NUCLEAR POLYHEDROSIS AND GRANULOSIS VIRUSES FOR THE CONTROL OF THE COMMON CUTWORM, *AGROTIS SEGETUM* (LEPIDOPTERA: NOCTUIDAE)

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SUMMARY

A nuclear polyhedrosis virus (NPV) and a granulosis virus (GV) of *Agrotis segetum* were compared in two field trials, in Spain and the UK, for efficacy and speed of kill of *A. segetum* larvae in artificially infested plots of maize or beetroot. Aqueous spray and bran bait formulations were compared. AsNPV killed larvae more rapidly (9.4 versus 11.3 days, and 177.4 versus 210.4 hours) and at earlier instars than AsGV. A mixed inoculum (AsNPV plus AsGV) produced intermediate results. AsNPV is therefore likely to be a more efficient biocontrol agent than AsGV for this species, which is most destructive in its later larval instars.

Keywords: baculovirus, cutworms, *Agrotis*, formulation, biocontrol

INTRODUCTION

The term "cutworm" is used to describe the larvae of several species of noctuid moths which share a common behaviour pattern. In their later instars larvae feed on plants at ground level, often severing the stems of seedlings or seriously damaging root crops. Unlike defoliators, cutworms destroy far more plant material than they actually consume, and losses can be rapid and unexpected, since early instar larvae are difficult to detect in the field.

The common cutworm, *Agrotis segetum* (Lepidoptera: Noctuidae), is a widespread, highly polyphagous pest, occurring in many high value crops such as vegetables, and even damaging tree seedlings in forestry nurseries. The closely-related greasy cutworm, *A. ipsilon*, occurs in New Zealand, and is also highly polyphagous.

A granulosis virus of *A. segetum* (AsGV) is effective in a spray formulation against both *A. segetum* and *A. ipsilon* in field trials (eg. Shah *et al.* 1979; Zethner *et al.* 1987), and has also been used in a bran bait in trials in Spain (Caballero *et al.* 1991). Preliminary studies in the UK had indicated that a nuclear polyhedrosis virus of *A. segetum* (AsNPV) may also have potential as a biocontrol agent for this species (de Oliveira 1988).

This paper describes a collaborative field trial (Trial A), carried out in southern Spain in conjunction with the University of Cordoba, to compare the two viruses in both spray and bait formulations (Bourner *et al.* 1992), and a second trial (Trial B) in the UK, using adjusted dose rates and a reduced application rate of bran bait. Trial B additionally investigated the effects of a mixed AsNPV / AsGV inoculum (Bourner 1993).

METHODS

Trial A

This trial was carried out in the Badajoz region of Spain in summer (June). Open plots (2.25 x 2.4 m) of 60 maize seedlings were infested with second instar *A. segetum* larvae from a disease-free laboratory stock at a rate of three larvae per seedling. On the same day, the plots were treated in a six by six Latin square design with the following treatments: AsNPV spray, AsNPV bait, AsGV spray, AsGV bait, control spray, and control bait. AsNPV was applied at 4×10^{12} polyhedral inclusion bodies (PIBs)/ha and

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AsGV at 4×10^{13} inclusion bodies (IBs)/ha in each formulation. Sprays were applied as an aqueous suspension containing 0.1% Agral as a wetting agent, using a backpack compression sprayer, at a rate of 463 litres/ha. The bait consisted of 800 g wheat bran mixed with 1.5 litres aqueous virus suspension per six plots, and was applied by hand. Treatments were applied in the late afternoon to avoid high levels of ultra-violet light, which rapidly destroys baculovirus activity. The virus suspensions used were bulked up from experimental isolates.

Twenty-four hours after treatment, 10% of seedlings from each plot plus the surrounding soil to a diameter of 10 cm and a depth of 2 cm, were randomly sampled. *A. segetum* larvae were retrieved using soil sieves, and maintained individually on artificial diet at $26^\circ\text{C} \pm 2^\circ\text{C}$ until death or pupation. Dead larvae were examined for the presence of virus using DNA:DNA hybridization techniques (Bourner 1993). The date and instar of death was recorded for each virally-infected larva. The final mortalities due to viral infection in each treatment were compared by analysis of variance using generalized linear models. The instar distributions of larvae killed by virus infection were compared between treatments using chi-squared tests.

Trial B

This trial was carried out near Oxford, UK in late summer (August), using 1 x 1 m plots of beetroot seedlings. Each plot was protected with a corrugated plastic sloping roof, approximately 75 cm from the ground, to prevent heavy rainfall from interfering with the treatments. A plastic barrier was placed around the central 50 x 50 cm of each plot to prevent the escape of *A. segetum* larvae. This area contained 49 beetroot seedlings, which were each infested with four second instar larvae from a clean laboratory stock. On the same day, the following treatments were applied in a 5 x 5 Latin square design: AsNPV at 4×10^{12} PIBs/ha; AsGV at 4×10^{14} IBs/ha; 4×10^{12} PIBs/ha AsNPV plus 4×10^{14} IBs/ha AsGV; infested control; uninfested control. All treatments were applied as a bait of wheat bran and aqueous virus suspension plus locust bean gum. The addition of gum allowed the bait to be made into pellets, approximately 0.5 cm in diameter. Two pellets were placed at the base of each seedling. Again, the virus suspensions were bulked up from experimental isolates.

Twenty-four hours after treatment, eight plants from the infested area of each plot, plus the surrounding soil (7 x 7 x 2 cm deep) were randomly sampled. *A. segetum* larvae were retrieved, reared and examined for infection as for Trial A. The time of death and instar at death were recorded, and the statistical analysis performed as before.

TABLE 1: Trial A. Percentage mortality due to viral infection, and time to death of *A. segetum* larvae.

Treatment	No. of larvae retrieved	Mortality (%)	Time to death (Days) Mean	SE ¹
NPV spray (4×10^{12} PIBs / ha)	24	83.3 a ²	9.4 a	0.285
NPV bait (4×10^{12} PIBs / ha)	23	87.0 a	9.4 a	0.334
GV spray (4×10^{13} IBs / ha)	24	12.5 b	10.5 a	0.500
GV bait (4×10^{13} IBs / ha)	33	57.6 ab	11.5 b	0.320

¹ Standard error

² Different letters after results indicate significant differences ($P < 0.05$)

RESULTS

Trial A

Overall, *A. segetum* mortality due to virus infection in NPV-treated plots was higher than in GV-treated plots ($P < 0.05$), but there was no significant difference between the bait and spray formulations ($P > 0.05$) (Table 1). The time taken for larvae to die of viral infection was shorter for NPV-infected versus GV-infected larvae (9.40 versus 11.32 days; $P < 0.001$). This was reflected in the instar attained by infected larvae

before death (Table 2); most NPV-infected larvae died at third instar, while most GV-infected larvae reached fourth instar. This difference was highly significant in a chi-squared test ($P < 0.001$). There was no mortality from viral infection in larvae retrieved from control plots.

TABLE 2: Trial A. Instars of virus-infected larvae at death.

Treatment	2nd	Instar at Death		
		3rd	4th	5th
NPV spray	2	15	3	0
NPV bait	0	17	3	0
GV spray	0	1	2	0
GV bait	0	6	9	4

Trial B

There was no difference in larval mortality from viral infection between those plots treated with the mixed virus inoculum and those treated with AsNPV alone ($P > 0.05$) (Table 3). However, the mixed inoculum caused a higher mortality than AsGV alone ($P < 0.05$). Infected larvae treated with AsGV alone died more slowly than those from the other two treatments ($P < 0.05$) (Table 3). This was reflected in the instar attained by the larvae before death (Table 4). There were no deaths from viral infection in larvae retrieved from control plots.

TABLE 3: Trial B. Percentage mortality due to viral infection, and time to death of *A. segetum* larvae.

Treatment	No. of larvae retrieved	Mortality (%)	Time to death (Hours)	
			Mean	SE ¹
NPV (4×10^{12} PIBs / ha)	70	90.0 ab ²	177.4 a	2.9
GV (4×10^{14} IBs / ha)	86	80.2 b	210.4 b	5.6
NPV + GV (4×10^{12} PIBs / ha plus 4×10^{14} IBs / ha)	55	90.9 a	185.7 a	5.1

¹ Standard error

² Different letters after results indicate significant differences ($P < 0.05$)

TABLE 4: Trial B. Instars of virus-infected larvae at death.

Treatment	2nd	Instar at Death	
		3rd	4th
NPV	44	19	0
GV	31	38	0
NPV + GV	35	15	0

DISCUSSION

The results from Trial A indicated that there may be a difference between AsNPV and AsGV in terms of the speed of kill of *A. segetum* larvae in the field. However, since the mortality in the NPV-treated plots was far higher than in GV-treated plots, this could simply have been a dose rate effect. Trial B, however, revealed the same difference in speed of kill between these two viruses under conditions which produced similar levels of mortality in each virus treatment. Therefore this effect appears to be a real difference between the viruses, and is great enough to be manifested as a difference in the developmental stage attained by infected larvae before succumbing

to infection. No synergistic effects between the two viruses were observed in the mixed inoculum treatment.

Other published accounts of field trials using baculoviruses to control cutworms have relied entirely on damage assessment data to evaluate the success of the treatments (Shah *et al.* 1979; Zethner *et al.* 1987; Caballero *et al.* 1991). None had retrieved larvae from the field in sufficient numbers to allow the course of infection to be followed.

Given the destructive nature of cutworms from third instar onwards, a biocontrol agent which rapidly stops the pest from feeding or with a rapid speed of kill is preferable to one which enables the larvae to develop to a later instar. Although further studies are required to see if there is a lower level of cutworm damage in crops treated with AsNPV compared to AsGV, we feel that AsNPV should have greater potential as a biocontrol agent for *A. segetum* larvae than the hitherto more widely-used AsGV.

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