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Does Elevated Body Ni Concentration Protect Insects Against Pathogens? A Test Using Melanotrichus boydi (Heteroptera: Miridae)

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ABSTRACT.—I hypothesized that the naturally elevated Ni concentration of *Melanotrichus boydi* (718–789 μg g^{-1}) might protect this insect species against pathogen attack. I contrasted survival of *M. boydi* against survival of the low-Ni mirid *Lygus hesperus* when treated with three entomopathogenic biocontrol agents. Biocontrol agents used were the fungus, *Beauveria bassiana*, and infective juveniles of two nematode species (*Steinernema carpocapse* and *Heterorhabditis bacteriophora*). Biocontrol agents suspended in water were sprayed onto insects placed in petri plates in the laboratory. Control treatments consisted of spraying water onto comparable replicates. Separate experiments examined effects of biocontrol agents on nymphs and adults of each species. I monitored insect survival for 4 d after treatment and used survival analysis to contrast survival of individuals receiving control and biocontrol treatments for each species. Biocontrol agents significantly reduced the survival of *L. hesperus* in all but two trials and reduced the survival of *M. boydi* in all experimental trials. Both insect species were susceptible to these pathogens and I rejected the hypothesis that elevated body Ni concentration protects *M. boydi* from pathogen attack.

INTRODUCTION

Hyperaccumulators are plants with unusually elevated concentrations of metals in their tissues (Brooks *et al.*, 1977). Ni hyperaccumulators are defined by Reeves and Baker (2000) as species which contain >1000 μg Ni g^{-1} dry weight in the aboveground parts of at least one specimen collected from the wild. At the time of their review, Reeves and Baker (2000) estimated that 418 hyperaccumulator taxa had been discovered, with the majority (76%) hyperaccumulating Ni. The remaining taxa hyperaccumulated other metals, such as Zn, Pb, Cd, Cu, Co, Mn or Se (Reeves and Baker, 2000).

The elevated metal content of hyperaccumulator plants creates a chemically unusual food source for herbivores that has probably contributed to plant/herbivore coevolution (Pollard, 2000). Generalist insect folivores offered leaves containing elevated metal concentrations may be deterred from feeding (Pollard and Baker, 1997) or killed after ingesting them (Boyd and Martens, 1994; Boyd and Moar, 1999), suggesting that metals constitute an ''elemental'' plant defense (Martens and Boyd, 1994). However, Boyd and Martens (1998) predicted that some herbivore species consume hyperaccumulator tissues without harm because they have evolved metal tolerance. They further suggested that some insect herbivores which feed on hyperaccumulators might sequester metal for their own defense, just as some insects do with certain organic plant defensive chemicals (*e.g.*, the moth *Utetheisa ornatrix* and pyrrolizidine alkaloids; Eisner and Meinwald, 1995). Indeed, metal-based defense of animal species has been suggested in scattered cases in the literature (*e.g.*, Gibbs *et al.*, 1981; Hopkin and Martin, 1984; Capon *et al.*, 1993). Thus, in naturally occurring populations of metal hyperaccumulating plants, Boyd and Martens (1998) postulated that: (1) a metal tolerant insect fauna feeds on hyperaccumulators, (2) some of these species would themselves have relatively elevated body metal concentrations and (3) high-metal insect species may be protected against attack by predators.

Wall (1999) surveyed the arthropod fauna associated with a California Ni hyperaccumulator in search of high-Ni herbivores. The hyperaccumulator, *Streptanthus polygaloides* Gray (Brassicaceae), may contain as much as 14,800 μg Ni g^{-1} in its tissues (Reeves *et al.*, 1981). Only one of the 33 arthropod taxa studied by Wall (1999) had a body Ni concentration >200 μg Ni g^{-1}. This insect species proved to be undescribed, and was later named *Melanotrichus boydi* (Heteroptera: Miridae) by Schwartz and Wall (2001). This species was remarkable among the arthropod fauna associated with *S. polygaloides* because of its greatly elevated body Ni concentration, which ranged from 718 to 789 μg Ni g^{-1} (Schwartz and Wall, 2001). It also appeared to be monophagic on *S. polygaloides* and, because its Ni concentration was much higher than that of other hemipteran species collected from and apparently feeding upon *S. polygaloides*, Wall (1999) concluded that *M. boydi* sequesters Ni from its host plant.

Discovery of this high-Ni insect species provided an opportunity to test the hypothesis that sequestered Ni can defend insect herbivores. Boyd and Wall (2001) conducted an initial test of this hypothesis using four species of arthropod predators. They collected high-Ni *Melanotrichus boydi* from the wild and also collected low-Ni insect species. Predators were fed either high- or low-metal insects and predator survival was compared for the two diets. Survival of three of the predator species used did not differ between high- and low-Ni diets, but the crab spider *Misumena vatia* suffered decreased survival when fed *M. boydi*. Boyd and Wall (2001) concluded that a defensive function of Ni was possible for *M. boydi*, but that generalist arthropod predators probably would be unaffected under natural conditions because their broad diet ranges diluted total Ni intake below the toxic threshold. Boyd and Wall (2001) suggested that a defensive effect of elevated body Ni concentration was more likely against pathogens because pathogens inhabit the tissues of a single host individual. Thus, they would be unable to use dietary dilution to avoid exposure to elevated host Ni levels.

Herbivore diets may affect the pathogens of herbivores. For example, Hunter and Schultz (1993) found improved survival of gypsy moth (*Lymantria dispar*) larvae exposed to a viral disease when the larvae consumed leaves containing elevated levels of gallotannins. I tested the hypothesis that elevated body Ni concentration defends *Melanotrichus boydi* against pathogens, using pathogens that have been developed as biocontrol agents against agricultural and horticultural insect pests (Hagler, 2000; Hall and Hall, 1999). I contrasted survival of *M. boydi* with that of a widespread generalist mirid species (*Lygus hesperus*) to determine if *M. boydi* has greater pathogen resistance.

MATERIALS AND METHODS

Study organisms: plant species.—*Streptanthus polygaloides* is a winter annual endemic to serpentine soils on the western slope of the Sierra Nevada in California, USA (Hickman, 1993). It ranges from Butte Co. in the north to Fresno Co. in the south (Munz and Keck, 1968), spanning approx. 350 km. All populations hyperaccumulate Ni (Kruckeberg and Reeves, 1995) and all plant organs hyperaccumulate Ni, ranging from 1100 μg Ni g^{-1} in fruits to as much as 14,800 μg Ni g^{-1} in leaves (Reeves *et al.*, 1981).

Study organisms: insect species.—*Melanotrichus boydi.*—Schwartz and Wall (2001) reported a close association between *M. boydi* and *Streptanthus polygaloides*, visiting 10 *S. polygaloides* populations across the species' geographic range and encountering *M. boydi* each time. Wall (1999) also conducted feeding trials that demonstrated preference of *M. boydi* for *S. polygaloides*. Wall (1999) observed *M. boydi* feeding primarily on flowers and young leaves with its piercing/sucking mouthparts.

Individuals of *Melanotrichus boydi* used in these experiments were collected from *Strep-*

tanthus polygaloides at two sites reported by Schwartz and Wall (2001). The first, in Placer Co. on the western slope of the Sierra Nevada, is located on a north-south trending serpentine belt centered at the town of Washington (elev. 796 m) on the South Fork of the Yuba River. *Streptanthus polygaloides* grows on serpentine areas on both north and south sides of the river and *M. boydi* is also found in both areas. Elevations of the serpentine locations used for collecting *M. boydi* on the south side of the river ranged between 1000 and 1220 m, whereas the location used on the north side of the river ranged between 800 and 915 m. The second *M. boydi* population was located in El Dorado Co. This site is a serpentine exposure located in a small basin at 1270 m on the western slope of the Sierra Nevada. Thinly populated by conifers, it is located about 1 km south of Sugar Pine Reservoir.

Streptanthus polygaloides grows in open areas between trees and on rocky outcrops and slopes on both sites. Individuals of *Melanotrichus boydi* were collected directly from *S. polygaloides*, placed into plastic containers along with cut *S. polygaloides* plants and refrigerated at approx. 4 C until used for experiments.

Lygus hesperus (Knight) (Heteroptera: Miridae) is well known as a pest in agricultural situations, with a relatively broad plant host range (Scott, 1977). It is similar in size to *Melanotrichus boydi*, can be cultured on artificial diet and is raised commercially for use in research in controlling pest outbreaks. *Lygus hesperus* is also a good choice for comparison with *M. boydi* because *Streptanthus polygaloides* hosts both bug species in the wild. Wall (1999) reported collecting *L. hesperus* adults from *S. polygaloides* at the same locality as one population of *M. boydi*. He found the Ni concentrations of these *L. hesperus* were elevated (131 $\mu g\ g^{-1}$), but much less than those of *M. boydi* collected from *S. polygaloides* at the same site (777 $\mu g\ g^{-1}$).

For our experiments, *Lygus hesperus* was obtained from a commercial supplier (BioTactics, Riverside, CA) as a mixture of late instar and adult stages. These insects had been reared on artificial diet (Debolt, 1982) and were refrigerated at approx. 4 C until used for experiments. During storage, cut fresh green beans (*Phaseolus vulgaris* L.: Fabaceae) were added to containers to supply additional food.

Study organisms: pathogen species.—Beauveria bassiana (*Balsamo*) *Vuillemin* (*Hyphomycetes*: *Moniliales*).—This imperfect fungus (Phylum Deuteromycota) has a relatively wide host range that includes insect species from most orders (Boucias and Pendland, 1998). As such, it can be an effective insect biocontrol agent (*e.g.*, Wraight *et al.*, 2000) under proper environmental conditions. *Beauveria bassiana* was a particularly appropriate choice for our experiments, as it has been reported to be an effective pathogen against mirid bugs in the genus *Lygus*. Examples include several species of Canadian *Lygus* (Bidochka *et al.*, 1993), *L. lineolaris* in Arkansas (Steinkraus and Tugwell, 1997) and *L. rugulipennis* and *L. lineolaris* in Europe (Riba *et al.*, 1986). As with other mycoinsecticides, *Beauveria* spores bind to the host cuticle, germinate and penetrate into the body. Death from mycoinsecticides can occur within a few days (Rechcigl and Rechcigl, 1999); Steinkraus and Tugwell (1997) reported that *B. bassiana* killed 89% of *Lygus lineolaris* within 5 d after exposure to spores.

Steinernema carpocapse (Weiser).—Steinernematid nematodes are obligate parasites of insects (Burnell and Stock, 2000). They have a remarkably broad host range in laboratory tests; Poinar (1979) reported they could attack more than 250 insect species from 11 orders. Found in soil on all continents except Antarctica, the infective juvenile stage seeks out insect larvae and enters through natural body openings (*i.e.*, mouth, anus and spiracles). Once in the host, the juvenile penetrates into the hemocoel and releases cells of a symbiotic bacterium carried in the juvenile's intestine (Burnell and Stock, 2000). The bacteria multiply rapidly in the hemocoel and release toxins that rapidly kill the host (usually within 24–48 h).

Heterorhabditis bacteriophora Poinar.—Like *Steinernema carpocapse*, *H. bacteriophora* is also globally distributed and infects a wide variety of insects (Grewal and Georgis, 1999). This nematode species is similar to *S. carpocapse* in mode of action and effect (Burnell and Stock, 2000). It possesses a different obligate bacterial symbiont, *Photorhabdus luminescens*, which is released into the hemocoel after infective juveniles penetrate the host and kills the host in about 48 h (Boucias and Pendland, 1998).

Several commercial suppliers sell both *Heterorhabditis bacteriophora* and *S. carpocapse* for insect biocontrol use. A recent quality assessment of these suppliers (Gaugler *et al.*, 2000) showed that the company which supplied our cultures (Integrated BioControl Systems) has a good record for delivering accurate numbers of infective juveniles.

EXPERIMENTAL PROCEDURES

Insects were maintained in small (5 cm diam.) plastic petri plates with a piece of filter paper placed in the bottom. Several small pieces of plant material were placed in each plate as food for the insects. Plates with *Melanotrichus boydi* contained 3 or 4 short (3–4 cm long) terminal portions of *Streptanthus polygaloides* inflorescence. This plant material was collected from the same site as the *M. boydi* used in each experimental trial. Plates containing *Lygus hesperus* contained 3 or 4 short (3–4 cm long) sections of fresh green beans, cut into halves lengthwise.

Experimental treatments were applied after insects and food materials had been placed into the petri plates. Pathogen treatments were applied using a hand-operated spray bottle to thoroughly wet insects and food materials. Excess moisture not absorbed by the filter paper was removed with a paper towel. Control treatments consisted of spraying similar amounts of water into control treatment plates. Plates were maintained at room temperature during each experiment. Data were the number of live insects in each petri plate counted daily for 4 d.

Beauveria bassiana.—Two experiments were conducted using *B. bassiana*, one using nymphs and the other using adults of *Melanotrichus boydi* and *Lygus hesperus*. Both nymphs and adults of *M. boydi* were obtained from the El Dorado County population. Spores of *B. bassiana* were applied using BotaniGard™ ES, an emulsifiable suspension produced by Mycotech Corporation (Butte, MT). Stock suspension (containing 2.1×10^{13} viable spores/liter) was diluted to make a suspension containing about 2.4×10^{7} viable spores/ml. The diluted suspension was sprayed onto petri plates and insects receiving the pathogen treatment.

For the nymph experiment each petri plate contained 3 nymphs. Each species/treatment combination was represented by 10 petri plates, for a total of 30 individuals per species/treatment combination. Food materials were replaced with fresh materials two Days After Treatment (DAT). In the adult experiment each petri plate contained five adults. Ten plates were used for each species/treatment combination. *Streptanthus polygaloides* inflorescence sections used as food for *Melanotrichus boydi* appeared to be in good condition during all four days of the experiment, but the cut bean slices given *Lygus hesperus* aged rapidly and so were replaced with fresh slices two DAT.

Steinernema carpocapse.—Three experiments were conducted using *S. carpocapse*. The first used nymphs of *Melanotrichus boydi* from Placer Co., whereas the second used nymphs from El Dorado Co. For each nymph experiment five nymphs were placed in each petri plate with five replications for every species/treatment combination. For the first experiment nematodes from a sponge containing approximately 2 million nematodes (estimated by the commercial supplier) were suspended in about 2 liter of water, whereas for the second experiment the same procedure was followed using a sponge containing about 5 million

nematodes. In each case the nematode suspension was immediately sprayed onto the pathogen treatment plates and insects. Water was used as a control spray treatment. Plant materials placed into the petri plates as food for the insects were replaced with fresh materials two DAT for the first experiment and three DAT for the second experiment.

The third experiment examined the response of adult insects to *Steinernema carpocapse*. Adult *Melanotrichus boydi* were obtained from El Dorado Co. Each petri plate contained eight individuals of either insect species, with five petri plates used for each species/treatment combination to yield a total of 40 individuals of each species per treatment. A nematode suspension was made using a sponge containing 5 million nematodes and applied to the pathogen treatment plates as described above. Water was used as a control spray treatment. Inflorescence sections of *S. polygaloides* appeared in good condition during the experiment, but the cut bean slices aged more rapidly and were replaced with fresh slices two DAT.

Heterorhabditis bacteriophora.—Three experiments also were conducted using *H. bacteriophora*. As with the *Steinernema carpocapse* experiments, one used nymphs of *Melanotrichus boydi* from Placer Co. whereas the other used nymphs from El Dorado Co. For each nymph experiment, three nymphs were placed in each petri plate with ten replications of each species/treatment combination. In both experiments, nematodes from a sponge containing 5 million nematodes were suspended in about 2 liter of water and the suspension was immediately sprayed onto pathogen treatment plates and insects. Water was used as a control spray treatment. Plant food materials placed into petri plates were replaced two DAT for both experiments.

A third experiment examined the response of adult insects to *Heterorhabditis bacteriophora*. Adult *Melanotrichus boydi* were obtained from the El Dorado County population. Each petri plate contained five individuals of either species, with ten petri plates used for each species/treatment combination yielding a total of 50 individuals of each species per treatment. A nematode suspension was made from a sponge containing 5 million nematodes and applied to the pathogen treatment plates and insects as described above for the nymph experiments. Water was used as a control spray treatment. Inflorescence sections of *Streptanthus polygaloides* were in good condition for the duration of the experiment, but the cut bean slices were replaced with fresh slices two DAT.

STATISTICAL ANALYSIS

Survival data were compared between pathogen-treated and control (water-treated) individuals of each species to determine if mortality differed significantly due to treatments. Data were analyzed by survival analysis (Abacus Concepts, 1994) using the Kaplan-Meier estimate, with treatment significance determined by the Peto-Peto-Wilcoxon rank test at alpha ≤ 0.05.

RESULTS

Beauveria bassiana.—Nymphs of the two insect species responded differently when inoculated with *B. bassiana*. *Lygus hesperus* (Fig. 1) survival did not differ between pathogen and control treatments (chi square = 0.005, df = 1, P = 0.946). In contrast, 97% of *Melanotrichus boydi* nymphs from the control treatment remained alive four DAT, compared with only 23% of those treated with *B. bassiana* (Fig. 1). These *M. boydi* survival curves differed significantly from each other (chi square = 27.8, df = 1, $P < 0.0001$). Survival of adults of both species significantly declined when exposed to *B. bassiana*. By four DAT, only 8% of pathogen-treated adults of *L. hesperus* remained alive, compared with 68% of control treatment insects (chi square = 26.5, df = 1, $P < 0.0001$; Fig. 1). Survival of *M. boydi*

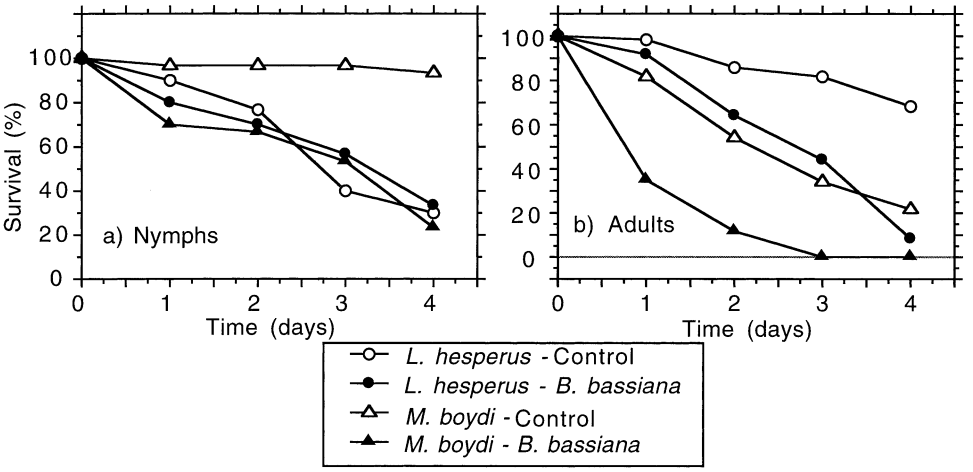

FIG. 1.—Survival of *Lygus hesperus* and *Melanotrichus boydi* inoculated with spores of *Beauveria bassiana*. a) Experiment using nymphs. b) Experiment using adults

(Fig. 1) also differed significantly between treatment groups (chi square = 37.2, df = 1, P < 0.0001).

Steinernema carpocapse.—In the nymph experiment using Placer Co. *Melanotrichus boydi*, survival of both species was significantly reduced by nematode treatment. Survival of *Lygus hesperus* controls (Fig. 2) was 72% vs. 16% for the nematode treatment by four DAT (chi square = 14.1, df = 1, P = 0.0002). Survival of *M. boydi* showed a similar significant contrast (Fig. 2), with 96% of control individuals alive at the end of the experiment vs. 42% of nematode-treated individuals (chi square = 17.7, df = 1, P < 0.0001). The nymph experiment using *M. boydi* from El Dorado Co. showed similar results. Survival of *L. hesperus* was significantly affected by treatments (chi square = 13.4, df = 1, P = 0.0003), with decreased survival for those treated with nematodes (Fig. 2). Survival of *M. boydi* nymphs also declined significantly for the *S. carpocapse* treatment (chi square = 15.3, df = 1, P < 0.0001).

Adults of both species responded similarly to nymphs, showing greater mortality in response to nematode treatment (Fig. 2). Survival of *Lygus hesperus* differed significantly (chi square = 34.2, df = 1, P < 0.0001) as did survival curves for *Melanotrichus boydi* (chi square = 27.1, df = 1, P < 0.0001).

Heterorhabditis bacteriophora.—Results of the first experiment using nymphs differed between insect species (Fig. 3). Treated nymphs of *Lygus hesperus* survived equally compared with the controls (chi square = 0.032, df = 1, P = 0.858) but survival of *Melanotrichus boydi* was significantly reduced (chi square = 4.91, df = 1, P = 0.0268). The nymph experiment using *M. boydi* from El Dorado Co. (Fig. 3) showed significantly reduced survival of both insect species when treated with *H. bacteriophora* (*L. hesperus*; chi square = 17.6, df = 1, P < 0.0001: *M. boydi*; chi square = 24.5, df = 1, P < 0.0001).

When treated with *Heterorhabditis bacteriophora*, adults of both species also showed decreased survival (although the effect was only marginally significant for *Lygus hesperus*). Survival curves for *L. hesperus* differed between treatments only in the final two days of the experiment (Fig. 3) and the curves were only marginally different (chi square = 2.92, df

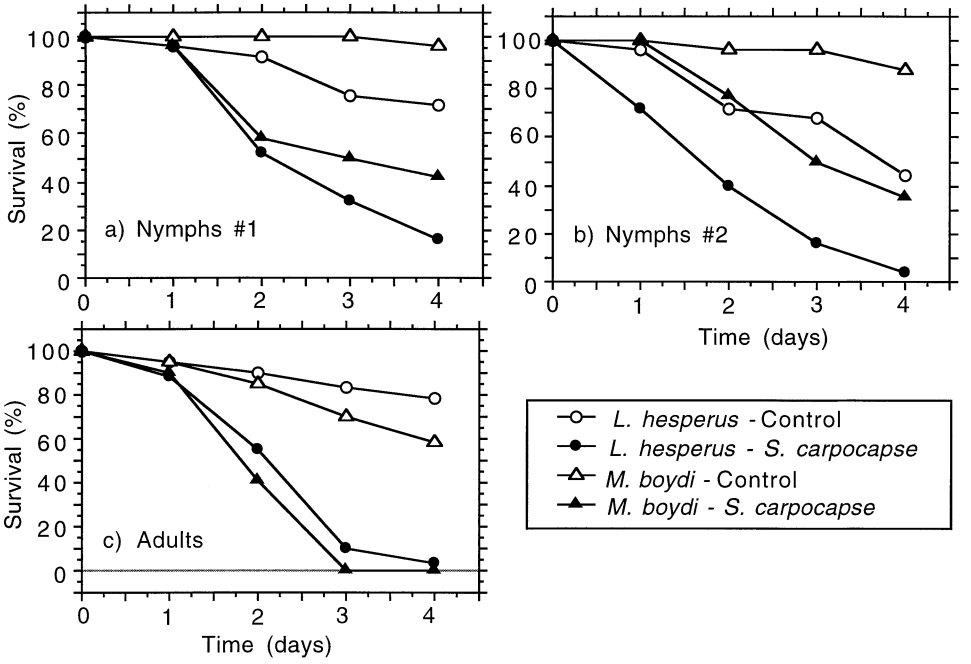

FIG. 2.—Survival of *Lygus hesperus* and *Melanotrichus boydi* inoculated with infective juveniles of *S. carpocapse*. a) Experiment using nymphs of *M. boydi* from Placer Co. b) Experiment using nymphs of *M. boydi* from El Dorado Co. c) Experiment using adults (*M. boydi* from El Dorado Co.)

= 1, P = 0.0874). Results for *Melanotrichus boydi* were more distinct, as survival was significantly less for treated adults (chi square = 8.16, df = 1, P = 0.0043).

DISCUSSION

Host-plant chemicals sequestered by herbivores may protect the herbivores from predators. Examples include certain aphids (Harborne, 1988), monarch butterflies (Harborne, 1988), buckeye butterflies (Strohmeyer *et al.*, 1998) and chrysomelid leaf beetles (Hartmann *et al.*, 1997; Ehmke *et al.*, 1999). It is also possible that sequestered chemicals provide a defense against pathogens (as suggested by the results of Hunter and Schultz, 1993), but this effect is less well studied. Boyd and Wall (2001) hypothesized that elevated body Ni concentration might provide *Melanotrichus boydi* with a defensive benefit. In particular, they suggested that metal might protect against pathogens because pathogens could not use diet dilution to circumvent a metal-based defense. The results reported here failed to provide evidence supporting the hypothesis that elevated body Ni concentration protects *M. boydi* against pathogen attack. Survival of *M. boydi* was decreased by exposure to pathogens in every experimental trial. *Lygus hesperus* was similarly affected, excepting two experiments in which a survival difference between pathogen and control treatments was either marginally significant (*Heterorhabditis bacteriophora* adult experiment) or not significant (*Beauveria bassiana* nymph experiment). Lack of significant effects in the latter two cases might have stemmed from the low survival of control treatment insects. However, it is clear from these results that *M. boydi* is not resistant to the negative affects of the three pathogenic organisms used.

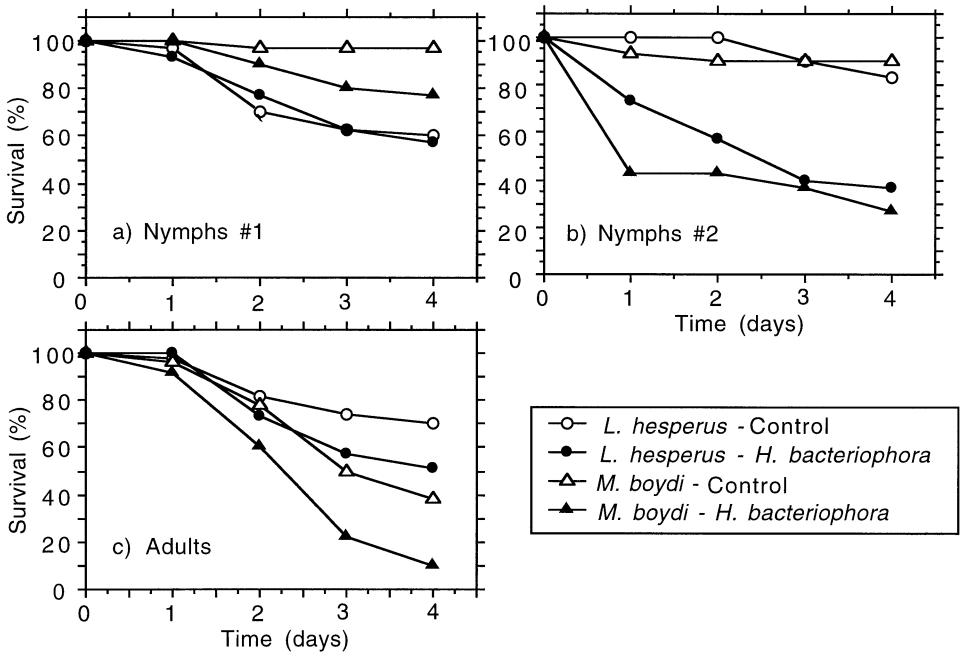

FIG. 3.—Survival of *Lygus hesperus* and *Melanotrichus boydi* inoculated with infective juveniles of *H. bacteriophora*. a) Experiment using nymphs of *M. boydi* from Placer Co. b) Experiment using nymphs of *M. boydi* from El Dorado Co. c) Experiment using adults (*M. boydi* from El Dorado Co.)

Melanotrichus boydi is susceptible to pathogens because the Ni dose present does not prevent the pathogens from negatively affecting *M. boydi*. There are two possible explanations for this: either the pathogens are relatively Ni tolerant or the Ni concentrations of the *M. boydi* tissues they infect are below the pathogens' sensitivity threshold to Ni. Because I used generalist pathogens obtained from standard commercial cultures (and hence low in Ni), it seems unlikely that these pathogens were unusually Ni tolerant. Therefore, it is probable that the Ni dose they experienced was too low to negatively affect them. The pathogens I used are in intimate contact with the hemolymph of infected insects (Tanada and Kaya, 1993; Burnell and Stock, 2000). This in turn suggests that the Ni dose in hemolymph of *M. boydi* was not elevated enough to be toxic. This could be due to concentration of Ni in other locations of the body, such as the gut or the exoskeleton. Unfortunately, no information on tissue-level Ni concentrations is yet available for *M. boydi* to explore this explanation. Indeed, there are relatively few reports of tissue-level metal distributions in arthropods generally (*see* Hopkin, 1989). Recent efforts to document the distribution of metals in cells and tissues of hyperaccumulator plants (*e.g.*, Mesjasz-Przybylowicz *et al.*, 1996; Heath *et al.*, 1997; Küpper *et al.*, 2000; Psaras *et al.*, 2000) need to be extended to insects such as *M. boydi* so that this question can be definitively addressed.

These results also contribute to our understanding of elemental plant defenses. Metal-based plant defenses have been termed "elemental defenses" (Martens and Boyd, 1994; Boyd, 1998) to differentiate them from plant chemical defenses synthesized by plants' biochemical machinery. As with organic defenses, elemental defenses protect plants from some herbivores and pathogens but are less effective against others. For the Ni hyperaccumulator

Streptanthus polygaloides, examples of herbivores or pathogens against which Ni is relatively ineffective include *Melanotrichus boydi* (Schwartz and Wall, 2001), the pea aphid *Acyrthosiphon pisum* (Boyd and Martens, 1999), the parasitic plant *Cuscuta californica* var. *breviflora* (Boyd *et al.*, 1999), and *Turnip mosaic virus* (Davis *et al.*, 2001). In general, these studies show that the Ni concentration of herbivore or parasitic plant bodies increases when hyperaccumulating tissues are tapped. However, the Ni concentration in the herbivore or parasitic plant is always less than that of the host. This fact, and our failure to demonstrate a defensive function of Ni against pathogens, strengthens the hypothesis that elevated body Ni concentration is simply a consequence of a high metal diet. Thus, a further difference between elemental and organic defenses may be the lack of a defensive benefit of the sequestered element.

Boyd (1998) and Pollard (2000) suggested that metal hyperaccumulation provides unique opportunities for the study of plant/herbivore coevolution. Similar opportunities to study herbivore/predator and herbivore/pathogen coevolution are provided by the existence of high-metal herbivores. To my knowledge, the research described here and in Boyd and Wall (2001) are the first tests of the defense hypothesis for an insect species naturally feeding upon a metal hyperaccumulator. Despite the lack of a defensive effect for *Melanotrichus boydi*, Boyd and Martens' (1998) prediction of a defensive role of metals for some insect herbivores may still prove correct as other examples are studied. A major obstacle to progress in this area is a general lack of knowledge regarding arthropod faunas of serpentine areas (Kruckeberg, 1984). Currently, some researchers are examining the metal concentrations of serpentine insects (*e.g.*, Davison *et al.*, 1999) and surveys targeting insect herbivores associated with hyperaccumulators are being attempted (*e.g.*, Wall, 1999). These efforts will probably identify appropriate insect species if they target high biomass hyperaccumulator plant species, because these species may host extensive herbivore faunas. Promising high biomass Ni hyperaccumulator species include the tree *Sebertia acuminata* Pierre *ex* Baillon from New Caledonia (Jaffré *et al.*, 1976), numerous shrub species recently documented from Cuba (Reeves *et al.*, 1996, 1999), and the relatively high biomass herbaceous perennial *Berkheya coddii* Roessler from South Africa (Anderson *et al.*, 1997). Tests using additional examples of high-Ni insects will show if the lack of defensive effects for Ni in *M. boydi* is a generality or an exception for elemental defenses.

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