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Flavonoids in potato cyst nematodes

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Summary – Eight compounds from a total of 23 phenolics present in both species of potato cyst nematodes (PCN) are flavonoids. Three of them – a hydroxyflavone, an aurone and a chalcone – being yellow in colour, are responsible for the coloration of PCN. The aurone (the strongest in colour) which is absent in *Globodera pallida* and present in *G. rostochiensis,* is the golden yellow pigment which is responsible for the vernacular name of the " golden nematode ".

Résumé – ***Flavonoïdes des nématodes à kystes de la pomme de terre*** – Sur 23 composés phénoliques présents chez les deux espèces de nématodes à kystes de la pomme de terre, huit sont des flavonoïdes. Trois d'entre eux, de couleur jaune – une hydroxyflavone, une aurone et une chalcone – sont responsables de la coloration de ces nématodes. L'aurone – le composé le plus coloré – absent chez *Globodera pallida* et présent chez *G. rostochiensis,* représente le pigment jaune doré responsable du nom vernaculaire du « nématode doré » donné à *G. rostochiensis.*

Key-words : Flavonoids, *Globodera,* nematodes, pigments.

The two species of potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida* differ in the colour sequence, or chromogenesis, that occurs as the female matures. In *G. rostochiensis,* chromogenesis is from white through yellow to brown. By contrast *G. pallida* has no yellow phase. Since Chitwood (1951) coined the name " golden nematode " for *G. rostochiensis,* many workers have investigated the chromogenesis during maturation (Ellenby, 1963; Wilski & Giebel, 1966; Guile, 1967; Smith & Ellenby, 1967; Wilski *et al.,* 1968; Voinilo, 1975; Hominick, 1983). Using ethanolic extracts of yellow cysts, Smit (unpubl.) was able to demonstrate that there were a number of phenolic compounds present in the extracts that had the properties of flavonoids. Further studies (Vlachopoulos, 1986) using two dimensional TLC showed conclusively that there were a large number of such compounds présent, some in quite large quantities. Although TLC analysis demonstrated the presence of 23 compounds with a phenolic basis, HPLC analysis indicated that there are many more (Vlachopoulos, 1986). However, spectra obtained from many of these compounds suggest that they are not flavonoids but of a more simple phenolic structure.

At first the presence in animals, including nematodes, of such secondary plant metabolites as the flavonoids was considered to be rather unusual (Harborne, 1967). However, subsequent studies have demonstrated the presence of a wide range of secondary plant metabolites, particularly flavonoids in insects (Morris & Thomson, 1963, 1964; Wilson 1986, 1987). Plant parasitic nematodes, and in particular the monophagous types or forms with a restricted host range such as PCN, are similar to monophagous insects as they are closely associated with and dependent on plants and must, therefore, come into contact with these secondary plant substances. As some of these substances are highly toxic they must be metabolised by the nematodes.

This study examined the properties of the phenolic coumpounds, previously isolated from PCN (Vlachopoulos, 1986), to determine their possible involvement in chromogenesis and to compare the presence of flavonoid compounds in the contrasting chromogenesis in the two species in PCN.

Materials and methods

Pot cultures of the international pathotypes Ro1, Ro2, Ro3, Ro4 and Ro5 of *Globodera rostochiensis* and Pa1, Pa2 and Pa3 of *G. pallida* were established on the potato cultivars Majestic and Arran Banner and kept in the greenhouse. All stages of the females were collected. The females were removed from the roots with the aid of a dissecting needle and placed in a watch containing tap water at 4 °C. Cleaned females and early cysts were placed in small (5 ml) snaptop vials containing approximately 1.5 ml of 96 % ethanol. Thus extracts of all developmental stages of females were made and separated by two dimensional thin layer chromatography (TLC). The support material for the TLC procedure was cellulose without binder (Scheicher & Schuell Type F1440, 20 × 20 cm). For spot detection, chromatograms were examined in an ultraviolet (UV) light cabinet (emitting

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at 254 and 365 nm) in the presence or absence of NH_3 vapor (880 sp. gr.).

Pilot studies showed that the compounds were separated most effectively in benzene-acetic acid-water (125:72:3 v/v) and 4 % acetic acid for the 1st and 2nd dimension respectively. The solvent systems, isopropanol-ammonia-water (8:1:1 v/v) and 4 % acetic acid, and chloroform-acetic acid-water (13:6:1 v/v) and 4 % acetic acid were also used in order to isolate components more distinctly from adjoining spots or to produce more compact areas in the chromatograms for the purpose of spectroscopy. The UV absorption spectra were scanned using a Shimadzu scanning and recording spectrophotometer (Model UV 240). All samples were scanned from 500 nm – 190 nm.

Results

From the 23 phenolic compounds detected by TLC in both species of PCN (Vlachopoulos, 1986) only eight showed spectra with the properties of flavonoids. The spectral analysis of these with their Rf values in benzen-acetic acid-water and 4 % acetic acid for the 1st and 2nd solvent systems respectively, are presented in Table 1. The λ max values in nm are followed by the shoulder values in brackets.

Compounds 1, 2, 3, 4 and 5 were eluted from chromatograms developed in chloroform-acetic acid-water; compounds 6 and 8 were eluted from chromatograms developed in benzene-acetic acid-water and compound 7 from chromatograms developed in isopropanol-ammonia-water.

Table 1. Rf values of PCN flavonoids separated by TLC in benzene-acetic acid-water (125:72:3 v/v) and 4 % acetic acid for the 1st and 2nd solvent systems, respectively, and λ max values of spectral curves of the same compounds in methanol. Compounds 7 and 8 were not present in Pa_1 and Pa_3 of *G. pallida* and Pa_2 lacks 6, 7 and 8.

Spot	Rf values		λ max (nm)	
Spot No.	1st solv.	2nd solv.	Band II 240-280 nm	Band I 300-380 nm
1	00	57	283	311, (330)
2	00	36	226, 277, (283), (293)	
3	08	80	(250), (264), 284	308, (330)
4	51	00	(218), 275, (291)	313, (353)
5	56	43	224, 276, 284	315
6	83	32	(233), (279), 288	338
7	87	12	238	308, (329),(350)
8	89	03		300, 348, 368

Shoulder values are given in brackets.

In chromatograms heavily loaded (about 100 λ) with the ethanolic extract from yellow females, it was apparent under visual light that three of the eight flavonoids – those numbered 6, 7 and 8 – appeared in various shades of yellow and are likely to be responsible for the coloration of the cysts of the " golden nematode ". No other pigment was observed in all stages of cyst development of all pathotypes of both species examined.

All flavonoids were present in all pathotypes of *G. rostochiensis*. However compounds 7 and 8 were not present in pathotypes Pa1 and Pa3 of *G. pallida* and pathotype Pa2 lacks all three (6, 7, 8) pigments.

From the characteristics of the UV absorption spectra we grouped the compounds listed in Table 1 according to the scheme of Marby *et al.* (1970) as follows : compounds no. 1, 3, 4, 5 and 6 which absorb in both band regions are flavones and/or flavonols. Compound no. 2 with an obvious band II but with an absence of band I absorption belongs to isoflavones or flavonones or dihydroflavonols group. Compound no. 7 with no band II but with band I in the range 370-430 nm is an aurone. Compound no. 8 which also lacks band II absorption but has an intense band I in the range 340-390 nm, is a chalcone.

This general classification of the compounds is supported by their chromatographic behaviour. Comparing the map of distribution of flavonoids in two dimension paper chromatograms (Marby *et al.*, 1970) with the distribution of PCN flavonoids (in similarly developed chromatograms) the nature of Table 1 compounds is approximately as follows : no. 1 is a flavone or flavone 7-0-monoglycoside, no. 2 is an isoflavone 7-0-diglycoside, no. 3 is a flavonol 3-7-0-diglycoside, no. 4 is a flavone or flavonol, no. 5 is a flavonol 3-0-monoglycoside, no. 7 is an aurone and no. 8 is a chalcone. Compound no. 6 was identified as quercentagetin 3, 5, 6, 3′, 4′ – pentamethyl ether or 3, 5, 6, 3′, 4′ – pentamethoxy-7-hydroxyflavone due to its characteristic behaviour in UV light and UV + NH_3, its Rf values in various solvent systems and its UV absorption spectrum (Fig. 1).

Discussion

The presence of three coloured compounds (6, 7 and 8) in *G. rostochiensis* is significant. Compound 6 is a hydroxyflavone, specifically the pentamethyl ether of quercetagetin, was very concentrated and present in both PCN species with the exception of Pa2. The same compound has also been isolated from the plant *Proposis spicigena* L. by Sharma *et al.* (1964) and its chromatographic data and spectra are given by Marby et *al.* (1970). Since this is the first record of this compound in animals and mainly because this is the first record of a flavonoid in nematodes, attempts were made to discover its origin. Thus, analysis of potato roots – infected and uninfected with nematodes – have demonstrated a number of compounds with phenolic properties (Vlacho-

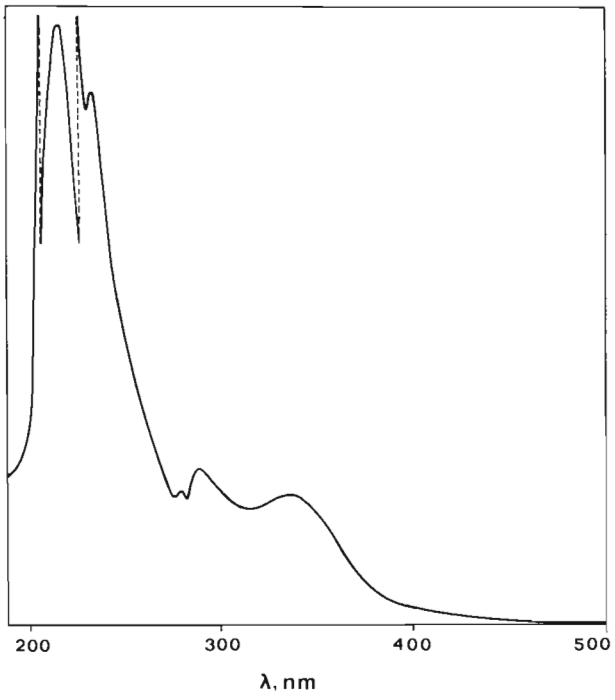

Fig. 1. UVspectrum in methanol of compound no. 6 (see Table 1) of potato cyst nematodes obtained by TLC.

poulos, unpubl.) but no traces of the above mentioned ether were found. Quercentagetin is not present in potatoes although quercetin and kaempherol are common. The pentamethyl derivative must therefore be synthesized by the nematode. The origin of this substance must await the results of experiments using labelled compounds.

Of greater significance from an evolutionary point of view is the distribution of compounds 6, 7 and 8 which are a hydroxyflavone, an aurone and a chalcone respectively. All of these compounds are yellow in colour; compound 7 (aurone) being the strongest, the other two are very pale yellow. The yellow colour of *G. rostochiensis* cysts is due to the presence of compounds 6, 7 and 8 while *G. pallida*, with the exception of Pa2, lacks 7 and 8 and Pa2 lacks them all. The most significant absence is that of compound No. 7 (the aurone) since this imparts the majority of the cyst colour.

Pathotype Pa2 of *G. pallida* is clearly different in that it lacks all three compounds. Cysts of this pathotype appear much paler than the other pathotypes. Pa2 is unique in other respects since differences can be detected by electrophoresis (Fox & Atkinson, 1984; Salame, 1985); Reynolds (pers. comm.) considers Pa2 differs with respect to catecholase distribution. These observations, while explaining the difference in colouration of *G. rostochiensis* and *G. pallida*, may also be of significance in relation to the metabolic pathways involved. The actual precursor of the flavonoids is a chalcone, often a dihydroxychalcone which is synthesized from p-coumaric acid. The present study demonstrates the presence of an aurone in *G. rostochiensis*. Aurones are unusual products; the presence of an aurone indicates that there is a block in the biosynthetic pathway from chalcone to flavone which can be by passed by the production of aurones (Siegelman, 1964). These can then enter the biosynthetic pathway for flavonoid production.

It is possible that variations in the biosynthetic pathway, depending on the presence or absence of the aurone, gives rise to the yellow and non-yellow forms of PCN. The presence of aurones may be central to the variations in chromogenesis in other cyst forming nematodes. Thus, apart from other differences in cyst types, there may be a major dichotomy in the flavonoid composition.

It is considered by Evans *et al.* (1975) that *G. pallida* originated from the more mountainous regions of South America. There has been a tendency to assume that *G. rostochiensis* was the typical PCN. This assumption probably arose because of the predominance of *G. rostochiensis* in earlier studies in Europe. The implications from this study are that the aurone-containing *G. rostochiensis* is not the " normal " condition and that *G. pallida* is more closely adapted to the host plant. Using the dihydroxychalcone, phlorisin, Salame (1985), Showed that *G. rostochiensis* did not possess the enzymes necessary to metabolize this substance, while *G. pallida* did. Additionally Pa2 had a different complement of isozymes than the other pathotypes. The inference of these findings is that *G. pallida* is earlier in origin and that *G. rostochiensis* has arisen later as a separate line. The marked uniformity of the *G. rostochiensis* pathotypes in many biochemical taxonomic studies compared to the variability of *G. pallida* would appear to support this observation.

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