

The Metabolism of Sunflower Phytoalexins Ayapin and Scopoletin

PLANT-FUNGUS INTERACTIONS

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ABSTRACT

The coumarin phytoalexins ayapin and scopoletin accumulate in longitudinal stem sections of sunflower (*Helianthus annuus* L., Compositae) following inoculation with fungi both pathogenic (*Alternaria helianthi*) and nonpathogenic (*Helminthosporium carbonum*) to this plant. Both compounds were induced more rapidly, and they attained higher levels in tissue inoculated with the heterologous pathogen *H. carbonum* as compared with the sunflower pathogen *A. helianthi*. Similarly, scopoletin and ayapin accumulated to comparatively low concentrations following inoculation with a second sunflower pathogen, *Phoma macdonaldii*. Scopoletin was biosynthesized *de novo* following inoculation, although levels of its glucoside scopolin exceeded those of the aglucone in both infected and control tissues. Both scopoletin and scopolin were routinely detected in trace amounts in uninoculated tissue. In contrast, ayapin was not detected as a component of uninfected plants. When [¹⁴C]scopoletin was supplied to induced sunflower stem sections about 36% of the recovered radioactivity was in the form of ayapin. *In vitro* studies demonstrated that *A. helianthi* possessed the ability to rapidly degrade both scopoletin and ayapin, whereas *H. carbonum* was much less efficient in these traits. The differential degradation of these compounds by phytopathogenic fungi which do not attack sunflower is also discussed.

Phytoalexins are low mol wt, antimicrobial compounds which are biosynthesized by plants *de novo* following exposure to microorganisms (1, 10). Such compounds have now been identified as products in a broad range of plant families including the Compositae (1, 2, 15). In a previous communication (13) we reported the production of two coumarin phytoalexins, ayapin and scopoletin (Fig. 1), by sunflower (*Helianthus annuus* L., Compositae) in response to inoculation with conidia of the fungus *Helminthosporium carbonum* Ullstrup., which does not attack this plant but which is pathogenic to maize (*Zea mays* L.). An organism nonpathogenic to sunflower was selected as inducing agent since it has been reported for other plant-microbe interactions that phytoalexins accumulate more slowly and/or are present at lower levels following inoculation with homologous pathogens as compared with heterologous pathogens or nonpathogenic microorganisms (7, 18). Accordingly, in our initial investigation of sunflower for phytoalexin production, we utilized the maize pathogen *H. carbonum* to induce the biosynthesis of ayapin and scopoletin (13). In that study (13) scopoletin was found to accumulate rapidly to relatively high levels. During the

same investigation the antifungal activity of both ayapin and scopoletin was demonstrated against two fungal species, *Pyrenochaeta terrestris* and *H. carbonum*, which are nonpathogens of sunflower (13). In contrast, neither coumarin derivative expressed significant activity against the sunflower pathogen *Alternaria helianthi*, and the closely related organism *Alternaria chrysanthemi* was also insensitive to both compounds (B Tal, DJ Robeson, unpublished data). There is a growing body of evidence which indicates that pathogen-mediated detoxification of phytoalexins constitutes a mechanism by which pathogenic microorganisms may overcome the plant's defenses (12, 14, 19). In the present paper we describe the results of comparative experiments performed to determine the kinetics of ayapin and scopoletin accumulation in host tissue induced by inoculation with the sunflower pathogens *A. helianthi* and *Phoma macdonaldii* and the nonpathogen *H. carbonum*. These data are discussed in relation to the ability of pathogenic and non-pathogenic fungi to degrade these compounds *in vitro*.

MATERIALS AND METHODS

Fungal and Plant Materials. *Alternaria helianthi* was kindly supplied by Dr. Rama Raje Urs, Dahlgren and Co., Crookston, MN. *Helminthosporium carbonum* (perfect state *Cochliobolus carbonum* Nelson) and *Phoma macdonaldii* were gifts from Dr. D. E. Mathre and Dr. G. A. Strobel, respectively, Department of Plant Pathology, Montana State University. Fungal cultures were maintained on V-8 juice agar. Sunflower plants, cv Mammoth Grey Stripe, were grown in the greenhouse as previously described (13).

Phytoalexin Induction. Excised sunflower stem segments were sectioned longitudinally and the exposed cell layer was inoculated with a conidial suspension of *A. helianthi* at a concentration of 10⁵/ml or *H. carbonum* conidia at the same concentration as previously described (13). Control tissue received sterile deionized H₂O. At various time intervals after inoculation, the upper 1 to 5 mm of tissue was removed and extracted with methanol prior to HPLC analysis.

Chromatography. Normal phase TLC on silica gel using diethyl ether as solvent and reverse phase HPLC with MeCN²-H₂O, 9:5

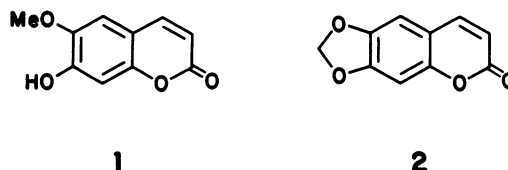


FIG. 1. Structures of scopoletin (1) and ayapin (2).

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² Abbreviations: MeCN, acetonitrile.

was performed as described (13). During HPLC analysis both compounds were detected by fluorometry and quantified by comparison of peak areas with standard curves.

¹⁴C-Labeling of Scopoletin and Ayapin. (a) *Preparation of Labeled Scopoletin.* Three sunflower leaves were excised under water and their petioles immediately immersed in distilled H₂O. The excised leaves (about 6 g fresh weight) were placed in a sealed transparent chamber under illumination ($1200 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) in the presence of ¹⁴CO₂ (1 mCi) at 28°C for 1 h. Scopoletin biosynthesis was then induced by exposure to short wavelength UV irradiation (254 nm) for 30 min. Treated leaves were incubated at 25°C and $400 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for 48 h. Induced tissue was extracted with methanol for 18 h and [¹⁴C]scopoletin was prepared by TLC, first on normal phase silica gel in diethyl ether and second on reverse phase C₁₈ plates in MeCN-H₂O, 8:2. The scopoletin band was eluted and final purification was performed by HPLC as described above to give about 15 μg of scopoletin with a specific activity of $0.83 \mu\text{Ci}/\mu\text{M}$.

(b) *Feeding of Labeled Scopoletin to Induced Tissue and Isolation of Labeled Ayapin.* The ¹⁴C-labeled scopoletin ($3.75 \mu\text{g}$, $0.0164 \mu\text{Ci}$) was applied to the surface of the upper 1.5 mm layer of a longitudinal section of sunflower stem which had been induced by inoculation 24 h previously with a conidial suspension of *H. carbonum* as described in Tal and Robeson (13). After incubation at 25°C for a further 24 h, the tissue was extracted with methanol and the extract partitioned against hexane. The aqueous methanol fraction was subjected to TLC in diethyl ether, and the developed chromatogram was scanned for radioactivity with a radio-scanning device (Berthold Automatic TLC-Linear Analyzer). The bands which gave the major radioactive peaks and which corresponded in R_F with ayapin and scopoletin standards were eluted and further purified by HPLC as described above prior to liquid scintillation counting of each compound. A polar fraction which did not migrate during TLC (Sigel, diethyl ether) was also associated with radioactivity. Subsequently, this fraction was refluxed with 2 N HCl for 2 h to release any scopoletin which may have been bound by glucosidation. The reaction mixture was diluted with H₂O and partitioned against diethyl ether prior to chromatography to afford ¹⁴C-labeled scopoletin as the aglucone.

In Vitro Fungal Degradation of Scopoletin and Ayapin. Sunflower extract medium, composed of the aqueous extract of 100 g fresh weight of sunflower leaf tissue (cv Mammoth Grey Stripe) and 5 g of sucrose per L, (20 ml in 125 ml Erlenmeyer flasks) was inoculated with various species of phytopathogenic fungi. After incubation at 28°C and 170 rpm for 24 h, scopoletin or ayapin was added in DMSO solution to a final concentration of 0.5 mM and 2% DMSO. Scopoletin or ayapin in DMSO was also added to uninoculated (control) culture media incubated as above. At various time intervals 0.5 ml aliquots were aseptically removed, filtered (0.2 μm Nalgene), and residual scopoletin or ayapin was quantified by HPLC as described above.

RESULTS

Time-Course of Phytoalexin Accumulation in Response to *A. helianthi* and *H. carbonum*. When longitudinal sections of sunflower stem segments were inoculated with conidial suspensions of *A. helianthi* or *H. carbonum*, tissue inoculated with the former fungus was softened after 24 h and by 72 h the tissue had developed what appeared to be a soft rot. In contrast, *H. carbonum*-inoculated tissue remained firm and apparently healthy throughout the experiment, and the growth of germ tubes emanating from conidia was restricted. At various time intervals after inoculation, small pieces of inoculated and control tissue were harvested, the upper 1.5 mm of tissue was excised, and the tissues were extracted separately. The concentration of scopoletin which accumulated in such tissue 18 h after inoculation with *H. car-*

bonum (Fig. 2) was about 8 times that found in equivalent uninoculated control tissue. In the case of the homologous pathogen *A. helianthi*, comparatively low levels of this compound accumulated in inoculated tissue after this time period (Fig. 2). The average concentration of scopoletin in tissue inoculated with *H. carbonum* showed a marked increase between 48 and 72 h, at which time it attained its highest recorded level (Fig. 2). In stem sections inoculated with *A. helianthi* the peak of scopoletin production was recorded at an earlier time and, in contrast with levels in *H. carbonum*-inoculated tissue, showed a steep decline between 48 and 72 h. Within 72 h of inoculation with *A. helianthi* the level of scopoletin had declined below that of ayapin. Seven d after inoculation, only relatively trace amounts of scopoletin were detected in stem tissue inoculated with *A. helianthi*. In contrast, comparatively high levels of this compound were maintained in *H. carbonum*-inoculated tissue (Fig. 2).

Although detectable amounts of ayapin were not observed at time zero, it was induced by both *H. carbonum* and *A. helianthi* within 24 h. Similar levels of ayapin were induced after 48 h by both fungi, and in each case maximum concentrations were recorded after 72 h. However, in stem sections inoculated with *H. carbonum*, the maximum concentration of ayapin was twice that in those inoculated with the pathogen *A. helianthi* (Fig. 3). As noted above for scopoletin, the concentration of ayapin

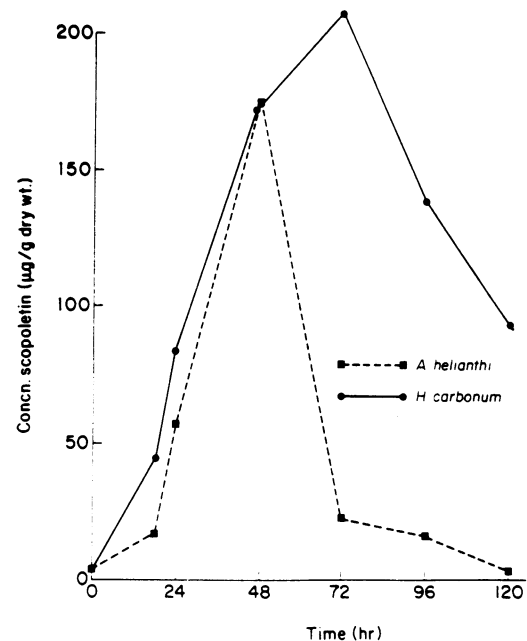


FIG. 2. Accumulation of scopoletin in sunflower stem tissue following inoculation with *A. helianthi* or *H. carbonum*.

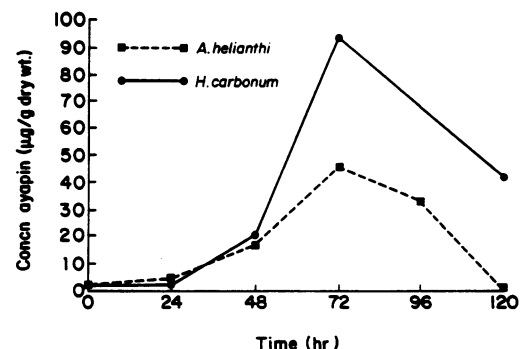


FIG. 3. Accumulation of ayapin in sunflower stem tissue following inoculation with *A. helianthi* or *H. carbonum*.

diminished with time after 72 h, but in the *A. helianthi*-inoculated stem the decrease was more rapid than in that inoculated with *H. carbonum* (Fig. 3).

Phytoalexin Accumulation in Response to *P. macdonaldii*. A second fungal pathogen of sunflower, *P. macdonaldii* (black stem) was also investigated for its ability to induce scopoletin and ayapin biosynthesis in sunflower stem tissue and to degrade these compounds *in vitro*. Inoculation of longitudinal sections of sunflower stems with this organism caused rapid development of necrotic spreading lesions and a severe infection within 48 h. Levels of both scopoletin and ayapin which were induced following inoculation with this fungus were much lower than those induced by either *H. carbonum* or *A. helianthi* (Table I). The maximum concentration of scopoletin was a mere 13.1 $\mu\text{g/g}$ dry weight. In addition, this maximum level was reached after only 24 h. Seventy two h after inoculation ayapin was more abundant than scopoletin by a factor of about 5-fold (Table I). The low levels of scopoletin can be accounted for by the ability of *P. macdonaldii* to rapidly degrade this phytoalexin, degradation of which was virtually complete within 48 h of inoculation (Fig. 4). Furthermore, since scopoletin is rapidly degraded, relatively little precursor is available for ayapin biosynthesis by the plant; hence, ayapin would be expected to accumulate to relatively low levels.

Biosynthesis of Ayapin from Scopoletin. From a consideration of the structural similarity of the two compounds and the fact that scopoletin accumulates prior to ayapin, it seemed likely that the former compound serves as precursor of the latter. Indeed, when ^{14}C -labeled scopoletin was fed to induced sunflower stem tissue, labeled ayapin was produced and accounted for 36% of the total recovered label; 45% of the recovered label was ac-

counted for as scopoletin itself. This represents 34% of the labeled scopoletin added to the induced stem tissue. The remainder of the recovered label was in a polar fraction which included scopolin. This was demonstrated by treatment of this fraction with acid to yield free scopoletin from its glucoside. The biosynthetic route to scopoletin, which is finally glucosidated to give scopolin, has been elucidated using tobacco callus tissue (3). The biogenesis of both scopoletin and its glucoside in sunflower is presumed to take place via the same pathway.

Degradation of Ayapin and Scopoletin by *A. helianthi* and *H. carbonum*. In considering the accumulation of phytoalexins in plant tissues, turnover by the plant must be considered as well as the rate of biosynthesis (17, 18). A third factor which may be important in determining phytoalexin concentrations in tissue induced by microbial inoculation is degradation by the microorganism itself. In an attempt to gain some insight into the possible effect of fungal degradation on the levels of scopoletin and ayapin in inoculated sunflower tissue, *in vitro* degradation of these compounds by fungi both pathogenic and nonpathogenic to this plant was investigated. Scopoletin was degraded much more rapidly by the pathogen *A. helianthi* than by *H. carbonum* (Fig. 5). Thus, scopoletin could no longer be detected within 24 h of inoculating medium, containing this compound at an initial concentration of 0.5 mM, with the pathogen. In contrast, the same medium inoculated with *H. carbonum* contained 50% of the original level of scopoletin after incubation for 24 h (Fig. 5). Ayapin was also degraded by *A. helianthi* but more slowly than was scopoletin, while the heterologous pathogen *H. carbonum* degraded ayapin to a very limited extent (Fig. 6). These results suggest that the lower levels of scopoletin and ayapin accumulating in sunflower tissues induced by *A. helianthi* as compared with *H. carbonum* are due, at least in part, to the greater capacity of the former, pathogenic fungus to degrade these compounds.

Phytoalexin Degradation by Other Fungi Nonpathogenic to Sunflower. A comparative study of fungi nonpathogenic to sunflower for their ability to degrade ayapin and scopoletin provided interesting results with possible plant-pathogen co-evolutionary significance. Of three other *Alternaria* species examined, two of them, *A. macrospora* and *A. chrysanthemii*, degraded both phytoalexins rapidly such that in each instance the compounds were essentially absent from the fungal cultures after incubation for 12 to 18 h. Data showing the kinetics of degradation by the latter fungus are given in Figure 7. *Alternaria chrysanthemii*, which is reported to be morphologically indistinguishable from *A. helianthi* (8) as well as pathologically related (16), is pathogenic to

Table I. Accumulation of Scopoletin and Ayapin in Sunflower Stem Sections Inoculated with *P. macdonaldii*

Time h	Concentration	
	Scopoletin	Ayapin
	$\mu\text{g/g dry wt}$	
Control	1.4	ND
24 h	13.1	14.8
48 h	5.0	7.2
72 h	4.7	24.5

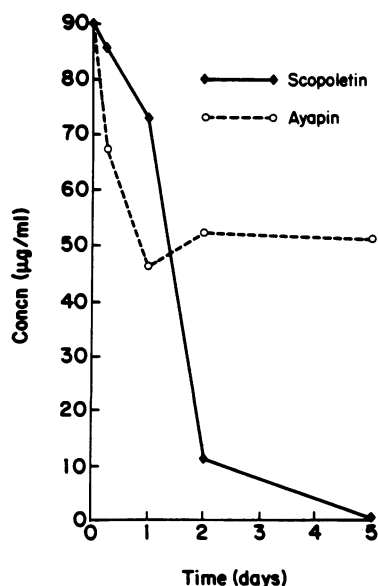


FIG. 4. Degradation of scopoletin and ayapin by *P. macdonaldii* *in vitro*.

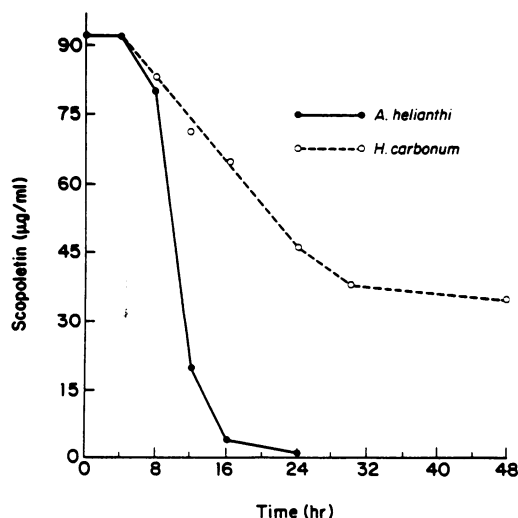


FIG. 5. Degradation of scopoletin by *A. helianthi* and *H. carbonum* *in vitro*.

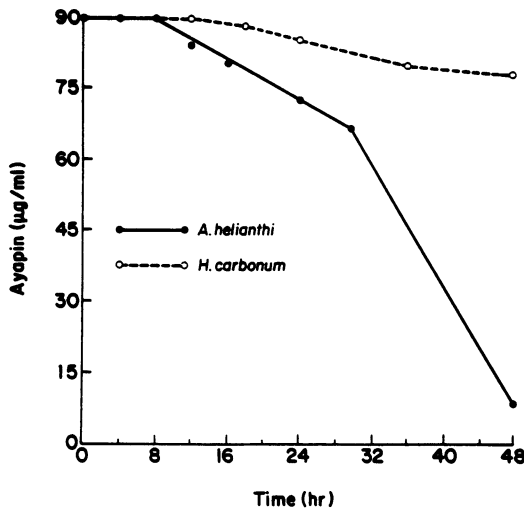


FIG. 6. Degradation of ayapin by *A. helianthi* and *H. carbonum* in vitro.

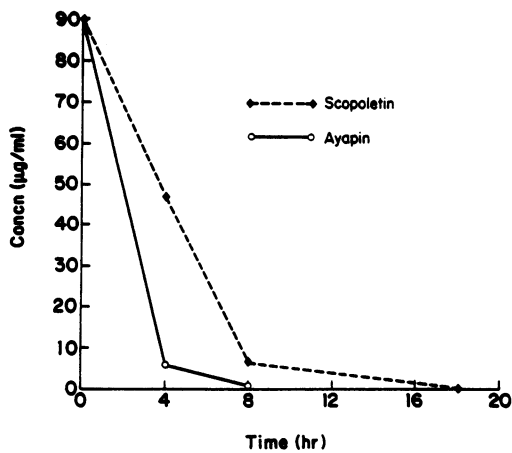


FIG. 7. Degradation of scopoletin and ayapin by *A. chrysanthemi* in vitro.

Chrysanthemum maximum (Ram.) DC (11). We have tentatively identified both scopoletin and ayapin as inducible metabolites of this plant (B Tal, DJ Robeson, unpublished data). The strain of *A. macrospora* used in the present study was isolated from cotton (*Gossypium hirsutum* L.), a plant known to accumulate increased levels of scopoletin following treatment with fungal elicitors (20). The third *Alternaria* species examined, *A. brassicicola* which is a pathogen of cabbage, was apparently unable to degrade ayapin, and degraded scopoletin very slowly after a lag period of about 72 h. Cabbage (*Brassica oleracea* L., Cruciferae) is not known to produce either ayapin or scopoletin and, furthermore, our attempts to induce these compounds in the leaves of this plant species by UV irradiation gave uniformly negative results. Similarly, *Pyrenochaeta terrestris*, the etiological agent of pink root disease of onion (*Allium cepa* L.) did not degrade scopoletin and degraded ayapin only slowly. Degradation of the latter commenced 3 to 4 d after inoculation (unpublished results, this laboratory). Taken as a whole, the results indicate that certain fungi pathogenic to plants which accumulate the coumarin derivatives scopoletin and ayapin possess enzymes capable of degrading these compounds rapidly as compared with other phytopathogenic fungi which are specialized parasites of plant species which do not have the capacity to produce scopoletin.

In the case of *A. helianthi*, which rapidly metabolized both compounds, the mycelium was thoroughly extracted by homogenization in methanol after added scopoletin and ayapin had

been reduced to very low final concentrations in the growth medium. Significant amounts of these compounds were not recovered in this manner, suggesting that both compounds were subjected to catabolic action by the fungus and were not merely bound to mycelial components. Similarly, in the case of *H. carbonum* which degraded both compounds relatively slowly, no evidence for mycelial binding of phytoalexins was obtained after thorough extraction of the harvested mycelium.

During HPLC analysis of samples obtained from experiments to study the kinetics of fungus-mediated degradation, in which a fluorometer detector was employed, no other fluorescent compounds were detected. All simple coumarins likely to be derived from scopoletin and ayapin are fluorescent. Therefore, the data suggest that these compounds undergo complete degradation in the presence of the fungus. The ability of certain fungi to utilize coumarin itself or dihydro-coumarin as sole carbon source has been demonstrated (9). Furthermore, when filtrate from cultures grown in the presence of scopoletin was harvested at a time when the concentration of this compound was reduced to relatively trace amounts as a result of fungal action, and subjected to acid hydrolysis in order to break glycosidic bonds, a significant increase in the concentration of free scopoletin was not realized. This indicates that glucosidation to form scopolin is not a factor in reducing the levels of scopoletin in fungal cultures.

It is worthy of note that the mycelium of *A. brassicicola* and *A. macrospora* was coagulated by incubation in ayapin, as previously reported for *H. carbonum* (13). None of the fungi cultured in the presence of scopoletin were induced to aggregate.

Determination of the Relative Concentrations of Free Scopoletin and Its Glucoside Scopolin in Control and Induced Tissue. Both uninoculated, apparently healthy, sunflower stem tissue and that inoculated with conidial suspensions of *A. helianthi* or *H. carbonum* were analyzed by HPLC to determine the concentration of free scopoletin present. Duplicate pieces of tissue treated in the same manner were subjected to acid hydrolysis as described above to release scopoletin bound as the glucoside scopolin prior to HPLC analysis. In all cases the ratio of free scopoletin to that present as the glucoside scopolin was less than one, although the total amount of scopoletin present in inoculated tissue was many-fold above that of the control (Table II). This demonstrates that the increased level of scopoletin in inoculated, as compared with control, sunflower tissue is the result of *de novo* biosynthesis in accordance with the phytoalexin concept (1, 6, 10) and is not simply due to hydrolytic release from preformed scopolin. This is consistent with the findings of Fritig *et al.* (3) on the biosynthesis of scopoletin and scopolin in tobacco callus tissue. They reported that scopolin is formed as the end product of the biosynthetic pathway resulting from the glucosidation of scopoletin.

DISCUSSION

Marked differences were noted between the induction of the phytoalexins scopoletin and ayapin by the sunflower pathogen *A. helianthi* and the heterologous pathogen *H. carbonum*. In the case of each compound induction of biosynthesis to detectable

Table II. Concentrations of Scopolin, Scopoletin and Ayapin in Sunflower Stem Tissue Inoculated with *A. helianthi* or *H. carbonum*

	Concentration ^a		
	Scopolin	Scopoletin	Ayapin
	µg/g dry wt		
Control	21	6	ND
<i>H. carbonum</i>	244	97	19
<i>A. helianthi</i>	99	102	15

^a 40 h postinoculation.

levels occurred earlier, maximum concentrations attained were higher, and the levels declined more slowly in tissue inoculated with the nonpathogen as compared with *A. helianthi*. Of the two compounds, scopoletin was present in uninoculated control tissue in trace amounts and accumulated more rapidly than ayapin, which in general was not detected in healthy sunflower tissue. The high levels previously recorded for scopoletin in excised cortical tissue (13) were not observed in the present investigation. This is best explained in terms of the highly localized nature of phytoalexin accumulation as well as the fact that in the present investigation medullary and cortical tissues were extracted together. In the case of the sunflower stem pathogen *Phoma macdonaldii*, levels of the two coumarin derivatives which accumulated in sections of stem segments inoculated with this organism were even lower than those induced by *A. helianthi*. This was associated with a severe spreading infection of the inoculated stem tissue. In contrast to induction by the fungi *A. helianthi* or *H. carbonum* as well as UV irradiation (B Tal, DJ Robeson, unpublished data), levels of ayapin exceeded those of scopoletin following induction by *P. macdonaldii* (Table I). This may be related to the differential degradation of these compounds by *P. macdonaldii* (Fig. 4). This pathogen would not be expected to be inhibited by the maximum levels recorded for scopoletin and ayapin, which were equivalent to 7 and 13 μM , respectively, on a fresh weight basis (Table I).

During bioassays in which ayapin and scopoletin were incorporated into solid medium as described in (13), neither compound expressed significant activity against the sunflower pathogen *A. helianthi* nor the closely related species *A. chrysanthemi*, in the concentration range 0.25 to 1.0 mM (B Tal, DJ Robeson, unpublished data). These results are consistent with the ability of *A. helianthi* and *A. chrysanthemi* to rapidly degrade both compounds (Figs. 5–7). The sunflower stem pathogen *P. macdonaldii* was not assayed for its sensitivity to scopoletin and ayapin. However, from a consideration of the rapid degradation of both compounds by this pathogen *in vitro*, little activity against *P. macdonaldii* is likely to be expressed at concentrations of 0.5 mM or below.

The fungi examined for their ability to degrade scopoletin and ayapin can be divided into two groups: those which degraded these compounds rapidly after a minimal or no lag period, and those which did not cause degradation or only at a relatively slow rate after an extended lag period. The former group included the two sunflower pathogens *A. helianthi* and *P. macdonaldii* as well as *A. chrysanthemi* and *A. macrospora*. *Alternaria chrysanthemi* is closely related to *A. helianthi* being described as morphologically indistinguishable (8), and is pathologically similar in that the two fungi share a common host in the Japanese chrysanthemum (16). In addition, during a preliminary investigation of *C. maximum* (the usual host of *A. chrysanthemi* [11]) we have tentatively identified scopoletin and ayapin as inducible metabolites. Furthermore, the isolate of *A. macrospora* employed in these studies was isolated from cotton, a plant known to produce scopoletin inducibly (20). In contrast, the latter group of phytopathogenic fungi were all nonpathogens of sunflower, and moreover at least two of their host species (onion and cabbage) are not known to produce scopoletin or ayapin. These data are consistent with the hypothesis that during the course of plant-pathogen coevolution, fungi pathogenic to plants which accumulate certain inducible coumarin derivatives have evolved enzymes capable of effectively degrading such metabolites.

The ratio of scopoletin to scopolin found in tissue inoculated with *A. helianthi* was considerably greater than that in analogous tissue inoculated with *H. carbonum*. Glucosidation of scopoletin to scopolin represents the terminal step in the biosynthetic sequence of this glucoside in tobacco tissue, and the glucosidation of exogenously supplied coumarins by plant tissues has been

demonstrated (4) as has the hydrolysis of scopolin to scopoletin and its subsequent reglucosidation (5). Therefore, in healthy tissue an equilibrium between glucoside and aglycone is expected to prevail. In this regard it may be significant that the ratio of scopoletin to its glucoside in control tissue is more similar to that found in *H. carbonum*-inoculated tissue than in that inoculated with the pathogen *A. helianthi*. Tissue inoculated with *H. carbonum* remained essentially healthy during the course of analyses for coumarin content, whereas that infected with *A. helianthi* was grossly disrupted. Under these conditions enzymic activity, especially of glucosidases which may be bound to the cell wall, can be expected to depart from the norm.

Labeling studies in which [^{14}C]scopoletin was fed to *H. carbonum*-induced tissue demonstrated that ayapin is biosynthesized from the former compound. This was as anticipated from a consideration of the structures of the two coumarin derivatives and the kinetics of their accumulation in induced tissue. *In vitro* studies demonstrated that *H. carbonum* was incapable of transforming scopoletin to ayapin. The high rate of incorporation of label supplied as scopoletin into ayapin, and the structures of the two compounds, suggest that the former compound behaves as an immediate precursor of the latter. In addition to serving as precursor for ayapin biosynthesis, exogenously supplied [^{14}C]scopoletin was also glucosylated to give scopolin, as demonstrated by hydrolysis of a polar fraction in the extract to yield [^{14}C]scopoletin once again. Therefore, scopoletin may be envisaged to represent a branch point in the biogenetic sequence, which by analogy with its biosynthesis in tobacco (*Nicotiana tabacum*, L.) proceeds from *t*-cinnamic acid via intermediates to ferulic acid, which is finally lactonized to form scopoletin (3). Glycosidation of relatively large amounts of scopoletin, which is produced following inoculation with the heterologous pathogen, may represent a detoxification mechanism for the plant and, in addition, may provide a reservoir from which fungitoxic free scopoletin may be released by the action of plant glucosidases. In contrast to scopoletin, its glucoside scopolin was inactive against *H. carbonum* in bioassays aimed at identifying additional sunflower phytoalexins.

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