EFFECTS OF APHID FEEDING ON FOLIAR ANTIOXIDANT ENZYMES IN COTTON

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RESEARCH PROBLEM

Conflicting results exist regarding the effects of aphid feeding on cotton (*Gossypium hirsutum* L.). It is still not clear if aphids themselves or their association with biotic stresses are responsible for physiological changes that could lead to decreased cotton yields. In previous studies, we found no significant change in photosynthesis or increased photosynthesis after exposing cotton plants to 9 days of aphid feeding. Very little information is available regarding the interactions between the aphid and cotton system. Moreover scarce information exists about the antioxidant responses in plants to phloem-feeding insects. In order to understand the biochemical changes induced by a phloem-feeding insect such as *Aphis gossypii* on cotton, we determined the activity of antioxidant enzymes after aphid feeding on cotton leaves.

BACKGROUND INFORMATION

Living organisms face a variety of internal and external stresses to which they must respond in order to maintain equilibrium. Numerous studies have reported that organisms may be stressed by biotic factors (e.g., nematodes, insects, and fungal, bacterial or viral pathogens) or abiotic factors (e.g., temperature extremes, drought, UV irradiation, high salt concentrations, herbicide exposure, nutrient deficiency) (in a review by Yu and Rengel, 1999). Life in an atmosphere containing oxygen has led to the evolution of biochemical adaptations that exploit the reactivity of active oxygen species (AOS) (Noctor and Foyer, 1998). Plants continuously produce AOS even under optimal conditions. AOS are involved in all major areas of aerobic biochemistry (e.g., respiratory and photosynthetic electron transport; oxidation of glycolate and glucose)

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and are produced in large quantities by several enzyme systems. These AOS attack lipids, proteins and nucleic acids, causing lipid peroxidation, protein denaturation and DNA mutation (Yu and Rengel, 1999; Noctor and Foyer, 1998). On the other hand, a defense system exists in plants that serves to detoxify these potentially dangerous reactive molecules. Most of these detoxification reactions are mediated by antioxidant enzymes. There is little doubt that arthropod herbivory induces biochemical and physiological changes in the host plants. In barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.), glutathione reductase activity increased after herbivory by aphids (*Sitobium avenae* F.) (Argandoña, 1994). Phloem-feeding insects seem to induce responses similar to pathogen infection and activate the salicylic acid (SA)-dependent and jasmonic acid (JA)/ethylene-dependent signaling pathways (Walling, 2000). Determination of antioxidant enzyme levels in host plants would serve as a quantitative indicator of stress following aphid herbivory.

MATERIALS AND METHODS

Three identical experiments were conducted in a growth chamber at the Altheimer Laboratory, University of Arkansas at Fayetteville. The growth chamber was programmed for 14:10 hours (day/night), with day/night temperatures ranging from 28°C to 16°C, and 75% relative humidity. Cotton cultivar Stoneville 474 was planted in 2-L pots filled with sunshine mix (soilless horticultural media). All pots were watered with half-strength Hoagland's nutrient solution. Plants were maintained in a well-watered status to avoid drought stress. Cotton aphids were collected from cotton fields at Lonoke, Arkansas, and reared in the laboratory. At 14 days after planting (DAP) the first unfurled leaf from the apex of each plant was tagged. Plants were divided into two groups, one group receiving aphids and the other one without aphids. At 20 DAP, 50 aphids (wingless adults + nymphs) were individually transferred to the selected leaf with a moist paintbrush. In addition, the rest of the leaves were infested with 5 aphids per leaf. Aphids were allowed to increase in numbers. Averages of 137 aphids per tagged leaf after 6 days of exposure and 255 aphids per tagged leaf after 9 days of exposure were recorded. After the plants had been exposed to 6 days of aphid feeding (26 DAP), three plants per treatment were sprayed with 1% (v/v) sodium dodecyl sulfate (SDS) and rinsed with deionized water one hour prior to collecting the leaves to remove the aphids. The tagged leaves were frozen in liquid nitrogen and kept at -70°C for subsequent protein extraction. The same procedure was repeated on the second leaf sampling in which leaves were exposed to 9 days of aphid feeding (29 DAP). For the protein extraction we followed the protocol used by Anderson et al. (1992), with slight modifications. Approximately 1 g of frozen tissue was used for the protein extraction, followed by centrifugation and desalting. The first antioxidant enzyme was catalase which had to be measured immediately due to its instability. The remaining eluate was frozen at -70°C for subsequent assays. All enzyme analyses were performed with a BioSpec

1601 UV/VIS spectrophotometer (Shimadzu, Columbia, Maryland). For the Catalase (CAT) assay, we followed Beers and Sizer (1952) protocol. We measured the disappearance of H_2O_2 by a decrease in absorbance at 240 nm for 1 min. at 25°C. A total of thirty-six tissue samples were processed, and each data point represents the mean of 27 values per treatment per sampling time. For the Peroxidase (POX) assay, the protocol used by Nickel and Cunningham (1969) was followed. We measured the hydrogen peroxide-dependent oxidation of 2, 3', 6 trichloroindophenol at 675 nm for 1 min. at 25°C. For the Ascorbate Peroxidase (APX) assay, we followed Anderson et al. (1992) protocol. We measured the ascorbic acid dependent reduction of H_2O_2 at 265 nm for 1 min. at 25°C. for the Glutathione Reductase (GR) assay, the assay used by Shaedle and Bassham (1977) was followed. We measured the glutathione dependent oxidation of NADPH+H at 340 nm for 1 min. at 25°C.

RESULTS AND DISCUSSION

An initial aphid infestation of 50 aphids per leaf increased to 137 aphids per leaf after 6 days and 255 aphids per leaf after 9 days. However, these populations of aphids had no significant effect on catalast, peroxidase, or ascorbate peroxidase activity. The activity of glutathione reductase was significantly higher in aphid-infested leaves than in non-infested leaves on day 6. This indicates that cotton plants were experiencing some stress caused by aphid herbivory. It has been shown that in this cascade of reactions, the levels of some antioxidant enzymes decrease as compared to others. It has been reported that antioxidant enzymes act almost immediately after the stress, and when the AOS are under control, the levels of antioxidant enzymes decrease. This could explain the unaltered levels of CAT, POX and APX.

PRACTICAL APPLICATION

Overall, an initial population of 50 aphids and 137 aphids per leaf on the sixth day did not alter the activity of foliar antioxidant enzymes, except for glutathione reductase. Probably cotton plants were experiencing some stress as indicated by higher levels of GR. An aphid infestation of 255 per leaf on the ninth day did not change the activity of foliar antioxidant enzymes in cotton. In general, this research demonstrated that cotton plants were only slightly altered physiologically or biochemically by the levels of aphid infestation and feeding duration used in this study.

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