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Influence of host plant nitrogen fertilization on hemolymph protein profiles of herbivore *Spodoptera exigua* and development of its endoparasitoid *Cotesia marginiventris*

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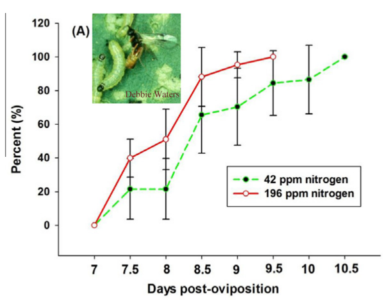
HIGHLIGHTS

- Low N fertilization of cotton plants slowed parasitoid development.
- Parasitoid size was inversely related to N fertilization of plants.
- 2 proteins of ca. 84 and 170 kDa, respectively, dominated host hemolymph proteins.
- Parasitism reduced some hemolymph protein concentrations in *Spodoptera exigua.*
- Nitrogen-poor plant diets of herbivores may significantly alter parasitoid fitness.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrogen has complex effects on plant–herbivore–parasitoid tritrophic interactions. The negative effects of low nitrogen fertilization in host plants on insect herbivores can be amplified to the higher trophic levels. In the present study, we examined the impact of varying nitrogen fertilization (42, 112, 196, and 280 ppm) of cotton plants (*Gossypium hirsutum* L.) on the interactions between the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), and the hymenopteran endoparasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). We predicted that the development and fitness of *C. marginiventris* would be adversely affected by low host plant nitrogen fertilization through the herbivore *S. exigua*. The percentage of *C. marginiventris* offspring developing to emerge and spin a cocoon, and total mortality of parasitized *S. exigua* larvae were unaffected by nitrogen level. The developmental time of *C. marginiventris* larvae in *S. exigua* larvae feeding on low (42 ppm) nitrogen cotton plants was approximately 30% longer than that of those feeding on higher (112, 196, and 280 ppm) nitrogen plants. Parasitoid size (length of right metathoracic tibia), a proxy for fitness, of *C. marginiventris* males was positively affected by nitrogen level. Total amounts of *S. exigua* hemolymph proteins were not affected by nitrogen level, but were reduced by parasitism by *C. marginiventris*. Two proteins with molecular weights of ca. 84 and 170 kDa dominated the *S. exigua* larval hemolymph proteins. Concentrations of the 170 kDa hemolymph protein were unaffected by nitrogen treatment, but parasitism reduced concentrations of the 170 kDa protein. Concentrations of the 84 kDa protein, on the other hand, were interactively affected by parasitism and nitrogen treatment: higher nitrogen fertilization (112, 196, and 280 ppm) increased

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protein concentrations relative to the 42 ppm treatment for unparasitized *S. exigua* larvae, whereas nitrogen treatment had no effects on parasitized larvae. For *S. exigua* larvae feeding on 42 ppm nitrogen plants, parasitism increased concentration of the 84 kDa protein, while for those feeding on 112, 196, and 280 ppm nitrogen plants, parasitism decreased concentrations of the protein. Possible mechanisms and ecological consequences for the extended development of *C. marginiventris* on *S. exigua* hosts grown on low-nitrogen plants are discussed.

1. Introduction

Nitrogen has profound effects through plants that can extend across trophic levels. In plant–herbivore interactions, low nitrogen availability decreases plant quality as a food resource for herbivores in many cases, which can be further exacerbated by increases in plant defensive compounds (Stout et al., 1998; Chen et al., 2008a,b). Herbivores fed on host plants with limited nitrogen access consequently tend to suffer detrimental effects (Loader and Damman, 1991; Kaneshiro and Johnson, 1996; Glynn et al., 2003). The negative effects can further extend to natural enemies of these herbivores (Campbell and Duffey, 1979; Duffey et al., 1986; Kester and Barbosa, 1991; for a review, see Turlings and Benrey, 1998). For example, Kaneshiro and Johnson (1996) found that intermediate nitrogen levels in plants yielded the greatest developmental and reproductive benefit for the leafminer parasitoid *Chrysocharis oscinidis* (Ashmead) relative to lower and higher levels, although the impact of varying nitrogen on the host, *Liriomyza trifolii* (Burgess), was less pronounced. The antibiotic effect of nicotine absorbed in tobacco hornworm, *Manduca sexta* (L.), hemolymph on survival of the gregarious parasitoid *Cotesia congregata* (Say) provides an example (Gilmore, 1938; Thurston and Fox, 1972). Increased nitrogen fertilization in tobacco, *Nicotiana attenuata* Torr. ex S. Watson, increased nicotine content in the plants (Lou and Baldwin, 2004). *M. sexta* is a specialist herbivore of tobacco that can process tobacco's nicotine effectively, mostly through excretion. However, some nicotine is sequestered in the *M. sexta* hemolymph without any negative effect on the herbivores (Self et al., 1964). The parasitic wasp *C. congregata*, however, is more sensitive to nicotine than its host, which reduces parasitoid survival when nicotine levels in the plant are elevated by increased nitrogen fertilization (Parr and Thurston, 1972; Thorpe and Barbosa, 1986; Barbosa et al., 1991).

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is an important crop pest and a generalist herbivore with over 90 known host plant species (Pearson, 1982). Its populations in the southeastern United States are often suppressed by the generalist parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) and other natural enemies (Ruberson et al., 1994; Bianchi et al., 2002). For example, mortality inflicted by feral *C. marginiventris* in the field can reach up to 45% for *S. exigua* larvae exposed only 2 d in the field (Chen and Ruberson, 2008). *C. marginiventris* is a koinobiont larval endoparasitoid, although it can also function as a facultative egg-larval parasitoid (Ruberson and Whitfield, 1996). The parasitoid can complete larval development in 6–10 d at 30 °C, with most parasitoid emergence from the hosts occurring 7 d after oviposition (Boling and Pitre, 1970).

Studies mechanistically linking plant nitrogen effects to organisms of the third trophic level are limited when compared to the somewhat fuller literature on plant nitrogen effects on life history of organisms of the third trophic level (Kaneshiro and Johnson, 1996; Nevo and Coll, 2001). The current study addressed both aspects by examining the impact of nitrogen fertilization of cotton plants, *Gossypium hirsutum* (L.), on *S. exigua* and one of its endoparasitoids, *C. marginiventris*. We examined the hypothesis that increased plant nitrogen would enhance life history traits of the parasitoid. Also, because *C. marginiventris* larvae are exclusively hemolymph feeders (Gauld and Bolton, 1988; Wharton, 1993; Strand, 2000), and hemolymph proteins are major nutritional and regulatory sources for many parasitoids (Beckage et al., 1987; Pennacchio et al., 1993, 1999), we examined hemolymph protein profiles of *S. exigua* in relation to host plant nitrogen levels and parasitism. We hypothesized that (1) the total hemolymph protein contents of *S. exigua* larvae will increase with increasing fertilization levels, and (2) the hemolymph protein profiles of parasitized *S. exigua* larvae will differ from those of unparasitized hosts.

2. Methods

2.1. Plants and nitrogen regimens

Cotton plants (cv. FiberMax 989) were grown using the method described elsewhere (Chen et al., 2008a). Briefly, cotton plant seedlings were fertilized daily with 100 ml of 112 ppm nitrogen nutrient solution for ca. 2 wk, at which time four cotton plants of the same height and with similar-sized leaves at the same leaf positions were assigned to a block. Blocks were arranged so that environmental conditions (e.g., light intensity) within a block were generally homogenous. The four plants within each block were each randomly assigned to four nitrogen levels (42, 112, 196, and 280 ppm nitrogen). Cotton plants were fertilized with corresponding nitrogen solutions daily for ca. 2 weeks, until the initiation of the experiment. Leaching (watering without nutrients) followed every fourth nitrogen solution application in order to reduce salt (salinity) buildup. All experimental plants were at the 3- to 5-mature-leaf stage (~3 weeks post-emergence) when experiments were initiated.

Leaf chlorophyll levels were determined between 1000 and 1200 h with a chlorophyll meter (SPAD-502;Konica Minolta Sensing, Inc., Japan) immediately before using plants for rearing *S. exigua* larvae. To determine consistency of nitrogen effects on leaf chlorophyll at different leaf positions, mature true leaves 1–4 were assayed. Two measurements were made (one on each side of the mid-vein at the base of the leaf blade) on each leaf blade and the average of the two measurements was used in statistical analyses. SPAD readings were later converted to leaf chlorophyll equivalents using the formula $Y = 10^{X0.265}$, where Y is leaf chlorophyll content ($\mu mol\ m^{-2}$) and X is SPAD reading (Markwell et al. 1995). The experiment was a randomized complete block design with eight blocks and four treatments (i.e., nitrogen levels) in each block.

2.2. Insects

Neonates of *S. exigua* and adults of *C. marginiventris* were from laboratory colonies maintained in the Biological Control Laboratory at the University of Georgia in Tifton, GA.

2.3. Development of C. marginiventris in S. exigua

This experiment was designed to investigate effects of nitrogen fertilization levels on development of *C. marginiventris*. Groups of