ADDITIVES TO ENHANCE BIOPESTICIDES

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Summary

Bioassays were conducted to investigate the effect of milk powder additives on the performance of a heliothis nucleopolyhedrovirus (Gemstar®) and *Bacillus thuringiensis* (DiPel SC®) on mungbeans and cotton. Larval mortality increased when the calf feeding supplement, Denkavit®, was added at 1 kg/ha to both Gemstar and DiPel SC. Other powder additives and a liquid formulation, Amino Feed®, were mostly equivalent to Denkavit. While Envirofeast® and Pred Feed® would not be considered for use solely as an additive to Gemstar or DiPel SC, the data indicate that improved performance from Gemstar could be expected if added to these food sprays. The mode of action of these additives has not been determined.

Introduction

Many additives have been evaluated in an effort to improve the performance of biopesticides (Hunter-Fujita *et al.* 1998). The local history of the current use of milk powder additives dates back to 1996 when Teakle and Monsour (Unpublished data) showed in glasshouse experiments on chickpea that the addition of skim milk powder improved the kill of heliothis larvae from nucleopolyhedrovirus (NPV) (Gemstar®). Field trials confirmed their findings. The addition of skim milk powder at 1 kg/ha boosted NPV mortality from 70% to 90%, and culminated in the registration of Gemstar on chickpea. This use of milk powder additives has extended to other crops, and to mixtures with *Bacillus thuringiensis* (DiPel SC®). Cheaper and more readily available calf feeding supplements such as Denkavit® and CalfPab®. Have replaced skim milk powder. Concerns about mixing issues and nozzle blockages from powder formulations have seen liquid formulations such as Amino Feed® enter the marketplace. Our studies were aimed at generating more data on mungbean and cotton to substantiate the use of milk powder additives mixed with either Gemstar or DiPel SC®. We also compared Amino Feed with the standard Denkavit, and included two food sprays, Envirofeast® and Pred Feed® in some comparisons.

Materials and Methods

Bioassays were carried out at Kingsthorpe Research Station (20 km north west of Toowoomba) on raingrown mungbeans (variety Emerald) and cotton (variety Siokra V16) sown in 1 m rows. Small, unreplicated plots were treated with test solutions using a hand-held rotary cage atomiser delivering 30 L water/ha. All NPV treatments used Gemstar produced by Thermo Trilogy in USA at 375 mL/ha. Gemstar was a liquid formulation of *Helicoverpa zea* NPV containing greater than 2 x 10^9 polyhedra/mL. All Bt treatments used DiPel SC produced by Abbott Laboratories (now Valent Biosciences) at 1.5 L/ha.

Gemstar

Various treatments (Tables 1 and 3) were applied to test plots 20 m long x 3 m wide. Immediately after treatment, 6 and 24 h after treatment (HAT), 16 leaves of plants were selected at random from the central row in each plot and placed into labelled paper bags. These were returned to the laboratory where a 50 mm diameter leaf disc was cut from each leaf and placed into a 50 mm diameter Falcon petri dish with five 1-day old *H. armigera* larvae. This procedure was repeated 16 times for each treatment so that a total of 80

larvae/treatment were fed plant material. After 24 h, four of the five larvae from each Falcon dish were transferred singly to artificial diet in 32 well trays. Only larvae that had fed on leaf material, as indicated by leaf feeding and larval growth, were transferred onto diet. Larvae were inspected daily for NPV infection.

Data for each bioassay were analysed by logistic regression (for binomial data) (GenStat 5 Release 4.1) and differences tested at $P<0.05$. Due to the high standard deviation of values near 0 and 100%, the logistic regression analysis will not detect differences for these values.

DiPel SC

Various treatments (Tables 2 and 4) were applied to test plots 20 m long x 4 m. Immediately after treatment, 24 and 48 HAT, 50 leaves of plants were selected at random from the two central rows in each plot and placed into labelled paper bags. These were returned to the laboratory where a 50 mm leaf disc was cut from each leaf. Each leaf disc was placed into a 50 mm diameter Falcon petri dish with one 1-day old *H. armigera* larva. Using this procedure, a total of 50 larvae/treatment were fed plant material. Mortality of larvae was recorded daily for 4 days. The length (mm) of surviving larvae after 4 days was measured as an indication of larval development. At 4 days, the developmental stage (instar) of surviving larvae was determined by head capsule size. Mortality data were analysed as previously described. Length of surviving larvae were compared by ANOVA (Genstar 5) and differences tested at $P<0.05$.

Results and Discussion

Gemstar

Mungbean

As determined by NPV infection levels, Gemstar alone was very active on mungbean and activity persisted beyond 24 HAT (Table 1). There was no apparent explanation for the decline in NPV activity recorded at 6 HAT that subsequently rebounded at 24 HAT. The addition of Denkavit increased Gemstar performance beyond that of Gemstar alone, but this was significant only at 6 and 24 HAT. Pred Feed and Amino Feed were the only other additives that significantly increased activity of Gemstar. Coaton ILP® and Envirofeast made no significant difference to Gemstar activity. It was surprising that Envirofeast did not result in dramatic improvement of Gemstar activity. This may in part be explained by some problems encountered with blockages when using this product.

Table 1. Percentage NPV infection levels following exposure to mungbean foliage treated with Gemstar and different adjuvants at various intervals after treatment.

Hours after treatment	Control	Gemstar	Gemstar + Denkavit @ 1 kg/ha	Gemstar + Coaton @ 1.25 kg/ha	Gemstar + Amino Feed @ 1 L/ha	Gemstar + Envirofeast @ 2.5 kg/ha	Gemstar + Pred Feed @ 2.5 kg/ha
0	0	72.6 a	90.0 ab	85.2 ab	93.6 b	83.9 ab	100.0
6	0	25.9 a	65.0 b	42.4 a	62.7 b	31.6 a	78.7 b
24	0	80.7 ab	98.2 c	74.6 a	92.9 bc	76.4 a	96.7 c

Means in a row followed by different letters are significantly different at $P<0.05$. Logistic regression analysis will not detect differences for values near 0 and 100%.

Cotton

As determined by NPV infection levels, some NPV infection was recorded on the unsprayed at 0 and 6 HAT (Table 2). In a field situation, natural NPV infection can not be excluded and may account for these low infection levels. Unsprayed plots were always located upwind at

the time of treatment to avoid drift. Gemstar alone was initially very active on cotton and lower activity persisted at 24 HAT. The addition of Denkavit increased Gemstar performance beyond that of Gemstar alone, and this difference was significant at 0, 6 and 24 HAT. At 0 HAT, all additives increased activity of Gemstar beyond that of Gemstar alone, and these were the equivalent of Denkavit. All additives indicated equivalent NPV activity at 6 HAT, and these were significantly different to Gemstar alone. At 24 HAT, Denkavit recorded the highest NPV infection levels, but this was not different to Coaton ILP. NPV infection levels for Pred Feed, Amino Feed and Envirofeast were equivalent, but significantly lower than for Denkavit.

Table 2. Percentage NPV infection levels following exposure to cotton foliage treated with Gemstar and different adjuvants at various intervals after treatment.

Hours after treatment	Control	Gemstar	Gemstar + Denkavit @ 1 kg/ha	Gemstar + Coaton @ 1.25 kg/ha	Gemstar + Amino Feed @ 1 L/ha	Gemstar + Envirofeast @ 1 kg/ha	Gemstar + Pred Feed @ 1 kg/ha
0	4.8 a	83.9 b	100	100	100.0	98.4 c	98.4 c
6	3.5 a	25.4 b	79.4 c	85.9 c	87.1 c	78.1 c	81.3 c
24	0.0	34.9 a	77.4 d	67.7 cd	52.4 bc	44.4 ab	54.8 bc

Means in a row followed by different letters are significantly different at P<0.05. Logistic regression analysis will not detect differences for values near 0 and 100%.

DiPel SC

Mungbean

For the 0 HAT bioassay, similar mortality levels were recorded for all treatments (Table3). There was a consistent trend where mortality was higher for all DiPel SC treatments containing additives compared to the DiPel SC alone. At 24 HAT, mortality was significantly higher for all DiPel SC treatments containing additives than for DiPel SC alone. There was no difference in mortality between DiPel SC and DiPel SC + Denkavit at 48 HAT.

Larvae from the unsprayed treatment were double the size of those survivors on the DiPel SC treatments (Table 4). These data reflect anti-feedant or sub-lethal effects from the DiPel SC. The surviving larvae were not reared beyond the 4 days of the bioassay to determine their subsequent survivorship. Where larvae were confined to leaf discs, their option was to feed on treated leaf or starve. In the field it is more likely that larvae would seek out 'untreated' sites on which to continue feeding. This may result in greater survivorship than recorded in this bioassay method. The data stress the need for thorough spray coverage to achieve maximum efficacy. At 24 HAT, all except two larvae on the unsprayed had moulted to become second instars after 4 days. In contrast, only two surviving larvae from treatments containing DiPel SC has become second instar.

Table 3. Percentage mortality after 4 days exposure to mungbean foliage treated with DiPel SC and different adjuvants at various intervals after treatment.

Hours after treatment	Control	DiPel SC	DiPel SC + Denkavit @ 1 kg/ha	DiPel SC + Coaton @ 1.25 kg/ha	DiPel SC + Amino Feed @ 1 L/ha
0	6 a	86 b	90 b	90 b	94 b
24	12 a	44 b	64 c	64 c	66 c
48	6 a	48 b	46 b	-	-

Means in a row followed by different letters are significantly different at P<0.05.

Table 4. Mean length ± standard error of larvae surviving 4 days exposure to mungbean foliage treated with DiPel SC and different adjuvants at various intervals after treatment.

Hours after treatment	Control	DiPel SC	DiPel SC + Denkavit @ 1 kg/ha	DiPel SC + Coaton @ 1.25 kg/ha	DiPel SC + Amino Feed @ 1 L/ha
0	4.5 ± 0.2 a	2.4 ± 0.1 b	2.7 ± 0.5 b	2.3 ± 0.1 b	2.7 ± 0.2 b
24	5.0 ± 0.1 a	2.5 ± 0.1 b	2.6 ± 0.1 b	2.5 ± 0.1 b	2.5 ± 0.1 b
48	5.3 ± 0.1 a	3.3 ± 0.2 b	2.9 ± 0.1 c	-	-

Means in a row followed by different letters are significantly different at P<0.05.

Cotton

For the 0 HAT bioassay, significantly higher mortality levels were recorded for all treatments containing additives than for DiPel SC alone (Table 5). There was a consistent trend at 24 and 48 HAT where mortality was higher for all DiPel SC treatments containing additives compared to the DiPel SC alone. There was no difference in mortality between DiPel SC and the unsprayed treatment at 48 HAT.

For all samples, larvae from the unsprayed treatment had grown significantly longer than survivors on the DiPel SC treatments (Table 6). These data reflect anti-feedant or sub-lethal effects from the DiPel SC. The surviving larvae were not reared beyond the 4 days of the bioassay to determine their subsequent survivorship. At 24 HAT, larvae on the unsprayed were mostly second instar (72%) or third instar (26%) after 4 days. In contrast, surviving larvae from the DiPelSC treatments were either first instar (38%) or second instar (62%).

Table 5. Percentage mortality after 4 days exposure to cotton foliage treated with DiPel SC and different adjuvants at various intervals after treatment.

Hours after treatment	Control	DiPel SC	DiPel SC + Denkavit @ 1 kg/ha	DiPel SC + Coaton @ 1.25 kg/ha	DiPel SC + Amino Feed @ 1 L/ha
0	4.1 a	72.0 b	91.8 c	93.9 c	92.0 c
24	6.0 a	34.7 b	47.9 bc	55.3 c	40.0 bc
48	10.0 a	24.0 ab	34.0 b	30.0 b	32.0 b

Means in a row followed by different letters are significantly different at P<0.05.

Table 6. Mean length ± standard error of larvae surviving 4 days exposure to cotton foliage treated with DiPel SC and different adjuvants at various intervals after treatment.

Hours after treatment	Control	DiPel SC	DiPel SC + Denkavit @ 1 kg/ha	DiPel SC + Coaton @ 1.25 kg/ha	DiPel SC + Amino Feed @ 1 L/ha +
0	6.0 ± 0.1 a	3.8 ± 0.2 b	3.4 ± 0.2 b	3.3 ± 0.3 b	3.8 ± 0.3 b
24	6.0 ± 0.2 a	3.8 ± 0.1 b	3.7 ± 0.1 b	3.8 ± 0.2 b	4.0 ± 0.1 b
48	5.4 ± 0.1 a	3.6 ± 0.2 b	3.2 ± 0.1 c	3.5 ± 0.1 b	3.5 ± 0.1 bc

Means in a row followed by different letters are significantly different at P<0.05.

Conclusion

These bioassays demonstrated increased larval mortality when Denkavit was added to either Gemstar or DiPel SC and applied to mungbeans and cotton. Other powder additives and the liquid formulation, Amino Feed, were mostly equivalent to Denkavit. While Envirofeast and Pred Feed would not be considered for use solely as an additive to Gemstar or DiPel SC, the data indicate that improved performance from Gemstar could be expected if added to these food sprays.

It is still not clear what these additives are doing to improve the performance of biopesticides. Suggestions are that the additives -

- act as attractants and feeding stimulants leading to greater uptake of the microbe particles
- protect the microbes from harmful chemicals on plant surfaces and possibly in the insect gut
- protect the microbes from the sun's harmful ultra violet rays

Further detailed studies are needed to identify the mode of action of these additives.

While the bioassays were conducted under field conditions, the results can not be assumed to translate directly into improved field performance. Commercial evaluation is the ultimate test, and experience during the 1999/2000 season generally supported these findings.

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References

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