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ORIGINAL CONTRIBUTION

Chemical and biological stress factors on the activation of nucleopolyhedrovirus infections in covertly infected *Spodoptera exigua*

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Abstract

Following the consumption of baculovirus occlusion bodies (OBs), insects may succumb to lethal disease, but the survivors can harbour sublethal covert infections and may develop, reproduce and transmit the infection to their offspring. The use of different chemical and biological stressors was examined to determine whether they could be used to activate covert infections in populations of *Spodoptera exigua* larvae infected by the homologous nucleopolyhedrovirus (SeMNPV). Treatment of covertly infected *S. exigua* second instars with Tinopal UNPA-GX, hydroxylamine, paraquat, *Bacillus thuringiensis* var. *kurstaki* crystals, spores or mixtures of crystals + spores, or a heterologous nucleopolyhedrovirus (*Chrysodeixis chalcites* SNPV) did not result in the activation of SeMNPV covert infections. Similarly, virus treatments involving permissive NPVs did not result in greater mortality in covertly infected insects compared with the virus-free controls. In contrast, 0.1% copper sulphate, 1% iron (II) sulphate and 1 mg/l sodium selenite treatments resulted in 12–41% lethal polyhedrosis disease in covertly infected larvae. A greenhouse trial using copper sulphate and sodium selenite as activation factors applied to covertly infected *S. exigua* larvae on sweet pepper plants resulted in very low levels of SeMNPV activation (<3%). These results highlight the important roles of copper, iron and selenium in insect immunity and baculovirus-induced disease. However, these substances seem unlikely to prove useful for the activation of covert SeMNPV infections in *S. exigua* larvae under greenhouse conditions.

Introduction

Nucleopolyhedroviruses (NPV, family Baculoviridae) are used as biological control agents of lepidopteran pests because of their notable insecticidal properties, high host specificity and their outstanding biosafety characteristics (Eberle et al. 2012). Following the application of a NPV-based insecticide, many of the target insects die from lethal polyhedrosis disease, normally in a period of several days after the virus insecticide treatment. However, a fraction of the target insect population may survive, either because certain individuals did not consume a lethal number of virus occlusion bodies (OBs) present on the crop foliage (Evans 1999) or because the insect does not develop a lethal infection after having consumed virus inoculum (Burden et al. 2002). The later may be due to inhibition of the viral infection by the host immune system (Marques and Carthew 2007; Pascual et al. 2012), or due to the virus adopting a low virulence

non-lethal infection strategy (Sorrell et al. 2009; Moreno-García et al. 2014), or a combination of both.

The host and pathogen factors that modulate variation in baculovirus virulence in particular insects remain poorly understood (Myers and Rothman 1995; Erlandson 2009; Cory 2010). Nevertheless, it is clear that in many cases, insects that survive following the consumption of virus OBs may acquire an inapparent infection that is not lethal and that can only be detected using molecular techniques (Burden et al. 2002). Such infections have been termed sublethal or covert and are likely widespread in lepidopteran populations (Kouassi et al. 2009; Burand et al. 2011; Cabodevilla et al. 2011a; Kemp et al. 2011; Virto et al. 2014). Furthermore, this type of infection can be transmitted vertically from parents to their offspring across several generations, both in natural insect populations (Burden et al. 2003; Vilaplana et al. 2010), and in laboratory colonies of insects that have consumed a sublethal dose of virus OBs (Burden et al. 2002; Cabodevilla et al. 2011b).

The nucleopolyhedrovirus of *Spodoptera exigua* (SeMNPV) forms the basis for a number of highly effective biological insecticide products that have been commercialized in developed and developing countries (Smits and Vlak 1994; Kolodny-Hirsch et al. 1997; Lasa et al. 2007; Pudjianto et al. 2016). Certain genotypes of SeMNPV are highly effective at inducing sublethal infections in survivors of OB inoculum. These genotypes were isolated from the progeny of sublethally infected *S. exigua* adults collected in the field in greenhouses in southern Spain and have reduced pathogenicity, in terms of concentration-mortality metrics, and a greater tendency for vertical transmission compared to genotypes associated with horizontally transmitted infections (Cabodevilla et al. 2011a,b).

SeMNPV-based insecticide applications are almost invariably performed using an inundative strategy of biological control within an integrated pest management programme (IPM) targeted at *S. exigua* larvae in greenhouse or field crop settings (Kolodny-Hirsch et al. 1997; Cunningham 1998; Lasa et al. 2007). However, knowing that a fraction of the pest population is likely to be covertly infected by NPV, the use of substances that trigger covert infections into lethal polyhedrosis disease may offer the possibility to initiate virus epizootics of disease in pest populations; a strategy that has received very little empirical attention to date.

The molecular and physiological mechanisms behind the transition from covert to overt infection in insect-baculovirus pathosystems remain unknown. Early studies suggested that stressful conditions related to nutrient availability or the presence of other pathogens may trigger the activation of covert infections across many species of Lepidoptera (Longworth and Cunningham 1968; Smith 1976; Jurkovicova 1979; Podgwaite and Mazzone 1986). More recently, more systematic approaches have focused on rearing conditions such as high population densities (Fuxa et al. 1999; Opoku-Debrah et al. 2013), high relative humidity (Fuxa et al. 1999) or ingestion of chemical compounds (Il'inykh et al. 2004). Additionally, a number of studies have reported the activation of covert infections following inoculation with viruses that naturally infect a different species of host insect, known as heterologous viruses (Hughes et al. 1993; Fuxa et al. 2002; Cooper et al. 2003; Kouassi et al. 2009).

The purpose of this study was to evaluate the effects of a selection of chemical and biological stressors that might trigger the activation of the covert SeMNPV infections in *Spodoptera exigua* larvae, under laboratory and greenhouse conditions, with the aim of evaluating the value of this strategy in the control of *S. exigua* populations on greenhouse crops.

Materials and Methods

Establishment of a covertly infected *S. exigua* population

A virus-free colony of *S. exigua* originating from Andermatt Biocontrol was maintained at 25 ± 1°C, 50 ± 5% RH and 16 : 8 h L:D photoperiod using a wheatgerm-based semi-synthetic diet (Elvira et al. 2010). A group of adults from the first generation were checked by qPCR and proved negative for SeMNPV infection, confirming that the colony did not harbour any inapparent infection (results not shown). To establish covert infections, groups of 200 fourth instar larvae from the colony were allowed to drink from a suspension of 9×10^3 OBs/ml of the vertically transmitted isolate VT-SeAl1 (Cabodevilla et al. 2011a), using the droplet feeding technique (Hughes and Wood 1981). This concentration of OBs was designed to kill ~50% of insects. A similar group of larvae were treated with water as a control. Larvae that drank from the droplets in a 10-min period were reared individually in plastic cups, containing a block of semisynthetic diet, until death or pupation. Insects that died from polyhedrosis disease were discarded. The surviving pupae were sexed, 20 couples were placed in male and female pairs in a paper bag,

provided with water in a small container with cotton wool wick. Mating and oviposition occurred in the bag, and eggs were collected daily and placed in 300-ml plastic bags with a slice of diet. Offspring (F_1) were reared in groups during the first instar. Then, groups of larvae were allowed to moult overnight without food. The resulting second instar insects were used to test for possible activation of covert infection. The procedure was performed on three occasions (replicates).

Activation of SeMNPV infections under laboratory conditions

To test for virus activation, groups of 24 second instar larvae were orally treated by droplet feeding with one of the following chemical compounds: 1% or 0.1% (wt./vol.) copper sulphate, 1% iron (II) sulphate, 0.1% hydroxylamine, 2% Tinopal UNPA-GX (Sigma Chemical CO, St. Louis, MO, USA), 1 mg/l (1 part per million, ppm) sodium selenite and 1 mg/l (ppm) paraquat dichloride. Similar groups of larvae were treated with one of the following biological insecticides: (i) *Chrysodeixis chalcites* single nucleopolyhedrovirus (ChchSNPV) at a concentration of 1×10^8 OBs/ml; ChchSNPV is a heterologous virus that does not cause a productive infection in *S. exigua* (Murillo et al. 2000), (ii) *Mamestra brassicae* multiple nucleopolyhedrovirus (MbMNPV) at a concentration of 4.9×10^4 OBs/ml (MbMNPV is a heterologous virus that can lethally infect *S. exigua*), (iii) OBs of a reference genotype of SeMNPV named SeMNPV-US2 (Muñoz et al. 1998) at a concentration of 1.7×10^4 OBs/ml, (iv) *Bacillus thuringiensis* spores (HD-1 strain, 0.5 absorbance at 600 nm), (v) *B. thuringiensis* crystals (HD-1 strain, 10 μg/ml), (vi) a mixture of *B. thuringiensis* spores and crystals described in treatments (iv) and (v) in a 1 : 1 ratio. Identical treatments were performed using virus-free control insects. The concentrations used in each treatment were designed to be non-lethal or lethal to a maximum of 50% of the experimental insects, based on the results of preliminary tests (data not shown). Treated insects were individually reared in plastic cups with semisynthetic diet at 25 ± 1°C. Mortality was registered daily until all insects had died or pupated. Pupae were reared to adulthood and frozen at −80°C for further analysis by qPCR. Death due to NPV infection was confirmed by the observation of OBs of insect cadavers using a phase-contrast microscope. Three replicates of the experiment were performed.

Frequencies of virus mortality after MbMNPV and SeMNPV-US2 application were compared between virus-free and infected insect lines using Pearson's chi-square test in the SPSS Statistics package (v.19 IBM).

Activation of SeMNPV infections under semi-field conditions

Two treatments that had proved to be effective in the laboratory experiment were tested on pepper plants in greenhouse conditions during September 2011. Three experimental greenhouses of 110 m^2 area (one per repetition) in the facilities of the Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Almeria, Spain, were planted with sweet pepper crops (*Capsicum annuum* var. Melchor). Plants were arranged in 5 rows, each comprising 20 plants, with a distance of 1.1 m between rows. Pepper crops were grown following the standard agricultural practices used in commercial greenhouses of this region, including the release of predators (*Amblyseius swirskii*, *Nesidiocoris tenuis* and *Orius laevigatus*) for biological control of whiteflies and thrips. Each greenhouse was split into four plots of ~27 m^2 containing 25 plants. Three central plants per plot were chosen for insect release and treatment applications to avoid border effects and cross-contamination among plots. When pepper plants reached a height of 80–120 cm and an average of 10 fruits per plant, one of the following treatments was applied using a 2 litre compressed-air sprayer (Pamex, Valencia, Spain): (i) 0.1% copper sulphate, (ii) 1 mg/l (1 ppm) sodium selenite, (iii) *B. thuringiensis*-based insecticide (0.3 g/l, var. kurstaki, FlorBac, Bayer) and (iv) water control. All treatments included 0.15% (v/v) Agral 90 (Syngenta Agro, Madrid, Spain) as a wetting agent. The production of sublethally infected *S. exigua* adults was performed as described above, and the eggs produced by these insects were used to artificially infest pepper plants. Egg masses were placed on the underside of randomly selected leaves in the middle section of the plant at a rate of 200 eggs per plant. At 5 days later, once most of the eggs had hatched and larvae had reached the second instar, treatments were applied. At 48 h post-treatment, a total of 30 larvae per plot were collected randomly, taken to the laboratory and reared individually in plastic cups with semisynthetic diet at 25 ± 1°C. Larvae were checked daily until death or pupation. Groups of 10 larvae per treatment were individually reared to adults and individually frozen at −80°C for subsequent analysis by qPCR. All dead larvae were smeared onto a microscope slide and observed under a phase-contrast microscope to determine the presence of OBs.

Detection of sublethal infections

Adults from the F_1 generation of both laboratory and field experiments were analysed for the evidence of transgenerational transmission of the infection according to the methodology described by Virto et al. (2013). For this, total insect DNA was purified from the abdomen of frozen adults using a Master Pure Complete DNA Purification kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's recommended protocol for tissue samples. DNA was resuspended in 20 μl sterile Milli-Q water. Blank extraction samples containing only water were processed in parallel to control for cross-contamination during the extraction. Quantitative PCR (qPCR) was carried out using SYBR green fluorescence in 96-well reaction plates in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA). Specific primers targeted at the SeMNPV *DNA polymerase* gene (Virto et al. 2013) were used to amplify a 149-bp region based on the complete genome sequence of the VT-SeAl1 genotype (Theze et al. 2014). These primers had been tested previously against genomic DNA of other alphabaculovirus species that we have in our laboratory to confirm primer specificity, including *Spodoptera frugiperda* MNPV, *Autographa californica* MNPV, *Spodoptera littoralis* NPV and *Helicoverpa armigera* NPV (Cabodevilla et al. 2011b). The qPCRs were performed in a volume of 20 μl, containing 10 μl SYBR Premix Ex Taq (2×), 0.4 μl ROX Reference Dye (50×), 0.4 μl of both forward and reverse primers (10 μM) and 1 μl of DNA template. The amplification reaction consisted in a denaturation step at 95°C for 30 s, followed by 45 amplification cycles at 95°C for 5 s and 60°C for 30 s. Finally, a melting curve analysis, involved a dissociation stage of 95°C for 15 s, 60°C for 15 s and 95°C for 15 s, was added to confirm the presence of a single peak of the target product.

Results

Of the larvae initially treated with VT-SeAl1 genotype OBs, a total of 62% succumbed to lethal polyhedrosis disease, whereas no viral mortality was registered in mock-infected control larvae. Survivors were reared to adults and allowed to mate to produce the offspring (F_1 generation) in which virus activation was tested.

Following treatment of F_1 larvae with different chemical compounds, virus-induced mortality was only observed in larvae that ingested 0.1% copper sulphate, 1% iron sulphate or 1 ppm sodium selenite, which resulted in 12%, 15% and 41% virus mortality, respectively (table 1). Virus-killed larvae did not show the typical symptoms of polyhedrosis disease, specifically tissue liquefaction (Hawtin et al. 1997), although abundant viral OBs were observed under phase-contrast microscope in insects from these three treatments. Unfortunately, the quantity of OBs that could be collected from these larvae was not enough to perform a restriction endonuclease analysis, which would have confirmed whether the virus of the progeny was the same as one that was originally inoculated in the previous generation. Nevertheless, as we started with a virus-free population and used a highly sensitive and specific qPCR technique to detect covert infections, we presume that the activated virus was that of VT-SeAl1. No post-treatment virus mortality was registered in virus-free control larvae.

In contrast, none of the biological treatments applied to F_1 larvae were capable of acting as activation factors in sublethally infected larvae. Virus-induced mortality observed in the F_1 larvae treated with SeMNPV-US2 OBs (17%) or MbMNPV OBs (64%) was in both cases similar to the levels of virus mortality in the insects that did not have a covert infection (SeMNPV-US2 = 23% mortality, χ^2=0.382,

Table 1 Percentage of virus-induced mortality and mortality due to other causes in virus-free and covertly infected *S. exigua* larvae after treatment with stressors in the laboratory (n = total number of treated larvae)

	Mortality of virus-free larvae (%)		Mortality of covertly infected larvae (%)	
Treatment	Other causes (n)	Virus (n)	Other causes (n)	Virus (n)
Control (H_2O)	0 (21)	0 (21)	0 (46)	0 (46)
Chemicals				
Copper sulphate (1%)	35 (37)	0 (37)	6 (37)	0 (37)
Copper sulphate (0.1%)	0 (23)	0 (23)	0 (58)	12 (58)
Iron (II) sulphate (1%)	0 (24)	0 (24)	0 (71)	15 (71)
Hydroxylamine (0.1%)	0 (24)	0 (24)	0 (63)	0 (63)
Tinopal UNPA-GX (2%)	0 (19)	0 (19)	0 (72)	0 (72)
Sodium selenite (1 ppm)	2 (24)	0 (24)	2 (61)	41 (61)
Paraquat dichloride (1 ppm)	0 (20)	0 (20)	0 (70)	0 (70)
Entomopathogens				
ChchSNPV (1×10^8 OBs/ml)	0 (23)	0 (23)	0 (67)	0 (67)
MbMNPV (4.9×10^4 OBs/ml)	0 (22)	50 (22)	0 (64)	64 (64)
SeMNPV-US2 (1.7×10^4 OBs/ml)	0 (22)	23 (22)	0 (71)	17 (71)
Bt crystals: HD-1 (10 μg/ml)	0 (43)	0 (43)	0 (70)	0 (70)
Bt spores: HD-1 (0.5 Abs_{600})	13 (22)	0 (22)	9 (22)	0 (22)
Bt crystals + spores (1 : 1 ratio)	26 (31)	0 (31)	0 (24)	0 (24)

d.f.=1, P = 0.538; MbMNPV= 50% mortality, χ^2=1.354, d.f.=1, P = 0.245) (table 1). Larval mortality due to causes other than virus varied between zero and 35%, probably due to the intrinsic toxicity of some compounds (table 1). All F_1 larvae that reached adulthood (n = 27, 100%) were confirmed by qPCR to harbour a VT-SeAl1 covert infection, suggesting a high prevalence of persistent infection in larvae subjected to activator treatments. None of the control insects proved positive for VT-SeAl1 DNA by qPCR.

Larvae collected from plants treated with copper sulphate or sodium selenite showed very low levels of virus-induced mortality: one larva (1.4%) and two larvae (2.4%), respectively (table 2). A *B. thuringiensis*-based pesticide was included as a control because this pesticide is widely used in greenhouse crop integrated pest management systems in Almeria, Spain. Of the insects recovered from this treatment, 2 larvae (2.8%) died from polyhedrosis disease (table 2). No virus mortality was observed in larvae from control plants. The level of mortality due to other causes was low and varied between 1.2 and 8% (table 2). All (100%) adult survivors from the F_1 generation (n = 36) that were analysed by qPCR harboured a VT-SeAl1 covert infection, indicating a high percentage of covert infections in insects used to infest greenhouse pepper plants.

Discussion

In the present study, we evaluated the potential of a selection of chemical compounds and entomopathogens as triggers for converting a covert infection into lethal polyhedrosis disease in *S. exigua* larvae. The ability to activate covert infections varied significantly according to the type of chemical and biological entities being tested, as well as the conditions under which larvae consumed these substances (laboratory or greenhouse conditions).

Table 2 Percentage of virus mortality after stressor treatments in covertly infected *S. exigua* larvae under field conditions (n = total number of larvae sampled at 48 h post-treatment and reared in the laboratory until death or pupation)

Treatment	Mortality from other causes (%) (n)	Mortality from virus (%) (n)
Control (H_2O)	8 (71)	0 (71)
Chemicals		
Copper sulphate (0.1%)	2.7 (74)	1.4 (74)
Sodium selenite (1 ppm)	1.2 (82)	2.4 (82)
Entomopathogens		
Bt (0.3 g/l)	5 (72)	2.8 (72)

Early studies on *Bacillus thuringiensis* and hydroxylamine as activators of covert NPV infections gave variable results, depending on the species of insect tested (see reviews by Aruga 1963; Il'inykh and Ul'yanova 2005), but neither were useful in the present study. Paraquat, a potent generator of superoxide anion, likely interacts with the viral superoxide dismutase enzyme which, if not functional, delays the systemic phase of infection and the production of OBs (Katsuma et al. 2015). Residues of this herbicide may be frequently present in agroecosystems. Stilbene optical brighteners, such as Tinopal UNPA-GX, degrade the larval peritrophic matrix and reduce sloughing of infected midgut cells (Washburn et al. 1998; Wang and Granados 2000). These compounds have been patented for their use as potentiators of baculovirus-based insecticides (Shapiro et al. 1992) and natural baculovirus populations present on crop plants (Black 1999). However, neither paraquat nor the stilbene brightener activated covert infections in *S. exigua* larvae.

In the laboratory, three chemicals, copper sulphate, iron sulphate and sodium selenite, triggered lethal polyhedrosis disease in 12–41% of larvae. Trace elements such as copper, iron and selenium are essential for immune system function in insects (Popham et al. 2012a). Considering each of these metals in turn, virus infection significantly altered copper titres in the haemolymph of *Heliothis virescens*, but not in *Helicoverpa zea*, compared with those of healthy larvae (Popham et al. 2012a). Indeed, excess or deficiency of copper can strongly influence insect responses to infection. For example, Il'inykh et al. (2004) previously reported activation of *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) in up to 18% of gypsy moth larvae that fed on diet containing 0.6% copper sulphate. In contrast, injection of *H. zea* larvae with a copper chelating compound increased the virulence of the homologous NPV (Washburn et al. 1996), possibly via its role at the active site of the phenoloxidase enzyme, a key component of the insect immune response to pathogens (Shelby and Popham 2006).

Despite the importance of iron availability in insects during infection by bacterial pathogens (Dunphy et al. 2002; Watson et al. 2010), the role of this metal during baculovirus infection is poorly understood. Divalent and trivalent iron cations markedly reduced the pathogenicity of LdMNPV OBs when administered simultaneously with OB inoculum (Shapiro 2001). In a later detailed study, Popham et al. (2012b) demonstrated that dietary iron was retained in larval tissues and increased significantly in the plasma late in infection in *Heliothis virescens* larvae. In the present study,

iron (II) sulphate was as effective as copper sulphate in VT-SeAl1 activation, albeit at a tenfold higher concentration.

Selenium in the form of sodium selenite was the most effective compound tested. Previous studies have reported that supplementation of selenium in the diet at concentrations of 5–60 ppm was associated with larval resistance to baculovirus infection following both peroral and injected inoculum challenge (Popham et al. 2005; Shelby and Popham 2007). This effect could be related to the role of this microelement in the modulation of the host immune system, as dietary levels of selenium were closely correlated with levels in the haemolymph. The fact that very low levels of selenium in our study (1 ppm) triggered activation of covert infections was therefore quite unexpected and clearly requires further investigation to identify selenium modulated physiological or immunological processes that may be involved in baculovirus virulence.

Peroral inoculation with heterologous viruses has been shown to be effective in activating covert baculovirus infections (Hughes et al. 1993; Fuxa et al. 2002; Cooper et al. 2003; Kouassi et al. 2009). For example, wild-type SeMNPV and MbMNPV-like genomes were detected in the OBs collected from virus-killed *S. exigua* larvae that had previously been injected with SeMNPV recombinant DNA, suggesting the presence of covert infections by both the heterologous virus and the homologous wild-type virus in the insect population (Murillo et al. 2011). In contrast, Yang et al. (2015) were unable to activate covert infections in *Lymantria dispar* larvae that had consumed OBs of a heterologous virus. In the present study, we were unable to activate infection in covertly infected larvae by inoculating them with homologous or heterologous viruses. The reason behind this result is unclear, although each virus-host pathosystem differs in the quantity of OBs required to initiate infection, such that activation of covert infections may also be dose-dependent, or virus-dependent if certain virus-specific genes are responsible for triggering activation.

Despite consistent results in the laboratory, copper sulphate and sodium selenite treatments failed to produce important levels of NPV-mortality in greenhouse trials. Larval feeding behaviour may have reduced the overall dose of each chemical consumed if insects were able to taste and avoid treated foliage, a behaviour that was not possible under laboratory conditions. Also, a number of biotic and abiotic factors such as host plant chemistry, insect nutrition or insect density could have influenced the vigour of the developing pest larvae and their susceptibility to virus activation. For example, the nutritional quality of a semi-synthetic insect diet clearly differs from that of crop plants, with numerous effects on insect physiology and immune system function (Ojala et al. 2005; Shikano et al. 2010; Vogelweith et al. 2011). In this respect, the survival of *Spodoptera littoralis* larvae varied according to the protein and carbohydrate content of the diet following inoculation with the homologous nucleopolyhedrovirus (Lee et al. 2006). The same applies to the microbiota present on host plants and in the gut of phytophagous insects, leading to diverse interactions, the outcome of which are difficult to predict when pathogenic viruses are present (Elliot et al. 2000; Bixby and Potter 2010). Susceptibility and immune response can also vary with rearing density. *Spodoptera exempta* and *S. littoralis* larvae reared gregariously were more resistant to NPV infection, likely due to the higher levels of phenoloxidase activity in the haemolymph of gregariously reared insects, suggesting a stronger immune response (Reeson et al. 1998; Wilson and Graham 2015). It is therefore clear that the results of our laboratory studies could not be directly extrapolated to field conditions for a number of reasons that require further study.

Baculovirus covert infections are common in natural lepidopteran populations (Burden et al. 2003; Vilaplana et al. 2010; Cabodevilla et al. 2011a; Virto et al. 2014), and we were able to induce such infections in the survivors of baculovirus OB inocula (Virto et al. 2016). The examination of factors involved in virus activation allowed us to explore this as an alternative strategy for pest control. The possibility of triggering lethal diseases and initiating viral epizootics could improve the effectiveness and reduce the cost of baculovirus-based control methods and could also contribute to the design of alternative or complementary strategies of microbial control instead of, or in addition to, the inundative use of baculoviruses as insecticides. However, further work is required to identify useful virus activation substances under field conditions.

We conclude that trace metals such as copper, iron and selenium can clearly modulate baculovirus induced disease, probably due to their different roles in insect immune functions. However, these substances are unlikely to prove useful for the activation of covert SeMNPV infections in *S. exigua* larvae under the greenhouse conditions described here.

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