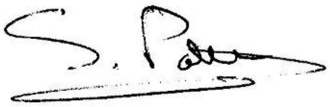
Project title	Integrated control of bean seed beetle (Bruchus rufimanus)
Project number:	FV322
Project leader:	Anthony Biddle Processors and Growers Research Organisation
Report:	Annual Report September 2008
Previous report	
Key staff:	Dr A J Biddle Miss S A Atkin
Location of project:	PGRO ,Rothamsted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

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.

Salvador Potter
CEO
Processors and Growers Research Organisation

Signature:  Date .23/12/08

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Grower Summary

Headline

- Improved control of bean seed beetle in beans was achieved by timing insecticides with temperature and crop development.
- Crop penetration by sprays was improved by angled nozzles and early work indicates that insects can be attracted by bean flower volatiles.
- Work on screening of *Vicia faba* germplasm is looking for possible sources of resistance.

Background and expected deliverables

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. However, 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

In the first year of this LINK project sponsored by Defra, Spray trials were carried out in commercial crops of field and broad beans to evaluate a range of insecticides for the control of the pest. Timing of application was either based on current recommendations for crop growth stage or was timed in relation to maximum daily temperature coupled with crop growth stage – a method suggested by French researchers. Where insecticides had been applied without reference to temperature, there was no apparent reduction of beetle larvae damage, however in two field scale trials, 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.

Farm scale trials also indicated 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 indicated that the beetles and the bean flowers released compounds that could be useful as attractants in a monitoring system.

In a field screen, over 600 *Vicia faba* lines were collected from International germplasm banks and grown to maturity. Early assessments in damage levels showed some lines with low or high levels of damage and this is to be investigated further. An image analysis method was evaluated for use in identification and measurement of bean seed beetle damage in produce.

Financial benefits

At this stage, an improvement in the timing and application of insecticides would reduce damage and reduce the risk of crop rejection of broad beans grown for processing or fresh market.

Action points for growers

- 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

Summary of Project Progress

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

WP1 Insecticide application and timing

1. Application (Syngenta Crop Protection and PGRO)

Field trials were carried out at 7 sites to assess the effectiveness of different volumes of spray and different 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 commercial crops of spring field beans using standard sprayers. The treated areas were unreplicated in large field scale plots and an unsprayed area was left in each field for comparison. In addition, some of the field trials compared the effects of different water rates.

Table 1: The sites and treatment details

Site	Variety	Nozzle/volume
Rothamsted	Fuego and Wizard	200L Standard vertical nozzle 110-05
		400L Twincap 2x110-05
FarmCare Essex	Fuego	400L Standard vertical nozzle 110-08 200L Standard vertical nozzle 110-05
		100L Standard vertical nozzle 110-03.
Richard Hinchliffe Farms	Wizard	200L Standard vertical nozzle 110-05
		100L Standard vertical nozzle 110-03
Phillimore Farms	Wizard	75L Airtec Med at 80cm
		75L Airtec Med at 50cm
Ranston Estate	Wizard	200L Standard vertical nozzle 110-05
		400L Standard vertical nozzle 110-08
		100L Standard vertical nozzle 110-03
		300L Standard vertical nozzle 110-05.
David Felce Farms	Fuego	100L Amistar nozzle (angled)
		200L F110-04
		200L potato nozzle 04 (angled)

Beans at all sites were harvested. Samples from each subsection of each plot were analysed for bruchid damage. The results were statistically analysed using GENSTAT for all trial data.

Overall the results of the damage assessments indicated that higher volumes of water, especially those at 400L are not effective at controlling damage. The most effective water volumes were 100-200L. At the Rothamsted site, it was found that standard vertical nozzles at 200L showed significant reductions in damage. Although twin cap nozzles were not significantly effective in reducing damage levels the number of live bruchid in the crop following application using these nozzles was lower. At the Hinchliffe site, sprays at 100L and 200L produced significantly better control than the untreated plots, the 100L treatment being more effective. The trial of David Felce showed that the Amistar nozzle at 100L was the least effective when compared to the untreated but both the standard nozzle at 200L and potato nozzle at 200L/ha produced a statistically significant reduction of damage

2. Insecticide timing (Velcourt Ltd and PGRO)

Experiments were carried out in commercial crops at two sites to examine the validity of a model used in France at Aylmer Hall, Kings Lynn the trial investigated the effectiveness of one or two applications of Hallmark applied at the small pod growth stage following two consecutive days where temperatures reached 20°C.

Damage was highest on the unsprayed area of the crop, with only a small level of reduction by the single application of Hallmark. Although no significant differences were found between plots in many cases, egg and damage assessments showed a high level of control where two applications of Hallmark were received after two consecutive days at 20°C.

At the Dover trial, there were significant differences between numbers of eggs per node and level of larval damage per node and overall bean damage after harvest between treatments. The lowest level of bruchid damage occurred where two applications of Hallmark were made following two consecutive days of 20°C at small pod growth stage.

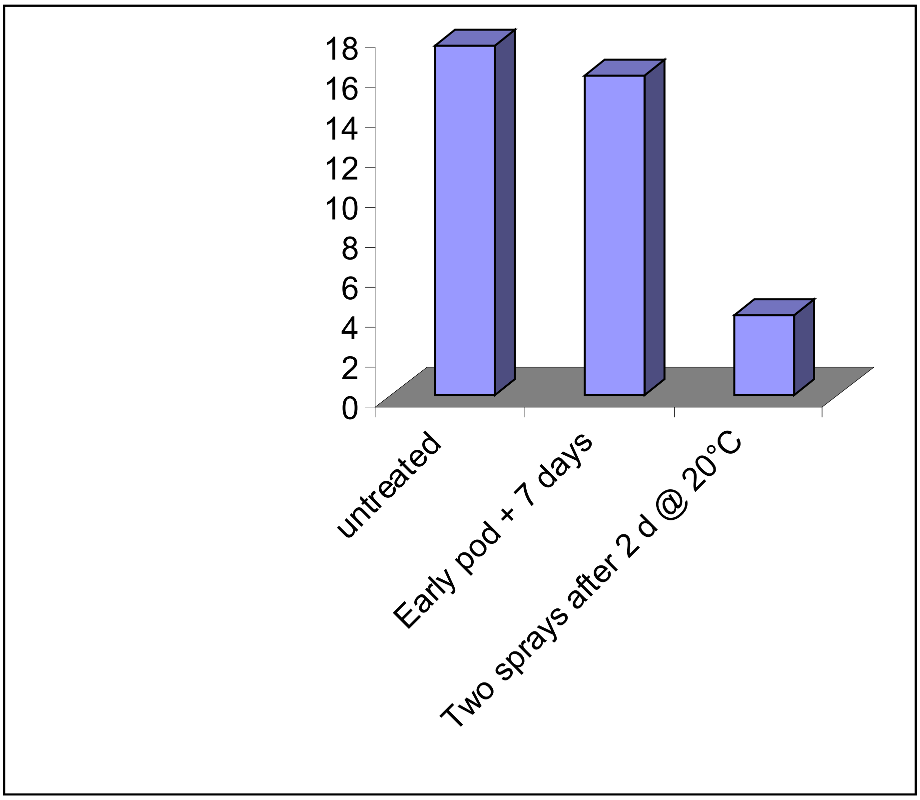

Figure 1. Damaged beans after treatment – Dover 2007

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

To determine the efficacy of different treatments and different drilling dates on the impact of bruchid beetle in broad beans a trial consisting of 6 drillings of approx 2.0 ha of the same variety of broad beans (cv Listra) was drilled at weekly intervals. Each drilling was divided into plots of three sections each of which received an insecticide application made by a farm sprayer. Treatments included deltamethrin applied in 200L/ha water with standard flat fan nozzles. The plots were further divided to allow comparisons of bruchid population distribution. Egg counts were made on pods in each area throughout the period and a final assessment of damaged beans was made at the stage normally taken for canning.

There appeared to be no differences in egg numbers or damage between the treatments, but there were differences between the drilling dates. The second drilling period suffered the greatest level of bean damage than any of the other drilling periods and this was reflected by the higher egg numbers found on the pods over the period. This sowing was at a susceptible growth stage when bruchid activity was at its peak. Temperatures reached 20°C for three days from 22nd to 24th May 2007 but there were no pods present in any of the plots. It also reached 20°C for two days on 1st and 2nd June when pods would have been present in the earliest drilling period.

Despite the spray applications, there was no indication that damage was reduced by any of the spray applications made to any of the six drillings.

WP2 Insect biology

1. Insect distribution in the crop (PGRO)

At the Dover site, the bean damage was assessed at the mature stage just prior to harvest for bean damage from plants taken at six locations along transects across the field. The distance between sampling sites was approximately 25 m. Assessments were made in the unsprayed and the two sprayed areas of the field as previously described in section 2. The damage distribution across the field is shown in the graph. The insecticides had reduced the damage compared with the unsprayed area. The damage distribution of the unsprayed area appeared to be relatively even over the length of the field although one outer margin of the crop appeared to have lower damage. There was however no clear indication that the damage was unevenly distributed (Figure 2)

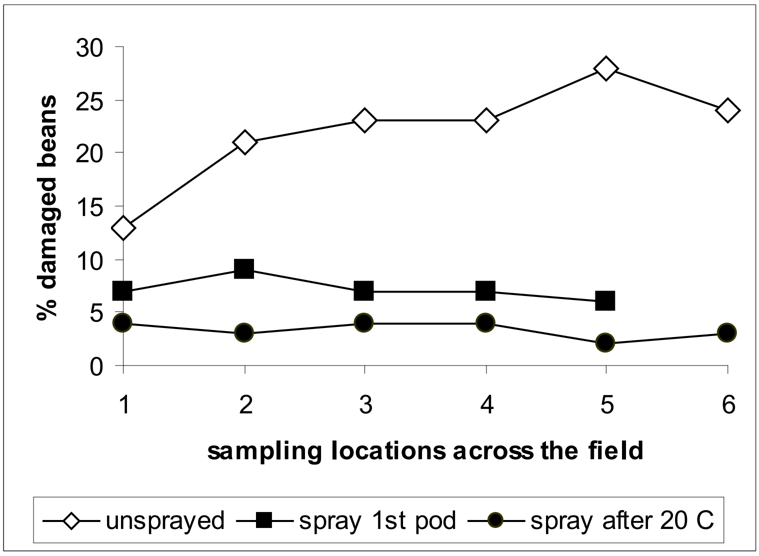

Figure 2. Damage distribution across commercial crop

2. National pest distribution (Frontier and NIAB)

A record of damage levels was made on all commercially produced bean crops marketed by Frontier in 2007. The data were correlated with production locality and areas of high and low incidence were mapped.

WP 3. Semiochemical studies

Four areas of study commenced in 2007:

1. First year study of bean flower volatiles
2. Initial study of bruchid specific volatiles
3. Laboratory study of plant repellents
4. Field experiment with prototype trap at overwintering sites and bean fields. (Rothamsted Research)

The volatiles produced by flowers of intact glasshouse grown field bean plants vars. Wizard and Fuego and broad bean plants var. the Sutton was collected in situ by air entrainment. The natural ratios of volatiles released by the flowers were determined by Gas Chromatography (GC) and GC-Mass Spectrometry. The composition of volatile chemicals from flowers of the different intact plants showed surprisingly little variation when compared to each other and to flowers of cut plants that had been entrained previously. Some of the identified chemicals, particularly cinnamyl alcohol, trans-cinnamyldehyde and linalool, were among those perceived by bruchids in GC-linked electrophysiological studies and were shown to be attractive when released individually in field traps. To mimic the natural ratio in which the chemicals are released, experimental dispensers, consisting of cellulose sponge and polyethylene tubing of various sizes and thickness, were designed to release these compounds. Release rates were determined under controlled air speed and temperature conditions in the laboratory and multiple baits were developed for use in field experiments to improve the performance of a prototype trap (see below).

Air entrainments were made of volatiles produced by post-diapause male and female bruchids collected from the field in early May 2008. The behavioural responses of field collected male and female bruchids to these entrainment samples were investigated using a Perspex four-arm olfactometer, a device which enables quantification of insect responses to different odour fields in terms of time spent and number of entries into different areas. The objective of this was to determine the presence of any pheromone. 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. Males were tested with female entrainment samples and females were tested with male entrainment samples. 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) and 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. Such a compound, once structurally identified, could be useful not only in the monitoring of this pest but could even be used for direct control as it is attractive to the female insects. Responses of male and female bruchids to the entrainment samples were investigated in GC-linked electrophysiological studies and female antennae responded to a small number of compounds from the male sample. These have been tentatively identified by GC-MS and dispensers will be devised to test these compounds in field traps in spring 2009.

Several volatile chemicals, collected from bean plants that had been damaged by feeding adult bruchids, were identified by GC-electrophysiology and included (*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. Formulations of these compounds were investigated and developed for field use and will be tested in small plot trials next season.

Field trapping trials were carried out on possible bruchid overwintering sites (i.e. sites of beans in 2007) and on winter and spring sown field bean sites in 2008 to evaluate the attraction of multibaits releasing three different ratios of the flower volatiles cinnamyl alcohol, trans-cinnamyldehyde and linalool. Two trap types were used initially, a prototype cone trap, which consisted of a modified boll-weevil trap, and a yellow sticky trap angled at 45°. The multibait releasing the three compounds in the most natural ratio proved to be the most attractive overall. Assessments of adult bruchid numbers, eggs and larval damage were made on transects across field sites where the traps had been placed to relate trap catch to bruchid colonisation and damage. The multibait will be field tested with further trap types and at different sites in the next season.

WP 4 Genetic Resistance and Analysis Methodology

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

Under this heading, the original target which was to gather and multiply seed, for more than 500 accessions from ICARDA and JIC collections was exceeded with advanced breeding lines and cultivars in development being supplied by the seed companies. Table 2 shows the final tally of ICARDA, varietal and pre-breeding materials gathered from 6 different sources which came to a total of 642 unique lines. Since all donors provided c. 70 seed per line, no multiplication was required prior to an initial field evaluation which was carried out from April-September 2008.

Table 2: Genotype material submitted for screening

Donor Institute	# lines	Type	Purpose in screen
ICARDA	600	ILB landrace accessions	high genetic diversity
JIC	6	Former PBI spring varieties	old UK-adapted diversity
KWS	12	Breeding lines	proprietary material
Limagrain	12	Breeding lines	proprietary material
INRA-Dijon	4	Varieties	controls from INRA screen
Goettingen Uni	3	Inbred research lines	closed flower mutants
CSIC Cordoba	1	Inbred research line	reference paucijuga line
NIAB	2	RL spring controls	to reference to current RL

All seed donations were weighed and photographed, and the resulting data captured in a customised project database. Each line was assigned a unique reference id, which will be frequently referred to from here on.

The second task, a field-based screen at the NIAB trial-ground was sown in a randomised block design with two replicates of 30-plant rows on 6th April 2008. Initially, emergence rates were good but subsequently, plants across approximately half the field in which the trial was being run died off at the seedling stage or stems broke off at soil level once stems had started elongating. Closer inspection of the plants and their root systems indicated that soil compaction was the cause of the poor establishment of the trial, probably due to difficulties with seed bed preparation in the wet spring conditions. Since the plant number and condition were severely affected, the scoring of non-target characters (e.g. flowering date) was postponed to a future trial. The plots have now been harvested and phenotypic analysis for bruchid damage is in progress. In case data quality is low from some plots, we have already put measures in place to multiply all 642 lines in preparation for a repeat screen in spring 2009.

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. 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.

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.

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Discussion

In the first year of this three year project, useful indications where control in the field can be achieved have been found. The next phases will further evaluate these findings.

Conclusions

Early indications have given some confidence in modifying the current control recommendations to take into account the temperature influence in insect activity.

Technology transfer

Dissemination and Communications

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

Date	Event/publication	Venue	Type	Organisation
November 2007	Pea and Bean Progress	publication	Article	PGRO
9, 16, 21,24th January 2008.	Pulse Roadshows	4 meetings throughout eastern England	talk	PGRO
January	Assured Produce Crop protocol- broad beans	publication	update of control measures	PGRO
23rd January	Training course	PGRO HQ	talk	PGRO
29th January	Growers meeting	Framlingham	talk	PGRO
31st January	Oilseeds and Pulses Conference	Peterborough	talk	PGRO
February	Pea and Bean Progress	publication	article	S-A.Atkin PGRO
6th February	Growers meeting	Dengie, Essex	talk	PGRO
8th February	Technical meeting	Quy, Cambs	talk	PGRO
26th February	Technical meeting	Bicester	talk	PGRO
April	Information Sheet		Technical update	PGRO
May	TAG News	publication	article	A Biddle and S-A Atkin PGRO
8th June	Open Day	PGRO	discussions	PGRO
11th-12th June	Cereals 2008	Lincoln	farmer discussions	PGRO

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.