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# Application technology of SfMNPV-6nd in the control of fall armyworm

**Abstract** – The objective of this work was to evaluate the application technology and the compatibility of the SfMNPV-6nd baculovirus with surfactants and markers in the mortality of *Spodoptera frugiperda* second-instar larvae. The compatibility studies were carried out with the brilliant blue (BB) dye and copper oxychloride (CO) markers and with the polyalkylene oxide heptamethyltrisiloxane (PH) and ethoxylated alkylphenol (EA) surfactants mixed with the SfMNPV-6nd biological insecticide. Droplet dispersion was assessed by spraying a SfMNPV-6nd solution on corn plants using flat fan and hollow cone nozzles. The evaluated parameters were: spray deposits, volumetric median diameter (VMD), droplet size, pH of spray solution, and mortality of second-instar *S. frugiperda* larvae. The PH and EA surfactants present synergism with SfMNPV-6nd and increase *S. frugiperda* control. The addition of the EA and CO surfactants to the SfMNPV-6nd solution reduces pH values. Droplet density, VMD, spray deposition of the SfMNPV-6nd solution, and mortality of *S. frugiperda* second-instar larvae do not differ among treatments regardless of the type of nozzle used.

**Index terms**: *Spodoptera frugiperda*, biological control, entomopathogenic virus, spray deposit.

## Tecnologia de aplicação de SfMNPV-6nd no controle da lagarta-do-cartucho

**Resumo** – O objetivo deste trabalho foi avaliar a tecnologia de aplicação e a compatibilidade do baculovírus SfMNPV-6nd com surfatantes e marcadores na mortalidade de larvas de segundo instar de *Spodoptera frugiperda*. Os estudos de compatibilidade foram realizados com os marcadores corante azul brilhante (AB) e oxicloreto de cobre (OC) e com os surfatantes heptametiltrisiloxano óxido de polialquileno (HP) e alquilfenol etoxilado (AE) misturados ao inseticida biológico SfMNPV-6nd. Avaliou-se a dispersão de gotas por meio da pulverização de solução de SfMNPV-6nd em plantas de milho, com uso de pontas de jato plano e cônico vazio. Os parâmetros avaliados foram: depósito da pulverização, diâmetro mediano volumétrico (DMV), tamanho de gotas, pH da solução e mortalidade de larvas de segundo instar de *S. frugiperda*. Os surfactantes HP e AE apresentam sinergismo com SfMNPV-6nd e aumentam o controle de *S. frugiperda*. A adição dos surfactantes AE e OC na solução de SfMNPV-6nd reduz os valores de pH. Densidade de gotas, DMV, depósitos da pulverização da solução de SfMNPV-6nd e mortalidade de larvas de segundo instar não diferem entre os tratamentos, independentemente do tipo de ponta utilizado.

**Termos para indexação**: *Spodoptera frugiperda*, controle biológico, vírus entomopatogênico, depósito da pulverização.

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## Introduction

*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), commonly known as fall armyworm, is a destructive agricultural pest worldwide (Rwomushana, 2019) and the most serious corn pest throughout Latin America (Nagoshi et al., 2012). Although the polyphagous *S. frugiperda* pest may feed on 353 different plant species, including rice, wheat, and soybean, it especially prefers corn (Montezano et al., 2018).

For the control of insect pests, an alternative to the use of chemicals are baculoviruses, an invertebrate pathogen (Possee et al., 1997). Among the viruses used to control insect pests, baculoviruses are the most studied and widespread group in agriculture, being important pathogens of a wide range of insect pests (Williams, 2018; García-Banderas et al., 2020). Since baculoviruses must be ingested by the target pest to be effective, the optimization of the technology for their application is fundamental for a successful control without economic impacts (Possee et al., 1997).

Baculoviruses act as a selective insecticide, which is beneficial since it does not contaminate other microorganisms, arthropods, and humans (Herniou et al., 2012). Moreover, that group of viruses can also be sprayed using the same equipment for the application of chemical insecticides, which points out the importance of its compatibility with other pesticides and of the pH and distribution over the plant of the mixed spray solution.

Several populations of *S. frugiperda* that are resistant to synthetic insecticides (Almeida et al., 2017; Gutiérrez-Moreno et al., 2019; Boaventura et al., 2020), as well as to Bt corn, are used to control caterpillars (Bernardi et al., 2016; Boaventura et al., 2020). However, further researches are necessary to find other efficient ways for controlling that pest, in order to obtain acceptable and economical levels of agricultural yield in a sustainable way.

The SfMNPV-6nd baculovirus is a variety that is highly efficient in causing the mortality of *S. frugiperda* (Diniz et al., 2018; García-Banderas et al., 2020). A precise technology for its application to pathogens, specially to entomopathogens, may contribute to a better control of the insect pest, allowing the deposition of the activated baculovirus in a sufficient amount on the desired target. In this scenario, the synergism and compatibility among products are important factors in the enhancement of chemical pesticides.

The objective of this work was to evaluate the application technology and the compatibility of the SfMNPV-6nd baculovirus with surfactants and markers in the mortality of *S. frugiperda* second-instar larvae.

## Materials and Methods

The experiments were carried out from February to August 2018 at the laboratory for integrated pest management in agriculture and at the laboratory for pesticide application technology, both located at the Plant Protection Department of Universidade Estadual Paulista Júlio de Mesquita Filho (Unesp), in the municipality of Botucatu, in the state of São Paulo, Brazil.

The used moths of *S. frugiperda* were obtained from the laboratory for integrated pest management in agriculture, being previously collected from corn fields in a farm from Unesp. To obtain eggs, the moths were kept in cylindrical polyvinyl chloride (PVC) cages with 21.5 cm in height × 10 cm in diameter. The cages were covered with voile fabric and were internally coated with kraft paper for oviposition. The moths were fed with an aqueous solution of commercial honey (10%) provided through moistened cotton, which was replaced every 48 hours. The eggs were removed daily, placed in a plastic cup of 300 mL, and kept in a room with controlled conditions – temperature of 25±1°C, relative humidity of 70±10%, and a 2 hours dark:12 hours light photoperiod.

The newly hatched caterpillars of *S. frugiperda* were separated from each other, transferred to plastic capsules with 5.0 g of an artificial diet, and kept in the room with controlled conditions until pupa formation. The pupae were kept in PVC cages to the onset and laying of the eggs. The diet was based on beans, wheat germ, and yeast, adapted from Kasten Jr. et al. (1978).

The corn plants used in the experiment were obtained from seeds from the non-Bt 2B587Hx genetically modified cultivar, which shows resistance to glyphosate. Three seed were sown at a depth of 3.0 cm in 3.8 L pots containing a substrate made up of a mixture of soil, cattle manure, and sand at a ratio of 1:1:1. After sowing, the pots were placed in a greenhouse, with temperature of 24±1°C but without control of relative humidity and light, and were irrigated daily as needed by micro-sprinklers. Sowing fertilization consisted of 2.0 g per pot of $N$-$P_2O_5$-$K_2O$,

meeting the nutrient requirements of the corn plants. After plant emergence, only one was kept per pot.

The following two markers were used in the solutions that were sprayed on the plants: brilliant blue (BB) dye (Duas Rodas Industrial Ltda, Jaraguá do Sul, SC, Brazil) at a concentration of 1.500 ppm (Palladini et al., 2005; Cerqueira et al., 2017) and copper oxychloride (CO) (Cobox DF, Basf S.A., Maipú, Santiago, Chile) at a concentration of 2.500 ppm (Christovam et al., 2010; Cerqueira et al., 2017). Two surfactants, both at 0.5% v/v, were also used: Silwet L-77 (Momentive, Londrina, PR, Brazil), with polyalkaleneoxide heptamethyltrisiloxane (PH) as active ingredient; and Agral (Syngenta, Paulínia, SP, Brazil), with ethoxylated alkylphenol (EA) as active ingredient. The biological insecticide used at 3.500 ppm was CartuchoVIT, whose technical name is *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) (Vitae Rural Biotechnologia, Uberaba, MG, Brazil).

The compatibility of the markers, surfactants, and of the mixture of markers, surfactant, and SfMNPV-6nd was evaluated separately by verifying the effect of each on the mortality of *S. frugiperda* second-instar larvae. First, the compatibility of markers was assessed, followed by that of surfactants, both mixed with the SfMNPV-6nd insecticide. Then, the surfactant with the best compatibility was selected and evaluated in the mixture with markers.

The following four treatments were used to assess the compatibility of the markers with the SfMNPV-6nd insecticide: T1, control (distilled water); T2, SfMNPV + distilled water; T3, BB marker + SfMNPV + distilled water; and T4, CO marker + SfMNPV + distilled water. Both used markers do not change the surface tension of the solution, are stable, are not photosensitive, and are recommended to evaluate spray deposition on natural and artificial targets (Cerqueira et al., 2017).

Four treatments were also used to assess the compatibility of the surfactants with SfMNPV-6nd: T1, control (distilled water); T2, SfMNPV + distilled water; T3, PH surfactant + SfMNPV + distilled water; and T4, EA surfactant + SfMNPV + distilled water. The chosen surfactants did not have insecticidal effects.

Corn leaves with 2.0 cm of length at the V3 stage were cut and washed in sodium hypochlorite and then in distilled water. The leaves were immersed in the spray solution of each treatment, dried (Evans & Shapiro, 1997), stored with white cotton soaked in distilled water, and placed in a flat-bottom glass tube (2.0×8.0 cm). Seven days after inoculation, a second-instar larva of *S. frugiperda* (< 1.5 cm) was placed on the corn leaves and all tubes were closed with voile. Each treatment consisted of 40 replicates (a glass tube with one leaf and one caterpillar). Two days after inoculation, the caterpillars were removed from the tubes and placed in plastic capsules with 5.0 g artificial diet (Vieira et al., 2012). The tubes were kept in a room with controlled conditions – temperature of 25±1°C, relative humidity of 70±0%, and a photoperiod of 12 hours light:12 hours dark.

The dead caterpillars in each test were counted. The treatments with higher levels of mortality were chosen for the study of the compatibility between the marker and the surfactant. The mortality data of *S. frugiperda* were subjected to exploratory analyses to evaluate the assumptions of normality of the residues and the additivity of the model for the application of the analysis of variance (Anova) or the Kruskal-Wallis H-test, and means were compared by Tukey's or Dunn's tests, at 5% probability, using the R software (R Core Team, 2020).

The influence of the surfactants on the pH of the solutions was also verified. For this, a study was performed in a factorial arrangement with five spray solutions (the same ones used to evaluate the compatibility with SfMNPV) × four times of assessment (0, 2, 4, and 6 hours after solution preparation) using the DM-20 pH-meter (Digimed, São Paulo, SP, Brazil). The pH values were subjected to the exploratory analysis of assumptions of normality of residuals and of the additivity of the model for ANOVA application, and means were compared by Tukey's test, at 5% probability, using the R software (R Core Team, 2020).

The influence of the nozzle in the application of the solution of SfMNPV-6nd + PH + EA on spray quality and *S. frugiperda* control was also studied. For this, an experiment with three treatments and 25 replicates was carried out. The treatments were: T1, control; T2, spraying with the AXI 11002 flat fan nozzle (Jacto, Pompéia, SP, Brazil); and T3, spraying with the JA-2 hollow cone nozzle (Jacto, Pompéia, SP, Brazil). Both spray nozzles produce fine-sized droplets according to the manufacturer.

To detect spray deposition on the corn plant at the V3 stage, one paper filter (2.0×2.0 cm) per plant (25 paper filters per treatment) was fixed on the adaxial surface of

the middle-third of the leaves. To assess spray quality (droplet density) and volumetric median diameter (VMD, μm), two water-sensitive cards (76×26 mm) were inserted in a metallic support placed at plant high.

Spraying was performed using an indoor boom sprayer moving on a track with speed and pressure control. Spray volume delivery was 110 L $ha^{-1}$ with a pressure of 207 kPa for AXI 11002 and of 414 kPa for JA-2, travelling at 7.0 km $h^{-1}$. The spray boom with six nozzles was positioned at 0.50 m above the corn plants. Two sprayings were performed at intervals of seven days. The replicates consisted of one corn plant per plot and were conducted under the same conditions described previously. The corn plants were sprayed at the V3 stage (Ritchie et al., 1993).

After the first spraying, one second-instar larva of *S. frugiperda* was placed on the corn whorl, and the plant was kept in PVC cages (10 cm in diameter × 21.5 cm in height) covered with voile. The cages were kept in the same greenhouse conditions aforementioned. The second spraying was performed with the larva of *S. frugiperda* already inside the corn whorl, and the presence of live or dead caterpillars was observed.

Spray deposits were assessed during the first and second sprayings following the methodology of Christovam et al. (2010). For each spraying, the water-sensitive cards were removed, scanned in a 600-dpi resolution scanner, and then analyzed in the Gotas software (Chaim et al., 2002). Fall armyworm mortality was evaluated out on the sixth day after the first and second sprayings.

The data were subjected to exploratory analyses in order to evaluate the assumptions of normality of the residuals and of model additivity to allow the use of the ANOVA or Kruskal-Wallis H-test. The mortality of the caterpillars was analyzed by Tukey's and Dunn's tests, at 5% probability. Spray deposits, VMD, and droplet size were compared by Tukey's test, at 5% probability. All analyses were performed using the R software (R Core Team, 2020).

## Results and Discussion

The assessment of the compatibility of the BB and CO markers with SfMNPV-6nd showed a difference in the mortality levels of *S. frugiperda* larvae (Figure 1 A). The lowest and highest mortalities were of 17.5 and 62.5% for the control and the treatment with SfMNPV-6nd alone, respectively. The treatment with CO and SfMNPV-6nd presented caterpillar mortality of 42.5%, whereas that with SfMNVP-6nd and BB caused an average mortality of 35%. The evaluation of the compatibility of the PH and EA surfactants with SfMNPV-6nd also showed significant differences among treatments (Figure 1 B). The average values of mortality were 85, 80, 55, and 25% for EA + SfMNPV-6nd, PH + SfMNPV-6nd, SfMVPV-6nd alone, and control, respectively.

Based on the highest mortality results, the marker and surfactant chosen for analysis were CO and EA, respectively. Fall armyworm mortality differed in the treatments with SfMNPV-6nd, EA, and CO (Figure 1 C). The lowest level of mortality was 15% for the control, the second lowest was 64.4% for SfMNPV-6nd alone, and the highest was 87.5% both for SfMNPV-6nd with EA and the mixture of SfMNPV-6nd, EA, and CO. The mixture of SfMNPV-6nd with the BB and CO markers did not show differences in *S. frugiperda* mortality, with similar results to those of SfMNVP-6nd alone.

In the literature, CO has been chosen as a marker for compatibility assessments due to the higher average number of dead caterpillars when compared with BB. Durigan (1993) evidenced that using the PH and EA surfactants together with SfMNVP-6nd in the spray solution improved droplet spreading and retention on the target. This is important for *S. frugiperda* mortality since the main way for SfMNPV-6nd to infect *S. frugiperda* is through ingestion; therefore, a satisfactory coverage of the leaves may increase the amount of virus consumed by the insect. In the present study, the surfactant associated with the virus provided a higher coverage of the target, leading to higher mortality rates than the control and the virus applied alone, with no surfactant in the spraying mixture.

In the last compatibility assessment, the mixture with CO, which was used to evaluate spraying coverage, did not affect the action between SfMNPV-6nd and the EA surfactant because both presented the same levels of larvae mortality. As CO is a copper inorganic fungicide with contact action and a low toxicological classification, it did not show incompatibility with the baculovirus and with the surfactant, which is indicative of its possible use in mixtures with this insecticide for the control of pests and diseases (Agrofit, 2020).

In the pH assessment, treatments ($F=42.86$; $p=0.001$), time intervals ($F=16.13$; $p=0.001$), and the interaction between treatments and time intervals ($F=90.2$;

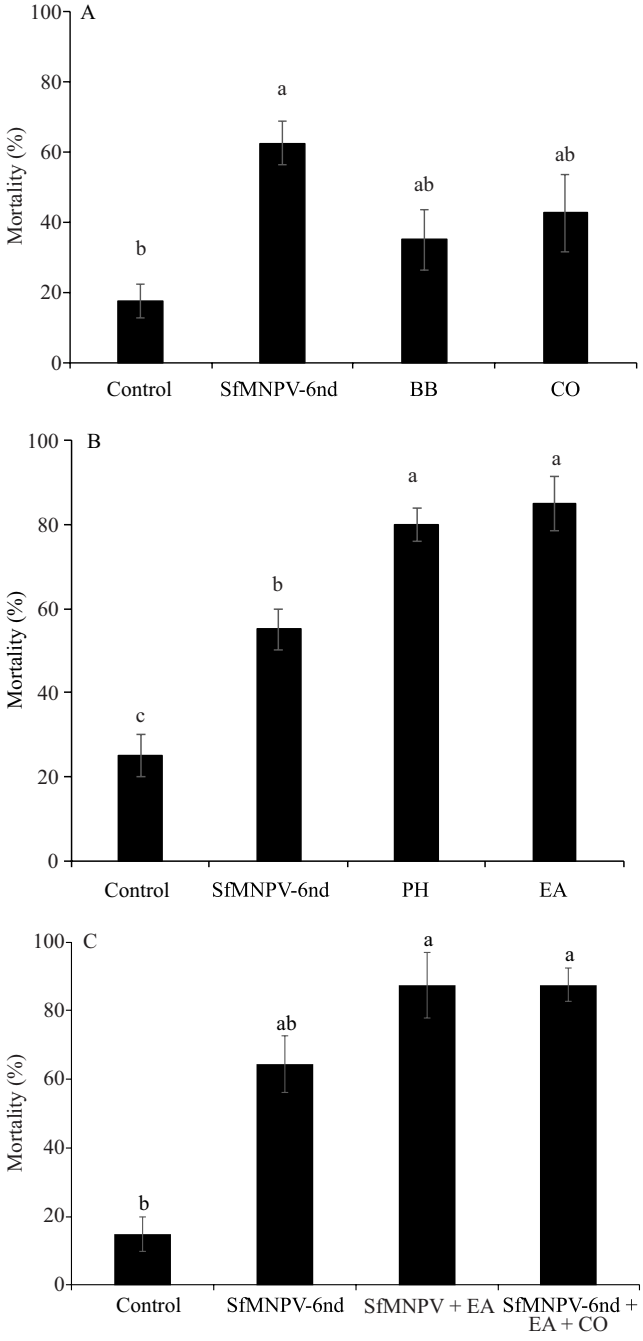


**Figure 1.** Average mortality of *Spodoptera frugiperda* caterpillars when sprayed with the solutions used to assess the compatibility of the SfMNPV-6nd baculovirus with: the brilliant blue (BB) dye and copper oxychloride (CO) markers (A), the polyalkylene oxide heptamethyltrisiloxane (PH) and ethoxylated alkylphenol (EA) surfactants (B), and marker and surfactant (C). Control, water. Equal letters do not differ statistically among treatments. Tukey test's, at 5% probability, was used to evaluate the compatibility of markers and surfactants, and Dunn test's, at 5% probability, that of the CO + EA + SfMNPV-6nd mixture.

p=0.001) differed (Figure 2). Regarding treatments, the stock with only SfMNPV-6nd in distilled water presented the highest pH of 7.11, followed by that of the control, SfMNPV + EA, and SfMNPV + EA + CO, with a pH of 5.5, 4.61, and 4.39, respectively (Figure 2).

Regarding time intervals, 0 hour was the one with the highest pH of 5.71, followed by 2, 4, and 6 hours, with a pH of 5.47, 5.21, and 5.21, respectively, values that did not differ significantly from each other (Figure 2).

SfMNPV-6nd is a virus enveloped by a simple protein called polyhedrin. This protein is activated only in alkaline medium (pH ≥ 8), which occasionally occurs in the midguts of caterpillars (Tanada, 1993). In this medium, the polyhedron is quickly dissolved and the viral particles, responsible for infecting the insect, are released, causing the infection of caterpillar cells (Volkman & Keddie, 1990). Therefore, the pH of the spray solution mixture is an important factor related to the efficiency of any baculovirus, including SfMNPV-6nd, in the control of pests in the field.

In the present study, a pH close to neutrality (pH=7.0) was observed in the SfMNPV-6nd solution, which can be attributed to the formulation of the insecticide (wettable powder), obtained from an alkaline rock. The proximity with the basic pH is important since it is responsible for insecticide activation. However, the most basic water can also provide a more alkaline pH in the solutions

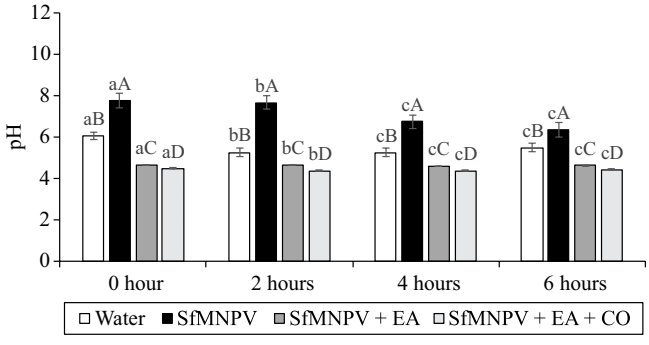


**Figure 2.** Hidrogenionic potential (pH) of the mixtures of the SfMNPV-6nd baculovirus with surfactant and marker at different time intervals (0, 2, 4, and 6 hours after the preparation of the solutions). EA, ethoxylated alkylphenol; and CO, copper oxychloride. Uppercase letters represent the group of treatments, and lowercase letters represent the groups among time intervals. Equal letters do not differ by Tukey's test, at 5% probability.

with SfMNPV-6nd, resulting in the dissolution of the polyhedrin and, consequently, in the inactivation of the viral capability of SfMNPV-6nd when exposed to environmental conditions. Therefore, it is important to take into account the pH of the spraying mixture in the application technology of this biological insecticide for a better pest control efficiency.

Treatments SfMNPV-6nd + EA and SfMNPV-6nd + EA + CO showed a pH close to 4.0 during the entire experimental period (Figure 2). Maintaining the pH of spraying mixtures throughout the application period is important, considering the buffer effect. This effect represents the capacity of a solution to keep its pH stable, i.e., not varying along time. In the present study, a reduction in the pH of the SfMNPV-6nd, SfMNPV-6nd + EA, and SfMNPV-6nd + EA + CO solutions was observed along time. However, since the problem in the use of SfMNPV-6nd is the alkalinity of water, a decrease in pH over time does not affect the dissolution of polyhedrin and the virulence of SfMNPV-6nd. In Brazil, there are extensive agricultural areas that require long periods of spraying when this is the chosen method for pest control. The used equipment have spray tanks to store the mixture and a capacity for use in large areas according to each type of application (stock volume, application rate, and spraying pressure and speed).

In the assessment of spraying deposition, there was no difference among treatments (Figure 3 C). In the first spraying (F=0.92; p=0.35), the average deposition values were 0.268 and 0.22 µL cm$^{-2}$ when using the

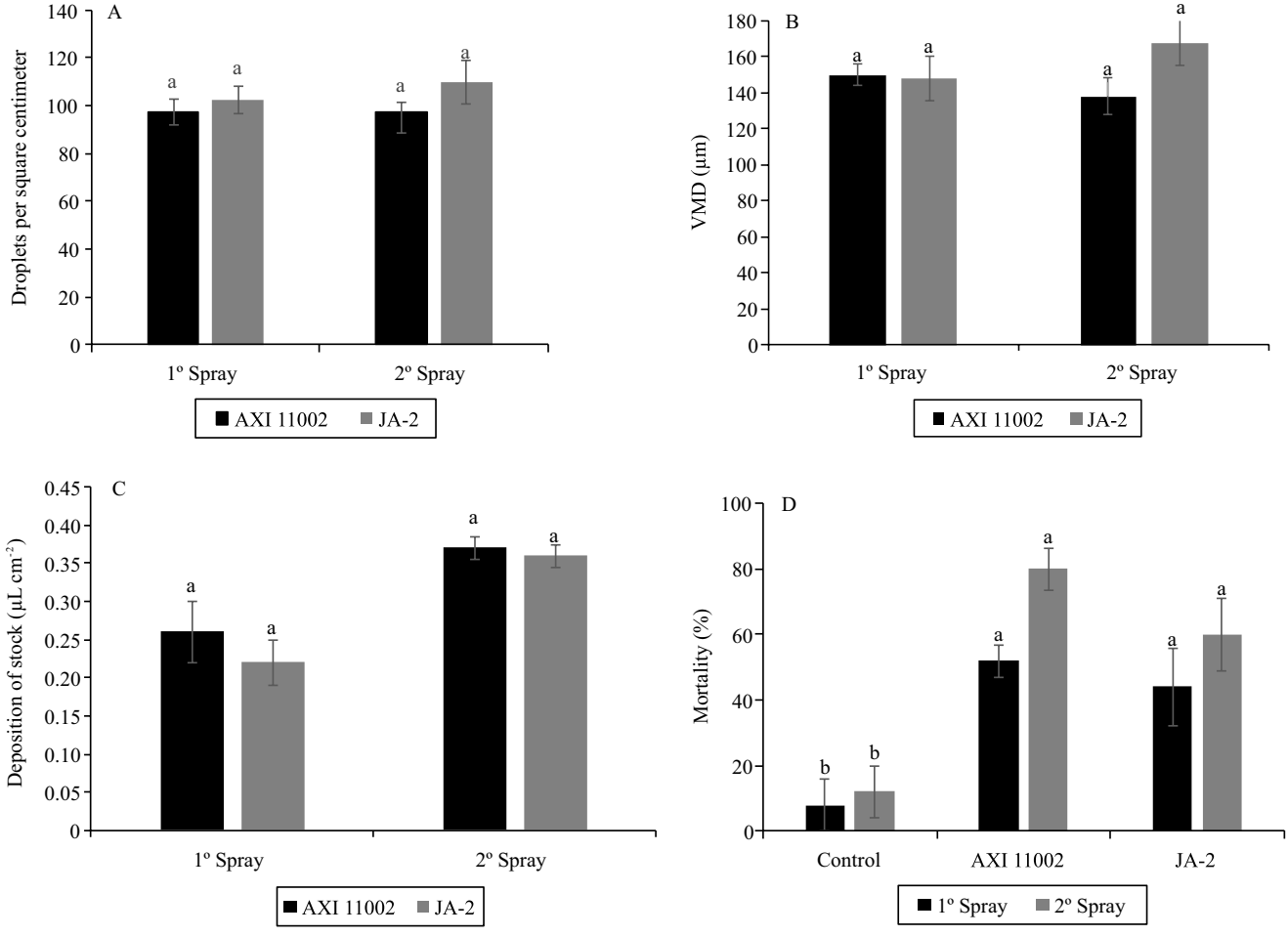


**Figure 3.** Parameters used for application technology of SfMNPV-6nd baculovirus in two sprayings using flat fan (AXI 11002) and hollow cone (JA-2) nozzles, showing density of droplets on water-sensitive paper (A), volumetric median diameter (VMD) on water-sensitive paper (B), and deposition of stock on filter paper (C), as well as mortality of *Spodoptera frugiperda* larvae in corn plants (D). Equal letters do not differ by Tukey's and Dunn's tests, at 5% probability.

flat fan and hollow cone nozzles, respectively. In the second spraying (F=0.04; p=0.82), the average values were 0.36 and 0.37 μL $cm^{-2}$ for the flat flan and hollow cone nozzles, respectively.

The number of droplets per square centimeter deposited on the water-sensitive cards did not differ in the first (F=0.42; p=0.52) or second (F=0.73; p=0.4) spraying, regardless of the used nozzle (Figure 3 A). The average values obtained with the hollow cone nozzle were 9.9 and 11.03 droplets per square centimeter in the first and second sprayings, respectively. The average values with the hollow cone nozzle were 10.2 and 10.01 droplets per square centimeter in the first and second sprayings, respectively.

The VMD also did not differ between the first (F=0.02; p=0.87) and second (F=3.32; p=0.08) sprayings both with the flat flan and hollow cone nozzles (Figure 3 B). The values obtained for VMD were: 149.6 and 138.0 μm in the first and second sprayings, respectively, with the flat fan nozzle; and 147.5 μm and 167.5 μm in the first and second sprayings, respectively, with the hollow cone nozzle.

The mortality of *S. frugiperda* differed among treatments in the first (F=7.35; p=0.008) and second (H=9.84; p=0.01) sprayings (Figure 3 D). In the first spraying, a mortality of 52 and 44% was observed when using the flat fan and hollow cone nozzles, which did not differ significantly from each other but differed from the control with 8% mortality. In the second spraying, the flat fan and hollow cone nozzles had a similar effect, causing caterpillar mortality of 80 and 60%, respectively, differing from that of the control, which was 12%.

The tools used in the spraying analyses, such as spray deposition and droplet density and size, did not differ among spray nozzles. Likewise, no differences were observed regarding fall armyworm mortality when using both spray nozzles for the two sprayings. This result may be attributed to the amount and form of the spraying stock that contributed to the deposition of the viral particles necessary for an effective mortality. The greater coverage and good distribution of the virus on the plant increases the chances of the insect ingesting the insecticide at feeding.

Since there may be a low droplet retention on plants due to the surface tension of pure water (72.6 m N $m^{-1}$), the use of surfactants becomes necessary specially when applying coarse droplets. When entomopathogenic viruses are applied, the rate of plant coverage must be high. In the literature, efficient virus applications validate that spray coverage is directly affected by the used application technology and other factors (Smith & Bouse, 1981).

The values obtained for VMD and droplets per square centimeter in the present study were similar to those of other researches on entomopathogenic viruses that presented a high efficiency in the control of pests, confirming the need of a high coverage of the applied virus (Bell & Kanavel, 1977; Entwistle et al., 1990). Although these authors studied other types of insect viruses, all observed that the use of fine droplets resulted in a greater number of droplets per area. A VMD between 90 and 150 μm causes high epizootics in insects (Bell & Kanavel, 1977; Entwistle et al., 1990), and these epizootics are responsible for the maintenance and spread of the virus in the crop area.

In the present study, both the flat fan and hollow cone spray nozzles caused second-instar *S. frugiperda* mortality over 60% and may be used for the control of this insect pest. Future studies in field conditions using the SfMNPV biological insecticide should be carried out in order to obtain more information on the control efficacy of this product.

## Conclusions

1. The polyalkylene oxide heptamethyltrisiloxane and ethoxylated alkylphenol surfactants present synergism with the SfMNPV-6nd baculovirus and increase the mortality of *Spodoptera frugiperda* second-instar larvae.

2. The addition of ethoxylated alkylphenol and copper oxychloride to the SfMNPV-6nd insecticide reduces the pH values of the spray solutions.

3. Droplet density, volumetric median diameter spray deposition of the SfMNPV-6nd solution, and *S. frugiperda* second-instar larvae mortality do not differ whether the flat fan or hollow cone nozzle is used.

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