Biochemical Changes in Terminal Root Galls Caused by an Ectoparasitic Nematode, Longidorus africanus: Phenols, Carbohydrates and Cytokinins[1]

E. EPSTEIN[2]

Abstract: Biochemical changes occurred in roots of bur marigold and grapevine infected with *Longidorus africanus*. Phenols of infected root tips differed quantitatively from those of healthy root tips (optical density of 0.68 and 0.32, respectively). Column chromatography of the phenol extracts resulted in more fractions in the infected than in the healthy root extract. Of the enzymes involved in phenol metabolism, three were tested. No polyphenol oxidase activity was detected and no difference in catalase activity was found between healthy and infected roots. Peroxidase activity, however, was much higher in infected roots. The extra peroxidase was associated mainly with the cortical cells of the infected root tips.

Galled roots contained twice the amount of simple sugars and inulin as did the healthy roots. A cytokinin-active fraction was found only in the noninfected roots.

Longidorus africanus has been found to be a serious pathogen of a number of economically important crops such as lettuce, sugarbeet (20) and grapevine (3). In grapevine, growth has been shown to be reduced by 18% as compared with healthy plants (5). The symptoms of infection are swelling of root tips, retardation of meristematic activity, hyperplasia or cortical parenchyma and a maturation of the root tip region (5). In the hope of elucidating the mechanism of gall formation, several biochemical parameters were examined following nematode infection. Previous work from this laboratory (6) showed that infection with *Longidorus* caused changes in the amino acid content of the plants. In the present study, phenols, carbohydrates, peroxidase, polyphenol oxidase, catalase and cytokinins were examined after infection.

MATERIALS AND METHODS

Plants of bur marigold (*Bidens tripartita* L.) and grapevine (*Vitis vinifera* L.) were inoculated with hand-picked specimens of *Longidorus africanus* as described by Cohn and Mordechai (4), and harvested 1 month and 5 months later, respectively. Root tips of infected (inoculated) and healthy (uninoculated) plants were excised, freeze-dried, and analyzed.

Phenols. Two grams of infected and healthy dried roots of bur marigold were macerated separately three times with an Ultra Turrax blender in 10% methanol. The methanol extract was filtered, brought to 1 N HCl, and refluxed for 1 hr. The volume was reduced to 10 ml with a flash evaporator, and 0.25 ml was used to measure total phenols with Folin-Ciocalteu reagent (9). Phenols were extracted from the hydrolysate by the addition of 5 g polyvinylpyrrolidone (pvp) (17). The phenols were eluted from pvp with 1 N KOH; the solution was neutralized with 1 N HCl and evaporated to 3 ml with a flash evaporator at 40 C. The extract was put on a 10 X 1 cm column of MN-polyamid-SC-6 and eluted with 25 ml water, 25 ml 50% acetone and 50 ml 0.01 N KOH, successively. Effluent was collected in 1-ml fractions, each fraction was chromatographed on cellulose thin-layer plates with 1 N acetic acid, and phenols were determined by the method of Keith et al. (13). The remaining acetone and 0.01 N KOH elutes were pooled together, reduced in volume, and put on top of a 10 X 1 cm Whatman CC 31 cellulose column. Phenols were eluted with 1% acetic acid, and aliquots of 1 ml of the elute were collected and tested for phenols with Folin-Ciocalteu reagent (9). All data represent an average of three replicates.

Sugars. Simple sugars and polysaccharides were extracted from the roots by the method of McCready et al. (18). One gram of freeze-dried roots of infected and of healthy bur marigold was macerated eight times with an Ultra Turrax blender in 80% ethanol until there was no more reaction with 2,3,5-triphenyl-tetrazolium chloride (7). All the macerates were pooled together and brought to 250 ml.

The root material remaining after ethanol maceration was macerated two times more with 52% perchloric:water (1:1). This solution,

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[2] Division of Nematology, Agricultural Research Organization, Volcani Center, Bet Dagan, Israel.

containing the inulin, was brought to 250 ml with water. The amount of simple sugars and inulin was determined by anthrone reagent (18), and by a standard curve of glucose and fructose, respectively.

Similarly, sugars were extracted from 1 g of infected and of healthy freeze-dried roots of grapevine. Glucose was used as a standard curve for the extracted starch. All data represent an average of four replicates.

Cytokinins. Cytokinins were extracted from 1.2 g of freeze-dried roots of bur marigold by the method of Gazit and Blumenfeld (11), and their activity was assayed by Miller's soybean bioassay technique (19). Data represent an average of three replicates.

Peroxidase, catalase and polyphenol oxidase. Five grams of fresh infected and of healthy roots of bur marigold were macerated twice for 2 min in the cold with an Ultra Turrax blender in 30 ml of 0.1 N sodium phosphate buffer, pH 7.5. The solution was centrifuged at 12,000 *g* after each maceration and the supernatants were combined. An equal volume of cold acetone was added, and the solution was left in the refrigerator overnight, then centrifuged for 30 min at 20,000 *g*. The precipitates (0.482 g from the infected roots and 0.433 g from the healthy roots) were used for the enzyme assays.

Total peroxidase activity was determined in 6 μliters sap using an equimolar guaiacol-H_2O_2 system at pH 5.8. Readings were taken with a Spectronic 20 colorimeter at 470 nm at intervals of 15 sec (16). Histochemical localization of peroxidase in bur marigold was performed by the method of Veech and Endo (24). Catalase was assayed by the paper disc method (10) with 0.19 g of acetone powder.

Polyphenol oxidase was assayed spectrophotometrically. The spectrophotometric reaction mixture (1) was composed of 0.10 g of the acetone powder and the change of optical density of pyrocatechol was recorded every 15 sec at a wavelength of 390 nm. Data represent an average of three replicates.

RESULTS

Phenols. The phenols of the infected root tips differed from those of the healthy tips both quantitatively and qualitatively. The amount of phenols in the infected roots was more than double that in the healthy roots (optical density of 0.68 and 0.32, respectively).

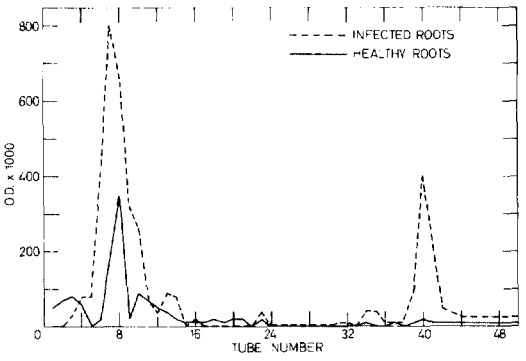

FIG. 1. Fractionation on a cellulose column with 1% acetic acid of the 50% acetone-soluble phenols of healthy and *Longidorus*-infected roots of bur marigold from a polyamid column.

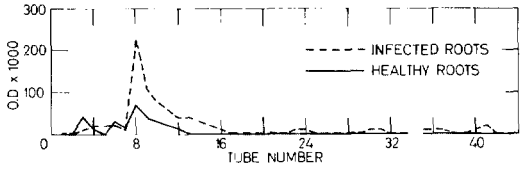

FIG. 2. Fractionation on a cellulose column with 1% acetic acid of the 0.01 N KOH-soluble phenols of healthy and *Longidorus*-infected roots of bur marigold.

The water-acetone-soluble phenols of the infected roots were separated on the cellulose column into two fractions, as compared with only one fraction in the healthy roots (Fig. 1). Of the two fractions, one (3-14 ml) was similar in both extracts, but the infected root fraction contained more phenols than did the healthy one. The elution of the 1 N KOH-soluble phenols of both infected and healthy roots on a cellulose column resulted in only one fraction (5-11 ml) (Fig. 2); again, the infected root fraction contained more phenols.

Sugars. The galled roots contained twice the amount of simple sugars and inulin as did the healthy roots (Table 1). The polysaccharide, which was extracted with 52% perchloric acid, gave a negative reaction with iodine-potassium iodide reagent. Chromatography of this compound before and after hydrolysis with fructose and inulin as markers proved it to be inulin. The amounts of simple sugars and of starch were almost equal in the galled and healthy grapevine roots (Table 2).

Cytokinins. The only cytokinin-active fraction was found in R_f 0.7 of the uninfected roots and was equal to about 0.003 ppm

TABLE 1. Amounts of simple sugars and inulin per gram of galled and healthy root tips of bur marigold.

Roots	Sugars (mg)	Fructose (mg)	Inulin[a] (mg)	% of dry weight: Sugars	% of dry weight: Inulin
Galled	100	32.7	29.4	10	2.9
Healthy	50	15.5	13.9	5	1.4

[a] mg inulin = mg fructose × 0.90.

TABLE 2. Amounts of simple sugars and starch per gram of galled and healthy root tips of grapevine.

Roots	Sugars (mg)	Glucose (mg)	Starch[a] (mg)	% of dry weight: Sugars	% of dry weight: Starch
Galled	70	218	196	6.3	17.8
Healthy	67	231	208	6.0	18.9

[a] mg starch = mg glucose × 0.90.

kinetin. All the other fractions showed a complete lack of activity.

Peroxidase, catalase and polyphenol oxidase. No activity of polyphenol oxidase was found in either infected or healthy roots. No differences in catalase activity were found between infected and healthy roots. The average time for disc flotation was 2 min 45 sec for both. Overall peroxidase activity was higher in the infected than in the healthy roots (Fig. 3); the difference was already very noticeable in 7-day-old plants, but was more pronounced in 1-month-old plants. The pattern of peroxidase activity in the infected and healthy roots is shown in Fig. 4. The highest peroxidase activity in the healthy roots (Fig. 4A) was associated with epidermal and vascular tissues. In the infected roots (Fig. 4B, C) the activity was associated mainly with cortical cells.

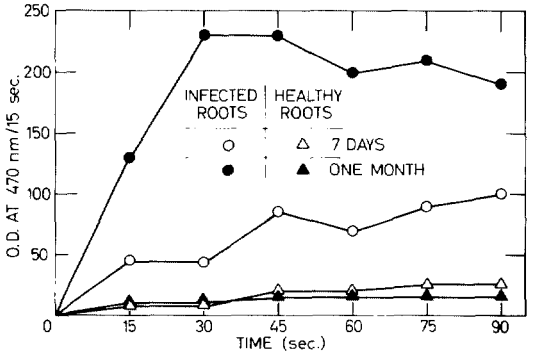

FIG. 3. Relative peroxidase activity in extracts of *Longidorus*-infected and healthy bur marigold roots.

DISCUSSION

An increase in simple sugars occurred in bur marigold roots infected with *Longidorus africanus*. This increase could be attributed to hydrolysis of polysaccharides by enzymes secreted by the nematode (15). However, an increase was also observed in the amount of inulin, which is the reserve material of this plant. This might have resulted from induction of enzymes involved in inulin biosynthesis. A similar case was reported by Schuster et al. (21), who observed an increase in the formation of starch granules in several plants following infection by *Nacobbus batatiformis* Thorne.

The present study demonstrated a marked increase in pre-existing phenols as well as synthesis of a small amount of new phenols in *Longidorus*-infected roots. These results are consistent with previous findings that the amount of phenolics increases in infected and wounded plants (8, 14), possibly by the depression of enzymes involved in phenol synthesis and release from complex compounds (14).

In bur marigold-infected roots a change in peroxidase was noticeable as early as 7 days after infection, and was much more pronounced after 1 month. This large increase in the galled tissue might be attributed to the wounding of the root by the nematode stylet and to the high rate of cell division which follows (5). Similar findings were reported by Van Fleet (23) in root tips of *Allium cepa*, and by Hussey and Krusberg (12) and Veech and Endo (24) in soybean roots after infection with *Meloidogyne incognita acrita*.

The results indicate that cytokinin activity in *Longidorus*-infected roots is markedly reduced. Similarly, Vaadia and Chanan (22) report that cytokinin activity was absent from the *Rf* 0.6-0.8 region in cases of root stress (drought and salinity), and Brueske and Bergeson noticed cytokinin reduction in tomatoes infected with root-knot nematodes (2). It is likely that the nematodes, by disturbing the vascular system, interfere with cytokinin synthesis.

These results demonstrate the complexity of the biochemical mechanisms involved in the response of the plant to nematode infection. Further studies of this kind may clarify the

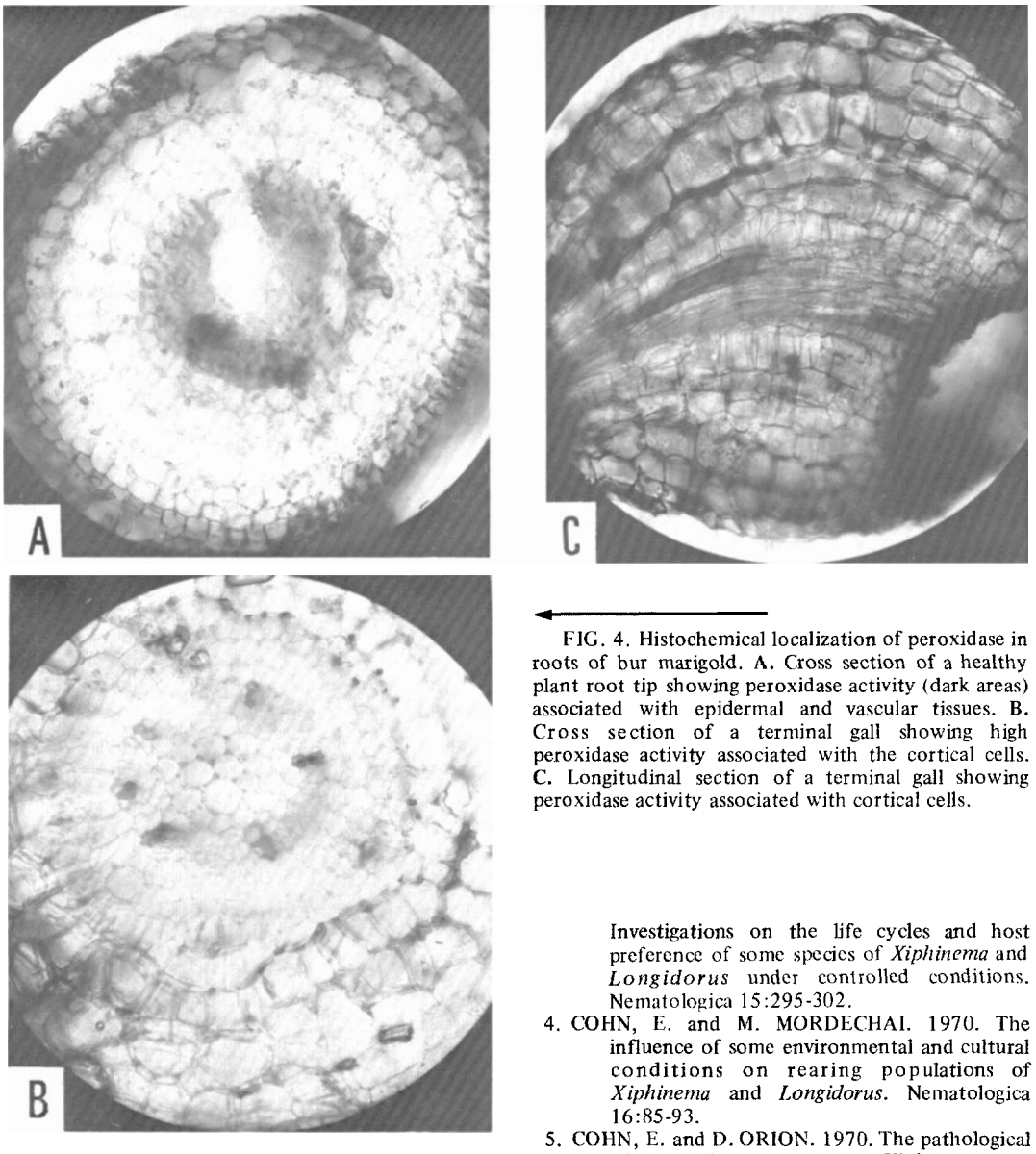

FIG. 4. Histochemical localization of peroxidase in roots of bur marigold. **A.** Cross section of a healthy plant root tip showing peroxidase activity (dark areas) associated with epidermal and vascular tissues. **B.** Cross section of a terminal gall showing high peroxidase activity associated with the cortical cells. **C.** Longitudinal section of a terminal gall showing peroxidase activity associated with cortical cells.

critical processes which are responsible for gall formation.

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