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Biological Control 32 (2005) 419–426
Biological Control
www.elsevier.com/locate/ybcon

Effect of dietary selenium supplementation on resistance to baculovirus infection

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Received 10 September 2004; accepted 26 December 2004

Abstract

We have examined the effects of dietary selenium (Se) supplementation on larval growth and immunocompetence of the lepidopteran pest, the cabbage looper, *Trichoplusia ni*. Supplementation of the diet of *T. ni* larvae with 10–20 ppm Se resulted in a 1 day delay in pupation. The effects of the addition and/or removal of dietary Se on total Se bioaccumulation and sequestration were determined by neutron activation analysis of pupae. Early penultimate instar larvae moved from selenium containing diet to basal diet lost total pupal Se content down to the level of those fed basal diet. Conversely, larvae moved from basal diet to diet containing additional Se rapidly attained pupal Se levels comparable to larvae fed Se throughout larval development. Therefore, dietary Se is rapidly accumulated or lost during larval development, but significant amounts are sequestered from diet into pupae. Larvae were reared on diet supplemented with 5 or 10 ppm Se until the onset of the penultimate instar then infected per os with increasing concentrations of the fatal baculovirus *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV). Larvae fed Se in the penultimate and ultimate instars were more resistant to viral infection than larvae not fed Se in the final instars. This study indicates that dietary Se levels rapidly impact Se assimilation and sequestration and that tissue Se levels are an important factor in resistance to AcMNPV infection in larval *T. ni*.
Published by Elsevier Inc.

Keywords: *Trichoplusia ni*; *Autographa californica* nucleopolyhedrovirus; Selenium; Biological control; Immunocompetence; Nutritional immunology

1. Introduction

Herbivorous insects encounter a range of dietary nutrients, antioxidants, co-factors, and toxic plant secondary metabolites which affect their development, reproduction, and behavior (Schultz, 2002). In their natural environment, insect populations are subjected to a withering onslaught of microbial pathogens and parasites. Maintaining a vigorous immune defense against pathogens may come at a significant cost to fitness via the diversion of nutritional resources from growth and reproduction (Rolff and Siva-Jothy, 2003). Although many plant derived compounds have demonstrated adverse effects on insect fitness, no plant derived compounds have been identified which impact the immunocompetence of herbivorous insects. Abiotic and biotic stress factors, fasting or specific nutritional deficiencies (i.e., malnutrition) may weaken the immune system enabling opportunistic infection (Rolff and Siva-Jothy, 2003). For example, the micronutrient selenium (Se) has been demonstrated to play a vital role in the immunocompetence of vertebrates (Beck et al., 2004). Se is a cofactor required for the activity of a number of selenoenzymes involved in the stress response, and the maintenance of high tissue antioxidant levels, which may contribute to a more robust antimicrobial and antiviral defense (Beck et al., 2004). This suggested to us that dietary Se may also play a role in insect immune responses. Our artificial diet used to rear *Trichoplusia ni*

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1049-9644/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2004.12.011

(Hübner) at our laboratory for three decades contained no added Se, resulting in a colony naturally depleted of this element.

Immunocompetence in insects is generally inferred from the survival of an infective challenge, from the circulating levels of antimicrobial peptides, lysozyme, encapsulation by immune cells, or melanization by the enzyme phenoloxidase (Rolff and Siva-Jothy, 2003). The latter two activities have been implicated in resistance to baculovirus infection (Clarke and Clem, 2003; Popham et al., 2004; Trudeau et al., 2001; Washburn et al., 1996). We report that dietary Se levels available to herbivorous insects may play a pivotal role in the resistance of lepidopteran larvae to a normally fatal baculovirus challenge.

2. Materials and methods

2.1. Insects

Trichoplusia ni (Lepidoptera: Noctuidae) larvae obtained from the BCIRL Insectary were reared on an artificial wheat germ based diet under a photoperiod of 14 h:10 h (L:D) at 55% relative humidity at 28 °C. The colony of *T. ni* has been maintained at BCIRL on a meridic diet for over three decades. This diet formulation contains Wesson's salt mixture minerals (Wilkinson et al., 1972) with no added Se and will subsequently be referred to as "basal diet." The low amount of Se present may be contributed predominantly by the wheat germ added to the diet (Anon, 2001). Thus, a viable colony of experimental insects naturally depleted of Se for many generations was fortuitously available for experimentation.

2.2. Selenium content determination

Groups of three pupae or larvae were killed by freezing, placed in pre-tared vials, and oven-dried at 65 °C. Dry weights were calculated. Se determinations were performed by the University of Missouri Research Reactor by instrumental neutron activation analysis using a modification of the method described in McKown and Morris (1978). Pupae were chosen for analysis because larvae completely void midgut contents prior to pupation, eliminating any contribution of diet contents within the digestive system to measured Se levels. Due to the expense of this technique, a single group of three pupae was used for each analysis. Se concentrations are expressed as μg Se/g dry weight (ppm).

2.3. Weight determination studies

Larvae were exposed to Se by the incorporation of differing concentrations of Na_2SeO_3 (Sigma Chemical, St. Louis, MO) directly into the diet during mixing. Three feeding regimes were tested at each of five concentrations of Se: (1) Se-supplemented diet present throughout the entire larval stage until pupation (Se/Se), (2) Se-supplemented diet present until the early fourth instar followed by transfer to basal diet until pupation (Se/NonSe or depletion), or (3) basal diet before the onset of the fourth instar followed by transfer to Se-supplemented diet until pupation (NonSe/Se or repletion). Control larvae were reared on basal diet containing no additional Se. Prior to the onset of the fourth instar, all larvae were fed diet dispensed into individual wax cups in 125 ml aliquots. A sheet of freshly oviposited *T. ni* eggs was stapled to the lids and larvae were allowed to develop in groups of approximately 70–100. At the onset of the fourth instar, larvae were manually transferred to fresh diet in wax cups with the appropriate Se regime and allowed to develop until pupation. Ten larvae were weighed from each of five cups for each Se concentration beginning in the second instar and the weights averaged. Groups of larvae were weighed daily and then discarded (to minimize the effect of handling on growth and survival) until more than 50% of the larvae had pupated. Statistical comparisons were made with the Tukey multiple comparison procedure when a significant ANOVA value was found ($P < 0.05$) (SigmaStat, Systat Software, Point Richmond, CA).

2.4. Viral infections

Virus was produced by per os infection of *T. ni* larvae with polyhedra from the L1 variant of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) (O'Reilly et al., 1992) and the resulting polyhedra were isolated and sucrose gradient purified (McIntosh and Ignoffo, 1983). For the larval bioassays, newly molted fourth instar larvae were infected per os by the droplet feeding method (Hughes et al., 1986) as modified (Slavicek et al., 1999) with concentrations ranging from 1×10^3 to 1×10^6 polyhedra/ml of AcMNPV. Larvae were presented with a 5 μl droplet consisting of virus suspended in water, sucrose, and blue food-coloring. After imbibing, larvae were placed in individual cells with diet. Larvae were monitored twice daily for death for 10 days and the times were recorded. Two bioassays were conducted for each of the three Se regimes.

2.5. Statistical analysis of bioassays

For the six experiments (EXP), two for each combination of Se concentration (SC) and a range of viral concentrations (VC), the cumulative number of dead insects and the elapsed time (T) were computed for each time of observation. An additional variable hour class (HC) was created whereby each observation was classified in 24 h increments, i.e., 0–36 was 24, 36–60 was