Variation among Accessions of *Pisum fulvum* for Resistance to Pea Weevil

S. L. Clement,* D. C. Hardie, and L. R. Elberson

**ABSTRACT**

The pea weevil, *Bruchus pisorum* (L.) (Coleoptera: Bruchidae), is one of the most intractable pest problems of cultivated pea, *Pisum sativum* L. The availability of resistant cultivars would give growers more pest management options. Searches for plant resistance to pea weevil were expanded to the *Pisum* secondary gene pool (*P. fulvum* Sm.) because seed resistance had not been located in *P. sativum* and subspecies. The objectives of this study were to determine the extent of pod and seed resistance to pea weevil in *P. fulvum*, and to use the life table format to characterize weevil stage-specific mortality and survivorship on different *P. fulvum* accessions. Mortality of first instar larvae on pods, mortality of all weevil stages within seed, adult emergence from seed, and seed damage levels were quantified. In two greenhouse trials, more larvae died (14 to 50% averages) on pods of *P. fulvum* accessions than on pods of ‘Alaska 81’ (6% average), and mortality of first instar larvae entering seed of *P. fulvum* accessions averaged 83.7%. Seed damage ratings (1 = feeding scar on seed testa, 0-1% cotyledon tissue eaten, dead first instar larva; 5 = extensive damage, live adult) averaged < 3.0 for 26 *P. fulvum* accessions, compared with mean ratings of 4.9 for Alaska 81. Using weevil mortality and survivorship values in life tables and adult emergence rates, entries were classified as susceptible (two controls and five accessions), moderately resistant (14 accessions), and resistant (12 accessions). Antibiiosis resistance was based on the death of weevil larvae on pods and seed testa and cotyledon tissues. The results identify sources of natural weevil resistance in the *Pisum* genome (26 moderately resistant and resistant accessions of *P. fulvum*) to endow pea cultivars with pod and/or seed resistance to *B. pisorum*.

**INSECT PESTS** are a major problem in the worldwide production of field pea, with the pea weevil being one of the most destructive pests of this grain legume (Clement et al., 2000). Adult weevils leave winter hibernation sites and invade pea fields in the spring to feed on pea pollen and other parts of the pea flower (Brindley, 1933; Clement, 1992). Females must feed on pollen before they can lay eggs and pea pollen is most effective in promoting oogenesis (Peshe and Van Houten, 1982). Eggs are laid on the surface of immature pea pods and hatch to produce first instar (neonate) larvae that chew through the pod wall from the underside of eggs. Once a neonate larva bores through a pod wall and reaches the inside of a pod, it soon locates and starts to feed on a developing seed (Brindley, 1934). There are four larval instars, with second, third, and fourth instars consuming a large part of the cotyledon contents of seed (Brindley, 1933; Smith, 1990). Before pupating, a larva chews a circular window in the seed testa that is pushed open by the emerging adult. Larval consumption of seed contents reduces yield, while feeding scars and holes on testa reduce the quality and marketability of pea seed. In addition, weevil-damaged seed has lower germination rates and is prone to structural weakening during harvest (Brindley and Hinman, 1937; Baker, 1990). High levels of weevil-infested seed have been reported in Australia (10.6 to 71.5%; Horne and Bailey, 1991), Spain (12.2 to 25.7%; Marzo et al., 1997), and the USA (up to 64%; Peshe et al., 1977; Bragg and Burns, 2000).

Worldwide, pea producers rely mainly on contact insecticides to control adults in pea fields before females lay eggs on pods (Horne and Bailey, 1991; O’Keefe et al., 1992; Clement et al., 2000). However, timing chemical applications to coincide with female egg laying is difficult. More than one application may be required if weevil invasions continue for 2 to 4 wk in a pea field (Michael et al., 1990). The development and use of cultivars with pod and seed resistance to *B. pisorum* would reduce control costs and provide an environmentally safer option than contact insecticides for adult weevil control.

Some *P. sativum* lines with the *Np* gene respond to the presence of pea weevil eggs on pods by forming callus (neoplastic pod trait) that reduces larval entry into the pod (Hardie, 1990; Berdnikov et al., 1992; Doss et al., 2000). In a field trial, this pod-based resistance was responsible for a lower rate of weevil infested seed (62.2%) in *Np* plants compared with that in a susceptible line (85.4%) (Doss et al., 2000). In addition, plant biotechnology has the potential to protect peas from *B. pisorum* damage, as evidenced by the development of transgenic *P. sativum* for resistance to *Callosobruchus* weevils (Shade et al., 1994) and *B. pisorum* (Schroeder et al., 1995; Morton et al., 2000). This resistance is based on the insecticidal activity of the α-amylase inhibitor in seeds of bean, *Phaseolus vulgaris* L., which blocks the action of the starch-digesting enzyme α-amylase and thus prevents weevil larvae from digesting starches in seed. More research is required before weevil-resistant transgenic peas can be released to growers (Morton et al., 2000). Moreover, any concerns about the biosafety of genetically engineered peas, as voiced by consumer and environmental groups for transgenic crops (Stewart et al., 2000), must be addressed by researchers and producer groups before transgenic peas are commercially deployed for weevil protection.

An alternative to genetically engineered crops is the development and deployment of crop cultivars with natural insect resistance from primary and secondary gene pools. In the absence of seed resistance to pea weevil in the *Pisum* primary gene pool (*P. sativum* and all subspecies) (Hardie, 1990; Clement et al., 1994), searches for resistance were expanded to the secondary gene

---

*S.L. Clement and L.R. Elberson, USDA–ARS Plant Germplasm Introduction and Testing Research Unit, Washington State Univ., Pullman, WA 99164-6402; D.C. Hardie, Entomology Section, Dep. of Agriculture Western Australia, 3 Baron-Hay Court, South Perth, WA, 6151 Australia. Received 3 Aug. 2001. *Corresponding author (slelement@wsu.edu).


**Abbreviations**: GRIN, Genetic Resources Information Network.
pool in the late 1980s, resulting in the discovery of seed resistance in *P. fulvum* during field evaluations (Hardie et al., 1995; 1999). This wild species from the eastern Mediterranean and Near East areas, where pea domestication took place (Zohary, 1973) and where *B. pisorum* is native (Clement et al., 1999), is the only species in the secondary gene pool (Muehlbauer et al., 1994). Because *P. fulvum* is cross-compatible with *P. sativum* when it is used as the pollen parent (Muehlbauer et al., 1994), conventional plant breeding could potentially transfer *P. fulvum* resistance traits to domesticated pea. Further consideration of *P. fulvum* as a potential source of weevil resistance genes for cultivar development requires detailed knowledge of the level and extent of seed resistance and identification of pod-based resistance in this species.

Data from life tables (Poole, 1974, p. 11–15; Southwood, 1978, p. 366–369) may provide insight into the genetics of plant resistance to insects if the resistance through high insect mortality is expressed by different plant parts. Indeed, the expression of resistance by different plant parts (i.e., a pod factor and seed abortion) can be under the control of different genes (Rusoke and Fatunla, 1987; Kennedy and Barbour, 1992). The objectives of this research were (i) to determine the extent of pod and seed resistance to pea weevil in *P. fulvum* accessions, and (ii) to use the life table format to characterize weevil stage-specific mortality and survival on *P. fulvum* accessions.

**MATERIALS AND METHODS**

**Plants and Insects**

Research was conducted in the USA (Pullman, WA) and Australia (South Perth, WA). Seed of *P. fulvum* accessions for a South Perth trial was acquired by D.C. Hardie in the late 1980s from several seed repositories (Hardie et al., 1995). Seed of 29 *P. fulvum* accessions were acquired from several sources (D.C. Hardie, Agriculture Western Australia, South Perth, Australia; J.G. Waines, University of California, River-side, CA, USA; John Innes Institute, Norwich, UK; Nordic Gene Bank, Alnarp, Sweden) for Pullman trials. Before this study, the *Pisum* germplasm collection at the USDA-ARS Western Regional Plant Introduction Station, Pullman, WA, USA contained viable seed of only two accessions of *P. fulvum* (PI accessions 343955 and 531199). The Genetic Resources Information Network (GRIN) (http://www.ars-grin.gov/npgs/) lists the original collection sites for only 13 of the accessions (10 from Israel, two from Turkey, one from Jordan) in this study.

Field-infested pea seeds were harvested at Farmington, WA, USA (46°43′ N, 117°9′ W) and York, WA, Australia (31°52′ S, 117°48′ E) and stored at 4°C (Pullman) or 10°C (South Perth), for 7 to 12 mo before greenhouse trials were conducted at each location. Adults emerging from infested peas were sexed using the dimorphic character described by Pesho and Van Houten (1982). Five to ten pairs were placed in cages and allowed to feed on pollen, which was in the form of pellets from commercial honey bee hives (Healthy Life Carousel, Cammington, WA, Australia) in South Perth cages (130 × 200 × 80 mm3 clear plastic). Fresh flowers of greenhouse-grown *P. sativum* ‘Alaska 81’ provided pollen in Pullman cages (125 × 175 × 45 mm3 clear plastic) at a rate of 10 to 15 flowers every 2 to 3 d for 10 to 14 d per cage. After 7 to 10 d, two or three flat or swollen pea pods from greenhouse-grown *P. sativum* were placed each day in the Pullman (Alaska 81) and South Perth (‘Pennant’) cages as oviposition substrates. In both environments, mated females began to lay fertile eggs on excised pea pods after feeding on pollen for 7 to 14 d. Cages were held at 25 ± 2°C and a 12- to 14-h photophase during feeding, reproduction, and egg laying on pea pods.

**Experimental Protocol**

In Pullman, seeds of wild *P. fulvum* accessions were germinated following methods described by Kaiser et al. (1997) for wild *Cicer* seed. Seeds were scarified by scoring testsa with sandpaper before they were placed in labeled cheese cloth bags in 1000-ml beakers filled with distilled water that was aerated with laboratory-supplied air. Every 2 d, the water in each beaker was changed and any germinated seeds were removed for planting. *P. sativum* ‘Alaska 81’ seeds were germinated in a similar fashion except they were not scarified.

Newly germinated seeds were planted individually in 15-cm pots containing a commercial soil mix and grown in a greenhouse between March and July 1995 and 1996, without any fertilizer. No pesticides were applied. The 1995 and 1996 trials evaluated 22 and 12 *P. fulvum* accessions, respectively. However, a total of 31 accessions were evaluated because three accessions were included in both trials. A pea weevil-susceptible cultivar (Alaska 81) of *P. sativum* (Clement et al., 1996; Hardie et al., 1999) was included in each trial. Entries (PI accessions and susceptible control) were arranged on two greenhouse benches in a completely randomized design with five replications (potted plants).

Both greenhouse trials were conducted under prevailing natural light/dark cycles and 15 to 21°C nighttime and 23 to 28°C daytime temperatures.

Mature weevil eggs (dark brown head visible through chorion) were transferred from oviposition cages with a moistened fine-tipped brush to surfaces of pods in the late flat and early swollen pod stages (Meichenheimer and Muehlbauer, 1996; Hardie et al., 1999) was included in each trial. Entries (PI accessions and susceptible control) were arranged on two greenhouse benches in a completely randomized design with five replications (potted plants). Both greenhouse trials were conducted in Pullman (south of greenhouse pool in the late 1980s, resulting in the discovery of seed resistance in *P. fulvum* during field evaluations (Hardie et al., 1995; 1999). This wild species from the eastern Mediterranean and Near East areas, where pea domestication took place (Zohary, 1973) and where *B. pisorum* is native (Clement et al., 1999), is the only species in the secondary gene pool (Muehlbauer et al., 1994). Because *P. fulvum* is cross-compatible with *P. sativum* when it is used as the pollen parent (Muehlbauer et al., 1994), conventional plant breeding could potentially transfer *P. fulvum* resistance traits to domesticated pea. Further consideration of *P. fulvum* as a potential source of weevil resistance genes for cultivar development requires detailed knowledge of the level and extent of seed resistance and identification of pod-based resistance in this species.

Data from life tables (Poole, 1974, p. 11–15; Southwood, 1978, p. 366–369) may provide insight into the genetics of plant resistance to insects if the resistance through high insect mortality is expressed by different plant parts. Indeed, the expression of resistance by different plant parts (i.e., a pod factor and seed abortion) can be under the control of different genes (Rusoke and Fatunla, 1987; Kennedy and Barbour, 1992). The objectives of this research were (i) to determine the extent of pod and seed resistance to pea weevil in *P. fulvum* accessions, and (ii) to use the life table format to characterize weevil stage-specific mortality and survival on *P. fulvum* accessions.
but was germinated on moistened filter paper. Five germinated seeds per entry were planted in a 25-cm pot containing washed river sand and top dressed with a slow release granular fertilizer (Osmocote, The Scotts Company, Marysville, OH). No pesticides were applied. One trial was conducted in 1993 with 23 *P. fulvum* accessions (also in Pullman trials) and the Pennant control. The 24 pots were randomly arranged on a greenhouse bench. The greenhouse trial was conducted under the prevailing natural light/dark cycle and temperatures ranged from 10 to 32°C.

Each pod on South Perth plants received eight mature eggs, but a variable number of pods per entry were infested (5 to 28 pods per entry 1 to 6 pods per plant). Pods were harvested at maturity and stored at room temperature in labeled seed envelopes. After 60 d, when adult weevils began emerging from Pennant seeds, all seeds were inspected and assessed for adult emergence. A seed was classified as infested if a live pupa or adult was found in the seed or an adult exit hole was found.

### Data Analysis

A few eggs on pods did not hatch in the Pullman trials, leaving a starting cohort of 7 to 10 on plants (range of 46 to 50 eggs per entry). The absence of feeding and penetration marks on testa was used to calculate the number of larvae dying en route to the outer surfaces of developing seeds inside green pods. Larvae on each infested pod were categorized as 0, 1, or 2 dead if feeding damage was recorded on 2, 1, or 0 seeds, respectively. Within-seed mortality of *B. pisorum* was compartmentalized, as follows: first instars consuming 0 to 5% of the cotyledon tissue (Damage Ratings 1 and 2); second to fourth instars consuming >5% of the cotyledon tissue (Damage Rating 3); and development to prepupa or adult with extensive damage to seed contents (Damage Ratings 4 and 5).

The life table format (Poole, 1974, p. 11–15; Southwood, 1978, p. 366–369) was previously used to record numerical changes in arthropod populations on cotton, *Gossypium hirsutum* L., (Trichilo and Leigh, 1985) and cowpea, *Vigna unguiculata* (L.) Walp., cultivars (Messina, 1984) and was used to quantify pea weevil mortality and survival on *P. fulvum* accessions and a susceptible pea cultivar (Alaska 81) in the Pullman trials. Parameters estimated in the life table analysis for pea weevil on each plant (replicate) of each entry were: *l*, the number of individuals entering stage *x*; *d*, the number of individuals dying in stage *x*; 100*q*, the percentage mortality in stage *x*; and 100*S*, the percentage surviving stage *x*. Instead of presenting these values in complete life tables for all pea weevil-entry interactions, tables are presented for *B. pisorum* on three PI accessions of *P. fulvum* in which values were generated from starting entry-cohorts of 48 or 50 neonate larvae (Table 1). This approach was used to conserve space while illustrating the structure of three representative life tables. To simplify the presentation of all life table results and to facilitate assessment of pod and seed resistance among entries, entry means of three parameters were statistically analyzed: (i) mortality [100*q*] of first instar larvae before reaching seed; (ii) mortality [100*q*] of all weevil stages within seed; and (iii) survival [100*S*] to the adult stage (Tables 2 and 3).

The *F*<sub>max</sub> test, a sample variance ratio procedure (Sokal and Rohlf, 1981, p. 403), was used to ensure that the assumption of homogeneity of variances was met before data were analyzed. Nonsignificant heterogeneity of variances justified analysis of variance on all original data sets (seed weights, life table parameters [100*q*], seed damage ratings, and adult weevil weights). Entry means for life table parameters and seed damage ratings were computed from replicate (*n* = 5 plants) means, whereas mean seed weights were based on 20 undamaged seeds per entry and mean adult weevil weights were calculated from all emerging adults per entry (Tables 2 and 3). General linear model procedures were used for unbalanced data and entry means were compared by Fisher’s least significant difference (LSD0.05). Pearson’s correlation coefficients were calculated to examine relationships between mean seed weights and mean within-seed mortality rates and between mean seed weights and mean seed damage ratings of *P. fulvum* accessions (SAS Institute, 1987). Since the entries differed among years (except for four entries), data from each year were analyzed separately. Results from the South Perth trial were expressed as percentage adult weevil emergence from seed of all egg-infested pods per entry.

### RESULTS AND DISCUSSION

There was significant (*P* < 0.05) variation among the entries in each of the Pullman trials for larval mortality before reaching seed (100*q*<sub>1</sub>) and within-seed (100*q*<sub>2</sub>) seed damage rating, adult emergence (100*S*<sub>1</sub>), and weight of emerging adults. As expected, Alaska 81 was...
Table 2. Mortality and survivorship of *Bruchus pisorum* on *Pisum sativum* ‘Alaska 81’ and accessions of *P. fulvum*, and levels of weevil-inflicted seed damage from a greenhouse study at Pullman, WA, in 1995.

<table>
<thead>
<tr>
<th>Entry†</th>
<th>Seed wt.‡</th>
<th>Larval mortality (100q,§)</th>
<th>Seed damage rating¶</th>
<th>Emergence (100q),§</th>
<th>Wt.#</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before reaching seed</td>
<td>Within seed</td>
<td>%</td>
<td>%</td>
<td>mg</td>
<td>%</td>
</tr>
<tr>
<td>‘Alaska 81’</td>
<td>240.0a††</td>
<td>6.0a</td>
<td>8.9a</td>
<td>4.8a</td>
<td>86.0a</td>
<td>12.8a</td>
</tr>
<tr>
<td>PI 560065</td>
<td>77.4e</td>
<td>29.1bcdef</td>
<td>14.4a</td>
<td>4.5ab</td>
<td>60.4b</td>
<td>8.7b</td>
</tr>
<tr>
<td>PI 595941</td>
<td>78.3d</td>
<td>22.7abcd</td>
<td>37.1b</td>
<td>4.1bc</td>
<td>46.4bc</td>
<td>7.0cede</td>
</tr>
<tr>
<td>PI 595938</td>
<td>77.2de</td>
<td>20.0bcdef</td>
<td>46.4bc</td>
<td>3.6cd</td>
<td>42.2bc</td>
<td>8.5bc</td>
</tr>
<tr>
<td>PI 595953</td>
<td>73.8e</td>
<td>18.4abc</td>
<td>53.8c</td>
<td>3.3cd</td>
<td>38.7c</td>
<td>8.0bcde</td>
</tr>
<tr>
<td>PI 595943</td>
<td>69.1ef</td>
<td>22.7abcd</td>
<td>59.5c</td>
<td>3.3cd</td>
<td>32.4c</td>
<td>6.4bcde</td>
</tr>
<tr>
<td>PI 595939</td>
<td>90.4b</td>
<td>26.0bcde</td>
<td>84.4d</td>
<td>2.1fgh</td>
<td>8.0d</td>
<td>8.0bcde</td>
</tr>
<tr>
<td>PI 595982</td>
<td>81.7cd</td>
<td>24.0bcde</td>
<td>85.4d</td>
<td>2.3fgh</td>
<td>8.0d</td>
<td>6.3ef</td>
</tr>
<tr>
<td>PI 560064</td>
<td>60.2g</td>
<td>18.7abc</td>
<td>92.7d</td>
<td>2.3fgh</td>
<td>6.0d</td>
<td>4.8f</td>
</tr>
<tr>
<td>PI 595942</td>
<td>55.9gh</td>
<td>34.0def</td>
<td>90.0d</td>
<td>2.2fgh</td>
<td>6.0d</td>
<td>5.5ef</td>
</tr>
<tr>
<td>PI 595951</td>
<td>58.7g</td>
<td>36.0cdef</td>
<td>92.5d</td>
<td>2.2fgh</td>
<td>6.0d</td>
<td>5.5ef</td>
</tr>
<tr>
<td>PI 595948</td>
<td>94.9b</td>
<td>14.7ab</td>
<td>95.0d</td>
<td>2.2fgh</td>
<td>6.0d</td>
<td>5.5ef</td>
</tr>
<tr>
<td>PI 343955</td>
<td>87.9bc</td>
<td>30.4cdef</td>
<td>96.0d</td>
<td>2.2fgh</td>
<td>6.0d</td>
<td>5.5ef</td>
</tr>
<tr>
<td>PI 595937</td>
<td>73.7de</td>
<td>46.5f</td>
<td>97.5d</td>
<td>2.1fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 560061</td>
<td>69.5f</td>
<td>32.4cdef</td>
<td>97.1d</td>
<td>2.0fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 595932</td>
<td>60.5g</td>
<td>29.3cdef</td>
<td>96.0d</td>
<td>1.9fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 595936</td>
<td>76.0de</td>
<td>28.0bcdef</td>
<td>100.0d</td>
<td>1.9fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>NGB 102148</td>
<td>49.4h</td>
<td>30.4cdef</td>
<td>96.0d</td>
<td>1.9fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 595950</td>
<td>49.4h</td>
<td>35.1cdef</td>
<td>97.0d</td>
<td>1.9fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 560066</td>
<td>55.6gh</td>
<td>38.5cdef</td>
<td>97.0d</td>
<td>1.9fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 560062</td>
<td>80.9cd</td>
<td>20.0baced</td>
<td>100.0d</td>
<td>1.9fgh</td>
<td>4.0§§</td>
<td>8.9, 10.7¶¶</td>
</tr>
<tr>
<td>PI 595933</td>
<td>59.7g</td>
<td>14.0ab</td>
<td>96.0d</td>
<td>1.7gh</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>PI 560063</td>
<td>63.9fg</td>
<td>43.1ef</td>
<td>100.0d</td>
<td>1.6h</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>LSD</td>
<td>8.59</td>
<td>19.17</td>
<td>14.21</td>
<td>0.24</td>
<td>18.26</td>
<td>1.67</td>
</tr>
</tbody>
</table>

† Cultivar, PI accession, and Nordic Gene Bank (NGB) accession.
‡ ‡ Means computed from five replicate (plant) means.
§ Means computed from five replicate (plant) means where 0 = little or no damage and 5 = extensive damage.
# Means computed from weights of all emerging adults from five replicates (plants).
†† Means within a column followed by the same letter are not different at P < 0.05 on the basis of LSD.
‡‡ Too few adults emerged and therefore not statistically analyzed.
¶¶ Weights of one or two adults.
### Moderately resistant because a few adults emerged in the Australian trial (see text).

Although there was statistical concordance (P > 0.05) between first instar mortality rates on pods of *Alaska 81* and pods of nine *P. fulvum* accessions in 1995 and pods of two accessions in 1996, the overall results reveal the presence of pod-based resistance in the *Pisum* secondary gene pool. The percentage of first larval larvae that died before reaching seeds of *P. fulvum* accessions averaged 14 to 43% and 22 to 50% in 1995 and 1996, respectively, compared with an average larvae larval mortality rate of 6% on pods of *Alaska 81* in both Pullman trials (Tables 2 and 3). We observed larvae wandering about on the pod surfaces of *P. fulvum* before attempting to bore into the pod wall, which suggested that pods of this wild species may be less hospitable to neonate larvae than are pods of *P. sativum*. Ninety-nine out of 100 neonates bored directly into the pod walls of *Alaska 81* from the underside of seeds in an earlier study (Clement, 1992, unpublished data). This normal neonate behavior on *P. sativum* pods may protect newly hatched larvae from predators, parasitoids, and dessication, as well as shield them from contact insecticides in agricultural settings.

On the basis of within-seed mortality rates of larvae, seeds of all *P. fulvum* accessions except PI 560065 (1995 trial) were significantly more (P < 0.05) resistant to larval feeding than seeds of *Alaska 81*. Pea weevil larvae sustained 88.4 to 100% within-seed mortality on 17 of 22 *P. fulvum* accessions in 1995 and 85.3 to 100% on all accessions in 1996. This high level of seed resistance in *P. fulvum* is also revealed by the seed damage ratings in both trials, which for all accessions except PI 560065 (1995 trial) were significantly lower (P < 0.05) than the average ratings of 4.8 (1995) and 5.0 (1996) for *Alaska 81*. Seed damage ratings averaged less than 3.0 for 17 *P. fulvum* accessions in 1995 and for all accessions in 1996 (Tables 2 and 3).

Seed of *P. fulvum* accessions in both Pullman trials weighed significantly less than seed of *Alaska 81* (Tables 2 and 3). However, resistance in small seeded *P. fulvum* accessions did not appear to be the result of limited food for larval development, as evidenced by nonsignificant (1995: r = 0.29, P = 0.2105; 1996: r = 0.40, P = 0.1994) correlation coefficients between *P. fulvum* seed weights and damage ratings and nonsignificant (1995: r = −0.25, P = 0.2692) or weakly significant (1996: r = 0.57, P = 0.0507) correlation coefficients between seed weights and within-seed mortality rates. Moreover, 81.8% (n = 768) and 87.5% (n = 384) of the first instar larvae that reached seed of *P. fulvum* entries in 1995 and 1996, respectively, died before consuming >5% of the cotyledon tissue of seed and before developing into second instars. This is further evidence that starvation was not responsible for high mortality
of first instars on *P. fulvum* seeds of weights that averaged 49.4 to 94.9 mg in 1995 and 52.1 to 87.9 mg in 1996. We speculate that insecticidal properties are responsible for seed resistance, although the absence of α-amylase inhibitor in seed of *P. fulvum* (Schroeder et al., 1995) precludes associating this seed protein with weevil toxicity. Schoonhoven et al. (1983) speculated that factors other than seed size and weight were responsible for resistance in small seeded and uncultivated bean accessions to the bean weevil, *Acanthoscelides obtectus* (Say), and Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae).

Mean adult emergence values (100x,) from *P. fulvum* were significantly lower (*P* < 0.05) than those from Alaska 81, which had emergence values of 86% (1995) and 94% (1996). However, emergence rates were relatively high (32.4 to 60.4%) for five *P. fulvum* accessions. Adults that emerged from *P. fulvum* weighed significantly less (*P* < 0.05) than those from Alaska 81 seed in both Pullman trials (Tables 2 and 3), prompting us to speculate that these very small adults would have reduced survivability and reproductive fitness. No large departure from a 1♀:1♂ sex ratio was recorded for adults emerging from Alaska 81 in 1995 and 1996 (49.51%) and from all *P. fulvum* accessions in both trials (52.48%).

In the South Perth trial, 15 *P. fulvum* accessions produced no adults (PI 595939, PI 560064, PI 595942, PI 595948, PI 560061, PI 595932, PI 595946, PI 595934, PI 595935, PI 595933, PI 560062, PI 560067, PI 595940, PI 560063). However, adults emerged from eight accessions with values averaging between 4 and 6% for three accessions (PI 595936, PI 595944, PI 343955) and between 15 and 47% for five accessions (PI 595938, PI 595941, PI 595943, PI 595945, PI 595947). Adult emergence from the susceptible *P. sativum* control (Pennant) averaged 85%. There was good concordance between the Pullman and South Perth results. A few adults emerged (2 to 8%) from seven accessions (PI 595939, PI 560064, PI 595942, PI 595948, PI 560061, PI 595932, PI 595937) in Pullman (Table 2), but none emerged from these entries in South Perth, and a few adults emerged (4 to 15%) from three accessions (PI 595947, PI 595944, PI 595936) in South Perth, but none emerged from these entries in Pullman (Tables 2 and 3).

Any variation among weevil populations (including *B. pisorum*) for virulence to resistant genotypes could minimize the ability of a resistant cultivar developed in one country to confer resistance to weevil populations in other countries (Dick and Credland, 1986; Shade et al., 1999). However, differences in adult emergence rates between Pullman and South Perth trials were not of a magnitude for us to conclude that significant variability exists among pea weevil populations in eastern Washington and Western Australia for virulence to resistant *P. fulvum* accessions. Thus, the resistant accessions in this study could be used to develop pea cultivars in different geographical areas with durable pea weevil resistance.

Three distinct patterns of *B. pisorum* mortality and survival on the entries in the Pullman trials emerged from the construction of life tables, illustrated by the values for the weevil on PI 560065, PI 595942, and PI 595940 (Table 1). The PI 560065 life table characterizes pea weevil susceptibility in *P. fulvum*, a pattern that was exhibited by four other accessions (PI 595941, PI 595938, PI 595953, PI 595943) in the 1995 trial. These susceptible accessions had within-seed larval mortality
rates of 14.4 to 59.5%, seed damage ratings >3.0, and adult emergence percentages of 32.4 to 60.4% (Table 2). Although the remaining accessions could not be separated into resistant classes on the basis of statistically different mean values for within-seed mortality and seed damage ratings (Tables 2 and 3), their adult emergence values yielded two distinct patterns. First, 13 *P. fulvum* accessions from the Pullman trails and two accessions from the South Perth trial produced a few adults, albeit only one or two small adults or emergence percentages of 8% or less (exemplified by PI 595942 in Table 1), and thus were classified as moderately resistant. Second, 14 accessions shared by the Pullman and South Perth trials produced no adults and were classified as resistant (exemplified by PI 595940 in Table 1) (Tables 2 and 3). A wide range in first instar mortality rates on pods (Tables 2 and 3) precluded the use of this parameter for classifying entries. Conclusions about the geographical distribution of susceptibility and resistance in *P. fulvum* accessions are not possible because of a paucity of passport information in GRIN.

Results from this study revealed the presence of low to complete levels of *B. pisorum* resistance in germplasm stocks of *P. fulvum*. This antibiosis resistance is based on the death of larvae on different plant parts (pod and seed) of *P. fulvum*, as revealed by life tables. Such patterns of insect resistance in plants can be complex in nature and controlled by more than one gene (Rusoke and Fatunla, 1987; Kennedy and Barbour, 1992). Indeed, this seems to apply to pea weevil resistance in *P. fulvum* because initial crosses between *P. sativum* and a weevil-resistant accession in Western Australia which produced resistant recombinant progeny indicate that resistance is likely controlled by two or more genes (Byrne et al., 2000). Success in transferring bruchid resistance in a wild crop relative to agronomically acceptable cultivars via traditional plant breeding has been achieved. In this example, single gene resistance in a wild mungbean (*Vigna radiata* L. Wilczek) accession of the subspecies *sublobata* (Kitamura et al., 1988; Fujii et al., 1989) was used to develop mungbean cultivars with resistance to *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) in many countries (Kaga and Ishimoto, 1998).

Our findings identify sources of natural resistance in the *Pisum* genome to develop pea cultivars with pod and/or seed resistance to *B. pisorum*. The best sources of resistance are the resistant accessions, although some of the moderately resistant accessions should not be overlooked because they exhibited high within-seed larval mortality rates (>95%) and low seed damage ratings (<2.2) (Tables 2 and 3). With different *P. fulvum* parts and tissues expressing resistance, it may be possible to develop a multi-tiered defense against *B. pisorum* in one pea cultivar to provide long-term stability of resistance to the weevil. In addition, strong pod resistance from *P. fulvum*, or from *P. sativum* lines with the neoplastic pod trait (Doss et al., 2000), would prevent many larvae from reaching seeds and causing damage. Finally, *P. fulvum* resistance traits could be transferred into transgenic peas containing the α-amylase inhibitor protein to provide durable protection against *B. pisorum* in one cultivar (Schroeder et al., 1995).

**ACKNOWLEDGMENTS**

We thank J. Burns, F. Muehlbauer, and E. Zakarison for manuscript review, H. Collie for technical assistance, and M. Evans for statistical advice. Research was supported in part by grants from the USDA-Foreign Agricultural Service-International Cooperation and Development-Research and Scientific Exchanges Division (AS37) in the USA and the Grains Research and Development Corporation (GRDC) in Australia.

**REFERENCES**


Clement, S.L., M.A. Evans, and D.G. Lester. 1996. Settling and feeding responses of pea weevil (*Coleoptera: Bruchidae*) to flowers of selected pea lines. J. Econ. Entomol. 89:775–779.


complex inform the breeding of new resistant varieties. Appl. Ento-
Hardie, D.C. 1990. Pea weevil, Bruchus pisorum (L.), resistance in-
shop, Melbourne, Australia. 9–10 May 1990. Dep. of Agric. and
Rural Affairs, Melbourne, Australia.
of Pisum accessions to evaluate their susceptibility to the pea weevil
Hardie, D.C., and S.L. Clement. 2001. Development of bioas-
says to evaluate wild pea germplasm for resistance to pea weevil
evaluations of wild peas against pea weevil. Arthropod Manage.
Bruchidae) control by knockdown pyrethroid in field peas. Crop
Prot. 10:53–56.
resistance gene and its relationship to insecticidal cyclopeptide
alkaloids, the vagnic acids, in mungbean (Vigna radiata L. Wäl-
Growing techniques and conservation of wild perennial Cicer spe-
cies in the U.S. Pacific Northwest. Int. Chickpea Pigeonpea Newsl.
4:7–8.
турal and managed systems. p. 13–41. In R.S. Fritz and E.L. Simms
(ed.) Plant resistance to herbivores and pathogens: Ecology, evolu-
tion, and genetics. Univ. of Chicago Press, Chicago.
Kitamura, K., M. Ishimoto, and M. Sawa. 1988. Inheritance of resis-
tance to infestation with azuki bean weevil in Vigna sublobata
Marzo, F., A. Aguirre, M.V. Castiglia, and R. Alonso. 1997. Fertiliza-
tion effects of phosphorus and sulfur on chemical composition of
seeds of Pisum sativum L. and relative infestation by Bruchus
Meichenheimer, R.D., and F.J. Muehlbauer. 1982. Growth and develop-
ment stages of Alaska peas. Exp. Agric. 18:17–27.
Messina, F.J. 1984. Influence of cowpea pod maturity on the oviposi-
tion choices and larval survival of a bruchid beetle Callosobruchus
Michael, P.J., D.C. Hardie, G.P. Mangano, T.P. Quinn, and L.A. Pritch-
ard. 1990. The effectiveness of chemicals against the pea weevil,
Bruchus pisorum (L.), and native budworm, Helicoverpa punctigera
Wallengren, on field peas, Pisum sativum, in Western Australia.
Melbourne, Australia. 9–10 May 1990. Dep. of Agric. and Rural
Affairs, Melbourne, Australia.
Morton, R.L., H.E. Schroeder, K.S. Bateman, M.J. Chrispeels, E.
Armstrong, and T.J.V. Higgins. 2000. Bean α-amylase inhibitor 1
in transgenic peas (Pisum sativum) provides complete protection
from pea weevil (Bruchus pisorum) under field conditions. Proc.
wild species in cool season food legume breeding. p. 531–539. In
F.J. Muchlbaumer and W.J. Kaiser (ed.) Expanding the production
and the use of cool season food legumes. Kluwer, Dordrecht,
The Netherlands.
and its control. Idaho Current Information Serial 885. Idaho Cooper.
Ext., Univ. of Idaho, Moscow, ID.
of pea introductions to the pea weevil. J. Econ. Entomol. 70:30–33.
Am. 75:439–443.
Poole, R.W. 1974. An introduction to quantitative ecology. McGraw-
Hill, New York.
resistance to the cow-pea seed beetle (Callosobruchus maculatus
2. SAS Inst., Cary, NC.
the bean weevil and the Mexican bean weevil (Coleoptera: Bruchi-
dae) in noncultivated common bean accessions. J. Econ. Ento-
mol. 76:1255–1259.
Schroeder, H.E., S. Gollasch, A. Moore, L.M. Tate, S. Craig, D.C.
α-amylase inhibitor confers resistance to the pea weevil (Bruchus
107:1233–1239.
Shade, R.E., L.L. Murdock, and L.W. Kitch. 1999. Interactions be-
tween cowpea weevil (Coleoptera: Bruchidae) populations and
Vigna (Leguminosae) species. J. Econ. Entomol. 92:740–745.
Shade, R.E., H.E. Schroeder, J.J. Pueyo, L.M. Tate, L.L. Murdock,
T.J.V. Higgins, and M.J. Chrispeels. 1994. Transgenic pea seeds
expressing the alpha-amylase inhibitor of the common bean are
London, UK.
plants and biosafety: Science, misconceptions and public percep-
varietal resistance of cotton to spider mites. Entomol. Exp. Ap-
plic. 39:27–33.
Zohary, D. 1973. The origin of cultivated cereals and pulses in the
Near East. p. 307–320. In J. Wahrman and K.R. Lewis (ed.) Chro-