SASKATCHEWAN
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ALFALFA LEAFCUTTING BEE MORTALITY ASSOCIATED WITH USE OF DICHLORVOS FOR CHALCID PARASITE CONTROL

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Research undertaken in an experimental system has shown that when dichlorvos is used for control of parasites during the alfalfa leafcutting bee incubation period, mortality in developing bee pupae increases as dichlorvos rate and treatment time are increased. The bee mortality level also rises as the number of bees per unit area in the incubator is decreased; as well, developing bee pupae in the top layer of cells in the incubation tray are more susceptible to dichlorvos poisoning due to increased exposure. Research has also indicated that bee mortality during dichlorvos treatment is related to relative humidity in the incubator; mortality caused by dichlorvos increases as incubation humidity is decreased.

INTRODUCTION

The chalcid parasite, *Pteromalus venustus*, is the most common parasite associated with populations of the alfalfa leafcutting bee in Saskatchewan. Alfalfa seed producers currently use the chemical dichlorvos (2, 2 - dichlorovinyl dimethyl phosphate), in the resin strip formulation commonly known as Vapona^{R}, for control of chalcid parasites during spring incubation of alfalfa leafcutting bees. Annual surveys undertaken over the period 1985 to 1990 indicated that while parasitized bee cells comprised 0.84% of cells evaluated during 1985-1987, a trend to increased parasitism was noted in 1988-1990 surveys, with parasitized bee cell levels at 1.64%. Over the same period, the level of parasite-free bee populations dropped from 51.2% (1985-1987) to 19.8% (1988-1990) of populations analysed.

This substantial increase in parasitism caused many producers to begin using dichlorvos for spring parasite control at rates exceeding the recommended rate of 75g/28.3m^3 (0.75 resin strip/1000 ft^3). Some producers also began using dichlorvos treatment early in the incubation cycle rather than restricting its use to the day 7-14 period as generally recommended.

DICHLORVOS RESEARCH

DICHLORVOS RATE / TREATMENT TIME

To examine the effect of increased dichlorvos rates over various time periods on bee development and emergence during incubation in an experimental system, preliminary tests were undertaken involving treatment of groups of 100 bee cells with various rates of dichlorvos (recommended rate, 0.5x, 2x, 4x, and 8x recommended rate, and untreated control) at several time regimes (day 0-7, 7-14, 14-21, and 0-21). Bee cells were incubated in a system of 0.75 ft^3 mini-incubators within a large temperature-controlled structure (30^{o}C/50% R.H.). Bee cells containing healthy prepupae, as determined by x-ray analysis, were held under various treatments and incubated to determine percent adult emergence.

Emergence of adults from control treatments was uniformly high (98.9-100.0%), while emergence in all dichlorvos treatments was low, even at 0.5x (54.0%) and 1x (28.0%); bee mortality increased with increasing dichlorvos rates. The low emergence at 0.5x and 1x rates was determined to be due to the relatively small number of bee cells used per treatment, resulting in an exaggerated dose effect. Further research on the effect of dichlorvos on pupal development during bee incubation was therefore undertaken. The object of this series of tests was to investigate bee mortality in relation to dichlorvos rate, treatment time, and number of bee cells treated (Table 1).

Table 1. Effect of dichlorvos rate, treatment time, and number of bee cells on percent adult emergence.

Treatment		Percent emergence	
Dose	Time	300 bee reps.	3000 bee reps.
Control	-	94.8%	93.7%
1 x	Day 0-14	3.1	55.2
1 x	Day 7-14	4.2	70.8
4 x	Day 0-14	0.0	24.0
4 x	Day 7-14	0.0	24.0

Dichlorvos rates of 1x and 4x were utilized, along with controls; all treatments were replicated with 300 and 3,000 bee cells containing healthy bee prepupae. Treated cells were incubated to determine percent adult emergence. Dichlorvos rate was once again shown to be directly related to bee mortality; at 1x the recommended rate, bee emergence in the incubator averaged 70.8%, while at 4x the recommended rate, emergence was reduced to 24.0%. Treatment time was also an important factor; treatment at 1x the recommended rate during day 7-14 of the incubation period resulted in bee emergence of over 70%, while use of the same rate during day 0-14 of incubation resulted in emergence of about 55%.

BEE CELL NUMBER / CELL POSITION

Since dichlorvos treatment is undertaken based on the volume of the incubator (i.e. 0.75 resin strip/1000 ft^3) the number of leafcutting bees placed in the incubator is also critical. In treatments of 300 and 3000 bee cells at the 1x dichlorvos rate during day 7-14 of incubation, emergence was significantly higher in 3,000 bee cell replicates (70.8%) than in 300 bee cell replicates (4.2%), indicating the importance of a minimum critical amount of organic material (i.e. number of cells in the incubator) to normal bee development. As well, percent emergence at the 1x (recommended) rate, was higher in the day 7-14 group than in the day 0-14 group, supporting previous data on the deleterious effect associated with day 0-7 dichlorvos treatment.

Work was also done in this experimental system to determine the effect of various dichlorvos treatments in relation to cell position in incubation trays. A comparison of emergence from cells on the surface of trays and cells randomly selected from throughout the 2.5cm (1 inch) depth of cells in the trays indicated that emergence from cells in the top layer was significantly lower than emergence from randomly selected cells, even in dichlorvos treatments undertaken at the recommended treatment rate and time period (Table 2).

Table 2. Effect of dichlorvos rate, treatment time, and cell position on percent adult emergence.

Treatment		Percent emergence	
Dose	Time	Top layer	Random sample
Control	-	96.0%	93.7%
1 X	Day 0-14	28.0	55.2
1 X	Day 7-14	32.0	70.8
4 X	Day 0-14	4.0	24.0
4 X	Day 7-14	12.0	24.0

Dissection of unemerged bee cells indicated that a teratogenic effect (i.e. one causing abnormal pupal development) had occurred. The characteristic teratogenic phenotype was a late-stage pupa or pre-emergent adult with poorly differentiated head, thorax, and abdomen, little or no apparent development of appendages, and retarded wing development (Figure A). This phenotype was observed in all dichlorvos treatments but was more apparent with increasing dichlorvos rate and in time regimes which included the day 0-7 period. Observations of the same type of abnormal pupal development were also made during dissections of cell samples from the incubators of producers who had utilized excessive rates of dichlorvos for extended time periods.

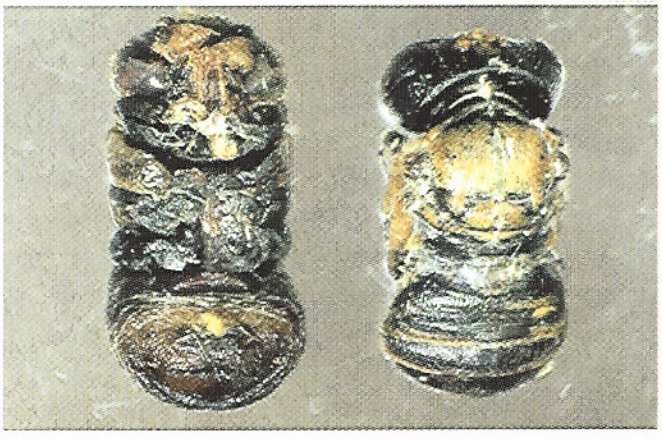

Figure A.

Dichlorvos-induced mortality in developing alfalfa leafcutting bee pupae.

DICHLORVOS AND RELATIVE HUMIDITY

The most recent research undertaken on dichlorvos-induced mortality in developing alfalfa leafcutting bees in the incubator has involved an examination of the relationship between dichlorvos and relative humidity. This work utilized treatment of bees in an experimental system (4000 bee cells/ft^3) at the 1x dichlorvos rate during day 7-14 of incubation under low (28%), ambient (59%), and high (84%) humidity conditions. An emergence profile of control and treated cells is given in Table 3. In the control cell treatment, incubation was undertaken at the ambient humidity (59%).

Table 3. Percent emergence in dichlorvos-treated bee cells at various humidities.

Treatment		Percent emergence
Control	(untreated)	72.0%
Low	(28% RH)	27.0
Ambient	(59% RH)	28.0
High	(84% RH)	79.0

Cumulative emergence data are presented in Figure B below. The effect of relative humidity in the incubator on dichlorvos-induced bee mortality was significant in the low to moderate humidities examined in this study. Adult emergence from cells treated with dichlorvos at the recommended rate from day 7-14 was very low at humidities of 28% and 59%. Dichlorvos-induced mortality in developing leafcutting bee pupae apparently increases with lower humidities due to increased dichlorvos release in a low humidity atmosphere and due to increased dichlorvos uptake by the developing bee pupa across the bee cell surface.

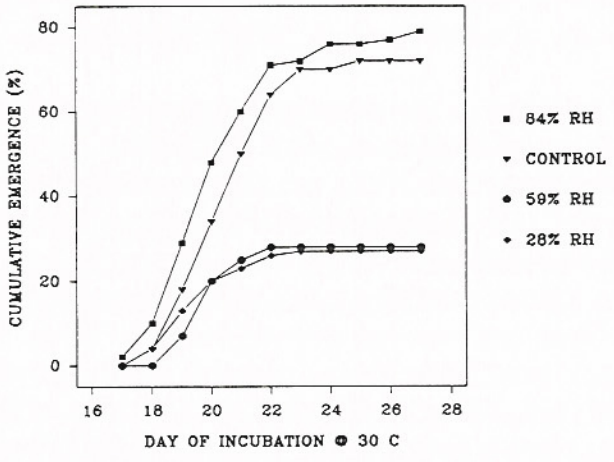

Figure B.

Emergence in alfalfa leafcutting bees treated with dichlorvos at 1x the recommended rate (0.75 resin strip/1000 ft^3) under low (28%), ambient (59%) and high (84%) relative humidity conditions.

Alfalfa seed producers incubate leafcutting bees at various relative humidities, and incubation humidity is often affected by weather conditions (i.e. wet or dry / warm or cool). The research data generated from work undertaken in an experimental system and presented here indicate that dichlorvos rate, treatment time, number of bees treated, and position of bees in the incubation tray may all significantly impact upon dichlorvos-induced mortality and subsequent emergence of alfalfa leafcutting bees. Relative humidity is an additional factor which appears to play a crucial role in dichlorvos-induced mortality in developing leafcutting bees.

CONCLUSION

Producers are cautioned to avoid using dichlorvos if possible, and when the resin strips are used, to strictly adhere to the currently recommended rate of 0.75 strip/1000 ft^3 and treatment time of day 7-14 during the incubation period. Care should also be taken to monitor humidity in the incubator; a relative humidity in the 60-70% range is likely high enough to prevent excessive bee mortality if recommended dichlorvos rate and treatment time are utilized. Use of dichlorvos in the incubator should be followed by an extended period of ventilation (i.e. 48-72 hour period of active fresh air intake and exhaust).

In addition to the potentially damaging effects that use of dichlorvos resin strips may have on developing leafcutting bee pupae, recent studies by the U.S. Environmental Protection Agency have demonstrated that dichlorvos is a carcinogen (i.e. cancer-causing agent) and is also implicated in liver and nervous system damage. There is thus a possibility that production of dichlorvos resin strips may be halted in the near future. To date, there is no alternative to the use of dichlorvos for parasite control in alfalfa leafcutting bee populations.

Research is currently underway to identify possible alternatives to dichlorvos for control of chalcid parasites in alfalfa leafcutting bee populations. Work to screen candidate compounds, test them for efficacy in parasite control and safety to developing and emerging bees, and then field test and register the compounds is aimed at providing alternative parasite control technology to alfalfa seed producers in the near future.

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