**ORIGINAL PAPER**

# Water stress decreases the biocontrol efficacy of a nucleopolyhedrovirus against the fall armyworm on maize

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## Abstract

Drought events are expected to limit crop productivity in the context of current climate change. Drought is also likely to affect multitrophic interactions such as those involving plants, phytophagous insects and their natural enemies. We designed a two-phase experiment to test the effect of water availability and fertilizer treatment on the interaction between maize, the fall armyworm and its nucleopolyhedrovirus pathogen. Plants grown in soil with high irrigation and mineral fertilization had the highest shoot and root dry weight. Furthermore, plant nitrogen levels were higher in plants with fertilization and a low irrigation scheme compared to other treatments. Low irrigation of maize plants reduced virus-induced mortality of armyworm larvae. Insects did not feed on plants with high irrigation and without fertilization. We conclude that water stress and plant nutrition can affect virus performance and thereby affect the efficiency of biological control and pest management, especially as water and nutrient limitation will likely increase under climate change.

**Keywords** Baculovirus · Drought · Fertilization · *Spodoptera frugiperda* · *Zea mays*

## Introduction

Maize, *Zea mays* L. (Poaceae), is one of the most important staple crops in the world and is used for food, animal feed and numerous industrial products (Ranum et al. 2014). This plant is attacked by pests that decrease the yield and can affect the quality of the grain (de Lange et al. 2014). The fall

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armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is a major pest of maize and a number of other crops including rice, sorghum and others (Nagoshi et al. 2011). This insect is widely distributed across the Americas, particularly in subtropical and tropical regions (Lafontaine and Schmidt 2010), but has recently invaded and become a devastating pest in peripheral regions of the European Union such as the Canary Islands, Africa, Asia and Oceania (Goergen et al. 2016; Baloch et al. 2020; Nagaratna et al. 2021; Gilioli et al. 2022; Wang et al. 2023).

Infestations of *S. frugiperda* are usually controlled by the application of synthetic insecticides (Chimweta et al. 2020) or by using genetically modified plants that express insect toxins (Carzoli et al. 2018) or the use of new genetic modification methods such as the self-limiting insect and germline transformation techniques (Chen and Palli 2021; Reavey et al. 2022). Nevertheless, there is a growing incidence of resistance to common pesticides (Van den Berg and du Plessis 2022) and genetically modified maize (Huang 2021). Excessive use of pesticides can also have consequences for human and animal health, an adverse environmental impact and significantly increased crop production costs (Mahmood et al. 2016). Biological insecticides based on entomopathogens represent an effective and environmentally benign

alternative to conventional insecticides as they are highly selective and compatible with other insect natural enemies, and fit well into integrated pest management programs (Lacey et al. 2015; Lacey 2017).

Lepidopteran nucleopolyhedroviruses (*Baculoviridae*; genus *Alphabaculovirus*) are DNA viruses that are embedded in a protein matrix to form large occlusion bodies (OBs) that allow them to remain viable on plant surfaces or in the soil for extended periods (Harrison et al. 2018). Infection occurs when larvae consume OB-contaminated foliage and death follows several days later with the release of large numbers of OBs into the environment for the following cycle of transmission (Williams 2018). These viruses do not pose any risk for human or animal health, beneficial insects or the environment, and they are considered to be safe for use as biological insecticides (Hokkanen and Hajek 2003). Several strains of the Spodoptera frugiperda multiple nucleopolyhedrovirus (SfMNPV) are being developed as the basis for biological insecticides targeted at this pest (Haase et al. 2015; Barrera-Cubillos et al. 2017; García-Banderas et al. 2020; Hussain et al. 2021).

Plants can influence phytophagous insects by modulating the nutritional content in their tissues and bodily fluids, thereby affecting positively or negatively their longevity, reproductive performance and physiology (Kariyat and Portman 2016). It is important to understand how plants influence the immune response, survival, mobility and reproduction of insects in order to predict the spread of invasive pests (Neubert and Parker 2004). These traits can also affect the interactions of herbivores with their natural enemies (Gols 2014).

Water stress and nutrition have a critical role in the development of plants in both natural habitats and agricultural conditions (Morales et al. 2008). Plants require water and nutrients to perform biochemical reactions and physiological functions, including photosynthesis, solute transport and defense (Hopkins and Hüner 2004; Galmés et al. 2007; Da Ge et al. 2010). Nevertheless, in the context of current global climate change, it is expected that heat waves and drought events will increase and have a negative impact on plants (Ploughe et al. 2019), which may affect the interactions between herbivorous insects and their natural enemies (Thomson et al. 2010; Anderegg et al. 2015; Díaz-Álvarez et al. 2020; Noman et al. 2020). Therefore, future pest control strategies will have to consider the aspects of crop development that interact with the development of insect pests and their natural enemies, such as their susceptibility to biological insecticides based on viruses.

There are very few studies on the efficiency of entomopathogens in phytophagous insects feeding on plants under extreme conditions such as water stress (drought) and nutrient limitation (Devi et al. 2005; Borisade and Magan 2015; Dara et al. 2017; Real Santillan et al. 2019). The objective of this study was to evaluate the effect of water stress and soil nutrients on the interaction between the fall armyworm on maize and its nucleopolyhedrovirus. Our hypothesis is that the limitation of water and nutrients will affect the performance of this baculoviruses toward its host, the fall armyworm.

## Materials and methods

### Biological materials

The maize hybrid line NB9 (Novasem Innovaciones, Jalisco, Mexico) has been developed to have water stress tolerance and rapid initial development traits (https://www.novasem.com.mx). Larvae of the fall armyworm were collected in maize fields in the municipality of Queréndaro, Michoacán State, Mexico. Larvae with signs of polyhedrosis disease were not observed in the field-collected insects or any of their descendants during laboratory rearing. Insects were reared at room temperature on semisynthetic diet (Poitout and Bues 1974), and the adults were given continuous access to 15% honey solution. The SfMNPV strain originated from Nicaragua and has been characterized in detail (Escribano et al. 1999; Simón et al. 2008). This isolate was produced in fourth instar *S. frugiperda* larvae, purified and OBs were quantified (Christian et al. 2001), using a Neubauer counting chamber mounted on a phase-contrast light microscope at 40X magnification in triplicate.

### Lethal concentration assay

A preliminary test was performed to estimate the median lethal concentration ($LC_{50}$) of SfMNPV OBs. Groups of 30 newly molted larvae in the second instar were inoculated with 10, 30, 100, 300 and 1000 OBs/mm$^2$ using the diet surface contamination technique which consists of inoculating the insect diet surface with OBs at known concentrations (Cisneros et al. 2002). Disks of semisynthetic diet (2 cm diameter) were treated with 100 µL of each OB concentration that was spread uniformly over the surface to ensure a homogeneous distribution of the inoculum. Control larvae were reared on diet without OBs. Larvae were reared at room temperature (normally 20–25 °C) and checked daily until 10 days after inoculation. Lethal polyhedrosis disease was confirmed by microscopic examination of OBs extracted from larval tissue. The assay comprised three replicates. Probit analysis indicated that the concentration of 30 OBs/mm$^2$ corresponded to the median lethal concentration ($LC_{50}$). The regression equation was: $y = 0.999x - 0.98$.

## Experimental design

The experiment had a completely randomized multifactorial design with three factors: (1) irrigation with two levels [low and high irrigation (40 and 85%) of soil moisture, representing 76 and 163 mL per kg soil, respectively], (2) fertilization [with or without fertilizer application, as described below] and (3) virus with two inoculation levels [with 30 OBs/mm$^2$ and without OBs]. Six replicates of each treatment combination were performed. The experiment consisted of two phases: The first was the feeding of the fall armyworm on the plants, carried out in a greenhouse, and the second phase was the laboratory experiment in which insects were inoculated with viral OBs. The two phases were carried out at room temperature and with a photoperiod of 12 h:12 h (light:dark).

## Soil and mineral fertilization

Plastic pots contained 800 g (dry wt) of an agricultural Luvisol soil collected from Morelia, Michoacán, Mexico (19°41′09″ N; 101°14′17″ W, 1900 m elevation), with pH = 7.28, 53.2% clay, 19.5% sand and 27.3% silt, 5.8 mg kg$^{-1}$ of phosphorus and 23.2 mg kg$^{-1}$ of nitrogen. The soil was mixed 1:1 (w/w) with river sand. The fertilizer used (and hereinafter called NPK) comprised the following nutrients (mg kg$^{-1}$ dry soil): $KH_2PO_4$ (30), $K_2SO_4$ (30), $CaCl_2.2H_2O$ (30), $CuSO_4.5H_2O$ (2.1), $ZnSO_4.7H_2O$ (5.4), $MnSO_4.H_2O$ (10.5), $MgSO_4.7H_2O$ (45), $Na_2MoO_4.2H_2O$ (0.18). NPK was applied by placing it on top of the soil of the fertilized treatments, allowing it to dry and thereafter mixing the fertilizer into the soil. Also, $NH_3NO_4$ (30 mg kg$^{-1}$) was added weekly to the fertilized treatments from the second week onwards during the experiment.

## First phase: plant growth and insect placement

Three maize seeds were planted in each pot to ensure germination and were watered with 124 mL tap water (65% of field capacity). When the maize plants were established, two plants were carefully removed so that only one plant remained in each pot and plants were as homogeneous as possible in size. Ten days after the establishment of the seedlings, the irrigation treatments were started. Field capacity was determined to be 192 mL/kg of soil by using the gravimetric method (Llorca and Bautista-Carrascosa 2004).

Six weeks after planting, 30 recently hatched *S. frugiperda* larvae with no previous feeding experience were placed on each plant. Plants were covered with an enclosure made of fine mesh fabric with a 0.2 mm pore size to prevent insect escape. The larvae were left to feed until they reached the second instar ($L_2$). Five days after the larvae were placed on the plants, 12 $L_2$ larvae were collected from each plant in the fertilizer and no fertilizer treatments. This group of 12 larvae was used in the second phase to test their susceptibility to SfMNPV OBs under laboratory conditions.

## Harvest

After 8 weeks, the plants that had not been exposed to insects were harvested. The aboveground part of the plant (stems and leaves) was separated from the roots, and both parts were dried in an oven for 72 h at 80 °C and weighed. The dried shoot or root parts of each plant were ground in a Thomas Scientific® mill and sieved through a 0.42 mm mesh and the nitrogen and phosphorus concentrations were quantified after Micro-Kjeldahl digestion, followed by colorimetric analysis as described previously (Murphy and Riley 1962 for P; Bremner 1996 for N).

## Second phase: Inoculation of *S. frugiperda*

Suspensions of 1.4 mL of SfMNPV OBs at a concentration of 30 OBs/μL were prepared for each well of a 12 well cell-culture plate (Nunc™ ThermoFisherScientfic, https://www.thermofisher.com/mx/es/home/life-science/cell-culture/cell-culture-plastics/cell-culture-plates.html). A square of approximately 1.5 × 1.5 cm was cut from a fully expanded young maize leaf of each of the treatments, avoiding the central vein. Each leaf square was placed in a well, and below this, a filter paper that had the shape of the wells was placed and moistened with 0.2 mL of distilled water at 24 h intervals. The leaves were previously disinfected with a 6% (v/v) solution of domestic bleach (0.9% sodium hypochlorite), washed thoroughly with water and afterward treated with 100 μL of 0.01% sodium dodecyl sulfate (SDS) solution containing OBs to produce a concentration of 30 OBs/mm$^2$ on each leaf square. Leaf squares without OBs were treated with 100 μL of 0.01% SDS alone. A second instar larva (collected from maize plants as described in Sect. 2.4) was placed in each well with a leaf square of the corresponding treatment. The larvae were left to feed on the virus for 48 h at room temperature, to 12:12 (light:dark) and a lid was placed on top of the box, with a damp towel. The leaf squares were then removed and exchanged for a new leaf square without virus that was replaced daily for 8 days. After 8 days, the larval mortality was quantified.

## Statistical analysis

A two-way ANOVA was used to examine water stress and fertilization effects. The assumptions of homogeneity of variances and a normal data distribution were confirmed by Bartlett's test and the Anderson–Darling test, respectively.

The LSD test (least significant difference) was used for mean comparisons. Spearman correlations were used to explore the relation between all variables. The median lethal concentration was estimated by Probit regression using the Polo Plus program (https://leora-software.com/). A principal component analysis (PCA) was performed with all variables, and the Mahalanobis distance was verified to detect outliers (Jolliffe 2002). The PCA was performed using R version 4.0 (https://www.r-project.org/) and plotted with the "FactoMineR" package. The correlations graphs were produced with the "ggplot2" package. Other graphics were prepared using SigmaPlot™ 14.0 (https://systatsoftware.com/products/sigmaplot/).

## Results

### Effect of low irrigation and nutrients on plant growth

The interaction between irrigation and fertilization treatments had a significant effect on shoot and root dry weight. Plants grown in soils with high irrigation and NPK fertilization had a significantly higher shoot and root dry weight when compared to low irrigated plants, regardless of fertilization and with high irrigated plants without fertilization (Fig. 1a and b). The root/shoot weight ratio was higher in unfertilized plants with low irrigation compared to plants that grew with higher irrigation, but not in the fertilizer treatment (Fig. 1c).

The plants that grew with low irrigation had a higher concentration of N compared to those grown with high irrigation, both in unfertilized plants (35.3% higher in the low irrigation treatment) and in fertilized plants (54.3% higher in the low irrigation treatment) (Fig. 2a). The concentration of P in plants did not vary significantly (Table 1). The mean ($\pm$ SE) concentration of P (mg g$^{-1}$) in plants with low irrigation was $0.104 \pm 0.004$ without NPK and $0.112 \pm 0.002$ with NPK and with high irrigation was $0.112 \pm 0.002$ without NPK and $0.104 \pm 0.003$ with NPK.

### Effect of SfMNPV on S. frugiperda larval mortality

Insect mortality could not be analyzed by two-way ANOVA because very few insects were recovered from the no fertilizer treatment with high irrigation. The few larvae collected, in addition, did not feed on the leaf squares inoculated with the nucleopolyhedrovirus. Therefore, this variable was analyzed with a one-way ANOVA with the remaining three treatments.

The larvae that died in the virus treatment displayed the characteristic signs of polyhedrovirus disease, which was confirmed by observing the presence of abundant OBs in
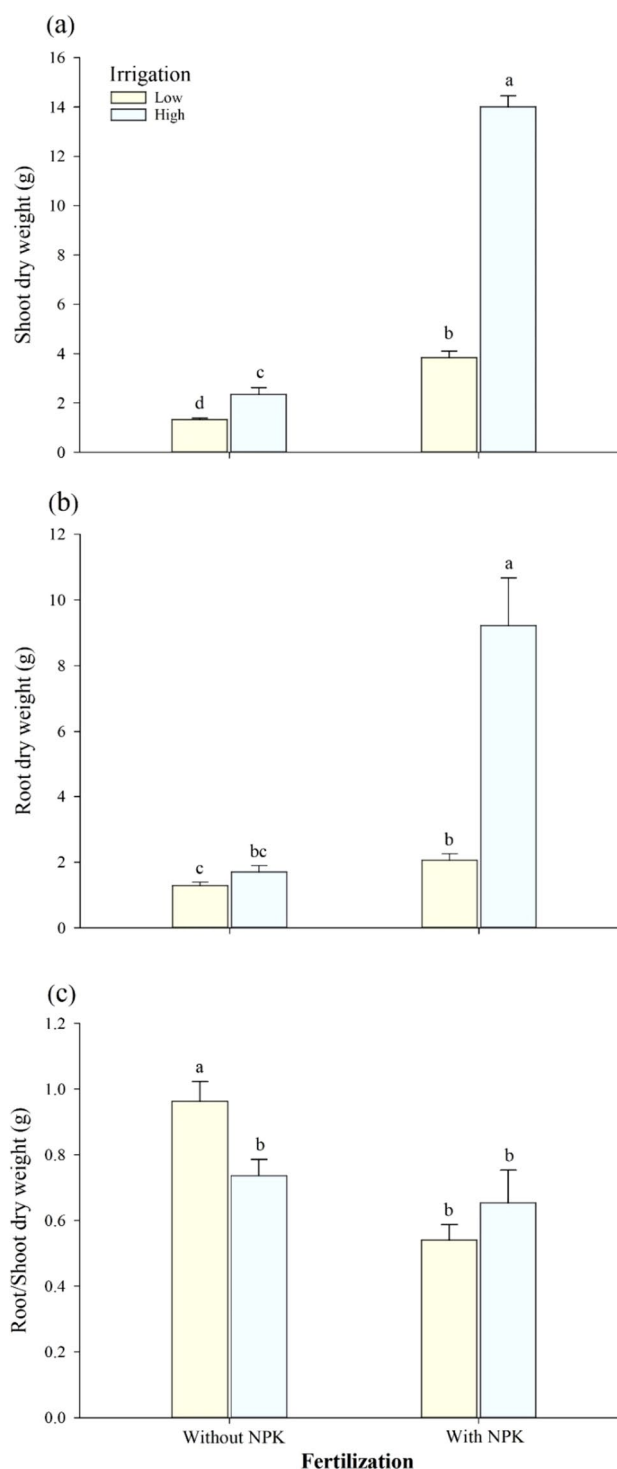


**Fig. 1** Means ($\pm$ SE, $n=6$) of shoot dry weight (**a**), root dry weight (**b**) and the root/shoot weight ratio (**c**) of the maize plants at 8 weeks of growth with or without NPK fertilizer treatment. Identical letters in the columns indicate that treatments did not differ significantly ($p > 0.05$)
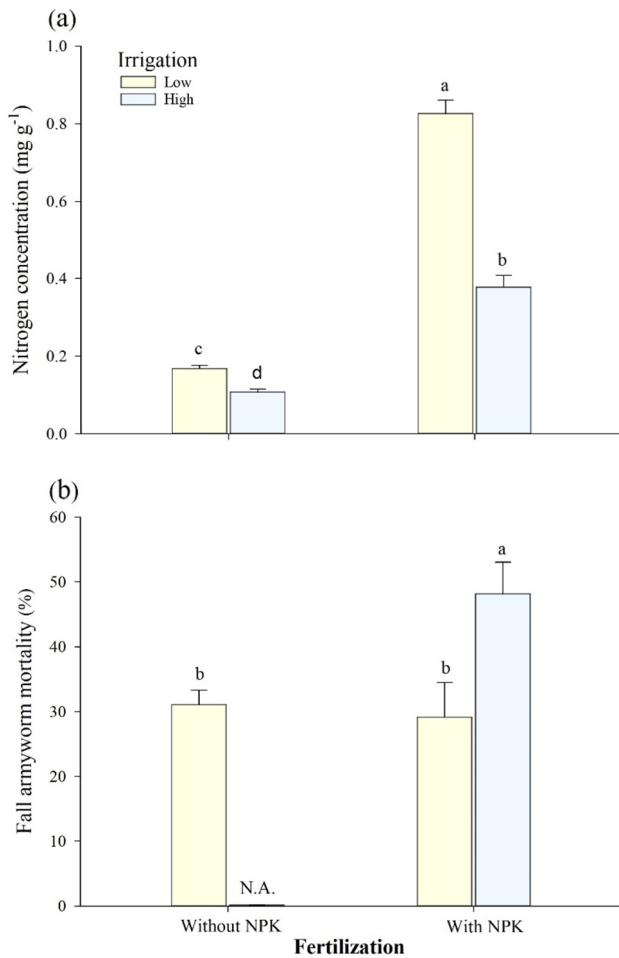
**Fig. 2** Means ($\pm$ SE, $n=6$) of the shoot nitrogen concentration (**a**) and the percentage of larval mortality (**b**). N.A. are missing data. Identical letters in the columns indicate that treatments did not differ significantly ($p > 0.05$)

smears of larval tissues under a phase-contrast microscope (Olympus BX41) at $\times 400$.

Larval mortality was observed in the control treatments, and these were: low irrigation without fertilization ($12.1 \pm 5.1\%$), low irrigation with fertilization ($0.7 \pm 0.7\%$) and high irrigation with fertilization ($3.8 \pm 1.8\%$). Therefore,

Abbott's formula was used to adjust for mortality in the control treatment (Rosenheim and Hoy 1989).

We found significant differences between the treatments inoculated with SfMNPV ($F_{2,15} = 5.70$, $P = 0.014$). The insects that fed on plants with high irrigation and fertilization, presented significantly higher mortality than those that fed on fertilized plants but with low irrigation, or unfertilized plants with low irrigation (Fig. 2b). Significant correlations were detected between shoot dry weight and root dry weight ($r=0.92$, $p < 0.001$), shoot dry weight and percentage of larval mortality ($r=0.57$, $p < 0.01$), i.e., when the aerial biomass of the plant increased, larval mortality also increased (Fig. 3). The same trend was observed in the relationship between root dry weight vs percentage of larval mortality ($r=0.49$, $p < 0.05$), shoot dry weight vs ratio root/shoot dry weight ($r= -0.50$, $p < 0.05$), N concentration vs ratio root/shoot dry weight ($r= -0.53$, $p < 0.01$), percentage of larval mortality vs N ($r=0.55$, $p < 0.01$) and shoot dry weight vs N ($r=0.56$, $p < 0.01$) (Fig. 3).

The principal component analysis explained 74.5% of the variability for all variables of experiment (Fig. 4). The first component included shoot and root dry weight and larval mortality (48.6% variability), which are related to the treatment of fertilized plants with high irrigation. In the second component, shoot nitrogen and phosphorus concentrations explained 25.9% of the variability and were related to fertilized plants with low irrigation. High root/shoot ratio was more related to unfertilized plants and those with low irrigation (Fig. 4).

## Discussion

Water shortage reduced the prevalence of nucleopolyhedrovirus-induced mortality in the fall armyworm on plants that received fertilizer. Low irrigation affected maize plants even when they were fertilized. This meant that nutrients were more concentrated in the leaves of low irrigation plants, especially nitrogen.

It is known that the nutritional quality of the foodplant can affect the capacity of phytophagous insects to defend themselves and resist an infection by pathogens (Lochmiller

**Table 1** Summary of linear model ANOVAs for all plant response variables and factors: Irrigation (I), Fertilization (F) and their interaction (I×F)

| | df | Sdw | | Rdw | | Rdw/Sdw | | Nitrogen | | Phosphorous | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P | F | P | F | P |
| Irrigation | 1,20 | 365.1 | **<0.001** | 46.56 | **<0.001** | 0.697 | 0.4101 | 97.29 | **<0.001** | 0.88 | 0.359 |
| Fertilization | 1,20 | 585.7 | **<0.001** | 72.17 | **<0.001** | 13.92 | **0.001** | 514.9 | **<0.001** | 0.876 | 0.36 |
| I×F | 1,20 | 244.1 | **<0.001** | 22.56 | **<0.001** | 6.255 | **0.021** | 7.78 | **0.011** | 3.21 | **0.088** |

Numbers in bold show significant differences ($P < 0.05$)

*Sdw and Rdw* shoot and root dry weight, respectively; *df* degrees of freedom; *F* Fisher's F statistic; *P* probability value

**Fig. 3** Spearman correlation indices for all variables. Blue colors indicate correlations close to 1 and orange color close to −1
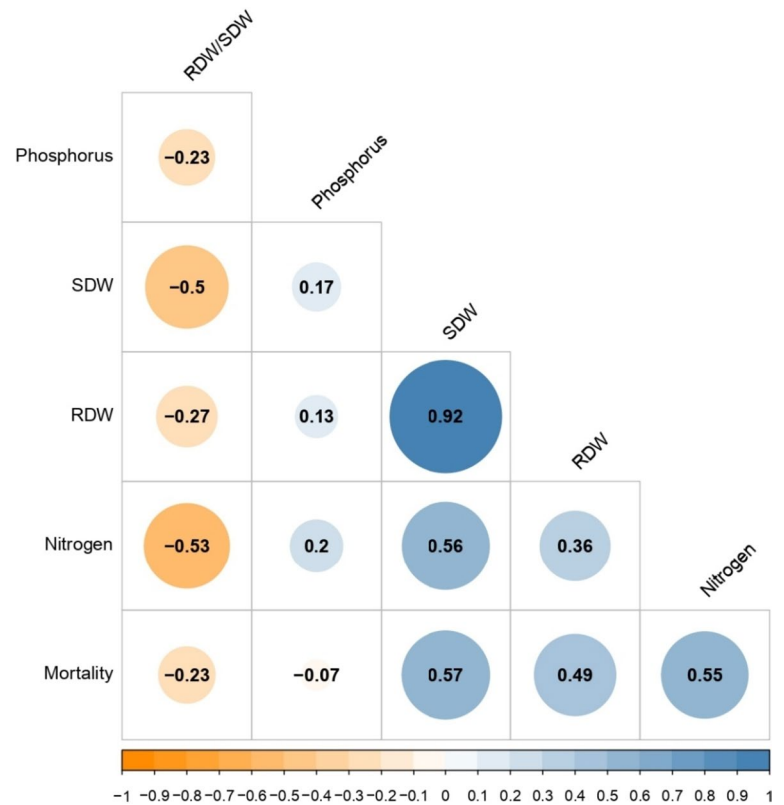


**Fig. 4** Principal component analysis including all variables measured in the experiment. Treatment abbreviations: LI-NPK (low irrigation without fertilization), LI+NPK (low irrigation with fertilization), HI-NPK (high irrigation without fertilization) HI+NPK (high irrigation with fertilization). The ellipses were drawn to indicate a 95% confidence interval
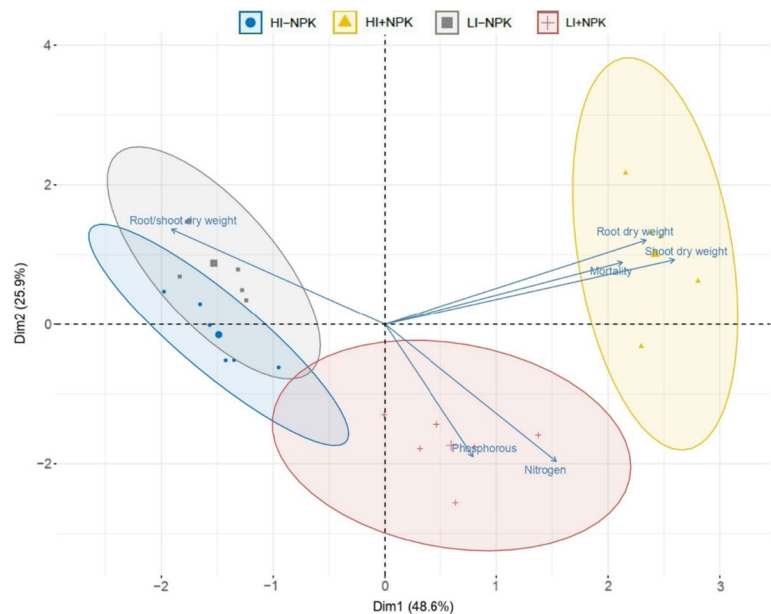


and Deerenberg 2000). For example, in a recent study, García-Gómez et al. (2021) reported that the mortality of *S. frugiperda* exposed to nucleopolyhedrovirus diminished as the concentration of nitrogen in maize leaves increased. In addition, experiments with *Spodoptera exempta* Walker and *Spodoptera littoralis* Boisduval larvae infected with their respective nucleopolyhedroviruses showed high survival

rates when insects fed on a protein-rich diet during the first 24 h post-infection (Lee et al. 2006; Povey et al. 2014). Those reports suggest that an adequate nutrition is likely required to trigger efficient defensive mechanisms and resist viral infection because almost all the defensive mechanisms of the immune arsenal require large amounts of amino acids for the production of proteins (Beisel 1977).

In this study, we did not perform protein analyses of plants that could have provided information on the nutritional status of *S. frugiperda* and their ability to resist viral infection. However, nitrogen is an integral constituent of plant of proteins, nucleic acids, chlorophyll, coenzymes, phytohormones and secondary metabolites (Hawkesford et al. 2012). Increased availability of nitrogen increases the concentration of crude protein in plants (Gallais et al. 2006; Wiesler 2012), which is of nutritional benefit to insects (Siva-Jothy and Thompson 2002) and may strengthen their immune response and increase survival following an entomopathogen challenge (Povey et al. 2014). In contrast, when the virus was applied onto leaves of water-stressed plants, it was difficult to disperse the OB suspension homogeneously on the leaf surface. This was possibly a consequence of the induction of physiological and chemical defensive mechanisms. Commonly, trichomes on leaves are associated with the physical defenses of the plant against herbivores (Björkman et al. 2008). However, such structures can also participate in water balance and can exert photoprotection by reflecting sunlight (Molina-Montenegro et al. 2006). Other physiological mechanisms that might modify the dispersion of OBs on the leaf surface include stomatal closure, since the stomata remain closed in response to water stress (Hopkins and Hüner 2004; Rodriguez-Dominguez and Brodribb 2020), or the presence of wax produced as a physiological response to environmental stress (Ludlow and Muchow 1990; Lewandowska et al. 2020).

The treatment involving high irrigation and fertilization resulted in the highest larval mortality, likely because the plant leaves in this treatment had adequate moisture and moderate nitrogen content, and the larvae consumed a greater quantity of OB-contaminated leaf tissue than occurred in the other treatments. Although this seems to be a contradiction, the larvae that fed on plants with low irrigation had lower mortality because the nutrients like nitrogen in the treatments with high irrigation were found scattered throughout the plant or less concentration. Thus, we postulate that the insects had to feed on more plant tissue which increased their consumption of inoculum OBs, resulting in higher mortality than in the larvae that fed on water-stressed plants where the insects consumed less tissue to satisfy their physiological demand due to the more concentrated nitrogen. Therefore, mortality did not change due to fertilization, but due to water stress, which increased or decreased the concentration of nutrients in the plant.

Another result was observed in the high irrigation with low nutrient treatment in which plants became unappetizing and were avoided by larvae. As mentioned before, it was not possible to recover more than a few of the individuals from this treatment, and they did not become infected by the virus. Intriguingly, very low nitrogen concentrations were detected in this treatment, and plants also showed a yellowish tinge and semitranslucent foliage, which is a sign of chlorosis.

Compared to other families of herbaceous plants, the concentration of proteins in grasses and in the family Poaceae in general tend to be low (Lyttleton 1973; Holechek 1984). Hence, it is possible that the nutritional quality of the plants in this treatment was so low that insects were unable to develop through the larval instars and undergo pupation. In addition, extended periods of time on plants represent an increased risk for insects because of a greater possibility of being preyed upon by natural enemies or of becoming infected by pathogens. In this regard, Loader and Damman (1991) observed higher predation of lepidopteran larvae when they fed on plants with little nitrogen content. In addition, Lepidoptera have a hundred times greater risk of being predated when they feed, compared to periods when they do not (Bernays 1997).

## Conclusion

Here we show that water stress interfered with nucleopolyhedrovirus infection of the fall armyworm. We conclude that water stress can affect the performance of the nucleopolyhedrovirus and thereby reduce the biological control of fall armyworm. As climate change continues to generate water stress and nutrient limitations, it is necessary to understand the effect of these factors on the ecology of the insects that feed on crops. This study may help to identify crop plant factors that influence the performance of biocontrol agents and improve pest management strategies that include the use of entomopathogens. However, more studies are needed on the role of water stress and plant nutrition in the efficacy of entomopathogens used for biological control. It is also necessary to study the effect of the secondary metabolites produced by the plant when there is water stress, such as phenolic compounds and their effect on the performance of baculoviruses in the control of the fall armyworm.

**Data availability** The data will be available on request.

## Declarations

**Conflict of interest** No potential conflict of interest was reported by the authors.

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

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