occurred as a result of close linkage of the genes for resistance to the chloroplast incompatibility gene responsible for plant variegation. This would mean that resistance would rarely be expressed because most plants carrying the trait died as seedlings.

The inheritance of the antibiosis mechanism requires further investigation. Only 6% of the infested seed of PIG 49 produced an adult weevil in this trial. To study the mechanism fully, larger samples of material are required which will only be available if weevil preference can be overcome. The screening procedure would be enhanced if a

simple chemical test could be used, but this requires the identification of the compound(s) responsible for resistance.

CHAPTER 7

POD PREFERENCE FOR OVIPOSITION AND TIMING OF EGG HATCH IN THE FIELD BY THE PEA WEEVIL

7.1. Introduction

The difficulty of using field trials to screen for antixenosis and other mechanisms was emphasised in Chapter 6. Many plants which appear resistant in a choice situation can in fact be "escapes", whereas the occurrence of a seed antibiosis mechanism is not revealed if the weevils do not lay their eggs on some genotypes as a result of antixenosis for oviposition. The effects of preference that occur when there is a choice of genotypes were eliminated in experiments described in Chapters 4 and 5. However using whole plants in these tests limited the number of genotypes that could be screened. Therefore tests which are easy to use, repeatable and which provide evidence of mechanisms are required when screening large numbers of plants for resistance.

An understanding of weevil behaviour in the field is necessary if tests are to be useful. The results for the susceptible cv. Pennant indicated that more eggs were laid on longer pods and that few eggs were laid on pods after seeds had filled the pod (Chapter 6). Brindley (1933) and Smith (1990) observed a similar behaviour in the field. Smith (1990) also noticed that most eggs hatched when the pods were mature and contained filled seeds, a matter which could be important when designing a test for pod or seed mechanisms. These observations on weevil preference and larval hatching need to be substantiated before tests are developed. This can best be achieved by following individual pods as they develop and by examining the distributions of oviposition and adult emergence. Therefore the aim of this experiment was to determine whether pea weevils have a preference for pods of different lengths and developmental stages, and at which pod developmental stage the pea weevil eggs begin to hatch.

7.2. Materials and Methods

A field plot (17 x 3 metres) of the susceptible cv. Pennant was sown at the Waite Institute. By sowing only one cultivar, differential weevil oviposition that might occur if several cultivars were sown was avoided. The plants began flowering on 28 August 1991 and weevils became evident on 6 September. Eggs were found on pods on 16 September but were not abundant for another week and tagging of flowers commenced on 24 September. Twenty unopened flowers were tagged on five occasions at six-day intervals to cover most of the flowering period. Pods developed quickly and were scored every third day until they began drying off, for length, morphological appearance (development), the number of eggs per pod, and the date on which the first egg hatched. The categories for normal pod length and development of Pennant are shown in Plate 7.1.

There was variation in the length of time it took pods to pass through the flat stage of development with some pods taking only six, most nine, and some twelve days. The analysis of oviposition on flat pods was confined to those pods that took at least nine days to pass through this stage, thereby excluding pods which were earliest to swell but allowing flat pods of all lengths to be included.

Several assumptions were made when analysing the field data: (1) pods were at the same development stage at which they were scored for most of the period since last sampled, (2) no egg was dislodged from a pod until the eggs hatched, and (3) pods scored for larval entries were at the same development stage as when the larvae had entered them.

7.3. Results

On nearly all occasions more eggs were laid on pods in the flat stage than in the later stages when the seeds began to grow and fill the pod (Figure 7.1). The slight increase in the eggs laid on swollen pods on 18 October could indicate a limit to the number of pods available to the insects towards the end of the fruiting period in the field. Fewer eggs were laid on filled pods than in preceding stages, although on most occasions the pods

passed more quickly through this stage. Weevils laid few eggs on pods after they began to wrinkle, though the pods were at this stage for 6-8 days. Between 48 and 63% of all eggs were laid on pods in the flat stage of development and between 80 and 99% of eggs were laid on the pods before they had filled with seed. These results suggest that the greater number of eggs laid on flat pods is not just a consequence of a greater length of time available for egg laying. This stage on average was only marginally longer than the latter stages where progressively fewer eggs were laid.

The number of eggs laid could also have been affected by pod area. The number of eggs laid on pods always increased with time and pod area (Figure 7.2). However for flowers tagged on 24 September, 6 and 12 October few eggs were laid on the pods until they had a large area, that is after day six.

The mean number of days it took for the first larvae to hatch was variable and ranged from 10.8 to 15.4 days (Table 7.1). The range in days to first larval hatching, for pods tagged on the same date, also varied considerably. The 13 days on average that it took for the first egg to hatch meant that although the eggs had been laid on flat pods, the larvae penetrated the pod when they were in a later stage of development (Figure 7.3). No eggs hatched before the pods began to swell and most pods were entered by a larva before they developed to the yellow-wrinkled stage.

The developmental stage at which most pods were first entered ranged from the pod swelling stage to the green-wrinkled stage. The number of eggs laid on pods was greater on all occasions than the number of seeds in the pods (Table 7.2). Not all seeds became infested because on most occasions more than 50% of larvae failed to penetrate the pod wall. However enough larvae did manage to enter seeds for the majority of seeds to become infested. The number of eggs laid on pods increased as the season progressed and so did the number of pod wall entries and the emergence of adult weevils. The number of

seeds produced in each pod and the number of weevils produced per pod was consistent throughout the experimental period.

Table 7.1. Number of days from oviposition to hatch of the first larvae attacking pods, for flowers tagged on Sept 24, 30 and Oct 6, 12 and 18 1991. Pods were scored for hatching larvae every three days.

Date flowers tagged	Mean (days)	Range (days)
24 Sept	15.4	9 - 21
30 Sept	12.5	6 - 18
6 Oct	10.8	6 - 15
12 Oct	13.9	6 - 21
18 Oct	12.4	6 - 15

Table 7.2. Eggs per pod, seeds per pod, number of weevils emerging from each pod and the percentage emergence of weevils from seed, for flowers tagged on Sept 24, 30 and Oct 6, 12 and 18 1991. Pods were scored for eggs laid every three days.

Date flowers tagged	Eggs laid/pod	Pod wall entries	Seeds/pod	Weevils produced/pod	Weevils/seed
24 Sept	8.65	4.65	4.24	3.13	0.73
30 Sept	8.11	5.83	4.39	3.50	0.78
6 Oct	8.50	4.75	4.38	3.09	0.68
12 Oct	11.69	5.44	4.69	3.44	0.71
18 Oct	14.90	8.30	3.70	3.40	0.90
		Weevil emergence from all harvested pods			0.76

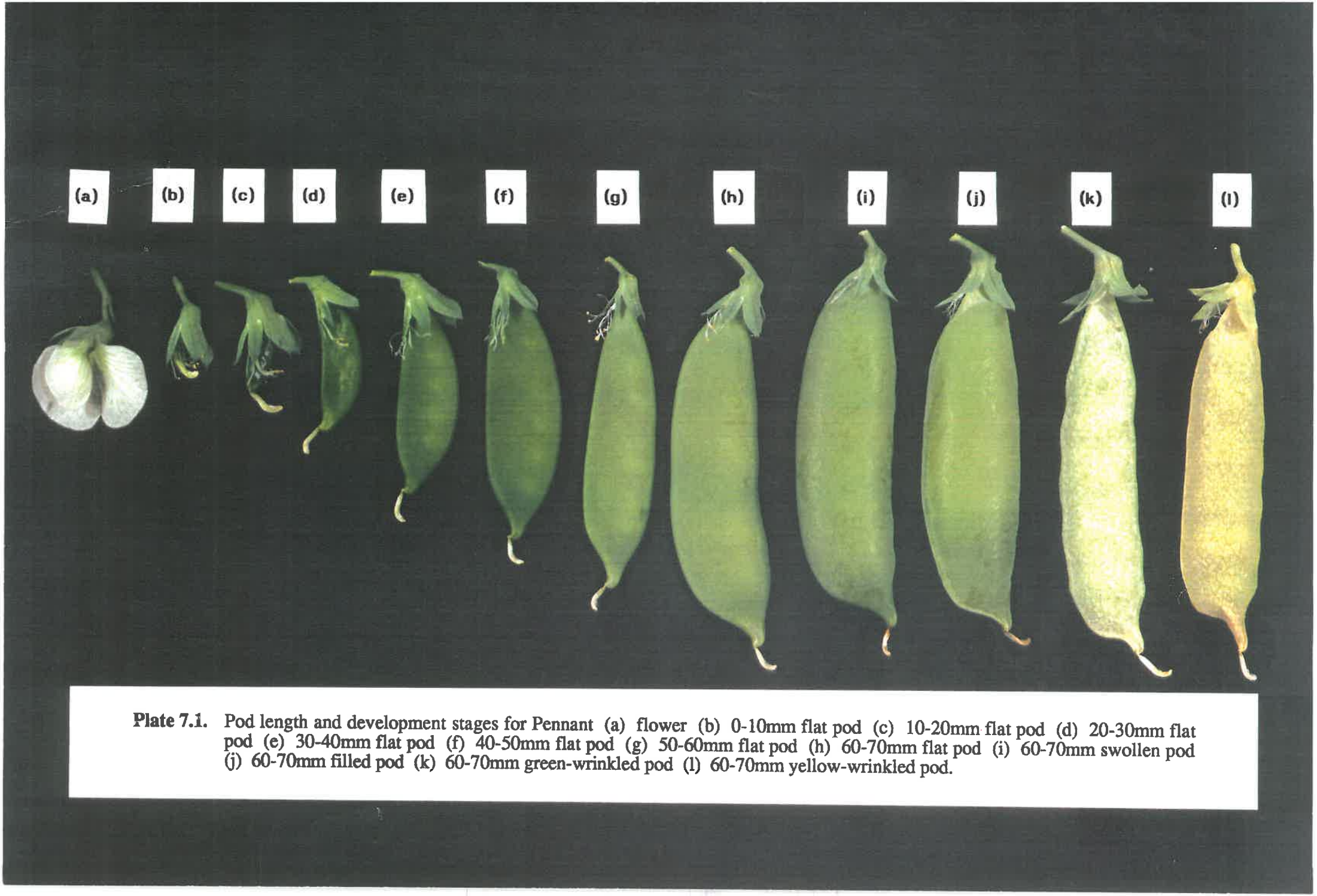


Plate 7.1. Pod length and development stages for Pennant (a) flower (b) 0-10mm flat pod (c) 10-20mm flat pod (d) 20-30mm flat pod (e) 30-40mm flat pod (f) 40-50mm flat pod (g) 50-60mm flat pod (h) 60-70mm flat pod (i) 60-70mm swollen pod (j) 60-70mm filled pod (k) 60-70mm green-wrinkled pod (l) 60-70mm yellow-wrinkled pod.

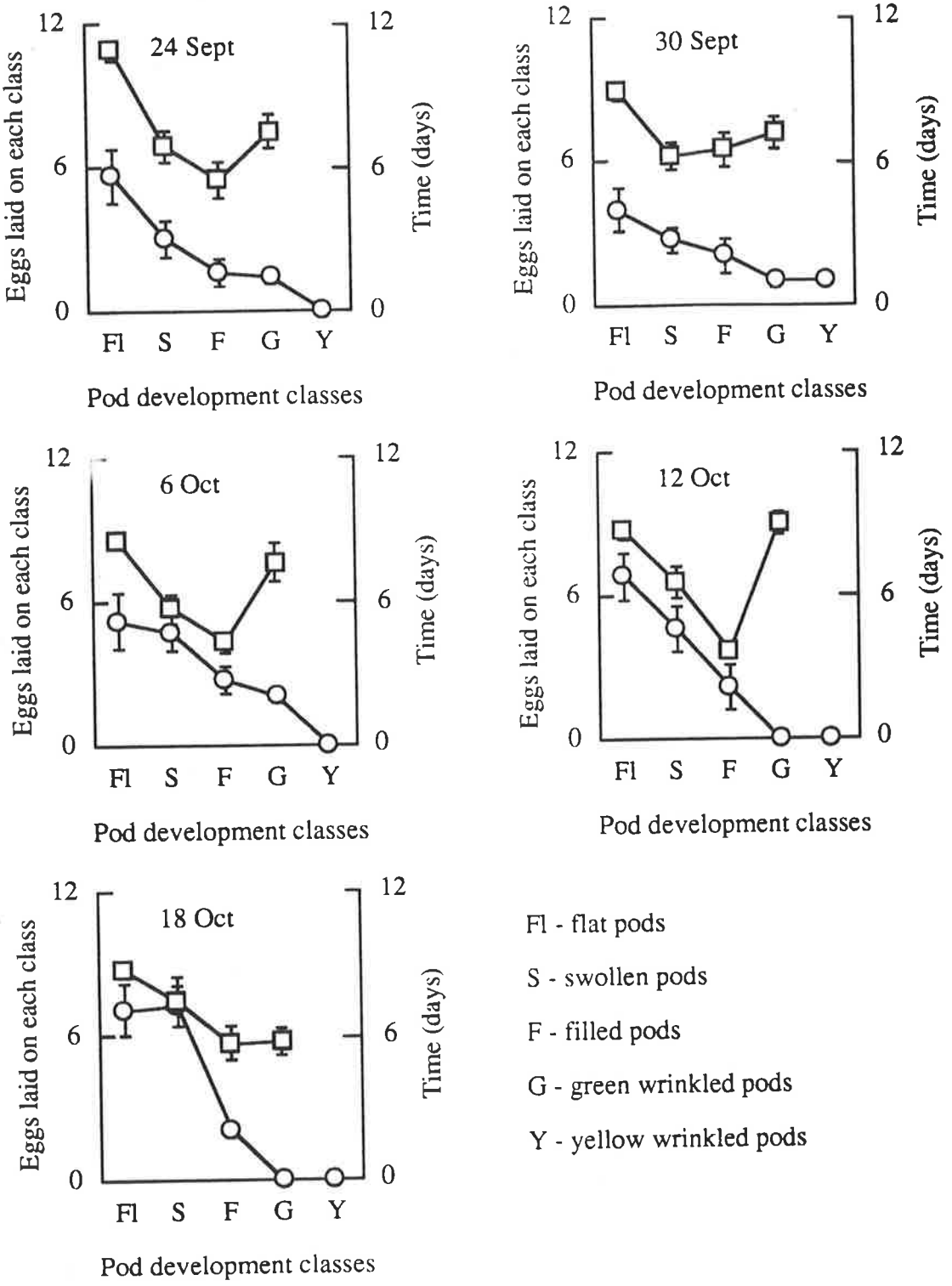


Figure 7.1. The number of pea weevil eggs laid (O) and days pods were at different stages of development (□), for flowers tagged on Sept 24, 30 and Oct 6, 12 and 18 1991. The number of days pods were at the yellow-wrinkled stage (Y) were not recorded.

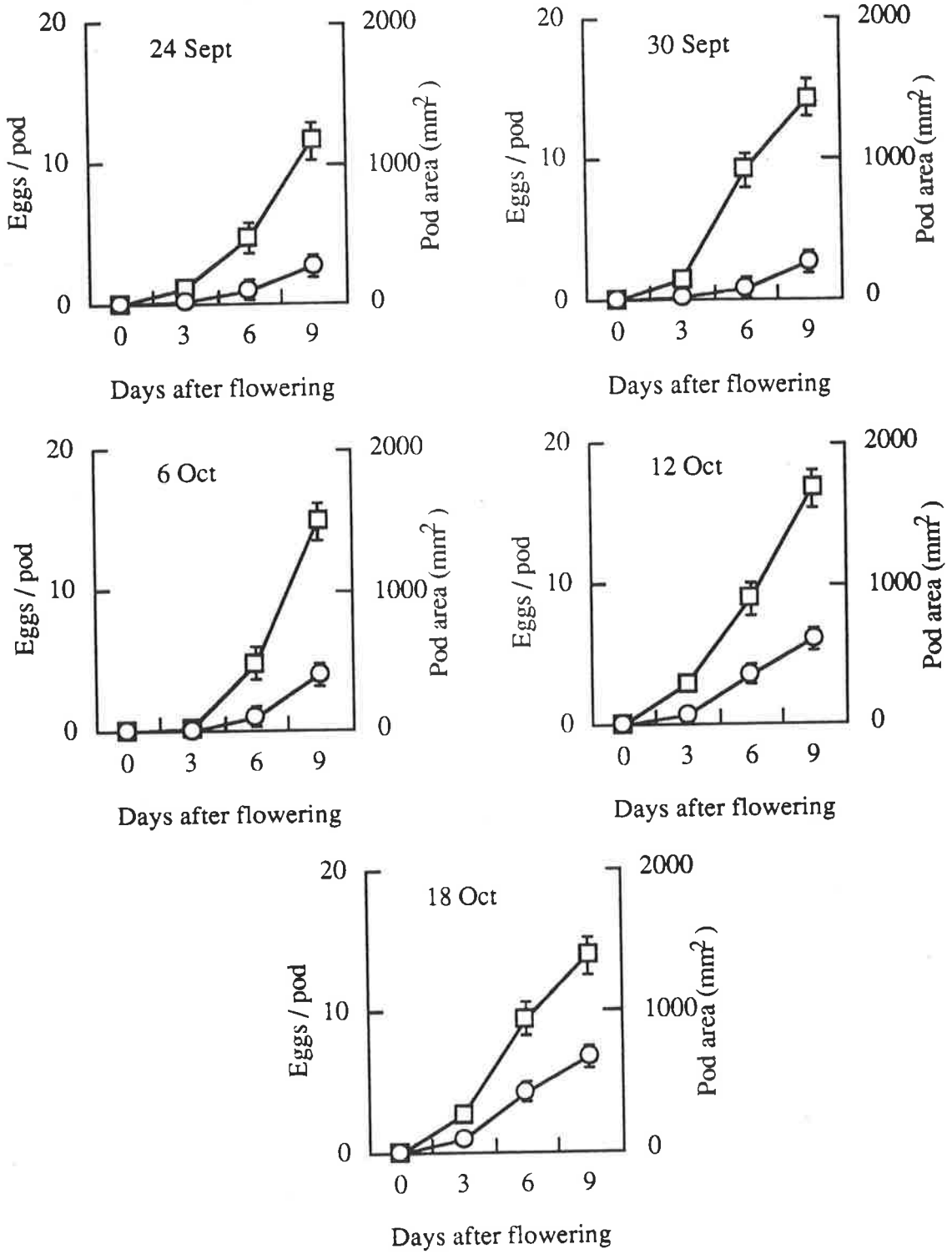


Figure 7.2. The cumulative number of pea weevil eggs laid (O) on flat pods and the area of these pods (□), for flowers tagged on Sept 24, 30 and Oct 6, 12 and 18 1991. Only eggs laid on pods which took at least nine days to progress through the flat (Fl) stage of development were included.

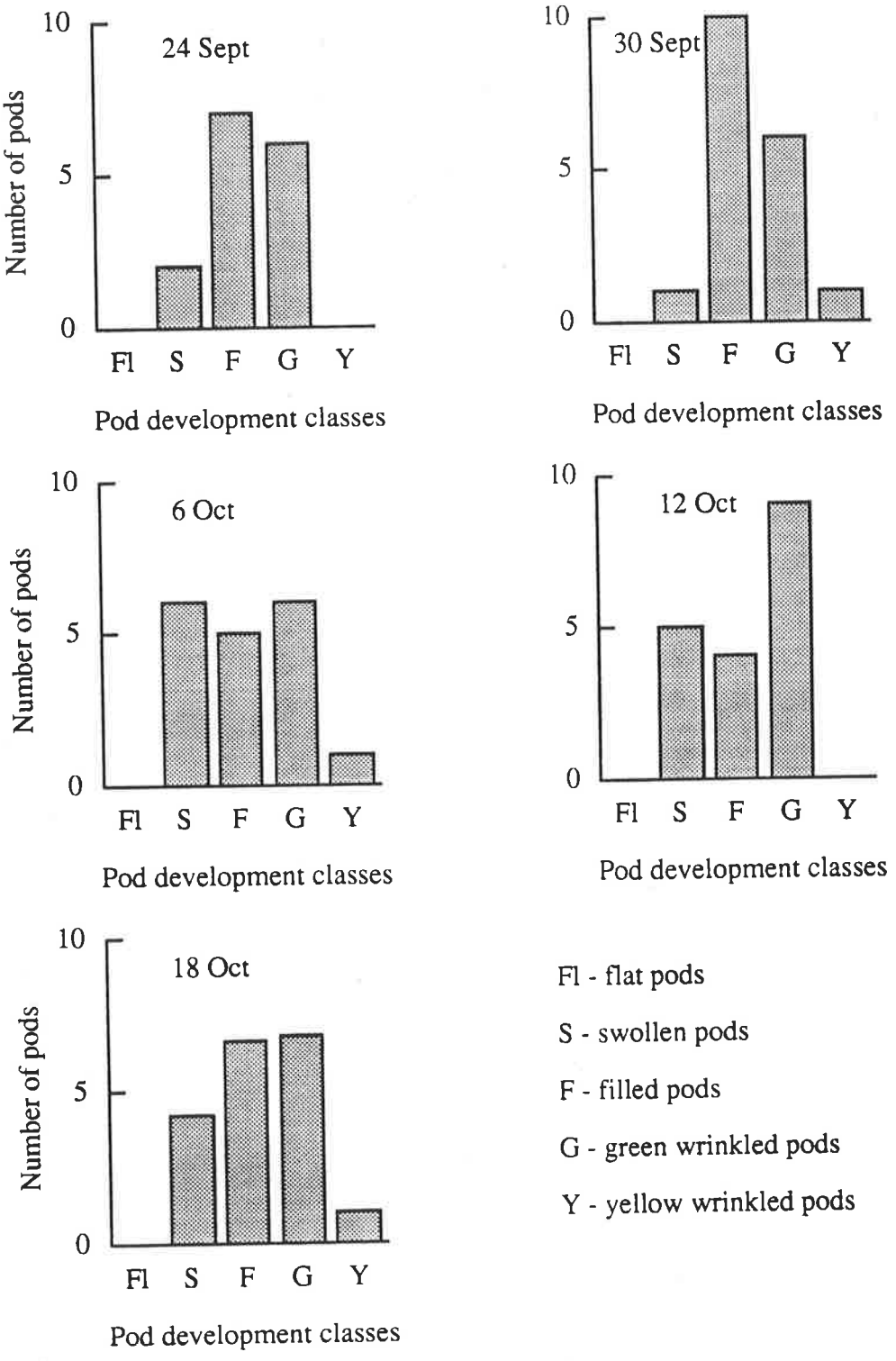


Figure 7.3. The stage of pod development at which the first pea weevil egg hatched from flowers tagged on Sept 24, 30 and Oct 6, 12 and 18 1991.

7.4. Discussion

The field observations showed that the pea weevil has a strong oviposition preference for pods that are flat or swelling over more mature pods. Although the number of eggs laid was affected by time, in this case the length of time spent at the flat and swelling stages and at the more mature stages was similar, fewer eggs were laid on the latter. At the flat stage of pod development the number of eggs laid was also governed by the length of the pod. These points should be considered when testing material for oviposition antixenosis.

By laying its eggs on long, flat or swelling pods the pea weevil may favour the hatching of eggs at a time when seeds are present, but the pod wall and seeds are easy to penetrate. When eggs hatch too early, before the pod begins to swell, the larvae do not encounter developing seeds. After the green-wrinkled stage newly hatched larvae could find it difficult to penetrate the pod wall and seed, which by then are drying off. The range in length of time taken for the first larvae to hatch emphasises this point. Any investigation into sources of resistance and selections for resistant progeny in the *P. fulvum* and *P. sativum* crosses should take into account pod length and the developmental stage of the pod as both affect the number of eggs that are laid.

Aspects of the results were similar to those of Brindley (1933) and Smith (1990). However neither of these workers showed how pod length (area) and the time of various developmental stages influenced the number of eggs laid. They both indicated that weevils lay very few eggs on filled pods. But pods pass through this developmental stage quickly and it is not until they reach the green-wrinkled stage of development that they lose their attractiveness to the weevil. Smith (1990) indicated that most eggs hatch once the pods have filled with seed although variability in the results was not given. It appears that eggs begin to hatch at all stages of development except on flat pods. Neither author mentioned that many of the eggs did not produce larvae that penetrated pods.

The results over time for the field observations were variable, but the daily changes in the important biotic and abiotic factors like temperature, wind speed, pod production and weevil numbers at the field site did not cause much variation from sample to sample. There was also considerable competition for oviposition sites, which was reflected in the high number of weevils that emerged from harvested seed (76%) and the number of eggs laid on pods on each occasion. This should have put pressure on weevils to lay eggs on less favourable pods, increasing the variability of the results.

CHAPTER 8

A BIO-ASSAY USING EXCISED PODS TO DETERMINE OVIPOSITION ANTIXENOSIS BY THE PEA WEEVIL TO *PISUM* GERMPLASM

8.1. Introduction

The results of the trial described in Chapter 6 indicated that field screening could not adequately identify antixenotic lines because of the high number of escapes. On 50% of the susceptible control plants, the same number of eggs, or fewer, were laid than on the resistant parent, despite the fact that weevils were released several times. A test was needed that would allow large numbers of plants to be screened for oviposition antixenosis. Annis and O'Keeffe (1984a) found significant differences in the number of eggs laid by pea weevils on pods of *P. sativum* and *L. sativus* placed in plastic vials. They concluded that the reduced oviposition on the pods of *Lathyrus* species was due to the presence of deterrents on the pods. If their conclusions are correct then an assay using pea pods would be useful.

Procedures using excised plant parts have been developed to evaluate segregating populations of plants for resistance to insects (Sams *et al.* 1975). The use of excised pods would allow the comparison of single pods from different accessions in choice and no-choice situations. The pea weevil has already been shown to be capable of discriminating between pods of different lengths and development stages (Chapters 6 & 7), these effects should be taken into account in any tests. The aim of this experiment was to confirm the pod preferences shown by the pea weevil in Chapters 6 and 7 and to develop a bio-assay for the antixenosis mechanism.

8.2. Materials and methods

The cultivar Pennant was used as the susceptible control and the *P. fulvum* accessions PIG 49, PIG 111, PIG 112, ATC 114, NGB 1256 and the *P. sativum* ssp. *humile* accession NGB 936 were chosen as test lines. The plants were grown using the procedures described in Chapter 4.

Clear plastic cages were used as cages in the experiment. Weevils were removed from storage and sexed, and five pairs were placed in each cage provisioned with pollen and water available to them (Plate 8.1). Weevils in trial cages were left in the growth room for a week to allow the weevils to mate before pods were placed in the cages. Testing was carried out in a growth room maintained at 25±1.0°C with a photo-period of 12L:12D. Four replicates of a choice test and two replicates of a no-choice test were set up at a time.

A preliminary investigation was made to compare Pennant pods of different lengths for confirmation of the results obtained in Chapters 5, 6 and 7, and to determine if it was necessary to account for pod length in the assay. Flat pods of 30-40mm in length were compared to flat pods of 10-20mm and 60-70mm long. The results to be presented confirm that it is essential to use pods of similar lengths in a choice or no-choice assay to allow a valid assessment of genotypes.

Following the preliminary test, pods chosen for use were flat or swelling if available, and between 30-40mm in length. This length of pod is not optimal for oviposition (Chapter 7), but it is a length that most *P. fulvum* pods can be expected to attain. The length and stage of development of each pod was recorded before it was placed in the cages. The pods were attached to corks with mapping pins and inserted through holes in the lids of the cages. Control pods were paired with test pods of the same length or slightly longer to ensure that oviposition was not biased towards the control. The matching of pods provided results for antixenosis that were independent of pod length.

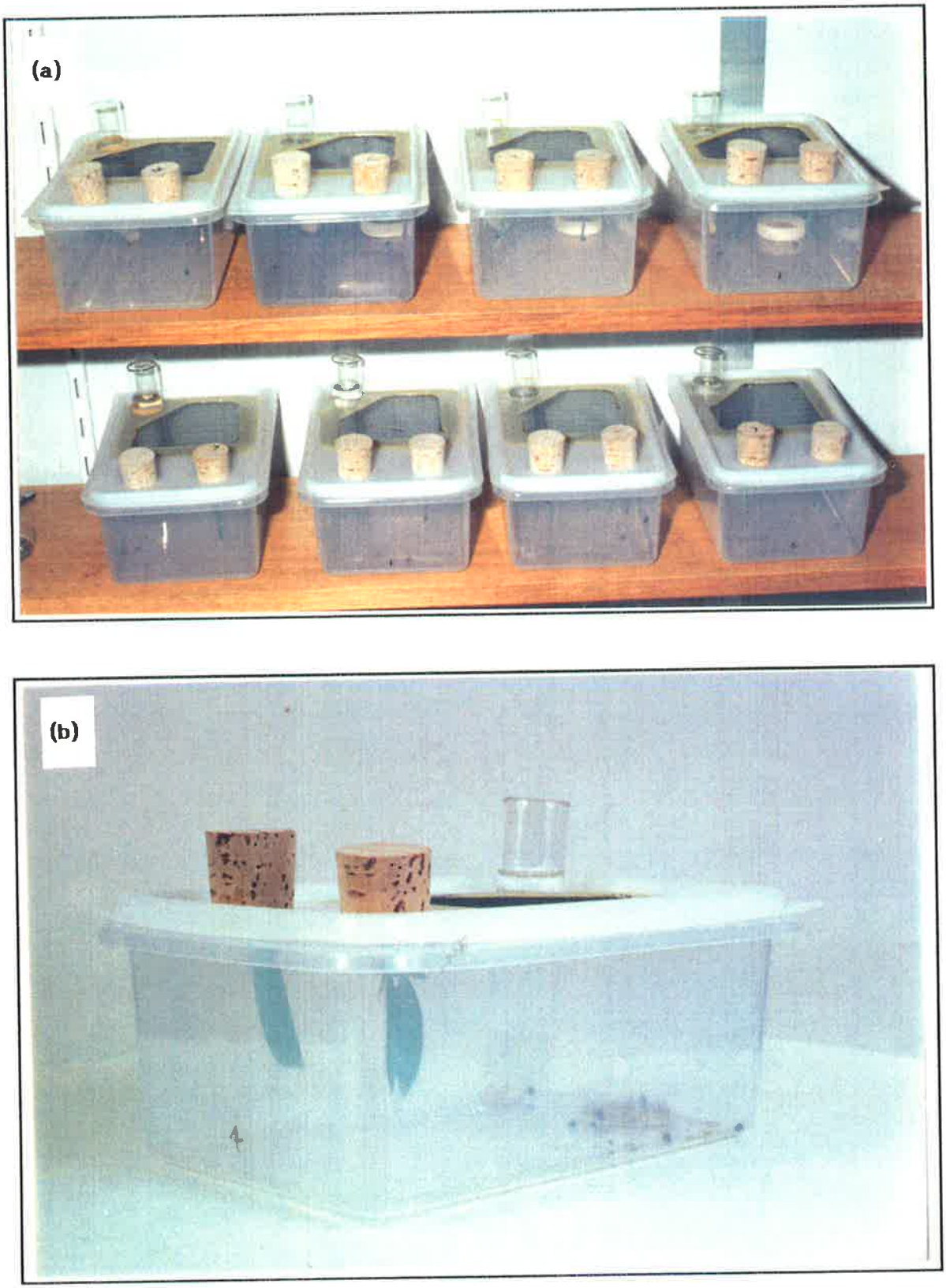


Plate 8.1. Plastic trial cages used in the antixenosis assay showing (a) a trial set up and (b) a close-up of a trial cage with pods.

The surface area of a control pod was similar to that of a test accession of the same length (Table 8.1). The surface area of NGB 936 pods was not estimated, but was assumed to follow the trends already mentioned.

In all experiments pods were tested for one day only. A test consisted of one control pod and one test pod per cage in the choice experiments, and two control or two test pods per cage in the no-choice assays. Position effects can occur in experiments when the number of choices is limited (Stanton 1979) and were alleviated by alternating, within the choice cages, the position of control and test pods daily. The number of eggs laid on each pod was recorded. After ten days the weevils were discarded and the cages were washed and stocked with fresh weevils.

An analysis of variance was conducted for the number of eggs laid and pod length for the control and test accessions. Results for each day and for the entire trial period were analysed.

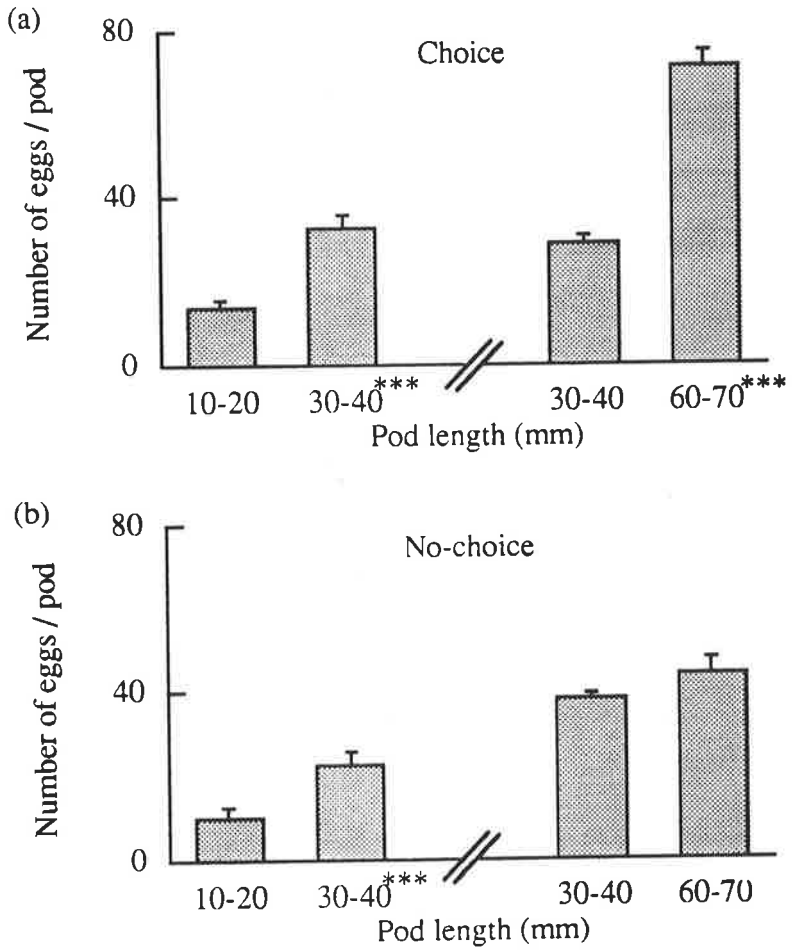
Table 8.1. Estimated surface areas of pods for the *P. sativum* cv. Pennant and the *P. fulvum* accessions PIG 49, PIG 111, PIG 112, ATC, 114 and NGB 1256.

Genotype	Pod length (mm)	Estimated surface area of pod (mm^2)*	r^2 of fitted line*
Control	34	469	0.98
Control	35	496	0.98
PIG 49	35	536	0.94
PIG 111	35	489	0.98
PIG 112	35	538	0.97
ATC 114	35	493	0.99
NGB 1256	35	478	0.97

*Estimates for pod surface area and the r^2 values were calculated using quadratic equations from the regression analysis in Chapter 5.

8.3. Results

A size-related oviposition response was demonstrated by the pea weevil on different length Pennant pods in the preliminary experiment. When pods 30-40mm were tested against flat pods 10-20mm or 60-70mm long in a choice situation, significantly more eggs were laid on the longer pods (Figure 8.1a). This result was similar to those described in Chapters 6 and 7. More eggs were also laid on the longer pods in the no-choice test. The 30-40mm pods still received a significantly higher number of eggs than on 10-20mm pods (Figure 8.1b). However oviposition was the same on the 30-40mm and the 60-70mm pods. The comparison of different size pods indicated how sensitive this assay was to the length of pods.



***$p<0.001$.

The break in the x axis indicates the results are from two separate tests.

Figure 8.1. The number of eggs per pod laid on different length flat pods of the cv. Pennant. (a) Choice. (b) No-choice.

There were also differences between the cv. Pennant and test accessions in day to day variation in the number of eggs laid on pods for the choice and no-choice tests. Each test lasted one day and was repeated daily for ten days. With the exception of ATC 114 the results obtained were similar for the five *P. fulvum* accessions. To simplify the presentation, only the results for PIG 49 are illustrated and compared to the Pennant control (Figure 8.2a &b). All results for other accessions are presented as means for the ten day test period. The lengths of the Pennant and PIG 49 pods were similar on most days, however, on a few occasions the difference in length between accessions was significant (Figures 8.2c & d). Overall, pod length was unlikely to have influenced the number of eggs laid on the control and test material because on days when pods were of a similar length the number of eggs laid on each still differed significantly. Again results for other accessions will be presented as the mean value from ten days of testing.

8.3.1. Choice assays

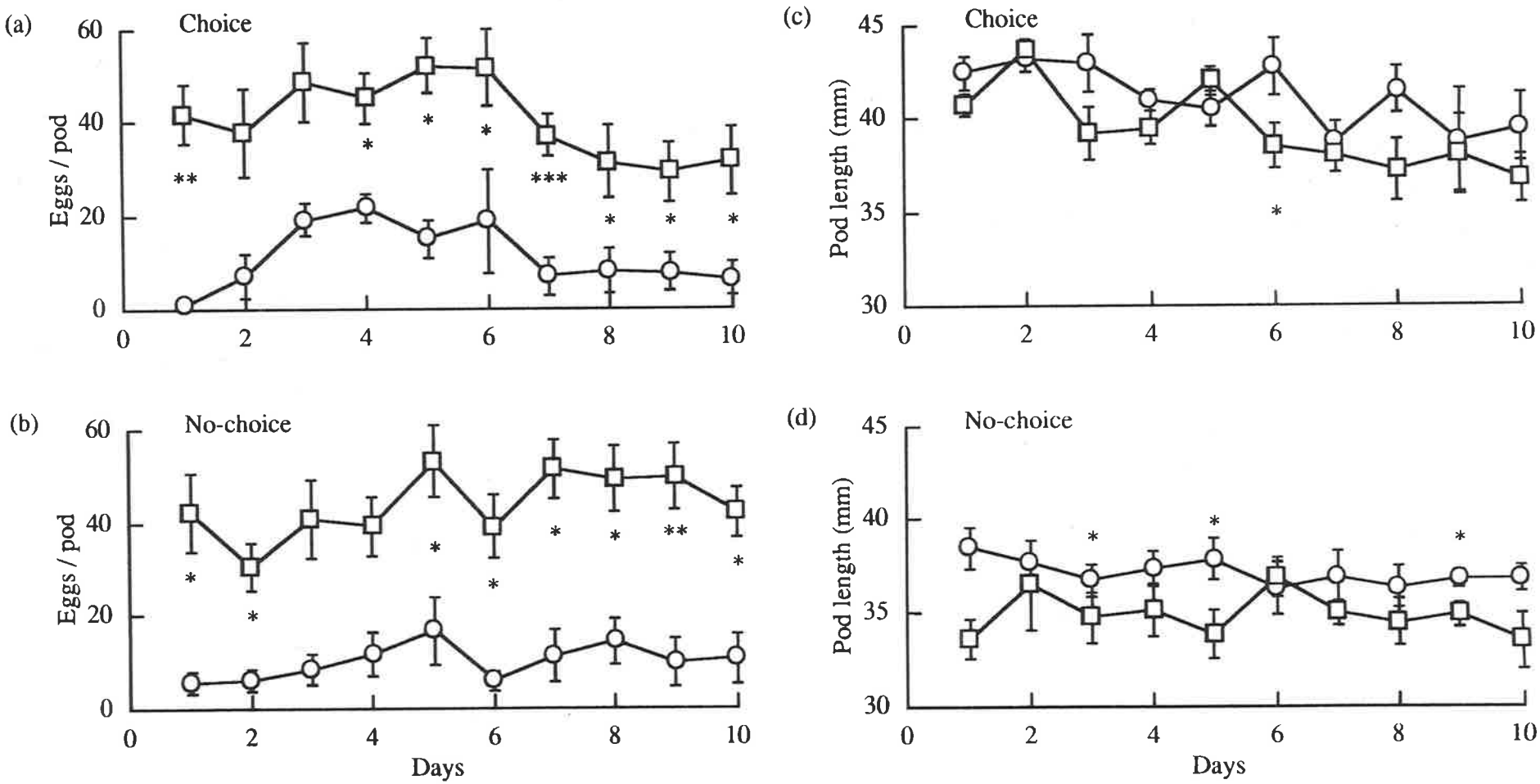
Choice assays of the Pennant control versus the five *P. fulvum* accessions demonstrated the weevils preference for pods of the cv. Pennant (Figures 8.3a, 8.4a, 8.5a, 8.6a & 8.7a). The number of eggs laid on pods of cv. Pennant differed significantly from the number laid on *P. fulvum* in every comparison. However there was no significant difference between the number of eggs laid on pods of Pennant, and on the pods of NGB 936, the *P. sativum* ssp. *humile* accession (Figure 8.8a). Pod development stage was consistent between tests, with nearly all control pods being flat and the majority of test pods being flat or swollen, though pod shortages made it necessary to use some filled and green-wrinkled pods (Figures 8.3c, 8.4c, 8.5c, 8.6c 8.7c & 8.8b).

8.3.2. No-choice assays

When the weevils had no choice and were confined to single genotypes they still laid fewer eggs on the *P. fulvum* accessions than the Pennant pods (Figures 8.3b, 8.4b, 8.5b, 8.6b & 8.7b). The difference was significant for all of the *P. fulvum* accessions except for ATC 114. NGB 936 was not subjected to a no-choice test because the choice test gave a

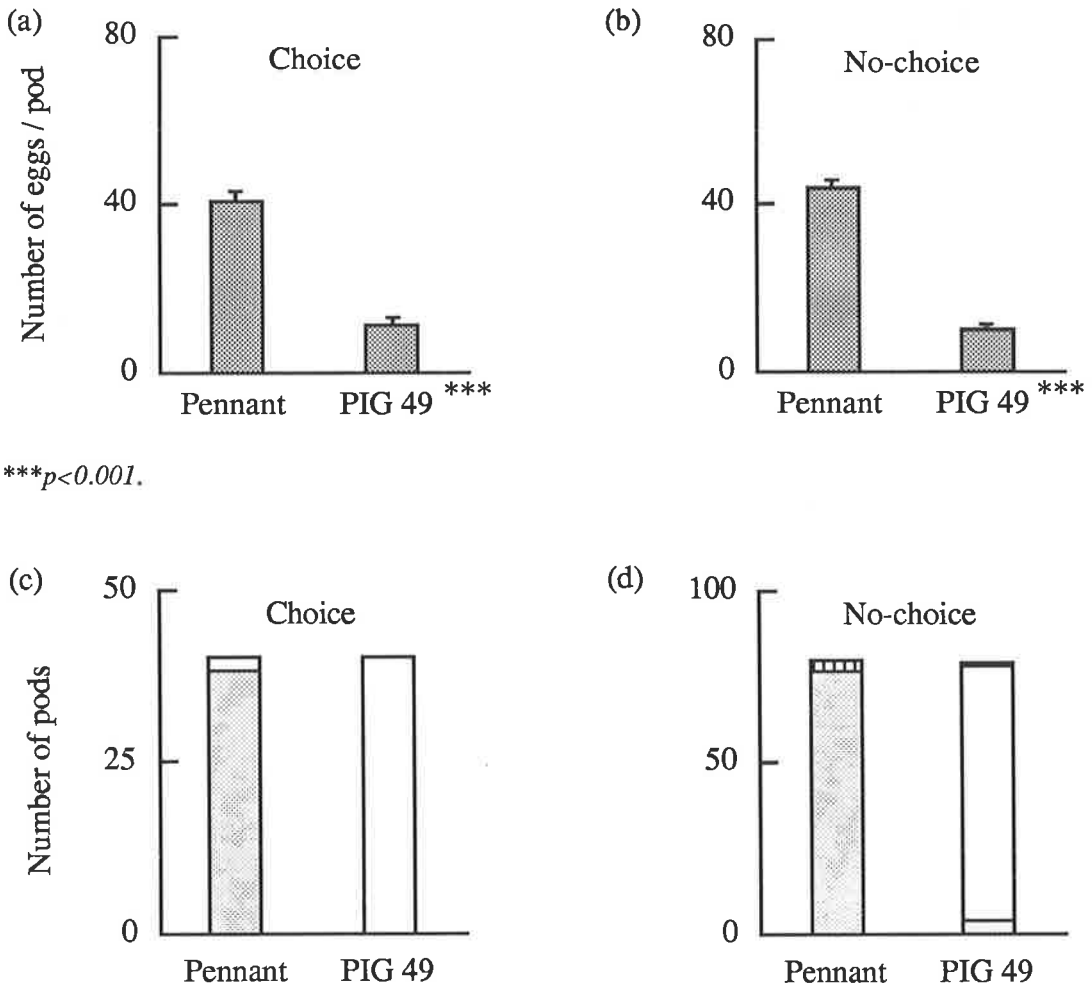
non-significant result. The range in the developmental stage of pods was also similar to that used in the choice tests (Figures 8.3d, 8.4d, 8.5d, 8.6d & 8.7d).

Choice tests were carried out to compare the number of eggs laid on pods of PIG 49 to PIG 111, and PIG 49 to NGB 1256 so that the levels of antixenosis in *P. fulvum* accessions could be assessed (Figure 8.9). Because of the shortage of pods not all the possible comparisons of the five *P. fulvum* accessions could be made. The two comparisons that were made demonstrated that the weevils had no preference for one accession over another (Figures 8.9a & b). This was evident for both daily and overall comparisons. These results were obtained from pods which varied slightly in length, although significantly, between accessions. Daily differences in pod lengths were significant on two occasions for the PIG 49/PIG 111 comparison and on six occasions for the PIG 49/NGB 1256 comparison. The majority of pods used in the tests were either flat or swollen, though some filled pods of PIG 111 were used in the PIG 49/PIG 111 comparison (Figures 8.9c & d).



*p = 0.05-0.01; **p = 0.01-0.001; ***p<0.001.

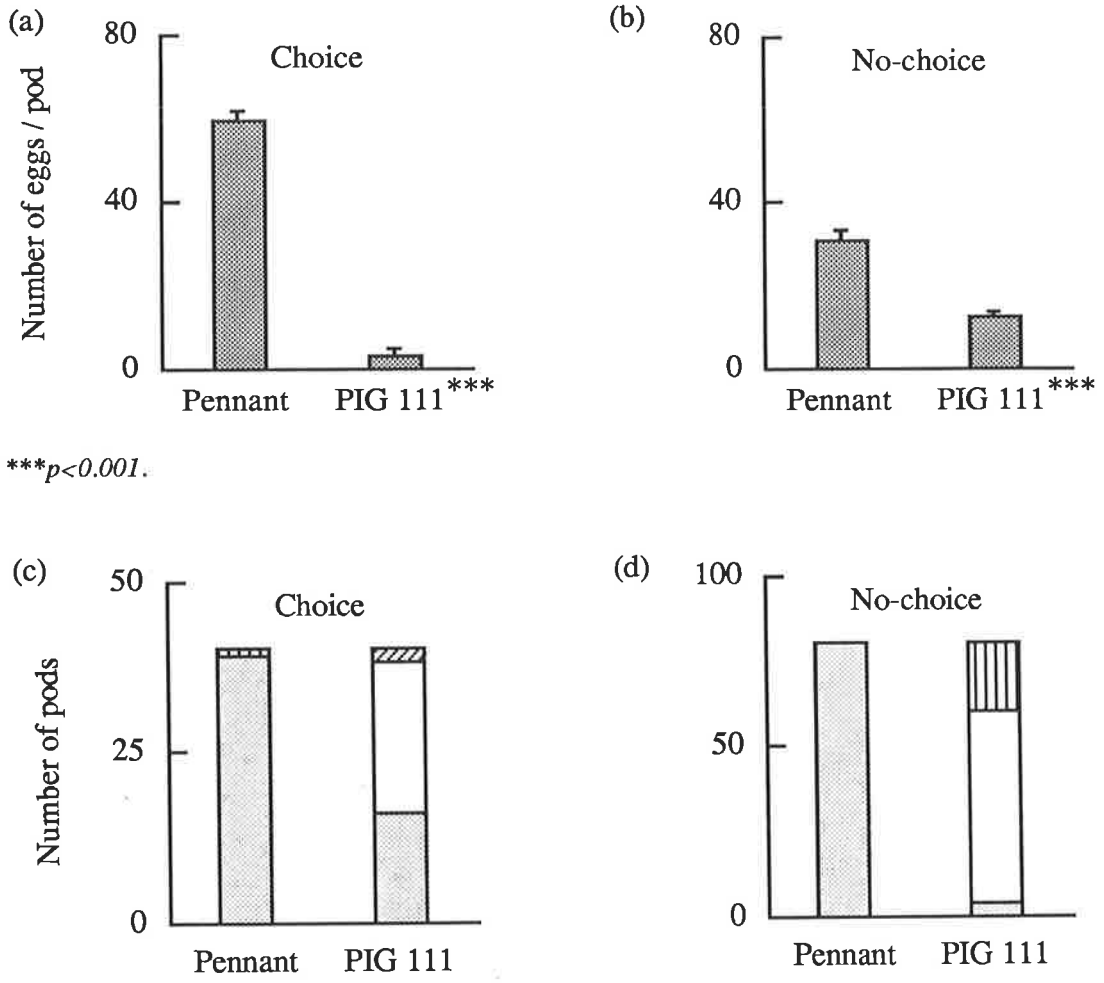
Figure 8.2. Eggs laid on similar length pods of the control cv. Pennant (□) and the *P. fulvum* accession PIG 49 (○). (a) Choice. (b) No-choice. (c) Length of pods in choice test. (d) Length of pods in no-choice test.



****p<0.001.*

Figure 8.3. The number of eggs per pod laid on the control cv. Pennant and the *P. fulvum* accession PIG 49. (a) Choice. (b) No choice. Development stages of pods in (c) choice test. (d) no-choice test. Development stages of pods in (c) and (d) are represented by the following legend:

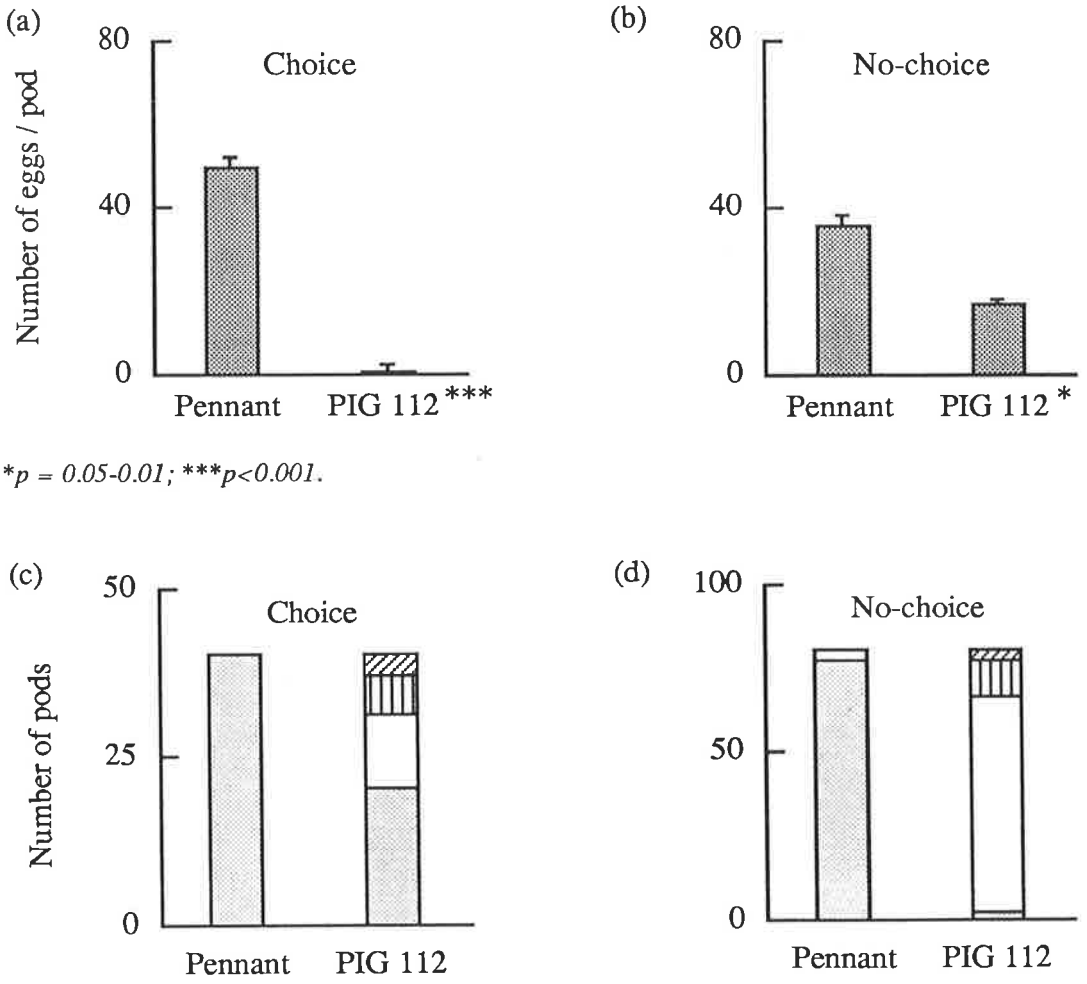
Flat pods Swollen pods Filled pods Green wrinkled pods



***$p<0.001$.

Figure 8.4. The number of eggs per pod laid on the control cv. Pennant and the *P. fulvum* accession PIG 111. (a) Choice. (b) No choice. Development stages of pods in (c) choice test. (d) no-choice test. Development stages of pods in (c) and (d) are represented by the following legend:

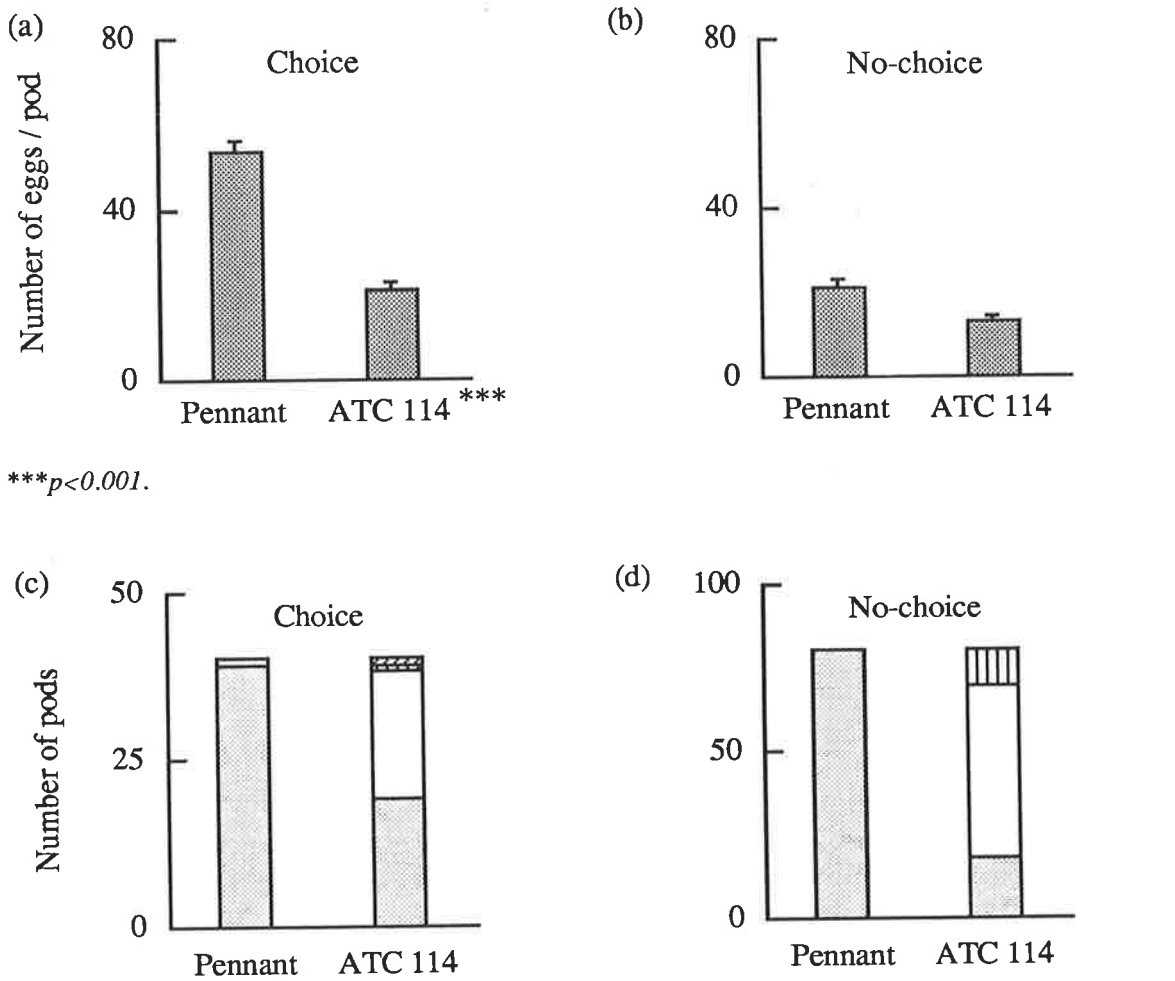
Flat pods Swollen pods Filled pods Green wrinkled pods



*p = 0.05-0.01; ***p<0.001.

Figure 8.5. The number of eggs per pod laid on the control cv. Pennant and the *P. fulvum* accession PIG 112. (a) Choice. (b) No choice. Development stages of pods in (c) choice test. (d) no-choice test. Development stages of pods in (c) and (d) are represented by the following legend:

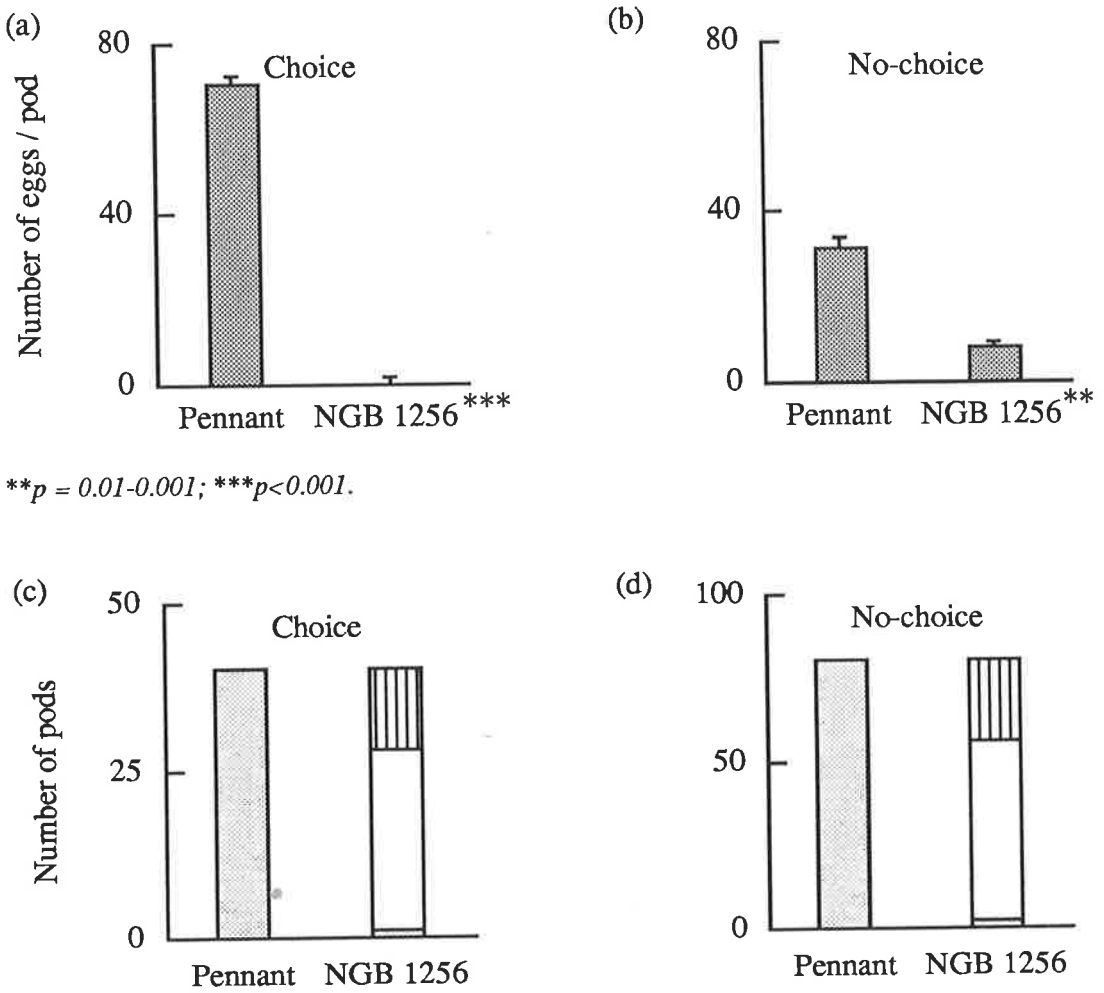
Flat pods Swollen pods Filled pods Green wrinkled pods



****p<0.001*.

Figure 8.6. The number of eggs per pod laid on the control cv. Pennant and the *P. fulvum* accession ATC 114. (a) Choice. (b) No choice. Development stages of pods in (c) choice test. (d) no-choice test. Development stages of pods in (c) and (d) are represented by the following legend:

Flat pods Swollen pods Filled pods Green wrinkled pods



***p = 0.01-0.001; ***p<0.001.*

Figure 8.7. The number of eggs per pod laid on the control cv. Pennant and the *P. fulvum* accession NGB 1256. (a) Choice. (b) No choice. Development stages of pods in (c) choice test. (d) no-choice test. Development stages of pods in (c) and (d) are represented by the following legend:

Flat pods Swollen pods Filled pods Green wrinkled pods

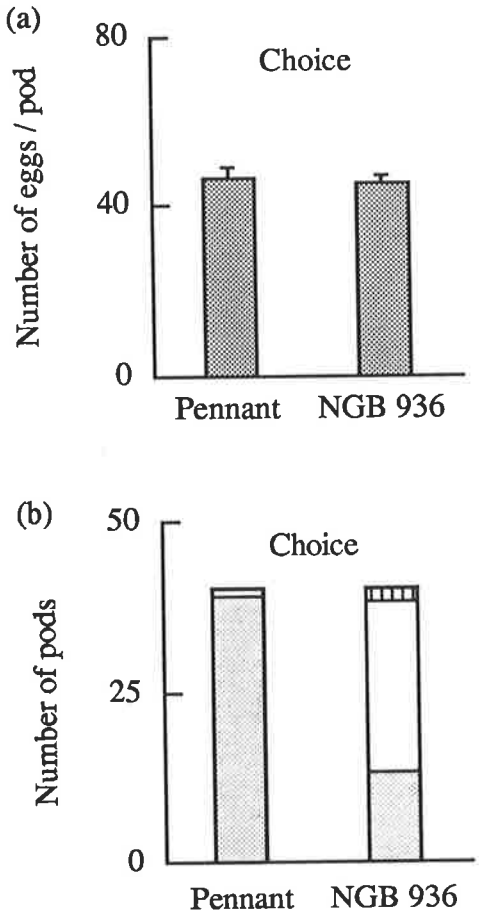


Figure 8.8. The number of eggs per pod laid on the control cv. Pennant or the *P. sativum* ssp. *humile* accession NGB 936. (a) Choice . (b) Development stages of pods in choice test. Development stages of pods in (b) are represented by the following legend:

Flat pods Swollen pods Filled pods Green wrinkled pods

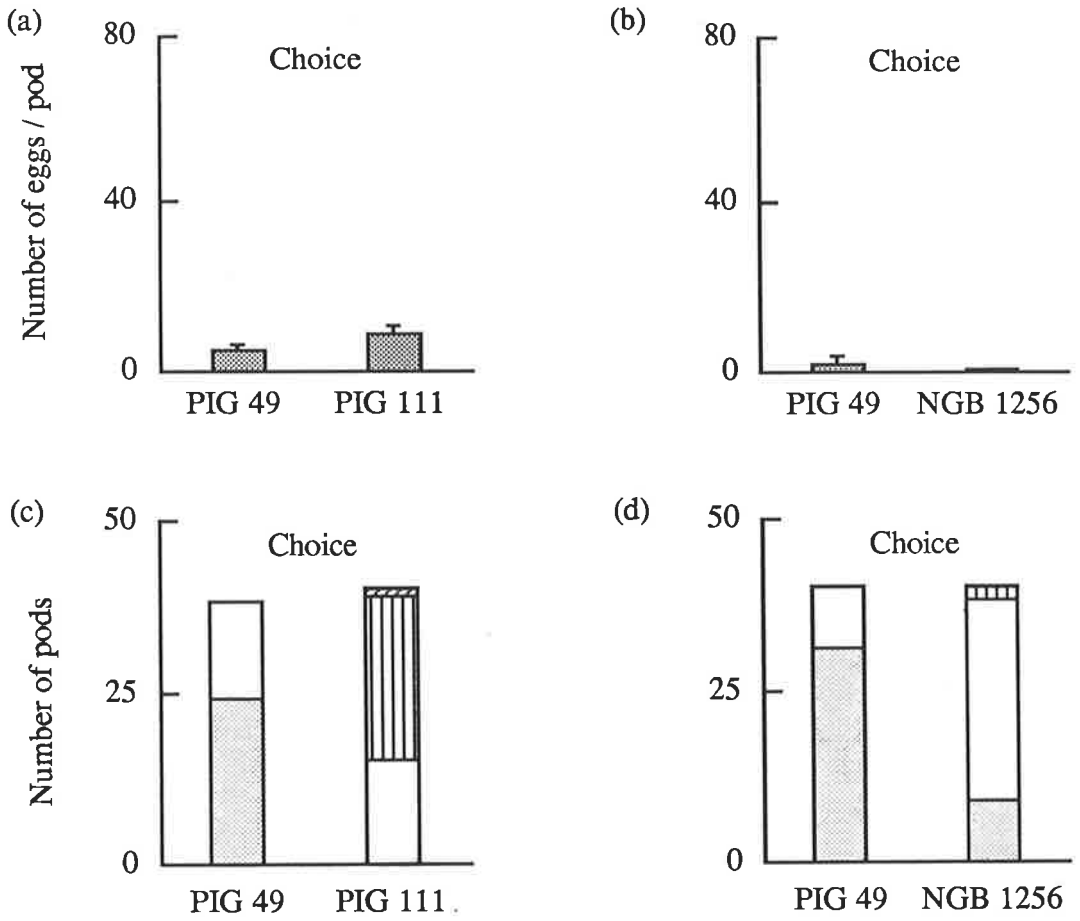


Figure 8.9. The number of eggs per pod laid on the *P. fulvum* accessions PIG 49 with PIG 111 and PIG 49 with NGB 1256. (a) and (b) Choice. (c) and (d) Development stages of pods in choice tests. Development stages of pods in (c) and (d) are represented by the following legend:

Flat pods Swollen pods Filled pods Green wrinkled pods

8.4. Discussion

Pea weevils prefer to lay their eggs on the control cv. Pennant when given the choice between Pennant and the *P. fulvum* accessions thus demonstrating that pods of *P. fulvum* exhibit antixenosis. The differences found in the choice assays were reduced in all no-choice assays except for one, the Pennant/PIG 49 comparison. The results from the Pennant/*P. fulvum* comparisons also suggested that all *P. fulvum* accessions tested would be useful for breeding resistance to the pea weevil. The *P. fulvum*/*P. fulvum* comparisons showed that PIG 111 and NGB 1256 were as antixenotic as PIG 49 (Figure 8.9). These results also suggest pod antixenosis might be effective in the field as egg lay was reduced in the no-choice test which simulates the conditions of a single cultivar crop.

The results obtained for the choice and no-choice assays demonstrated that either procedure could be used to screen *P. fulvum* derived material for oviposition antixenosis. A choice procedure provides the better test once antixenosis has been established, because the control pods provide a sink for oviposition and weevil fecundity can be estimated from the total number of eggs laid in a cage. In no-choice assays there are no internal controls, and if egg lay on the control pods is low a non-significant difference may be produced. This could explain the result obtained for ATC 114 in the no-choice assay. Egg lay on ATC 114 pods was similar to that of the other *P. fulvum* accessions in no-choice assays, however the control value of 21.0 eggs per pod was considerably lower than those from the others tests which ranged from 30.8 for PIG 111 to 43.7 for PIG 49.

The use of excised pods provided a simple method of testing the effectiveness of the antixenosis mechanism against the pea weevil. However excision of plant parts has been linked to changes in plant metabolism that can affect the expression and magnitude of resistance (Tingey 1986). Thomas *et al.* (1966) found that excised plant parts underestimated the resistance of lucerne accessions to the spotted alfalfa aphid. Conversely, van Emden and Bashford (1976) found that two aphid species grew more slowly on leaf discs cut from plants than on leaves still attached to plants. These findings

reinforce the need to ensure that a bio-assay reflects characteristics of whole plants in the field. The inheritance study presented in Chapter 6 was analogous to a large choice assay. The results obtained for the ratios of eggs laid on the test accessions to those laid on Pennant in the inheritance study compared the ratios obtained in the present choice assay (Table 8.2). The ranking of ratios is similar but the choice assay was less discriminating; this is desirable in a screening test because it identifies material which will be resistant in the field to the pea weevil.

Table 8.2. The ratio of eggs laid on pods in the inheritance trial and the present choice assay.

Genotype of test accession	Inheritance trial ratio[1] *P. fulvum* : Pennant	Choice assay ratio *P. fulvum* : Pennant
PIG 49	1 : 8.1	1 : 3.6
PIG 111	1 : 4.1	1 : 2.6
PIG 112	1 : 2.3	1 : 2.2
ATC 114	-	1 : 2.6
NGB 1256	1 : 9.0	1 : 3.9

[1] Ratios calculated from pods of all lengths and development stages

The result obtained for the *P. sativum* ssp. *humile* accession NGB 936 in the choice assay indicates that differences found between it and the control in Chapter 4 were solely related to the development of pod callus. It appears the surface chemistry of the NGB 936 pod does not influence oviposition by the weevil.

The preference for Pennant over *P. fulvum* accessions began on the first day pods were introduced into a cage and continued for the duration of the trial (Figure 8.2). This suggests that some aspect of surface chemistry of the pod may inhibit oviposition on the *P. fulvum* accessions. The significant daily difference in eggs laid on pods between

accessions also suggests the testing period could be reduced if this technique is used in the screening of breeding lines.

In the choice tests pod length influenced the number of eggs laid. This was demonstrated in the preliminary trial undertaken on Pennant pods, although the influence of length was not as pronounced in the no-choice 30-40/60-70mm comparison. Differences in the number of eggs laid were significant for the 30-40/10-20mm no-choice comparison. The probable cause for this was competition between female weevils for space to oviposit on the 10-20mm pods. The effect on egg lay of using pods of different stages of development in the assays is unknown. However the results for the different *P. fulvum* accessions are consistent and suggest that more mature pods have a minor influence on the result when used intermittently. If a wider range of pod developmental stages could be used, it would increase the number of plants that could be screened and the precision of the comparison.

CHAPTER 9

AN IN-VIVO TECHNIQUE FOR IDENTIFYING AND QUANTIFYING MECHANISMS OF RESISTANCE TO THE LARVAE OF THE PEA WEEVIL

9.1. Introduction

No-choice testing with individually caged pea plants (Chapter 4) showed that *P. fulvum* accessions had a significant level of resistance to the larvae of the pea weevil. However, the number of seeds infested depended on the level of antixenosis displayed to each accession by the weevil. In addition, many eggs were deposited on unsuitable mature pods which dried off before the larvae had time to enter a seed and develop. An inheritance study (Chapter 6) indicated that the mechanism was most likely to be a form of antibiosis located in the cotyledons, but again antixenosis influenced the rate of infestation, leaving the mode of inheritance and effectiveness of this mechanism unresolved. A reliable screening method for pods and seeds is needed to determine the mode of inheritance and to evaluate the mechanism of inheritance. The procedure would have to overcome the oviposition antixenosis of the weevil. It was achieved by transferring eggs laid on susceptible pods to pods being evaluated. The objective was to identify the best sources of larval resistance and to determine which tissues in the pea pod are involved in resistance.

9.2. Materials and methods

The susceptible *P. sativum* cv. Pennant was grown as a control and *P. fulvum* accessions PIG 49, PIG 111, PIG 112, ATC 114 and NGB 1256 were chosen as test lines. Resistance had already been demonstrated in PIG 49, PIG 111 and ATC 114 to larvae in a no-choice experiment using whole plants (Chapter 4). Control and test plants were sown in a glasshouse when space was available. Seedlings were trained on wire trellises. When both the control and test plants began to set pods, pods from control plants were placed in

cages with mated weevils to obtain eggs as in Chapter 8. Fresh pods were placed in the cages each day and the egg-laden pods were stored at room temperature in ventilated plastic boxes. When eggs developed black spots indicative of the developing larval heads, they were transferred to test pods.

Pea weevil eggs were transferred as the test pods began to swell and the larvae emerged when the pods were filled with soft seed (Plate 9.1). This mimicked what occurred in the field (Chapter 7). At this stage the pod wall, seed coat and seed were soft and did not act as a physical barrier to the larvae. Pods grow to an optimum length before they begin to fill. The length varies with genotype and environment. The length was 60-70mm or 30-40mm in length respectively and *P. fulvum* pods for the control.

A moistened fine tipped brush was used to transfer eggs to the pods. Twelve eggs per pod, or about two eggs per seed, were transferred. Eggs were spaced down one side of the pod. A new control was used for each test. The number infested each day depended on availability of eggs and pods. Larvae took up to a week to hatch depending on the glasshouse temperature which ranged between 7 and 26°C. Pods were harvested as they matured as the pods of *P. fulvum* shatter. Harvested material was stored in seed envelopes at 25°C until adult weevils emerged.

Mechanisms of resistance to pea weevil larvae can be located in three pod tissues; the pod wall, seed coat (testa) or the cotyledons (Figure 9.1). Differences between accessions in the proportion of larvae which fail to penetrate each of these can indicate the presence of a resistance mechanism. The number of larvae that entered the wall of each control and test pod was scored to determine the proportion of pod entrances. This provided a measure of resistance in the pod wall of each accession.

The testas of all the seeds were scored for larval penetrations. Having scored the pod wall and the seed coats of seed in each pod for larval entries the proportion of pod entrances

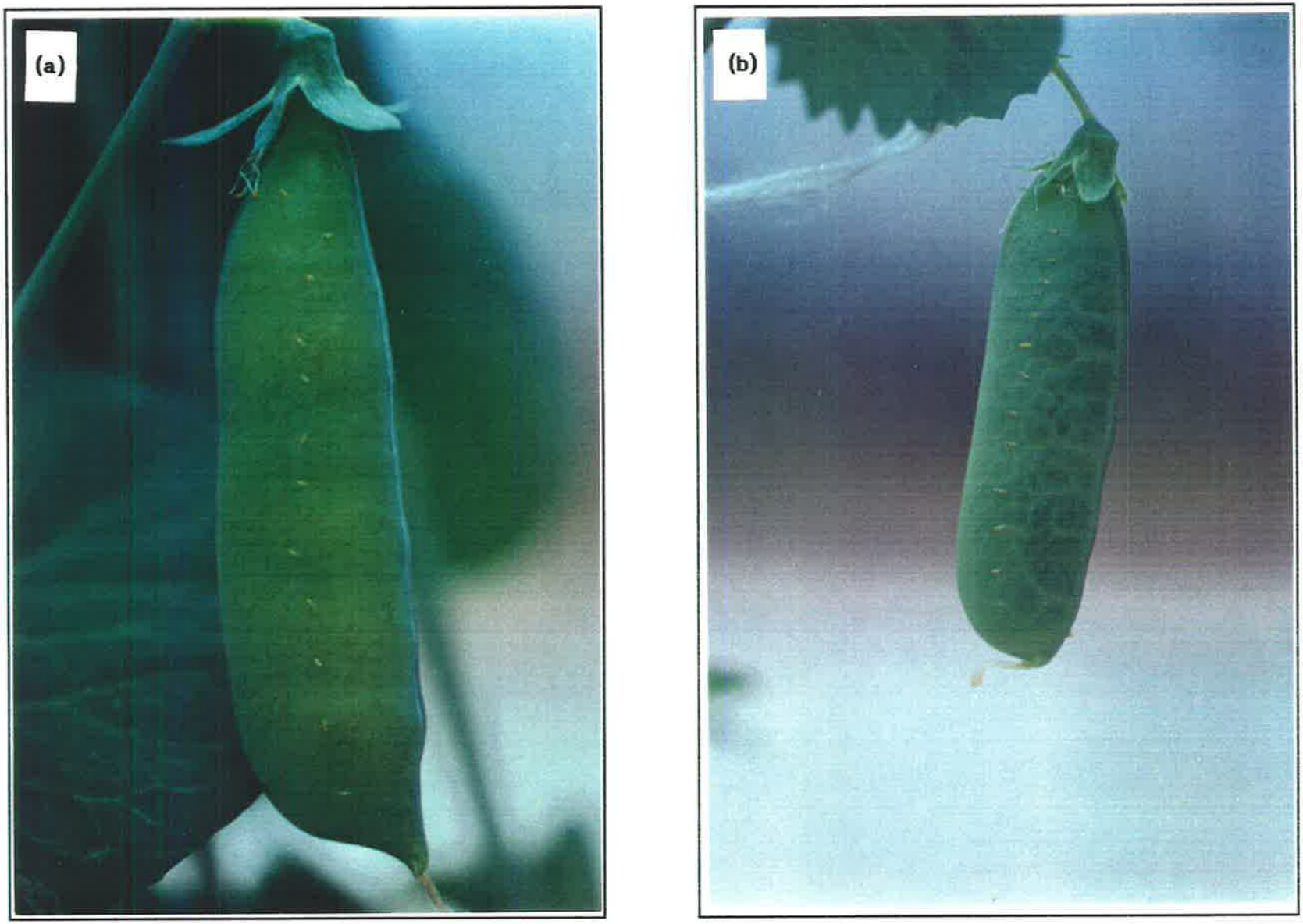


Plate 9.1. Pods with the transferred eggs (a) Pennant pod (b) *P. fulvum* accession.

that resulted in seed coat entrances by larvae per pod was calculated. This was used to indicate if a seed coat mechanism was present.

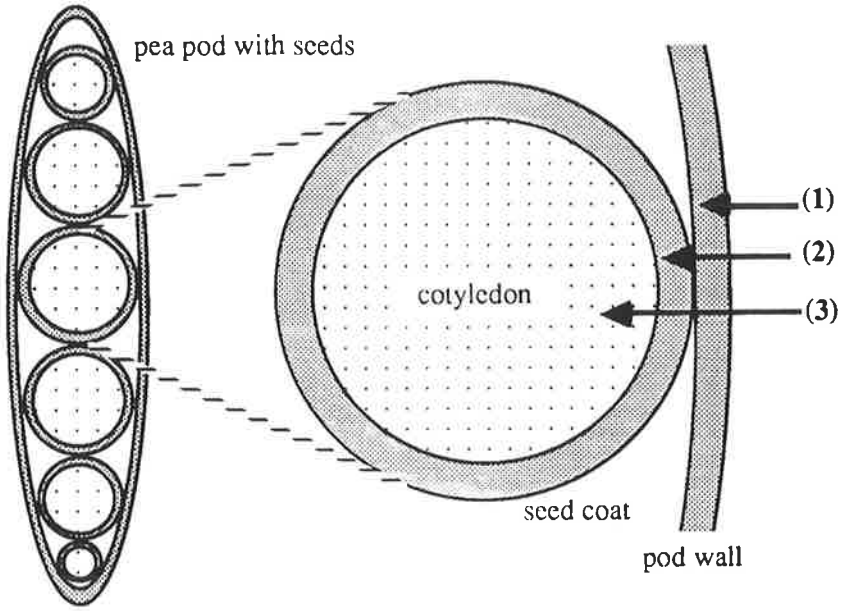


Figure 9.1. Longitudinal section of a pea pod with peas and a magnified section through a pea seed showing three sites where resistance mechanisms could be located; (1) a pod wall mechanism preventing larvae reaching the seed, (2) a seed coat mechanism preventing larvae reaching the cotyledons and (3) a cotyledon mechanism preventing the development of the larvae which enter the seed.

The cotyledons of every seed were scored for larval entry and the emergence of adults. The proportion of weevil emergence per seed entered was used as a measure of a resistance mechanism in the cotyledons. Only seeds where a larva had penetrated the seed coat and begun to chew the cotyledons were included in this category. This separated the effects from any resistance mechanism associated with the seed coat and made the result independent of the seed number in each pod.

The number of seeds per pod and the mean weight of weevil-free seed from each accession were also recorded. Seed number and seed size could influence the level of competition between larvae in the pod and in each seed respectively. The weight of each seed also determines the amount of food available to a weevil larva and some small-seeded accessions may limit weevil development.

The five *P. fulvum* accessions with 52 pods (replicates) per accession were compared with control pods. A test pod was compared with a control pod on each occasion because of the extended duration of the transfer procedure from June-October 1991. The following parameters were analysed using Genstat 5:

(a) The proportion of larvae to enter pods

(b) The proportion of pod entrances that resulted in seed coat entrances

(c) the proportion of seed exits from seed cotyledons entered

(d) The number of seeds per pod

Parameters a, b and c were fitted to a binomial model of the form $y_{ij} = B(p_i; n_{ij})$. For example parameter (a) was fitted to this model where:

i = 1, 2 (number of lines)

j = number of replicates

y_{ij} = number of weevil larvae which entered a pod

n_{ij} = number of weevil eggs per pod (12)

p_i = probability of a weevil larva entering the pod of accession i

Parameter (d) follows a Poisson distribution (Snedecor & Cochran 1989) and a test performed to see if the number of seeds between the control line and line i differed. The model, in the form of $y_{ij} \sim Po(\lambda_i)$ was fitted where:

i = 1, 2 (number of lines)

j = number of replicates

y_{ij} = number of seeds per pod

λ_i= mean number of seeds belonging to line i

The resulting analyses of deviance (ANODE) is analogous to an analysis of variance. The mean deviance ratios are approximately distributed as F-statistics.

9.3. Results

There were no differences between the cv. Pennant and test accessions in the proportion of larvae that penetrated the pod wall except for ATC 114 which had a higher proportion than the control (Figures 9.2a, 9.3a, 9.4a, 9.5a & 9.6a). This demonstrates that none of the *P. fulvum* lines tested possess a mechanism for resistance in the pod wall.

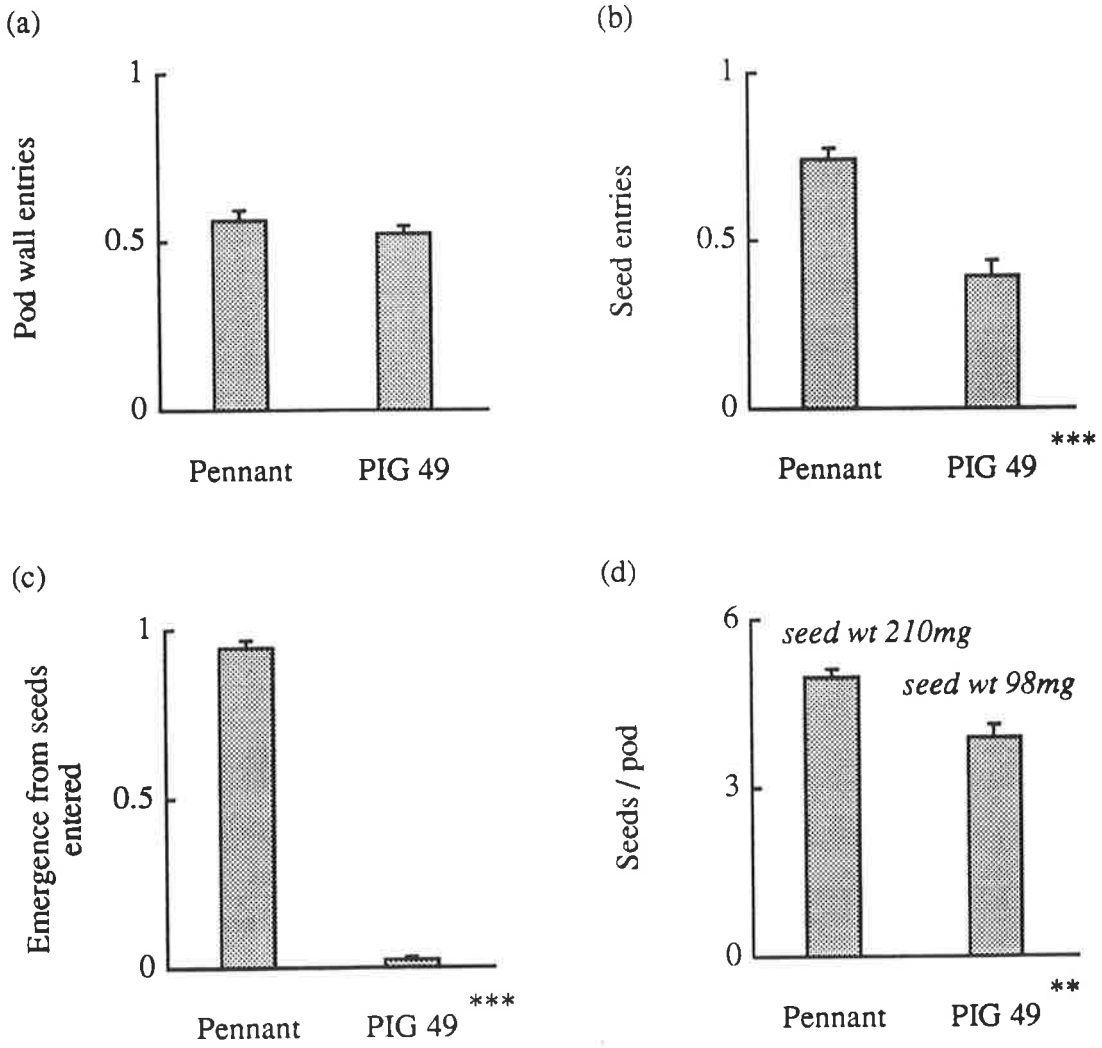
The proportion of seed coat entrances resulting from pod entrances was smaller in all the *P. fulvum* accessions than in the controls (Figures 9.2b, 9.3b 9.4b 9.5b & 9.6b). This smaller proportion of seed entrances suggests that a seed coat mechanism inhibited larval penetration. The proportion in seeds of the control ranged from 0.66-0.74 and in the test accessions ranged 0.32-0.39. PIG 111 and ATC 114 had the lowest proportion and PIG 49 and NGB 1256 the highest. The reduction in seed coat penetrations of *P. fulvum* suggests the same mechanism was responsible in each accession.

The level of adult weevil emergence from seed where the cotyledons had been entered was low for seed of all the *P. fulvum* accessions and high in the controls.(Figures 9.2c, 9.3c, 9.4c, 9.5c & 9.6c). The proportion of seed exits per seed entered for the control ranged from 0.89-0.95 and for the test accessions from 0.00-0.14 with ATC 114 lowest and PIG 112 highest. These results indicate the presence of a major mechanism for resistance in the seed cotyledons of the test accessions. When the cotyledons were scored for the presence of weevil damage it was found that all weevils in control seed were adults or had emerged from the seed, while in the test accessions many weevils were still present as larvae.

All test accessions produced significantly fewer seeds per pod than the control (Figures 9.2d, 9.3d, 9.4d, 9.5d & 9.6d). The number of seeds per pod ranged from 4.94-5.31 in the control, and in test lines from 2.93 seeds in NGB 1256 to 4.14 seeds in PIG 111. Seed weights also differed. PIG 49 produced the largest seed (98 mg) of any

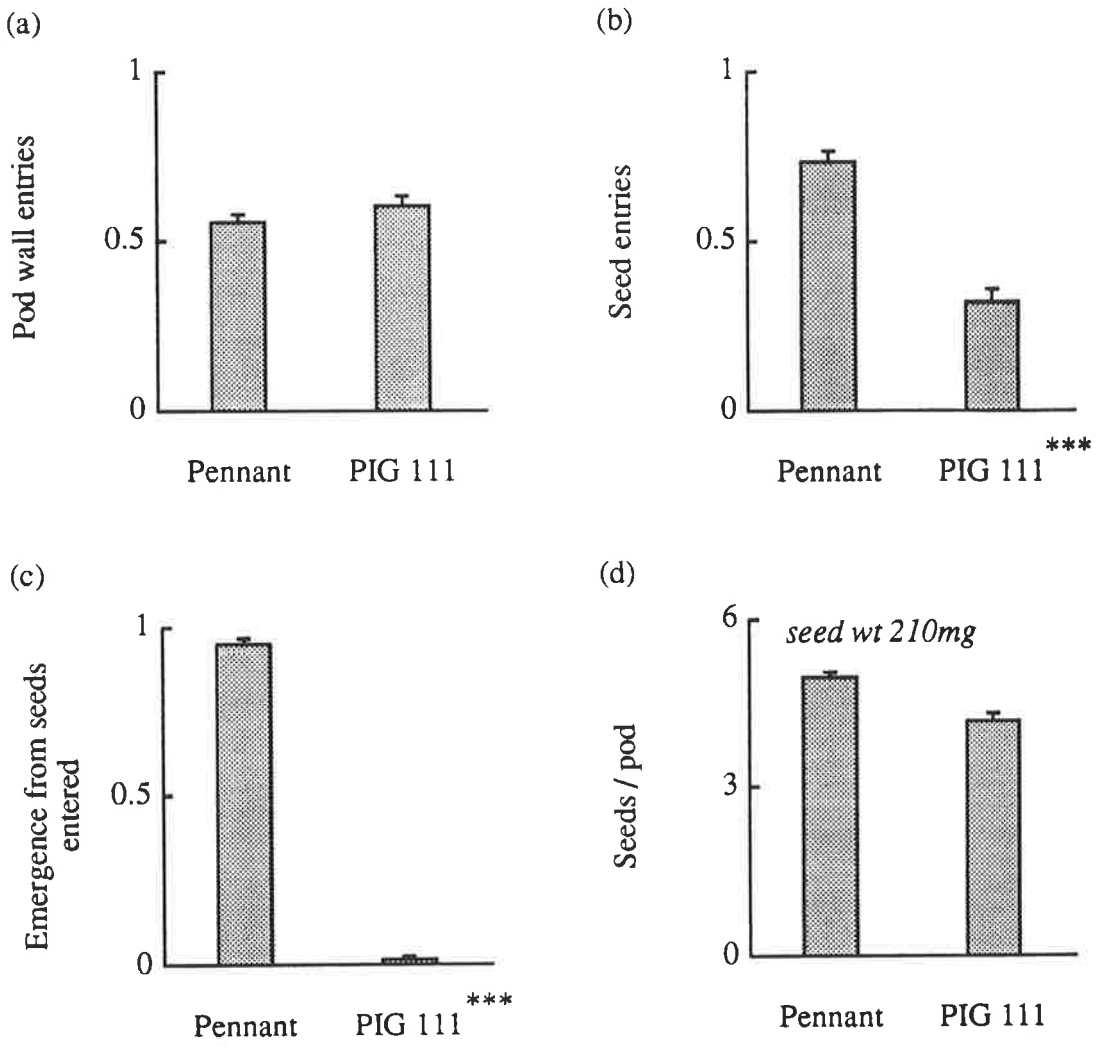
P. fulvum accession but it was less than half the weight of the control seed (210 mg). NGB 1256 produced the smallest seed (39 mg).

The *P. fulvum* accessions with the lowest level of adult emergence per seed entered ATC 114, PIG 49 and PIG 111 were tested at the same time on 16 occasions and a comparison of these accessions was made for all the test parameters except seed number. The analysis showed no difference between these accessions for any of the parameters.



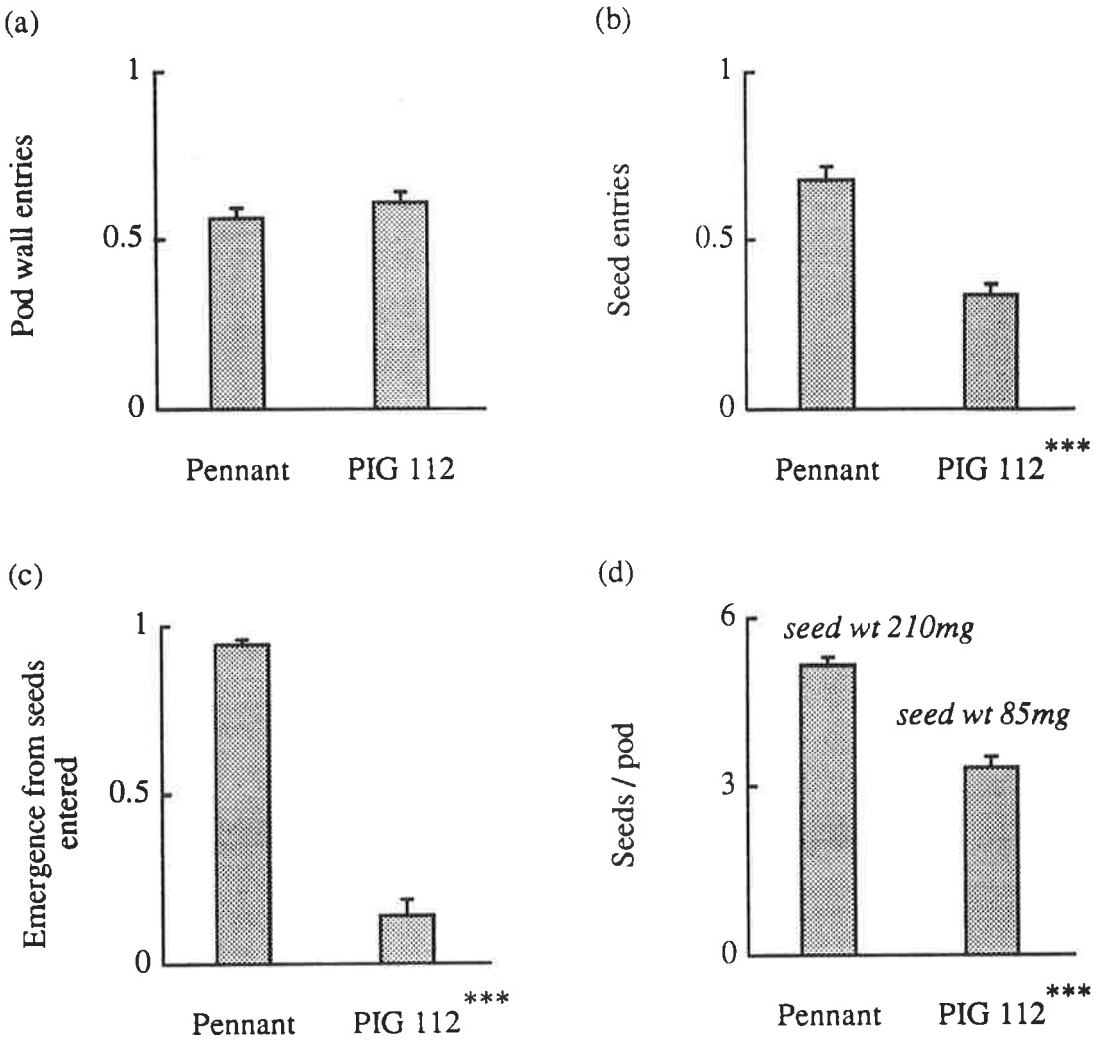
***p = 0.01-0.001; ***p<0.001.*

Figure 9.2. Comparison of larval survival in pods of *P. sativum* cv. Pennant and the *P. fulvum* accession PIG 49. (a) Proportion of larvae to enter pods. (b) Proportion of pod entrances that resulted in seed coat (testa) entrances. (c) Proportion of seed exits from cotyledons entered. (d) Number of seeds per pod.



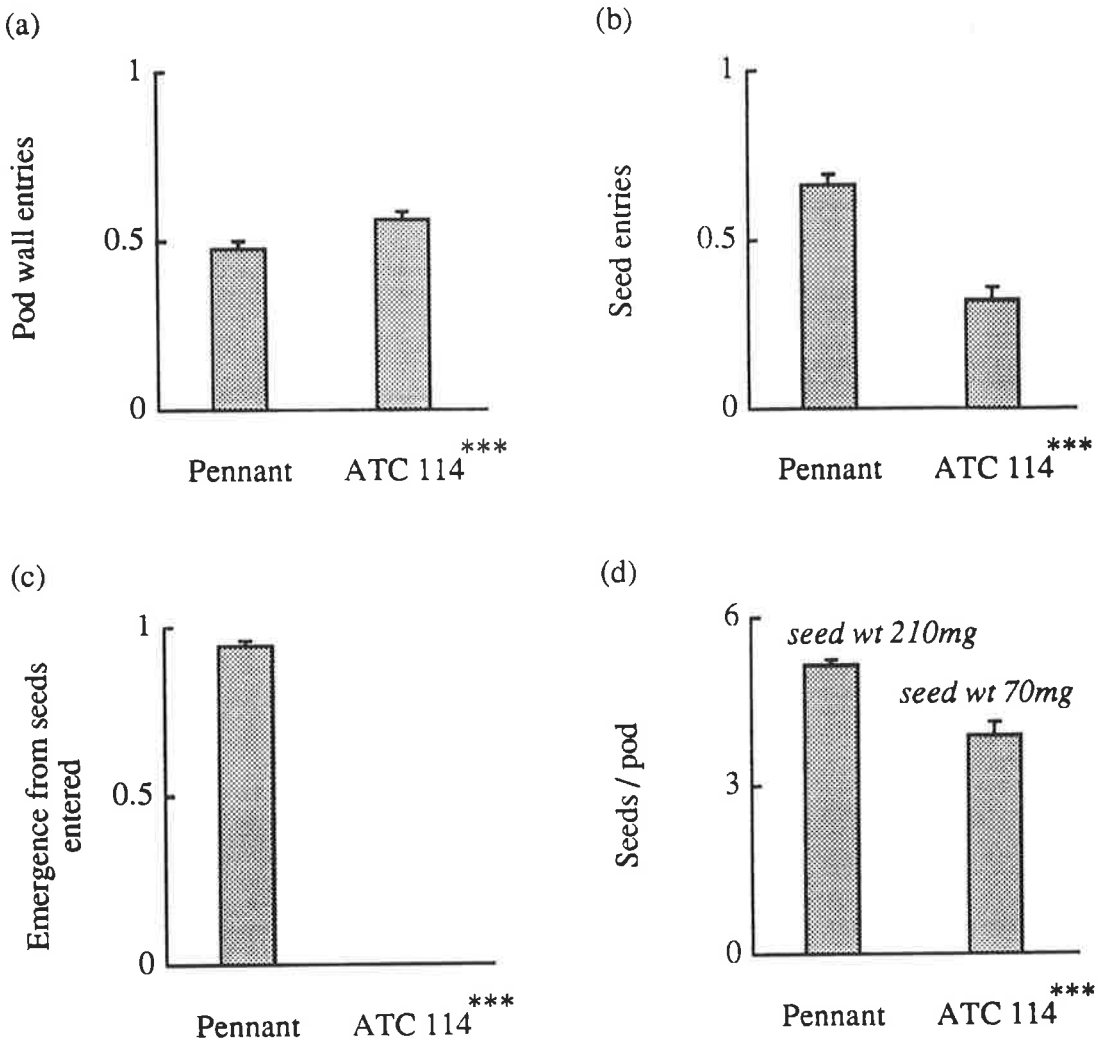
***$p<0.001$.

Figure 9.3. Comparison of larval survival in pods of *P. sativum* cv. Pennant and the *P. fulvum* accession PIG 111. (a) Proportion of larvae to enter pods. (b) Proportion of pod entrances that resulted in seed coat (testa) entrances. (c) Proportion of seed exits from cotyledons entered. (d) Number of seeds per pod.



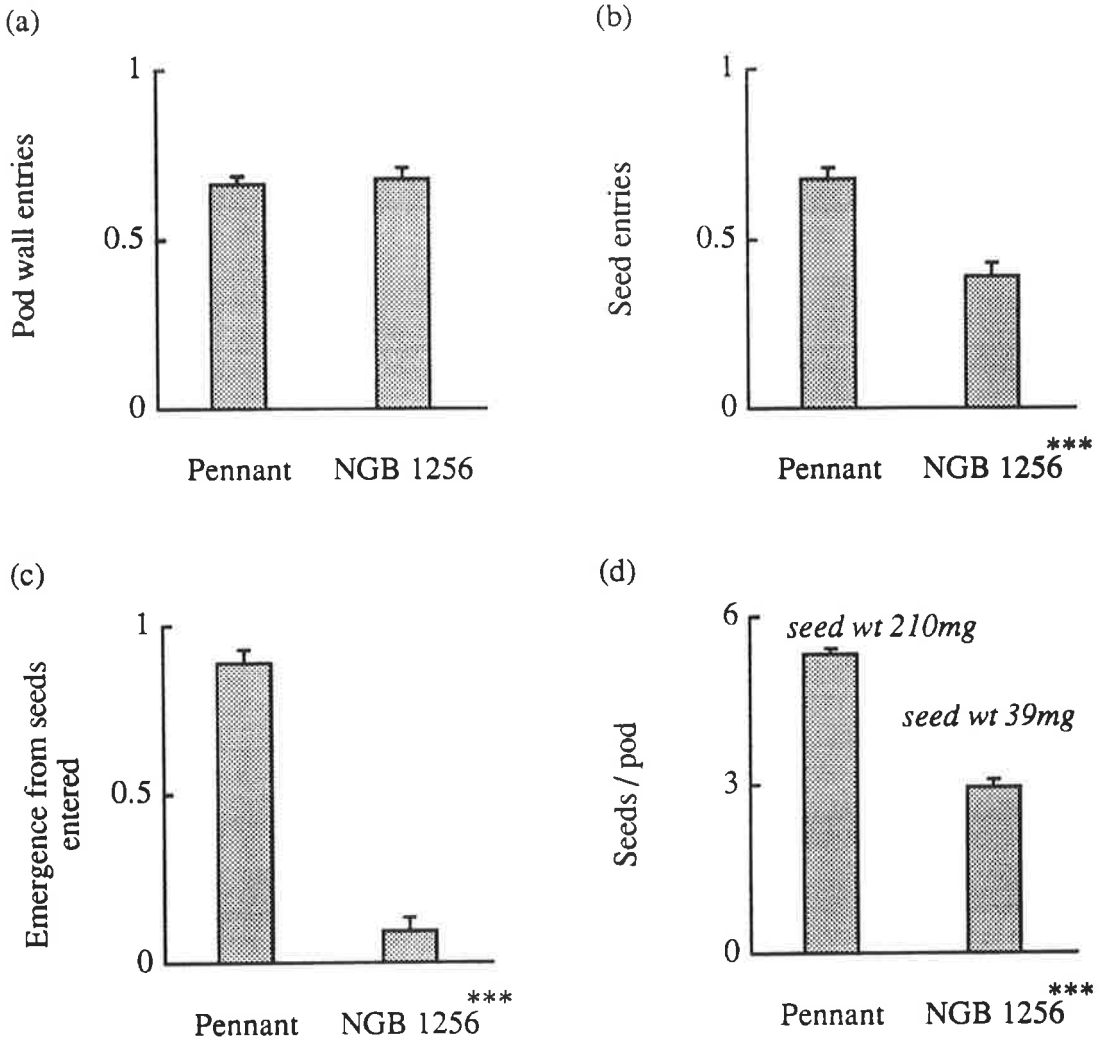
***$p<0.001$.

Figure 9.4. Comparison of larval survival in pods of *P. sativum* cv. Pennant and the *P. fulvum* accession PIG 112. (a) Proportion of larvae to enter pods. (b) Proportion of pod entrances that resulted in seed coat (testa) entrances. (c) Proportion of seed exits from cotyledons entered. (d) Number of seeds per pod.



****p<0.001.*

Figure 9.5. Comparison of larval survival in pods of *P. sativum* cv. Pennant and the *P. fulvum* accession ATC 114. (a) Proportion of larvae to enter pods. (b) Proportion of pod entrances that resulted in seed coat (testa) entrances. (c) Proportion of seed exits from cotyledons entered. (d) Number of seeds per pod.



***$p<0.001$.

Figure 9.6. Comparison of larval survival in pods of *P. sativum* cv. Pennant and the *P. fulvum* accession NGB 1256. (a) Proportion of larvae to enter pods. (b) Proportion of pod entrances that resulted in seed coat (testa) entrances. (c) Proportion of seed exits from cotyledons entered. (d) Number of seeds per pod.

9.4. Discussion

The individual comparisons between the control and the *P. fulvum* test accessions indicated the presence of two mechanisms of resistance to the pea weevil, a seed coat and a cotyledon mechanism. Both of these mechanisms had an impact on the survival of larvae in the seed. The consistency in the results suggest the egg transfer procedure can be used when screening for resistance among progeny of crosses and should allow the genetics of resistance to be determined.

The use of seed entrances as a proportion of pod entrances removed a possible bias arising from the different number of seeds per pod. The results clearly show that a mechanism for larval resistance is present in the testa of all the *P. fulvum* accessions. A seed coat mechanism can act by causing either a feeding antixenosis or an antibiotic response in the larvae. The results do not exclude the possibility that morphological differences between the control and test material rather than a chemical resistance mechanism caused the observed response. Another possibility is the larvae have no difficulty feeding on the seed testa but perish when they feed on the cotyledons.

Adult emergence from all seed harvested, has been used successfully in bean breeding programs as a measure of resistance to storage bruchids, where a given number of dried seeds are tested for oviposition antixenosis and or larval antibiosis (Redden & McGuire 1983; Rusoke & Fatunla 1987). It provides a measure of seed resistance, but does not distinguish between seed testa and cotyledon mechanisms. In my experiments, differences in seed number and the seed coat mechanism were isolated from the cotyledon mechanism by only scoring seeds for adult emergence where a larva had penetrated the seed coat. The large and highly significant differences between the survival of larvae in the control and test material indicate that this is the major mechanism of resistance in the seed. Most of the young larvae which penetrated the seed coat of a resistant accession died without doing much damage to the still green cotyledons and the few larvae which

emerged took twice as long as those from susceptible seed. The longer period of development, in the few seeds where a weevil did successfully develop in the cotyledons of a *P. fulvum* accession, reinforces the argument for a separate cotyledon mechanism rather than the effect of an artefact of the seed coat mechanism. The slow development on *P. fulvum* indicates that either a biochemical form of antibiosis or a nutritional deficiency is affecting the development of larvae.

As PIG 49, PIG 111 and ATC 114 were similar in their resistance response they would be equally suitable when breeding for resistance. PIG 112 and NGB 1256 were nearly as resistant, but when sampled many of the pods contained a fungal mycelium which could have been responsible for much of the larval mortality. All the *P. fulvum* accessions produced smaller seeds than the control and it was noticed on some occasions a seed contained a dead larvae and the total contents of the seed had been consumed. The death of the larvae may have resulted from starvation rather than the cotyledon mechanism. This could be a factor in larval mortality in some of the smaller seeded *P. fulvum* accessions such as NGB 1256 and needs to be taken into account when selecting resistant material.

The pod wall is not a barrier and as many pea weevil larvae entered the *P. fulvum* pods as entered the controls. The proportion of larvae entering the pod wall was never higher than 0.70 and this could indicate the eggs were damaged while being transferred between pods or that pods had begun to harden before some of the larvae hatched. The similar value for both test and control material indicates that eggs were being transferred at a similar stage of pod development, but the technique needs to be monitored continuously to maintain the high level of pod wall penetration by larvae. While the pod wall does not preclude entry of larvae in *P. fulvum* accessions, it could be of low nutritive or some other cryptic value which may increase the impact of the seed coat and cotyledon mechanisms.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS

10.1. Identification of Pea Weevil Resistant Germplasm

It is probable that the majority of the 69 *Pisum* accessions tested in the field in 1989-91 (Chapter 3) were not resistant to the pea weevil. Most were probably temporal or spatial escapes as is evident from the results of no-choice testing with whole plants (Chapter 4). A significant level of resistance to the pea weevil was demonstrated in some of the *P. fulvum* accessions but no resistance was found in the domesticated *P. sativum*. Previous attempts to identify pea weevil resistance have concentrated on cultivars and landraces of *P. sativum* (Chapter 2) and this could explain why they were not successful.

The no-choice testing (Chapter 4) identified two mechanisms of resistance in the *P. fulvum* accessions. These were an oviposition antixenosis and a pod based mechanism, which was later found to consist of a seed coat and a cotyledon factor (Chapter 9). More detailed studies on antixenosis with pods on whole plants (Chapter 5) and with excised pods (Chapter 8) provided conflicting results on the existence of the mechanism. The results presented in Chapter 5 were obtained from a sampling procedure similar to that used in Chapter 4, but this time there were no differences between accessions. This is important because only the trial environment and the scoring method were changed between the two trials. However the choice and no-choice pod assay (Chapter 8) provided clear evidence for antixenosis in the majority of the *P. fulvum* accessions tested. These results illustrate that field screening alone cannot adequately identify resistant material and that trial design, trial environment, sampling method, insect behaviour and phenotypic aspect of the host species are all important.

10.2. Mechanisms of Resistance to the Pea Weevil

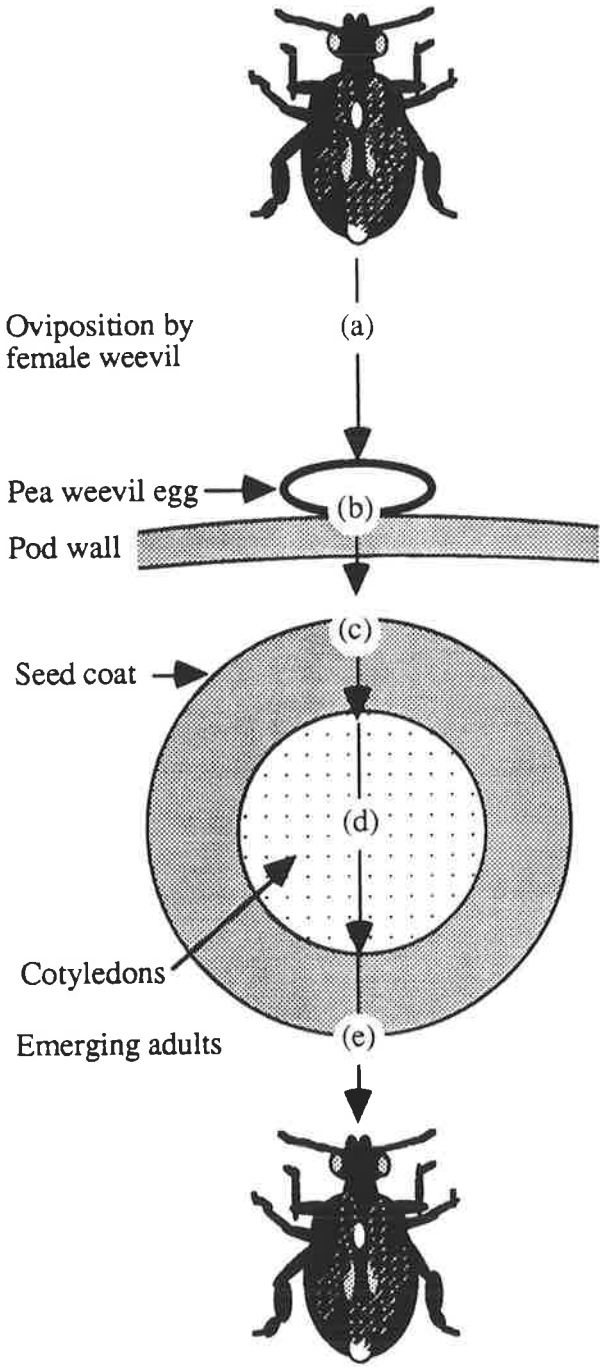
This investigation of resistance to the pea weevil identified four resistance mechanisms. They were oviposition antixenosis on the pods (Chapters 4 & 8), a seed testa mechanism (Chapters 4 & 9), a cotyledon antibiosis mechanism (Chapters 4, 6 &9) and a pod callus mechanism (Chapter 4). All appear to be useful except for the pod callus mechanism which requires a specific environment. Other factors such as pod length, thickness of the pod wall and the rate at which pods mature may also reduce oviposition and infestation. The effect of pod length was demonstrated in the no-choice test (Chapter 5) and the antixenosis assay (Chapter 8). The effect of the pod wall was evident for cv. Pennant in the pod preference experiment (Chapter 7) and for all trial accessions used in the antibiosis assay (Chapter 9). Given the results obtained in the choice and no-choice assays (Chapter 8), and the antibiosis assay (Chapters 9) a, model can be developed to predict the impact of the resistance mechanisms and the pod wall effect for each accession.

10.3. A Model for Resistance to the Pea Weevil

An idealised comparison is presented in a model that demonstrates the influence of defence mechanisms in the resistant accessions PIG 49 and ATC 114 when compared to the susceptible cv. Pennant (Table 10.1). The model uses the results obtained and discussed in Chapters 8 and 9. The number of weevils to emerge from seeds of each accession was calculated for a female with the ability to produce 100 eggs and on the assumption each larva entered a separate seed. Antixenosis was calculated from the no-choice data of Chapter 8 for pods of similar length and development.

In the model, PIG 49 represents the influence of high levels of all resistance mechanisms, while in ATC 114 the influence of antixenosis is substantially reduced. For each 100 eggs produced the model illustrates that 39.0, 0.1 and 0.0 weevils would emerge from seeds of Pennant, PIG 49 and ATC 114 respectively (Table 10.1 section (e)). The differences between predicted values are comparable for the emergence values of *P. fulvum* accessions and cultivars presented in Chapter 3 (Figure 3.1). A similar difference for weevil

Table 10.1. Model showing egg lay and survival of larvae (number of weevils and the percentage to survive from the previous step) in the *P. sativum* cv. Pennant and the *P. fulvum* accessions PIG 49 and ATC 114. (a) Effect of the oviposition antixenosis mechanism. (b) Pod wall effect. (c) Effect of the seed coat mechanism. (d) Effect of the cotyledon mechanism. (e) Emergence of adults from seed.



	Control	PIG 49	ATC 114
	100 eggs per female	100 eggs per female	100 eggs per female
(a)	100.0 eggs (100.0%)	23.1 eggs (23.1%)	61.9 eggs (61.9%)
(b)	56.0 larvae (56.0%)	12.0 larvae (52.0%)	34.7 larvae (56.0%)
(c)	41.5 larvae (74.0%)	4.7 larvae (39.0%)	11.1 larvae (32.0%)
(d)	39.0 larvae (94.0%)	0.1 larvae (2.0%)	0 larvae (0.0%)
(e)	39.0 adults	0.1 adults	0.0 adults

emergence was also found in the field (Chapter 6) for Pennant (23%) and PIG 49 (0.05%), but a comparison was not available for ATC 114 because it was not sown in the field trial. It needs to be emphasised that the field results are from a choice environment, and that the model does not allow for competition between larvae in the same seed.

In the model the proportions of larvae that hatch from eggs and successfully penetrate the pod wall come from the glasshouse experiment (Chapter 9) and were 0.56, 0.52 and 0.56 for Pennant, PIG 49 and ATC 114 respectively (Table 10.1 section (b)). These appear to be severe mortality figures, especially for the susceptible cv. Pennant. However, the proportion of larvae that entered pods from eggs laid on Pennant in the field trial (Chapter 7) ranged from 0.46 to 0.70 over the period the weevils laid eggs, suggesting that the glasshouse values are suitable for the model.

The proportion of larvae that survive in the cotyledons of each accession and emerge as adults were similar for both the bio-assay (Chapter 9) and the field data (Chapter 6). Emergence from infested Pennant seed in the bio-assay and in the field was 94.0 and 99.5% respectively, and for PIG 49 it was 2.0 and 5.9% respectively. ATC 114 was not used in the field trial, but no weevil reached maturity in the cotyledons of this accession in the bio-assay. The cotyledon mechanism(s) present in PIG 49 and ATC 114 represents the plant's last line of defence and was very effective, but only accounts for a small percentage of larval deaths in PIG 49 and ATC 114 (Table 10.1 section (d)).

Field data were not available to confirm the effectiveness of the seed coat mechanism and oviposition antixenosis in a no-choice environment. However there is no reason to believe that the seed coat mechanism (Table 10.1 section (c)) identified from the pod assay will act differently to the cotyledon mechanism in field grown plants. The only results included in the model not verified by field data were the results of the antixenosis assay (Table 10.1 section (a)) . The effectiveness of antixenosis has been referred to previously

(Chapters 4,5 & 8) and its possible value in protecting a crop must be resolved before any efforts are made to incorporate this trait by breeding.

Even if there was no antixenosis mechanism in the *P. fulvum* accessions, the seed coat effect and the seed mechanism would virtually eliminate all weevils. The level of adult emergence from ATC 114 remained unchanged at 0.0 per 100 eggs due to the cotyledon mechanism, while adult emergence from PIG 49 would increase marginally from 0.1 to 0.4 if antixenosis was removed. The number of adults to emerge from Pennant in the model remained at 39 per 100 eggs produced. Though antixenosis of *P. fulvum* accessions in the model exercises little influence over adult emergence, it can reduce selection pressure against the other mechanisms and should be part of any breeding strategy if it is found to be effective.

10.4. Inheritance of Resistance to the Pea Weevil

Some progress was made towards understanding the inheritance of two mechanisms of resistance to the pea weevil.

Results obtained in Chapter 6 for F_2 plants indicated that antixenosis is recessive and could be simply inherited, although the low number of eggs laid on some of the susceptible parent plants indicated that there were probably many escapes in the F_2 population. Also, the presence of variegated and non-viable albino seedlings implies that the segregating ratio could have been modified by mortality of plants. Seedling mortality is a common feature of interspecific crosses (Sears 1944; Gerstel 1954) and could indicate that other individuals were eliminated at the meiotic, gametic or zygotic stages. These effects might be overcome by testing plants in the F_3 generation, and by testing for antixenosis in a bio-assay which will reduce the chance of escapes occurring.

The mode of inheritance of the antibiosis mechanism in the F_2 and F_3 seed cotyledons is unresolved, however emergence from cotyledons entered in the susceptible parent was

higher than in some of the cross material (Chapter 6). This may at least indicate that the mechanism is maternally inherited. The use of the pod bio-assay will allow the inheritance of this mechanism to be determined as it removes the effect of the antixenosis mechanism, which also allows the seed coat mechanism to be investigated.

10.5. The Use of Bio-assays in Breeding for Pea Weevil Resistance

It is evident from the three years of mass screening trials, that many of the accessions thought to be resistant were escapes, as discussed in Chapter 3. Numerous escapes were also evident in the inheritance trial (Chapter 6) indicating the difficulty of trying to select resistant progeny in a field environment. This was further complicated by there being several mechanisms present within accessions, and that screening is being attempted against a highly mobile insect.

There is a need for bio-assays for each resistance mechanism, which are simple, repeatable and can be carried out in a short time, so that they can be used in breeding programs. Both assays (Chapters 8 & 9) were easy to use and the results were repeatable, but they required plants that were old enough to produce pods. An antixenosis assay requires up to 9-12 pods per plant for testing and the seed antibiosis assay requires 4-6 weeks post harvest storage before scoring. However if the chemical components causing antibiosis could be identified, then a quick chemical assay of individual seeds for the testa and cotyledon mechanisms would increase the number of progeny that could be screened and reduce the time taken for screening. The development of a chemical assay for antixenosis may be difficult and hard to justify because of its doubtful efficacy. But antixenosis could be selected using the pod assay in later generations once other characters have been fixed in the population.

10.6. Conclusions

From the many studies undertaken the following conclusions are drawn:

1. *P. fulvum* exhibits resistance to the pea weevil and is possibly the only source of resistance in the *Pisum* gene pool.

2. There are at least three mechanisms of resistance in the *P. fulvum* accessions: antixenosis for oviposition, antibiosis in the seed coat and antibiosis in the cotyledons.

3. The prospects of developing pea weevil-resistant cultivars are good.

4. The antixenosis mechanism requires field testing in a no-choice environment to confirm its effectiveness.

5. The inheritance of each resistance mechanism requires clarification.

6. Bio-assays for antixenosis and antibiosis developed during this study were effective.

7. Inheritance studies and the screening of progeny would be more effective in the laboratory than in the field.

8. If compounds responsible for resistance can be identified, then the effectiveness of screening will be increased.

10.7. Further Research

The present studies have indicated that there is a potential for breeding of peas resistant to the pea weevil. The issues that remain and require further research are:

1. To determine the genetics of the resistance mechanisms identified in *P. fulvum* in this study. It would be advantageous if the reciprocal cross to the one studied could be made and F_1 and F_2 populations analysed.

2. To identify the compounds responsible for each mechanism and develop appropriate chemical assays.

3. To establish, using isolated field sites, the effectiveness of the antixenosis mechanism.

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APPENDICES

Appendix 1

Models used to investigate the relationship between number of adults to emerge and eggs per cage of the eleven accessions in Chapter 4.

The maximal linear model fitted was $y_{ij} = \alpha_i + \beta_i x_{ij} + R_{ij}$

where i = 1...11 (number of genotypes) and j = 1...n_i for:

$y_{ij} = \sqrt{\text{number of adults to emerge per cage}+0.5}$ for j^{th} cage for genotype i

$x_{ij} = \sqrt{\text{estimated eggs per cage}+0.5}$ for j^{th} cage for genotype i

α_i = intercept for i^{th} genotype

β_i = linear regression coefficient for i^{th} genotype

where R_{ij} is identical and independently normally distributed with a mean of zero and a common variance of σ^2

n_i = number of cages measured for the i^{th} genotype

As this maximal model allows the estimation of different α_i and β_i coefficients for each genotype, distinct linear regressions can be described. To establish the simplest model which adequately described the relationship between eggs per cage and adults to emerge progressively simpler models were fitted. First the model

$$y_{ij} = \alpha_i + \beta x_{ij} + R_{ij}$$

was fitted to all genotypes (i.e different intercept but same slope, giving parallel lines). Comparing these models allowed the testing of the null hypothesis (H_0)

$$H_0: \beta_1 = \beta_2 ... = \beta_{11}$$

to be tested against

$$H_a: \beta_r \neq \beta_s \text{ where r and s belong to } 1...11.$$

A significant variance ratio would have indicated that at least one line was distinct from another, therefore we would reject H_0 and conclude the maximal model was preferable. Paired comparisons of the control with test lines enabled the determination of the genotypes that had distinct slopes from the control. If the above H_0 was not rejected we would have concluded a model involving parallel lines was adequate.

We then fit the model

$$y_{ij} = \alpha + \beta x_{ij} + R_{ij}$$

which fits coincident lines (same intercept and slope, i.e common line) to each genotype. Comparing these models allowed the testing of the null hypothesis (H_0)

$$H_0: \alpha_1 = \alpha_2 ... = \alpha_{11}$$

to be tested against

$$H_a: \alpha_r \neq \alpha_s \text{ where r and s belong to } 1...11.$$

A significant variance ratio indicated that at least one line was not coincident to another and therefore we reject H_0. Paired comparisons of the control with test genotypes enabled determination of the genotypes that had different intercepts to the control.

The final model considered was

$$y_{ij} = \alpha + R_{ij}$$

which then allowed testing of

$$H_0: \beta = 0$$

against

$$H_a: \beta \neq 0$$

A significant variance ratio indicated that the slope of the common line was significantly different from zero.

In each case when comparing one model to another, the observed variance ratio was calculated using the following formula:

$$\text{variance ratio}_{obs} = \frac{(\text{Regression SS}_{(complex)} - \text{Regression SS}_{(simple)})/(\Delta \text{ in df})}{\text{Residual MS}_{(complex)}}$$

Appendix 2

Models used to investigate the relationship between the pod surface area and pod length of the six accessions in Chapter 5.

The maximal quadratic model fitted was $y_{ij} = \alpha_i + \beta_i x_{ij} + \gamma_i x_{ij}^2 + R_{ij}$

where i = 1, 2, 3, 4, 5, 6 (number of genotypes) and j = 1...n_i for:

y_{ij} = surface area of j^{th} pod from genotype i

x_{ij} = length of j^{th} pod from genotype i

α_i = intercept for i^{th} genotype

β_i = linear regression coefficient for i^{th} genotype

γ_i = quadratic regression coefficient for i^{th} genotype

R_{ij} = residual error associated with j^{th} pod from genotype i

where R_{ij} is identical and independently normally distributed with a mean of zero and a common variance of σ^2

n_i = number of values for the i^{th} genotype

As this maximal model allows the estimation of different α_i, β_i and γ_i coefficients for each genotype, distinct quadratic curves can be described. To establish the simplest model which adequately describes the relationship between area and length of pods, progressively simpler models were fitted. First, the model

$$y_{ij} = \alpha_i + \beta_i x_{ij} + \gamma x_{ij}^2 + R_{ij}$$

was fitted to all genotypes. Comparing these models allowed the testing of the null hypothesis (H_o)

$$\gamma_1 = \gamma_2 = \gamma_3 = \gamma_4 = \gamma_5 = \gamma_6$$

against the alternative hypothesis (H_a)

$\gamma_r \neq \gamma_s$ where r and s belong to 1...6.

A significant variance ratio would have indicated that at least one curve was distinct from another and thus the more complicated maximal model would be preferred to the simpler model. However when this test was applied, the variance ratio was non-significant; thus the H_o was retained indicating the simpler model with a common quadratic coefficient was adequate.

Next the model

$$y_{ij} = \alpha_i + \beta_{ij}x_{ij} + R_{ij}$$

which fits distinct (separate) linear regressions to each genotype was fitted. Comparison of these two models tested

$$H_o: \gamma = 0 \text{ versus } H_a: \gamma \neq 0.$$

The appropriate variance ratio for this comparison of models was highly significant, thus the first, more complicated quadratic model was retained. The relationship shows curvature and cannot be adequately represented by straight lines.

The next model considered was

$$y_{ij} = \alpha_i + \beta x_{ij} + \gamma x_{ij}^2 + R_{ij}$$

which then allowed

$$H_o: \beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta_5 = \beta_6$$

to be tested against

$$H_a: \beta_r \neq \beta_s \text{ where r and s belong to 1...6.}$$

The test variance ratio here was found to be non-significant; thus H_o was retained which indicates this simpler model, with a common linear coefficient adequately represents the relation.

The final model fitted was

$$y_{ij} = \alpha + \beta x_{ij} + \gamma x_{ij}^2 + R_{ij}$$

which is one common quadratic curve. Comparison of these models tested the

$$H_o: \alpha_1 = \alpha_2 = \alpha_3 = \alpha_4 = \alpha_5 = \alpha_6$$

versus

$H_a\ \alpha_r \neq \alpha_s$ where r and s belong to 1...6.

On this occasion the observed variance ratio was highly significant; thus H_o was rejected and the relationship between area and pod length over the six genotypes was best described by six parallel curves.

In each case when comparing a complex to a simpler model, the observed variance ratio was calculated using the following formula:

$$\text{variance ratio}_{obs} = \frac{(\text{Regression SS}_{(complex)} - \text{Regression SS}_{(simple)})/(\Delta \text{ in df})}{\text{Residual MS}_{(complex)}}$$