The measurement of resistance to *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) in seeds of *Phaseolus vulgaris* L.

C.J Moss and P.F Credland*

Abstract

Acanthoscelides obtectus is a cosmopolitan insect pest infesting leguminous seeds in the field and especially within storage. Control of this pest is usually based on the use of insecticides but opinion now favours a shift towards methods which incorporate varietal resistance. Effective and useable resistance has been detected in the hosts of many bruchid pests and can be used to contribute towards their control. However, in the case of *A. obtectus*, its behaviour, which is unique among bruchid pest species, has inhibited the development of an effective bioassay and thereby complicated the identification of resistant seeds.

A. obtectus differs from all other bruchid pest species in that its eggs are not firmly attached to individual seeds but are scattered loosely amongst potential hosts. On hatching, the larvae are then free to move from seed to seed. Seed choice is not therefore exclusively the domain of the ovipositing female. Useful resistance could be expressed at oviposition, larval penetration of seed, development within the seed or as reduced fitness of emerging adults.

This contribution attempts to define the problems to be overcome in developing a useable bioassay for the assessment of seed resistance to *A. obtectus*, and to provide a simple and reliable protocol for comparative studies.

Introduction

Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) is a cosmopolitan insect pest of leguminous seeds in the field and, especially, within storage. The feeding of adult bruchids is of no economical importance; it is the larval stages which consume parts of the seed, causing considerable damage. The main and original host of *A. obtectus* is the common bean, *Phaseolus vulgaris* L. The beans play a vital role in the diet and economy of many countries and are of particular importance as a subsistence crop in Central and South America and in parts of Africa. Control measures differ widely in Africa, Asia and the Americas, ranging from large scale applications of granular insecticides (Cardona and Karel 1990) to traditional cultural practices used by subsistence farmers. Effective insect control in developing countries has certain limitations; insecticides are expensive, sometimes hazardous, and often unavailable to the small farmer. Opinion now favours a shift towards integrated methods which incorporate varietal resistance as opposed to those based on insecticide use alone.

* Department of Biology, Royal Holloway, University of London, Egham Hill, Egham, Surrey, TW20 OEX, U.K.

The assessment of resistance to *A. obtectus* requires an effective and reliable bioassay, which must be relatively straightforward to execute and flexible enough to accommodate different sized and shaped seeds. The behaviour of *A. obtectus*, which is unique among pest species of bruchid, has inhibited the development of an effective bioassay and consequently the identification of resistant seeds. It differs from all other bruchid pest species in that its eggs are not firmly attached to individual seeds but are scattered loosely amongst potential hosts. On hatching the larva is then free to wander from seed to seed before penetrating an acceptable host. Seed choice or resource partitioning is not therefore exclusively the domain of the ovipositing female. For the purpose of developing satisfactory bioassays, this behaviour leads to difficulties in ensuring that a viable larva comes into contact with the seed and, having done so, is able to adopt the necessary posture for penetration to occur. Neither of these problems needs to be overcome in the case of other bruchid pest species.

Useful resistance could be expressed at several stages in the insect's life cycle: oviposition, larval acceptance and penetration, development of larvae within the chosen seed, or as reduced fitness of the emerging adults. Two bioassays are required to incorporate all these biological stages, one for oviposition and the other to measure the remaining variables. This contribution attempts to define the problems to be overcome in developing bioassays for the assessment of seed resistance to *A. obtectus* and to describe a simple and reliable protocol for comparative studies.

Materials and methods

Origin and maintenance of insects

The insects used in the experiment were obtained from a stock culture of *A.obtectus* collected in Colombia. The insects have been in culture for some years, initially at CIAT and then the Natural Resources Institute, where they are known in the laboratory as stock culture 101. They are reared in large glass jars of approximately 2.5 L capacity, sealed with filter paper and wax, each containing approximately 600 mL of commercially available North American red kidney beans, *Phaseolus vulgaris* L. Six jars, set up one week apart, are operational at any one time. New subcultures are established by removing approximately 300 beetles from each of the two oldest jars, the oldest then being discarded. The culture is kept under controlled conditions at 27 ± 1°C and 70 ± 10% r.h. with a regime of 14 hours of dim illumination and 10 hours darkness.

All seed investigated was equilibrated in muslin covered glass containers, under the controlled conditions previously described, for a minimum of three weeks before use. All experiments were carried out under the controlled conditions and used the same commercially available red kidney beans as a susceptible control of known performance.

Oviposition bioassay

Newly emerged adults were obtained from culture seeds (containing only one or two larvae). The adults were removed and placed in a clean container for 24 hours. The adults were then sexed and a single male and female were placed in a glass tube with three seeds. A sponge bung prevented escape while still allowing the passage of air. Eggs were counted daily for 8 days (until the adults were 10 days old), and again on the death of the female, to obtain a total oviposition value. Twenty replicates of each seed accession were investigated. This is a realistic number since many wild collections, or samples of newly bred cultivars, are small and comprise few useable seeds.

Larval penetration and development bioassay

The experiments were carried out using a variety of flat bottom microtitre plates (MERC Ltd. U.K.) to accommodate different sized seeds. The plates were covered with individual glass plates to prevent larval escape. Plastic lids tended to warp and did not provide a satisfactory alternative to glass.

Glass beads were used during some bioassays to provide additional contact points for larval penetration (Dobie et al. 1990). The glass beads were of a similar size to the seed being investigated except in the case of red kidney beans where 6.5 to 7.5 mm beads were used. The size and shape of the seed determined the need for glass beads (see below). Beads were added to small seeds which did not fill the bottom of the well. Spherical seeds also required additional beads to secure the seed, preventing movement during larval penetration.

A single seed was placed in an appropriate sized well of a microtitre plate. Glass beads were added as required. The seeds of each accession were placed in a separate microtitre plate and spaced apart to minimise the risk of any movement by larvae between wells. Eggs were obtained from 3-day old females. A single 5-day old egg was added to each seed and checked 3 days later for hatching and larval penetration of the seed. If the egg had not hatched or the hatched larva had died or escaped from the well, another egg was supplied. The procedure was repeated until a total of three replacement eggs, after the initial application, had been made, if necessary. The seeds were examined and adult emergence recorded daily, beginning 28 days after the first egg was introduced; recording continued for a period of 42 days. Twenty replicates of each seed accession were investigated.

The newly emerged adults were sexed and weighed. Where possible female fecundity was also investigated. Each female was placed in a glass tube with three control seeds and a newly emerged male from the stock culture. The total oviposition of each female was recorded on day 10; oviposition after day 10 is negligible (Parsons, per. comm. 1993).

Three different experiments are described to illustrate the development and use of the procedure.

1. The effectiveness of glass beads was investigated using the small and spherical seeds of three species: *Vigna angularis* (Willd.) (adzuki bean), *Vigna radiata* (L.) Wilczek. (mung bean) and *Lupinus* sp. (lupin). The larval penetration and development bioassay procedure was followed with:

i. A single seed in a well.
ii. Addition of glass bead(s) to a single seed in a well.
iii. Addition of an extra seed (of the same species) to the single seed in a well.

Twenty replicates of each treatment were investigated.

2. The effectiveness and reliability of the procedure was determined by replication on a susceptible host, red kidney beans. Three complete repeats of the experiment were undertaken.
3. To demonstrate its value for comparing seeds, the bioassay procedure was also applied to *Vigna unguiculata* (L.) Walp. (black-eye bean), RKB (control) and eight wild accessions of *P. vulgaris* from Colombia.

Determination of resistance

Resistance was defined using the following criteria:

Larval penetration: The seed was considered resistant if larval penetration occurred in 25% or less of the replicates. If penetration occurred in at least 50%, then the seed was considered susceptible.

Larval development: Only if penetration occurred in at least 50% of the seeds, could resistance within the seed be assessed. If the number of adults emerging from the seed was 25% or less of the number of larvae which penetrated, the seed was considered resistant. If at least 50% of those larvae which penetrated emerged, then the seed was considered susceptible.

Any seed falling outside these categories was considered of intermediate resistance.

Data analysis

Analysis of the data from both assays was undertaken with analysis of variance and subsequent Student-Newman-Keuls multiple range tests if differences among the samples were indicated.

Results

Oviposition bioassay

Total oviposition data for adults on the eight wild accessions of *P. vulgaris*, *V. unguiculata* (black-eye) and red kidney beans are given in Figure 1. Total oviposition differed little between all the accessions, except on black-eye bean. A one-way ANOVA revealed a significant difference among the accessions ($F_{(9,181)}= 3.99$; $P < 0.001$). A Student-Newman-Keuls test confirmed that oviposition on black-eye bean was significantly less ($P < 0.05$) than on other accessions.

Red kidney beans, black-eye bean and the wild accessions G10000 and G12949 were chosen to illustrate the rate of oviposition (Fig. 2). In each case eggs were laid at a steadily decreasing rate for 8 days after which no significant oviposition occurred on all the accessions investigated.

Larval penetration and development bioassay

Effectiveness of glass beads

The presence of glass beads increased penetration rates into all small and spherical seeds investigated (Table 1). A test was undertaken to compare an 'ideal' situation (where larval penetration occurred in all 20 replicates) and the actual data observed for each of the three separate treatments: penetration with a single seed, penetration with a single seed and glass beads, and penetration with extra seed of the same species. The only significant difference was in the case of penetration into a single isolated seed ($\chi^2 = 28$; $P = 0.0000$). No difference in penetration was found between using glass beads or extra seed of the same species.

Repetition of the bioassay on red kidney beans

Consistently high penetration and emergence rates were observed in each of the three repeat experiments using red kidney beans (Table 2).

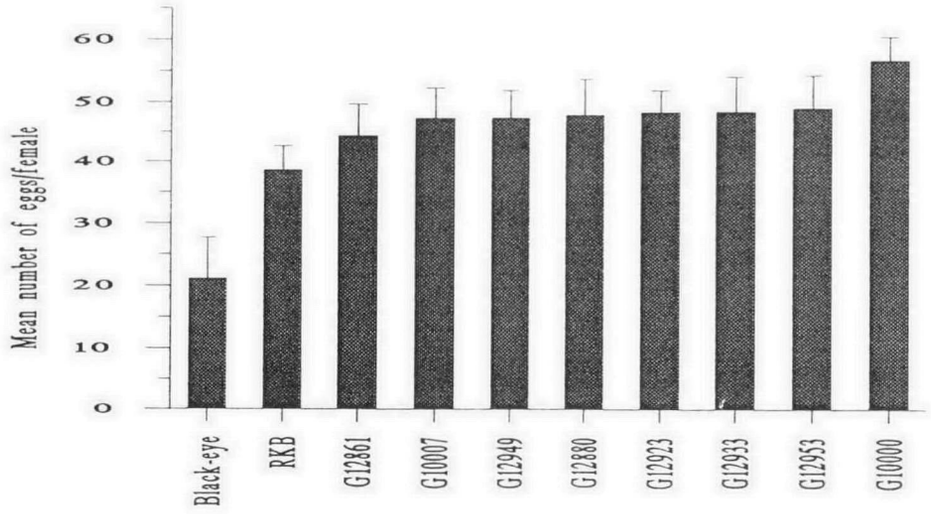

Fig. 1. Total oviposition on *V. unguiculata*, red kidney bean (RKB) and eight wild accesions of *P. vulgaris*.

Table 1. The effects on penetration of adding glass beads to small and spherical seeds.

Species	Mean weight (g)	Penetration with single seed	Penetration with glass beads	Penetration with extra seed
Adzuki bean	0.078	1	15	17
Mung bean	0.057	5	20	19
Lupinus sp.	0.024	0	15	12

All data are derived from 20 replicates.

Table 2. Penetration and emergence from red kidney beans in three separate assays. (Mean weight of seed was 0.60 g in each experiment).

Experiment	Total penetration	Total emergence
1	20	20
2	20	18
3	20	18

All data are derived from 20 replicates.

Bioassay using wild accessions of P. vulgaris

Accessions G12861, G12880 and G12953 were determined to have resistant properties using the criteria previously described (Table 3). There was little or no penetration into seeds of G12861 or G12880. More than 25% of larvae penetrated G12953 but emergence was only 6.67%. G10007 was considered to be of intermediate resistance as larval penetration exceeded 25% but was lower than 50%. The remaining accessions were all considered susceptible to *A. obtectus*.

Development period and fitness

The longest development period was observed in G12953 (60 days) and the shortest was in the control, red kidney beans (31.42 days) (Table 4). A one-way ANOVA was undertaken on data from those accessions from which more than five emergences occurred: G12949, G12933, G10000, G12923 and red kidney beans. A significant difference was determined among the accessions tested ($F_{(4, 106)} = 52.434$; $P = 0.0000$). A Student-Newman-Keuls test, showed that the only pair of accessions not significantly different ($P < 0.05$) from each other were G10000 and RKB.

Mean weights of both male and female adults emerging from all the wild accessions except G10000 were lower than those observed in the control (Table 4). A one-way ANOVA was carried out on the accessions: G12933, G10000, G12923, red kidney beans (all those with $n > 5$), for male and female weights separately. A significant difference in both the male and female weight was found among the adults emerging from all the accessions tested ($F_{(3, 44)} = 12.536$; $P = 0.0000$ and $F_{(3,37)} = 27.008$; $P = 0.0000$ respectively). A Students-Newman-Keuls test confirmed that the weights of females from all accessions were significantly different from each other ($P < 0.05$) except those from G10000 and red kidney beans, which did not differ from each other. Males from all accessions had significantly different mean weights except that those from G12933 were the same as G12923, and those from G10000 the same as those from red kidney beans.

Mean oviposition was greatest among females that emerged from the control seeds (43.72) and G10000 (54.33) (Table 4). A one-way ANOVA was carried out on data from G12933, G12923 and red kidney (all those with $n > 5$). A significant difference in total oviposition was found among the samples ($F_{(2,44)} = 4.667$; $P < 0.05$). A Student-Newman-Keuls test on the same accessions determined that the only significant difference ($P < 0.05$) was between G12933 and red kidney beans.

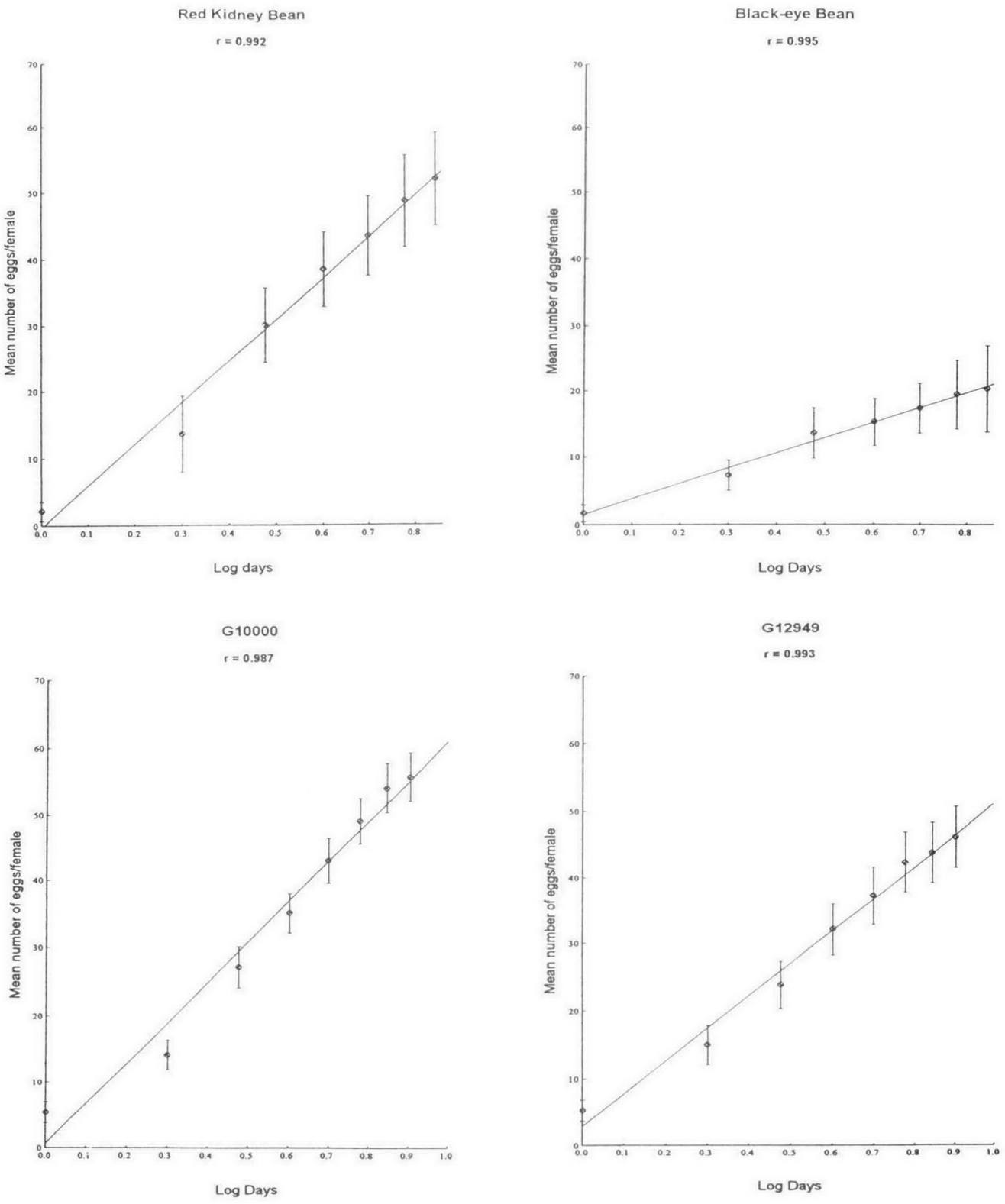

Fig. 2. The cumulative rate of oviposition on red kidney bean, black-eye bean, G10000 and G12949.

Table 3. Estimation of resistance at larval penetration and development..

Accession	Mean weight of seed (g)	Total penetration	Total emergence	Resistance/ susceptibility (possible site of resistance)
G12861	0.0458	0	0	Resistant (testa/cotyledon)
G12880	0.0331	1	0	Resistant (testa/cotyledon)
G12953	0.0487	15	1	Resistant (cotyledon)
G10007	0.0633	6	4	Intermediate (testa)
G12949	0.0569	10	7	Susceptible
G12933	0.1352	19	17	Susceptible
G10000	0.1729	19	17	Susceptible
G12923	0.1576	20	20	Susceptible
Red kidney beans	0.589	20	20	Susceptible
Black-eye bean	0.240	19	13	Susceptible

All data are derived from using 20 replicates.

Table 4. Developmental period, weight and fecundity of insects emerging from six wild accessions of *P. vulgaris* and red kidney beans.

Accession	Development period (Mean no. of days ± SE)	Mean weight (mg) ± SE		Fecundity (Mean total no. of eggs ± SE)
		Male	Female	
G12953	60 (n=1)	3.287 (n=1)	–	–
G10007	35.60 ± 0.678 (n=4)	3.94 ± 0.465 (n=2)	5.77 ± 1.011 (n=2)	31 (n=1)
G12949	46.71 ± 2.02 (n=7)	2.81 ± 0.143 (n=4)	3.98 ± 0.293 (n=3)	18.5 ± 8.995 (n=3)
G12933	38.94 ± 0.915 (n=17)	4.46 ± 0.169	4.38 ± 0.289	22.166 ± 6.156
G10000	32.88 ± 0.283 (n=17)	5.44 ± 0.440	6.22 ± 0.369 (n=3)	54.33 ± 6.936 (n=3)
G12923	36.65 ± 1.013 (n=20)	4.30 ± 0.109	5.28 ± 0.910	33.44 ± 6.301
Red kidney	31.42 ± 0.364 (n=58)	5.04 ± 0.116	6.63 ± 0.104	43.72 ± 2.259

All data are derived from original larval penetration and development bioassays with 20 replicates. Replicate numbers in the development period column refer to the total number of insects emerging. In other columns n > 5 unless stated otherwise.

Discussion

Oviposition bioassay

In stores, a female beetle is most commonly faced with a single species or variety of host. For this reason, a no-choice experimental protocol was chosen to investigate oviposition. The oviposition bioassay was adapted from the procedure described by Howe and Currie (1964). Care was taken to obtain newly emerged adults from seeds producing only one or two adults rather than seeds from which greater numbers emerged. Increased competition for resources within the seed is known to result in lower adult body weights and reduced fecundity in other bruchids (Credland et al. 1986). The newly emerged adults were left together for 24 hours to mate. Oogenesis and mating may need the stimulus of a host seed (Huignard and Biemont 1981); a single male was included in tube with the female and seeds in case mating had not taken place. The recording of oviposition began on day 3 because significant oviposition is known to begin then and continue until adults are 10 days old (Parsons, pers. com. 1993). For practical purposes, the delaying of oviposition will not prevent infestation during long periods of storage, and therefore a resistant accession should stimulate little or no oviposition. For this reason the total oviposition was chosen as the parameter to determine resistance. It can be seen in Figure 2 that rates of oviposition do not provide more useful information than can be gathered from total oviposition data. Measuring rates over only the first 4 or 5 days does give a rapid assessment of the final total and could be useful for a quick estimation of acceptability.

The total oviposition did not differ significantly between the wild and cultivated accessions of *P. vulgaris* although oviposition on black-eye bean was significantly lower (Fig. 1). Black-eye bean is not the original host of *A. obtectus* but is susceptible to infestation (Southgate 1978; Jarry and Bonet 1982; Singh 1990). Further investigation, with the use of multi-choice oviposition experiments may indicate lower total oviposition to be comparable with non preference.

Oviposition acceptance does not appear to be host specific in strain 101. Females have oviposited in substantial numbers on all seeds investigated to date, including a large variety of legume species (Moss, unpublished data). Further investigations on seed from different families is required to determine if this behaviour is common on all seeds or just those within the Leguminosae, but the likelihood of effective and useable resistance being expressed at this stage in the life cycle of the beetle is remote. However, strain 101 has been kept under laboratory conditions for many years; the possibility that host acceptance in the laboratory biotype has diversified from the original wild population cannot be excluded. It is interesting to note that suitability for oviposition and the capacity to support larval acceptance do not always correspond (Birch et al. 1989). Females will oviposit on seeds which are totally unsuitable for larval development (Moss, unpublished data).

Larval penetration and emergence bioassay

The larval penetration and emergence bioassay was adapted from several procedures (Howe and Currie 1964; Schoonhoven et al. 1983; Gatehouse et al. 1987; Shade et al. 1987). The use of microtitre plates allows convenient separation and confinement of a single seed and egg in each well. Eggs were obtained from 3-day old females which are known to produce the highest proportion of viable eggs (Parsons, pers. comm. 1993). The earliest stage at which the viability of eggs can be accurately determined under a low power microscope is when they are 4–5 days old; this is why eggs of this age are used in the assay (Moss, unpublished data).

The movement of larvae before penetration is a unique problem associated with *A. obtectus*. Larvae can survive for up to 120 hours without food (Credland and Dendy, unpublished data). Ensuring that only a single larva has penetrated a seed is difficult. A single penetration hole is not enough; it is common for 'pioneer' larvae to bore the hole and other 'follower' larvae then to use this as means of entering (Labeyrie 1960). It is important to eliminate interlarval competition for the food reserves within a seed for the reasons described in the oviposition bioassay.

Legume seeds come in many shapes and sizes. Wild accessions of *P. vulgaris* are typically small (approximately 5 mm in length and 3 mm in diameter), reniform and flattened. These seeds do not fit tightly in the well (7 mm dia) of the smallest microtitre plate (96 well); often only the bottom of the seed is in contact with another surface. Larger seeds, which are able to touch the sides, will therefore provide more potential contact points for larval penetration. In stores, contact points are provided by neighbouring seeds and the addition of extra seed could simulate this situation in the laboratory. Unfortunately, many samples of wild accessions are small and consequently few seeds can be used in each assay. Glass beads provide the most suitable alternative (Gatehouse et al. 1987). The addition of glass beads, which were as effective as extra seeds, gave greatly increased penetration into all accessions investigated.

Larvae often wander for a long time (3–4 days) in the well and subsequently may not have enough energy to penetrate; others escape into neighbouring wells. Physical separation of accessions and individual replicates (as described above) minimised the risk of multiple infestation. Alterations to the apparatus, however, were not sufficient to eliminate all the variation. Re-applying eggs in the event of eggs failing to hatch, or death or disappearance of the larvae, provided a partial solution to the problem. A significant decrease in the variation attributable to these problems was observed when this replacement technique was used (Moss, unpublished data).

Repeating the bioassay technique on the culture bean, red kidney beans, proved the bioassay to be effective and consistent. High penetration and emergence values were recorded in all three experiments. The development of a repeatable bioassay on a susceptible host provides a means of screening for resistance in wild accessions of unknown host suitability. The accessions G12861, G12880 and G12953, using the

Table 3. Estimation of resistance at larval penetration and development..

Accession	Mean weight of seed (g)	Total penetration	Total emergence	Resistance/ susceptibility (possible site of resistance)
G12861	0.0458	0	0	Resistant (testa/cotyledon)
G12880	0.0331	1	0	Resistant (testa/cotyledon)
G12953	0.0487	15	1	Resistant (cotyledon)
G10007	0.0633	6	4	Intermediate (testa)
G12949	0.0569	10	7	Susceptible
G12933	0.1352	19	17	Susceptible
G10000	0.1729	19	17	Susceptible
G12923	0.1576	20	20	Susceptible
Red kidney beans	0.589	20	20	Susceptible
Black-eye bean	0.240	19	13	Susceptible

All data are derived from using 20 replicates.

Table 4. Developmental period, weight and fecundity of insects emerging from six wild accessions of *P. vulgaris* and red kidney beans.

Accession	Development period (Mean no. of days ± SE)	Mean weight (mg) ± SE		Fecundity (Mean total no. of eggs ± SE)
		Male	Female	
G12953	60 (n=1)	3.287 (n=1)	–	–
G10007	35.60 ± 0.678 (n=4)	3.94 ± 0.465 (n=2)	5.77 ± 1.011 (n=2)	31 (n=1)
G12949	46.71 ± 2.02 (n=7)	2.81 ± 0.143 (n=4)	3.98 ± 0.293 (n=3)	18.5 ± 8.995 (n=3)
G12933	38.94 ± 0.915 (n=17)	4.46 ± 0.169	4.38 ± 0.289	22.166 ± 6.156
G10000	32.88 ± 0.283 (n=17)	5.44 ± 0.440	6.22 ± 0.369 (n=3)	54.33 ± 6.936 (n=3)
G12923	36.65 ± 1.013 (n=20)	4.30 ± 0.109	5.28 ± 0.910	33.44 ± 6.301
Red kidney	31.42 ± 0.364 (n=58)	5.04 ± 0.116	6.63 ± 0.104	43.72 ± 2.259

All data are derived from original larval penetration and development bioassays with 20 replicates. Replicate numbers in the development period column refer to the total number of insects emerging. In other columns n > 5 unless stated otherwise.

criteria previously described, were determined to be resistant. The possible location of resistant factors was estimated by comparing the penetration and emergence data. Little or no penetration occurred in G12861 and G12880; resistance could then be presumed to be situated within the testa, although the additional presence of cotyledon resistance cannot be excluded. More than 25% of larvae penetrated into seeds of G12953 but resulting emergence was 6.67%, suggesting that resistance is possibly situated within the seed. Resistance has been previously identified in G12953 (Gatehouse et al. 1987; Dobie et al. 1990) but its nature has not been defined. Resistance was attributed to the soluble carbohydrate fraction, but the precise component could not be isolated (Minney 1990). G10007 was considered to be of intermediate resistance as penetration was greater than 25% but lower than 50%. The resulting emergence was high (80%) indicating that the site of resistance is most likely to lie within the seed testa. Confirmation of the site of resistance requires further investigation with artificial seeds (based on Shade et al. 1986).

Development time and fitness

Development times and parameters of fitness are other sites at which resistance may be manifested. The development period, mean weight of male and female and mean oviposition data were collected. Large replicate numbers are obviously desirable but the very nature of many resistant accessions resulted in low emergence and hence low replicate numbers. Increasing the frequency of emergence from resistant seeds is difficult; large amounts of seed would be required to provide more replicates and this is not usually available. Obtaining large quantities of seed from wild sources is often impractical. Accessions susceptible to larval penetration and development do, however, give adequate emergence numbers. If resistance was manifested in adult fitness alone, then this difference would indicate it. G12933 and G12923 were both susceptible to larval penetration and development, but mean male and female weights and total oviposition were significantly lower and mean development time in G12933 was significantly longer than among adults emerging from RKB. Therefore, it is possible that adult fitness may be affected, to some degree, by resistant factors within the seeds of both accessions. G10000 did not significantly differ from red kidney beans as a host for *A. obtectus* and can be considered susceptible, although it is much smaller and a similar number of seeds would produce fewer adults if larval density was not regulated. The determination of resistance criteria for accessions of intermediate or high resistance to larval penetration and development is a difficult task. Insects from G12949, for example, had a significantly longer development time than those from red kidney beans but emergence numbers were too small to provide adequate replicates for measurement of mean adult weights and total oviposition. The assay, therefore, has to be described as a preliminary screen which identifies susceptible seeds. Accessions which are not recognised as susceptible may need to be reassessed in larger experiments with more replicates before detailed assertions of their resistance qualities can be made.

Conclusions

A. obtectus, like most organisms, is inherently variable and responsive to changes in the environment. The bioassays described have attempted to reduce the variation observed in the physical environment, apparatus and the beetles under investigation, to enable the effect of the host on the insects to be observed. Variation is, however, part of the real world experienced by bruchids. The merits of reducing variation are often contested but, in practice, a worthwhile bioassay cannot reflect the complex interactions experienced in the field.

The search for resistance to *A. obtectus* in the seeds of *P. vulgaris* demands a pair of bioassays to investigate all the possible stages at which resistance could be manifested in the insect's life cycle: oviposition acceptance, larval penetration and development, and adult fitness. The necessity to use multiple criteria was clearly illustrated because accessions deemed susceptible at oviposition were subsequently discovered to be resistant to larval penetration and development. Furthermore, because of the requirement to standardise procedure, the separate assays cannot be compounded into a simple test.

For an accession to be determined as resistant, a set of arbitrary criteria had to be defined. The criteria are not absolute but their prior definition is essential to avoid terms like 'resistant' and 'susceptible' becoming trivialised. No population of wild *Phaseolus* is likely to contain all the possible genetic variants that might exist and enhance resistance. Natural selection is unlikely to lead to the evolution of the 'perfect' resistant individual since there is probably a trade-off diminishing other elements of plant fitness.

The challenge is to provide a practical, realistic means of identifying the most resistant plants, recognising that many accessions may need screening, and that numerous factors affect the behaviour and physiology of *A. obtectus*. The procedures described in this paper provide such a means and enable rapid assessments to be made. It is recognised that statistically valid data may not always be obtained, but, as explained, this outcome has a meaning in its own right. The necessity for repetition and further assays at a later date is not precluded by an initial screen.

Acknowledgments

Caroline Moss is grateful to the Biotechnology and Biological Sciences Research Council and the Natural Resource Institute for providing a post graduate studentship. She also appreciates the additional financial help from the BBSRC and the Royal Society which enabled her to attend this conference.

References

Birch, A. N. E., Simmonds, M. S. J. and Blaney, W. M. 1989. Chemical interactions between bruchids and legumes. Advances in Legumes Biology. Monograph of Systematic Botany, Missouri Botanical Gardens 29, 781–809.

Cardona, C. and Karel, A. K. 1990. Key Insects and other invertebrate pests of beans. In: Insect pests of tropical food legumes. (ed. Singh, S. R.), John Wiley and Sons, Chichester. pp. 157–191.

Credland, P. F., Dick, K. M. and Wright, A. W. 1986. Relationships between larval density, adult size and egg production in the cowpea seed beetle, *Callosobruchus maculatus*. Ecological Entomology, 11, 41–50.

Dobie, P., Dendy, J., Sherman, C., Padgham, J., Wood, A. and Gatehouse A. M. R. 1990. New sources of resistance to *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* Boheman (Coleoptera: Bruchidae) in mature seeds of five species of *Phaseolus*. Journal of Stored Product Protection 26(4), 177–186.

Gatehouse, A.M.R., Dobie, P., Hodges, R.J., Meik, J., Pusztai, A. and Boulter, D. 1987. Role of carbohydrates in insect resistance in *Phaseolus vulgaris*. Journal of Insect Physiology, 33(11), 843–850.

Howe, R. W. and Currie, J. E. 1964. Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. Bulletin of Entomological Research, 55(3), 437–477.

Huignard, J. and Biemont, J. C. 1981. Reproductive polymorphism of populations of *A. obtectus* from different Colombian Ecosystems. In: V. Labeyrie ed. The ecology of Bruchids attacking legumes (pulses). Proceedings of the International Symposium held at Tours (France), April, 1980. Volume 19, 149–164.

Jarry, M. and Bonet, A. 1982. La bruche de haricot. *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae), est-elle un danger pour le cowpea, *Vigna unguiculata* (L.) Walp. Agronomie, 2, 963–968.

Labeyrie, V. 1960. Nature de la répartition des larves d'Acanthoscelides *obtectus*. Comptes rendus des séances de l'Academie des Sciences, 251, 149–151.

Minney, B. H. P. 1990. Breeding *P. vulgaris* (common bean) for resistance to the major pest bruchids *Z. subfasciatus* and *A. obtectus*. Biochemical bases for seed resistance in wild lines. Ph.D. Thesis. University of Durham.

Schoonhoven, A. V. Cardona, C. and Valor, J. 1983. Resistance to the bean weevil and the Mexican bean weevil (Coleoptera: Bruchidae) in noncultivated common bean accessions. Journal of Economic Entomology, 76, 1255–1259.

Shade, R. E., Murdock, L. L., Foard, D. E. and Pomeroy, M. A. 1986. Artificial seed system for bioassay of cowpea weevil (Coleoptera: Bruchidae) growth and development. Environmental Entomology, 15, 1286–1291.

Shade, R. E., Pratt, R. C. and Pomeroy, M. 1987. Development and mortality of the bean weevil, A. *obtectus* (Coleoptera: Bruchidae), on mature seed of tepary beans, *P. acutifolius*, and common beans, *P. vulgaris*. Environmental Entomology, 16, 1067–1070.

Singh, S. R. 1990. Insect pests of tropical food legumes. John Wiley & Sons, Chichester.

Southgate, B.J. 1978. The importance of the Bruchidae as pests of grain legumes, their distribution and control. In: Singh, S. R., van Emden, H. F., and Taylor, T. A. eds., Pests of grain legumes: Ecology and Control. London/New York, Academic Press. 219–229.

van Emden, H. F. 1987. Cultural methods: the plant. In: A. J. Burn, T. H. Coaker and P. C. Jepson, eds, Integrated pest management. Academic Press. Pp. 27–68.