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Polyphenols and inhibitors of indolyl-3-acetic acid oxidase in carrot roots infested with northern root-knot nematode

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Abstract

It is suggested that IAA-oxidase inhibitors accumulate in plants infested by the nematodes. This leads to local accumulation of active auxins and causes proliferation of tissues near the place of nematode infection. T e carrot cv. Slendero seems to be less sensitive to nematode as the inhibitors of IAA-oxidase do not accumulate at early stages of infection.

INTRODUCTION

Polyphenols play an important role in the resistance of plants to fungal, bacterial and viral infections (F a r k a s and K i r á l y 1962; R o h r i n g e r and S a m b o r s k y 1967).

Phenol compounds also accumulate in the roots of many plants infested with nematodes. P i t c h e r et al. (1960) noted an increased phenol content in the roots of strawberries, celery and apple trees infested with *Pratylenchus penetrans*. In roots of various plant species infested with *Meloidogyne* spp. and *Heterodera* spp. the polyphenol level and particularly that of *ortho*-dihydroxyphenols increased (B a l a s u b r a m a n i a n and P u r u s h o t h a m a n 1972; P i and R o h d e 1967; W i l s k i et al. 1971; K n y p l and C h y l i ń s k a 1974). The free-living nematode *Longidorus africanus* doubled the phenol content in the roots of *Bidens tripartita* (E p s t e i n 1972). G i e b e l (1970) found that *Solanaceae* spiecies resistant to *Heterodera rostochiensis* were characterized by a lower monophenol to polyphenol ratio than susceptible species.

The total phenol, chlorogenic acid and phenol inhibitors of IAA-oxidase conterts increased in galls and side roots of carrot cv. Perfekcja infested with *Meloidogyne hapla* Chitw. as compared with those in healthy plants (K n y p l et al. 1975). The present paper describes

changes in total phenol, chlorogenic acid and IAA-oxidase inhibitors contents in the roots of two cultivars infested with this nematode. Bisides the susceptible cultivar Perfekcja, the resistant one Slendero (Brzeski 1974) was used. The aim in view was to estabilish whether the degree of resistance of carrot to *Meloidogyne hapla* Chitw. is correlated with changes in total phenol compounds and inhibitors of IAA-oxidase contents.

MATERIAL AND METHODS

1. Plants

The plant material (*Daucus carota* L.) cv. Perfekcja and cv. Slendero and the culture conditions were described in the previous paper (Janas 1976). The seeds of both cultivars were sown at the same time into soil sterilized with steam and the plants were grown in a glasshouse. From 3-, 4- and 5-month-old plants side roots as well as secondary phloem and secondary xylem of the storage root were taken. In the case of 2-month-old plants the secondary phloem and xylem were analysed jointly. The material for the analyses was collected and sterilized surface with chloretone (Janas 1976). Biochemical analyses of both carrot varieties were performed simultaneously on even-aged plants. Fresh tissues collected immediately after taking the plants out of the soil were used.

2. Extraction of IAA-oxidase inhibitors

Fresh tissue of the carrot storage root, side roots and galls was homogenized with cold 0.2 M phosphate buffer pH 6.1. The homogenate was left to stand 15 min at 4°C and centrifuged for 15 min (3400 x g at 0°C). The sediment was discarded and the buffered extract was divided into two parts. One part was used for IAA-oxidase assays, the other one was boiled for 2 min to inactivate the enzyme, cooled and centrifuged. The denaturated protein sediment was discarded and the supernatant frozen (—20°C) and used for 2-4 following days for the tests on IAA-oxidase inhibitor activity. For these tests a standard IAA-oxidase extract from cucumber seedlings was used.

3. Standard IAA-oxidase from cucumber seedlings

Cucumber seedlings (*Cucumis sativus* L.) cv. Delicates were grown for 5 days in darkness at 25°C. The hypocotyls, cotyledones and roots were cut off and sterilized with 0.1 per cent chloretone for 15 min

followed by washings with sterile distilled water. The tissues were blotted with filter paper, homogenized in a pre-chilled mortar and pestle with cold phosphate 0.2 M buffer, pH 6.1 and hydrated PVP (Polyclar AT); 5 ml of buffer and 1 g of hydrated PVP were used per 1 g of fresh tissue. After 15 min extraction in the refrigerator the homogenate was filtered through three layers of Miracloth and centrifuged at 3400 x g for 20 min at 0°C. The supernatant was frozen (—20°C) and after dilution used for determination of IAA-oxidase inhibitors activity (Knypl and Chylinska 1974).

4. Determination of IAA-oxidase inhibitors activity *in vivo*

The method of determination of IAA-oxidase activity *in vivo* in carrot tissue was described in the previous paper (Janas 1976). As measure of endogenous IAA-oxidase inhibitors activity served the duration of the induced lag phase, in minutes, preceding the moment of initiation of the IAA oxidation process.

5. Determination of IAA-oxidase inhibitors activity *in vitro*

The reaction mixture (Galston and Dalberg 1954) contained: 1 ml 2.4-dichlorophenol (1 mM), 1 ml $MnCl_2$ (1 mM), 2.5 ml phosphate buffer (0.2 M, pH 6.1), 2 ml IAA (1 mM), 0.1-0.3 ml inhibitor extract or the spots scraped off TLC plates and water to 9.5 ml. The reaction was started by adding of 0.5 ml aliquots of the standard IAA-oxidase extract from cucumber. The enzyme was diluted before use with 0.2 M phosphate buffer (pH 6.1) so that it would oxidize about 150 μg IAA per 30 min. Incubation was run at 30°C in a water bath with continuous shaking (100 oscillations min^{-1}) in diffuse daylight. The decrease in IAA content was measured colorimetrically at 15 min intervals (Pilet and Chollet 1970; Janas 1976).

6. Determination of chlorogenic acid and total phenols

Fresh tissues of secondary phloem, secondary xylem, side roots and galls were boiled with 80 per cent ethanol for 60s, cooled and homogenized in a pre-chilled mortar and pestle (Davies 1972; Knypl et al. 1975). After centrifuging off the sediment the supernatant was used for further analyses; 3 ml of 80 per cent ethanol were used per 1 g of fresh tissue.

The total phenol content was determined colorimetrically according to the method of Swain and Hillis (1959): 0.5 ml of the ethanol extract were made up with distilled water to 7 ml and than 0.5 ml

of the Folin-Denis reagent was added with rapid strring. After 3 min 1 ml saturated Na_2CO_3 solution was added. After thorough mixing, the solution was made up with distilled water to 10 ml and left to stand for 1 h. Absorption was measured at 720 nm. Chlorogenic acid was used as a standard.

Chlorogenic acid content was determined by the colorimetric method of Zucker and Ahrens (1958). On a column (12×250 mm) filled with Al_2O_3 (Woelm basic cationotropic, activity grade I) 5-10 ml of ethanol extract of phenols was applied. The column was successively washed with 5 ml distilled water, 4 ml freshly prepared mixture of 0.5 per cent $NaNO_2$ and 5 per cent acetic acid (1:1, v/v), 20 ml distilled water and, finally, 15 ml 5 N KOH. The chlorogenic acid fraction (red colouring) was collected up to 10 ml. Absorbancy of eluates was determined at 520 nm. Chlorogenic acid subjected to analogous chromatographic analysis as the tissue extracts, as a standard.

7. Thin-layer chromatography of polyphenols

The phenol extract in 80 per cent ethanol prepared as described above was evaporated to dryness in vacuo (40-50°C). The sediment was dissolved in 50 per cent methanol in the proportion of 1 ml solvent per equivalent of 1.5 g fresh weight.

The extract (200 μl equivalent to 133.3 mg fresh weight) was spotted on glass plates (20×20 cm) coated with cellulose MN 300 G (0.3 mm). The plates were developed two-dimensionally (I) in 6 per cent CH_3COOH solution and (II) in the organic fraction of the mixture n-butanol: CH_3COOH:H_2O (4:1:5, v/v/v) (BAW). For identifiaction were viewed in ultraviolet light (emission max. 366 nm), and than kept over NH_3 vapours and inspected again both in UV and visible light. Spray colour reactions were run for the particular groups of phenol compounds. Phenols were visualized with: [1] 1 per cent potassium ferricyanide mixed with an equal volume of 1 per cent $FeCl_3$ solution (Ościk 1957), [2] solution of silver nitrate in ammonia (Burke et al., 1960). [3] 1 per cent $AlCl_3$ solution in ethanol (Forest and Bendall 1969). *Ortho*-dihydroxyphenols were revealed with: Arnow mixture (Johnson and Schaal 1957), and buffered titanium-potassium oxalate mixture (PTO) (4.5 ml Tris 0.078 M mixed with equal volume 0.044 M PTO) (Davies 1972). Chlorogenic acid was visualized with Hoepfner's mixture (Craft et al. 1962).

Some spots eg. (No. 5 and 12) were eluted with 3 ml 30 per cent methanol (equivalent to 254 mg fresh weight), centrifuged and the absorption spectrum was determined in the UV using UV-Vis (C. Zeiss, Jena) spectrophotometer.

For enzymatic tests the phenol spots revealed in the UV were scraped off the plates and added to the standard mixture for IAA-oxidase assay supplemented with the standard IAA-oxidase of cucumber. To the control sample containing standard IAA-oxidase from cucumber seedlings cellulose scraped off the side of the plates was added.

Reagents: IAA (Fluka AG), chlorogenic acid (Serva Feinbiochemica), PTO (BDH Chemicals Ltd.).

RESULTS

1. Activity of IAA-oxidase inhibitors *in vivo*

In the tissue of side roots and storage roots of healthy carrot of the cultivar Slendero IAA-oxidase inhibitors were more active than in the

Table 1

Activity of IAA-oxidase inhibitors *"in vivo"* in roots of healthy (H) and *M. hapla* (M) infested carrot plants cv. Perfekcja and cv. Slendero

Tissue	Lag-phase, minutes		
	Age of plants, months		
	3	4	5
cv. Perfekcja			
Secondary phloem (H)	0	0	0
Secondary phloem (M)	18	15	0
Secondary xylem (H)	75	0	0
Secondary xylem (M)	9	30	6
Side roots (H)	90	30	6
Side roots (M)	150	60	18
cv. Slendero			
Secondary phloem (H)	0	0	0
Secondary phloem (M)	25	10	7
Secondary xylem (H)	5	2	0
Secondary xylem (M)	23	5	40
Side roots (H)	120	105	90
Side roots (M)	360	180	180
Galls	—	210	195

Tissue samples (ca. 500 mg fr. wt.) were placed in 10 ml reaction mixture for IAA-oxidase *in vivo* test. Values in the Table represent duration lag phases, in minutes, preceding the moment of initiation of IAA oxidation. Kinetics subsequent oxidation of IAA were presented earlier (cf. Fig. 1 in Janas, 1976).

corresponding roots of the cultivar Perfekcja. The only exception was the secondary xylem of 3-month-old carrot of cv. Perfekcja where the relation was reversed (Table 1). In secondary phloem of the storage roots of healthy plants in both carrot cultivars IAA-oxidase inhibitors could not be detected *in vivo* (the lag-phase is lacking). The highest IAA-oxidase inhibitor activity was found in the side roots of 3-month-old plants in both carrot cultivars. The activity of these inhibitors rapidly decreased as the plants aged (Table 1). For instance in the side roots of 3-month-old healthy carrot of the Perfekcja the duration of the lag-phase was 90 min, while after 5 months of growth it was reduced to 6 min.

In both carrot cultivars the IAA-oxidase inhibitors activity was higher in the tissues infested with nematodes than in healthy ones. An exception was the secondary xylem of the 3-month-old carrot of the Perfekcja variety where the activity of the inhibitors in healthy tissue was higher than in the infected tissue. This effect disappeard in the 4-month-old plants. IAA-oxidase inhibitors activity in the side roots cv. Slendero infested with *M. hapla* was higher than in the analogous tissue of the variety Perfekcja, notwithstanding the age of the plants.

After 4 and 5 months of growth the activity of IAA-oxidase inhibitors was also determined in the galls formed on the storage root of infected carrots of the cv. Slendero. These galls exhibited the highest activity of the inhibitors.

2. Activity of IAA-oxidase inhibitors *in vitro*

The activity of IAA-oxidase inhibitors in 2-month-old carrot was analysed only in the storage root and side roots. The storage roots at this age are so small that it is difficult to separate secondary phloem from secondary xylem. The inhibitor content in the healthy storage root of the carrot cultivar Perfekcja and Slendero was similar (Table 2). In the side roots of healthy plants cv. Perfekcja the inhibitor activity was not much higher than in the corresponding tissue of healthy cv. Slendero plants.

In the side roots cv. Perfekcja infested with *M. hapla* the activity of IAA-oxidase inhibitors was twice higher than in the analogoues tissue cv. Slendero. In the storage root of young plants of both cultivars the inhibitor activity doubled under the influence of the pathogen. An analogous phenomenon was observed in the case of side roots of the carrot Perfekcja. On the other hand in the side roots of the carrot Slendero the activity of IAA-oxidase inhibitors was slightly higher in healthy tissue in infected ones (Table 2).

The slope of the curves illustrating the oxidation of IAA in the presence of the inhibitors investigated was the same as the curve for the control samples containing the standard IAA-oxidase alone (Fig. 1).

Table 2

Activity of IAA-oxidase inhibitors "*in vitro*" in roots of 2-month-old healthy (H) and *M. hapla* (M) infested carrot cv. Perfekcja and cv. Slendero

Tissue	Lag-phase, minutes	
	cv. Perfekcja	cv. Slendero
Storage root (H)	14	12
Storage root (M)	30	31
Side roots (H)	60	52
Side roots (M)	105	46

Fresh tissues were ground with sodium-phosphate buffer, 0.2 M, pH 6.1. Extracts were boiled and 0.3 ml aliquots were added to 9.7 ml IAA-oxidase activity reaction mixture containing 0.5 ml of IAA-oxidase from cucumber cotyledons.

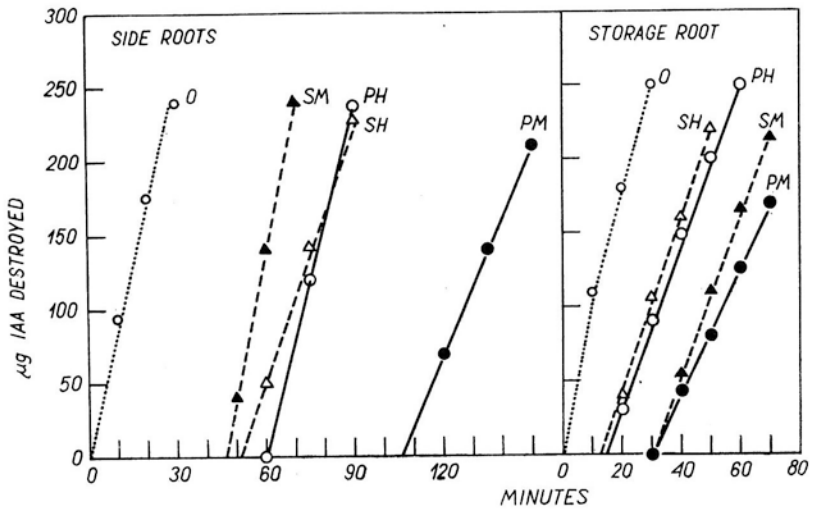

Fig. 1. Kinetics of IAA destruction by the standard IAA-oxidase of cucumber cotyledons in the presence of 0.3 ml aliquots of 0.2 M phosphate buffer, pH 6.1 extracts of the side roots and storage roots of healthy (H) and *M. hapla* (M) infested 2-month-old carrot plants

SM, SH — cv. Slendero, healthy and *M. hapla* infested, respectively. *PM, PH* — cv. Perfekcja, healthy and *M. hapla* infested, resectively. 0 — enzyme control containing no inhibitor
Other details see in Table 2.

Thus, we are possibly dealing here with inhibitors which do not inactivate the enzyme, but only postpone the initiation of oxidation IAA (H a r e 1964).

The IAA-oxidase inhibitors activity decreases with the age of the plants (Table 3).

Table 3

Activity of IAA-oxidase inhibitors *"in vitro"*, in roots of healthy (H) and *M. hapla* (M) infested carrot plants cv. Perfekcja and cv. Slendero

Tissues	Lag-phase, minutes		
	Age of plants, months		
	1	2	3
	IAA-oxidase inhibitor, ml		
	0.3	0.1	0.1
cv. Perfekcja			
Secondary phloem (H)	15	16	2
Secondary phloem (M)	30	70	31
Secondary xylem (H)	40	13	24
Secondary xylem (M)	65	46	44
Side roots (H)	50	44	15[a]
Side roots (M)	100	75	23[a]
cv. Slendero			
Secondary phloem (H)	45	6	7
Secondary phloem (M)	98	35	11
Secondary xylem (H)	12	9	0
Secondary xylem (M)	120	12	6
Side roots (M)	53	88	3[b]
Side roots (H)	203	100	7[b]
Galls	—	—	11[c]

IAA-oxidase inhibitors were extracted with 0.2 M sodium-phosphate buffer (pH 6.1), extracts were boiled for 2 min, cooled and centrifuged. The supernatant fraction was stored in a deep-freeze for 2-4 days before tests.

a 0.03 ml, b 0.02 ml and c 0.012 ml aliquots of extracts of IAA-oxidase inhibitors were used for tests. When 0.1 ml aliquots of the extracts were used, the lag phase was longer then 3 hours.

In side roots of 3-month-old carrot cv. Slendero infested with the nematodes the inhibitor activity was about 4 times higher as compared with that in the healthy tissue. An analogous increase for the variety Perfekcja was of the order of two times. In older plants these differences are less pronounced (Table 3).

3. Contents of chlorogenic acid and total phenols

In the storage root and side roots of the carrot cv. Perfekcja the total amount of phenols present was higher than in cv. Slendero (Table 4). In both cultivars the total phenol content did either not change with age (secondary phloem) or was slightly lower (side roots).

Table 4

The content of phenolics in healthy (H) and *M. hapla* (M) infested carrot plants cv. Perfekcja and cv. Slendero

Tissues	Phenolics, μg g^{-1} fr. wt. Age of plants, months		
	3	4	5
cv. Perfekcja			
Secondary phloem (H)	329	357	343
Secondary phloem (M)	422	373	441
Secondary xylem (H)	351	186	284
Secondary xylem (M)	210	152	242
Side roots (H)	509	444	442
Side roots (M)	659	476	587
cv. Slendero			
Secondary phloem (H)	274	284	253
Secondary phloem (M)	344	221	287
Side roots (H)	446	365	389
Side roots (M)	543	551	481

Phenolics were extracted with 80% ethanol, and measured by the method of Swain and Hillis (1959).

Under the influence of the nematode the content of phenols increased in 3-month-old plants of both varieties in the tissues of the storage root and side roots with the exeption of the secondary xylem in the cv. Perfekcja (Table 4). In older plants, 4- and 5-month-old, a significant increase in phenol content was observed only in side roots.

In side roots and storage roots of carrot cv. Perfekcja the amount of chlorogenic acid was higher than in the variety Slendero. Older plants of both cultivars contained less chlorogenic acid than did the 3-month-old plants (Table 5). A particularly marked 7-fold fall of the chlorogenic acid level with age was noted in the case of the secondary xylem of the storage root cv. Perfekcja. In the side roots characterized by the highest chlorogenic acid content this decrease with age of the plants was smaller, of the order of two times.

Chlorogenic acid content in tissues of plants infected with the nematode was in most cases higher in both varieties than in healthy tissues. The only exception was again the secondary xylem of 3-month-old healthy carrot cv. Perfekcja in which two times more of the chlorogenic acid was detected than in analogous tissue of infected plants. The largest quantities of chlorogenic acid accumulated in the tissues directly attacked with nematode. For istance in the side roots of 3-month-old carrot of cv. Perfekcja its content under the influence of *M. hapla* increased from 353 to 446 $\mu g \cdot g^{-1}$ fresh weight.

Table 5

The content of chlorogenic acid in healthy (H) and *M. hapla* (M) infested roots of two cultivars of carrot, cv. Perfekcja and cv. Slendero

Tissue	Chlorogenic acid, μg g^{-1} fr. wt.		
	Age of plants, months		
	3	4	5
cv. Perfekcja			
Secondary phloem (H)	147	87	84
Secondary phloem (M)	194	88	174
Secondary xylem (H)	220	19	31
Secondary xylem (M)	117	13	11
Side roots (H)	353	218	172
Side roots (M)	446	268	315
cv. Slendero			
Secondary phloem (H)	99	62	59
Secondary phloem (M)	179	74	71
Secondary xylem (H)	22	11	17
Secondary xylem (M)	58	11	26
Side roots (M)	288	131	213
Side roots (H)	387	365	250

Chlorogenic acid was extracted with 80% ethanol and measured by the method of Zucker and Ahrens (1958).

4. Chromatographic analysis of the phenols

On the chromatograms of extracts from side roots of healthy and infested plants and from galls, 11 spots of phenol compounds were detected. Additional spots No. 5 and No. 9' were detected in the extracts of infested side roots and galls, respectively (Fig. 2*A-C*). Spots Nos 9 and 10 in BAW have Rf 0.60 and 0.66, respectively. Both spots react with the Hoepfner's reagent giving a red colouring. They were identified as chlorogenic acid and *iso*-chlorogenic acid. Identification was confirmed by determination of the spectrum in UV light and comparative tests with authentic chlorogenic acid.

The remaining spots were not identified. All of them with the exception of spot No. 1 reacted with the mixture of $FeCl_3$ with potassium ferricynide.

On the other hand, in secondary xylem and secondary phloem of both healthy and infected plants 6 spots occurred. Spots Nos II and III appeared as diffuse bands. Spot No. V was identified as chlorogenic acid (Fig. 2*D-F*).

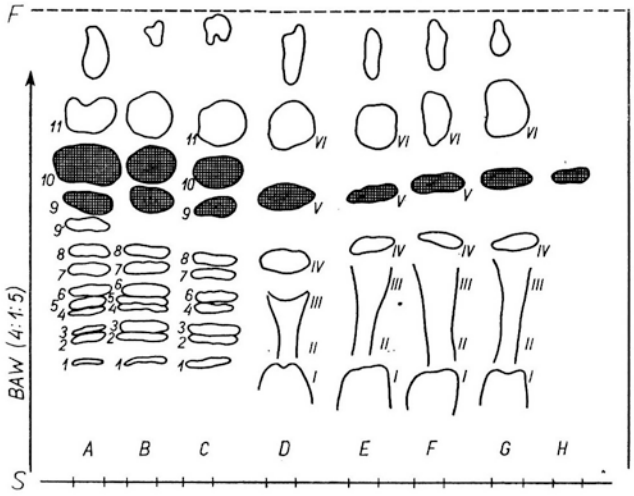

Fig. 2. One-dimensional TLC of phenolics from galls, side roots and storage roots of healthy (H) and *M. hapla* (M) infested carrots cv. Perfekcja

Phenolics from 4-month-old carrots were extracted with 80 per cent ethanol. Cellulose MN 300 G plates (0.3 mm) were development in BAW. *A* — galls (equiv. 66 mg fr. wt.); *B* — side roots M (equiv. 133 mg fr. wt.); *C* — side roots H (equiv. 133 mg fr. wt.); *D* — secondary phloem M (equiv. 133 mg fr. wt.); *E* — secondary phloem H (equiv. 166 mg fr. wt.); *F* — secondary xylem M (equiv. 166 mg fr. wt.); *G* — secondary xylem H (equiv. 166 mg fr. wt.); *H* — chlorogenic acid; *S* — start; *F* — front. Spots Nos 9, 10 and V are chlorogenic acids

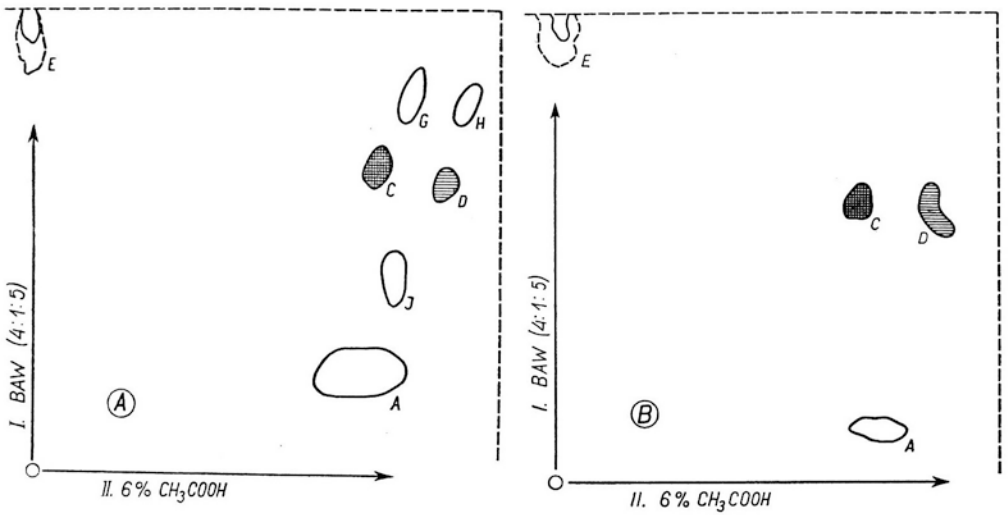

Fig. 3. Two-dimensional TLC of phenolics from secondary xylem (equiv. 200 mg fr. wt.) of the storage root of healt y (A) and *M. halpa* (B) infested carrot cv. Perfekcja

Phenolics from 4-month-old carrots were extracted with 80% ethanol. Cellulose MN 300 G plates were developed in (I) BAW followed by (II) 6% acetic acid

In secondary xylem of healthy carrots 7 spots appeared on two-dimensional TLC: A, C, D, E, J, G and H (Fig. 3*A*). Spot C was identyfied as chlorogenic acid, whereas spot D proved to be *ortho*-dihydroxyphenol since it reacted with PTO giving a yellow colouring (D a v i e s 1972). Under the influence of infestation with nematodes the spots J, G and H disappeared in secondary xylem (Fig. 3*B*).

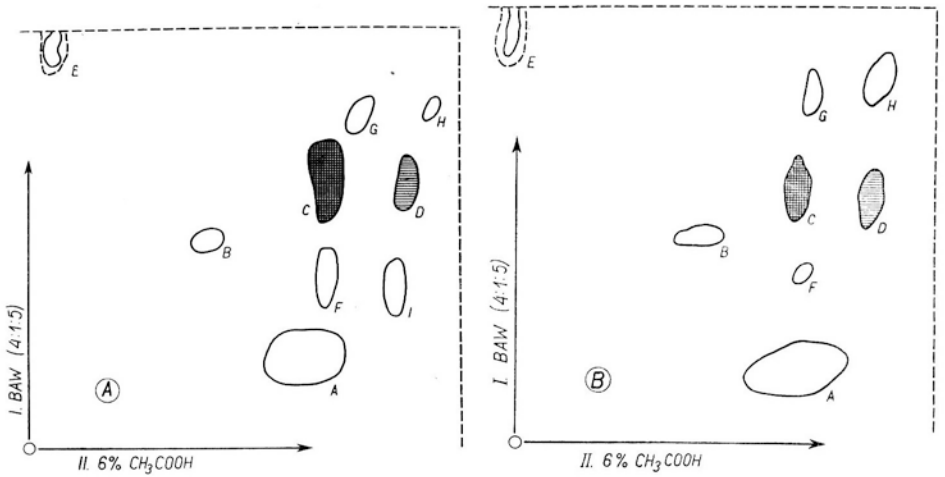

Fig. 4. Two-dimensional TLC of phenolics from secondary phloem (equiv. 200 mg fr. wt.) of the storage roots of healthy (A) and *M. hapla* (B) infested carrot cv. Perfekcja

Details as in Fig. 3

The secondary phloem of the storage root of healthy carrot of the cv. Perfekcja also gives more spots than does analogous tissue of infected plants (Fig. 4*A*). Spot I disapears as the result of infestation (Fig 4*B*).

33 spots were detected on two-dimensional chromatograms of the exstracts of side roots of healthy plants cv. Perfekcja (Fig. 5*A*), whereas analogous tissue of infested plants gave an additional spot No. 21', while spots Nos 3, 7, 11, 12, 16, 20, 22, 26, 27, 28, and 33 are missing (Fig. 5*B*). Spots Nos 14 and 15 were identified as chlorogenic and *iso*-chlorogenic acids.

20 spots of phenol compounds were detected in the extracts of the galls (Fig. 6). Eleven of them corresponded to *ortho*-dihydroxyphenol which react with Arnow's reagent giving a red colouring. Spots Nos 5 and 12 were identified as chlorogenic and *iso*-chlorogenic acids (Fig. 7). Previously 13 spots of phenol compounds had been detected in extracts from galls (K n y p l et al. 1975). In the present study much more spots were revealed by changing the sequence of solvents (D a v i e s 1972).

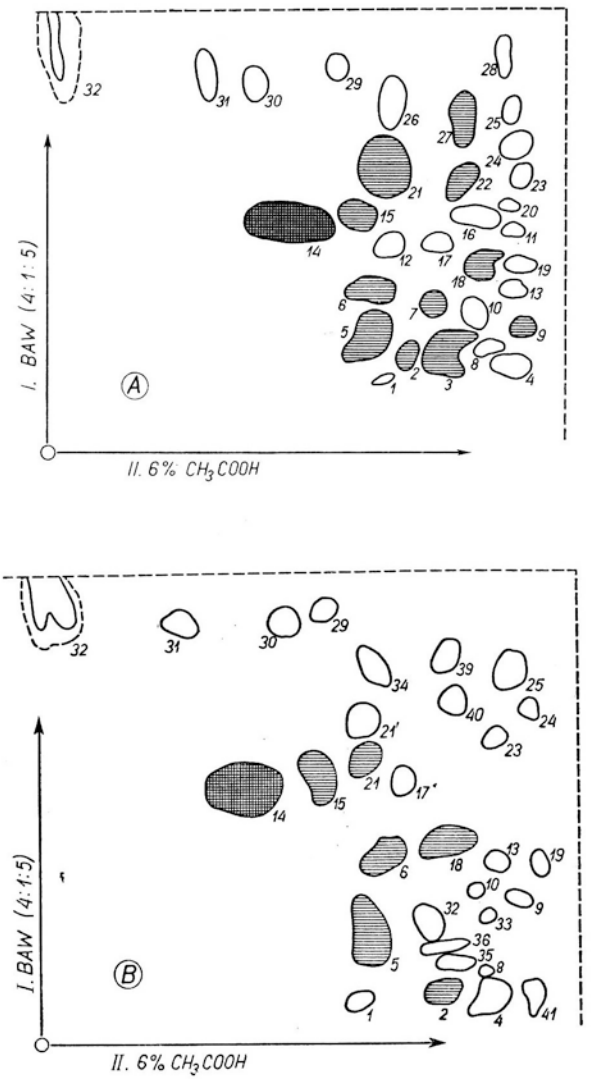

Fig. 5. Two-dimensional TLC of phenolics from side roots (equiv. 133 mg fr. wt.) of healthy (A) and *M. hapla* (B) infested carrot cv. Perfekcja

Details as in Fig. 3

All phenol compounds detected on chromatograms of extracts from galls inhibited IAA-oxidase activity (Fig. 6). Most active as IAA-oxidase inhibitors were spots Nos 5 and 12, that is chlorogenic and *iso*-chlorogenic acids and spots Nos 8 and 9 corresponding to spot No. 9' in one-dimensional chromatograms (Fig. 2). These spots represente *ortho*-dihydroxyphenols occuring in much smaller amounts than did chlorogenic acids. These compounds were not found in tissues of healthy plants.

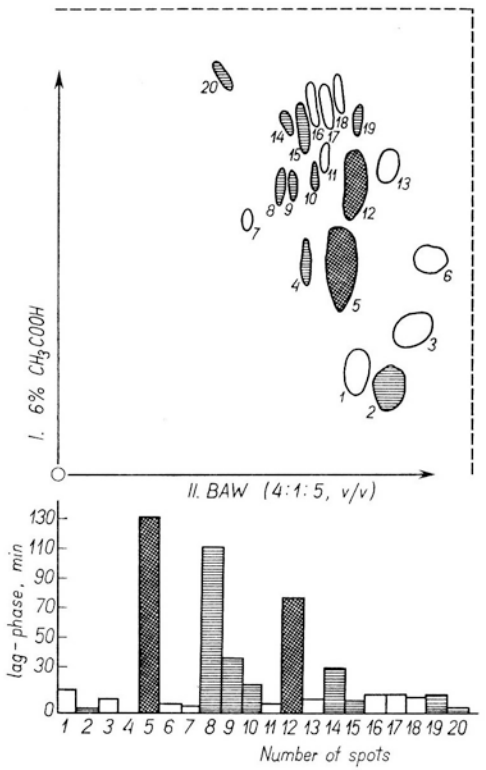

Fig. 6. IAA-oxidase inhibitory activity of phenolics detected in galls of storage roots of *M. hapla* infested carrot cv. Perfekcja

Phenols were extracted from 5-month-old plants. Equivalent of 133 mg fr. wt. was subjected to TLC details in Fig. 3. Spots, detected in UV, were scrapped off and transferred to vails containing 9.5 ml of buffered co-factors for the standard IAA-oxidase activity test Reaction was rated by adding 0.5 ml of the standard IAA-oxidase (cf. Material and Methods). Upper part of the Figure: Two-dimensional TLC chromatogram of the phenols. Lower part of the Figure: IAA-oxidase inhibitory activity of each spot represented in the upper part. Striped spots, *ortho*-dihydroxyphenols; squared spots, chlorogenic acids

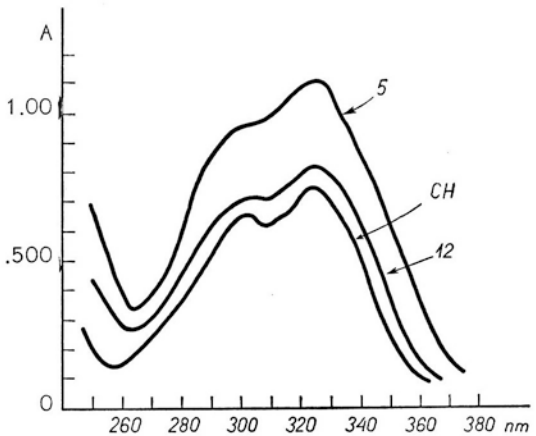

Fig. 7. UV absorption curves for chlorogenic acid (CH) and spots. No. 5 and No. 12 of the TLC chromatogram represented in F.g. 6. The compounds were solubilized in 50% methanol

DISCUSION

An external symptom of infestation of the plants by *M. hapla* is the formation of galls. It is the result of: [A] the action of the pathogen on the plant and [B] of the defence reaction of the plant. It is supposed that the appearance of galls may be the function of disturbances of the rate of synthesis of auxins and for their inactivation (Dropkin 1969; Viglierchio 1971).

Nematodes cause an increase in the content of phenol compounds in plants (Pi and Rohde 1967; Giebel 1970; Balasubramanian and Purushothaman 1972). In response to infestation by *Aphelenchoides* spp. chlorogenic acid and *iso*-chlorogenic acid accumulate in the leaves of *Ficus* and *Chrysanthemum* (Wallace 1973).

Phenol compounds may stimulate IAA-oxidase activity — [(e.g. 2.4-dichlorophenol (DCP), *p*-coumaric and *p*-hydroxycynammic acids, naphtol etc. (Furuya et al. 1962)], or they may inhibit the activity of the enzyme — [(e.g. chlorogenic acid at pH 6.1, ferulic acid and caffeic acid (Sondheimer 1958; Pilet 1964)]. Classical IAA oxidase inhibitors can, if pH changes, stimulate the activity of this enzyme as for instance chlorogenic acid at pH 4.4 (Sirju and Wilson 1974). This study revealed phenol compounds, particulary chlorogenic acids accumulate in the carrot (cf. Knypl and Chylinska 1974). It was also found that in the root tissues of carrots infested with *M. hapla* changes occur in the ratios between the phenols. Particularly interesting is the fact of appearance in the galls of a new unidentified substance No. 9' with Rf 0.53 (Fig. 2A). As shown by two-dimensional chromatographic analysis, spot No. 9' consists of two substances, of the spots 8 and 9 (Fig. 6). In spite of the increase in total phenol content, the number of various phenol compounds detected on the chromatograms of the tissues decreases owing to infection with *M. hapla*.

All phenols extracted from galls of cv. Perfekcja inhibit IAA-oxidase activity (Fig. 6). Most interesting are spots Nos 8 and 9, because they are most active as IAA-oxidase inhibitors, although they occur in much smaller amounts than do chlorogenic acids.

There is no simple correlation between the chlorogenic acids content and the inhibitory activity of the extracts towards IAA-oxidase since, beside chlorogenic acid, 19 other IAA inhibitors were detected (Fig. 6), for instance in side roots of 3-month-old carrot of the cv. Perfekcja the duration of the lag-phase in healthy tissues is 90 min, and in the infested ones 150 min, whereas the chlorogenic acid amount in analogous tissue of healthy plants is 353 and in the infected ones 446 μg g^{-1} fresh weight.

Brzeski (1974), on the basis of the phenomenon of storage root branching, established that the cultivar Perfekcja is susceptible to *M. hapla*, while cultivar Slendero is resistant.

Many factors determine the susceptibility of plants to pathogens. Among other factors of great importance is the ability of phytoalexins synthesis by the tissues (Ingham 1972), of phenol compounds and of balancing the processes of growth inhibitor and phytohormone synthesis and accumulation, particularly of auxins. In the ealier study (Janas 1976) it was found that carrot of the cv. Perfekcja exhibits a higher IAA oxidase activity than does the cv. Slendero. At the same time the cv. Perfekcja is characterized by a higher polyphenols content, their content increasing more conspicuously in response to infestation in this cultivar than in the cultivar Slendero (Tables 4 and 5). Of particular importance seems to be the fact that in young, 2-month-old side roots of the cv. Perfekcja the IAA-oxidase inhibitors activity is twice higher than in healthy plants. On the other hand, in analogous tissue of the cv. Slendero the acivity of these inhibitors in healthy tissues and those infected with *M. hapla* is similar (Table 2).

Wilski and Giebel (1970) suggested that inhibition of IAA breakdown by phenol compounds is stronger in susceptible potato varieties than in those resistant to *Heterodera rostochiensis*. In both potato cultivars, according to these authors, β-glucosidase secreted by the nematodes may release IAA, cytokinins and phenol compound from their complexed forms.

In the previous paper it has been demonstrated that the IAA-oxidase activity in healthy and infected tissues is the same (Janas 1976). The results of the mentioned work seem to confirm the suggestion (Dropkin et al. 1969; Viglierchio 1971; Knypl et al. 1975) that in carrots of the cv. Perfekcja inhibiton of IAA-oxidase activity occurs *in vivo* as a response to infestation by nematodes, owing to the accumulation of polyphenol inhibitors of this enzyme, particularly of the unidentified substance No. 9' (Fig. 6). This probably leads to a local increase in the concentration of physiologically active auxins stimulating tissue proliferation. The phenomenon of inhibitor accumulation does occur in early phases of infected of cv. Slendero by *M. hapla* (Table 2). It would seem that in these roots there does initially not occur disturbances in the process of auxin degradation, and in connection with this growth is almost normal and the later formed gall are small.

SUMMARY AND CONCLUSIONS

Experiments were carried out with 2-, 3-, 4- and 5-month-old carrot plants cv. Perfekcja and cv. Slendero, healthy and infested with northern root-knot nematode, *Meloidogyne hapla* Chitwood. Cultivar cv. Perfekcja is regarded to be very sensitive to the nematode whereas cv. Slendero is resistant. It has been revealed that:

1. Activity of inhibitors of IAA-oxidase is higher in the side roots of cv. Slendero as compared with those of cv. Perfekcja.

2. Inhibitors of IAA-oxidase accumulate in the response to the infestation.

3. However, the youngest plants cv. Slendero (2-month-old) contain the same quantity of the inhibitors irrespective if healthy or infested tissue is taken into consideration, whereas the quantity of the inhibitors in the youngest plants cv. Perfekcja is already doubled in response to the pathogen.

4. Carrot cv. Perfekcja contain more phenols and chlorogenic acid than carrot cv. Slendero.

5. TLC revealed the presence of two very active inhibitors of IAA--oxidase in galls (spots No. 8 and 9), that are absent in healthy tissues.

6. All the phenolics revealed in TLC are active as inhibitors of IAA-oxidase.

This suggested that IAA-oxidase inhibitors accumulate in the plants infested with the nematode. The inhibitors slown down the rate of oxidation of IAA. This possibly leads to local accumulation of active auxins and proliferation of the tissues nearby the site of nematode penetration. Finally, galls are formed. Carrot cv. Slendero seems to be less sensitive to the nematode because the inhibitors of IAA-oxidase do not accumulate in the side roots at early stages of infestation.

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Polifenole i inhibitory oksydazy kwasu indolilo-3-octowego w korzeniach marchwi porażonych przez mątwika północnego

Streszczenie

Materiałem do badań była marchew 2-, 3-, 4-, i 5-miesięczna odm. 'Perfekcja i odm. 'Slendero', zdrowa i porażona przez mątwika północnego *Meloidogyne hapla* Chitw.

Stwierdzono, że:

1. W korzeniach bocznych marchwi odm. 'Slendero' inhibitory IAA-oksydazy są bardziej aktywne niż w analogicznej tkance odm. 'Perfekcja'.

2. Pod wływem porażenia przez nicienie następuje akumulacja inhibitorów IAA-oksydazy. W korzenach bocznych 2-miesięcznej marchwi odm. 'Perfekcja' aktywność inhibitorów IAA-oksydazy jest dwa razy wyższa u roślin porażonych niż u roślin zdrowych, natomiast w marchwi odm. 'Slendero', w tkankach zdrowych i porażonych przez *M. hapla*, aktywność inhibitorów jest podobna.

3. Zawartość fenoli ogólnych i kwasu chlorogenowego jest wyższa u marchwi odm. 'Perfekcja' niż u odm. 'Slendero'.

4. Na jednokierunkowych chromatogramach ekstraktów fenoli z wyrośli stwierdzono występowanie plamy 9', która rozdziela się na dwie plamy nr 8 i 9 wykrywane na chromatogramach dwukierunkowych. Są one bardzo aktywne jako inhibitory IAA-oksydazy. Substancje te nie występują w ekstraktach z roślin zdrowych.

5. W wyroślach marchwi odm. 'Perfekcja' wszystkie fenole wykryte na chromatogramach hamują aktywność IAA-oksydazy. Wnioskuje się, że w korzeniach marchwi, w odpowiedzi na porażenie przez mątwika północnego, gromadzi się duża ilość inhibitorów IAA-oksydazy, które obniżają szybkość utleniania IAA.

Zahamowanie aktywności IAA-oksydazy *in vivo* wskutek gromadzenia się polifenolowych inhibitorów, zwłaszcza plamy 9', prowadzi prawdopodobnie do wzrostu zawartości fizjologicznie czynnych auksyn pobudzających proliferację tkanek.