Final Report for

HDC Project FV13c

Assessing new ways of controlling the cabbage root fly and the carrot fly

Entomology Section Horticulture Research International Wellesbourne, Warwick, CV35 9EF This project was studied in two parts under the following research topics:

Part 1

Determining the effectiveness of insecticide sprays applied against the second generation of carrot fly

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Part 2

Assessing strains of <u>Bacillus thuringiensis</u> as a possible method for controlling the cabbage root fly and the carrot fly

N.D. Pipe & S. Finch

Determining the effectiveness of insecticide sprays applied against the second generation of carrot fly

G. H. Edmonds & S. Finch

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Summary

Most of a large block of carrots drilled on 2 April was covered with Envirofleece to protect the plants from attack by the first generation of carrot fly. A second plot of carrots was drilled late on 26 May so that these plants also were not subjected to attack by the first generation of carrot fly. Attack by second generation carrot fly at the site was extremely high. On the "check" plots that were not treated with insecticide, 92% and 94% of the carrot roots were damaged when the plants were harvested on 15 October.

Chlorfenvinphos, quinalphos, pirimiphos-methyl, triazophos and diazinon were all applied at the recommended rates in 1000 l water/ha. In addition, to assess the effect of lower volume sprays, the recommended amounts of chlorfenvinphos and triazophos were applied in 500 l water/ha and triazophos was also tested using only 250 l water/ha.

Carrots on the earlier drilled block had larger roots and considerable amounts of foliage. The latter intercepted much of the spray and as a result reduced the effects of the treatments. Of the plots treated with 1000 l of spray/ha, triazophos at 0.53 kg a.i./ha gave the highest (82%) estimated reduction in numbers of maggots. The next most effective treatment was chlorfenvinphos at 2.35 kg a.i./ha, followed by diazinon at 1.12 kg a.i./ha, triazophos at 1.05 kg a.i./ha, pirimiphos-methyl at 2.1 kg a.i./ha, quinalphos at 0.74 kg a.i./ha, pirimiphos-methyl at 1.4 kg a.i./ha and diazinon at 2.24 kg a.i./ha. Reducing the volume sprayed to 500 ml/ha had no effect on the efficiency of triazophos, but reduced the effectiveness of chlorfenvinphos from 77% and 31%. Triazophos sprayed in 250 ml/ha gave only 47% reduction compared to 83% when applied in 500 ml/ha.

On the later drilled block, all chemicals applied in 1000 l/ha, gave moderately good control of carrot fly. Diazinon applied at 1.12 kg a.i./ha reduced the estimated numbers of carrot fly larvae by 96%, followed by triazophos at 0.53 kg a.i./ha, chlorfenvinphos at 2.35 kg a.i./ha, pirimiphos-methyl at 2.1 kg a.i./ha, triazophos at 1.05 kg a.i./ha, quinalphos at 0.74 kg a.i./ha, diazinon at 2.24 kg a.i./ha and pirimiphos-methyl at 1.4 kg a.i./ha. Reducing the volume sprayed to 500 l/ha had only a moderate effect on the efficiency of chlorfenvinphos and triazophos, but triazophos applied in 250 ml/ha gave only a 67% reduction, compared to a 93% reduction when applied in 500 ml/ha.

The present experiments were carried out to demonstrate the relative effectiveness of the various treatments currently applied to control this fly. High numbers of damaged roots, even after applying the most effective chemicals, are expected from this type of experiment.

In the present experiments, in which 92-94% of the plants in the untreated ("check") plots were damaged by the fly, the overall insect population was much too high for adequate control. In such situations, even a 95% effective insecticide treatment is not able to reduce crop damage below 16%. Remember, there is a limit above which damage to untreated carrots cannot be allowed to rise, if crop protection measures are to give adequate control.

Methods

Site and soil type

The field experiment was carried out in Long Meadow West at HRI(W) in 1992 on a freely-drained, coarse loamy soil of the Wick series with a pH of 6.8 and an organic carbon content of <1.0%.

Rainfall and temperature

Rainfall and soil temperature at 10 cm depth were recorded daily at 0900 GMT at the HRI(W) meteorological station.

Crop

The activity of the insecticides against carrot fly was investigated using Danvers 126, a carrot cultivar that is highly susceptible to maggot damage. The carrot plots were drilled in two contiguous areas of the same field. The first plot (12 x 41 m) was drilled on 2 April 1992 and was covered with Envirofleece. The second plot was drilled on 26 May, so that the plants did not need to be covered to avoid being attacked by the first generation of carrot fly. The carrot seed was sown in four rows per bed, at 30 cm between rows, using a Mk II Stanhay S-870 drill. Once the seedlings had emerged from the soil, areas were hoed out to produce 2 m long plots with 1 m between plots.

Site husbandry

Cultivation and fertilisers

The land used for the experiment was ploughed on 28 October 1991. A

base fertiliser application of 231 kg P₂O₅/ha and 231 kg K₂O/ha was applied on 17 March 1992. On 19 March, the whole area was crumble rolled and Nitram was applied at 80 kg N/ha to the area to be drilled first. This area was power-harrowed on 2 April immediately before drilling. On 26 May, the second area had Nitram applied at 80 kg N/ha and was then power-harrowed immediately before drilling.

Weed control

Weeds were controlled with a pre-emergence spray of 675 g linuron (Hoechst Afalon; 450 g/l)/ha applied to the first area on 6 April and to the second area on 3 June. Both areas were hand-weeded as necessary.

Aphid control

On 24 July both areas were sprayed with pirimicarb (Phantom; Bayer; 50% SG) at 140 g a.i./ha.

<u>Irrigation</u>

The second drilling only was irrigated, using fixed spray lines, on 17 June, 22 June and 31 July, applying 8 mm on each occasion.

<u>Treatments</u>

Plot covering

Carrots drilled on 2 April were covered on 29 April with a 4.7 m wide crop cover (Agralan Envirofleece), dug in round the edges, to exclude the first

generation of carrot fly. The covers were removed on 17 June.

Insecticides

Table 1: <u>Insecticide treatments and dates of application</u>

	Ra	te	Volume	
Insecticide	product/ha	kg a.i./ha	of water (l/ha)	Dates sprays applied
Chlorfenvinphos	9.8 1	2.35	1000	3 August
Chlorfenvinphos	9.8 1	2.35	500	3 August
Quinalphos	2.5 1	0.74	1000	3 August, 3 September, 1 October
Pirimiphos-methyl	2.8 1	1.4	1000	3 August, 3 September, 1 October
Pirimiphos-methyl	4.2 1	2.1	1000	3 August, 1 October
Triazophos	2.38 1	1.05	1000	3 August, 3 September, 1 October
Triazophos	1.19 l	0.53	1000	
Triazophos	1.19 1	0.53	500	3 August, 14 August, 28 August, 11 September,
Triazophos	1.19 1	0.53	250	28 September
Diazinon	2.8 kg	1.12	1000	
Diazinon	5.6 kg	2,24	1000	3 August

Formulations used were: - chlorfenvinphos, 24% e.c.; quinalphos, 245 g/l e.c.; pirimiphos-methyl, 50% e.c.; triazophos, 42% e.c.; diazinon, 40% w.p.

All treatments were applied using a CP3 knapsack sprayer. Each of the six replicated blocks in both halves of the trial included one plot for each insecticide treatment and three untreated check plots.

Assessments of carrot fly damage

On 15 October, all plots were sampled, taking 1 m from each of the two centre rows. Carrots were washed, and all roots greater than 1 cm diameter were separated into damaged and undamaged categories, and were counted and weighed.

Carrot fly numbers

During the course of the experiment, carrot flies were trapped on three sticky traps set up nearby. These were replaced each Wednesday and the trapped flies were counted (Appendix A).

Computation of results

The effect of the treatments on the numbers of carrot fly larvae were estimated using a log-log transformation of the % undamaged carrots from each plot, and the transformed data were examined by analysis of variance.

Results

Weather

The rainfall and 10-cm soil temperature recorded from 1 April to 15 October 1992 are shown in Figs. 1 & 2. The total rainfall during the experiment (2 April to 15 October) was 440 mm, 0.1 mm or more being recorded on 93 of the 197 days.

The mean soil temperature at a depth of 10 cm was 14.5°C. The weather prevailing at the time of each spray application is shown in Appendix B.

Carrot fly damage to carrots

Carrot fly damage on untreated plots was severe, with 92% and 94% of the roots being damaged when the two plots were harvested on 15 October (Tables 3, 4 & 5 numbers not significantly different).

Damage to carrot roots on the insecticide treated plots, was higher on the first than on the second drilling (Table 3). The relative efficiencies of the insecticide treatments were similar on both plots, but the range was greater on the first than on the second drilling.

Considering first only the treatments sprayed using 1000 l of water per hectare, a single application of chlorfenvinphos at 2.35 kg/ha, five applications of diazinon at 1.12 kg/ha and five applications of triazophos at 0.53 kg/ha gave good control on the second drilling and moderately good on the first drilling. A single application of diazinon at 2.24 kg/ha did less well than the five applications at 1.12 kg/ha, particularly on the plots drilled early. As the latter involved a total of 5.6 kg of insecticide rather than the 2.24 kg of chemical in the single treatment, it is not surprising that the latter regime was the more effective. However, three applications

of triazophos at 1.05 kg/ha (total = 3.15 kg/ha), performed less well than five at 0.53 kg/ha (total = 2.65 kg/ha). The former gave the same level of control as two applications of pirimiphos methyl at 2.1 kg/ha (total = 4.2 kg/ha). Three applications of pirimiphos-methyl at 1.4 kg/ha (total 4.2 kg/ha) and three of quinalphos at 0.74 kg/ha (total = 2.22 kg/ha) did less well.

When a single application of chlorfenvinphos at 2.35 kg/ha was made as a 500 l spray/ha, it performed less well, than when applied as 1000 l spray/ha. In contrast, the performance of 0.53 kg/ha of triazophos was similarly whether applied in 500 or 1000 l/ha. However, when applied in only 250 l of water/ha its performance was greatly reduced.

The foliage on the plants in the first drilling was much denser than that of the plants in the second drilling, and the weights of the carrot roots were also much higher. Mean weights of the carrot roots were 33 g and 9 g from the first and second drillings, respectively.

Numbers of carrots

On the first-drilled blocks of carrots, there were no differences in the numbers of roots harvested per plot and the overall mean was 86 roots from each 2 m plot. On the block of carrots drilled later, the overall mean was 81 roots/2 m plot. The numbers of roots from untreated ("check") plots and from plots sprayed with diazinon at 2.24 kg a.i./ha, averaged 71 roots/2m plot. These numbers were significantly less than those from the plots treated with diazinon at 1.12 kg a.i./ha and triazophos at 0.53 kg a.i./ha in 1000 l/ha and 500 l/ha, in which on average 94, 92 and 93 roots were recovered respectively from each 2 m plot.

Caveat

The present experiments were carried out to demonstrate the relative effectiveness of the various treatments currently applied to control this fly. High numbers of damaged roots, even after applying the most effective chemicals, are expected from this type of experiment.

As most growers wish to limit crop damage to a maximum of 5%, then there are limits above which damage to untreated carrots (i.e. the local fly infestation) cannot be allowed to rise. It is important to remember that insecticides kill only a percentage of the insects against which they are applied. Therefore, the effectiveness of the chemical treatment that is eventually applied, largely determines how high the background damage can rise before the grower will not get the expected level (95%) of control, no matter how accurately he applies his insecticide treatments. Work carried out at Wellesborne by Wheatley & Freeman in the early 1980s showed how to calculate the size of pest populations that can be controlled (5% crop damage) with insecticide treatments of various efficiency. For example, to limit fly damage to 5% of the crop, damaged on untreated ("check") plants should not be allowed to rise above the figures shown in Table 2. Data for 97% control (3% damage) are also included, to show how controlling the last few insects becomes progressively much more difficult.

In the present experiments, in which 92-94% of the plants in the untreated ("check") plots were damaged by the fly, the overall insect population was much too high for adequate control. In such situations, even a 95% effective insecticide treatment is not able to reduce crop damage below 16%.

Table 2:

	Maximum damage	on untreated crop
Effectiveness of pesticide treatment	For 95% fly control	For 97% fly control
75	19	12
80	24	14
85	30	18
90	40	26
95	65	46

If as Wheatley & Freeman (1982) suggest, carrot fly can consistently damage 40-60% of untreated carrots at a particular site, then it is obvious from the above table that the pesticide treatment would have to be 90-95% effective to give 95% fly control and that 97% fly control would probably be unachievable.

Further research

At present most growers dislike applying 1000 litres of water/ha, and many now apply only 500-600 litres/ha. The results of the present experiments indicate that while this practice might be acceptable with certain insecticides, it is not with others. Research is needed, therefore, to indicate the minimum amount of water that should be applied with each insecticide to ensure that each spray treatment is effective. Addition of a surfactant, like Codacide, may help to make the results from lower volume sprays more consistent.

Reference

Wheatley, G.A. & Freeman, G.H. (1982). A method of using the proportions of undamaged carrots or parsnips to estimate the relative population densities of carrot fly (*Psila rosae*) larvae, and its practical applications. *Annals of Applied Biology* 100: 229-244.

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Mean number of carrots per 2 m plot, percent of roots damaged and estimated % reduction in numbers of carrot fly larvae Table 3:

Insect	Insecticide treatment		Number of	Ä	First drilling (2 April)	April)	Sec	Second drilling (26 May)	26 May)
Active ingredient	Rate (kg/ha)	Volume (1/ha)	applications	Number of roots	% damage	% reduction	Number of roots	% damage	% reduction
Chlorfenvinphos	2.35	1000		81	43	1.1	73	91	94
Chlorfenvinphos	2.35	500		74	82	31	77	21	91
Quinalphos	0.74	1000	3	84	73	47	90	28	88
Pirimiphos-methyl	1.4	1000	3	85	76	42	82	32	98
Pirimiphos-methyl	2.1	1000	7	68	64	58	88	21	92
Triazophos	1.05	1000	8	93	63	09	82	22	16
Triazophos	0.53	1000	\$	06	36	82	92	14	95
Triazophos	0.53	500	\$	06	33	84	93	18	93
Triazophos	0.53	250	Ş	96	73	47	79	59	29
Diazinon	1.12	1000	S	87	52	70	94		96
Diazinon	2.24	1000		82	78	39	7.1	28	88
Untreated		ODDAY OF THE PERSON OF THE PER		84	92		71	94	
	L.S.D. $(P = 0.05)$	0.05)		17		1.9	22		0.7

The numbers of carrots damaged by carrot fly (CF) and total numbers (TOT) harvested per plot - first drilling (2 April)

Table 4:

							Numb	Numbers of carrots/2 m plot	ırrots/2	m plot		***************************************	***************************************	
Insecticide treatment	reatment		Repl	Replicate 1	Replicate	icate 2	Replicate	icate 3	Replicate	cate 4	Replicate	icate 5	Replicate	cate 6
Active ingredient	Rate (kg/ha)	Volume (1/ha)	CF	TOT	CF	TOT	CF	TOT	CF	TOT	CF	TOT	CF	TOT
Chlorfenvinphos	2.35	1000	15	89	25	17	51	94	26	62	57	26	36	77
Chlorfenvinphos	2.35	200	8.1	90	51	63	46	09	57	75	87		64	78
Quinalphos	0.74	1000	73	92	55	88	70	92	53	89	65	92	53	78
Pirimiphos-methyl	1.4	1000	76	95	53	73	65	76	59	78	52	82	74	66
Pirimiphos-methyl	2.1	1000	39	67	67	93	50	86	62	98	56		70	93
Triazophos	1.05	1000	75	101	63	101	43	72	55	92	09	113	57	81
Triazophos	0.53	1000	38	97	33	97	24	76	38	66	36	107	28	77
Triazophos	0.53	500	18	76	24	86	39	95	31	99	37	112	22	63
Triazophos	0.53	250	72	100	62	84	95	57	73	92	79	110	56	76
Diazinon	1.12	1000	32	69	44	103	09	84	32	98	52	85	52	83
Diazinon	2.24	1000	47	51	86	104	33	46	72	92	89	95	74	101
Untreated check (mean)			79	83	74	84	70	79	83	91	83	96	72	77.

Mean % damage on untreated plots = 92

The numbers of carrots damaged by carrot fly (CF) and total numbers (TOT) harvested per plot - second drilling (26 May) Table 5:

	WHAT HE WAS AND A STATE OF THE	Annual Annua					Numb	Numbers of carrots/2 m plot	ırrots/2	m plot				
Insecticide treatment	reatment		Replicate	icate 1	Replicate	icate 2	Replicate	icate 3	Repl	Replicate 4	Repl	Replicate 5	Replicate	cate 6
Active ingredient	Rate (kg/ha)	Volume (1/ha)	CF	TOT	CF	тот	CF	TOT	, CF	TOT	CF	TOT	CF	TOT
Chlorfenvinphos	2.35	1000	18	108	14	83	9	35	14	99	11	95	5	54
Chlorfenvinphos	2.35	200	14	104	18	72	20	95	17	61	14	55	19	89
Quinalphos	0.74	1000	25	62	37	111	14	80	13	77	17	77	44	97
Pirimiphos-methyl	1.4	1000	18	91	28	91	26	78	30	65	30	73	30	94
Pirimiphos-methyl	2.1	1000	16	109	14	67	17	95	Ξ	77	35	86	19	83
Triazophos	1.05	1000	14	103	7	64	27	66	15	72	21	78	26	76
Triazophos	0.53	1000		103	8	104	19	99	10	80	7	115	21	85
Triazophos	0.53	500	13	107	19	102	10	92	31	90	19	108	80	58
Triazophos	0.53	250	55	74	21	64	69	122	7.1	95	32	55	33	63
Diazinon	1.12	1000	10	94	8	81	3	120	9	70	14	90	20	108
Diazinon	2.24	1000	8	78	26	81	25	93	18	55	16	55	27	62
Untreated check (mean)			64	67	85	93	99	71	69	72	63	69	51	53

Mean % damage on untreated plots = 94

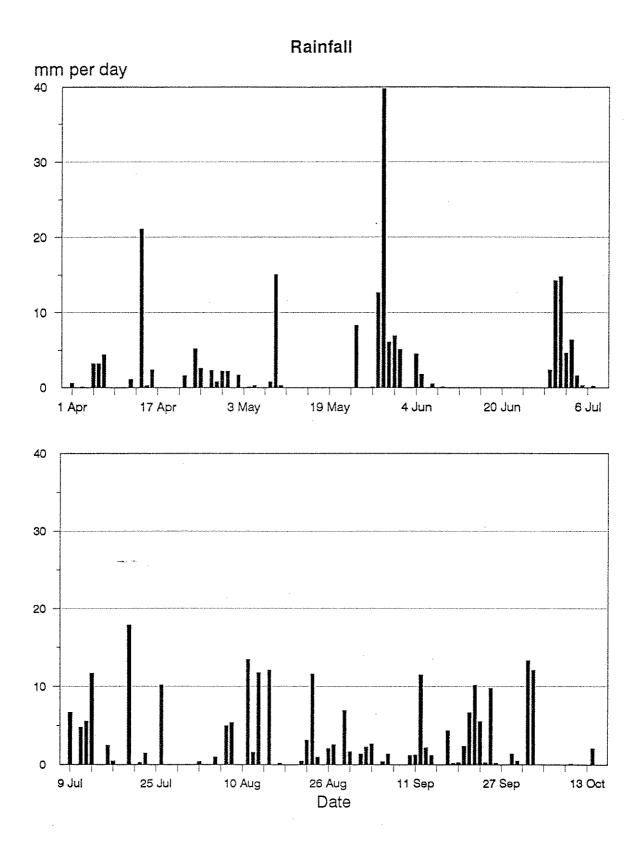


Figure 1: Daily rainfall recorded at the ${\sf HRI}({\sf W})$ weather station during the period of the experiment

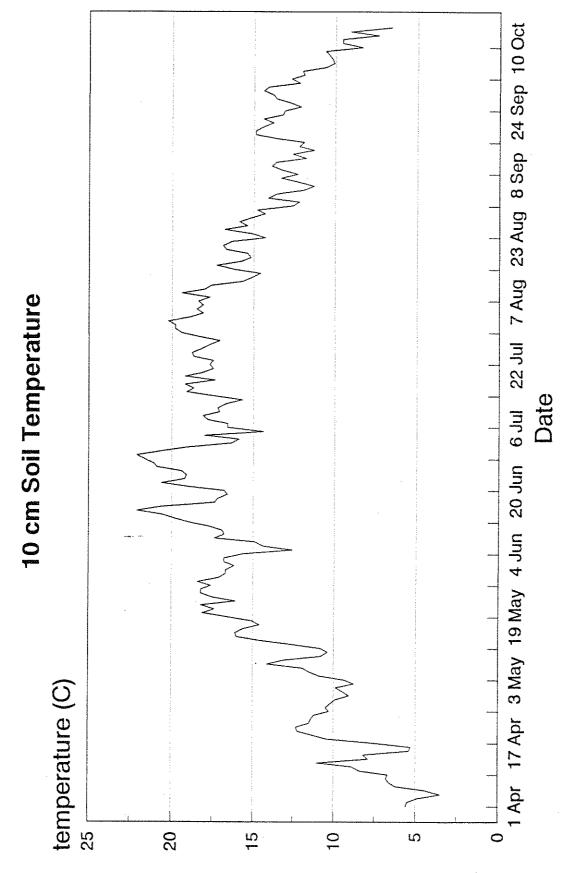


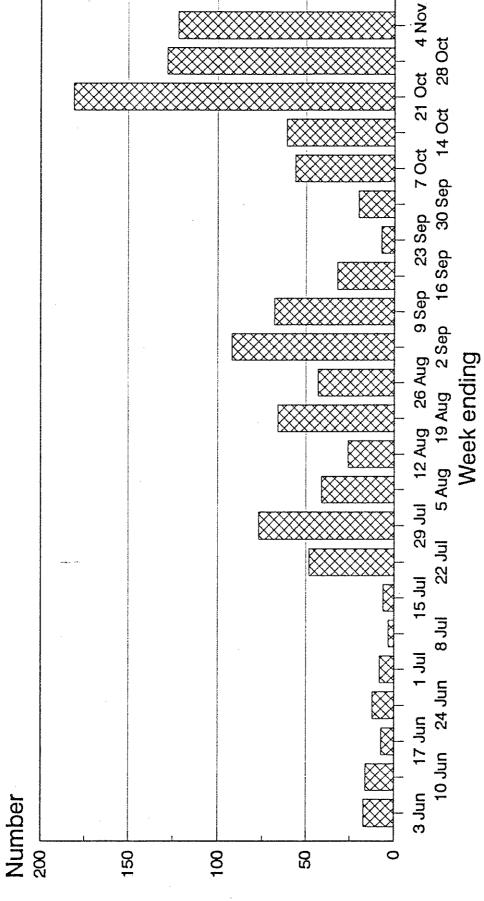
Figure 2: Soil temperature at a depth of 10 cm recorded at the ${\sf HRI}({\sf W})$ weather station during the period of the experiment.

Appendix A

Total number of carrot flies trapped in each seven day period

Seven days ending	Number
3 June	17
10 June	16
17 June	7
24 June	12
1 July	8
8 July	3
15 July	6
22 July	48
29 July	77
5 August	41
12 August	26
19 August	66
26 August	43
2 September	92
9 September	68
16 September	32
23 September	7
30 September	20
7 October	56
14 October	61
21 October	181
28 October	128
4 November	122





Appendix A.1: Weekly totals of numbers of carrot flies trapped near the experimental site.

Appendix B

Weather at time of spray applications.

	Cloud	W.	/ind	
Date	cover	Speed	Direction*	Rest of day
3 August 14 August 28 August 3 September 11 September 28 September 1 October	5/8 5/8 7/8 7/8 5/8 7/8 4/9	moderate light moderate light moderate calm calm	SSW NNW SSW WSW S	Dry, windy Squally with heavy showers Windy with rain later Dry, moderate winds Some rain, windy Misty with some rain Heavy rain later

^{*} plots were aligned N to S

On many days conditions were not ideal for spraying, but as unsuitable conditions prevailed for much of August and September spray applications had to be fitted in whenever they could be. On three occasions applications were delayed by adverse weather, from 1 August to 3 August, from 1 September to 3 September and from 25 September to 28 September.

Summary

Not one of the sixteen strains of *Bacillus thuringiensis* screened against adults of the cabbage root fly (*Delia radicum*) and the carrot fly (*Psila rosae*) was highly effective against the flies. The most effective strain, HD 293, caused 50% mortality of cabbage root fly after 5 days. Carrot flies were difficult to keep alive during the assays. Mutant strains of *B.t.*, that do not produce toxic crystals, produced comparable mortalities to similar strains of *B.t.* that do produce toxic crystals. Hence, fly death appeared to result from inhibition of feeding, rather than from the action of the D-endotoxin found in the protein crystals.

Assessing strains of *Bacillus thuringiensis* as a possible method for controlling the cabbage root fly and the carrot fly

N.D. Pipe & S. Finch

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Introduction

Bacillus thuringiensis (B.t.) produces two types of protein that are toxic to insects, the B-exotoxin, and the D-endotoxin. The D-endotoxins, which are regarded of greater interest for pest control, are produced in large quantities within the crystal proteins that the bacteria release at the time of sporulation (Crook & Jarrett, 1991). In the past, B.t. has generally been used to control caterpillar (Lepidopteran) pests, but strains showing activity against fly pests, including leaf-mining flies (Agromyzidae) and house flies (Musca domesticae), have also been identified (Feitelson, Payne & Kim, 1992). Havukala (1988) has already investigated the use of B.t. against the maggots of the cabbage root fly and the closely-related onion fly (Delia floralis). His results, and those of certain French workers on the actual flies (Brunel - personal communication), suggested that B.t. had some promise for field control of these flies. If a sufficiently active strain of B.t. could be identified flies might pick up a lethal dose, during the time they spend probing the leaves of their host plants, prior to laying eggs. The objective of this project was to produce a bioassay system for assessing the activity of B.t. against these two flies.

Materials and Methods

B. thuringiensis strains

Strains of B.t. (Table 1) were obtained from the culture collection at HRI Littlehampton and incubated on nutrient agar plates at 28°C in the dark.

Stocks of the various strains of *B.t.* were maintained in cryopreservatives (Prolab Diagnostics) under liquid nitrogen until required for bioassays. *B.t.* was grown in sterile 500 ml flasks containing 50 ml glucose/peptone media. The spore/crystal suspensions were harvested by centrifugation at 8,000 rpm for 15 min. To obtain pure spore/crystal preparations, all samples were washed three times by resuspending in 30 ml sterile distilled water after centrifugation at 8,000 rpm for 15 min.

Insects

The cabbage root flies used in the tests were reared in the Insect Rearing Unit at Wellesbourne. The carrot flies were obtained by collecting flies as they emerged into field cages covering parts of a highly-infested crop of carrots.

<u>Bioassays</u>

Strains of B.t. were bioassayed at a concentration about 5x as high as that applied in commercial preparations. Adult flies were placed in $30 \text{ cm } x \ 30 \text{ cm } x \ 30 \text{ cm} x \ 30 \text{ cm}$

B.t. added to fly food (Feeding assays)

A 2 ml suspension of the test strain of *B.t.* was pipetted gently onto the surface of the filter paper covering the 10% sucrose solution. In the "check" treatments, only 2 ml of water was pipetted onto the filter paper.

B.t. sprayed onto host-plants (Probing assays)

Carrot or cauliflower plants, approximately 10 cm high, were sprayed with 2 ml of a B.t. suspension that also contained 0.05% Triton as a wetting agent. A compressed air sprayer was used to apply the suspension to the plants. Plants were allowed to dry for 30 min before being placed in the bioassay cages. Plants in the "check" treatments were sprayed with 2 ml distilled water that contained only 0.05% Triton.

The numbers of dead insects were recorded daily in all experiments.

Results

Carrot fly

Control mortalities in both the probing and feeding assays were unacceptably high (Table 2). For example, 62% of the flies died within 7 days, even when the plants were sprayed only with water.

Cabbage root fly

The pathogenicities against the cabbage root fly of two strains of *B.t.* are shown in Table 3 for both the probing and the feeding assays. As expected, the strains were more pathogenic when presented in the food than when sprayed onto the host plants. However, none of the *B.t.* strains were highly active against the cabbage root fly (Table 4).

The most pathogenic strain, HD293, caused 50% mortality of the flies after 5 days. Mutant strains of *B.t.*, that do not produce toxic crystals gave similar levels of control to wild-type strains that do produce toxic crystals. This, combined with the high variability in the times for 50% mortality between replicates, indicated that the fly mortality from the strains of *B.t.* tested was unlikely to be the action of D-endotoxins, but more likely to be an anti-feedant effect.

Discussion

The objective of this project was to develop a bioassay for determining the activity of strains of *B.t.* against the cabbage root fly and the carrot fly. Cabbage root flies, but not carrot flies, have to feed to obtain sufficient sources of carbohydrate to enable them to mature even their first batch of eggs. Both flies, however, recognise their host plants by repeatedly probing the leaf surface of the host-plant with their mouthparts before laying their eggs in the soil alongside the plant. For fly control, therefore, it was important to determine if a lethal dose of *B.t.* could be ingested during this probing phase, as most other feeding is carried out on wild flowers in hedgerows and uncultivated areas, many of which may not be near to the host-crop.

The aim, therefore, was to identify strains of *B.t.* that were effective in feeding assays and then test these strains to determine if any gave reasonable control in the probing assays.

Carrot fly

High mortality within the field-collected flies, even in the "check" treatments prevented the *B.t.* strains being screened against the carrot fly. High mortality was not caused by handling the insects, as 62% of the flies died after 7 days even in a "no treatment" check.

This fly is one of those that continues to frustrate applied entomologists, as it is extremely difficult to breed and keep alive in reasonable numbers in the laboratory and yet remains such an effective pest under field conditions. Its ability to lay viable eggs within one or two days of emerging from the soil is one of the main factors behind its success. This ability, also means, that any non-chemical treatment for

controlling this fly must be similar to a commercial insecticide and work within hours of application.

Cabbage root fly

Not one of the strains of *B.t.* screened in the current experiments was highly active against the cabbage root fly. According to Jarrett (Personal communication), highly active strains of *B.t.* should produce 100% mortality in susceptible hosts within 3-4 days of application. The results indicate that even when presented in the food (10% sucrose = weak nectar), the flies did not ingest sufficient *B.t.* to give acceptable levels of control. As expected, the *B.t.* presented in the food, always killed more flies than the *B.t.* sprayed onto host-plants. An alternative target for *B.t.* is the actively feeding larvae. However, small-scale bioassays with individual larvae were found not to be suitable, as larval feeding is communal and so individual larvae rarely survive. A suitable system could be a whole plant bioassay. This would involve placing 100 eggs around the base of each test plant, applying the *B.t.* to the plant roots and then, rather than assessing larval mortality which is difficult with subterranean insects, counting the number of fly pupae recovered from the soil during a destructive harvest taken 4 weeks later.

Conclusions

- 1. Even when provided in the diet, only three of the strains of *B.t.* tested in the current experiments killed more than 40% of cabbage root fly within 5 days of application.
- 2. The three effective strains were HD754 (43%), HD137 (49%) and HD293 (50%).
- 3. The two crystal negative mutants gave similar levels of control to their parent (crystal positive) strains.
- 4. The effects of the *B.t.* preparations appeared to result from an anti-feedant effect and not from the action of a crystal D-endotoxin.
- 5. B.t. preparations sprayed onto host plants were much less effective than those included in the diet of the flies.
- 6. As the carrot fly is difficult to keep alive under laboratory conditions, it might be more appropriate to restrict future assays with this pest to small field cages.
- 7. Strains of *B.t.* with higher activity against flies might be identified if a more comprehensive strain screening programme was carried out within HRI.

8. Until strains of *B.t.* are isolated that are much more effective against fly pests than the strains tested in the current programme, it would be unwise for the HDC to support any further work on this aspect of biological control.

References

- Chauthani, A.R., Snideman, M. & Rehnborg, C.S. (1971). Comparison of commercially produced *Bacillus thuringiensis* var. *thuringiensis* with two bioassay techniques based on toxicity units. *Journal of Economic Entomology*, **64**: 1291-1293.
- Crook, N.E. & Jarrett, P. (1991). Viral and bacterial pathogens of insects. *Journal of Applied Bacteriology Symposium Supplement*, **70**: 91-96.
- Feitelson, J.S., Payne, J. & Kim, L. (1992). *Bacillus thuringiensis*; Insects and beyond. *Bio/Technology*, 10: 271-275.
- Havukkala, I. (1988). Non-chemical methods against cabbage root flies *Delia* radicum and *Delia floralis* (Anthomyiidae). Annales Agriculturae Fenniae, 27: 271-279.
- Li, R.S., Jarret, P. & Burges. (1987). Importance of spores, crystals and D-endotoxins in the pathogenicity of different varieties of *Bacillus thuringiensis* in Galleria mellonella and Pieris brassicae. Journal of Invertebrate Pathology, **50**: 277-284.

 Table 1:
 Strains of Bacillus thuringiensis used in the bioassays

Strain		Strain
HD1		P23
HD1-xtal	Crystal negative mutant	HD867
IPS78	Mosquito active	HD754
IPS78-xtal	Crystal negative mutant	HD395
PG14	Mosquito active	HD293
HD240		HD137
HD198		HD125
HD753		Btt

Table 2: Percentage mortality, 5 days after treatment, of carrot flies subjected only to the control (water) treatments

Bioassay	Control mortality	Control mortality
	Plant	Feed
1	52%	48%
2	72%	80%
3	44%	n/a*
4	n/a	46%

^{*} n/a = not assessed

Table 3: Percentage mortality, 5 days after treatment, of cabbage root flies provided with food or plants treated with two strains of *Bacillus thuringiensis*

Strain	Feed assay	Plant assay
HD1	38%	14%
IPS78	16%	10%
Control	8%	6%

Appendix 1: Percentage mortality of cabbage root fly 5 days after applying the B.t. treatment

Assay	1	2	3	4	5	6	7	8	Mean ± SE
HDI	38			20*		10	46	16	26 ± 7
HDI-xtal		48*			62*	22	48	18	29 ± 9
IPS78	16					14		12	14 ± 1
IPS78-xtal			16			12		10	13 ± 2
PG14	38					18		34	30 ± 6
HD240		50*		42*			44	10	32 ± 11
HD198		40*				16 14		26	19 ± 4
HD753		46*				6 20 8			11 ± 4
P23		30*				16 10		8	11 ± 2
HD867	į				68*	16		10 24	17 ± 4
HD754					44*		60 38	30	43 ± 9
HD395					68*		30 48	38	39 ± 5
HD293					58*		36 74	40	50 ± 12
HD137			***************************************		40*		76 50	22	49 ± 16
HD125					58*			16 28 22	22 ± 4
Btt			To the state of th	T-1-1 Carlos Anna	56*			28 10 26	21 ± 6
Control	8	40*	20	6**	38*	18	32	32	

^{*} Control values too high, data not used for calculation of percentage mean mortality

Appendix 2: The time (in days) for 50% mortality of cabbage root flies subjected to preparations of various strains of *B.t.*

Assay	1	2	3	4	5	6	7	8	Mean ± SE
HDI	9			23		29	5	8	15 ± 5
HDI-xtal		5*			2*	12	5	10	9 ± 2
IPS78	8					25		9	14 ± 6
IPS78-xtal			14			17		16	16 ± 1
PG14	7					9		6	7 ± 1
HD240		4*		6			5	12	8 ± 2
HD198		6*				9 18		7	11 ± 3
HD753		6*				8 26 10			15 ± 6
P23		13*				15 10		9	11 ± 2
HD867					3*	8		21 11	14 ± 4
HD754			A CANADA A		5*		4 5	6	5 ± 1
HD395					4*		8 5	7	6 ± 1
HD293					3*		6 3	7	5 ± 1
HD137					6*		3 5	7	5 ± 1
HD125					3*			10 10 8	9 ± 1
Btt					4*			8 11 9	9 ± 1
Control	8%	40%*	20%	6%	38%*	18%	32%	32%	

Control = percentage mortality 5 days after treatment.

*Control values too high, data not used for calculation of percentage mean mortality.