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ORIGINAL PAPER

The attractiveness of phagostimulant formulations of a nucleopolyhedrovirus-based insecticide depends on prior insect diet

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Abstract The multiply-enveloped nucleopolyhedrovirus (SeMNPV) of *Spodoptera exigua* (Hübner) is being employed on an increasing scale as the basis for bioinsecticidal products for control of this pest in greenhouse crops in Europe. The mortality of diet-reared *S. exigua* larvae was determined after feeding on lettuce leaf discs contaminated by mixtures of SeMNPV occlusion bodies and 1 of 13 substances reported to have phagostimulant properties. Of the substances tested, wheatgerm and soya flour resulted in significantly increased mortality compared to the virus alone. However, these preferences disappeared when larvae were reared on lettuce. We conclude that (1) the activity of potential phagostimulant substances depends on the prior feeding experience of *S. exigua* and, (2) laboratory tests using diet-reared insects require validation using plant-reared larvae before being developed for field testing.

Keywords Baculovirus · Feeding preferences · *Spodoptera exigua* · Virus formulation

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Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest of diverse field and greenhouse crops. This species is globally distributed across southern Asia, the Asian-Pacific region, northern and sub-Saharan Africa, southern Europe and the Neotropics from southern USA to Central America and the Caribbean (Hill 1983; Pérez et al. 2000: Ehler 2007). The *S. exigua* multiple nucleopolyhedrovirus (SeMNPV, Baculoviridae) is an effective microbial control agent against larvae of this pest, especially on greenhouse crops (Smits et al. 1987; Kolodny-Hirsch et al. 1997; Bianchi et al. 2002; Lasa et al. 2007a, b). However, the requirement to produce these pathogens in vivo means that nucleopolyhedrovirus (NPV) insecticides tend to be more expensive than synthesized chemicals or biopesticides produced in bioreactors. Formulation of biopesticides can improve their efficacy such that acceptable levels of pest control can be achieved with low doses of pathogen, representing an important reduction in the cost of each application (Bartelt et al. 1990; Castillejos et al. 2002). In the case of NPV-based insecticides, the use of feeding stimulants that encourage phytophagous larvae to consume foliage contaminated with viral occlusion bodies (OBs) can result in an increased prevalence of infection and improved pest control (Andrews et al. 1975; Bell and Kanavel 1977). However, the added cost of such formulations may not always be justified by corresponding improvements in crop yields (Farrar and Ridgway 1994; Ballard et al. 2000).

In this study, we determined the prevalence of mortality of *S. exigua* larvae that fed on lettuce leaves contaminated by mixtures of viral OBs and a range of economical natural substances, the majority of which were identified from previous reports of their phagostimulant properties (reviewed

by Hunter-Fujita et al. 1998; Burges and Jones 1998; Williams and Cisneros 2001). Unexpected results then caused us to examine the role of prior insect diet on the phagostimulant characteristics of a number of the candidate feeding stimulants.

Materials and methods

Larvae were obtained from a colony of *S. exigua* reared on a modified Hoffman's tobacco hornworm diet (Hunter-Fujita et al. 1998), incubated at 25 ± 2°C, 70 ± 5% R.H. and 16 h:8 h L:D photoperiod in the insectary of the Universidad Pública de Navarra, Spain. OBs of a Spanish isolate (SP2) of SeMNPV (Caballero et al. 1992) were obtained by purification from infected fifth instars (Muñoz et al. 1998) and were quantified by counting using a Neubauer chamber (Hawksley Ltd., Lancing, UK).

To evaluate phagostimulant effects, leaf disc bioassays were performed. Lettuces were purchased at a supermarket and foliage was disinfected in 0.10% (wt/vol) sodium hypochlorite for 5 min and then rinsed under running water for 10 min. A cork borer was used to cut 27-mm-diameter discs in parts of the leaves which showed the same texture and colour, and which lacked veins. Leaf discs were dipped to the half way point in a mixture containing 1×10^4 OBs/ml of SeMNPV, one of 13 candidate phagostimulant substances at the corresponding concentration (Fig. 1a), and 0.05% (vol/vol) of the commercial wetter-sticker Erfitos® (nonylphenylpolyethylene ether, Union Explosivos Rio Tinto, Madrid, Spain). Initial assays indicated that this concentration of virus would result in ~50% insect mortality. Previous studies using groups of 10 *S. exigua* second-instars fed on lettuce discs treated with 0.05% Ertfitos indicated that this compound had no effect on the rate of weight gain over a 24-h period (mean weight after 24 h ± SD, 5.64 ± 0.46 mg/group of larvae), an indicator of feeding activity, compared to groups of larvae fed untreated leaf discs (mean ± SD, 5.26 ± 0.33 mg/group of larvae) (ANOVA based on 13 replicates, $F = 0.46$; $df = 1, 24$; $P = 0.50$). Seven half dipped discs were carefully placed around the edge of a 140-mm-diameter plastic Petri dish, containing a 2-mm-deep layer of bacteriological agar, and were allowed to air dry for 40 mins. Control discs were treated identically but were half dipped in wetter-sticker solution alone. Virus controls were also performed in which leaf discs were half dipped in an OB suspension with wetter-sticker but without phagostimulant substances. When discs had dried, groups of 55 *S. exigua* second instars from the laboratory colony were placed in the centre of each Petri dish and were allowed to feed ad libitum for 24 h in the dark at 25 ± 1°C. In this way, larvae could choose between feeding on the half of the leaf disc treated with the feeding stimulant substance and OBs or the untreated half. After this time, 50 larvae were randomly selected, individually transferred to dishes containing artificial diet, reared at 25°C and examined for virus mortality 1 week later. Each assay was performed three times using different batches of larvae.

As some of the candidate phagostimulants were also components of the artificial insect diet used to rear the laboratory colony, namely brewer's yeast and wheatgerm, we decided to test the feeding preferences of larvae that had been reared exclusively on lettuce. The bioassay procedure described above was repeated for yeast, wheatgerm, or soya flour using second instar *S. exigua* that had fed on lettuce leaves from the moment of hatching. Groups of 55 larvae were placed in Petri dishes containing seven leaf discs that had been half dipped in virus suspension + wetter-sticker, or control larvae that fed on discs half dipped in wetter-sticker alone. Other groups of larvae were offered leaf discs half treated with virus suspension + yeast, wheatgerm, or soya flour. After a 24 h feeding period, these larvae were individually reared on artificial diet and checked for virus mortality after 7 days. All bioassays were performed three times.

For both diet-reared and lettuce-reared insects, the median percentage of virus mortality in larvae from treatments involving feeding stimulants was compared to that of larvae that fed on discs treated with virus alone by the non-parametric Mann–Whitney *U* test (Sokal and Rohlf 1995).

Results

The median percentage of virus mortality observed in larvae that fed on leaf discs half treated with SeMNPV OBs alone was 44% (Fig. 1a). No mortality was observed in control larvae that fed on untreated leaf discs. Of the 13 substances tested, wheatgerm and soya flour resulted in significantly increased mortality compared to the virus alone. Of the remaining substances tested, Sorbex, sucrose and starch resulted in no increased mortality, whereas brewer's yeast was borderline significant ($U = 0.50$; $P = 0.07$). In contrast, monosodium glutamate (MSG), albumin, vegetable oil, citrus extract, skimmed milk, maize flour and wheat flour resulted in significantly reduced mortality compared to the virus control.

Three substances that showed significant (wheatgerm and soya flour), or near-significant (yeast), phagostimulant activity in the previous experiment were tested using larvae that had fed on lettuce leaves from hatching to the second instar. Lettuce-reared larvae that fed on leaf discs treated with OBs alone suffered 46% median mortality, whereas control larvae that fed on untreated leaf discs suffered no mortality (Fig. 1b). In contrast to the findings based on

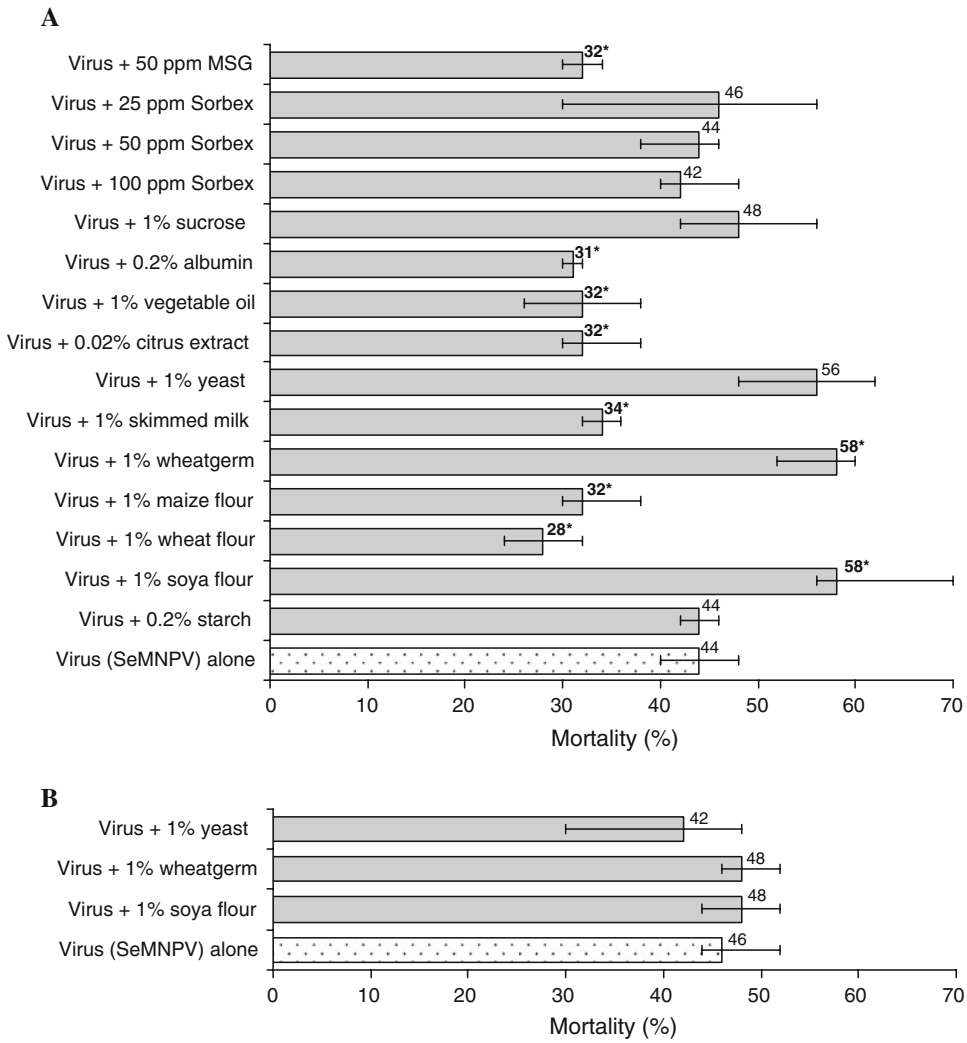

Fig. 1 Median percentage of mortality of *Spodoptera exigua* second instars (**a**) reared on artificial diet or (**b**) reared on lettuce, that fed on leaf discs half treated with SeMNPV occlusion bodies (OBs) alone (*dotted column*) or OBs in mixtures with different feeding stimulants and a wetter-sticker (*grey column*). *Error bars* indicate maximum–minimum range of mortality responses observed among replicates. Values beside columns indicate percentage of mortality. *Values in bold* indicate significant differences compared to the treatment involving virus OBs alone (Mann–Whitney *U* test, $*P \leq 0.05$). Concentrations of test substances are given as percentage wt/wt or parts per million (ppm)

diet-reared insects, none of the three substances tested resulted in significant increases in virus mortality in lettuce-reared insects compared to those that fed on leaf discs treated with virus OBs alone (Fig. 1b).

Discussion

In laboratory bioassays, wheatgerm and soya flour were identified as potential feeding stimulants in diet-reared *S. exigua* larvae. Surprisingly, 1% sucrose did not result in increased mortality of *S. exigua*, despite being considered as an important gustatory stimulant in other lepidopteran species (Bell and Kanavel 1978), although this effect may depend on the concentration of sugar used (Ballard et al. 2000). Indeed, the effectiveness of many feeding stimulants may be concentration dependent (Hostetter et al. 1982), but the large volumes of spray applications (500–1,000 l/ha) for greenhouse crops means that only feeding stimulants that function at low concentrations are likely to be economically viable. Similarly, the culinary flavour enhancer monosodium glutamate (MSG) and the more active product Sorbex (a mixture of MSG, inosinic acid and guanylic acid), that was tested at various concentrations (25, 50 and 100 parts per million, ppm), did not prove attractive to *S. exigua* larvae despite evidence that MSG has phagostimulant properties in tortricid larvae (Pszczolkowski et al. 2002) and can enhance the effectiveness of bioinsecticides (Pszczolkowski and Brown 2002; Pszczolkowski et al. 2004).

When larvae were reared exclusively on lettuce, the phagostimulant properties of wheatgerm, soya flour, and to a lesser extent yeast, disappeared. From this, it became evident that the attractiveness of these feeding stimulants depended on the previous dietary experience of the insect. Indeed, the host plant may contain natural gustatory stimulants that limit the effectiveness of formulation adjuvants both in *S. exigua* (Farrar and Ridgway 1994), and other species of Lepidoptera (Bartelt et al. 1990; Li and Fitzpatrick 1997; Chapman et al. 2003).

We arrived at three principal conclusions. First, most of the substances tested, including the flavour enhancers MSG and Sorbex, did not act as feeding stimulants in *S. exigua* despite published evidence of their attractiveness to phytophagous Lepidoptera. Second, the activity of potential phagostimulant substances depends on the prior feeding experience of *S. exigua*. Third, laboratory tests using insects reared on artificial diets require validation using plant-reared larvae before being developed for field testing. We hope these results will assist in the development of effective feeding stimulants for NPV-based insecticides for use in integrated pest management programs.

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