HONEY BEE REPELLENCY TRIALS ON FABA BEANS (*Vicia faba* L.) GROWN IN MANITOBA

S.C. Jay, R. Currie and D. Wright

Department of Entomology

University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Summary

Three chemicals, Demeton (®Systox), Disulfoton (®Di-syston) and Permethrin (®Ambush) are known to repel honeybees when applied as insecticides. These were tested as bee repellents to reduce, or eliminate, outcrossing between faba bean (*Vicia faba* *L*.) plots grown close together in hybrid breeding programs. Despite significant reductions in numbers of honeybees on plots sprayed every four days or less seed weight data suggest that enough bees were present that they could effect a high degree of cross-pollination. It is suggested, therefore, that large reductions or near total absence of honeybees, are necessary to offset cross-pollination in faba beans.

Introduction

Important studies have been undertaken in recent years to identify chemical repellents that might be added to insecticides to reduce the mortality of insect pollinators, in particular, honeybees, and/or to identify insecticides which might, by themselves, repel selected pollinators from insecticide-treated crops. Aspects of this subject have been reviewed by Atkins *et al*. (1975a,b), Jay (1986) and Rieth *et al*. (1986).

Atkins *et al*. (1975a,b) tested a large number of possible chemical additives in the laboratory for honeybee repellency before selecting candidate chemicals for field trials. However, none of these chemicals met all of his stated criteria for practical field usage, i.e. were harmless to selected crops, were commercially feasible and repelled honeybees for a minimum period of 24 hours. In subsequent studies Atkins *et al*. (1976) found that when certain insecticides were combined (e.g.

Demeton and methyl parathion) and used as sprays a significant reduction in pollinator mortality occurred. Later research (Atkins, 1981) showed that in addition to Demeton, Permethrin and Disulfoton also showed promise as honeybee repellents. If applied at the correct time, when bee flight is minimal or absent (i.e. at night or early morning), these three insecticides appear to be dependable repellents on such crops as alfalfa, cotton, and safflower (Atkins, pers. comm.).

Of interest also is the search for repellents among the various honeybee pheromones. Most of this research to date has involved the pheromonal properties of the mandibular gland secretions of worker bees (Simpson, 1966; Butler, 1966; Rieth et al., 1986). Free (1979) suggested that the pheromone 2-heptanone (found in the mandibular glands of honey bees by Shearer and Boch, 1965) be added to insecticides to reduce honeybee mortality. However, Rieth et al. (1986) have shown that although 2-heptanone does repel bees from certain flowering crops it cannot as yet be used satisfactorily on a commercial scale.

Our laboratory has been involved in a collaborative research program with the International Centre for Agricultural Research in Dry Areas (ICARDA) at Aleppo, Syria where it is important that interplot outcrossing due to insect pollinators be reduced, or eliminated, in the faba bean hybrid line breeding programs. Since certain chemicals added to insecticides and/or insecticides used alone have shown repellency characteristics for pollinators it seemed feasible that such repellents, if used properly in repeated applications, might reduce or prevent outcrossing (by pollinating insects) between plots of faba beans grown close together. With this in mind the candidate insecticides, Demeton, Disulfoton, and Permethrin were tested as repellents of honeybees, bumblebees and other possible pollinators on faba bean plots.

Methods

Repellent trials were conducted in 1985 and 1986 within fields of faba beans (cv. Hertz Freya) planted 15 cm apart within, and between, rows.

1985:

In one experiment (Experiment 1) four plots (each 2.4 m X 4.9 m) were staked out with string throughout a 1.6 hectare field of faba beans. Using a back-pack sprayer equipped with a 1.2 m boom extending to one side (so the plants in the plots would not be trampled) three repellents were applied as follows*: plot 1, Demeton (®Systox) at a rate of 280 grams ai per hectare; plot 2, Disulfoton (®Di-syston) at a rate of 560 grams ai per hectare; plot 3, Permethrin (®Ambush) at a rate of 112 grams ai per hectare; plot 4, (control) was left unsprayed. This experimental regime was replicated twice within the same field in a randomized block design.

All sprays were applied to the experimental plots on every second day throughout the flowering period. They were applied early in the morning when wind speeds were low, or non-existent (so that spray drift would not affect the control plots) and few, if any, pollinating insects were flying within the experimental plots. Precautions were taken to fully protect technical staff with safety clothing and respirators during spraying periods because of possible health hazards that might be associated with these chemicals.

A second experiment (Experiment 2) was conducted within a 1.6 hectare field of faba beans located 50 m from the field used in Experiment

(*rates suggested by Atkins, pers. comm.).

1. It was conducted at the same time and was similar to Experiment 1 in every respect except that the sprays were applied to the plots every fourth day instead of every second day.

1986:

In one experiment (Experiment 3) five plots (each 2.4 X 4.9 m) were staked out with string throughout a 1.6 hectare field of faba beans. Three repellents were applied with the same equipment and at the same rates as described in Experiment 1 in plots 1, 2 and 3 except that the sprays were applied every fourth day throughout the flowering period. Two control plots were used as follows: plot 4 (control 1) was left unsprayed while plot 5 (control 2) was caged to exclude pollinators and left unsprayed. This experimental regime was replicated three times within the same field in a randomized block design.

A second experiment (Experiment 4) was conducted in a 1.6 hectare field of faba beans located 50 m from the field used in Experiment 3. It was conducted at the same time and was similar to Experiment 3 in every respect except that the sprays were applied to the plots every eighth day instead of every fourth day.

In both 1985 and 1986 two colonies of honeybees were placed alongside each field of faba beans. Also, in both years the plants in test and control plots were examined at regular intervals to determine if there were any obvious signs that would suggest that any of the test sprays were phytotoxic.

No insect control measures were used on the control plots in 1985 but malathion sprays were used twice in 1986 to control aphids on both groups of control plots - once prior to the time when the repellents were sprayed on the test plots and once half way through the experimental period.

The total weight (gm) of seed produced in the plots that were treated with the various repellents, and in the uncaged plots, were recorded in both 1985 and 1986. Also, in 1986 the mean number of pods per 50 plants produced in each of the uncaged and caged plots was recorded.

Insect counts on the sprayed and control plots were recorded as follows: Between 1000 and 1100 h, 1100 and 1200 h, 1300 and 1430 h and 1430 and 1630 h the total number of honeybees, bumblebees and "other insects" that visited the flowers in each plot during two 60-second observation periods were recorded. The data was later combined to reflect only two time periods, i.e. 1000 to 1100 h and 1300 - 1630 h. Counts were done on the day when the spray was applied and the day before the next spray. An additional count was also done on the plots of the 4 and 8 day spray regimes mid-way through each time period. Each insect was also classified according to its behaviour relative to the flower it was visiting, i.e. whether it was making a "positive" visit (where it crosses over the stigma of the flower) while collecting nectar or pollen or whether it was making a "negative" visit (where it does not cross over the stigma) while standing on the flower or while collecting nectar from extra floral nectaries.

Results

The results appear in Tables 1, 2, 3 and 4.

The total weight of seed produced in plots sprayed with Demeton, Disulfoton, or Permethrin at different time intervals as well as for four unsprayed control plots are shown in Table 1. There was no significant difference in seed weight between control plots and each of the three sprayed plots when the spray frequency was every second day in 1985 and every fourth or eighth day in 1986. Although there was no significant difference in seed weight between plots sprayed with one or other of the three repellents every fourth day in 1985 each of the repellent-sprayed plots produced seed that was significantly greater in weight to that of seed produced in the control plots.

In 1985 there was a significant difference in weight of seed produced between the two control plots ($P<0.001$) and also between the Demeton plots ($P<0.01$) when sprayed every second day as compared to every fourth day. There were no significant differences in seed weight between the Disulfoton- or between the Permethrin- sprayed plots in 1985. In 1986 only the Permethrin-sprayed plots showed no significant difference in seed weight ($P<0.07$) when the plots were sprayed every fourth day as compared to every eighth day.

The mean number of pods per 50 plants produced in 1986 in each of six caged plots compared to six uncaged plots (where no repellents were applied) are shown in Table 2. Significantly ($P<0.05$) more pods per plant were produced in the uncaged plots than in the caged plots.

Counts of honeybees, bumblebees, and "other insects", along with their foraging behaviour relative to the flowers, were recorded in both 1985 and 1986 and appear in Tables 3 and 4.

In 1985 significantly ($P<0.05$) more honeybees foraged for nectar and pollen on the control and repellent-sprayed plots in the afternoon than in the morning (Table 3). There was no significant difference between the mean number of honeybees that collected nectar from the extra floral nectaries of flowers in the repellent-sprayed plots or between any of these and the control plots in either of the second or fourth day treatments. The mean number of honeybees that collected nectar or pollen did not differ significantly between the control plots (A and B), or between the plots sprayed every second day, and those sprayed every fourth day. Significantly more honeybees collected pollen ($P<0.02$), or nectar ($P<0.01$), in the control plots than in any of the repellent-sprayed plots but there were no significant differences between the number of honeybees observed in the various repellent-sprayed plots when one or other of the spray frequencies were compared.

In 1985 and 1986 there were no significant differences in the mean number of bumblebees observed on the control and repellent-sprayed plots for both the second and fourth day spray regimes. Most of the bumblebees collected pollen from the flowers.

"Other insects" observed on the plots belonged primarily to the families Syrphidae, Ichneumonidae, Vespidae, Bombyliidae, Chrysomelidae, Megachilidae, Tipulidae, and Pieridae. There were significantly more ($P<0.001$) of these insects foraging on the extra floral nectaries on the control plots than on the repellent-sprayed plots in 1985 but not in 1986. However, there were no significant differences between the mean numbers of these insects observed on the plots sprayed every second day compared to those sprayed every fourth day in 1985 or when sprayed every fourth day compared to every eight day in 1986.

In 1986 significantly ($P<0.05$) more honeybees foraged for pollen in the control and repellent-sprayed plots in the afternoon than in the morning. There were no significant differences in the number of honeybees foraging for nectar or pollen, or foraging for nectar on extrafloral nactaries, on the control and repellent-sprayed plots. However, significantly more ($P<0.05$) honeybees were observed on the control plots as well as on the repellent-sprayed plots of the experiments that were sprayed every fourth day rather than on every eighth day (except for the plots treated with Permethrin). Significantly more honeybees collected pollen ($P<0.05$) in the control plots than in the repellent-sprayed plots in the four day spray regime but there were no significant differences between the number of honeybees observed in the various repellent-sprayed plots when one or other of the spray frequencies were compared.

Discussion

Experiments, to reduce inter-plot outcrossing rates between increase plots of faba beans, being grown close together, and where insect pollinators are involved, have been undertaken by Robertson and Cardona (1986). In their trials faba bean plots were isolated by using triticale as a physical barrier, or Brassica napus as a competing attractive crop, for insect pollinators (also see Bond and Poulsen, 1983). Brassica, more so than triticale, reduced bee activity in faba bean plots but this reduction did not result in a corresponding reduction in interplot outcrossing rates. However, the use of Brassica shows promise as a means of reducing intra-plot outcrossing rates (Robertson and Cardona, 1986). We used three repellents, that are also insecticides, in an attempt to reduce the number of pollinating insects that visit small faba bean plots. Our ultimate objective was also to reduce, or eliminate, outcrossing between large numbers of faba bean plots grown close together in hybrid breeding programs.

Because most faba bean cultivars grown in Manitoba show a high degree of autofertility (including the cv. Hertz Freya used in this study) pod production, occurring between caged and uncaged plots of faba beans, were compared. Although significantly more pods were produced in uncaged plots (which suggests that insect activity is important in cross-pollination; see review by McVetty and Nugent-Rigby, 1984) it is possible that cage effects may have partially contributed to the differences in pod production.

Overall pod numbers were low in both the caged and uncaged plots compared to average counts usually observed by researchers and commercial growers. It has been found (Rowland and Bond, 1983) that faba bean pods are very sensitive to environmentally-induced stresses, and readily abort. All of our plots in this trial were subjected to a wet, cool period both during, as well as following flowering of the crop; this may have contributed to some abortion of the pods. Further, pollinator activity was also reduced during the flowering period in 1986 (compare bee counts in 1985, Table 3, when fine weather prevailed, with those in 1986, Table 4).

Insects, other than honeybees and bumble bees, were recorded in low numbers on all plots in both years. With few exceptions these insects made "negative" visits and thus it is not likely that they cross-pollinated the faba bean flowers. Bumblebees, which were also observed in low numbers, usually made "positive" visits to the flowers and therefore may have effected some cross-pollination. There are excellent methods available for rearing and maintaining various species of bumblebees in artificial domiciles (Plowright, 1984) and since they adapt well to cage and greenhouse conditions they could be used to cross-pollinate faba beans under both field and enclosed situations.

In 1985 and 1986 there were no significant differences between the number of honeybees making "positive" visits to faba bean plots regardless of the repellent used and no significant reduction in bees occurred when the plots were sprayed every second day instead of every fourth day. However, except for Permethrin, it appeared that in 1986 spraying the plots every eight, instead of every four, days significantly reduced bee numbers. Although phytotoxic effects did not appear to cause yield reductions in the repellent-sprayed plots in any experiment, considerable flooding occurred on the field containing the eight day repellent-sprayed plots which reduced both the number and the size of the plants within the plots. These flood-related factors probably account for the reduced number of bees recorded on the eight day spray plots as it is unlikely that such a spray frequency would retain any strong repellency effect this long in an outdoor environment.

It is important to note that in 1985 there was a significant reduction in the number of "positive" (both nectar and pollen collection) visits made to flowers by honeybees when the control plots are compared to each of the repellent-sprayed plots for both the two or four day spray regimes. This was also true for pollen collection in 1986 for the four day spray regime, and the eight day spray regime (except for the Permethrin-sprayed plots). Thus it appears that honeybees are repelled to a high degree from faba bean fields by these three chemicals when applied every four days or less. These data verify those of Atkins (see above) who found that these three chemicals repel honeybees from target fields when they are applied as insecticides (either early in the morning or at night) against pest species. Our data (Tables 3 and 4) show that significantly fewer honeybees forage on the crops in mid- to late morning (with probably much fewer before this time) so that insecticidal effects due to direct contact by the sprays at the time of application is reduced considerably.

Despite the significant reductions in numbers of honeybees making positive visits to repellent-sprayed plots compared to control plots these differences are not reflected clearly in the total weight of the seed obtained in the control and treated plots. These apparent differences may result from problems that occurred in the control plots. In 1985 the control plots (in particular those of the four day trials) which received no chemical treatments, developed a severe infestation of aphids that probably account for the low seed weight in these plots. In turn, in 1986 the control plots (along with the repellent-sprayed plots) in the eight day spray experiment were partially flooded and thus the reduction in number and size of the plants probably accounts for the reduction in seed weight obtained in these plots.

Despite these problems when the data are taken together they appear to indicate that although these three repellents reduce the number of honeybees visiting the plots sufficient numbers remain on the plots that they could probably effect a high degree of cross-pollination. This suggests, that honeybees are good pollinators of faba beans (see review by McVetty and Nugent-Rigby, 1984), and that perhaps only a few of them are required to enhance seed production even in the autofertile cultivars. Thus large reductions, or near total absence of honeybees, appear to be necessary to offset cross-pollination problems in faba bean plots. It will require further testing with these, or other candidate repellents, in conjunction with closely spaced test plots of faba beans carrying genetic markers, to verify this hypothesis.

Acknowledgements

The authors thank the International Development Research Centre of Canada for providing the funds to conduct this study. We also thank Dr. G.H. Hawtin for his direction in establishing priorities in this study and Dr. E.L. Atkins for critically reviewing the manuscript.

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Table 1 - Mean number of pods per plants in caged plots and in uncaged open pollinated plots (1986)

Replicate	Mean number of pods per 50 plants	
	Caged	Uncaged
1	4.16	8.62
2	4.60	5.82
3	5.82	5.70
4	3.30	4.22
5	2.36	5.12
6	1.84	4.06
Totals	3.68±0.60[1]	5.59±0.68[2]

1,2 Mean ± standard error. (These two figures are significantly different at $P<0.05$).

Table 2 - Total weight (gm) of seed from plots of faba beans treated with various insect repellents.

Year	Spray frequency	Treatments			
		Control plots (no repellents)	Demeton	Disulfoton	Permethrin
1985	2[1]	1954±54[4] a(a)[5]	2279±61a(a)	1941±288a(a)	2130±293a(a)
	4[2]	955±18a(b)	2869±84b(b)	2341±71b(a)	2808±199b(a)
1986	4	2922±265a(a)	3334±331a(a)	2907±237a(a)	2798±346a(a)
	8[3]	1811±186a(b)	1471±519a(b)	1311±372a(b)	1914±140a(a)

[1,2,3]Plots sprayed every second, fourth and eighth day respectively.

[4]Means ± standard error.

[5]Means followed by the same letter are not significantly different at $P<0.05$. Letters without brackets relate to data in rows; letters with brackets relate to data in columns.

Table 3 - Counts of insects on plots of faba beans treated with various repellents (1985)

Time of day[1]	Insect species	Treatments																							
		Control plots (no repellent)						Demeton						Disulfoton						Permethrin					
		For A[2]			For B[3]			A[2]			B[3]			A			B			A			B		
		N[4]	P[5]	O[6]	N	P	O	N	P	O	N	P	O	N	P	O	N	P	O	N	P	O	N	P	O
1000-1200h	Honey bees	7	14	1	9	6	3	1	5	3	1	8	4	2	7	4	3	4	1	5	8	4	0	16	4
	Bumble bees	2	1	0	0	3	0	0	2	2	0	4	0	1	3	0	0	7	1	0	5	0	0	4	0
	Other insects	1	0	15	0	0	17	1	0	3	0	0	1	0	0	1	0	0	6	0	0	2	0	1	1
1300-1630h	Honey bees	14	94	0	10	106	0	1	32	0	8	34	2	6	40	3	2	38	0	6	49	2	3	45	0
	Bumble bees	0	0	0	2	1	0	0	7	0	4	3	1	0	2	0	0	2	0	0	4	0	0	0	1
	Other insects	1	1	2	1	1	8	0	0	0	0	0	3	0	0	5	0	0	1	0	0	0	1	0	0
Overall totals	Honey bees	21	108	1	19	112	3	2	37	3	9	42	6	8	47	7	5	42	1	11	57	6	3	61	4
	Bumble bees	2	1	0	2	4	0	0	9	2	4	7	1	1	5	0	0	9	1	0	9	0	0	4	1
	Other insects	2	1	17	1	1	25	1	0	3	0	0	4	0	0	6	0	0	7	0	0	2	1	1	1

[1] Number of days counts were made = 8.

[2,3] Repellent applied every second and fourth day respectively.

[4] Nectar foragers, [5] Pollen foragers (these are "positive" visits where the bees cross over the stigmas).

[6] Other visits (these are "negative" visits where the bees collect nectar from extra floral nectaries or were observed on a flower but do not cross over the stigmas).

Table 4 - Counts of insects on plots of faba beans treated with various repellents (1986)

Time of day[1]	Insect species	Treatments																							
		Control plots(no repellent)						Demeton						Disulfoton						Permethrin					
		For A[2]			For B[3]			A[2]			B[3]			A			B			A			B		
		N[4]	P[5]	O[6]	N	P	O	N	P	O	N	P	O	N	P	O	N	P	O	N	P	O	N	P	O
1000-1200h	Honey bees	0	19	0	0	13	0	0	13	2	0	5	0	0	9	0	0	13	0	0	11	1	0	12	0
	Bumble bees	0	5	0	0	0	0	0	5	0	0	0	0	0	2	0	0	4	0	0	7	0	0	4	0
	Other insects	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1300-1630h	Honey bees	0	60	3	1	36	2	0	44	5	0	27	0	0	46	2	0	25	2	1	34	2	0	33	3
	Bumble bees	1	6	0	0	2	0	0	4	0	0	1	0	0	4	0	2	1	0	0	6	0	0	1	0
	Other insects	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Overall totals	Honey bees	0	79	3	1	49	2	0	57	7	0	32	0	0	55	2	0	38	2	1	45	3	0	45	3
	Bumble bees	1	11	0	0	2	0	0	9	0	0	1	0	0	6	0	2	5	0	0	13	0	0	5	0
	Other insects	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] Number of days counts were made = 8.

[2,3] Repellent applied every fourth and eighth day respectively.

[4] Nectar foragers
[5] Pollen foragers } (these are "positive" visits where bees cross over the stigmas)

[6] Other visits (these are "negative" visits where the bees collect nectar from extra floral nectaries or were observed on a flower but do not cross over the stigmas).