Project title	Integrated control of bean seed beetle (*Bruchus rufimanus*) LK09102
Project number:	FV322
Project leader:	Dr Anthony Biddle Processors and Growers Research Organisation
Report:	Annual Report October 2009
Previous report	Annual Report September 2008
Key staff:	Dr Anthony Biddle Miss Becky Ward
Location of project:	PGRO ,Rothamsted Research, NIAB
Project coordinator:	
Date project commenced:	1 August 2007
Date project completed (or expected completion date):	30 July 2010
Key words:	Bean seed beetle, spray timing, spray application, *Bruchus rufimanus*

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.

[Name] S P Potter
[Position] CEO
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Signature .... Date 16th October 2009

Report authorised by:

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CONTENTS

Science section

Grower Summary

Headlines

- An IPM programme for the control of bean seed beetle is under development. Timing pesticide application using temperature data and crop growth stage has been demonstrated. The use of angled nozzles when spraying to achieve maximum penetration is essential. Pest distribution in the UK has been mapped using damage data from commercial bean crops.

- Semiochemicals with potential for use in Bean Seed Beetle trapping and monitoring are under investigation and bean germplasm screening for resistance is underway.

Background and expected deliverables

The current control practices are not effective in reducing damage by the Bean Seed Beetle, *Bruchus rufimanus*. There is a large gap in knowledge regarding the biology and behaviour of the pest in locating host crops and of oviposition during the critical flowering and pod forming stages in early summer. Current recommendations are based on insecticide sprays applied during flowering. A lack of precision in 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 aimed to improve this situation by using a pheromone/semio-chemical system to monitor and risk assess as part of an integrated control method. This could provide a more sustainable approach to IPM which would also include resistant or tolerant varieties.

Effective control of *Bruchid* is essential to alleviate risks of poor returns due to unacceptable levels of pest damage, if growers are to expand the bean crop to be a valuable and break crop in both organic and conventional sustainable arable farming systems. A greater knowledge of the biology and behaviour of the pest particularly during flowering and early pod development stages of the crop, will allow the development of a more effective pest control method with insecticides. 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 spray, reducing the need for multiple applications and risk of resistance. In addition, improvements in pesticide application techniques will deliver more effective chemical control. In the long term, the delivery of identified genetic resources of resistance for future breeding programmes will help develop a package of integrated management approaches to improve insecticide timing, reduce the risk of pesticide resistance and ultimately reduce reliance on insecticide based control. This will enable sustainable bean production in the UK.

The specific aims of the project are to:-

1. fill the gaps in knowledge of the pest biology and the pest/host plant relationship to improve the chances of success in control
2. improve pesticide 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. provide a semio-chemical based trapping system for monitoring the pest in the crop.
4. utilise the trapping system together with meteorological data and crop development to provide a reliable indicator for pesticide application.
5. minimise pesticide usage by reducing the numbers of sprays applied to a crop to reduce pesticide resistance and to avoid unnecessary spraying.
6. examine varieties of beans for possible sources of genetic resistance to provide information for future breeding programmes.

Summary of the project and main conclusions

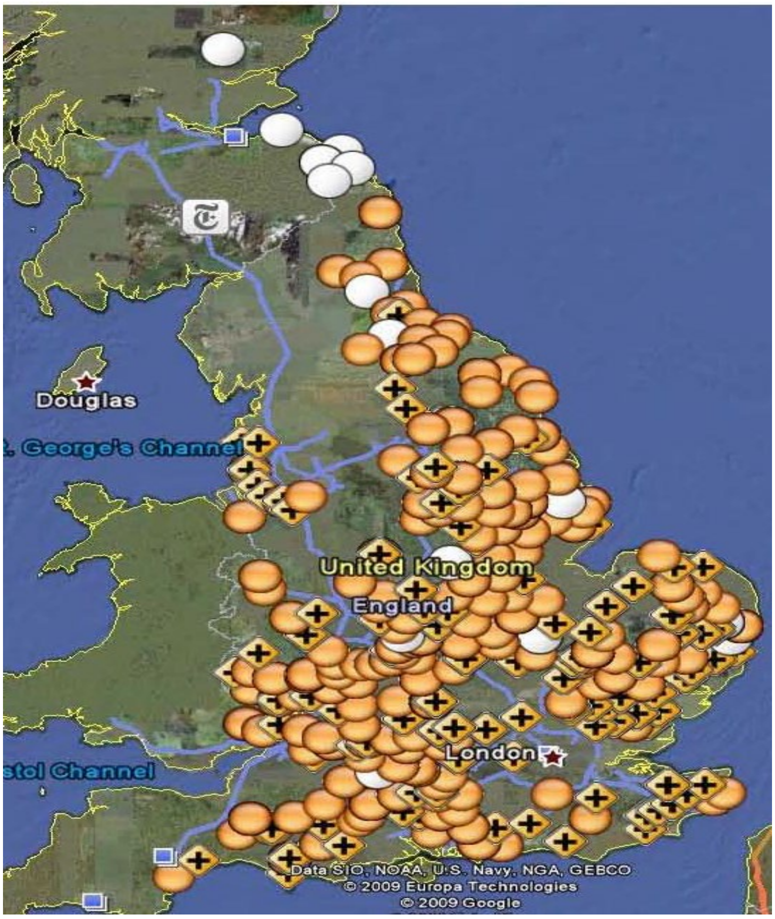
In the second year of the project, spray trials to evaluate a range of insecticides were carried out in commercial crops of field and broad beans. Timing of application was based on two days of a maximum daily temperature coupled with crop growth stage – a method suggested by French researchers. In two field scale trials, a significant level of control was achieved where sprays were applied at the early pod stage following two consecutive days when the air temperature had reached a maximum of 20ºC. This confirmed preliminary studies made in the previous year.

Farm scale trials showed improved control where spray penetration in the crop canopy had been improved by the use of angled nozzles at a normal water volume.

Laboratory studies identified the key components of flower volatiles that could be used in a monitoring system and preliminary field testing showed some response.

In a field screen, over 600 *Vicia faba* lines were collected from International germplasm banks and grown to maturity. There was an indication that at least one of the lines showed significantly less Bruchid damage than the standard current varieties.

Damage assessments made on commercial crop samples were correlated with their growing location to produce a map showing the distribution of the pest in the UK. This map is given below:



Bruchid damage (●) is present in most bean growing areas except for crops in the far north of England and Scotland (clean samples denoted by ○). High levels of damage (>5% of seeds, ◆) were seen predominantly in the south, east and west.

Financial benefits

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

Action points for growers

- Crops in Eastern and Western England are more at risk from Bruchid damage than northern 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

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

WP1 Insect biology

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

Damage levels were assessed using a standardised protocol on seed samples submitted to seed laboratories and on commercial crop samples submitted to merchants on beans produced in 2007 and 2008. In all around 800 samples were assessed in this way in each year. The data were correlated with production locality and areas of high and low incidence were mapped. These data have now been available from 2007 and 2008 and for the first time for *Bruchus rufimanus*, is now giving a clear indication of the distribution of the pest in the UK. The incidence of Bruchid damage among the samples examined during 2007 is shown on the map. Bruchid damage (●) is present in most bean growing areas except for crops in the far north of England and Scotland (clean samples denoted by ○). High levels of damage (>5% of seeds, ◆) were seen predominantly in the south, east and west.

WP2 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.

The sites and treatment details were as follows in Table 1.

Table 1. Site and treatments details.

Site	Variety	Nozzle/water volume
Rothamsted	Fuego and Wizard	200l IDK05 angled nozzle
		100l Amistar angled
		100l 03 Hawk angled nozzle
		100l 04 potato angled nozzle
Co-op Farms Essex	Fuego	200l Standard vertical nozzle 110-05
		100l Standard vertical nozzle 110-03.
Richard Hinchliffe Farms	Wizard	Amistar angled nozzle
		05 potato nozzle
Robert Spencer	Wizard	Hawk angled nozzle
		Amistar angled nozzle
JB Stevenson	Wizard	400l potato 05 angled nozzle
		200l potato 05 angled nozzle
EW Davies	Wizard	300l twin cap nozzle 2 x 04 db
		300l 05 potato angled nozzle

Beans at all sites were harvested. Samples from each plot were analysed for bruchid damage. The results were statistically analysed using GENSTAT where possible.
Results from the Davies site 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 Co-op Farms in Essex 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. The Spencer trial showed 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 the Rothamsted site (Gardner) compared to the untreated area, and in the spring beans standard nozzles at 200 l/ha also provided improved control.

There were no differences at the Stevenson site but Amistar and 05 potato nozzles provided improved control at the Hinchcliffe site compared to the untreated area.

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.

There were no strong indications that differences in water volume consistently affected control of bruchids.

2. Insecticide timing (Velcourt Ltd and PGRO)

Experiments were carried out in commercial crops of Fuego spring beans at two sites to examine the validity of a model used in France. The sites were at Aylmer Hall, Kings Lynn and Swanton Farms, Lydden near Dover. 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.

Samples were taken from the plots during the growing season. Records were made of egg numbers and damage caused by the pest at each podded node on 40 plants per plot. Samples were taken from Dover on 15th July 2008 and from Kings Lynn on 21st July 2008.

Samples were taken at harvest from each plot and analysed for bruchid damage. The data were statistically analysed using GENSTAT.

Plants that were assessed for egg numbers and damage at the fresh stage had very few eggs and no damage. The data were therefore not analysed.

Damage levels at dry harvest were analysed and the results are shown below.

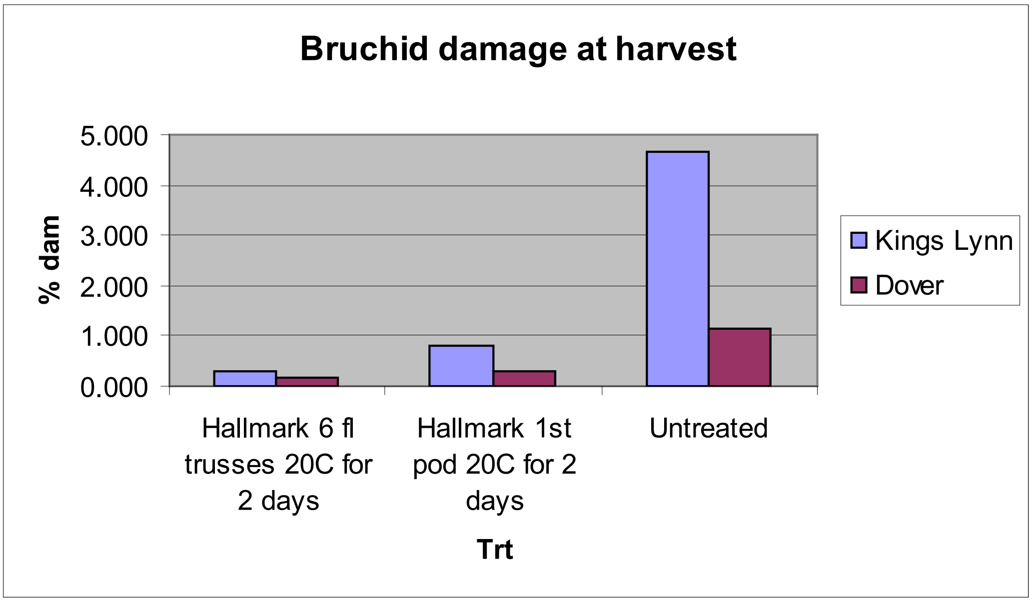
Table 2. Dry harvest damage levels at Kings Lynn 2008

	Treatment	Crop growth stage	Temperature	Total % damage
1.	Hallmark Zeon	203(6) + 7-10 DAT1	20°C for 2 days	0.31
2.	Hallmark Zeon	204(1) + 7-10 DAT1	20°C for 2 days	0.79
3.	Untreated			4.66
	Probability			< 0.01
	LSD @ p=0.05			0.570
	cv%			17.2

Table 3. Dry harvest damage levels at Dover 2008

	Treatment	Crop growth stage	Temperature	Total % damage
1.	Hallmark Zeon	203(6) + 7-10 DAT1	20° for 2 days	0.18
2.	Hallmark Zeon	204(1) + 7-10 DAT1	20° for 2 days	0.28
3.	Untreated			1.13
	Probability			0.024
	LSD @ p=0.05			0.665
	cv%			72.6

Figure 1. Bruchid damage at dry harvest 2008



Data 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.

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

An insecticide spray trial was conducted in a crop of Listra broad beans. The site was at East Raynham in Norfolk, OS: TF889254 and consisted of 2 plots of the same variety of broad beans drilled at different times. The first was planted on 4th April 2008 and the second on 17th April 2008. 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. Each drill plot measured approximately 90m x 170m and treatments were approximately 30m x 170m and not replicated. Sprays were applied in 200l/ha water with standard flat fan nozzles.

After flowering eggs were counted on all pods of randomly selected plants in each plot. The first assessment was carried out on 27th June 2008 on 20 plants per plot and the second assessment on 4th July 2008. The mean number of eggs per pod per node was calculated for each plant.

A sample of plants was taken from each plot and returned to the laboratory to be assessed. The first drilling was assessed on 24th July 2008 and the second on 30th July 2008. Ten plants per plot were assessed for egg numbers on the pods and larval damage on the bean seeds. 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.

Data were analysed using analysis of variance (Genstat 5)

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. Drilling 1 had higher levels of damage than drilling 2 across the three treatments.

WP 3. Semiochemical studies

Six areas of study continued in 2008:
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. (Rothamsted Research)
5. Trapping experiments with floral and/or bruchid derived volatiles
6. Field trial with repellents

1. Study of bean flower volatiles

Responses of female bruchid beetles to headspace samples of broad bean floral volatiles were investigated using gas chromatography (GC) coupled electrophysiology. In these studies, electrophysiological recordings from insect antennae revealed responses to six compounds in a sample of Sutton dwarf volatiles. The electrophysiologically active floral volatiles were subsequently identified, using GC coupled to mass spectrometry, as myrcene, limonene, ocimene, 4-allylanisole, cinnamaldehdye, caryophyllene and humulene. The natural emission ratio of these was quantified. As four of the six compounds are not in the synthetic blend currently used in traps in the field we will conduct experiments next year to evaluate if their inclusion can enhance trap catch.
Olfactometer bioassays with a natural sample of bean floral volatiles showed that the response was variable: less active insects were significantly attracted to it whereas very active insects were not. Perhaps insects in a dispersal mode temporarily switch off their response to host plant odours. This suggested that the physiological status of the insect could alter its response to a floral lure but that the insects affected were perhaps attempting to move away from the crop.

2. Bruchid mating behaviour

Adult bruchids 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 and some 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, females all had undeveloped ovaries. 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. If sufficient bruchids are available, this preliminary study will be repeated and expanded next year with PGRO.

3. Laboratory study of the effects of plant repellents

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 coupled to mass spectrometry. 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. 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. This work will be continued in field trials – see below.

4. Field testing of prototype trap
Traps containing the floral multi-bait developed in 2008 were evaluated on possible bruchid overwintering sites (i.e. sites of beans in 2008) and on winter and spring sown field bean sites in 2009 at Rothamsted and at PGRO sites. Assessments of adult bruchid numbers were made on transects across field sites where the traps had been placed to relate trap catch to bruchid colonisation. Assessments of eggs and larvae were made on some sites to relate trap catch to damage. Unlike 2008, no bruchids were caught on overwintering sites in 2009 probably due to lower temperatures. 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. Male bruchids arrived first in traps on both crops. As in 2008, the bruchids spread quickly throughout the crops.

5. Trapping experiments with floral and/or bruchid derived volatiles
1-undecene, the putative sex pheromone compound, was evaluated in field trapping experiments both in combination with the floral volatiles and on its own at different doses. Unfortunately, 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 floral volatiles 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. The implication is that this compound may not provide sufficient additional efficacy for use in a monitoring trap, however, further investigation of floral and bruchid produced compounds is planned for 2010.

6. Field trial with repellents**.**
The plant activator *cis*-jasmone and slow release formulations of (*E*)-ocimene and (*Z*)-3-hexen-1-ol were tested in a replicated small plot field trial in spring beans var. Fuego in 2009. 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 29th May and again on 16th June. The slow release dispensers were put out on 1st June and replaced as necessary. Numbers of bruchid eggs and larvae are currently being assessed on plants samples taken from each plot.

WP 4 Genetic Resistance

1. Screening for genetic resistance (NIAB, Frontier, Nickerson-Advanta, Wherrys, KWS (UK) Ltd)

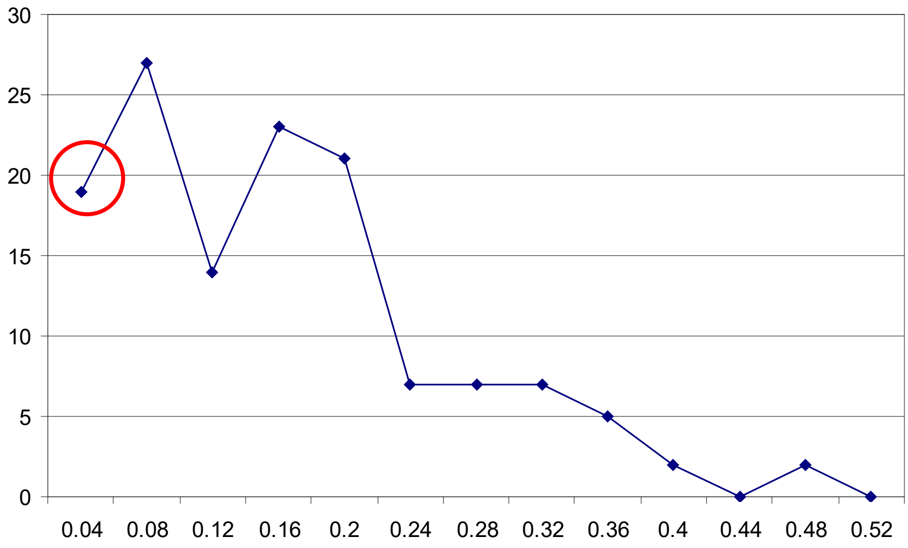
Previously, we reported on the collection of 642 faba bean accession and the establishment of a field-based screen for bruchid resistance at NIAB in 2008.

As the growth season progressed, the initially high rate of emergence was followed by a steady decline of the plants from stem elongation onwards. Typically, plants were chlorotic, prone to wilting, and eventually the stems fell over, breaking off from the root system at soil level. High *Sitona* weevil and bean aphid levels were also a problem, exacerbated by the necessity to avoid insecticide sprays which would have impacted on bruchid health and/or visitation levels. This general decline in plant population followed a clear spatial pattern with about 2/3 of the trial area encompassing all of Rep 2 and part of Rep1 affected severely together with surrounding bean crop over an area approximately half of the total field area. The generalised nature of the decline, without specific symptoms resembling known bean diseases, and its confinement to a particular section of the field, led to the conclusion (confirmed by an independent agronomist) that soil compaction was at fault.

One line – Borington Bulk - was notable in showing near normal vigour in marked contrast to the surrounding plots and the hypothesis that it has a root system capable of thriving in adverse soil conditions has led to its inclusion in a drought tolerance trial being conducted by PGRO at Brooms Barn in 2009.

Although growth was patchy and plant populations and general vigour were both extremely low, bruchids were observed visiting flowering plants and the trial was harvested and bruchid damage evaluated. The average percent of seeds showing bruchid emergence holes was relatively high at 14.2% and the distribution of holes/seed ratio for Rep1 is shown in Figure 1, where a clear peak in the distribution around the average holes/seed ratio of 0.142, but also a number of lines (highlighted by a red circle) where all harvested seed were free of bruchid damage. The low total number of lines reported on reflects the high number of plots where there was total loss. Due to the low seed recovery on the line reported on, lack of bruchid damage could be due entirely to a combination of chance and avoidance mechanism; however, it is probably valid to conclude that where non-negligible bruchid damage levels were observed, that true resistance (i.e. toxicity to the larvae) can be concluded to be absent, and that future trials should focus on lines which could not be excluded in this way.

Figure 2. Distribution of Bruchid emergence holes per seed ratio values in samples where more than 50 seeds were recovered from a plot.



Given that 60 out of 70 donated seed had been committed to the field trial, leaving a remnant sample of 10 seed per line at most, it was decided to undertake an overwinter multiplication of as large a part of the collection as available heated and lit glasshouse space permitted to replenish seed stocks. This has been carried out in insect-free winter glasshouse conditions between Dec 2008 and April 2009 and seed was harvested from each of 520 accessions (4 plants per line). Nineteen of these lines with the lowest bruchid damage scores were entered into a further round of multiplication in outdoor isolation cages in June 2009 (16 plants per line, all from a single selfed plant from the overwinter multiplication). This should result in production of enough selfed seed from the 19 lines to carry out a replicated field bruchid screen on a larger scale in 2010.

A side benefit from the unplanned multiplication of the collection overwinter was the chance to pick out lines carrying traits which may potentially impinge on bruchid behaviour or success, and to this end a waxy/glossy pod and a number of white flowered lines have been identified for multiplication and inclusion in future bruchid screening.

Conclusions

Currently, the pest is restricted to eastern southern and western parts of England. Where crops are at risk, spraying should take place following two days of maximum air temperature of 20ºC. Angled nozzles provide more effective crop penetration. Indications are promising for an effective Semio chemical monitoring system and early indications are that there may be some germplasm with some degree of resistance.

Technology transfer

Dissemination and Communications

1. A log of communications concerning the project is shown in Table 4

Table 4. KT log 2008-2009

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
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

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

3. Control measures were updated in the Assured Produce Crop Protocol for broad beans

Appendix

LK09102 Integrated control of bean seed beetle (Bruchus rufimanus) is sponsored by Defra through the Sustainable Arable LINK programme in association with PGRO, Rothamsted Research, NIAB with industrial partners, HDC, Frontier Agriculture, Wherrys, KWS(UK), Nickerson, Oecos, Velcourt and Raynham Farms