ADDITIVES THAT ENHANCE NUCLEOPOLYHEDROVIRUS PERFORMANCE ON CENTRAL QUEENSLAND COTTON

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Summary

A difficulty faced by growers using nucleopolyhedrovirus (NPV) products such as Gemstar® can be inconsistency of the products' performance for *Helicoverpa* spp. control. This problem has lead to the advent of additives developed to improve biopesticide performance. In this paper we investigated several liquid additives for their potential to augment the performance of Gemstar®. The results suggest that Aminofeed® and Aminofeed UV® both increase the efficacy of Gemstar.

Introduction

The use of additives to improve the performance of NPVs has been the subject of considerable research. The objective for using an additive with biopesticides such as NPV has been to improve field performance by increasing larval intake, improving the microbes' virulence or protecting the microbes and enabling them to persist for longer periods of time in the external environment (Cunningham *et al.* 1997; Morales *et al.* 2001). A number of liquid additives for use with NPVs have recently entered the Australian marketplace. Aminofeed® was one of the first products available and was subject to testing by Murray *et al.* (2000). In these tests Aminofeed® gave an equivalent response to the calf feeding supplement Denkavit® which had been widely used as an additive after initial work conducted by Teakle and Monsour (unpublished data). Due to a liquid formulation which eliminates the application difficulties associated with milk powders, Aminofeed® has become a popular choice as an additive for NPVs.

Since the successful introduction of Aminofeed®, several new liquid IPM tank mix products for use with biopesticides have also entered the Australian market. Aminofeed UV® by Agrichem is a new product that incorporates sunscreening agents with the original Aminofeed® formulation. Coaton ILP® by Biostarch Australia has been designed as a liquid alternative for calf milk powders and Mobait® by Nufarm has been promoted as a feeding attractant suited for addition to a variety of insecticides.

During the last two seasons we have conducted a series of experiments to compare the range liquid additives available and their ability to enhance the performance of NPVs on cotton under central Queensland conditions.

Materials and Methods

A series of experiments were conducted at the Biloela Research Station on irrigated cotton (Sicot 189) grown on 1 metre rows with sub-surface drip irrigation during 2000-02. A series of assays were conducted during this period to investigate the performance and persistence of NPVs when used with different additives on cotton.

A standard method was used to assess the persistence of Gemstar when used with different additives which will be referred to as a Field Based Laboratory Assay. This assay was used to assess the presence of virus on sprayed leaf surfaces for increasing time periods after an initial application. For each assay, twelve leaves were sampled from each treatment plot at 12 hourly intervals from 0-96 hours after application. The leaves for these assays were sampled from the terminals of the plants as this canopy section was fully exposed to sunlight throughout the day. Upon return to the laboratory, each sample of leaves was divided and 4 leaves were placed into individual disposable plastic containers (750 mL). 30-40 *Helicoverpa armigera* neonates were then introduced into each container. The larvae were left to feed at 25 ± 1°C, 65% RH and 12:12 photoperiod until they had become satiated (24-36 hours later). 30 larvae were then transferred from the leaves for each treatment replicate and placed onto artificial diet in 28 mL plastic portion cups with 8 pin holes in each lid for ventilation. Only larvae that had fed on the upper leaf surface were transferred to artificial diet as this leaf surface had been exposed to the maximum amount of sunlight whilst in the field. Larvae were then grown out and their fate determined :- healthy, NPV, or unknown.

All treatment results were corrected for control mortality using Abbott's formulae (Abbott 1925). The data was then converted to probits and the values of each slope and intercept were checked in Genstat 5 (Payne *et al.* 1989) after pooling the replicates for each experiment.

Aminofeed® as an Additive for NPV

This first experiment was conducted to quantify the potential benefit of using Aminofeed as an additive for NPV under central Queensland conditions. Two treatments of Gemstar® (500 mL/ha) together with Aminofeed® (1 L/ha) and Gemstar® (500 mL/ha) alone, were applied to cotton plots (20 m long x 3 rows wide) at 6:00a.m on 15 November 2000 together with an untreated control. Treatments were applied with hand held hydraulic equipment equipped with 0.02 110 flat fan nozzles operated at 3 bar delivering 150 L water/ha. Each treatment was replicated three times. Leaves were then collected from the plots for 0-96 hours and exposed to larvae as per the Field Based Laboratory Assay protocol outlined earlier. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 32-36°C.

Aminofeed®, NPV and Application Time of Day

A common usage concern for NPVs are their rapid breakdown in response to sunlight and alkaloids on leaf surfaces. Given the intensity of central Queensland's summer season, this

experiment was conducted to test whether or not the time of day that an application takes place makes a difference to the performance of Gemstar® when used with Aminofeed on cotton.

Gemstar® (500 mL/ha) together with Aminofeed® (1 L/ha) was applied to cotton plots (20 m long x 3 rows wide) at 6:00 a.m., 12:00 p.m. and 6:00 p.m on 10 December 2000 using the same application method and equipment outlined earlier. Leaves were again sampled from the plots and exposed to larvae using the Field Based Laboratory Assay protocol. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 30-34°C.

Laboratory Assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

This experiment was conducted to compare Aminofeed with Aminofeed UV, Coaton and Mobait as additives for NPV under central Queensland conditions. Treatments of Aminofeed® (1 L/ha), Aminofeed UV® (1 L/ha), Coaton ILP® (2 L/ha) and Mobait (250 mL/ha) were combined with Gemstar® (500 mL/ha) and applied to cotton plots (Sicot 189) at 6:00 a.m using 100 L water per hectare on 27 November 2001. An un-sprayed control and treatment of Gemstar® (500 mL/ha) alone were also applied for comparison. Each of the treatments were replicated four times within a randomised block experimental design. Leaves were sampled from the plots and exposed to larvae using the Field Based Laboratory Assay protocol. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 31-35°C.

Field Mortality assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

Treatments of Aminofeed® (1 L/ha), Aminofeed UV® (1 L/ha), Coaton ILP® (2 L/ha) and Mobait (250 mL/ha) were combined with Gemstar® (500 mL/ha) and applied to cotton plots at 5:00 a.m using 120 L water per hectare on 6 December 2002. An un-sprayed control and treatment of Gemstar® (500 mL/ha) alone were also applied for comparison. Each of the treatments were replicated four times within a randomised block experimental design. 4-6 small (>3 mm) *Helicoverpa armigera* larvae were present in the crop at the time of application.

30 larvae were field collected from each treatment plot 48 hours after application and transferred onto artificial diet in 28 mL plastic portion cups with 8 pin holes in the lid for ventilation. Larvae were then grown out in a constant climate laboratory at 25 ± 1°C and their fate determined. Data was corrected for control mortality using Abbots (1925) formulae and subject to ANOVA using the Genstat 5 computer program (Payne *et al.* 1989) with LSDs calculated to determine treatment differences at $P<0.05$.

Results & Discussion

Aminofeed® as an Additive for NPV

The effect of Gemstar® with and without Aminofeed® on larvae mortality over time is given in Figure 1. Regressions for the data (excluding data for 0 hrs) and a comparison of slopes indicated no significant differences between treatment slopes but a significant difference ($P<0.03$) for slope intercepts. What this suggests is that Aminofeed® delayed the onset of the viruses decline on the cotton foliage for a short period of time which in this case was 24 hours, before then proceeding to decay at a similar rate as for Gemstar® applied alone. The net result of this process was greater persistence of virus as indicated by higher larvae mortality over time (Fig 1).

Aminofeed®, NPV and Application Time of Day

The effect of each treatment being Gemstar® and Aminofeed® applied at 06:00, 12:00 and 18:00 hours on the rate of larvae mortality over time is given in Figure 2. Regressions for the data (excluding data for 0 hrs) and a comparison of treatment slopes and their intercepts suggested that time of day that the treatments were applied had no significant effect on the persistence of Gemstar® whether it had been applied during the morning, noon or night. Figure 2 shows that the virus was viable on the leaves for a considerable period of time and that mortality dropped off more rapidly after the first 36 hours.

Laboratory Assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

The effect of each of the additive treatments on larvae mortality over time is given in Figure 3. Regressions for the data (excluding data for 0 hrs) and a comparison of slopes suggested no significant differences between each of the treatments. However, a comparison of treatment slope intercepts suggested that Aminofeed UV® had a significantly different ($P<0.01$) intercept to all of the other treatments. In this experiment the addition of Aminofeed® did not provide a significant response ($P>0.11$) compared to Gemstar® alone. What the results suggest is that Aminofeed UV® initially delayed the onset of the viruses decline on the cotton foliage before then proceeding to decay at a similar rate as for Gemstar® applied alone. This resulted in an overall increase in persistence as indicated by larvae mortality (Fig 3). The original formulation of Aminofeed® in this experiment did not provide the same level of persistence as recorded in earlier experiments. However, conditions during this experiment were warmer and dryer than in previous tests. Coaton® and Mobait® did not provide a response that was different to Gemstar® applied alone in this experiment.

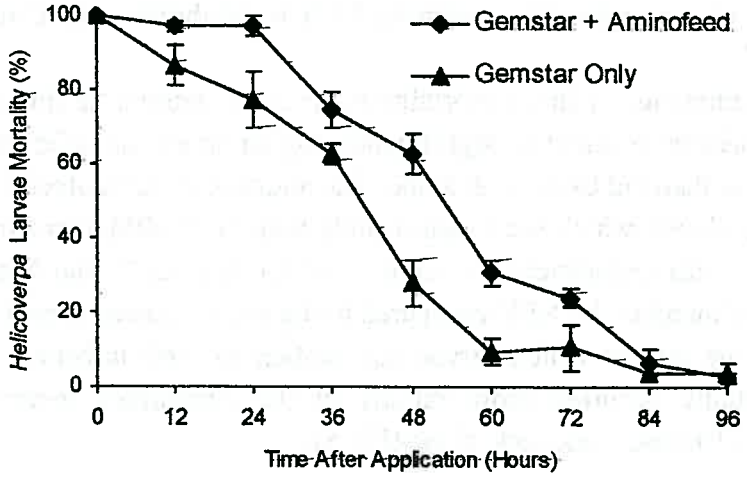

Figure 1. Mortality of *Helicoverpa* neonates exposed to NPV-treated foliage weathered in the field for increasing periods of time. There was a significant increase in larvae mortality when Aminofeed® was used with Gemstar®.

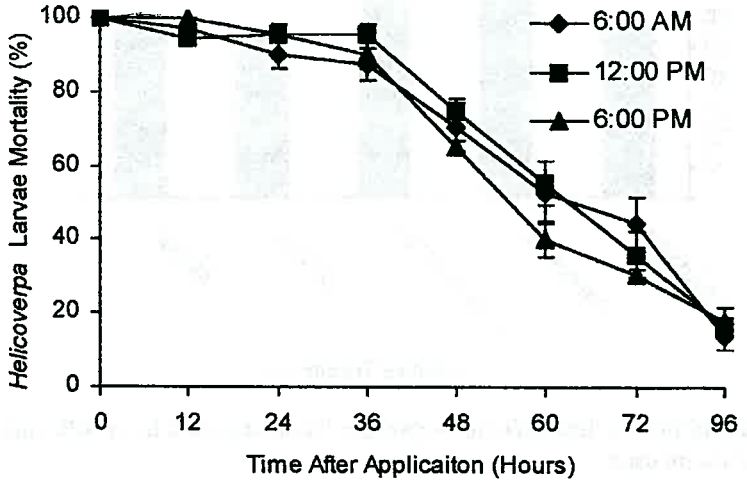

Figure 2. Mortality of *Helicoverpa* neonates exposed to NPV-treated foliage weathered in the field for increasing periods of time. There no significant difference between applications made at 6:00 a.m., 12:00 p.m. and 6:00 p.m.

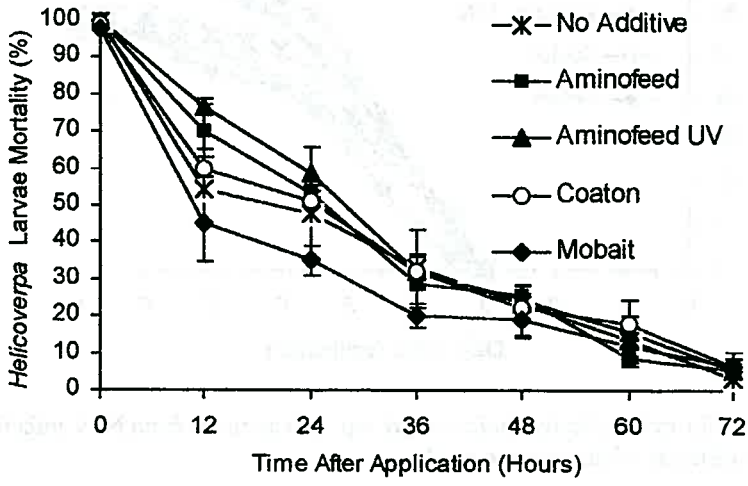

Figure 3. Mortality of *Helicoverpa* neonates exposed to foliage treated with Gemstar® and various additives that have been weathered in the field for increasing periods of time.

Field Assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

The effect of the treatments on larvae mortality is given in Figure 4. In this experiment the addition of Aminofeed® resulted in significantly higher levels of infection ($P<0.05$) in field collected larvae than did Gemstar® alone. The addition of Aminofeed UV® produced the highest infection levels which were significantly better ($P<0.05$) than Aminofeed® and Gemstar® alone. In this experiment the addition of Coaton ILP® and Mobait® did not improve the level of infection by NPV compared to the application of Gemstar® alone.

A comparison of the rate at which larvae succumbed to viral infection after spraying suggests that mortality occurred more rapidly in the Aminofeed treatments than for Mobait®, Coaton ILP® and Gemstar® alone (Fig 5).

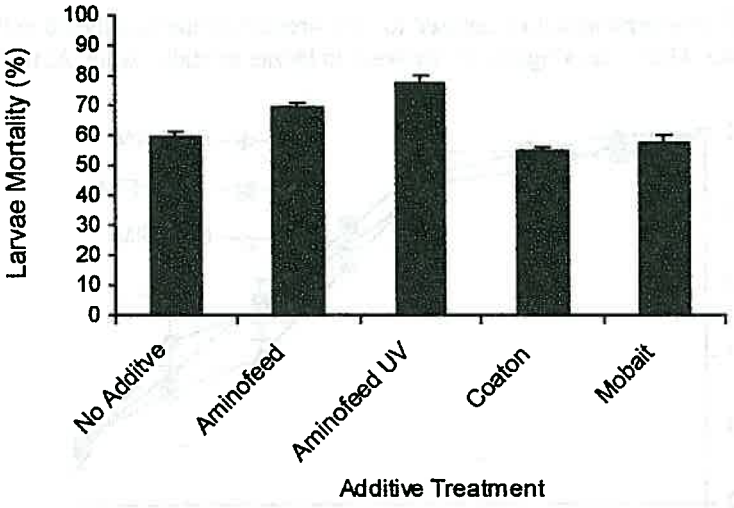

Figure 4. The percentage of field collected *Helicoverpa* spp. larvae that died from NPV infection where different additives were used.

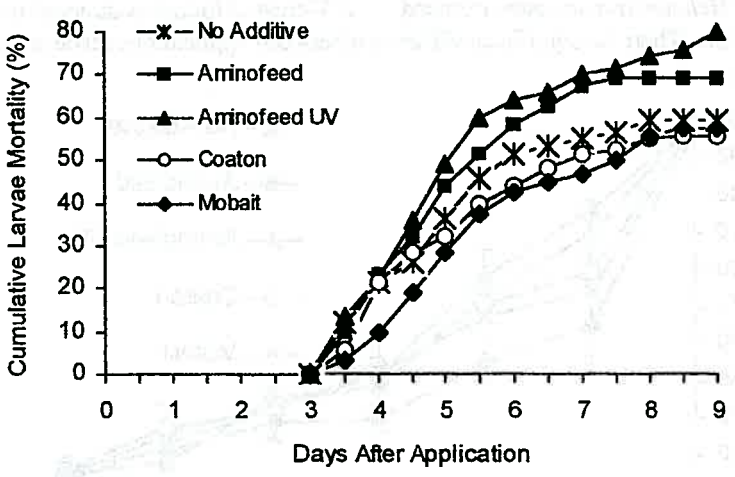

Figure 5. The time taken for field collected *Helicoverpa* spp. larvae to die from NPV infection after spraying (day 0) where different additives were used.

Conclusions

The data from these experiments strongly suggest that the use of additives is beneficial for NPV applications in cotton. When added to Gemstar®, Aminofeed® and Aminofeed UV® both increased larvae mortality compared to when Gemstar® was used alone. It is not entirely clear how these additives improve the performance of Gemstar® although it is likely that they serve to both protect the virus from the field environment as well as encourage rapid ingestion by larvae after application.

Acknowledgments

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