Project title:	Integrated control of bean seed beetle (Bruchus rufimanus)
Project number:	FV322
Project leader:	Dr Anthony Biddle PGRO
Report:	Final report November 2010
Previous report:	Annual report, October 2009
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Location of project:	PGRO, Rothamsted Research
Industry Representative:	Tim Mudge PVGA
Date project commenced:	1st August 2007
Date project completed (or expected completion date):	11th November 2010

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- Bruchid beetle damage can be reduced by using the recommended insecticide application and timing presented in this report.

Background

Current control practices are not effective in reducing damage by bean seed beetle and there is a large gap in the knowledge regarding the biology and behaviour of the pest in locating host crops and oviposition during the critical flowering and pod forming stages in early summer. Current recommendations are based on insecticide sprays applied during flowering, and a lack of precision in the timing of sprays is resulting in an increase in the number of spray applications being made to crops which in turn increases the risk of pesticide resistance. The project will improve this situation by using a pheromone/semio-chemical system for monitoring and risk assessment as part of an integrated control method and will provide a more sustainable longer-term approach to IPM which would include resistant or tolerant varieties.

In order for growers to further expand the bean crop as a valuable and break crop in both organic and conventional sustainable arable farming systems, risks of poor returns due to unacceptable levels of pest damage must be alleviated and effective control of Bruchid is essential. A greater knowledge of the biology and behaviour of the pest particularly during the flowering and early pod development stages of the crop will allow the development of a more effective pest control method with insecticides. The development of a semio-chemical based trapping system to monitor the pest in the crop will provide a reliable risk indicator and a means of determining the need or the optimum timing for sprays, reducing the need for multiple applications and risk of resistance. In addition, improvements in pesticide application techniques will deliver a more effective chemical control and in the longer term the delivery of identified genetic resources of resistance for future breeding programmes will develop a package of integrated management approaches to improve insecticide timing, reduce the risk of pesticide resistance and ultimately to reduce reliance on insecticide based control. This will enable sustainable bean production in the UK.

The specific aims of the project are:

1. To fill the gaps in the knowledge of the pest biology and the pest/host plant relationship to improve the chances of success in control
2. To improve the application techniques to provide a more effective level of control and to improve the method of damage assessment used within the project and for future wider usage.
3. To provide a semio-chemical based trapping system for monitoring the pest in the crop.
4. To utilise the trapping system together with meteorological data and crop development in providing a reliable indicator for pesticide application.
5. To minimise pesticide usage by reducing the numbers of sprays applied to a crop to reduce pesticide resistance and to avoid unnecessary spraying.
6. To examine varieties of beans for possible sources of genetic resistance to provide information for future breeding programmes.
7. The work will help avoid the problem in the longer term and hence the need to spray

Summary of the project and main conclusions

The distribution of *Bruchid rufimanus* as a pest of *Vicia faba* field and broad beans in the UK was mapped from data obtained from damage assessments of crops grown throughout the UK in each of the cropping years, 2007-2010. It was found that there was no detectable shift in the pest distribution over the four years. The most severe infections always seemed to occur in a band south of the Wash and stretching from the West Midlands to East Anglia and extending down to the south coast. From a small number of isolated incidences of crop damage in areas outside those referred, there is a suggestion if conditions are favourable, the pest will spread quickly and infest crops wherever they are grown

In spray trials carried out in field and broad beans to optimise insecticide application, an evaluation of a spray forecasting model used in France showed that under UK conditions, the model was successful in improving control of damage. Sprays applied at first pod development following two consecutive days where the temperature has reached 20ºC were the most effective but control was further improved using angled alternate facing nozzles. There was no advantage found by using increased water volumes.

Evaluation of flower volatiles as a potential lure for a trapping system to monitor insect activity identified a complex mixture of compounds which were then tested in traps in the field. In addition, some initial studies were carried out to investigate the use of chemicals produced by the plants defence system as a repellent to *B. rufimanus.*

To examine possible sources of varietal resistance to the pest, of 641 accessions screened for infestation in the UK, 390 were eliminated as potential sources of complete genetic resistance. A small proportion of the total number screened, were found to have lower levels of damage and these lines have been retained for future evaluation. As a rapid means of assessing the percentage of damaged beans in a harvested sample, an image analysis was evaluated but found to need further modification and development to provide very accurate results.

Important points for growers

- Crops grown in areas in a band south of the Wash and stretching from the West Midlands to East Anglia and extending down to the south coast are the most susceptible to bean damage.
- Evaluation under UK conditions of the spray forecasting model used in France showed similar positive results.
- Sprays should be applied at first pod development following two consecutive days where temperature has reached 20ºC
- Improved control can be achieved using angled nozzles.
- No advantage was found using increased water volumes.
- The efficacy of a prototype trap has been confirmed and a standard lure developed, however further field evaluation is necessary before commercialisation.
- No evidence of complete varietal resistance to Bruchid damage was found.

Financial benefits

The work has clearly provided a recommendation for the timing and application of insecticides to reduce damage and reduce the risk of crop rejection of broad beans grown for processing or fresh market.

Action points for growers

- Crops in eastern, southern and western England are more at risk from Bruchid damage than northern or south western Britain.
- Apply insecticides to crops which have reached the early pod stage following two consecutive days when temperatures have reached 20ºC.
- Use angled nozzles to improve canopy penetration.

SCIENCE SECTION

Introduction

The aims of the project are listed:-

1. To advance the knowledge of the biology of *Bruchus rufimanus* and to identify features in its life cycle and behaviour.
2. To improve the efficacy of existing insecticides targeting adult beetles and investigate the potential of alternative chemicals targeting eggs and larvae
3. To develop a monitoring system based on species specific sex pheromones or plant volatile mixtures for Bruchid attraction and a prediction model to optimise insecticide applications.
4. To investigate naturally occurring variation in Bruchid susceptibility of UK bean varieties and breeding lines from UK and international germplasm collections

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Methods and results

The project is focussed on several main elements contained in 4 Work packages

Work Package 1. Insect biology

1. National pest distribution (Frontier, NIAB, Wherry, PGRO) 2007- 2010

Commercial samples of harvested beans intended for export obtained from Frontier as part of the investigation of image analysis for damage assessment were also used to investigate the distribution of pest damage, and any changes which occurred during the course of the project. Pest distribution maps offer a potential route to understanding epidemiology of the organism, validating forecasting schemes, and providing further refinement to these. Though these aspects were outside the scope of the LINK project, pest distribution knowledge can provide growers with an indication of whether they are in a high risk area or not.

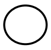
Data from Frontier samples (% of seeds with Bruchid damage) and a number of samples submitted to NIAB and PGRO for seed health tests in 2008 was mapped using post code data with Google Earth. The numbers of samples used is shown in Table 1.1. For each sample, 100 seeds were assessed.

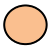
Table 1.1 Annual sample numbers used for mapping

2007	1396
2008	1388 (includes 93 lab samples ex PGRO & NIAB
2009	1914
2010	1604

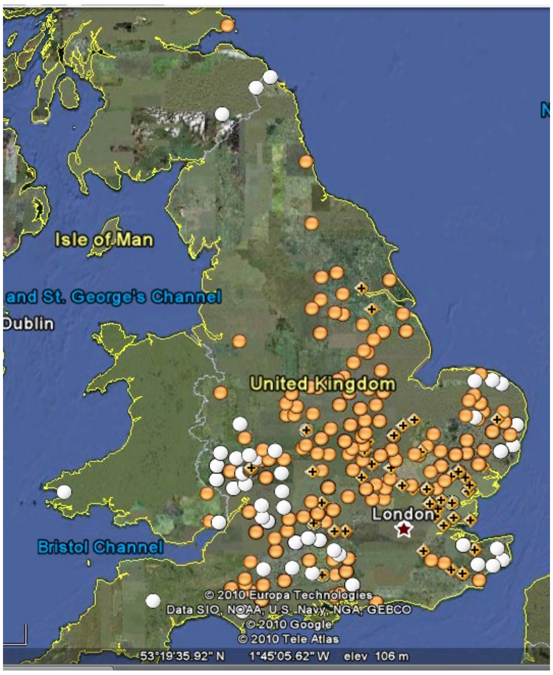
For each year, the samples were categorised as either zero infection, 1 to 5% seeds damaged, and greater than 5% damage, and each category mapped separately. These data from 2007, 2008, 2009 and 2010 have, for the first time for *Bruchus rufimanus*, provided a clear indication of the distribution of the pest in the UK.

Key to figures

 Zero infection

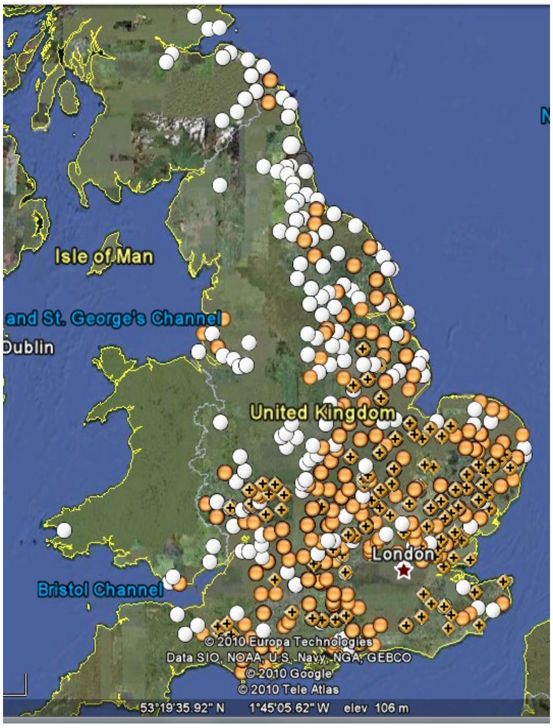
 1 to 5% infection

 Greater than 5% infection

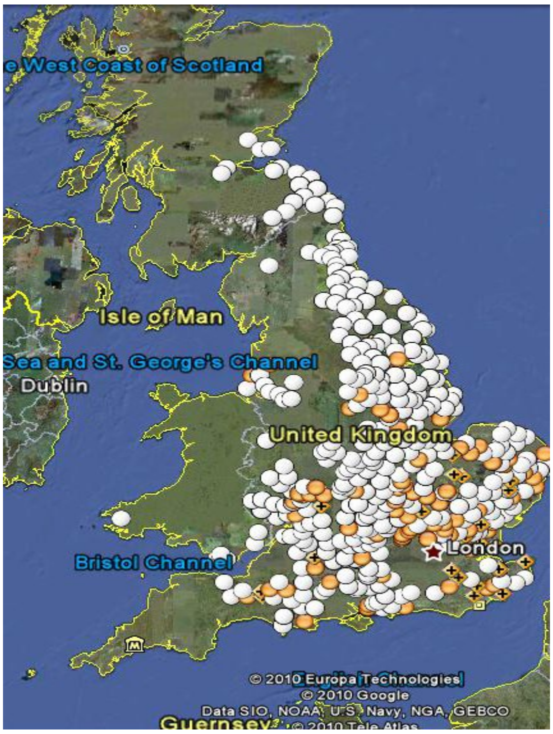


2007

2008



2009



2010

There are constraints in Google Earth mapping that bespoke software would avoid, the most obvious being that multiple samples with the same or proximal post codes do not show separately on the map, thus in 2010, where zero damage appears to be predominate, the zero points mask several positives at or near the same post code point. Nevertheless, the maps are accurate enough to reflect the higher overall damage levels seen in 2008, and the lower levels seen in 2010.

Conclusions of Insect Biology work

There is no detectable shift in the pest distribution over the four years shown here. It was found in the Scottish borders in three of the four years, in the west of England, and in the Cheshire/Lancashire area. The most severe infections always seemed to occur in a band south of the Wash and stretching from the West Midlands to East Anglia and extending down to the south coast. Changing conditions, whether shifts in climate or more extensive bean cropping, may extend the range of severe infections further north. Several isolated severe infections were seen in the Humberside area in 2008, whereas only one or two were seen in 2007, and suggest if conditions are favourable, the pest will spread quickly and infest crops wherever they are grown.

Work Package 2. Insecticide application and timing

1. Application 2008 (Syngenta Crop Protection and PGRO)

Field trials were carried out at 6 sites to investigate the effectiveness of different water volumes and nozzle types. The nozzles included standard flat fan and angled spray nozzles, e.g. Syngenta Amistar and Syngenta Potato nozzles. Each trial was carried out in a commercial crop of spring or winter field beans using standard sprayers. The treated areas were in large field scale plots, not replicated and an unsprayed area was left in each field for comparison. Some of the field trials compared the effects of different water rates. At all sites Hallmark with Zeon Technology was applied at 0.075l/ha at first pod when temperatures reached 20°C for two consecutive days.

Beans at all sites were harvested. Samples from each plot were analysed for Bruchid damage. Results showed improved control using angled potato 05 nozzles at 300 l/ha compared to the untreated plot or those where twin cap nozzles were used at 300 l/ha.

Data from other sites were not analysed due to lack of replication. Some trends could be seen however. At one site there was improved control using standard nozzles at 200 l/ha compared to plots where standard nozzles were used at 100 l/ha and untreated plots and at another site, there was improved control where Hawk and Amistar nozzles were used compared to the untreated area.

Hawk and Amistar nozzles at 100 l/ha provided good control in both winter and spring beans at a further site compared to the untreated area, and in the spring beans standard nozzles at 200 l/ha also gave improved control and in another trial, Amistar and 05 potato nozzles were more effective.

The results suggested that angled nozzles provided better control of Bruchid than standard nozzles when applications were made at first pod when temperatures reached 20°C for 2 consecutive days. Where standard nozzles were used damage was reduced where applications were made using 200 l/ha of water compared to 100 l/ha of water but there were no strong indications that differences in water volume consistently affected control of Bruchids.

2. Application trials 2009 (Syngenta Crop Protection and PGRO)

Field trials were established at 5 sites to further assess the effectiveness of different volumes of sprays and different nozzle types. The best control of Bruchid damage was achieved when angled nozzles were used. Variations in water volume did not consistently affect the results. Levels of damage in 2009 were variable and winter beans showed higher levels of damage than other sites.
New Hawk and Amistar spray systems, both with alternating nozzles pointing forwards and backwards, were used at a volume of 100l/ha and compared to a standard Lechler 120 IDK05 flat fan system at a volume of 200l/ha and an untreated plot. Applications of Hallmark were made when the beans had developed the first 2cm pods and when temperatures of 20°C or above had been recorded on two consecutive days. Applications were repeated after 7 days or when weather conditions permitted.
In each of the trials where angled nozzles wee used, there was significantly better control using potato nozzles and twin cap/Amistar nozzles. Results from water volume trials have been inconsistent over the three years of the project and failed to produce any statistically significant results.

3. Application trials 2010 (Syngenta Crop Protection and PGRO)

The work was repeated in 2010 to evaluate the benefit of angled nozzles and in 4 farm scale field trials the best control of Bruchid damage was achieved when angled nozzles were used. Variations in water volume did not consistently affect the results. Levels of damage in 2010 were variable and winter beans showed higher levels of damage than other sites.

4. Insecticide timing 2008 (Velcourt Ltd and PGRO)

Experiments were carried out in two commercial crops of Fuego spring beans to examine the validity of a spray timing model used in France. The trials investigated the effectiveness of two applications of Hallmark applied at the small pod growth stage following two consecutive days where temperatures reached 20°C. This was compared to applications starting at 5-6 flowering trusses when temperatures reached 20°C for two days, and an untreated plot. The second spray application followed 7 to 10 days after the first. Hallmark with Zeon Technology was applied at 0.075l/ha using a tractor mounted sprayer fitted with flat fan nozzles at 200l/ha when temperatures reached 20°C for two consecutive days.

Damage analysis showed statistically significant differences between the treated and untreated plots at both sites. The treatment applied at six flowering trusses showed slightly improved control compared to the treatment applied at first pod. This was not statistically significant. Both treatments provided statistically significant levels of control compared to the untreated plots.

5. Insecticide timing 2009 (Velcourt Ltd and PGRO)

Experiments were carried out at two sites, Kings Lynn and Dover. Both trials investigated the effect of spraying at different crop growth stages when temperatures had reached 20°C for two consecutive days. Hallmark was applied in one treatment at 6 flowering trusses and in the second treatment at first pod. Applications were repeated 10-14 days after the first spray. The results of final damage assessments at both sites showed significant differences between the treated plots and the untreated plot.
Damage analysis showed statistically significant differences between the treated and untreated plots at both sites. Both treatments provided significantly improved control compared to the untreated plots. At Dover the spray applied at 1st pod provided slightly better control of Bruchid damage than that applied at six flowering trusses. This was not statistically significant. Both treatments provided statistically significant levels of control compared to the untreated plots.

Temperature data from Kings Lynn showed that when the first spray was applied at six flowering trusses on 3 June temperatures had reached 20°C for two days. Bruchids would have been quite active and therefore susceptible to spray application. When the first spray of the second treatment was applied at first pod on 16 June temperatures also reached 20°C for two days. This was also the day that the second spray of treatment 1 was applied. This may explain why the first treatment achieved better control than the second. Trials carried out at Kings Lynn in both 2008 and 2009 showed that the spray at six flowering trusses provided better control than that applied at first pod.

Temperatures at Dover were generally slightly lower than those recorded at Kings Lynn. When the first spray was applied at six flowering trusses on 1 June the maximum temperature was 19°C and on 12 June, when the second spray of treatment 1 was applied, the maximum temperature was 17°C. When the first spray of the second treatment at first pod was applied on 15 June the maximum temperature was 18°C. When the second spray of the second treatment was applied on 24 June the maximum temperature was 23°C. This would explain why better control was achieved in the second treatment as a temperature threshold was reached at the second spray. Bruchids would have been more active and easier to target.

The temperature data from the two sites emphasised the importance of the temperature threshold when applying sprays to control Bruchids. There is a clear difference in levels of damage recorded at the two sites. Where temperatures at Dover were below 20°C for most of June, the time of greatest Bruchid activity and when crops were at the most susceptible crop growth stage, overall damage was very low. The untreated plot showed a mean damage level of just over 1%. At Kings Lynn, where temperatures in June reached and exceeded 20°C for three weeks of the month, levels of damage were higher. Damage on the untreated plot was 4.33%.

6.. Insecticide timing 2010 (PGRO and Velcourt)

Experiments were carried out at Aylmer Hall Farm, Kings Lynn. The trial investigated the effect of spraying at different crop growth stages when temperatures had reached 20°C for two consecutive days. Hallmark was applied at first pod, first pod plus 7 to 14 days and 26 days after first pod. An area was left untreated.
Plant samples were taken by hand just prior to harvest and returned to PGRO for evaluation. Data analysis showed significant differences between the treatments on nodes 1 to 5 with significantly higher levels of damage found on the untreated plot compared to all treatments. Damage levels were higher in 2010 than 2009 and this is due to high temperatures experienced throughout June and July. The average daily maximum temperature was 21.98°C in June and 24.92°C in July. This provided a protracted period where temperatures exceeded 20°C, the threshold for Bruchid egg-laying. Lower pods had highest levels of damage as would normally be expected. This is because bottom pods are exposed to attack for a longer period of time than pods at the top of the plant.

Samples were taken at harvest during combining and delivered to PGRO for evaluation. Data analysis showed statistically significant differences between the treatments, with treatment 4 (1 spray at seven days after T1) having the highest levels of damage. This may be due to the spray being applied 26 days after 1st pod when a significant amount of egg-laying would have occurred. However, the untreated plot had lower levels of damage than treatments 3 and 4.

Temperature data from Kings Lynn showed that when the first spray was applied at first pod on 23 June temperatures had reached 20°C for two days. Bruchids would have been quite active and therefore susceptible to spray application. When the second spray of the second treatment was applied on 6 July temperatures had also reached 20°C for two days. The spray was applied to treatment 4 on 19 July. Temperatures had been above the 20°C threshold for the preceding four weeks. The data from samples taken by hand at harvest supports the recommendation to spray at the threshold temperature of two consecutive days at 20°C. Control was significantly better on treated plots than on the untreated plot. This supports the recommendation to spray at the threshold temperature of two consecutive days at 20°C.

7. Insecticide timing in broad beans 2008 (PGRO and Raynham Farms):

An insecticide spray trial was conducted in a crop of Listra broad beans in Norfolk, and consisted of 2 plots of the same variety drilled at different times. The plots were divided into three treatments:

1. Untreated
2. Decis Protech (deltamethrin) at 1st pod and 7 – 10 days later
3. Biscaya (thiacloprid) at 1st pod and 7 – 10 days later

Spray applications were made when temperatures reached 20°C for two consecutive days and first pods were set, and second applications were made 7-10 days later.. When mature the plots were harvested and samples of produce from each plot were assessed for Bruchid damage and larval infestation. Damage was expressed as % by weight.
Damage levels and egg numbers for each treatment were very low or nil and data were not analysed. The data from damage levels at harvest were analysed and the results showed that were no significant differences in damage levels at harvest between the treatments for either of the drillings. There was, however, a statistically significant difference between the overall damage levels in each drilling. The earlier drilling had higher levels of damage than the later one, across the three treatments.

8. Insecticide timing in broad beans 2009 (PGRO and Raynham Farms):

The broad bean trial was repeated. Sprays were applied when temperatures reached 20°C for two consecutive days at first pod for each drilling. Egg data were not analysed due to numbers of eggs and levels of damage to the seed coat being nil or very low. There were no significant differences in damage levels at harvest between the treatments for the first drilling. The data does show a similar trend to that seen in the second drilling, where Biscaya provided the best control compared to Decis Protech and the untreated plot. Decis Protech showed greater control compared to the untreated. At drilling 2 the plot sprayed with Biscaya showed significantly less Bruchid damage than Decis Protech or the untreated plot. There was a slight improvement in control using Decis Protech compared to the untreated plot, but this was not statistically significant.

There was no significant difference in damage levels between the two drillings.

Temperature data from East Raynham for May, June and July showed that temperatures were relatively low for most of June. Maximum temperatures of more than 20°C were not reached on many occasions until the end of June. This caused overall levels of damage to be low and the untreated plots showed less than one per cent damage at harvest.

The temperature and damage data confirm that when temperatures do not reach 20°C for two consecutive days, Bruchid activity is reduced and damage levels are much lower. This would suggest that it may not be necessary to spray unless the threshold is reached.

Conclusions of Insecticide application and timing work

- Evaluation under UK conditions of the spray forecasting model used in France showed similar positive results
- Sprays should be applied at first pod development following two consecutive days where temperature has reached 20ºC
- Improved control can be achieved using angled nozzles
- No advantage was found using increased water volumes.

Work Package 3. Semiochemical studies (Rothamsted Research)

Six areas of study were carried out:

1. Study of bean flower volatiles
2. Bruchid mating behaviour
3. Laboratory study of effects of plant repellents
4. Field experiment with prototype trap at overwintering sites and bean fields.
5. Trapping experiments with floral and/or Bruchid derived volatiles
6. Field trial with repellents

1. Analysis of bean flower volatiles towards an optimal lure

In previous studies (Defra projects PI0339 and PI0341) a prototype Bruchid trap had been developed and the bean floral volatiles trans-cinnamaldehyde, cinnamyl alcohol and linalool, released in a crude 1:1:1 mixture, were shown to be significantly attractive to *Bruchus rufimanus* in this trap in the field compared to the same compounds released individually. However, this lure was less effective when the crop was in flower. The natural ratio of compounds released by the host plant is a crucial part of the signal to the insect (1). Thus, to develop a better attractant by reproduction of natural ratios, volatiles produced by intact glasshouse grown flowering field bean plants, vars. Wizard and Fuego, and broad bean plants, var. the Sutton, were collected *in situ* by air entrainment of the headspace around the plant (2). Natural ratios of volatiles released by the plants were determined by Gas Chromatography (GC) and GC-Mass Spectrometry (GC-MS). The composition of volatile chemicals from the different intact plants showed surprisingly little variation when compared to each other and to those of cut plants that had been entrained previously. The natural ratios of cinnamyl alcohol, trans-cinnamaldehyde and linalool were determined (approximately 1:4:20) and experimental dispensers, consisting of cellulose sponge with measured amounts of individual neat chemical applied and heat sealed into polythene sheet or enclosed in polyvials, were developed to release these compounds at a ratio approximating nature (3). The release rate was controlled by the size of the sponge, the amount of chemical and the thickness of the polythene and was determined by weighing replicated dispensers on a 5 decimal place balance and then hanging them in a wind tunnel at constant wind speed and temperature ($0.2m^{s-1}$, 20°C) and re-weighing them at intervals over at least 3 weeks. For chemicals with very low release rates, the amount released was determined by air entrainment of the volatiles and assessment by GC. The multiple baits were tested in field experiments with the prototype trap (see section 3).

Development of a more complex lure

Responses of female Bruchid beetles to headspace samples of broad bean floral volatiles were investigated using GC coupled electrophysiology (GC-EAG). In these studies, electrophysiological recordings from insect antennae revealed responses to eight compounds in a sample of Sutton dwarf volatiles. The electrophysiologically active volatiles were subsequently identified, using GC-MS, as myrcene, limonene, (*E*)-ocimene, linalool, 4-allylanisole, cinnamaldehyde, caryophyllene and humulene and the natural emission ratio of these was quantified. Trapping and field assessments in 2009 showed that Bruchids can arrive in the crop prior to the onset of flowering and therefore are probably responding to volatiles from beans at the vegetative stage as well as to the floral volatiles. Therefore in 2010, dispensers were developed for the other electrophysiologically active compounds not in the standard lure to evaluate if their inclusion, in natural ratios, could enhance trap catch further (see section 3). (*E*)-Ocimene was omitted as it was not possible to design a dispenser to release it at low enough levels and at high levels it acts as a repellent (see section 4).

Behavioural assays

The behavioural responses of field collected male and female Bruchids to these entrainment samples were investigated using a Perspex four-arm olfactometer (4), a device which enables quantification of insect responses to different odour fields in terms of time spent and number of entries into different areas. In each replicate, a single adult Bruchid was introduced into the central chamber and the time spent and number of entries into each arm was recorded using specialist software. A 10 µl aliquot of entrainment sample was applied to a filter paper strip placed at the end of the treated side arm. The three control arms were similarly treated with redistilled solvent (10 µl) alone on filter paper. The mean time spent in and number of entries into treated and control arms were compared using a paired *t*-test (Genstat). Olfactometer bioassays with a natural sample of bean floral volatiles showed that the behavioural response of Bruchids collected from the field in early June was variable. Less active insects were significantly attracted to it whereas very active insects were not suggesting that insects in a dispersal mode temporarily switch off their response to host plant odours. Thus, the physiological status of the insect could alter its response to a floral lure, but the insects affected were perhaps attempting to move away from the crop.

Figure 3.1 Response of female Bruchids to male produced pheromone 1-undecene in a four-arm olfactometer (P=0.016)

Air entrainments were made of volatiles produced by post-diapause male and female Bruchids collected from the field in early May 2008. Olfactometer bioassays tested the behavioural responses of field collected male and female Bruchids to these entrainment samples with the objective to determine the presence of any pheromone. Males were tested with female entrainment samples and females were tested with male entrainment samples. Results showed that female Bruchids were significantly attracted to volatiles from males. This is a novel finding and provides evidence that male *B. rufimanus* release a pheromone which attracts the females. Responses of male and female Bruchids to the entrainment samples were investigated in GC-EAG studies and female antennae responded to a small number of compounds from the male sample. The main active chemical in the male extract was identified by GC-MS as 1-undecene and its behavioural activity was confirmed in the olfactometer (Figure 3.1). Slow release dispensers were developed as described above and tested in the trap in the field (see section 3).

2. Bruchid mating behaviour

Adult *B. rufimanus* collected after harvest of spring bean crops in 2008 were sexed and the segregated sexes overwintered under controlled conditions (12°C then 5°C, 10h light per day) in the laboratory for at least 5 months. In April 2009 the Bruchids were brought out of cold conditions and gradually exposed to natural temperature and day length. Some adults were fed sucrose solution while others were given access to pollen from flowering bean plants. After several weeks under natural conditions, pairs of Bruchids were placed into small Petri dish arenas and observed. However, none of the pairs, either from the sucrose or pollen fed populations, mated. Dissected males from both sources were found to have terminated diapause, since the lateral glands of the reproductive system were enlarged. However, all the females had undeveloped ovaries suggesting that the diapause under laboratory conditions was sub-optimal. Female Bruchids collected from flowering field crops at the same time (late May – early June) had well developed ovaries with large numbers of eggs. Field collected pairs mated readily in Petri dish arenas and field collected males mated with laboratory overwintered females suggesting that sexually immature females will still mate. Since the Bruchids overwintered in the laboratory were unresponsive, all subsequent assays were performed with field collected beetles.

3. Investigation of semiochemical repellents

Laboratory study of the effects of plant activators

In previous studies (Defra projects PI0339 and PI0341) several volatile chemicals, collected from bean plants that had been damaged by feeding adult Bruchids, were identified by GC and GC-EAG including (*E*)-ocimene and (*Z*)-3-hexen-1-ol. These two compounds were tested in a field trapping trial where dispensers releasing the compounds individually were added to flowering bean plants in large bucket traps. Significantly fewer Bruchids were captured in traps baited with the compounds compared to those with flowering plants alone indicating that the compounds reduced the attraction to the flowering plants.

In laboratory experiments in this project, pre-flowering spring field beans, var. Fuego, were sprayed with the plant activator *cis*-jasmone or a blank formulation and, after 48h, headspace samples of volatiles were collected from the plants by air entrainment and were subsequently identified, using GC-MS. There were differences in the volatile profiles of plants from each treatment, but one of the main differences was the elevated levels of (*E*)-ocimene produced by the *cis*-jasmone treated plant (5). This compound has been shown to be repellent to Bruchids in previous studies. In addition, when pods on the winter bean variety Wizard were exposed to the natural Bruchid produced compound Bruchin B, a similar elevation of ocimene production was obtained. Slow release dispensers were developed (as described above in section 1) for (*E*)-ocimene and (*Z*)-3-hexen-1-ol for field use and these were tested along with applications of *cis*-jasmone in small plot trials against natural Bruchid populations in spring field beans.

4. Development of field trap

In 2008, field trapping trials were carried out on possible Bruchid overwintering sites (i.e. sites of beans in the previous season) and on winter and spring sown field bean sites to evaluate the attraction of multi-baits releasing different ratios of the flower volatiles cinnamyl alcohol, trans-cinnamaldehyde and linalool. In addition, four trap types were evaluated, the prototype cone trap, which consisted of a modified boll-weevil trap, a yellow sticky trap angled at 45°, a McPhail trap and a funnel trap. The yellow sticky trap, the McPhail trap and the funnel trap were ineffective even with the improved lure. The multi-bait releasing the three compounds in the most natural ratio in the cone trap proved to be the most attractive overall, capturing approximately 40% more Bruchids than the 1:1:1 mixture, and this combination was subsequently used as the standard monitoring trap during the rest of the project (Figure 3.2).

Cone traps containing the improved multi-bait were evaluated on overwintering sites and on winter and spring sown field bean sites in 2009 and 2010 at Rothamsted and at PGRO. At Rothamsted, traps were sited on all four field edges where possible to see if site was critical for trap catch. To compare trap catches with field infestation levels, assessments of adult Bruchid numbers were made by beating 50 pairs of plants into a white tray on transects across field sites where the traps had been placed. Assessments of eggs and larvae on 25 plants were made on some sites to relate trap catch to damage (also see Table 3.1). Unlike 2008, no Bruchids were caught on overwintering sites in 2009 probably due to lower temperatures (Figure 3.2B). Only small numbers were caught on the winter and spring bean sites, but in each case the trap catches preceded the Bruchids arrival in the crop showing that the trap can indicate early Bruchid activity. In 2010, Bruchid numbers were greater than in preceding years. Low temperatures in early May restricted trap catches, but as in 2009, the trap catches preceded Bruchid arrival in the crop (Figure 3.2C). Large numbers were trapped on spring bean sites as the temperature rose at the end of May suggesting a migration from winter bean sites, which were nearing the end of flowering. In all seasons male Bruchids arrived first in traps and on both crops and both sexes spread quickly throughout the crops.

In 2009 Bruchids were caught predominantly in traps on the SSW edge of the test fields with fewer on the ESE and WNW edges and none on the NNE edge. However, this was reversed in 2010 with the lowest catch in traps on the SSW edge. This could be due to a change in patterns of dominant wind direction in May 2010 from SW to NE compared to 2009, but could also be due to the topography and landscape surrounding the fields in the different years or variable levels of sunshine, which raises the temperature inside the trap and increases release rate of volatiles in the lure.

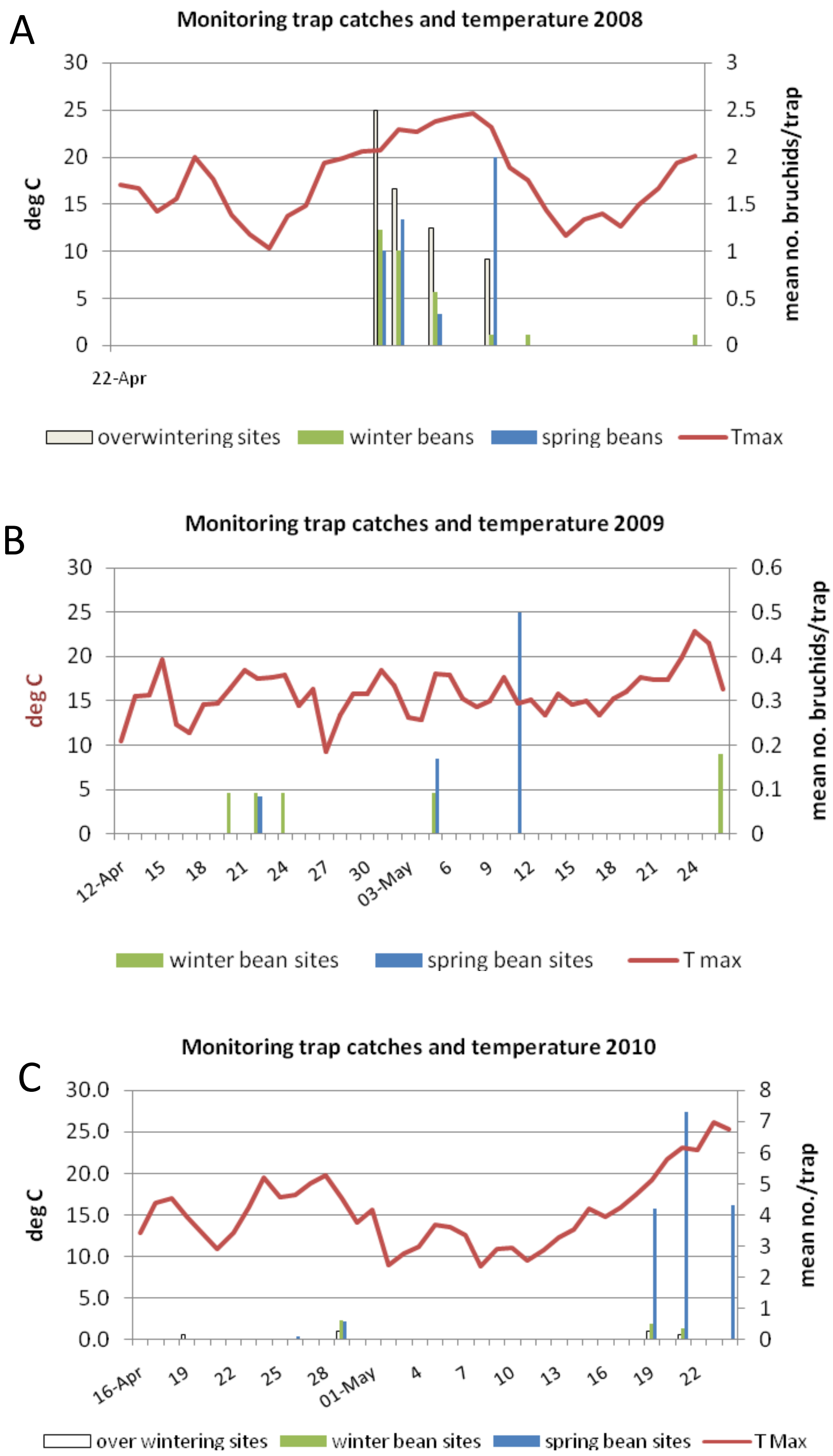


Figure 3.2. Monitoring trap catches and maximum temperature at Rothamsted 2008-10

5. Trapping experiments with Bruchid derived volatiles and more complex lures

The sex pheromone compound, 1-undecene, was evaluated in field trapping experiments both in combination with the standard 3 floral volatile multi-bait and on its own at different doses in 2009. Very few Bruchids were caught in traps baited with 1-undecene alone, but there was a very slight increase in trap catch when 1-undecene was released with the multi-bait compared to floral volatiles alone. It would appear that 1-undecene acts as a short-range mating cue but possibly does not attract over a long distance suggesting that it may not provide sufficient additional efficacy for use in the monitoring trap. In 2010, a more complex lure consisting of the standard 3 floral volatile multi-bait plus the extra bean components, identified by GC-EAG, released in a near natural ratio was developed. The efficacy of this lure, with and without the addition of 1-undecene, was field tested in replicated trapping trials and compared to the standard 3 component multi-bait and an unbaited trap. The complex lure was significantly more attractive than the blend of 3 components and with the addition of the 1-undecene caught significantly more females than the complex alone (Figure 3.3).

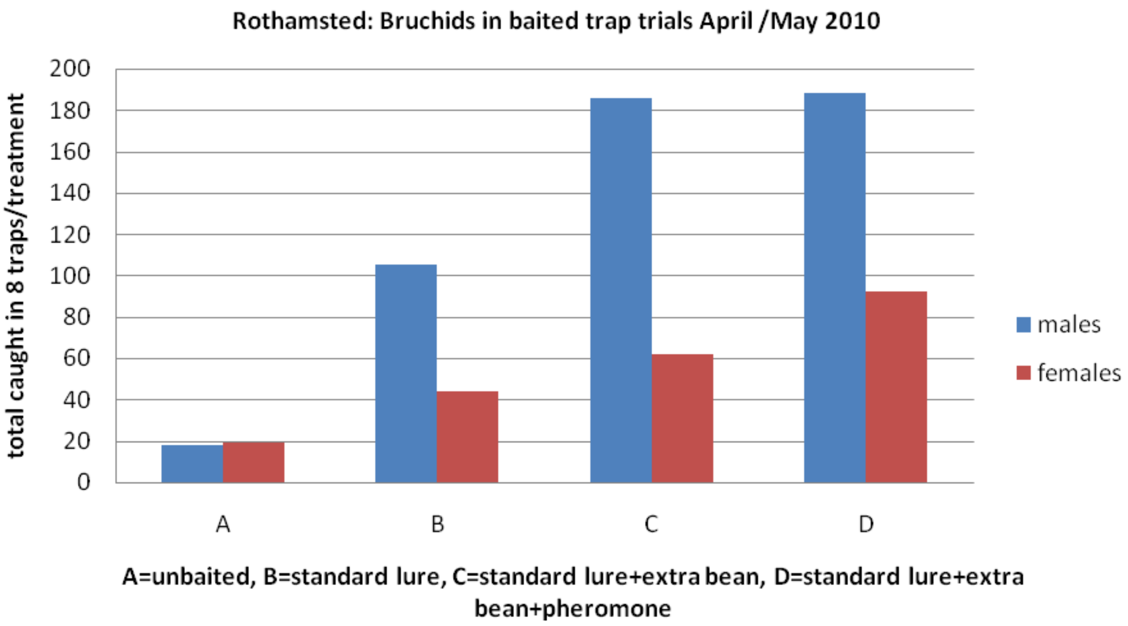


Figure 3.3 Field trapping trials with complex lures

6. Field trials with repellents

The plant activator *cis*-jasmone and slow release formulations of (*E*)-ocimene (releasing 20.2 mg/day/plot) and (*Z*)-3-hexen-1-ol (releasing 220 mg/day/plot) were tested in a 4 x 4 Latin square design replicated small plot field trial in spring beans var. Fuego in 2009 and 2010. The *cis*-jasmone (50ga.i./ha) was formulated with a 0.1% solution of the non-ionic surfactant Ethylan BV and sprayed onto the crop at a volume of 200l/ha on 29 May and 16 June 2009 and 29 May and 17 June 2010. The slow release dispensers were put out on 1 June 2009 and 27 May 2010 and replaced as necessary. Numbers of Bruchid eggs and larvae were assessed on 10 plant samples taken from each plot on 25 June and 27 July 2009 and 24 June and 23 July 2010 respectively. In 2009, there were no treatment effects on egg numbers per pod, but mean numbers of larvae per seed were lower in both *cis*-jasmone and (*Z*)-3-hexen-1-ol treated plots, although the differences were not significant to the untreated control (0.0237, 0.0183 and 0.04 respectively, ANOVA, P=0.08). In 2010, mean egg numbers per pod were lower in both *cis*-jasmone and (*Z*)-3-hexen-1-ol treated plots compared to the untreated plots (0.473, 0.305 and 0.703 respectively) and the difference was significant for (*Z*)-3-hexen-1-ol (ANOVA, P=0.03). Unfortunately, this difference was not maintained in the later assessment of numbers of larvae per seed, which showed no significant treatment differences.

Conclusions of Semiochemical studies

- The efficacy of the prototype trap has been confirmed and a standard lure developed
- The trap can indicate early Bruchid migratory activity
- A Bruchid specific male produced pheromone compound has been identified
- Further investigation of the lure components could improve the trap
- Work with experimental semiochemical repellents was promising, but efficacy needs to be improved

Work Package 4. Evaluation of *Vicia faba* germplasm for resistance to *Bruchus rufimanus* (NIAB, Frontier, Nickerson-Advanta, Wherrys, KWS (UK) Ltd)

1. Germplasm screening

All current commercial material of winter and spring sown beans in the UK is susceptible to infection by *B. rufimanus*. Different damage levels are recorded in seed harvested from Recommended List variety trials, though this variation appears to be associated with flowering time, with later flowering varieties generally showing lower levels of damage. Thus the lower levels of infection that have been observed are most probably due to escape rather than tissue resistance.

There has been significant effort in the past to screen a wide range of *V faba* accessions for resistance to Bruchid infection (Tahhan, PhD Thesis,1986 Bionomics of *Bruchus dentipes* Baudi and varietal resistance in *Vicia faba* L.)) at the ICARDA facility in Syria, where the pest is endemic. Some 972 lines (906 being Bean Pure Lines - BPLs) were screened, with no confirmed tissue resistance being found, and the low levels of infection found in some material were attributed to late flowering and late podding. However, a considerable proportion of the germplasm collection at ICARDA remained un-screened, particularly landrace material

Resistance to other Bruchid species in various legumes has been identified and crossed into commercial backgrounds where it has provided effective pest control. The identification of resistance to *Bruchus pisorum* in *Pisum fulvum* provides an example of where screening of a wide range of material has enabled the identification of a high level of tissue resistance which has subsequently been transferred to susceptible *Pisum sativum*. Though *Vicia faba* lacks any known wild relatives, more extensive screening of the species germplasm could yet reveal resistance sources. The aim of this workpackage was to access un-screened material from ICARDA and add any material from within current European programmes that was available from project partners and subject it to natural infestation in an area of high pest pressure. Any material showing evidence of low or zero Bruchid damage would then be further evaluated.

A total of 598 accessions were obtained during 2007 from the ICARDA collection in Syria. All lines which had been included in the previous Bruchid resistance screen there were eliminated. Accessions were selected with the help of ICARDA staff to focus on landrace material from the range of countries represented in the collection, though a small number of bean pure lines (BPLs) were included. A further 43 samples were added from project partners and several commercial varieties were included. All lines were allocated a new number (NV = NIAB Vicia). Seed was photographed digitally on arrival and images databased with available accession information, then stored at ambient temperature until planting the following spring.

A two replicate trial with each replicate consisting of 30 seeds sown at 15 cm spacing was established on 8th April 2008. The trial was sited on the NIAB farm, Cambridge, 12 m from a hedgerow, and surrounding by a spring bean crop. The trial ground has had a long history of including both winter and spring beans in the rotation to act as break crops for cereals. Major Bruchid infestations had occurred in at least the preceding two years. The spring bean variety Fuego was included at 23 locations in each replicate to provide an indication of overall variability in infection levels in the experimental area.

Establishment was initially good, but after a short period of growth, a considerable proportion of material appeared to suffer from wilting and stress symptoms related to soil conditions. It was thus difficult to collect comparable phenotypic data during the course of the experiment, but many lines set sufficient seed for harvest and assessment of Bruchid damage. Green cone traps set at 6 points in the trial area caught Bruchids between the end of May and end of June, though numbers declined to zero subsequently at a time when some accessions were still in flower.

Harvested seed numbers varied considerably between lines and between replicates of the lines, due to the growing problem encountered. Bruchid damage appeared to be relatively evenly distributed over the experimental area as indicated by the range of damage levels on the Fuego plots (Table 4.1), though replicate 2 appeared to have lower levels overall.

Table 4.1 % seeds with Bruchid holes in all harvested produce of 23 Fuego plots, replicate 1 and 2

Replicate 1	Replicate 2
6.4	6.3
13.5	0.0
6.3	1.4
20.8	3.3
5.3	3.8
14.7	8.4
5.4	5.4
16.3	11.5
13.0	2.9
33.3	12.5
0.0	20.0
14.0	0.0
10.2	0.0
8.3	0.0
0.0	0.0
7.7	0.0
4.2	0.0
0.0	0.0
10.7	0.0
14.3	13.9
7.5	3.0
33.3	0.0
0.0	9.7

Poor seed production, resulting in nil increase for some accessions, meant that no data was available for 97 of the accessions planted. Of the remaining 544, 203 lines produced fewer than 10 seeds in total from both replicates. Of the 203, 111 had no Bruchid damage. This result was not reliable because of the low seed number, and was disregarded. The remainder of the 203 all had some level of Bruchid damage, and in view of the objectives of the experiment, these lines were considered to be susceptible, despite low seed numbers.

Several lines produced higher numbers of seed and had zero or very low levels of Bruchid damage, and thus might include material with true resistance. Where necessary, seed of this material was increased during 2009 in a bee proof cage with artificial “tripping” of flowers to induce self fertilisation and obtain seed set. These “low Bruchid” lines were sown in a second field experiment on 6th April 2010 with three replicates each consisting of a row of 20 seeds. within a crop of spring beans at NIAB trial ground. Fuego, Tattoo and Maris Bead were included as commercial controls, and two lines exhibiting the “glossy pod” phenotype were also included in limited seed numbers (12 and 15 total) to test whether this pod characteristic had any effect on egg laying and subsequent damage. Bruchids were recovered from “tray beating” catches in the trial towards the end of June. Egg deposition was assessed on 5th and 13th July by counting eggs on one pod from each of three successive pod trusses on three plants in the middle of each row. Seed was harvested on 17th August, but delayed for some lines where pods were still very green until August 24th, and assessed for total Bruchid damage (holes and larval traces) a minimum of four weeks after harvest. Seeds with traces were cut open to confirm the presence of Bruchid larvae. Other insect damage (wasp parasitoids) causing small diameter holes was also recorded.

There were differences between lines in numbers of eggs deposited on both scoring dates (Figures 4.1 and 4.2)

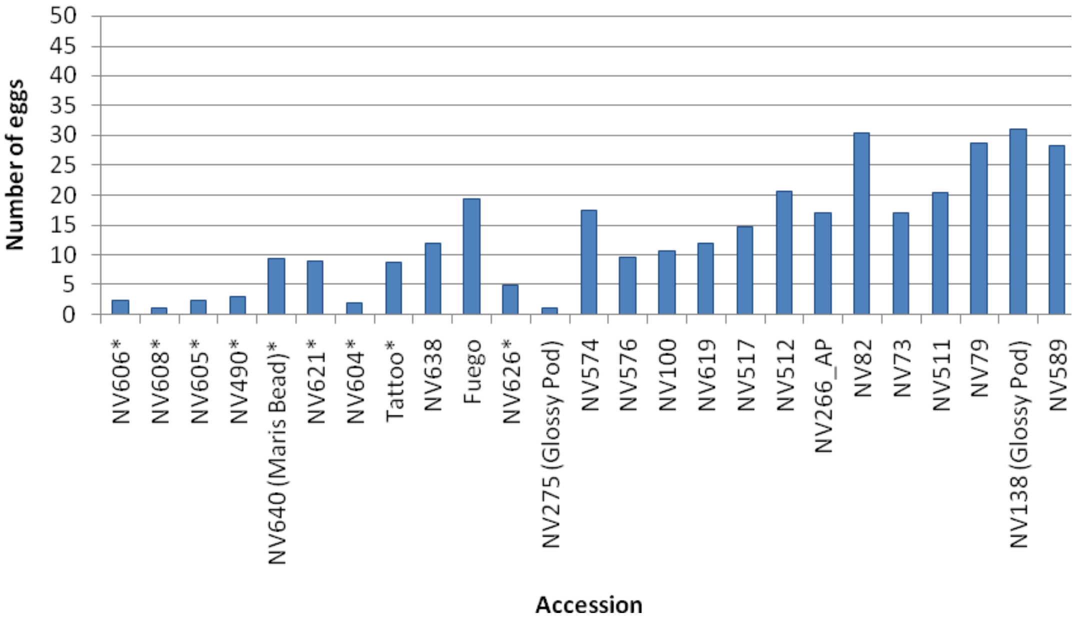


Figure 4.1 Mean egg number per pod (over nine pods) 5th July 2010

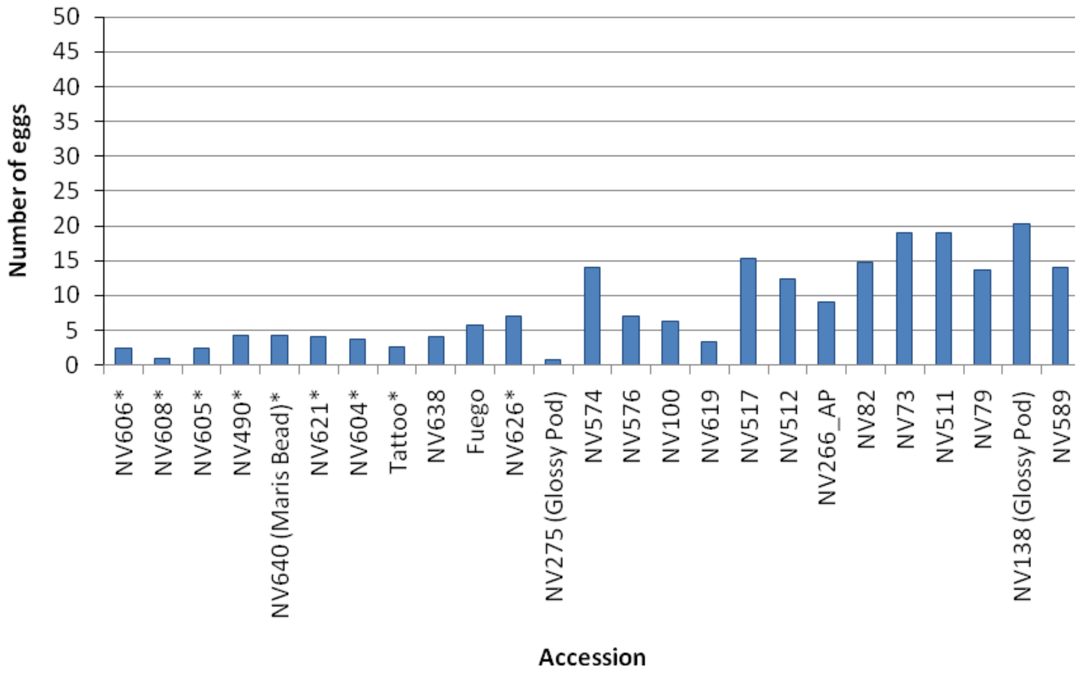


Figure 4.2 Mean egg number per pod (over nine pods), 13th July 2010

There were also large and significant differences ($p < 0.001$) in the level of Bruchid damage on harvested seed (Figure 4.3). Other insect damage, ie small diameter holes also differed significantly.

In Figures 4.1, 4.2 and 4.3, lines with an asterisk were late to mature and were harvested one week after other lines.

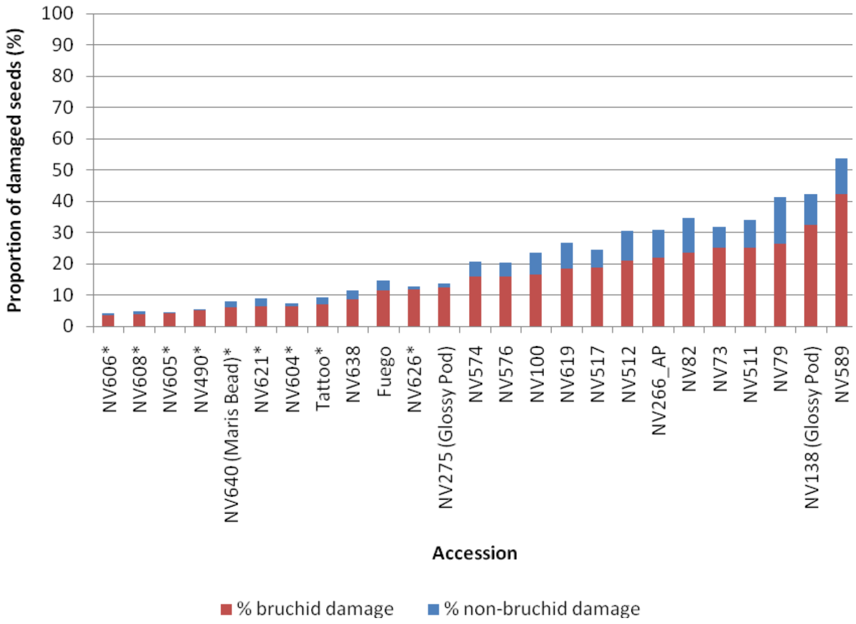


Figure 4.3 Bruchid and other insect damage after harvest

Conclusions of Evaluation of Vicia faba germplasm for resistance to Bruchus rufimanus

The low or zero Bruchid lines identified in the first large screen did not show any evidence of true resistance in the second experiment, and all lines had some degree of damage. Considerably more seed was harvested from the second experiment and estimations of Bruchid infestation were much more reliable. There was no indication that the "glossy pod" phenotype had either lower egg numbers or lower Bruchid damage, the two lines included differed significantly in damage level. It was clear that those lines which were harvested late due to late maturity were consistently less severely damaged, and also had lower egg counts. Late maturity would therefore offer a means of escaping damage, but escape was not complete and late maturing phenotypes are in general much less desirable agronomically. Even escape in one season would not always guarantee escape in other seasons as the timing of pest activity may change from year to year. It is still possible that lines with low Bruchid damage, even if they were late to mature, may contain some element of true resistance, and further work would need to include controlled experiments where captive pest populations are introduced onto putative partially resistant material.

Of the 641 accessions originally examined, 390 have been eliminated as potential sources of complete genetic resistance. There are small reserve portions, held at NIAB, of accessions which failed to produce enough seed for evaluation. These may be increased in the future and evaluated in the field for other traits as part of NIAB's *Vicia* pre-breeding initiative, and data may be obtained on Bruchid infestation.

World wide, some 38,000 accessions of *Vicia faba* are thought to exist (Global Strategy for the *Ex Situ* Conservation of Faba Bean (*Vicia faba* L., Global Crop Diversity Trust, Rome, 2009), though the level of uniqueness of these is unknown. However, the work within the LINK programme, together with the previous screening at ICARDA, and some smaller scale work funded by UNIP in France, has probably examined no more than 1500 accessions. There is thus still a very considerable genetic resource which has not been evaluated for Bruchid damage, and finding genetic resistance still remains a realistic possibility. Resistance to related Bruchid pests has been found in many legume species, and introduction of resistance genes through genetic transformation of *Vicia faba* also remains a possible, though challenging, route.

2. Image analysis (NIAB, Frontier)

The bean image analysis programme was developed from its pilot form in two further phases in 2008. First, a version of the programme that captured weight data from an electronic balance and image data from a digital camera was tested and used as described above. Secondly, some modifications to the programme to allow touching beans to be recognised and separately analysed was developed and introduced. Figure 5 shows how a sample presented with randomly touching beans was analysed by subsequent releases of the FabAnalysis programme before and after implementation of a touching bean detection and delineation algorithm.

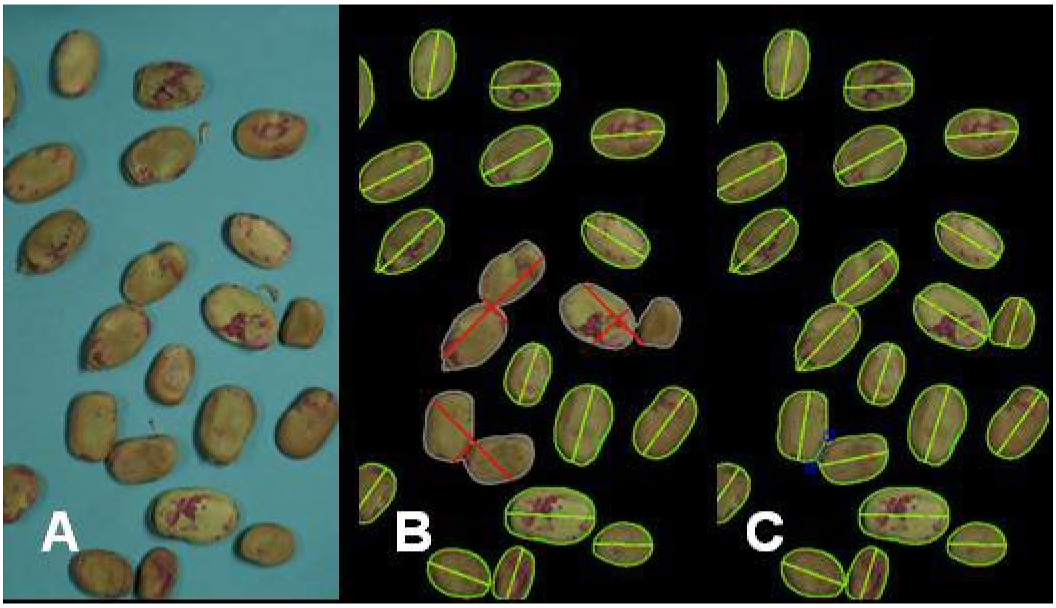


Figure 5. **A.** Original photo of a bean sample. **B.** Image analysis with FabAnalysis v2.1 results in touching bean 'objects' being excluded from the analysis as their shape does not fit the programmed parameters for objects to be analysed. **C.** Image analysis with FabAnalysis v.2.2 showing recognition and virtual separation of touching beans in the same image field.

Frontier supplied 400 samples from their quality labs 2007 harvest analysis to permit validation of the image analysis programme for the purposes of validating the Bruchid hole recognition capability of the NIAB IA software in 'real-world' samples and compared with the manual phenotype data gathered in Frontier quality labs.

Conclusions of Image Analysis

Image analysis was a useful tool for capturing and storing seed phenotype data but was not reliable for scoring Bruchid damage. In general, image analysis resulted in a twofold over estimation of holes when compared to manual assessments. In some individual samples, the correlation was much better, but these tended to be good quality seed, of a uniformly pale colour and free from depressions. The false positives were generally identified as dimples and patches of pigmentation in the seeds. A revision to the software may slightly improve the situation but as positive identifications were determined from areas of darker pixilation corresponding to Bruchid hole size, all objects conforming to this specification will be counted. One solution would be to identify holes by laser scanning but this option is expensive and was beyond the scope and funds of the project.

General Conclusions

The distribution of Bruchid rufimanus as a pest of *Vicia faba* field and broad beans in the UK was mapped from data obtained from damage assessments of crops grown throughout the UK in each of the cropping years, 2007-2010. It was found that there was no detectable shift in the pest distribution over the four years. The most severe infections always seemed to occur in a band south of the Wash and stretching from the West Midlands to East Anglia and extending down to the south coast. From a small number of isolated incidences of crop damage in areas outside those referred, there is a suggestion if conditions are favourable, the pest will spread quickly and infest crops wherever they are grown

In spray trials carried out in field and broad beans to optimise insecticide application, an evaluation of a spray forecasting model used in France showed that under UK conditions, the model was successful in improving control of damage. Sprays applied at first pod development following two consecutive days where the temperature has reached 20ºC were the most effective but control was further improved using angled alternate facing nozzles. There was no advantage found by using increased water volumes.

Evaluation of flower volatiles as a potential lure for a trapping system to monitor insect activity identified a complex mixture of compounds which were then tested in traps in the field. In addition, some initial studies were carried out to investigate the use of chemicals produced by the plants defence system as a repellent to *B rufimanus.*

To examine possible sources of varietal resistance to the pest, of 641 accessions screened for infestation in the UK, 390 were eliminated as potential sources of complete genetic resistance. A small proportion of the total number screened, were found to have lower levels of damage and these lines have been retained for future evaluation. As a rapid means of assessing the percentage of damaged beans in a harvested sample, an image analysis was evaluated but found to need further modification and development to provide very accurate results.

Knowledge and Technology Transfer

Date	Event/publication	Venue	Type	Organisation
November 2008	Pea and Bean Progress	publication	Article	PGRO
29, 30 October, 6, 17, 18, 20, 25 November 2008.	Pulse Roadshows	7 meetings across England	talk	PGRO
12 November	Meeting with UNIP France	Paris	Meeting/discussion of potential collaboration	PGRO/RRes/ UNIP
December	Meeting PC-GIN		Meeting/ presentation	NIAB
January 2009	Assured Produce Crop protocol-broad beans	publication	update of control measures	PGRO
January	Technical bulletin	publication	Technical update	PGRO
January	Pulse Agronomy Guide	publication	Technical update	PGRO
20 January	Growers meeting	PGRO	talk	PGRO
24 January	Meeting	Lincoln University	meeting	PGRO/ Lincoln Uni
11 March	Technical meeting	PGRO	talk	PGRO
April	The Pulse Magazine	PGRO	Article	PGRO

Date	Event/publication	Venue	Type	Organisation
June	Farmers Weekly, Farmers Guardian, Horticulture Weekly		Articles	PGRO
16 June	Open Day	PGRO	Discussions and demonstration	PGRO
10 – 11 June	Cereals 2009	Royston	farmer discussions and demonstration	PGRO
25 June	Post-graduate conference	Newcastle University	Poster	PGRO/ Newcastle Uni
30 June	SPDM Centre Research Day	RRes	Poster	RRes
January 2009	Assured Produce Crop protocol- broad beans	publication	update of control measures	PGRO
January	Technical bulletin	publication	Technical update	PGRO
January	Pulse Agronomy Guide	publication	Technical update	PGRO
20 January	Growers meeting	PGRO	talk	PGRO
24 January	Meeting	Lincoln University	meeting	PGRO/ Lincoln Uni
11 March	Technical meeting	PGRO	talk	PGRO
April	The Pulse Magazine	PGRO	Article	PGRO
June	Farmers Weekly, Farmers Guardian, Horticulture Weekly		Articles	PGRO
16 June	Open Day	PGRO	Discussions and demonstration	PGRO
10 – 11 June	Cereals 2009	Royston	farmer discussions and demonstration	PGRO
25 June	Post-graduate conference	Newcastle University	Poster	PGRO/ Newcastle Uni
30 June	SPDM Centre	RRes	Poster	RRes

Date	Event/publication	Venue	Type	Organisation
	Research Day			
November	PGRO/Syngenta Pulse Roadshows		talk	PGRO
November	Pulse Magazine	publication	article	PGRO
January 2010	2010 Pulse Agronomy Guide	publication	Technical update	PGRO
January	Assured Produce Crop protocol- broad beans	publication	update of control measures	PGRO
April	HDC Project News	publication	article	PGRO/HDC
June	Cereals 2010	Cambs	farmer discussions and demonstration	PGRO
July 8th	Pulse Trials day	Open day	demonstration	PGRO
July 2010	EntSoc Conference	RRes	Poster	RRes

2. A project web site was designed and outlines the aims and objectives of the project described. This is hosted by PGRO.

Publications

Ward RL and Smart, L (2011) The effect of temperature on the effectiveness of spray applications to control bean seed beetle (*Bruchus rufimanus*) in field beans (*Vicia faba*) Aspects of Applied Biology **106**, 2011 Crop Protection in Southern Britain 247-254