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Flavonoids Promote Haustoria Formation in the Root Parasite *Triphysaria versicolor*[1]

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Parasitic plants in the Scrophulariaceae develop infective root structures called haustoria in response to chemical signals released from host-plant roots. This study used a simple in vitro assay to characterize natural and synthetic molecules that induce haustoria in the facultative parasite *Triphysaria versicolor*. Several phenolic acids, flavonoids, and the quinone 2,6-dimethoxy-*p*-benzoquinone induced haustoria in *T. versicolor* root tips within hours after treatment. The concentration at which different molecules were active varied widely, the most active being 2,6-dimethoxy-*p*-benzoquinone and the anthocyanidin peonidin. Maize (*Zea mays*) seeds are rich sources of molecules that induce *T. versicolor* haustoria in vitro, and chromatographic analyses indicated that the active molecules present in maize-seed rinses include anthocyanins, other flavonoids, and simple phenolics. The presence of different classes of inducing molecules in seed rinses was substantiated by the observation that maize kernels deficient in chalcone synthase, a key enzyme in flavonoid biosynthesis, released haustoria-inducing molecules, although at reduced levels compared with wild-type kernels. We discuss these results in light of existing models for host perception in the related parasitic plant *Striga*.

Plants naturally produce more than 8000 different phenolic compounds for functions as varied as cell wall biosynthesis, flower pigmentation, and host defense (Harborne and Moss, 1993). The prevalence of plant phenolic molecules and the broad spectrum of potential structures and electrochemical forms make these effective signaling molecules for mediating interactions between plants and other organisms in the soil (Sisqueira et al., 1991).

Flavonoids released by legume roots activate a set of genes in *Rhizobium* sp. whose products are responsible for the biosynthesis of nodulation factors (Pueppke, 1996). The NodD protein binds to specific promoter sequences in *nod* (nodulation) genes and, when NodD perceives the appropriate flavonoids, activates their transcription (Fisher and Long, 1992). Although a single *nodD* gene can respond to multiple flavonoids, different *nodD* genes are optimally responsive to specific structures. This can represent one level of host-range specificity in the *Rhizobium*-legume interaction (Spaink et al., 1987). Host-plant phenolics released from wounded plant cells induce virulence genes in the soil pathogen *Agrobacterium tumefaciens* (Hooykaas and Beijersbergen, 1994). The phenolics are perceived by a two-component system composed of the VirA sensor protein and the VirG transcriptional regulator. VirA responsiveness to phenolic signals is further refined by synergistic association of signaling phenolics with specific monosaccharides. Different *virA* gene products sense multiple phenolic compounds, but preferences for particular compounds can in some cases affect host range (Heath et al., 1997).

Phenolic compounds are also important signaling molecules for mediating parasitic plant-host plant interactions in the rhizosphere (Musselman, 1980; Press and Graves, 1995). Seeds of the parasitic weeds *Striga* and *Orobanche* remain dormant in the soil until they sense specific hydroquinones that are released from potential host roots (Chang and Lynn, 1986). These germination stimulants become inactive at increasing distances from the root, thereby allowing the parasite to judge the availability and distance to a potential host root (Fate and Lynn, 1996).

Parasitic plants in the Scrophulariaceae also use host-encoded phenolic derivatives to signal the transition from vegetative to parasitic growth. In response to these host factors, parasitic plants develop haustoria near their root tips (Riopel and Timko, 1995). Haustoria serve several functions for the parasite: they attach the parasite and host roots, they invade host tissues through a combination of enzymatic and physical processes, and they serve as the physiological conduit through which the parasite robs the host plant of water and nutritional resources (Kuijt, 1969). A diverse array of phenolic derivatives that induce haustoria in *S. asiatica* and *Agalinis purpurea* has been identified (MacQueen, 1984; Riopel and Timko, 1995; Smith et al., 1996). When parasite roots are exposed to these HIFs in vitro, haustorium development is rapid, and highly synchronous morphological changes can be observed within hours (Baird and Riopel, 1984).

We studied the genetic mechanisms by which phenolic signals are perceived and interpreted by parasitic plants. The genus *Triphysaria* (previously *Orthocarpus*) of the Scrophulariaceae is composed of five cross-hybridizing species within the subtribe Castillejinae (Chuang and Heckard, 1991). *Triphysaria* is a common herbaceous annual in coastal fields and bluffs, inland grasslands, and serpentine

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Abbreviations: CHS, chalcone synthase; DMBQ, 2,6-dimethoxy-*p*-benzoquinone; HIF, haustoria-inducing factor.

slopes distributed along the Pacific Coast from Baja to British Columbia (Hickman, 1993). It is a facultative root parasite that can be grown without a host, but will parasitize a broad spectrum of host plants, including Arabidopsis, tobacco, and maize (*Zea mays*) (Atsatt and Strong, 1970; Estabrook and Yoder, 1998). *Triphysaria* sp. are diploid and have perfect flowers amenable to classical genetic analysis (Chuang and Heckard, 1982; Yoder, 1998). The generation time of *Triphysaria* sp. is 3 to 4 months, with each flower producing about 100 seeds.

We used a simple bioassay to examine the ability of different phenolic compounds to induce haustoria in the self-incompatible species *Triphysaria versicolor*. Several phenolic molecules were active in haustoria induction, although the concentrations at which they were active varied widely. We show, for the first time to our knowledge, that anthocyanins induce haustoria, and we discuss these findings in light of existing models of quinone recognition. Chromatographic analyses were consistent with multiple molecules that induce *T. versicolor* haustoria in vitro being released into aqueous and methanol rinses of maize kernels. The study of maize mutants deficient in CHS further confirmed that a redundancy of signaling molecules is released from maize kernels.

MATERIALS AND METHODS

Materials

Triphysaria versicolor (Fischer & C. Meyer) seeds were collected from grassland stands near Napa, CA, and stored at 4°C. Maize seeds (*Zea mays* cv B73) were kindly provided by Pioneer Hi-Bred International (Johnston, IA). Maize seeds bearing the two CHS mutations *c2* and *whp1* (Coe et al., 1981) were generously supplied by the Maize Genetic Cooperative Stock Center (stock no. 224H, University of Illinois, Urbana). This stock contains seeds of two genotypes: *C2/c2,whp1/whp1* and *c2/c2,whp1/whp1*. Expression of CHS in the aleurone layer of maize is encoded by the *C2* locus, whereas expression in other plant parts is encoded by *Whp* (Dooner et al., 1991).

Phenolic compounds were obtained from Sigma, DMBQ from Pfalz and Bauer (Waterbury, CT), and flavonoids from Indofine Chemical (Belle Mead, NJ). Chemicals and HPLC fractions were dissolved in 50% methanol and frozen at −80°C. Anthocyanidin stock solutions were kept in the dark. Dilutions were prepared in distilled water just before use.

Bioassay for Haustorial Induction

T. versicolor seeds were surface-sterilized for 5 min in 70% ethanol followed by 30 min in 50% bleach plus 2% Triton X-100 before rinsing with sterile water. Germination was carried out at 16°C under high-output, cool-white fluorescent lights with a 12-h photoperiod in 0.25× Murashige and Skoog medium (0.75 mM $CaCl_2$, 0.3 mM KH_2PO_4, 5 mM KNO_3, 0.2 mM $MgSO_4$, and 5 mM NH_4NO_3) supplemented with micro-nutrients (10 nM $CoCl_2$, 500 nM $CuSO_4$, 70 μM H_3BO_3, 14 μM $MnCl_2$, 10 μM NaCl, 200 nM $NaMoO_4$, and 1 μM $ZnSO_4$), 0.75% Suc, and solidified with 0.6% Phytagar (GIBCO-BRL).

Five to seven 3-week-old seedlings were aseptically transferred to the surface of 0.25× Hoagland agar (1.25 mM $Ca[NO_3]_2$, 1.25 mM KNO_3, 0.25 mM KH_2PO_4, 0.5 mM $MgSO_4$) with micronutrients, 1% Suc, and 1% Phytagar in 90- × 90-mm dishes. The dishes were sealed with Micropore tape (3M), and placed for 1 week at a nearly vertical angle at 25°C with a 16-h photoperiod. Under these conditions *T. versicolor* roots grew along the surface of the agar.

To assay haustoria-inducing activity, the candidate inducer in 3 mL of water was applied to *T. versicolor* roots. After the liquid absorbed into the medium (1–3 h), the plates were returned to the 25°C growth chamber. After 24 h each root tip was scored for the localized swelling and hair proliferation typical of developing haustoria. Results

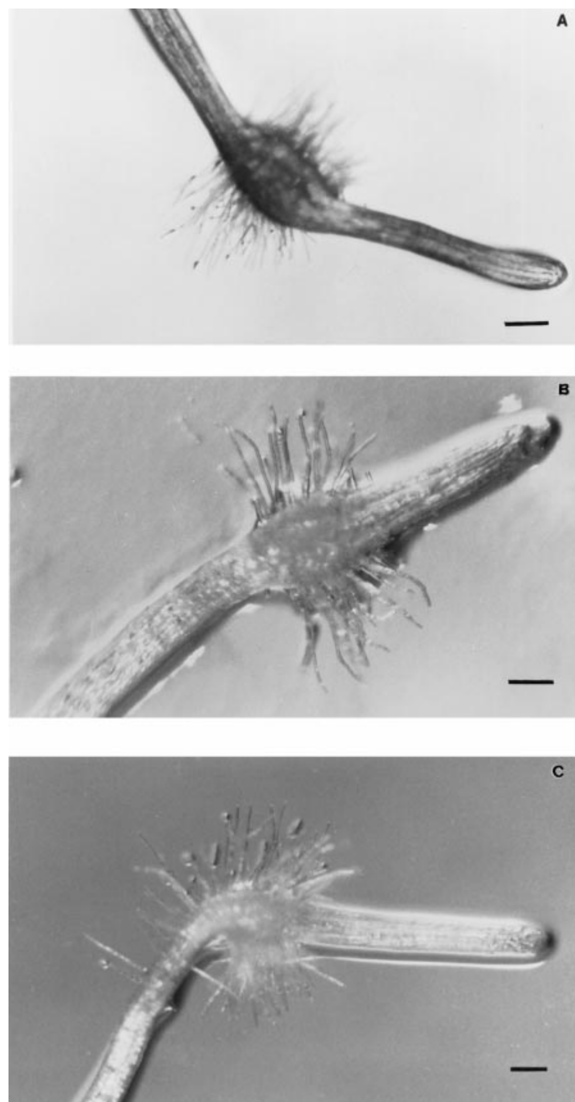
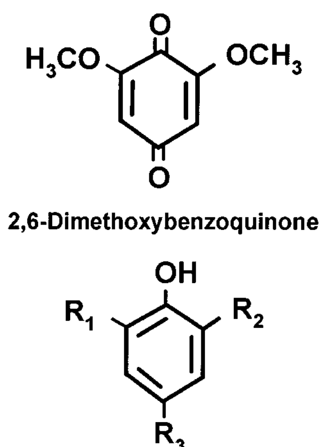


Figure 1. Secondary haustoria on *T. versicolor* roots. Haustoria were induced by a fraction (the 30-min eluant from Fig. 7B) of the purified methanolic rinse of maize seeds (0.04 g seed equivalents/mL) (A), by 10 μM peonidin (B), or by 50 μM DMBQ (C). Photographs were taken approximately 24 h after treatment. Scale bars = 100 μm.



Compound	R_1	R_2	R_3
p-coumaric acid	H	H	CH=CHCOOH
vanillic acid	OCH_3	H	COOH
vanillin	OCH_3	H	CHO
syringic acid	OCH_3	OCH_3	COOH
syringaldehyde	OCH_3	OCH_3	CHO
p-hydroxybenzaldehyde	H	H	CHO
ferulic acid	OCH_3	H	CH=CHCOOH
sinapinic acid	OCH_3	OCH_3	CH=CHCOOH
caffeic acid	OH	H	CH=CHCOOH
salicylic acid	COOH	H	H

Figure 2. Phenolics, phenolic acids, and quinones assayed for haustoria-inducing activity in *T. versicolor*.

are typically expressed as the proportion of root tips with haustoria.

Characterization of Maize-Seed Rinses

Maize kernels were swirled for 16 h at room temperature in either 50% methanol or water. Samples analyzed by HPLC were prepared by shaking 300 g of seeds for 4 h in 100% methanol (300 mL) at room temperature. The resulting seed rinse was concentrated under vacuum at 50°C, frozen as 30% methanol, and lyophilized to dryness. The dried material was dissolved in 50% acetonitrile, diluted with water to 5% acetonitrile, and injected into a HPLC system (Millipore) fitted with a RP-18 column (250 × 4.6 mm; Lichrosorb, Alltech Associates, Deerfield, IL). The column was rinsed for 2 min with 5% acetonitrile and then eluted with a 58-min linear gradient (5%–100% acetonitrile), followed by a 30-min rinse in 100% acetonitrile using a flow rate of 2 mL/min. Eluate was monitored (200–400 nm) with a photodiode array detector (model 996, Waters). In other tests, seed rinses were fractionated by open-column chromatography on preparative 125-Å C_{18} columns (Waters) using methanolic gradients.

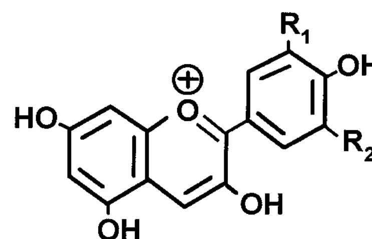
RESULTS

Purified Phenolics Induce Haustoria in Vitro

We used an in vitro system to bioassay various phenolic derivatives for their ability to induce haustoria in *T. versicolor* roots. Seedlings were grown in vertically oriented Petri dishes such that the roots grew along the surface of the agar. Candidate haustoria-inducing compounds were diluted in water and applied to the roots. With active HIFs, epidermal hairs began to proliferate and elongated near the root tip within 4 h after exposure. At about the same time, cortical cells underlying the proliferating hairs began to swell and divide, resulting in an observable swelling near the root tip (Estabrook and Yoder, 1998). The swelling and hair proliferation continued for approximately 24 h. Under these conditions only those cells that were near the root tip at the time of HIF treatment differentiated into haustorial cells. Within a few hours after exposure to HIFs, root-tip development reverted to its typical growth pattern, and normal roots grew out of the haustoria. This resulted in the globe-shaped haustoria being located proximal to the root tip (Fig. 1).

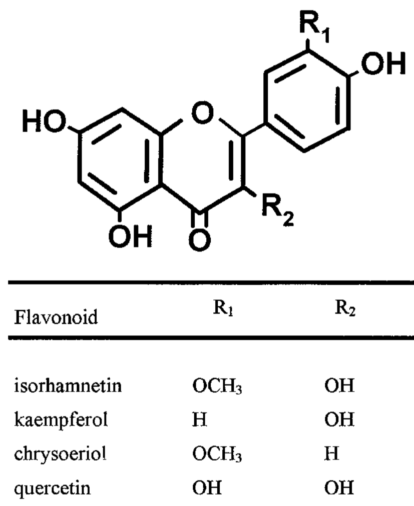
The ability of various phenolic derivatives to induce haustoria in *T. versicolor* was assayed (Figs. 2–4; Tables I and II). The most active HIFs were DMBQ and the anthocyanidin peonidin. Both DMBQ and peonidin were maximally active at a concentration near 10 μM (Table I; Fig. 5). At higher DMBQ concentrations some of the root tips became brown and necrotic, resulting in a smaller proportion of root tips with haustoria; this was not the case with peonidin.

Many of the simple phenolics evaluated induced haustoria to some degree over background; only caffeic acid, sinapinic acid, and salicylic acid were inactive at all the concentrations examined. In addition, three anthocyanidins, cyanidin, pelargonidin, and delphinidin (but none of the flavones or flavonols), had haustoria-inducing activity. However, unlike DMBQ and peonidin, none of these molecules was maximally active at 10 μM. All of the HIFs



Anthocyanidin	R_1	R_2
peonidin	OCH_3	H
cyanidin	OH	H
pelargonidin	H	H
delphinidin	OH	OH
malvidin	OCH_3	OCH_3

Figure 3. Anthocyanidins assayed for haustoria-inducing activity in *T. versicolor*.



Flavonoid	R_1	R_2
isorhamnetin	OCH_3	OH
kaempferol	H	OH
chrysoeriol	OCH_3	H
quercetin	OH	OH

Figure 4. Flavonoids assayed for haustoria-inducing activity in *T. versicolor.*

induced morphologically similar haustoria in *T. versicolor* (Fig. 1).

Maize-Seed Rinses Contain HIFs

Based on observations that *T. versicolor* parasitizes maize and that haustoria develop in response to different anthocyanins, we decided to test whether HIFs could be identified in maize kernels, a rich and abundant source of anthocyanins.

Maize kernels were rinsed with water or 50% methanol and the rinsates were assayed on *T. versicolor* roots. Both rinses induced haustoria in a concentration-dependent manner, but the activity of the methanol rinse was two to three times higher (Fig. 6). Under these conditions the water and methanol controls induced haustoria in less than 0.2% of the root tips.

Table I. *Induction of T. versicolor haustoria by simple phenolics and quinones*

Compounds were diluted in water to the concentrations shown. Values are the means ± SD of three plates with six *T. versicolor* plants each (50–90 total root tips per treatment).

Compound	Root Tips with Haustoria		
	1.0 μM	10 μM	50 μM
		proportion	
DMBQ	0.31 ± 0.11	0.73 ± 0.17	0.65 ± 0.19
p-Coumaric acid	0.23 ± 0.02	0.29 ± 0.14	0.35 ± 0.20
Vanillic acid	0.00 ± 0.00	0.35 ± 0.02	0.78 ± 0.16
Vanillin	0.01 ± 0.03	0.16 ± 0.08	0.74 ± 0.12
Syringic acid	0.03 ± 0.03	0.04 ± 0.04	0.48 ± 0.16
Syringaldehyde	0.05 ± 0.07	0.10 ± 0.05	0.38 ± 0.08
p-Hydroxybenzaldehyde	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.14
Ferulic acid	0.00 ± 0.00	0.03 ± 0.02	0.16 ± 0.19
Caffeic acid	0.05 ± 0.05	0.01 ± 0.02	0.02 ± 0.03
Sinapinic acid	0.00 ± 0.00	0.05 ± 0.03	0.00 ± 0.00
Salicylic acid	0.03 ± 0.04	0.00 ± 0.00	0.00 ± 0.00

Table II. *Induction of T. versicolor haustoria by flavonoids*

Flavonoids were diluted in water to the concentrations shown. Values are the means ± SD of three plates with six *T. versicolor* plants each (50–90 total root tips per treatment).

Compound	Root Tips with Haustoria		
	1.0 μM	10 μM	100 μM
		proportion	
Peonidin	0.12 ± 0.15	0.83 ± 0.15	0.65 ± 0.16
Pelargonidin	0.00 ± 0.00	0.04 ± 0.04	0.26 ± 0.10
Cyanidin	0.03 ± 0.04	0.01 ± 0.02	0.11 ± 0.14
Malvidin	0.02 ± 0.03	0.04 ± 0.07	0.11 ± 0.11
Quercetin	0.03 ± 0.04	0.00 ± 0.00	0.04 ± 0.06
Kaempferol	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.06
Delphinidin	0.00 ± 0.00	0.03 ± 0.05	0.03 ± 0.05
Isorhamnetin	0.00 ± 0.00	0.04 ± 0.06	0.04 ± 0.04
Chrysoeriol	0.01 ± 0.02	0.02 ± 0.02	0.01 ± 0.02

When maize-seed rinses were separated by HPLC (Fig. 7A), 6 of 14 fractions exhibited haustoria-inducing activity (Fig. 7B). Spectral characteristics of the active fractions were consistent with the presence of both flavonoids and simple phenolics. The color and photolability of three red bands separating on open C_{18} columns were consistent with the presence of anthocyanidins in the active fractions. The hydrophilicity of these fractions suggested that the molecules were present as anthocyanin glycosides (data not shown).

We then assayed haustoria-inducing activity in rinsates of maize seeds deficient in CHS, a key enzyme in flavonoid biosynthesis. The seeds segregated 1:1 for *C2/c2,whp1/whp1* and *c2/c2,whp1/whp1*. Kernels could be distinguished because *C2/c2* seeds are purple and *c2/c2* seeds are yellow. An equal number of kernels with approximately the same weight of each genotype were rinsed overnight in 50% methanol, and the rinsate was diluted 1:10 in water and

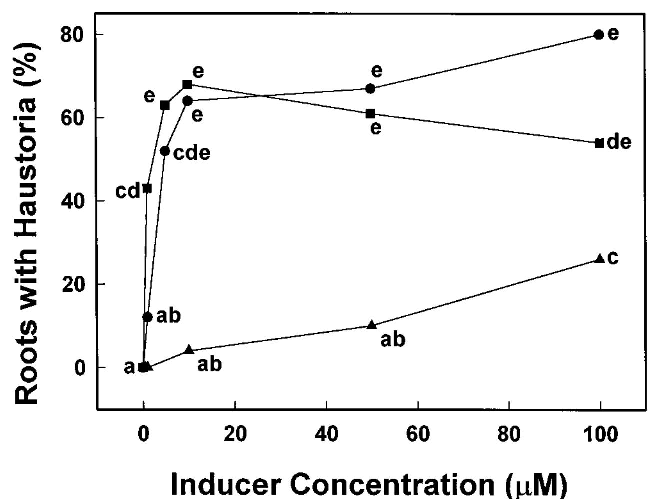


Figure 5. Dosage responses to haustoria-inducing anthocyanidins and phenolics. Haustoria-inducing activity in *T. versicolor* of two anthocyanidins, peonidin (●) and pelargonidin (▲), and DMBQ (■). Mean values associated with the same letter were not significantly different ($P \leq 0.05$).

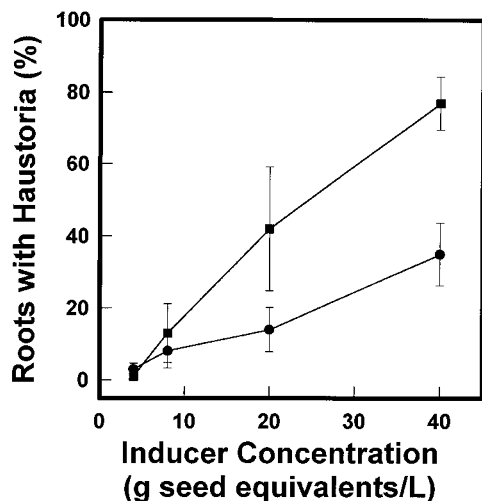


Figure 6. Haustoria-inducing activity of maize-seed rinses. Ten grams of B73 maize kernels was swirled overnight in 25 mL of water (●) or 50% methanol (■). The seed rinse was diluted in water and 3 mL was applied to the roots of in vitro-grown *T. versicolor*. Data are averages ± SD of three experiments, with about 18 plants treated in each experiment.

assayed in the roots of *T. versicolor*. Although less activity was recovered from *c2/c2* kernels than from those with a *C2/c2* genotype, rinses from both genotypes contained active HIFs (Fig. 8). This means that although some flavonoids were active HIFs, they were not the only HIFs released from maize kernels.

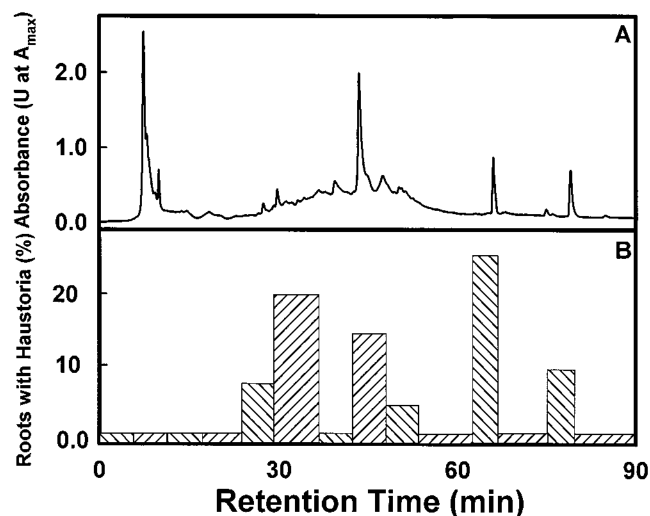


Figure 7. HPLC characteristics and haustoria-inducing activity of maize-seed rinse. A, A_{max} (200–400 nm) of 100% methanolic maize-seed rinse fractionated on a C_{18} column and eluted by a linear acetonitrile gradient of 5% to 100% for 2 to 60 min, followed by 100% acetonitrile for 60 to 90 min. B, Haustoria-inducing activity in *T. versicolor* for different HPLC fractions assayed at 0.9 g seed equivalents/mL. In the same assay, 50 μM DMBQ induced haustoria in 57% of the roots, but no haustoria were induced in the methanol-treated control.

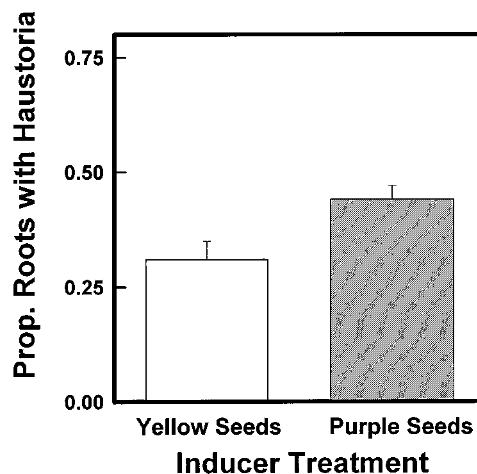


Figure 8. Haustoria-inducing activity of CHS-deficient maize seeds. Rinses from yellow (*c2/c2,whp1/whp1*) or purple (*C2/c2,whp1/whp1*) maize seeds were assayed on roots of *T. versicolor* at 40 g seed equivalents/L. Each value represents the mean ± SD of three tests. Seed weights of the yellow and purple genotypes did not differ significantly.

DISCUSSION

Molecular phylogenic studies indicate that all of the parasitic Scrophulariaceae species, including those in the genera *Triphysaria* and *Striga*, share common evolutionary origins (DePamphilis et al., 1997). This suggests that the fundamental mechanisms used by the two genera may be similar. Both *T. versicolor* and *S. asiatica* induce root haustoria in response to several different phenolic compounds (Riopel and Timko, 1995; Smith et al., 1996). Redox potential is a critical characteristic of haustoria-inducing molecules in *S. asiatica*. Structurally diverse quinones that induce *S. asiatica* haustoria have redox potentials within a narrow window, and related quinones that fall outside of the redox window are inactive as inducers (Smith et al., 1996). This suggests that haustoria development is initiated when the appropriate quinone associates with a parasite oxidoreductase to complete a redox circuit. Redox control of developmental programs in many organisms, including phototropism and defense in plants, is well documented (Hammond-Kosack et al., 1996; Huala et al., 1997).

Differences in the *S. asiatica* response to quinone and phenolic HIFs suggest how a common redox mechanism can use both structures. The phenol-exposure time for *S. asiatica* seedlings is longer and the concentrations higher than for analogous quinones (Lynn and Chang, 1990). When syringic acid, a common phenolic component of plant cell walls, is incubated with *S. asiatica* roots, DMBQ accumulates with kinetics similar to those seen during haustorium development (Kim et al., 1998). When hydrogen peroxide is removed from the reactions by the addition of catalase, haustorial induction with syringic acid, but not the quinone, is inhibited. These observations led to the hypothesis that root peroxidases convert inactive phenolic molecules to active quinones (Kim et al., 1998).

Apoplastic peroxidases have been identified in *S. asiatica* that catalyze the oxidation of *p*-hydroxy acids to quinones

with the same kinetics and pH dependence as haustoria induction in response to syringic acid (Kim et al., 1998). Because similar peroxidases were found in host roots, it was suggested that hydrogen peroxide is the limiting component in the system. In this model, *S. asiatica* roots supply the hydrogen peroxide oxidant required for the conversion of phenols to quinones. We are in the process of determining whether similar oxidation reactions are required for phenolic-acid induction of *T. versicolor* haustoria.

One novel aspect of this report is the observation that peonidin, an anthocyanidin that was apparently present in maize-seed rinses, induced haustoria in *T. versicolor*. Anthocyanidins are common in plants, and those released from legume seeds induce nodulation genes in *Rhizobium* bacteria (Hungria et al., 1991). A natural role for anthocyanins in the *Rhizobium*-bean system is supported by genetic and surgical variables showing that seed anthocyanins contribute to root-nodule formation at the top of the primary root (Hungria and Phillips, 1993). However, anthocyanins are not typically found in root exudates, and different *nod*-inducer molecules have been identified in seed effusates and root exudates (Schlaman et al., 1998). Thus, although anthocyanins induce haustoria in vitro, their role as natural signals for parasitic plants in the soil is not clear.

It is possible that the similarity in the activity of DMBQ and peonidin is related to the redox potential of different tautomeric forms of the anthocyanidin. Anthocyanidins exist in at least nine forms that change with pH and temperature (Cheminat and Brouillard, 1986). Several quinone forms of peonidin would likely be in equilibrium under our conditions, one of which would contain a quinone form of the methoxylated ring that could potentially satisfy the same electrochemical and/or structural requirements fulfilled by DMBQ (Fig. 9). One complicating factor for this model is that malvidin, the anthocyanidin that has a methoxylation pattern identical to that of DMBQ, showed essentially no inducing activity. Therefore, it seems reasonable to conclude that the remainder of the anthocyanidin molecule also contributes to biological activity, whether structurally (by changing equilibrium structures) or by changing redox states.

In addition to the anthocyanidins described here, other flavonoids have previously been identified as HIFs. For example, one of the first two HIFs identified was xenognosin B, which belongs to the isoflavonoid subclass of flavonoids (Lynn et al., 1981). CHS is the key enzyme in flavonoid biosynthesis and is therefore necessary for the synthesis of these compounds. The ability of *T. versicolor* to develop haustoria in response to rinses of maize kernels lacking CHS indicates that, in addition to phenolpropanoid biosynthesis, the pathways encode HIFs. Similarly, although flavonoids stimulate growth of arbuscular mycorrhizal fungi (Chabot et al., 1992), roots of CHS and wild-type maize are colonized by arbuscular mycorrhizal fungi to the same degree (Becard et al., 1995). These experiments demonstrate that multiple biosynthetic pathways generate a spectrum of signaling molecules that are active in vitro.

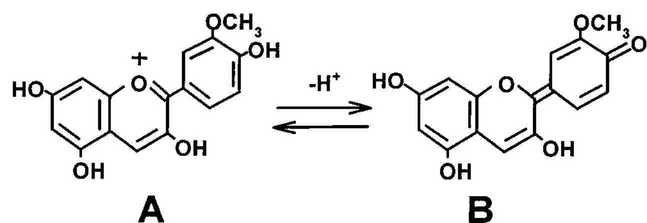


Figure 9. Two structures of the anthocyanin peonidin. As the pH was increased, the flavylium cation (A) was converted into several quinone structures, including that shown in B (Cheminat and Brouillard, 1986).

Although most of the phenolics that promote haustorium development in *T. versicolor* also stimulate haustoria in its close relative *A. purpurea*, there are exceptions. For example, coumaric acid is active in *T. versicolor* but not in *A. purpurea*, whereas sinapinic acid is active in *A. purpurea* but not in *T. versicolor* (Riopel, 1979). This suggests that different parasitic species distinguish different haustoria-inducing molecules. The quinone-dependent oxidoreductase receptors in different parasitic species may have different substrate affinities or redox optima, or peroxidases from different genera might be selective for particular structures. The amenability of *T. versicolor* to genetic analyses should help to clarify these possibilities.

The haustoria produced by *Triphysaria* and *Striga* are commonly distinguished as being primary and secondary, respectively (Kuijt, 1969). In the continued presence of DBMQ, haustorium development in *S. asiatica* results in a terminal differentiation of the radicle, giving rise to a primary haustorium. However, if DMBQ is washed from *S. asiatica* seedlings a few hours after exposure, normal root growth resumes. This cyclic reversion to normal root growth is observed when *S. asiatica* seedlings are exposed to syringic acid (Kim et al., 1998). The authors propose that hydrogen peroxide production is reduced in the presence of quinones, so during haustorium development phenols are not oxidized and normal roots develop.

The response of *T. versicolor* roots to DMBQ is different. Haustorium development is transient. Only those cells near the root tip when DMBQ is applied develop into (secondary) haustoria. Normal *T. versicolor* root growth commences after a few hours in the continued presence of DMBQ, mimicking the periodic response of *S. asiatica* to syringic acid. It may be that the phenol oxidation and quinone recognition mechanisms are different between *T. versicolor* and *S. asiatica*. Such differences may reflect the need for *S. asiatica* to infect a host soon after germination, whereas *T. versicolor*, being facultative, can be more opportunistic in its pursuit of host resources. Alternatively, later stages in the haustoria-development pathway may be autoregulatory in *T. versicolor* but not in *S. asiatica*.

Many of the active haustoria-inducing molecules are common constituents of plant cells, where they function in lignin biosynthesis, host defense, and other specialized physiological processes. Their recognition as HIFs allows the parasites to form haustoria in response to a broad spectrum of host plants. Host specificity in obligate parasitic Scrophulariaceae species such as *S. asiatica* is not defined at the haustoria-initiation stage, but earlier, at seed germination, or later, at haustorium penetration (Parker and Riches, 1993; Hood et al., 1998).

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