SHORT COMMUNICATIONS

Change of Phenylalanine Ammonia-Lyase Activity in Strawberry Leaves Infested with the Two-Spotted Spider Mite, Tetranychus urticae KOCH (Acarina: Tetranychidae)1,2

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Mite-induced physiological changes of host plants were reported in plum (AVERY and LAGEY, 1968) and strawberry (CHAPLIN et al., 1968; SANCÉS et al., 1979; SANCÉS et al., 1981). In a previous paper (INOUE and SUGIURA, 1984) we suggested that mite-induced physiological changes in host strawberry might influence the population of two-spotted spider mite. But there is no evidence of changes in secondary plant metabolite levels induced by infestation in the mite-strawberry system. Phenylalanine ammonia-lyase (EC. 4. 3, 1, 5), (PAL), demonstrated by KOUKOL and CONN (1961), catalyzes the deamination of phenylalanine to trans-cinnamic acid, one of the most important precursors of secondary plant metabolites. This report shows that PAL activity in mite-infested leaves increases rapidly.

MATERIALS AND METHODS

Plants of Fragaria x ananassa (Duch.) Hort cv. “Hokowase” were defoliated to 3 leaves and planted in polyethylene pots (12 cm in diameter) and were grown at 24°C under white fluorescent lamps for two weeks before inoculation. Most of the plants had 4 leaves at the beginning of the experimental period. Ten adult female mites were inoculated on each leaf (40 mites/plant) and plants were grown under the same conditions for a given period. Then inoculated leaves were cut from the plant and brushed with a brushing machine before washing with distilled water. Leaves were frozen and stored at -20°C for enzyme assay. Leaves were homogenized for 60 sec with a tissue homogenizer (Rabodispersa) in the presence of cold acetone (30 ml/g fresh weight) and filtered through a Toyo No. 2 filter paper. The resulting pulp was washed with cold acetone (100 ml/g fresh weight) and dried under reduced pressure. Crude enzyme was extracted from the resulting pulp with 0.05 mol sodium borate buffer (pH 8.8, 20 ml/g fresh weight) for 30 min and filtered through a Toyo No. 2 filter paper. The enzyme mixture consisted of 1 ml of 0.06 mol l-phenylalanine and 2 ml of enzyme solution. The mixture was incubated at 40°C for 120 min and the reaction was arrested by 0.3 ml of 6N HCl. The acidified mixture was extracted twice with 7 ml of ethyl ether and the ether was evaporated at room temperature under an air stream. The remaining residue was dissolved in 0.05 mol sodium borate buffer (pH 8.8) and the amount of trans-cinnamic acid formed was determined with a high performance liquid chromatograph (apparatus: HPLC, Shimadzu LC-2, column: Whatman Partisil-10 SAX, 100 mm x 4.6 mm, mobil phase: 0.02 mol borate acid (pH 8.5), flow rate: 2 ml/min, detector: UV 270 nm, retention time: 370 sec).

RESULTS AND DISCUSSION

The results of enzyme assay with 3 replication groups of plants are shown in Fig. 1. Uninoculated plants usually had a low activity of PAL. The average of uninoculated plants during the experimental period was 21.2 nmol cinnamic acid/g fresh weight/120 min. The activity in the leaves of the inoculated plants rapidly increased; two days after inoculation, the activity rose to eight times that of the uninoculated plants, and then decreased. The increasing mechanism, however, is not clear. CRESSY (1967) reported that PAL activity in leaf discs (10 cm in diameter) from leaves of fragaria vesca var. “Alpine” increased continuously for 60 hr. Various compounds are known as elicitors of PAL, for instance, chitosans, β-1,4 glucosamine polymers found in insect and fungal cell walls elicited both PAL activity and biosynthesis of pisatin, the phytalexin in pea pod tissue (LOCHKE et al., 1983; WALKER-SHIMONS et al., 1983). In our system physical injury such as mite

2 This research is a part of the studies on developing forecasting techniques of mite pests on vegetables (1981–1985) projected by the Ministry of Agriculture, Forestry, and Fisheries, Japan.

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feeding or a chemical compound such as chitosan may elicit the increase of PAL activity. Many reports suggested that PAL plays an important role in the resistance of plants to pathogens including viruses, fungi (Legrand, 1983) and nematodes (Giebel, 1973). The role of PAL in the mite-strawberry system should be determined.

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REFERENCES


