

Nodulation Reaction to Fungal Infections in Larvae of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) Mediated by Eicosanoids

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Abstract: Injecting larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), with eicosanoid biosynthesis inhibitor, phenidone (104 µg larva⁻¹), immediately prior to intrahemocoelic injections of the fungus *Beauveria bassiana* (Bals.) Vuill. (Deuteromycota: Hyphomycetes) (isolate No: HRI-215: 10 µg larva⁻¹ from 2 x 10⁶ blastospores ml⁻¹), sharply reduced (about 75 %) the nodulation reaction to the fungal challenges. Separate treatments with specific inhibitors of the major enzymes involved in eicosanoid biosynthesis, including phospholipase A₂ (PLA₂), cyclooxygenase, and lipoxygenase, reduced nodulation reactions to fungal infections. These findings support the view that nodule formation is a complex process involving lipoxygenase and cyclooxygenase products. The inhibitory influence of phenidone, the dual cyclooxygenase and lipoxygenase inhibitor, was apparent within each time. Phenidone-treated larvae formed about 14 nodules/insect at 0.5 h post injection (hpi), which increased to 54 at 8 hpi, whereas the ethanol-treated control larvae produced significantly more nodules at each time, from 49 nodules at 0.5 hpi to 147 at 8 hpi. The inhibitory effect of dexamethasone on nodulation was reversed by treating fungus-injected insects with the eicosanoid-precursor polyunsaturated fatty acid, arachidonic acid. These findings support our hypothesis that eicosanoids mediate insect cellular immune reactions to fungal infections in *L. decemlineata*.

Key Words: Nodulation, eicosanoid, *B. bassiana*, *L. decemlineata*, blastospore

Leptinotarsa decemlineata (Say) (Coleoptera:Chrysomelidae) Larvalarında Fungus Enfeksiyonuna Karşı Eikosanoidler Vasıtasıyla Gerçekleşen Nodülasyon Reaksiyonu

Özet: *Beauveria bassiana* (Bals.) Vuill. (Deuteromycota: Hyphomycetes) blastosporlarının (2 x 10⁶ blastospor ml⁻¹'den her larvaya 10 µl) enjeksiyonundan önce eikosanoid biyosentez inhibitörü phenidone (104 mg larva⁻¹) enjekte edilmesi fungus istilasına karşı patates böceği, *L. decemlineata* larvalarında oluşan nodül sayısını kontrol larvalarına oranla önemli ölçüde (yaklaşık %75) azaltmıştır. Eikosanoid biyosentezinin temel enzimleri phospholipase A₂, cyclooxygenase ve lipooxygenase enzim inhibitörleri larvalara uygulandığında fungal enfeksiyonlara karşı oluşan nodülasyon reaksiyonunda kontrol larvalarına oranla azalma görülmüştür. Bu bulgular nodül oluşumunda cyclooxygenase ve lipooxygenase enzimlerinin her ikisinin de rol aldığını göstermiştir. Hem cyclooxygenase hem de lipooxygenase inhibitörü olan phenidone'un nodülasyonu engelleyici etkisi her bir uygulama zamanında görülmüştür. Phenidone uygulanan larvalarda uygulamadan 30 dakika sonra ortalama 14 nodül ve uygulamadan 8 saat sonra ortalama 54 nodüle yükselirken, kontrol larvalarında (etil alkol uygulanan larvalar) uygulamadan 30 dakika sonra ortalama 49 nodül ve uygulamadan 8 saat sonra ortalama 147 nodüle yükselmiştir. Dexamethasone'un nodülasyon oluşumu üzerine engelleyici etkisi eikosanoid substratı olan doymamış yağ asidi arakidonik asit uygulanması ile ortadan kaldırılmıştır. Bu bulgular, *L. decemlineata* larvalarında fungal enfeksiyonlara karşı böcek hücresel bağışıklık reaksiyonunda eikosanoidlerin rol aldığını desteklemiştir.

Anahtar Sözcükler: Nodülasyon, eikosanoid, *B. bassiana*, *L. decemlineata*, blastospor

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Introduction

Two categories of defense responses to microbial infections have been seen in insects: humoral and hemocytic (Stanley, 2000). Humoral defense reactions take several hours for their full expression and involve induced biosynthesis of anti-microbial peptides and proteins (Leulier et al., 2003; Stanley and Miller, 2006).

Hemocytic reactions involve direct cellular interactions between circulating hemocytes and infecting microbes (Stanley and Miller, 2006). In contrast to humoral defense reactions, hemocytic responses are very quick; typically occur within minutes of an infection cycle. Specific cellular defense mechanisms include phagocytosis, nodulation, and encapsulation (Gupta, 1986, 1991). These immune functions are well known and previous studies reveal information on the signal mechanisms responsible for mediating and coordinating insect immunity.

Drawing on the background of signal transduction systems in mammalian immunity, Stanley-Samuels et al. (1991) suggested insect cellular immune reactions are mediated by eicosanoids. This suggestion prompted more detailed research meant to determine which of the several cellular defense reactions depend on eicosanoid biosynthesis. Because nodulation is the predominant cellular immune reaction to bacterial infections, it has been hypothesized that eicosanoids mediate nodulation reactions to bacterial infections (Miller et al., 1994). On the basis of these findings, Stanley and his colleagues developed the hypothesis that eicosanoids mediate nodulation reactions to bacterial infections in most, if not all, insect species, now known as the eicosanoid hypothesis (Stanley and Howard, 1998; Stanley, 2000). Several research groups have tested the hypothesis using over 20 insect species, as summarized in recent reviews (Stanley, 2006; Stanley and Miller, 2006). All experimental work has strongly supported the idea.

Eicosanoids influence several aspects of insect immunity. Mandato et al. (1997) found that cell spreading, a distinct phase of nodulation, and phagocytosis are mediated by eicosanoids in larval wax moths, *Galleria mellonella*. Another role of eicosanoids in insect cellular immunity was developed by Dean et al. (2002) and Lord et al. (2002). They suggested that besides bacteria, eicosanoids mediate *Manduca sexta* cellular response to the fungal pathogens *Beauveria bassiana* and *Metarhizium anisopliae*.

Although there are many works that eicosanoids mediate nodulation reactions to bacterial infections in insects, there are limited works that eicosanoids mediate nodulation reactions to fungal infections in insects. The fungus tested in this study, *Beauveria bassiana*, is a well-known entomopathogen with world-wide distribution and has a wide host range. To broaden the role of eicosanoids in nodulation reactions to fungal infection in insects, the larvae of *Leptinotarsa decemlineata* were tested. Nodulation reactions to fungal infections in larvae of Colorado potato beetle was observed, and it was found that these reactions depend on eicosanoid biosynthesis. This finding supports the idea that eicosanoids mediate nodulation in most insect species against different infecting microbes.

Materials and Methods

Organisms

Late-stage *L. decemlineata* larvae were collected from potato fields in Göksun/Kahramanmaraş, Turkey. The larvae were maintained in a container in a climatic room at 20 ± 1 °C with $60 \pm 5\%$ relative humidity.

Beauveria bassiana isolate HRI-215 (Horticultural Research Institution), an entomopathogenic fungus, was used in this study. The fungus was originally isolated from "Boverin" by E.A. Grula at Oklahoma State University, USA, in 1978. Culture slants under a layer of sterile mineral oil were prepared to preserve the fungus culture during the study. Fungal mycelia grown on potato dextrose agar (PDA) were used to inoculate blastospore culturing medium (10 g peptone, 20 g glucose, 2 g yeast extract, and 10ml 1% Tween 80 per liter). These cultures were incubated in an orbital shaker at 25 ± 1 °C in darkness for 10 days. The content was passed through 2 layers of cheese cloth to remove any large particles and hyphae, and then centrifuged. After removing the supernate, blastospores were dispensed in sterile saline (0.6 %). This stock suspension was then diluted to obtain the final concentration of 2×10^6 blastospores ml⁻¹.

Injections and assays for nodulation

The protocols formalized by Miller and Stanley (1998) were followed. Larvae of *L. decemlineata* were injected with either the phospholipase A₂ (PLA₂) inhibitor dexamethasone {(11 α , 16 β)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-dione}, one of the cyclooxygenase inhibitors ibuprofen { α -methyl-4(2-

methylpropyl) benzeneacetic acid}, indomethacin {1-P-(chlorobenzyl)-5-methoxy-2-methyl-3-indolyl-acetic acid}, naproxen {O-2-(6-methoxy-naphthyl) propionic acid}, or piroxicam {3,4-benzothiazine-3-carboxamide 1,1-dioxide}, the dual cyclooxygenase and lipoxygenase inhibitor phenidone {1-phenyl-3-pyrazolidinone}, or the 5- and 12- lipoxygenase inhibitor, esculetin {6,7-dihydroxycoumarin} (all inhibitors purchased from Sigma Chemical Co.). In rescue experiments, larvae were also injected with arachidonic acid {5,8,11,14-eicosatetraenic acid}, purchased from Sigma Chemical Company. Control insects were injected with 70% ethanol. Drugs and control substances were injected into the opposite side of the abdomen using a 50 µl Hamilton 701 micro-syringe. All injections of pharmaceuticals were in a standard dosage of 104 µg in 10 µl of ethanol, except in dose-response experiments. Before being injected, larvae of *L. decemlineata* were surface sterilized by swabbing their cuticle with 70% ethanol.

Immediately after the drug injections, larvae of *L. decemlineata* were infected by injecting 10 µl blastospore suspension at the concentration of 2×10^6 blastospores ml^{-1} into each larva using an insulin syringe, following injection protocols of Miller and Stanley (1998).

We assessed nodulation at selected times after injections. The larvae of *L. decemlineata* were anesthetized by chilling on ice, and then their hemocoels were exposed. Melanized, brownish black nodules were counted under a stereo microscope at 45 \times . The nodules were distinct and direct counting reliably reflected the extent of the nodulation response to infections (Miller and Stanley, 1998; Miller et al., 1999). After the first counting, the alimentary canal was removed. Nodules in the previously unexposed areas and remaining internal tissues were then counted.

Control Experiments

To determine the level of the background nodulation in larvae of *L. decemlineata*, several control experiments were conducted. To record the nodulation in unchallenged larvae, 10 larvae of *L. decemlineata* were taken from culture at various times in this project. We anesthetized larvae on ice for 5 minutes, and then assessed nodulation. To determine the influence of the drug vehicle, ethanol, on nodule formation, 10 larvae were injected with 10 µl of ethanol. Nodulation was assessed 4 hpi, following the same protocol. To assess the

effect of phenidone on nodulation in unchallenged larvae of *L. decemlineata*, a standard dosage (104 µg) of phenidone in 10 µl ethanol was injected into 10 larvae. Nodulation was assessed by standard methods 4 hpi. Injection of 10 µl saline into 10 larvae of *L. decemlineata* was used as control for nodulation by following the standard protocol.

Dose-response curve for *B. bassiana* on nodulation

Various doses of *B. bassiana* blastospores were injected into the hemocoel of *L. decemlineata* larvae. After injection, the larvae were kept on a potato diet and maintained individually in a container as described. Larvae of *L. decemlineata* were anesthetized, sterilized and injected as described. Nodulation was assessed 4 hpi.

Time course of nodulation - Influence of phenidone

Individuals in 2 groups of larvae of *L. decemlineata* were injected with ethanol or with 104 µg of phenidone. The larvae were immediately injected with fungus as described. At 0.5, 1, 2, 4 and 8 hpi, sub-groups of control and experimental insects were anesthetized, and nodulation was assessed.

Influence of other eicosanoid biosynthesis inhibitors on nodulation

We divided larvae of *L. decemlineata* into 2 groups and injected individuals in each group with the cyclooxygenase inhibitors indomethacin, naproxen, ibuprofen, or piroxicam and the dual cyclooxygenase and lipoxygenase inhibitor phenidone, or lipoxygenase inhibitor esculetin, all in standard dosages of 104 µg in 10 µl of ethanol. Control insects were injected with 10 µl of ethanol. Following injections, the larvae of *L. decemlineata* were infected with a standard dosage of *B. bassiana* blastospores as described. At 4 hpi, the larvae of *L. decemlineata* were anesthetized and nodulation was assessed.

Dose-response curve for phenidone

Individuals in 5 groups of *L. decemlineata* were injected with 10 µl of ethanol, or 0.104, 1.04, 10.4, 104 µg of phenidone in 10 µl ethanol, then infected with a standard dosage of *B. bassiana* blastospores. At 4 hpi, the *L. decemlineata* were anesthetized, and nodulation was assessed.

Fatty acid rescue experiment

Individuals in 2 groups of larval *L. decemlineata* were injected with either 10 µl ethanol or 104 µg of dexamethasone in 10 µl of ethanol and then infected with *B. bassiana* blastospores as described. Immediately after infection, the dexamethasone-treated larvae of *L. decemlineata* were divided into 2 sub-groups. Individuals in one sub-group were treated with 5 µg arachidonic acid in 5 µl of ethanol. Another sub-group was treated with 5 µl of ethanol to control for the effects of the extra injection on nodulation. At 4 hpi, larvae of *L. decemlineata* were anesthetized and nodulation assessed.

Statistical Analysis

Data were analyzed using the General Linear Models procedure, and mean comparisons were made using Least Significant Difference (LSD) test ($P \leq 0.01$) (SAS Institute Inc., 1989).

Results

Control experiments

Table 1 shows the results of several control experiments. We recorded no nodules larva⁻¹ ($n = 10$ larvae) in untreated insects taken directly from the culture. We observed about 67 nodules larva⁻¹ of the *L. decemlineata* injected with ethanol, and 46 nodules larva⁻¹ of the *L. decemlineata* injected with phenidone. Injections with saline resulted in about 15 nodules larva⁻¹. By comparison, infections with *B. bassiana* blastospores at the concentration of 2×10^6 blastospores ml⁻¹ resulted in about 120 nodules per larva.

Table. Outcomes of the background control experiments. Insects were treated as specified in the left column and nodulation was assessed at 4 hpi as described in materials and methods.

Treatment	Nodules per larvae (Mean ± SEM)
None (untreated)	0
Vehicle (ethanol only)	67.0 ± 6.1
Phenidone only	46.0 ± 6.2
Saline	15.3 ± 2.5
Fungal challenge	119.8 ± 7.9

Dose-response curve for *B. bassiana*

Figure 1 shows the relationship between *B. bassiana* dosages and nodulation number in larvae of *L. decemlineata*. The optimal dosage of *B. bassiana* was 2×10^6 blastospores ml⁻¹, which caused higher nodule formation in the insects. These findings informed the use of 2×10^6 blastospores ml⁻¹ in subsequent experiments.

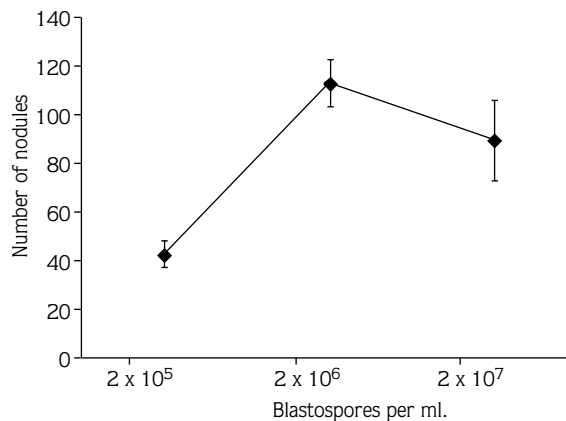


Figure 1. The influence of *B. bassiana* blastospore concentrations on nodulation reactions in larvae of *L. decemlineata*. The larvae were challenged with the injection of the indicated dosages of fungal suspensions, and then nodulation was recorded at 4 hpi. Each point indicated the mean number of nodules found in each insect ($n = 10$ individuals), and the error bars represent ± SEM.

Time course of nodulation

Figure 2 shows the time course of visible nodule formation in 2 groups of *L. decemlineata* larvae experimentals and controls. Phenidone-treated larvae formed about 14 nodules/insect at 0.5 hpi, which increased to 54 at 8 hpi, whereas the ethanol-treated control larvae produced significantly more nodules at each time, from 49 nodules at 0.5 hpi to 147 at 8 hpi (LSD, $P \leq 0.01$).

Influence of other eicosanoid biosynthesis inhibitors on nodulation

We considered the influence of 7 pharmaceutical inhibitors of eicosanoid biosynthesis on nodulation in response to fungal infections. Figure 3 shows that, compared to control (EtOH) larvae of *L. decemlineata*, except phenidone, all inhibitor-treated larvae of *L. decemlineata* exhibited small-reduced nodulation in

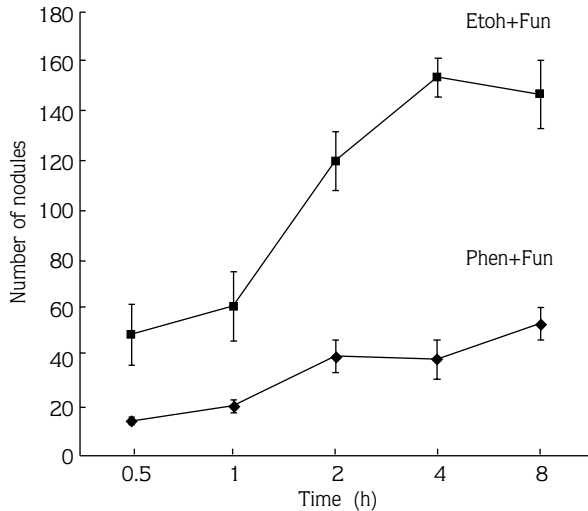


Figure 2. Time course of nodulation in larval *L. decemlineata* in response to infections with *B. bassiana*. Each point indicated the mean number of nodules found in each insect ($n = 10$ individuals) and the error bars represent \pm SEM.

response to fungal infections. We obtained significant differences between the influence of phenidone, which severely inhibited nodule formation, and the influences of other six inhibitors (LSD, $P < 0.01$).

Dose-response curve for phenidone

The influence of phenidone on nodulation in response to fungal infections was expressed in a dose-dependent manner. Nodulation declined from about 156 nodules larva⁻¹ in ethanol-treated control *L. decemlineata*, to about 36 nodules larva⁻¹ in *L. decemlineata* treated with the highest phenidone dosage (Figure 4).

Fatty acid rescue experiment

Dexamethasone inhibits eicosanoid biosynthesis through its effect on PLA₂. If this is true, then injecting the eicosanoid-precursor polyunsaturated fatty acid, arachidonic acid, into dexamethasone-treated infected adults should reverse the effects of dexamethasone on nodulation. To test this, we followed the following procedure: After injection with dexamethasone, larvae were infected with *B. bassiana* and then immediately treated with arachidonic acid. To control the influence of the third injection on nodulation, an additional control group of *L. decemlineata* was injected with ethanol. Figure 5 shows that the arachidonic acid treatments reversed the effects of dexamethasone on nodulation ($P < 0.01$). The ethanol-injected control *L. decemlineata*

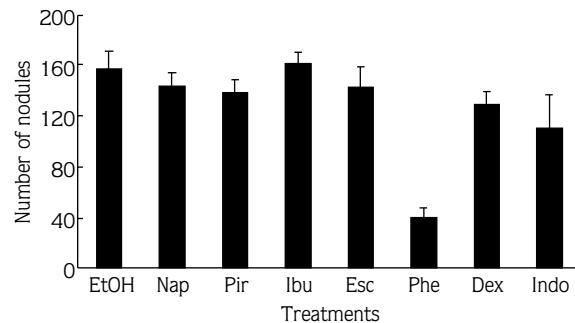


Figure 3. Effect of treating *L. decemlineata* larvae with individual eicosanoid biosynthesis inhibitors on nodule formation in response to infections with *B. bassiana*. Each point indicates the mean number of nodules found in each insect, and the error bars represent \pm SEM.

yielded about 124 nodules larva⁻¹ and dexamethasone-treated *L. decemlineata* about 75 nodules larva⁻¹, both in line with expectation. The arachidonic acid-treated *L. decemlineata* produced about 186 nodules per larva, also in line with expectation for control animals. The second control group, injected with a second dose of ethanol, yielded about 92 nodules larva⁻¹.

Discussion

This paper reports the outcomes of the experiments designed to test the hypothesis that eicosanoids mediate nodule formation in response to fungal infections in *L. decemlineata*. The results of all the experiments performed in this study support this hypothesis. First, treating the experimental *L. decemlineata* with phenidone prior to infecting them with *B. bassiana* significantly reduced nodulation at all points in the time course experiments. Second, the influence of the eicosanoid biosynthesis inhibitor, phenidone, on nodulation was expressed in a dose-dependent manner. Third, 1 of 7 different eicosanoid inhibitors, phenidone, significantly reduced nodulation when compared to the control treatments. Finally, the influence of dexamethasone on nodulation was reversed by treating infected *L. decemlineata* with arachidonic acid, an eicosanoid precursor polyunsaturated fatty acid. When taken

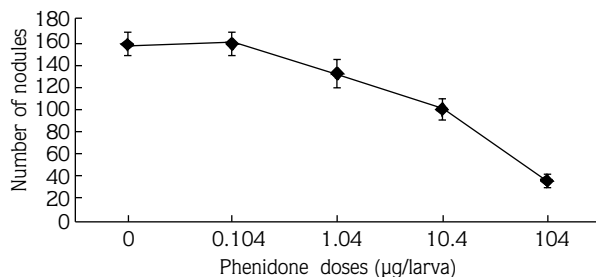


Figure 4. Dose-response curve for the effects of phenidone on nodule formation. *L. decemlineata* larvae were first injected indicated doses of phenidone and then infected with *B. bassiana*. Nodulation was assessed at 4 hpi. Each point indicated the mean number of nodules found in each insect (n = 10 individuals), and the error bars represent ± SEM.

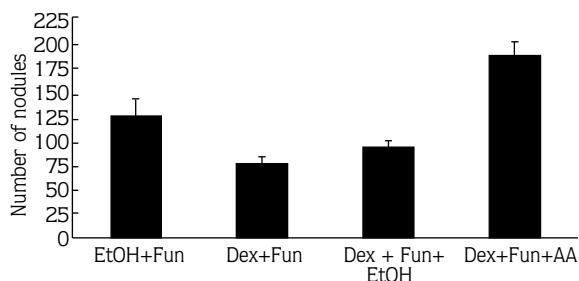


Figure 5. Eicosanoid-precursor fatty acids reverse the effect of dexamethasone on nodulation. *L. decemlineata* larvae were treated with ethanol (E+F) or dexamethasone (D+F) and then intrahemocoelically infected with *B. bassiana*. Immediately after infection, test insects were treated with 5 µg of arachidonic acid (D+F+AA). Control insects were treated with dexamethasone and ethanol (D+F+E). At 4 hpi, the insects were anesthetized on ice, and nodulation was assessed. The height of histogram bars represents the mean number of nodules found in each insect, and the error bars represent 1 SEM.

together, these 4 separate lines of evidence strongly support the overall hypothesis.

Time course experiment resulted that treating experimental *L. decemlineata* with phenidone produced significantly fewer nodules compared to the control *L. decemlineata* at all time points in the experiment. It could be inferred from this finding that inhibition of eicosanoid biosynthesis influences the cellular events involved in nodulation early in the infection process, and continues to exert a negative influence for a few hpi. The time course experiment also indicates the upper limits of nodulation reactions to the fungal infections in *L. decemlineata*. In the experiments, *L. decemlineata* produced a maximum of

about 150 nodules/individual within 4 hpi, which is in line with the outcomes of similar experiments with other insect species (Dean et al., 2002). The larvae of *M. sexta* produced about 108 nodules/individual by 24 h after infections with conidia of the fungal pathogen *M. anisopliae* (Dean et al., 2002). On the other hand, Lord et al. (2002) showed that the larvae of *M. sexta* produced about 600 nodules/individual by 2 h after infections with the fungal pathogen *B. bassiana* blastospores. Tunaz (2006b) showed that the time course experiment indicates also the upper limits of nodulation reactions to the bacterial infections in Colorado potato beetle. In the experiments, Colorado potato beetle produced a maximum of about 100-145 nodules/individual within 2 hpi, which is in line with the outcomes of our study. Büyükgüzel et al. (2007) found that nodulation in larvae of *Galleria mellonella* can be induced by *Bovine herpes simplex virus-1* (BHSV-1). They indicated that *Galleria mellonella* produced about 190 nodules/larva within 4 hpi, which is higher than the 150 or so nodules/individual seen in our work here.

Ratcliffe and Gagen (1976) showed that larvae of *Pieris brassicae* and *G. mellonella* were differentially competent to form nodules in response to several nonpathogenic bacteria but nodules were not evoked by the pathogen *Staphylococcus aureus*. Similarly, Howard et al. (1998) found that nodulation intensity in *M. sexta* is related to bacterial species. Our findings accord with the general idea that different microbial challenges evoke varying intensities of nodulation response.

It is considered the influence of phenidone dosage on nodulation reactions to fungal challenges. Dose-response relationship is basic to physiological research, and the approximately linear negative relationship we obtained for phenidone strongly supports the idea that eicosanoids are one of charged molecule for nodulation reactions to fungal infections in the *L. decemlineata*. Similarly, it was noted that the approximate linear relationship between phenidone dosages and decreasing nodulation to bacterium, *Serratia marcescens* (Tunaz, 2006b).

Eicosanoid biosynthesis inhibitors, which we refer to with the general term "eicosanoid biosynthesis inhibitors", exert different actions in cellular eicosanoid biosynthesis (Smith, 1989). For example, dexamethasone inhibits PLA₂. Dexamethasone exerts other actions, as well, including the influence on gene expression. Several compounds specifically inhibit cyclooxygenase, the first

step in prostaglandin biosynthesis, while esculetin is a specific inhibitor of 5- and 12-lipoxygenases (Schnitzer, 2001). Our experiments with different inhibitors showed that at least one of the compounds we tested resulted in similar reductions in nodulation. The observation that separate experiments with different inhibitors of eicosanoid biosynthetic pathways similarly retarded nodulation in larvae of *L. decemlineata* indicates that eicosanoids act in nodule formation. This makes sense because nodulation results from complex cellular physiology, which involves many separate cellular actions. Inhibiting one or more of the eicosanoid-mediated steps may impact the overall nodulation process. We interpret the results of our experiments to suggest that prostaglandins and various lipoxygenase products mediate various, still unknown, steps in nodulation.

Our background control experiments indicate that the nodules we recorded were due to the experimental treatments, and not to adventitious infections. *L. decemlineata* taken directly from the field did not have any nodulation. The inject treatments had low background of nodulation. We note that control experiments with ethanol yielded similar numbers of nodules with the European corn borer (Tunaz et al., 2003). On the other hand, our control experiments with ethanol yielded higher numbers of nodules than those recorded with other insect species (Miller et al., 1994, 1996), however, the key point is that the drug vehicle did not, in itself, diminish the *L. decemlineata*'s ability to form nodules. Hence the experimental protocols allow a physiological interpretation of the data, inhibition of eicosanoid biosynthesis impairs *L. decemlineata* immunity.

Outcomes of the rescue experiments strongly indicated that eicosanoids mediate nodulation in *L. decemlineata*. Dexamethasone is an inhibitor of PLA₂, the enzyme responsible for releasing arachidonic acid from cellular phospholipids. This is the first and rate-limiting step in eicosanoid biosynthesis. In this case, dexamethasone inhibits eicosanoid biosynthesis by inhibiting the release of substrate from cellular phospholipids, which in effect withholds substrate from cyclooxygenase and other eicosanoid biosynthesizing enzymes. If this is the case, then providing free, i.e. unesterified, arachidonic acid to the immunity-conferring cells within the *L. decemlineata* would be expected to reverse the influence of dexamethasone on nodulation. Indeed, the arachidonic acid treatments restored the *L.*

decemlineata's ability to produce nodules in response to fungal infections. We conclude that results of this experiment strongly support the hypothesis.

It has been reported that eicosanoids mediate nodulation reactions to bacterial, fungal and viral infections in insects (Stanley-Samuels et al., 1991; Tunaz et al., 1999; Dean et al., 2002; Lord et al., 2002; Büyükgüzel et al., 2007). Our study reports that eicosanoids mediate nodulation reaction to fungal infection in larvae of *L. decemlineata*. Therefore, it is reported in this paper, and in the previous studies cited above, that eicosanoids mediate insect cellular immune response to microbial infections.

Tunaz (2006a) showed that eicosanoid biosynthesis inhibitors influence the mortality of *Pieris brassica* larvae co-injected with fungal conidia. It was indicated that an increased and faster mortality of the larvae was seen when *B. bassiana* (ARSEF-1151) was co-injected with the eicosanoid biosynthesis inhibitors (dexamethasone, naproxen, phenidone, and esculetin) with different modes of action. Similarly, Connick et al. (2001) tested the role of eicosanoid biosynthesis inhibitors (EBIs) when co-applied with the pathogenic bacterium, *Serratia marcescens*, as a potential in insect pest control. They reported an increased mortality of the termites, *Coptotermes formosanus*, when the bacteria were co-applied with EBIs.

In conclusion, although there are few studies for a possible use of eicosanoid biosynthesis inhibitors with entomopathogenic microorganism for insect pest control, in the future it could improve the effectiveness of microbial control agents against insect pest. Hence, mediation of cellular immunity in *L. decemlineata* by eicosanoids takes on broader significance in mechanisms of signal transduction in invertebrate immunology. Eicosanoids were the first biochemical signal moieties discovered in insect cellular immune reactions (Stanley-Samuels et al., 1991); however, they are not the only mediators of cellular immune reactions in insects, nor other invertebrates. It was shown that biogenic amines are involved in hemocytic reactions, including phagocytosis, nodulation, and cellular movements (Baines et al., 1992; Baines and Downer, 1994; Diehl-Jones et al., 1996). Therefore, besides eicosanoids, amines, and other unknown biochemicals are also essential in overall cellular defense processes. However, according to the results of the present study, it could be speculated that

eicosanoids mediate nodulation in *L. decemlineata* against different infecting microbes in nature and use of eicosanoid biosynthesis inhibitors with entomopathogenic microorganism is possible for controlling this insect pest.

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