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The Effect of Temperature, Diet, and Larval Instar on the Susceptibility of an Aphid Predator, *Hippodamia convergens* (Coleoptera:Coccinellidae), to the Weak Bacterial Pathogen *Pseudomonas fluorescens*

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We tested the effects of larval age and stress on the susceptibility of the convergent lady beetle (*Hippodamia convergens*) to the weakly pathogenic bacterium *Pseudomonas fluorescens*. To test for the effects of larval age, the dose response to the bacterium was determined for each instar. The LC_{50}s for first, second, and third instars (4.8×10^9, 2.8×10^{10}, and 3.9×10^9 CFU/ml, respectively) were not significantly different from each other, but the LC_{50} for fourth instar larvae was 40 times greater at 3.2×10^{11} CFU/ml. To test for the effects of stress, first instar larvae were maintained at one of two temperatures (25 or 30°C) for 18 hr and were given one of three diets (water, sucrose solution, or aphids) for 24 hr before being exposed to the pathogen. Larvae maintained at 25°C and fed aphids (the optimum diet) had the highest susceptibility with an LC_{50} of 4.8×10^9 CFU/ml. The least susceptible larvae were those maintained at 25°C and fed only water, with an LC_{50} of 7.3×10^{11} CFU/ml, i.e., starvation increased resistance 150-fold. Temperature had a significant effect on the dose response only for the water diet treatment, decreasing the LC_{50} from 7.3×10^{11} CFU/ml at 25°C to 8.9×10^9 CFU/ml at 30°C. These results suggest that some stressors, such as poor diet, may decrease the susceptibility of *H. convergens* to *P. fluorescens*, whereas certain combinations of stressors, such as starvation and high temperature, have an interactive effect that then increases susceptibility.

KEY WORDS: ***Hippodamia convergens; Pseudomonas fluorescens;* microbial pesticides; temperature stress; diet stress; biological control.**

INTRODUCTION

Microbial pesticides are increasing in use because of their specificity and low mammalian pathogenicity but could be used more effectively in integrated pest management systems if their impact on beneficial insects was better understood. The U.S. Environmental Protection Agency (EPA) requires individuals applying for registration of a microbial pesticide to test for the presence of toxic or pathogenic effects on representative beneficial insects (Pesticide Testing Guidelines, 1989). The results of these bioassays may be affected by the conditions of the tests. Such conditions may be the age of the insect, or exposure to factors which stress the insect such as extremes in temperature, relative humidity, or diet. Past studies have shown that mortality due to bacterial infections can be affected by larval age (McGaughey, 1978; Beegle *et al.*, 1981; Wraight *et al.*, 1981; Rock and Monroe, 1983), temperature (Tashiro, 1957; Ignoffo, 1962; McLaughlin, 1962a,b; Hurpin, 1968; and Wraight *et al.*, 1981), and diet (Rinderer and Rothenbuhler, 1974; Kea *et al.*, 1978; Beegle *et al.*, 1981). However, environmental factors such as temperature, which affect the susceptibility of the insect, may also affect the pathogen. Few studies have addressed this, Lighthart *et al.* (1988) and Donegan and Lighthart (1989) exposed arthropods to different environmental conditions before exposure to an entomopathogen and found such factors as nutrition, temperature, and crowding to have a significant effect on susceptibility.

In a similar manner, we tested the effect of temperature, diet, and larval instar on the susceptibility of *Hippodamia convergens* Gue. (Coleoptera: Coccinellidae), to infection by the weak bacterial pathogen *Pseudomonas fluorescens*. *H. convergens* is a major predator of aphids and occurs throughout most of the United States and as such is an important biological control agent of aphids and other pest insects. This research was used to develop a standard bioassay for screening microbial pesticides for effects on nontarget, beneficial coccinellids (James and Lighthart, 1990).

MATERIALS AND METHODS

Insect culture. H. convergens larvae were laboratory reared, individually, in 20-ml glass scintillation

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vials with foam stoppers. They were fed live pea aphids (*Acyrthosiphon pisum* (Harris)) and maintained at 25°C, 70% RH, and 14:10 hr light:dark. Pea aphids were reared on fava bean seedlings. See James and Lighthart (1990) for a complete description of rearing procedures.

Microbiological culture. The original isolate of *P. fluorescens* was obtained from the hemolymph of dead adult beetles during a time when there was high mortality in our laboratory colony. This bacterium is an aerobic Gram-negative bacillus, glucose nonfermenter. It was identified using MicroScan's (Travenol Laboratories) Neg Combo Type 6/Neg ID test panel. *P. fluorescens* was cultured in Lurin–Bertani (LB) (Difco)[1] broth on a rotary shaker at 30°C for 18 hr to a concentration of ca. 10^9 CFU/ml. The bacterial cells were washed once in sterile saline (0.85% NaCl) and suspended in sterile distilled water at 1/100th the original volume to produce a concentration of approximately 10^{11} CFU/ml.

Experimental design. Ten treatments were set up: six diet and temperature combinations and four larval instars. For each test treatment, five to seven concentrations of the bacterium (in water) were used which ranged from 10^6 to 10^{11} CFU/ml; all dilutions were equally spaced on the $\log_{10}$ scale. The actual concentration of the solutions was determined using plate counts on LB agar. Twenty insects were used per concentration for a total of 120–160 insects per treatment. Insects were individually dipped in the bacterial solutions for 10 sec, so that each insect was a replicate. The entire bioassay was repeated twice. After exposure to the pathogen, insects in all treatments were maintained on aphids at 25°C, 70% RH, 14:10 hr light:dark. The effect of larval instar on susceptibility was determined by comparing the dose response of each of the four larval instars at 25°C on an aphid diet. First instar larvae were used to determine the effects of diet and temperature stress on susceptibility. Larvae ≤24 hr posteclosion were exposed to one of two temperatures (25 or 30°C) for 18 hr and fed one of three diets (water, 5% sucrose in water, or aphids) for 24 hr before they were exposed to the pathogen. Thus, the environmental conditions preceded the bacterial exposure to eliminate the direct effect of these variables on the pathogen.

Mortality is reported for Day 6 after inoculation because most mortality occurred by Day 3 and little or no mortality occurred after Day 5 except for fourth instar larvae. Fourth instars pupated before 6 days and so postinoculation mortality included insects that failed to emerge from the pupa. Larvae were considered dead when they would not move of their own accord, even when prodded.

A probit dose response was determined for each treatment using the statistical program SAS PROBIT (SAS, 1990). The data for each replicate were combined into one probit analysis since both replicates were conducted in the same manner. The different stress treatments were found to have different control mortalities; therefore, the data were standardized using Abbot's formula (Abbot, 1925). Comparisons of the slopes and *y*-intercepts of the probit lines were made using SAS PROBIT to determine whether or not there was a significant difference in dose responses among treatment groups. The slopes of the probit lines for all instars were not significantly different from each other, nor were the slopes of the probit lines for first instars given the different stress treatments. Therefore the slopes were considered equal to each other in the probit models and any significant differences in the *y*-intercepts were interpreted to mean a significant difference in the LC_{50}s (the concentration that causes 50% of the larvae to die), and are reported in that way. Probits were transformed back to percentage mortality for the figures.

RESULTS

Susceptibility of different instars. At 25°C with an aphid diet, there were no differences in susceptibility among the first three instars. However, these three instars were significantly ($P \leq 0.001$ for each instar) more susceptible than fourth instar larvae (Fig. 1) and the LC_{50} for fourth instar larvae was 40.7 times greater than the mean LC_{50} of the other three instars (Table 1). Most, if not all mortality caused by *P. fluorescens* in fourth instar larvae occurred before pupation, because 97% of those that pupated developed to adults.

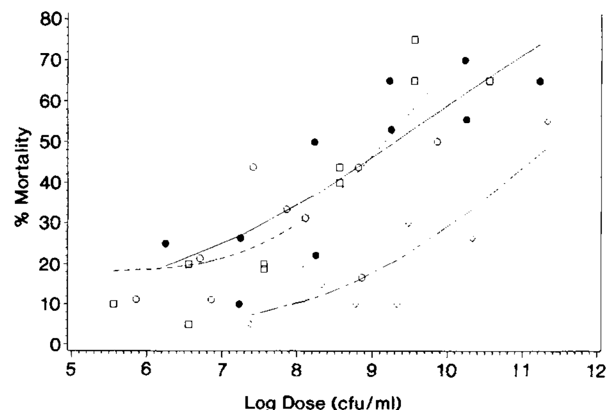

FIG. 1. Relative susceptibility of different instars of *H. convergens* to *P. fluorescens*. Larvae were maintained at 25°C, on an aphid diet. Points represent percentage mortality of 20 insects for first (●), second (○), third (□), and fourth (◇) instars. Lines represent the predicted probit line transformed back to percentage mortality for first (—), second (---), third (–·–) and fourth (–·–) instars.

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

TABLE 1

The Effect of Larval Instar and Stress Factors on the LC_{50} of *H. convergens* to *P. fluorescens*

Instar	Temperature pulse[a] (C°)	Pre-exposure[b] diet	LC_{50}[c] (CFU/ml)	Probit slope[d]	Control mortality[e]
4	25	Aphids	3.2×10^{11}a	0.41	2.5 (2.5)
3	25	Aphids	3.9×10^{9}b	0.66	9.2 (5.3)
2	25	Aphids	2.8×10^{10}b	0.27	5.9 (0.4)
1	25	Aphids	4.8×10^{9}bd	0.35	10.0 (0)
1	25	Sucrose	1.2×10^{11}c	0.38	12.5 (3.5)
1	25	Water	7.3×10^{11}c	0.33	15.0 (0)
1	30	Aphids	9.8×10^{10}d	0.19	10.4 (5.4)
1	30	Sucrose	3.3×10^{10}cd	0.42	13.0 (8.0)
1	30	Water	8.9×10^{9}e	0.51	25.5 (5.8)

[a] Insects were either maintained at 25°C or given an 18 hr, 30°C temperature pulse before being exposed to the bacteria.
[b] Diets were given for 24 hr before larvae were exposed to the bacteria.
[c] Values followed by the same letter are not significantly different from each other ($P > 0.05$).
[d] None of the slopes of the probit lines were significantly different.
[e] Mortality of larvae dipped in water after being exposed to the given temperature and diet and indicates the relative stressfulness of the temperature and diet conditions.

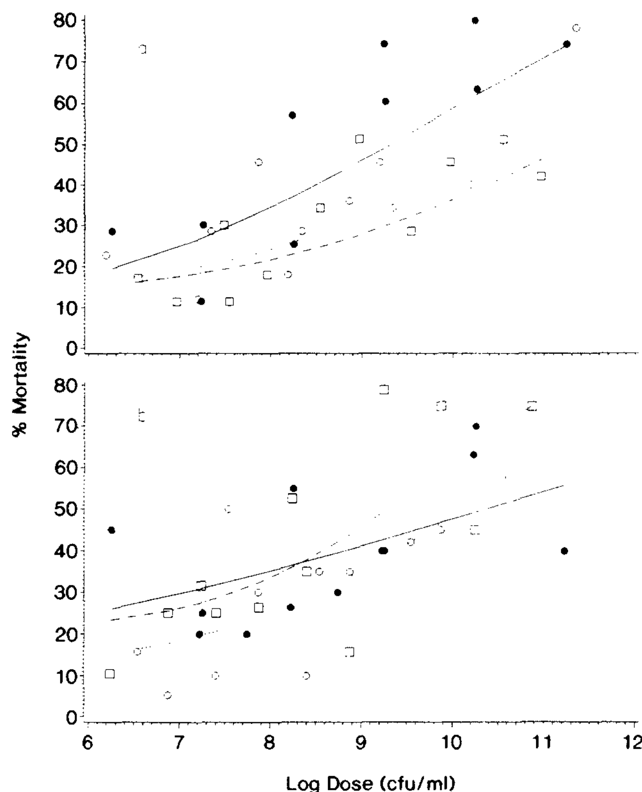

FIG. 2. The effect of diet on the susceptibility of first instar *H. convergens* to *P. fluorescens* (a) at 25°C and (b) after a 24 hr temperature pulse of 30°C. Points represent the percentage mortality of 20 insects for each diet as follows: aphids (●), 5% sucrose solution (○), and water (□). Lines represent the predicted probit line transformed back to percentage mortality for each diet as follows: aphids (—), 5% sucrose solution (---), and water (–-–). Temperature exposures were given for 18 hr and diets were given for 24 hr before exposure to the pathogen.

Effect of temperature and diet. The effects of diet under the two temperature conditions were analyzed separately. When no temperature stress was applied, larvae fed the aphid diet had the highest susceptibility and the susceptibilities of larvae fed the water and sugar diets were not significantly different from each other (Fig. 2a).

When the insects were exposed to the temperature stress of 30°C, there appeared to be no effects of the diet treatments on susceptibility; however, the relative order of the LC_{50}s were apparently the reverse of that found at 25°C (Table 1). The 30°C temperature stress caused a consistent increase in the variance about the probit lines which can be seen in the high degree of scatter of the points about the lines in Fig. 2b, as compared to Fig. 2a. This variability indicates that within a given diet treatment, some of the larvae were more affected by the temperature stress than were others.

When a comparison was made between the two temperature treatments for each diet, temperature did not appear to affect the LC_{50} of larvae fed either an aphid or sucrose diet. Temperature stress did have a significant ($P \leq 0.01$) effect on larvae fed water only, decreasing the LC_{50} approximately 10-fold (Table 1).

DISCUSSION

Fourth instar larvae of *H. convergens* were more resistant to *P. fluorescens* than earlier instar larvae. This effect was probably not due solely to a difference in size, because there was not a continuous decrease in larval susceptibility. There could be some size threshold; for example, once a certain weight is reached in ultimate instar *Manduca* larvae, the gut is purged in preparation for pupation (Chapman, 1982). If a similar purging of the gut occurs in *H. convergens* in preparation for pupation, the gut may be emptied of *P. fluorescens* before the bacterium can invade the haemoceol and cause septicemia.

It seems surprising that dietary stress would decrease susceptibility, such as was found here where the starvation diet increased the LC_{50} 151-fold at 25°C. However, Chernysh (1991) found that inducing stress in *Calliphora vicina* and *Barathra brassicae* results in increased ecdysteroid titers, which in turn activate anti-infection immune systems of the insect.

The effect of temperature stress appeared to have no effect on the susceptibility of *H. convergens* to *P. fluorescens* septicemia (other than to increase the variability of the response) except when larvae were starved, suggesting that an interaction occurred between the two stressors. This interaction could be due to the fact that temperature stress increases energy requirements in insects, with carbohydrates primarily being utilized, especially fat body glycogens, and starvation causes a decline in fat body glycogen (Ivanović, 1991). The resulting loss of energy may have weakened *H. convergens* larvae enough to increase their susceptibility to

bacterial infection. Supplying larvae with sucrose, however, appeared to provide a sufficient energy source, as there was no significant effect of temperature when larvae were given sucrose as a diet.

The effects of age and stress on insect susceptibility to microbial infection has direct implications for field applications of microbial pesticides. The timing of application of microbial pesticides is often based on the age of the target insect and environmental conditions, such as air temperature and precipitation, optimizing the effects of the pesticide and increasing its life in the field. It should be recognized that the environmental conditions that occur before and after the spray may also affect the susceptibility of both target and nontarget insects. To help understand responses found in the laboratory and field, more research is needed to determine the physiological mechanisms by which age and stress affect insect immune systems.

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