Field screening of *Pisum* accessions to evaluate their susceptibility to the pea weevil (Coleoptera: Bruchidae)

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Summary

Seventeen unreplicated field trials over nine sites and four years were used to classify *Pisum* germplasm (*P. sativum* L. & *P. fulvum* Sibth. & Sm) as potential sources of resistance to the pea weevil, *Bruchus pisorum* (L.). The emergence of adult weevils from < 10% of harvested seed was used as the selection criterion to indicate possible resistance. A total of 1900 *Pisum* accessions were assessed using the field trials and 1754 of the *P. sativum* accessions were eliminated. However in the 18 *P. fulvum* accessions screened, the level of infestation by pea weevil was always below the arbitrary resistance threshold selected. This suggests that *P. fulvum* accessions could be a valuable source of resistance to the pea weevil.

Introduction

Pea cultivars (*Pisum sativum* L.) developed for human consumption are threatened by one of the most damaging insect pests of field peas in Australia, the pea weevil (*Bruchus pisorum* (L.)). Pea weevil reduce the yield and quality of a field pea crop by consuming a large part of the seed they infest (Michael et al., 1993). Seed infested with pea weevil also has an increased like-lihood of shattering from the harvesting process and this also affects the appearance of the grain because of higher levels of shattered seed which in turn reveals the presence of weevil larvae in seed (Baker, 1990a).

The pea weevil is a univoltine species (Brindley et al., 1946). In Australia adult weevils leave sites where they have spent the cooler months and arrive in pea crops in late winter to early spring. They may arrive as early as mid-August, but most years they arrive in early September (Baker, 1990b). Estimates of fecundity range from three (Panji & Sood, 1976) to 735 (Brindley, 1939) eggs per female. Presumably the large variation in fecundity is related to the fitness of individuals used. The bright yellow-orange eggs are laid singly on the surface of pods and the eggs usually hatch in three

to five days, 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 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 (Smith, 1990). Adults emerge from the seed leaving a large exit hole. The emergence of adults from seed occurs over summer from unharvested crops and harvested seed in storage or in the following year from seek sheltered sites to spend the winter and remain there until the following spring.

The damage caused to a crop can be reduced by monitoring for the invasion of weevils and adopting a spraying strategy. This can minimise losses and ensure that most seed is acceptable for milling, though in some instances seed cleaning is still necessary. A high level of weevil control 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 difficult to see at harvest. The size and duration of the weevil invasion determines the number of types of sprays needed. Most chemicals registered in Australia provide protection for a maximum of seven days (Michael et al., 1993). Invasions can continue for many weeks in some seasons, thus requiring several sprays (Michael et al., 1990; Baker & Phillips, 1992). Chemical and application costs severely reduce the profitability of growing peas in Australia. There are also concerns about the impact of spray drift on the environment and the marketing of pea grains with insecticide residues.

The limited success of cultural methods of control, the failure of biological control (Wilson, 1960; Clausen, 1978) and the reliance on expensive insecticides demonstrates the need for alternative control measures. None of the field pea cultivars grown in Australia have any known resistance to pea weevil, but this remains one of the most attractive options for reducing the impact of this pest on field pea production (S.M. Ali, South Australian Department of Primary Industries, personal communication).

Resistance to the pea weevil in the genus *Pisum* has been reported in the literature from Russia and the USA (Aleksandrova, 1977; Pesho et al., 1977; Posylaeva, 1988). Accessions identified as being resistant have reportedly been incorporated into breeding programs in both countries and advanced pea lines were introduced into Australia from the USA program for resistance screening against the pea weevil (Ali, 1984). The subsequent field evaluation of this material indicated that none of the accessions imported from the USA were resistant (S.M. Ali, South Australian Department of Primary Industries, personal communication).

These field trials were an essential step in developing pea weevil resistant cultivars because they eliminated most of the susceptible germplasm in the *Pisum* gene pool and allowed the authors to direct their research at a small number of accessions for resistance to the pea weevil.

Materials and methods

Germplasm

Pisum germplasm was obtained from nine collections. All the *Pisum* germplasm available in the Australian collections was obtained, this included breeding lines as well as wild and landrace accessions. Many of the accessions received were later found to be duplicates, but were kept as separate accessions for field screening purposes in case the passport information accompanying them was incorrect. Germplasm used in trials by Pesho et al. (1977) was imported from the USDA, while wild pea types, landrace accessions and older cultivars were forwarded from the John Innes collection.

Trial details

A total of 1882 P. sativum and 18 P. fulvum accessions were evaluated for resistance in 17 field trials over four seasons and nine sites. Forty non-Pisum accessions from the tribe Vicieae were included in the 1989 trials because of a report that pea weevil has attacked the faba bean (Vicia faba L.) in Iraq (Al-rawy & Kaddou, 1971). A review by Johnson (1981) also lists V. faba as a host species along with Lathyrus sativus L., L. odoratus L. and Vicia leucantha Biv. The non-Pisum accessions used were from the following species: Lathyrus cicera L., L. ochrus (L.) DC, L. sativus L., L. tingitanus L., L. inconspicuus L., Lens culinaris Med., V. sativa L. ssp. cordata (Wulfen ex Hoppe) Asch & Graebner, V. Cracca L., V. ervilea (L.) Wild, V. faba, V. lathyroides L., V. lutea L., V. narbonensis L., V. sativa L. and two unnamed Vicia sp.

A unreplicated trial design was used to screen most of the pea germplasm because it enabled a large number of accessions to be tested using the small number of seeds available for most genotypes. The repetitive evaluation of genotypes over several years, or in more than one plot/year if seed was available was used as the basis for identifying the majority of susceptible germplasm.

Screening of germplasm in small unreplicated plots was an effective way of eliminating the greater proportion of accessions. Unfortunately it did not allow some accessions to be properly evaluated. Within a selffertilizing species, such as peas, there may be some genetic variation 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 ICARDA, USDA and John Innes because of the cost of processing accessions through quarantine in Australia, 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 the pot trials 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 seeds or fewer would probably not represent all the genotypes in a wild or landrace accession.

Two forms of unreplicated trials were used depending on the type of seed provided. They were the field plot and the pot trial.

Field plots

Field plots were used for accessions where additional seed could easily be obtained if necessary. In the 16 South Australian trials, 11 were hand sown into field plots consisting of one metre rows (20 seeds per accession), with half a metre space between accessions and a one metre row spacing. All seed sown in the South Australian trials received a fungicidal seed dressing of P-Pickle^(R) (480 g/kg thiram & 266 g/kg thiabendazole) to protect germinating seed from fungal attack. A single trial was sown in Western Australian to screen the majority of genotypes that were unique to the Western Australian Department of Agriculture collection. This trial was machine sown and without a seed dressing, using five metres of row per accession and a row spacing of one metre.

Pot trials

Five pot trials were set up to screen pea genotypes which were available in limited quantities and where additional seed could not be easily obtained. A maximum of ten seeds/genotype was sown in sterilised soil into a nine litre plastic pot. 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. All seed sown into pots received a fungicidal seed dressing of P-Pickle[®]. Water was supplied to the pots by overhead sprinklers or drippers. A slow release fertiliser (18% N, 4.8% P, 9.1% K & 3.7% S) was added to all potted material once it had germinated. Pots were spaced at about 750 mm centres to reduce inter-twining of plants of different accessions.

Seed from all 288 CSIRO pea accessions, many of which had been in storage for many years was sown into pots in a bird proof enclosure to protect it from pigeon attack. The seed was surface sterilised to reduce fungal and bacterial seed contamination, with a 10% sodium hypochlorite solution for five minutes, then rinsed in distilled water. Seed was placed on moist filter paper in petri dishes in an incubator set at 25° C to optimise germination. Only healthy seedlings were transplanted into pots. This procedure was also used with accessions which did not emerge in field plots. All accessions suspected of being hard-seeded were scarified prior to sowing to initiate the germination process in seed by allowing water uptake. It scarified accession did not germinate in the field, attempts were made to germinate the seed on filter paper in petri dishes before sowing. Accessions which did not germinate on filter paper were surface sterilised and grown under sterile conditions on a standard PDA nutrient agar plate containing 100 ppm streptomycin sulphate (R. Cook, South Australian Department of Primary Industries, personal communication). These procedures increased the number of accessions that were screened for pea weevil resistance.

Weevil releases

Three of the field sites used to screen pea germplasm in pea growing areas were less than two kilometres from paddocks where pea crops were attacked each year by pea weevil. This indicated that large numbers of adult pea weevil were likely to be attracted to each of these trials sites. All other sites had pea seed infested with adult weevils scattered in the trial area from the beginning of September until flowering ceased. It was estimated that around 2000 weevils emerged from each lot of infested seed used. This procedure provided weevils for the entire flowering and podding period.

Field observations

The flowering date of each accession used in a trial was recorded to determine if pods suitable for oviposition were available when sexually mature pea weevil were active in the field. Flower colour was also recorded to determine if this morphological character was linked to a possible resistance mechanism. Several Australian field pea cultivars were used as controls at each site to compare with the level of pea weevil infestation in the test accessions.

Germplasm assessment

All trials were hand harvested to conserve the limited quantities of seed, to maximise the collection of dehiscing accessions and to decrease splitting of infested seed. In 1989 the harvested seed was stored at room temperature in seed envelopes and two months after the adult weevils began emerging from the control acces-

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% emergence (x)	All accessions		P. fulvum	accessions	P. sativum accessions		
	Number	(plots sown)	Number	(plots sown)	Number	(plots sown)	
x = 0	33	(177)	9	(39)	24	(138)	
0 < x < 5	11	(82)	7	(58)	4	(24)	
5 < x < 10	12	(76)	2	(22)	10	(54)	
x = 10	14	(95)	0	(-)	14	(95)	
not categorised. ^A	76	(384)	0	(–)	76	(384)	
Totals	146	(814)	18	(119)	128	(695)	

Table 1. Number of Pisum accessions with 10% or less adult pea weevil emergence from all the sites they were sown at between 1989 and 1992

^A not enough seed was harvested to make a valid assessment on the level of weevil infestation.

sions assessed for the number of weevils that completed their life cycle. When this was repeated in 1990, a species of straw itch mite (*Pyemotes herfsi* Oudemans), a predator of pea weevil larvae 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 stored in the cool room was left for four months before being scored for the emergence of adult weevils. To reduce the likelihood of a mite infestation in 1991 and 1992 seed harvested from all plots was kept at 15° C until it could be assessed for the emergence of adult weevil.

A minimum of 20 seeds/plot was sampled if available, accessions were considered susceptible and discarded when more than two seeds in 20 or > 10% of a larger sample were infested with adult weevils. However accessions which yielded as few as three seeds per plot were discarded if all seeds were infested. A seed was classed as infested, if at the time of sampling a live larva or an exit hole was found.

The method of harvesting plots led to a bias against wild and landrace material because seed harvested from individual plants within plots were pooled to reduce the processing time for samples. The pooling procedure did not allow for the presence of a single resistant plant among non-resistant genotypes when the samples were evaluated. The use of unreplicated plots also rules out a rigorous statistical separation of accessions and only provides a result for the weevil's preference because of the highly mobile nature of this species. The > 10% limit was intended to remove the obviously susceptible accessions.

Results

Pisum accessions

After four years of screening trials 1754 of the original 1882 P. sativum accessions had been discarded and 76 could not be categorised because they did not flower or set sufficient seed. The remaining 52 P. sativum and all 18 P. fulvum accessions were categorised as potential sources of resistance to the pea weevil (Table 1). These results were obtained from all field plot and pot trials, and the mean infestation rate of control cultivars at these same sites was between 7.5 and 100%. It is important to note that of the 18 P. fulvum accessions obtained for screening purposes, adult weevils did not emerge from more than 10% of seeds sampled from any plot (Table 1). Furthermore adult weevils never emerged from seed of nine of the P. fulvum accessions across all sites (Table 1). When pea weevil emergence from seed is restricted to Pisum accessions harvested from a minimum of three sites, 21 accessions were found to have adult emergence levels of 10% or less and eleven of these were P. fulvum accessions (Table 2). Only seven of the Pisum accessions harvested from at least three sites were not infested and four of these seven were P. fulvum accessions (Table 2).

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. The *P. fulvum* germplasm also flowered and podded in the same period as the *P. sativum* accessions identified by Pesho et al. (1977) and as a Russian cultivar (VIR 4739) obtained from the South Australian Department of Primary Industries collection and designated as resistant to pea weevil. The individual

Table 2. Pisum accessions with adult pea weevil emergence not above 10% and evaluated in at least three trial sites between 1989 and 1992

Accessions number(s) ^A	Species	Highest recorded emergence (%)
PIG 49 (= PI 343955, CPI 62379)	P. fulvum	0.0
ATC 113	P. fulvum	0.0
JI 849	P. fulvum	0.0
JI 1006	P. fulvum	0.0
PIG 148	P. sativum	0.0
ATC 124	P. sativum	0.0
ATC 167	P. sativum	0.0
PIG 111	P. fulvum	0.1
PIG 296 (= NGB 1256, JI 1392)	P. fulvum	0.2
SA 1607 (= VIR 3397, JI 2204)	P. fulvum	1.0
ATC 114	P. fulvum	2.5
PIG 112	P. fulvum	3.3
PIG 277 (= CPI 53306)	P. fulvum	5.0
SA 1602 (= VIR 2523, JI 2203)	P. fulvum	5.0
NGB 1352 (= SA 659)	P. sativum	5.0
ATC 308	P. sativum	5.0
ATC 12	P. sativum	10.0
SA 516	P. sativum	10.0
SA 1408	P. sativum	10.0
ATC 315	P. sativum	10.0
PI 164758	P. sativum	10.0

^A PIG = Plant Industries Genetics, CSIRO, NGB = Nordic Gene Bank, ATC = Australian Temperate Field Crops Collection, JI = John Innes Institute, SA = South Australian Department of Primary Industries, PI = Plant Introductions, USDA, VIR = Vavilov Institute of Plant Industry (St Petersburg, Russia), CPI = Commonwealth Plant Introductions, CSIRO (Canberra, Australia).

adult emergence results for the American accessions showed that all except PI 164758 were discarded from the screening program. While PI 164758 was the best of the American accessions it was nearly discarded from the screening program in 1990 after pea weevil emerged from 10% of seed from this accession in the bird proof enclosure (Table 3). A similar result was obtained for these accessions when they were screened for pea weevil resistance in Chile in 1981 (M. Gerding, Instituto de investigaciones Agropecurias, personal communication) (Table 3). The effect of flower colour on the level of pea weevil infestation was inconclusive (data not shown). Flower colour varied greatly in the *P. sativum* accessions which were listed as possible sources of resistance to the pea weevil, but it was not correlated with infestation level. However all the *P. fulvum* accessions screened had orange-brown coloured flowers which is characteristic of this species.

Non-Pisum accessions

Seed from the non-*Pisum* genera in the tribe Vicieae was harvested from three sites in 1989. The majority of these accessions were from a lightly infested site, though accessions harvested from other sites were more heavily infested by the pea weevil. All seed from non-*Pisum* accessions was free of weevil damage and the evidence suggests these genera are not chosen for oviposition and are therefore resistant to the pea weevil.

Discussion

It is clear from the field screening trials that none of the Australian pea cultivars used have any resistance to the pea weevil and that material reported to be weevil resistant imported into Australia from the USA and the Russia does not have the required level of resistance either. The failure of P. sativum material classified by Pesho et al. (1977) in the USA as highly resistant when evaluated in Australia and Chile is puzzling, although Pesho and his colleagues indicated adult weevils had the choice of many accessions on which to oviposit. 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 suggested 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 weevil are searching for oviposition sites.

The trial results show that *P. fulvum* has 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 for the *P. fulvum* and *P. sativum* accessions indicated that pods of both species were available for oviposition when adult weevils were active in the field. Although many accessions, especially those of *P. fulvum*, perform well when a choice of genotypes is available, there is a need to determine how they will perform when a choice is not available. This would provide information on how the pea weevil might respond to cultivars derived from this material.

Accession	Seed supporting weevil development (%)									
	US ^A 1972	US ^A 1974	CH ^B 1981	BC 1990	GP 1990	TF 1990	WP 1991	NF 1991	NF 1992	
										PI 164304
PI 164758	1.3		42.2	10.0			5.0	9.3		
PI 165949	4.0		42.0	15.0	65.0 ^C		0.0			
PI 166051	0.0							32.5		
PI 174917	2.9			30.0, 60.0 ^C		5.9				
PI 174919	1.8			45.0, 20.0 ^C		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				

Table 3. The level of pea weevil infestation (%) from accessions screened in the USA, Chile and Australia

* no seed harvested.

^A Results published by Pesho et al. (1977).

^B Results obtained through M. Gerding, Instituto de investigaciones Agropecurias, Chile.

^C Adult emergence results from more than one plot at the same site.

US = United States of America. CH = Chile. Australian sites (BC = Waite campus bird proof enclosure, GP = Waite campus glasshouse plot, TF = Turretfield research station, WP = Waite campus field site, NF = Northfield field site).

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, unlike the reports of Al-rawy & Kaddou (1971) and Johnson (1981) who list the pea weevil as a pest of faba beans. This result was disputed by Tahhan & van Emden (1989), who believed the pea weevil had been confused with *Bruchus dentipes* Baudi on 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 remain resistant for years, because other legume species do not appear to be hosts for the pea weevil.

Taxonomists generally regard *P. fulvum* to be separate species from *P. sativum*. Ben-Ze'ev & Zohary (1973) have found *P. fulvum* growing along side *P. sativum* ssp. *elatius* and *P. sativum* ssp. *humile*, but have found no evidence of spontaneous hybridisation with *P. fulvum* or introgression of distinctly *P. fulvum* genes into other wild *Pisum* species. They believe 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. Pods of dehiscent accessions may shed their seed before being harvested in screening trials as a result of damage caused by weevil larvae. To avoid this, pods on dehiscent lines in these trials were harvested as they began to dry off.

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 in Australia or elsewhere. It is conceivable that fewer than 1000 accessions of the 1900 screened were distinct accessions. This is probably a small proportion of the total pea gene pool and could mean that any resistant genotypes identified were a small proportion of the number that exists. It is also possible that resistant genotypes could have been overlooked if they were included in a mixture, as often occurs in landraces. The findings presented in this paper indicate that there are possible sources of resistance to the pea weevil present in some *Pisum* accessions. However the mechanisms of resistance and their inheritance need to be understood before resistant varieties can be developed and an appropriate breeding strategy for pea weevil resistance established.

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