Control of the Diamondback Moth (Lepidoptera: Plutellidae) on Collard by a Nucleopolyhedrovirus with a Stilbene-based Enhancer and an Ultraviolet Light Protectant[1,2]

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Abstract When we evaluated the nucleopolyhedrovirus of the diamondback moth, *Plutella xylostella* (L.) (PxMNPV), in small plots of collard, *Brassica oleracea* L., artificially infested with this insect, numbers of larvae were reduced in all tests. Further reductions were obtained with the addition of a fluorescent brightener (Blankophor P167®) in both tests in which it was included. Addition of an ultraviolet light protectant (photostabilized titanium dioxide) to PxMNPV also improved control, but only when the test was conducted in May and June. Titanium dioxide had no effect when the test was conducted in September. Addition of both materials together did not improve results compared with addition of Blankophor P167 alone. No virus treatment, however, was as effective as the insecticide spinosad.

Key Words Diamondback moth, nucleopolyhedrovirus, fluorescent brightener, enhancer, ultraviolet, titanium dioxide

Diamondback moth, *Plutella xylostella* (L.), is a major pest of cabbage and other cruciferous crops (Brassicaceae) wherever these crops are grown. Annual costs of diamondback moth management are estimated to be approximately $1 billion worldwide. The situation is exacerbated by development of resistance to many insecticides, including biopesticidal formulations of *Bacillus thuringiensis* Berliner, in some populations of the diamondback moth (Talekar and Shelton 1993).

Nucleopolyhedroviruses (NPVs) are potential alternatives to chemical insecticides and *B. thuringiensis* (see reviews by Entwistle and Evans 1985, Granados and Federici 1986, Adams and McClintock 1991, Hunter-Fujita et al. 1998). Kariuki and McIntosh (1999) isolated a new NPV (PxMNPV) from the diamondback moth and showed it to be more potent against this species than were other NPVs known to infect the diamondback moth, including those of the alfalfa looper, *Autographa californica* (Speyer) (AcMNPV), and celery looper, *Anagrapha falcifera* (Kirby) (AfMNPV). This result was confirmed by Farrar et al. (2004).

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NPVs are used in production agriculture on only a limited basis for numerous reasons, such as short residual activity in the field (primarily due to inactivation by ultraviolet (UV) light), low potency, slow speed of kill, and high production cost (Entwistle and Evans 1985, Granados and Federici 1986, Adams and McClintock 1991, Hunter-Fujita et al. 1998). Various strategies have been proposed to overcome these limitations, including the use of derivatives of diaminostilbene disulfonic acid (fluorescent brighteners or optical brighteners) as spray adjuvants. These materials enhance the potency of many NPVs (Shapiro and Robertson 1992, Shapiro et al. 1992, Shapiro 1995, Farrar and Ridgway 1997, Hamm 1999, Monobrullah 2003). Because these compounds absorb UV light and reemit the energy as visible light (hence, the fluorescence), they can also protect NPVs from degradation by sunlight (Shapiro 1992, 1995, Farrar and Ridgway 2000). Farrar et al. (2004) showed that certain fluorescent brighteners enhanced the activity of PxMNPV, although other fluorescent brighteners inhibited this virus.

Many other materials have been tested as protectants for NPVs and other microbial control agents from UV light in the field (see reviews by Jaques 1977, 1985, Shapiro 1995, Burges and Jones 1998, Hunter-Fujita et al. 1998). Among these materials is photostabilized titanium oxide (TiO_2) (Farrar et al. 2003). This material blocks UV light, and photostabilization prevents TiO_2 from catalyzing the production of hydrogen peroxide, a chemical that can degrade NPVs. Farrar et al. (2003) showed that the addition of photostabilized TiO_2 to sprays of the NPV of the corn earworm, *Helicoverpa zea* (Boddie), extended the activity of this virus on lima bean, *Phaseolus lunatus* L., plants in the field.

We designed the study reported here to extend findings of laboratory studies of PxMNPV to a system that more closely approximates growers' fields, and to evaluate the potential of a fluorescent brightener and a UV protectant to improve control with this virus. Artificial infestation was used because natural infestations at the field site (Beltsville, MD, USA) were very low.

Materials and Methods

Insects. We obtained diamondback moths from a continuous culture maintained on artificial diet (Shelton et al. 1991) at the Insect Biocontrol Laboratory (USDA-ARS, Beltsville, MD, USA). For production of the virus (see below), we obtained eggs of the beet armyworm, *Spodoptera exigua* (Hübner), from the Crop Protection and Management Research Laboratory (USDA-ARS, Tifton, GA, USA).

Solar irradiance. Measurements of solar irradiance were obtained from a weather station maintained by the Beltsville Agricultural Research Center and located approximately 300 m from the field in which the tests were conducted.

Virus. We originally obtained PxMNPV from A. H. McIntosh (USDA-ARS, Columbia, MO, USA). The virus used in the tests reported here was produced in larvae of the beet armyworm as described previously (Farrar et al. 2004).

Rate response test. We first evaluated the effects of varying rates of PxMNPV with no additional enhancers or UV protectants. Collard transplants (*Brassica oleracea* L. *Acephala* group, cv. 'Champion') were obtained from a commercial supplier (Piedmont Plant Co., Albany, GA, USA). The transplants were placed in a walk-in growth chamber at 20°C and 16:8 (L: D) photoperiod. Approximately 2000 adult diamondback moths were released in the chamber and allowed to oviposit on the plants for 3 d.

The infested transplants were planted in the field at the Beltsville Agricultural Research Center, Beltsville, MD, USA, on 23 April 2001. Rows were 90 cm apart, with every third row skipped as a buffer between plots; plants were 30 cm apart within rows. The field was divided into four blocks of six plots with each plot measuring 7.5 m long by two rows wide and 2 m between plots within rows.

Six spray treatments were included. These were control (water); PxMNPV at 7.81×10^{10}, 2.47×10^{11}, 7.81×10^{11}, and 2.47×10^{12} occlusion bodies (OB)/ha; and spinosad (SpinTor®, Dow AgroSciences LLC, Indianapolis, IN, USA) at 52.7 g active ingredient (AI)/ha. Joint Venture® (Helena Chemical, Memphis, TN, USA), a spreader/sticker marketed for use with biological insecticides, was included in all treatments at the rate of 0.125% (v/v). All spray materials were prepared with deionized water. Treatments were randomly assigned to plots within blocks (randomized complete block design). A CO_2-pressurized backpack sprayer (KQ-25®, Weed Systems, Inc., Keystone Heights, FL, USA) was used to apply treatments. The sprayer had three Tee Jet 8003VS® flat fan nozzles (Spraying Systems, Dillsburg, PA, USA), one approximately 30 cm above the row, and two drop nozzles 60 cm apart aimed horizontally at the row; the spray fans were vertical. It was calibrated to deliver 280 L/ha.

The insects were allowed to complete one generation in the field. Based on observations made in the field, small larvae of the next generation were expected to be present by the end of May. Spray treatments were thus applied on 31 May, 4 June, 8 June, 11 June, and 18 June 2001. Plants were sampled on 11 June, 18 June and 26 June. On days on which both sampling and spraying were to be conducted (11 June and 18 June), sampling was done first. Samples consisted of three randomly selected whole plants from each plot, cut at the soil level, and placed in a single plastic bag. Samples were brought to the laboratory and held in a refrigerator (5°C) until they were dissected (within 2 d). Live diamondback moth larvae were counted, classified as small (first or second instars) or large (third or fourth instars), and placed individually in cells of plastic bioassay trays (Bio-BA-128©, CD International, Pitman, NJ, USA) with artificial diet (Shelton et al. 1991). Larvae were held at 27°C for 14 d to determine if they died of viral infection or were parasitized. Dead larvae that had characteristic symptoms of NPV infection (dark color and liquefied tissues) were classified as killed by PxMNPV.

Only 32 larvae of all sizes were found in all samples from the third sample date. Because this number was inadequate for analysis, only data from the first two sample dates were analyzed statistically and are presented here. Insects from the first two sample dates were assumed to represent a cohort consisting of the offspring of those insects that were on the plants when they were transplanted. The number of larvae among those held on artificial diet that died of PxMNPV was subtracted from the total number of larvae in each sample to obtain numbers of uninfected larvae. This variable was then subjected to analysis of variance (ANOVA) with sample date, block, and both linear and quadratic effects of rate of PxMNPV (Sokal and Rohlf 1981). The PROC GLM procedure (SAS Institute 1999) was used. Because the quadratic effect of rate was not significant (see Results and Discussion), data were reanalyzed without this term. The treatment with spinosad was not included in the analyses of rates of PxMNPV; data from this treatment are presented for reference.

Because relatively few large larvae were collected on some treatments (see Results and Discussion), data on the fates of large larvae collected were pooled across blocks for analysis. Small larvae were excluded because few of these larvae were

found on the second sample date (total of 11 small larvae). The percentage of larvae that died of viral infection and the percentage parasitized were calculated, normalized by arcsine √% transformation, and analyzed by ANOVA with sample date and rate of PxMNPV as independent variables.

Enhancer and UV protectant. These tests evaluated a fluorescent brightener and a UV protectant to improve levels of control by PxMNPV. Selection of these materials was based on prior results: Blankophor P167® (Bayer, Pittsburgh, PA, USA) as the fluorescent brightener (Farrar et al. 2004) and photostabilized TiO_2 (Cardre 72248®, Cardre Inc., South Plainfield, NJ, USA) as the UV protectant (Farrar et al. 2003).

Observations in 2001 indicated a decline in numbers of diamondback moths in the field, possibly due to parasitism (see Results and Discussion) and predation. Therefore, applications of the fluorescent brightener and TiO_2 were made against insects hatching from eggs laid on the transplants while they were in the growth chamber, rather than the offspring of these insects as in 2001.

Collard transplants (cv. 'Top Bunch') were infested as before, except that approximately 3,000 moths were released in the growth chamber. Infested plants were planted in the field on 21 May 2002. Plots were set up as in 2001, except with seven plots per block. Spray treatments were applied on 23 May, 26 May, 30 May and 3 June 2002.

Treatments were: control (water); PxMNPV at 7.81×10^{11} OB/ha; PxMNPV at 7.81×10^{11} OB/ha + 1% (wt/vol) Blankophor P167; PxMNPV at 7.81×10^{11} OB/ha + 0.5% (wt/vol) TiO_2; PxMNPV at 7.81×10^{11} OB/ha + both Blankophor P167 (1%) and TiO_2 (0.5%); PxMNPV at 2.47×10^{12} OB/ha; and spinosad at 52.7 g AI/ha. Joint Venture was included in all treatments at 0.125% (v/v). Cardre 72248 photostabilized TiO_2 will not mix directly with water. To disperse this material in water, a concentrate of 5% (wt/vol) TiO_2 was prepared by adding 5g TiO_2 and 1ml Triton X-155® (wetting agent; Union Carbide, Danbury, CT, USA) to 94 ml distilled water and mixing in a small blender. The concentrate was then diluted 10 fold to make the final spray material. Treatments were applied as in 2001, except that a lower volume (187 L/ha) was used because the plants were smaller.

Samples were collected on 3 June, 5 June, 10 June, and 12 June 2002. Plants were sampled as described before, except that six randomly selected plants from each plot were included in each sample because the plants were smaller. Large larvae and cocoons were assumed to represent the cohort of insects hatching from eggs laid on the plants while they were in the growth chamber. Only 54 small larvae were found in all samples from all dates. Because they were collected 13 - 22 d after the infested plants were transplanted, we assumed that these small larvae represented a low level feral population; they were not included in the statistical analyses.

In each sample the number of large larvae that died of viral infection was subtracted from the total number of large larvae. The number of uninfected large larvae was then added to the number of cocoons in each sample to obtain a number representing the total cohort of uninfected insects. (We assumed that insects that survived long enough to spin cocoons were not infected with PxMNPV.) The uninfected cohort was subjected to ANOVA with sample date, block, and spray treatment as independent variables. Treatment means were separated by Scheffe's test with a significance level of $P = 0.05$ (Sokal and Rohlf 1981). Data from only those treatments with PxMNPV at 7.81×10^{11} OB/ha were also analyzed factorially for the effects of Blankophor P167, TiO_2, and their interactions. Because relatively few larvae were

collected, especially from the more effective spray treatments (see Results and Discussion), data on the number of larvae that died of virus or were parasitized were pooled across all blocks and sample dates for analysis. The treatment with spinosad was omitted from this analysis because no live larvae were found on it, though three cocoons were found. Because the pooled data contained only one observation per treatment, these data were analyzed by the *G* test for heterogeneity (Sokal and Rohlf 1981).

The test of PxMNPV with Blankophor P167 and TiO_2 was repeated in 2003. Infested transplants (cv. 'Georgia', Davon Crest Farms, Hurlock, MD, USA) were planted on 8 Sept. 2003. Spray treatments were applied on 9 Sept., 12 Sept., 15 Sept. and 17 Sept. Samples were collected on 17 Sept., 22 Sept., 24 Sept., and 29 Sept. 2003, but only data from the first two dates were analyzed statistically because few insects were found on the last two dates (totals of 30 and 15 insects of all stages in all samples, respectively.) Small larvae were again excluded because few were found (totals of 15 and 8 small larvae on the first two sample dates, respectively). This test was otherwise similar to the 2002 test.

Results and Discussion

Rate response test. Numbers of uninfected larvae declined with increasing rates of PxMNPV (Fig. 1A). Quadratic effects of concentration of virus on numbers of larvae were not significant ($F = 0.18$; df = 1, 33). When the data were analyzed without the quadratic term, numbers of insects declined significantly with increasing rates of virus ($F = 7.37$; df = 1, 34), and fewer were found on the later sample date ($F = 14.64$; df = 1, 34). The percentage of collected large larvae that died of viral infection (Fig. 1B) increased with increasing rates of PxMNPV ($F = 6.78$; df = 1, 7) but was not affected by sample date ($F = 1.59$; df = 1, 7). The percentage of these larvae that were parasitized by *Diadegma insulare* (Cresson) (Fig. 1B) was not affected by either variable ($F = 0.16$; df = 1, 7 for rate; $F = 0.60$; df = 1, 7 for date).

Enhancer and UV protectant. In 2002, overall treatment effects were significant ($F = 15.09$; df = 6, 100) (Fig. 2A). Numbers declined over time ($F = 6.58$; df = 1, 100; data shown in Fig. 2A are pooled across dates). Scheffe's test indicated reduced numbers of insects on all virus treatments compared with the control. Factorial analysis of only those treatments with the lower rate of PxMNPV showed that numbers of insects were significantly reduced by Blankophor P167 ($F = 18.23$; df = 1, 55) and by TiO_2 ($F = 8.70$; df = 1, 55) and were affected by the interaction thereof ($F = 6.89$; df = 1, 55). Of large larvae that were collected, treatment did not affect the percentage dying from viral infection (Fig. 2B) ($G = 9.70$, df = 5) or the percentage that were parasitized ($G = 4.18$, df = 5).

In 2003, all virus treatments again reduced numbers significantly compared with the control (Fig. 3A) (treatment effect: $F = 7.33$; df = 6, 45) and numbers declined over time ($F = 12.26$; df = 1, 45; data shown in Fig. 3A are pooled across dates). Factorial analysis of those treatments with the low rate of PxMNPV showed that numbers were reduced by Blankophor P167 ($F = 5.45$; df = 1, 24) but were not affected by TiO_2 ($F = 0.01$; df = 1, 24) or by the interaction thereof ($F = 0.01$; df = 1, 24). The percentage of large larvae that were killed by PxMNPV was increased by virus treatment ($G = 49.42$, df = 5), whereas the percentage that was parasitized was reduced ($G = 12.78$, df = 5) (Fig. 3B).

PxMNPV reduced populations of the diamondback moth in all tests. In the test of

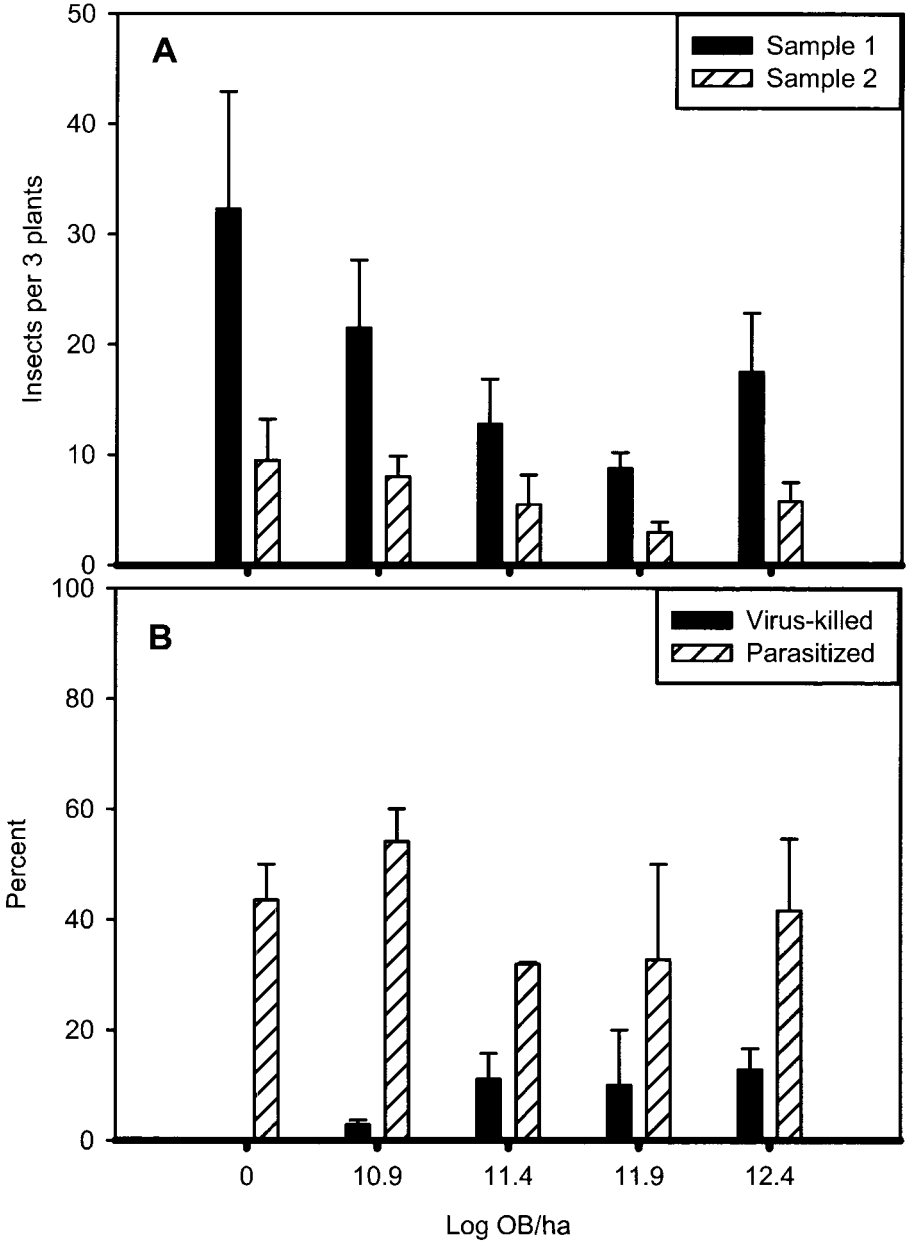

Fig. 1. (A) Numbers of uninfected diamondback moth larvae on artificially infested collard plants following treatment with PxMNPV. (B) Rates of parasitism by *D. insulare* and virus-induced mortality in insects collected from artificially infested plants, Beltsville, MD, June 2001. Results of statistical analyses are presented in the text.

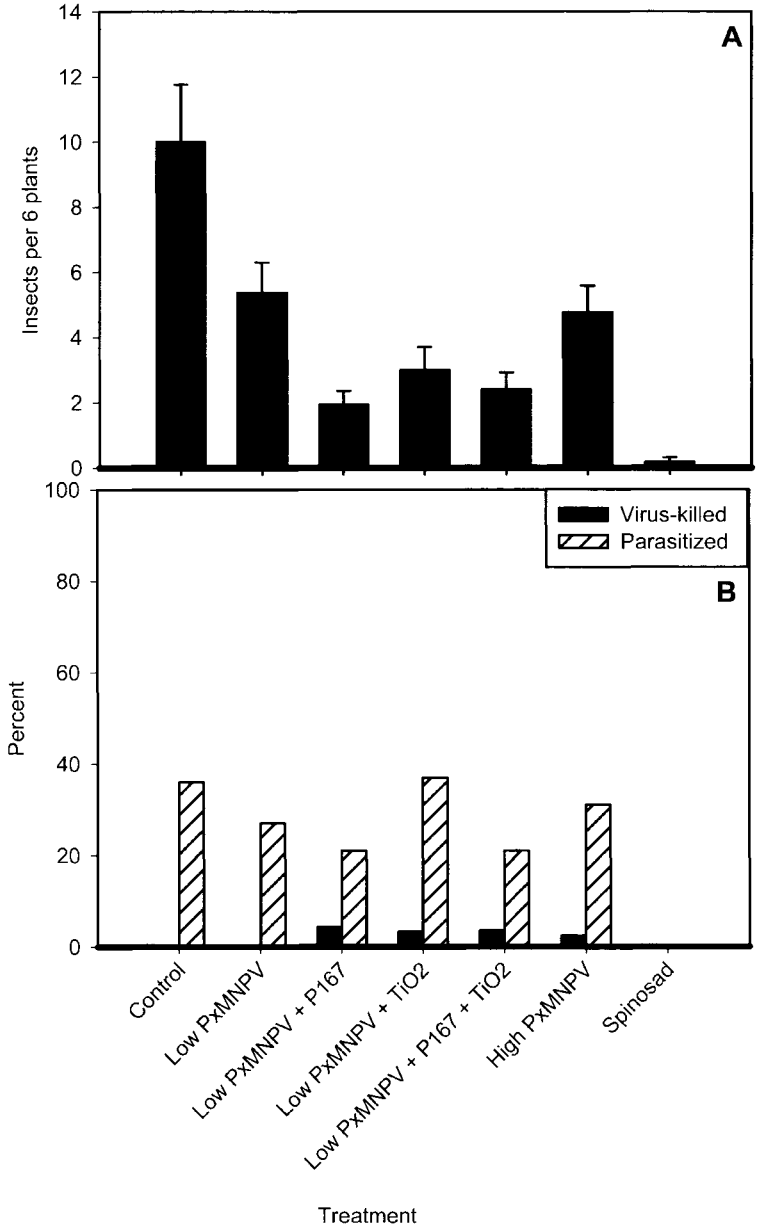

Fig. 2. (A) Numbers of uninfected large diamondback moth larvae and cocoons on artificially infested collard plants treated with PxMNPV with and without Blankophor P167 (P167) or titanium dioxide (TiO_2); data pooled across sample dates. (B) Rates of parasitism by *D. insulare* and virus-induced mortality in insects collected from artificially infested plants, Beltsville, MD, June 2002. Results of statistical analyses are presented in the text.

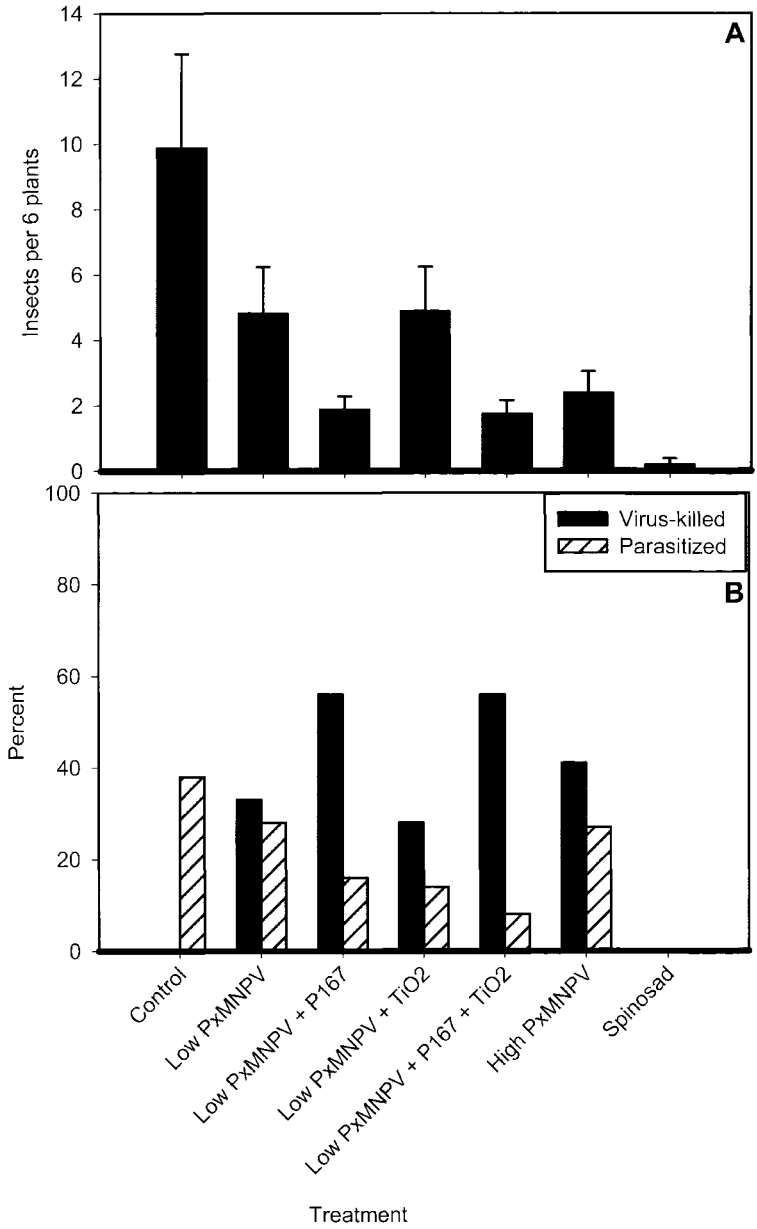

Fig. 3. (A) Numbers of uninfected large diamondback moth larvae and cocoons on artificially infested collard plants treated with PxMNPV with and without Blankophor P167 (P167) or titanium dioxide (TiO_2); data pooled across sample dates. (B) Rates of parasitism by *D. insulare* and virus-induced mortality in insects collected from artificially infested plants, Beltsville, MD, September 2003. Results of statistical analyses are presented in the text.

varying rates of the virus (Fig. 1A), the greatest reduction (up to 78%) was obtained with a rate of 7.81×10^{11} OB/ha. In 2001, more insects were found on the treatment with the higher rate of PxMNPV, 2.47×10^{12} OB/ha. This difference may reflect only experimental error, however, because the quadratic (nonlinear) component of the analysis of the effect of rate of PxMNPV on numbers of insects was not significant, and similar results were not obtained in 2002 or 2003.

The addition of the fluorescent brightener, Blankophor P167, to PxMNPV reduced numbers of insects compared with similar treatments with the virus only (Figs. 2A, 3A). This result is consistent with several previous studies that have shown an improvement in the control by NPVs of lepidopterous pests in the field through the addition of fluorescent brighteners (see reviews by Hamm 1999, Monobrullah 2003).

The addition of TiO_2 to PxMNPV significantly reduced numbers of insects in 2002 (Fig. 2A) but had no effect in 2003 (Fig. 3A). In 2002, the reduction in numbers of insects attributable to TiO_2 was comparable to that caused by Blankophor P167. However, combining both materials caused no further improvement in control. We found a significant statistical interaction between TiO_2 and Blankophor P167 in 2002, probably as a result of the combined effect being less than would be expected if the effects of the two materials together were additive. Results of the 2002 test were consistent with those of Farrar et al. (2003) who obtained increased persistence of an NPV with TiO_2.

In 2003, TiO_2 had no effect and did not interact with Blankophor P167. The difference in results in 2002 versus 2003 may be because the two tests were done at different times of the year, when levels of solar radiation are different. In 2002, the test was done in late May and June (mean daily solar irradiance during the test of 257.2 W/m^2). The test of 2003 was done in September (mean daily solar irradiance of 140.3 W/m^2). We would expect that a UV protectant such as TiO_2 would have a greater effect when solar irradiance is greater.

Many of the larvae that were collected were parasitized by *D. insulare* in all tests. In some samples, parasitism was > 50%. Parasitism was not related to virus treatment except in 2003 (Fig. 3B) when fewer parasitoids were reared from insects from those treatments on which higher percentages of larvae died of virus. Observations made in the field after the tests in 2001 and 2002 indicated low populations of diamondback moth larvae. The activity of *D. insulare* may have been a factor in preventing buildup of diamondback moth populations.

Virus treatments provided control of diamondback moth larvae, but in no case was this control comparable to that provided by the insecticide spinosad. However, some field populations of the diamondback moth have developed resistance to spinosad, as they have to many other insecticides (Zhao et al. 2002, Sayyed et al. 2004). PxMNPV could thus have a role where resistance has occurred or as a part of a strategy to delay development of resistance to insecticides such as spinosad.

We conclude that PxMNPV may have potential as a microbial control agent for the diamondback moth. Previous research (Kariuki and McIntosh 1999, Farrar et al. 2004) showed this virus to be more potent than other NPVs known to infect the diamondback moth. Our study shows that PxMNPV can reduce populations of the diamondback moth in the field, and that further reductions can be obtained by the addition of a fluorescent brightener, and (at least under certain circumstances) by UV protectants. More research, however, is needed to determine such factors as rates, application technology, treatment frequency and adjuvants if PxMNPV is to be developed into a practical pest management tool.

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