

CHAPTER 5

CHEMICAL SIGNALLING BETWEEN PLANTS

Mechanistic similarities between phytotoxic allelopathy and host recognition by parasitic plants

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Abstract. Parasitic plants in the *Orobanchaceae* use chemicals released from host-plant roots to direct developmental processes crucial to their heterotrophic lifestyle. An illustrative example is the development of haustoria; parasite root organs that function in host attachment and penetration, and in the establishment of a physiological conduit through which host resources are robbed. The facultative parasite *Triphysaria* develops haustoria only in the presence of host roots or host root factors. An *in vitro* assay was used to identify several phenolic derivatives that induce haustorium formation; the activity of multiple signalling molecules is consistent with a redundancy of active molecules in the rhizosphere triggering haustorium development. Haustorium-inducing factors are structurally related to phytotoxic allelochemicals released by some plants to inhibit the growth of neighbouring plants. We used genomic approaches to demonstrate that similar genetic pathways are up-regulated in parasitic roots upon contact with host plants as are regulated in response to allelochemical exposure. A parasite quinone oxidoreductase was identified that has properties suggesting that it functions in both allelochemical detoxification and haustorium signal transduction. These and other mechanistic similarities between allelopathic toxicity and haustorium signal transduction support the hypothesis that parasitic plants have recruited allelotoxin defence mechanisms for host-plant recognition.

Keywords: parasitic plants; allelopathy; plant–plant communication; haustorium development

INTRODUCTION

Parasitic angiosperms live in intimate associations with their plant hosts and by their very definition fulfil at least some of their nutritional requirements by directly invading other plants to rob them of water and nutrients (Kuijt 1969). In some species host-plant identification and invasion is orchestrated through chemical signalling between the host and parasite. Most notably, parasitic species of

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Orobanchaceae use molecules made by the host root to trigger various developmental programmes including seed germination, host attachment and invasion, and the establishment of physiological conduits through which nutrients are transferred from host to parasite (Press and Graves 1995). The effects of plant parasitism can be devastating for the host plants and some of the world's worst agricultural pests are parasitic weeds (Parker and Riches 1993; Matúšová and Bouwmeester in press).

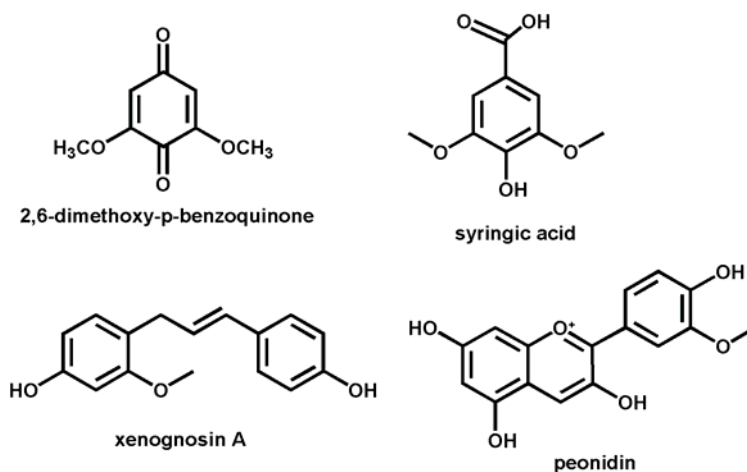
Almost thirty years ago Peter Atsatt drew parallels between insect herbivory and plant parasitism and suggested that parasitic plants, like specialist insect herbivores, may recruit plant defence molecules as 'feeding cues' (Atsatt 1977). This insightful analogy was proposed before most of the molecules used by parasitic plants for host recognition were identified. As seen in Figure 1, many of the molecules used by parasitic plants for host identification are structurally similar to phytotoxins produced by allelopathic plants to inhibit the growth of neighbouring plants (Conger 1999). We will show that not only are phytotoxic allelochemicals similar to host recognition cues, but in some cases the same molecules have both activities. We will also show that many of the genes activated in parasite roots after contact with host roots are similarly activated by exposure to allelotoxins. At least two parasite genes activated by host root contact encode quinone oxidoreductases that are known to function in xenobiotic detoxification in other biological systems. Biochemical and transcriptional experiments suggest that one of these may also function in host signal perception and transduction. While the molecular mechanisms of host identification by parasitic plants have yet to be fully elucidated, the current evidence suggests underlying similarities between host plant recognition and defence against allelopathic phytotoxins. The collective conclusions of these studies support Atsatt's hypothesis that parasitic plants have adapted host defence molecules as recognition cues.

PHYTOTOXIC ALLELOPATHY

For many years it was accepted that spatial patterning of plants in natural populations is established to a large extent by inherent properties of the plants themselves. There was, however, considerable debate about the role of chemical factors in establishing localized communities (Muller et al. 1964). While there were numerous publications of phytotoxic molecules being produced by plants, a phenomenon generally termed allelopathy, the ecological and/or agronomic effects of these molecules in field settings remained questionable (Conger 1999; Williamson 1990). Recently it was shown that the toxic flavonoid catechin is secreted into the soil by *Centaurea maculoso*, an exotic invasive weed of North America (Bais et al. 2002). Native grasses in North America are more susceptible to catechin than are their European relatives. Also, catechin concentrations are higher in North-American grasslands invaded by *C. maculoso* than in European grasslands where *C. maculoso* is native. The conclusion reached by these studies is that secretion of phytotoxic catechin contributes to the invasive success of this pernicious weed and established that allelotoxins exchanged between plants are of

ecological significance (Bais et al. 2003; 2002). However, our ability to exploit allelopathic phytotoxins in agricultural settings remains limited by a general lack of knowledge about mechanisms underlying plant–plant interactions.

Haustorium inducing factors



Phytotoxic allelochemicals

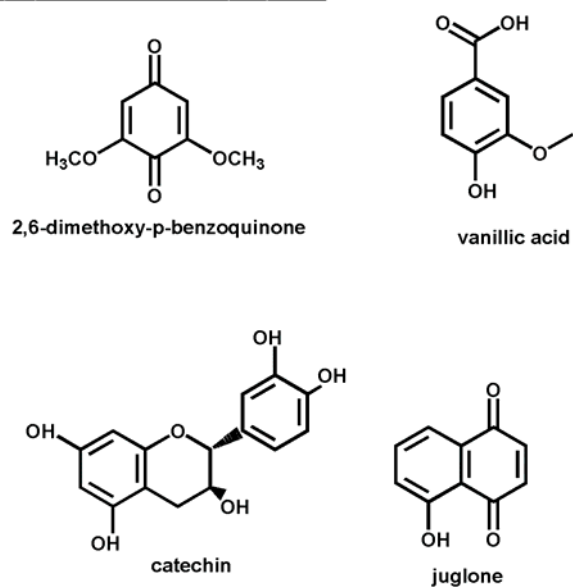


Figure 1. Common haustorial inducing and phytotoxic allelochemicals

It has been known for centuries that walnut trees poison the soil for underlying vegetation (Gries 1942). The allelochemical responsible for walnut toxicity is juglone (5-hydroxyl-1,4-naphthoquinone), a highly toxic quinone frequently used in pharmacological studies (Gries 1942; Inbaraj and Chignell 2004; Kamei et al. 1998) (Figure 1). We assayed the effects of juglone on *Arabidopsis* seed germination and root growth. Germination was assayed by plating the seeds directly into media containing various concentrations of juglone; root growth was measured by germinating the seeds in non-selective media and then transplanting the seedlings into juglone-containing media (Figure 2, top). As seen from the bars in Figure 3, there was a significant reduction (T test, $P \leq 0.05$) in both germination and root growth rates in juglone concentrations greater than 40 μM . The concentrations of juglone required for $\frac{1}{2}$ maximal germination or growth were similar, suggesting that phytotoxicity is associated with a common metabolic pathway shared by germination and root growth processes.

Quinones and phenolics are among the most commonly described classes of allelopathic phytotoxins (Inderjit 1996) (Figure 1). Quinones are oxidized phenols, and phenols are reduced quinones, and electrical transformations between these states account for much of their biological significance (Harborne 1989). Because quinones are widely used in medicine as anticancer agents, antibiotics and antimalarial drugs, the mechanisms of quinone cytotoxicity are well known (O'Brien 1991). Most significant are those mechanisms associated with free-radical formation during quinone reduction. Single electron reductions catalysed by enzymes such as quinone oxidoreductase or xanthine oxidase produce highly reactive semiquinone intermediates that directly bind to and inactivate nucleic acids, proteins, lipids and carbohydrates (Testa 1995). Semiquinone radicals also react with molecular oxygen leading to the generation of superoxide anions and hydroxyl radicals. These highly toxic radicals inactivate enzymes, break DNA strands, and cause membrane-lipid peroxidation. These molecules also play an integral role in the cytotoxicity associated with the hypersensitivity response of plants against microbial pathogens (Hammond-Kosack and Jones 1996).

There are good reasons to believe that juglone phytotoxicity results from similar mechanisms. Juglone is not synthesized by walnut trees, which rather synthesize the non-toxic reduced form 1,4,5-trihydroxynaphthalene (hydrojuglone) (Lee and Campbell 1969). Hydrojuglone is abundantly produced by roots, leaves and nuts and becomes oxidized to toxic juglone upon exposure to air or oxidizing agents from other organisms, including roots of other plants (Gries 1942). Free radicals formed during redox cycling between juglone and hydrojuglone have been identified in human and mouse cells and intact *Caenorhabditis elegans* (Chignell and Sik 2003; Noda et al. 1997; De Castro et al. 2004). While the cytotoxicity mechanisms of this and other phenolic allelotoxins have not been specifically elucidated, it is reasonable to propose that toxicity is to a large extent associated with free radicals produced during redox cycling.

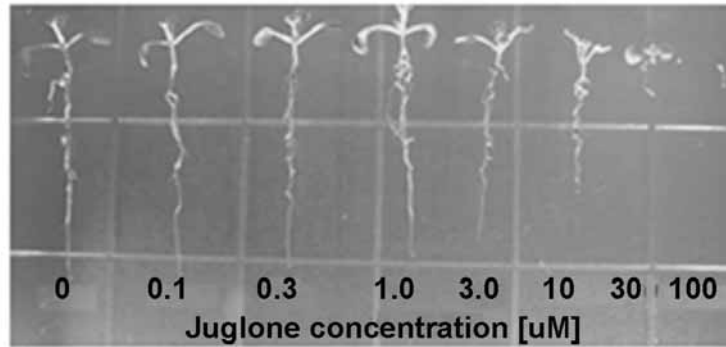
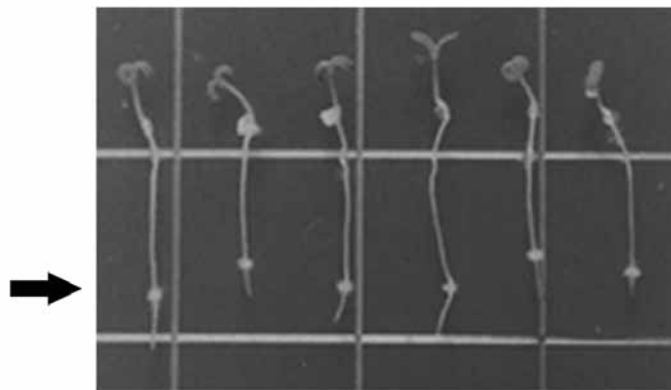
Phytotoxicity assay with juglone**Haustorium assay with exudate**

Figure 2. Phytotoxicity and haustorium induction assays

Top photo: Aseptic Arabidopsis seedlings were placed in media containing juglone at the concentrations indicated. After nine days the seedlings were removed, spread along the surface of an agar plate and photographed.

Bottom photo: Aseptic Triphysaria seedlings were germinated in agar, exposed to rice exudates, and photographed thirty hours later. The arrow approximately marks the single haustorium formed on every Triphysaria root.

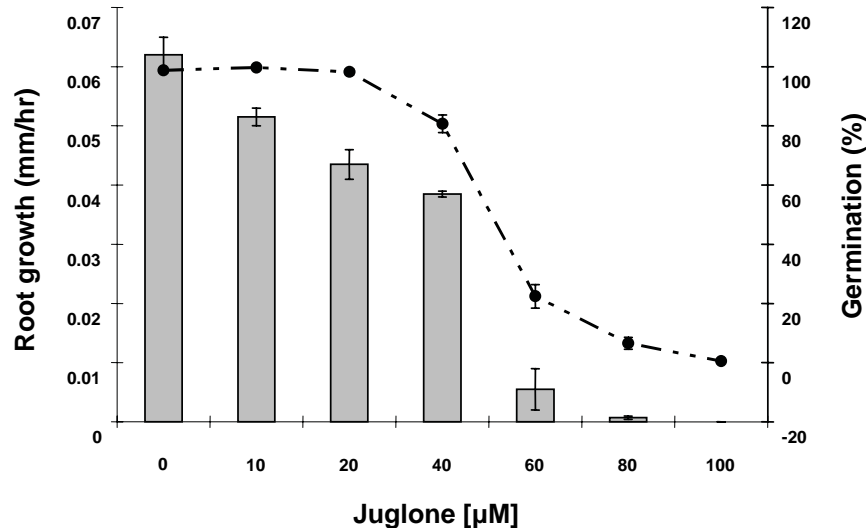


Figure 3. Toxicity of juglone on *Arabidopsis* germination and growth
 The bars indicate *Arabidopsis* root growth rates in different concentrations of juglone and are referenced by the primary axis. About 35 roots were measured in each of two experiments for each data point graphed. The dashed line shows the percent germination at the same juglone concentrations. The results are the averages of two experiments with about 300 seeds each. The error bars indicate the minimum and maximum values obtained.

HOST RECOGNITION AND HAUSTORIUM DEVELOPMENT IN THE PARASITIC PLANT *TRIPHYSARIA*

Over three thousand angiosperm species are parasitic and able to invade host plants to obtain nutrients (Nickrent 2005). Parasitic plants encompass a wide range of growth habits ranging from mistletoes that grow on the tops of conifers to root parasites, like *Striga*, that live a significant portion of their lives underground. Perhaps the most bizarre habit is displayed by *Rafflesia*, a rootless, stemless plant comprised of little more than the world's biggest flower (Brown 1822). The single morphological feature that all parasitic plants have in common is their ability to produce a haustorium, a structure able to invade host plant tissues and act as the physiological bridge through which host resources are translocated into the parasite (Kuijt 1969).

At least one family of parasitic angiosperms, the *Orobanchaceae*, develops haustoria in response to molecules secreted by host plant roots. This family is comprised of about thirty species of root parasites that rely on host resources to varying degrees. Representative of the obligate parasites that must attach to host plants within days of germination are the agriculturally devastating weeds *Striga* and *Orobanche* (Parker and Riches 1993). As described elsewhere in this volume, these plants have evolved host detection systems to identify host roots prior to committing

to germination (Matúšová and Bouwmeester in press). Other *Orobanchaceae* are facultative parasites that do not require host germination factors and can mature without attaching to a host. Facultative parasites do, however, require host factors to initiate the switch from autotrophic to heterotrophic growth.

Triphysaria is a facultative parasite that grows as a common springtime annual throughout the pacific coast of North America. *Triphysaria* is a small genus with five inter-hybridizing species, four of which are outcrossing and one autogenous (Yoder 1998). We are using *Triphysaria* to study the genetic factors that govern plant parasitism because unlike *Striga*, *Triphysaria* can be grown in the US without quarantine restriction or environmental concerns. This allows us to easily collect large numbers of seeds that represent a wide range of genetic variants. *Triphysaria* has a broad host range that includes at least 27 families of angiosperms ranging from *Arabidopsis* to maize (Thurman 1966; Goldwasser et al. 2002). Intriguingly, the only plant species apparently not infected by *Triphysaria* are other *Triphysaria* (Yoder 1997). The mechanism of vegetative self-recognition in *Triphysaria* is not currently known but is an active area of investigation because of its potential application for engineering host resistance against parasitic weeds.

Haustorium development in *Triphysaria* roots can be monitored *in vitro* by applying host roots, root exudates or purified root factors to aseptic *Triphysaria* seedlings (Jamison and Yoder 2001). In brief, *Triphysaria* seeds are surface-sterilized and germinated in agar plates at 16°C. One to two weeks after germination, aseptic seedlings are transferred to square Petri dishes containing nutrient agar, and incubated at 20°C at a near vertical angle so that the *Triphysaria* roots grow down along the agar surface. After additional one or two weeks of growth, host root exudates or aqueous solutions of purified haustoria-inducing factors (HIFs) are spread across the roots. The first morphological response to HIF exposure is an almost immediate cessation of root elongation (Baird and Riopel 1984). Within about five hours haustorial hairs begin to proliferate in a zone just behind the root tip. Concomitantly, cortical cells underlying the haustorial hairs begin to expand and by twelve hours a hairy, swollen knob appears distal to the root tip. In the presence of a host, haustorial hairs will attach themselves firmly to the host root and the haustorium will penetrate via a combination of enzymatic activity and physical pressure (Losner-Goshen et al. 1998). In the absence of a host root the swelling and hair proliferation continue for about 24 hours at which time the *Triphysaria* root reverts to its normal growth programme. Haustorium development is highly synchronous, and when several *Triphysaria* are treated together haustoria are observed at defined locations distal to the tip (Figure 2). Photographs of haustoria and a time-lapse animation of haustorium development can be seen at <http://www.plantsciences.ucdavis.edu/yoder/lab/>.

Using the *in vitro* assay we identified several phenolic derivatives that trigger haustorium formation when applied to *Triphysaria* roots including simple phenolics, flavonoids and quinones (Figure 1) (Albrecht et al. 1999). Similar molecules were previously identified as HIFs for *Striga* and *Agalinis* (Riopel and Timko 1995; Smith et al. 1996). Many of these molecules are commonly found in the rhizosphere and play signalling roles in the attraction and/or repulsion of microbial populations (Siqueira et al. 1991). The triggering of haustorium development by multiple

phytochemical signals suggests that there is a redundancy in HIFs functioning in the rhizosphere. This hypothesis is supported by our observation that inbred lines of *Triphysaria* selected for the inability to form haustoria when exposed to a specific HIF still form haustoria when exposed to complex host root exudates (Jamison and Yoder 2001).

Two general hypotheses can be proposed for the ability of *Triphysaria* to form haustoria in response to several different molecules. One hypothesis is that there are several specific receptors, each recognizing a different HIF, that trigger haustorium development. Alternatively there may be a single receptor that recognizes multiple inducing molecules. Because HIF receptors have not yet been isolated we cannot rule out either mechanism. However an informative set of experiments conducted by David Lynn and co-workers suggests a model for activation of a single receptor by multiple phenolics. This group assayed a number of natural and synthetic quinones for their ability to induce haustoria in *Striga* (Smith et al. 1996). Active haustorial-inducing quinones had similar redox potentials while inactive molecules generally fell outside the redox window. Lynn's group then designed spin trap molecules that acted as haustorium development inhibitors (Zeng et al. 1996). This work led them to suggest that haustorium signalling involves a redox-regulated signalling mechanism that is triggered by cycling between the reduced and oxidized states of the HIF. There is considerable precedent for redox regulation of development and many biological processes are under redox control, including DNA replication, transcription, translation, hormone reception, phototropism and defence responses (Huala et al. 1997; Allen 1993).

Redox cycling of quinones is catalysed by quinone oxidoreductases and, as will be discussed later, we have studied two *Triphysaria* quinone oxidoreductases that are active during haustorium initiation. The role of quinone oxidoreductases in haustorium signalling was examined using pharmacological inhibitors (Matvienko et al. 2001b). Dicumarol and Cibacron blue are specific inhibitors of quinone oxidoreductases, and these inhibit haustorium formation when applied to *Triphysaria* roots prior to host root factors. Root growth measurements taken before and after inhibitor exposure showed that the inhibitors did not affect overall root health. These experiments support the model that enzymatically catalysed quinone oxidation is a component of haustorium signalling (Matvienko et al. 2001b).

The current model for haustorium initiation predicts that semiquinone intermediates formed from the action of quinone oxidoreductase initiate haustorium signal transduction through a redox signalling pathway. This model has obvious parallels to the mechanisms of allelopathic quinone toxicity since both are dependent upon the generation of free-radical intermediates. The important roles of redox transformations in subterranean interactions between plants and other rhizosphere organisms have been previously highlighted (Appel 1993).

HAUSTORIUM INDUCING FACTORS CAN BE ALLELOTOXINS

Host root factors can be both phytotoxic and organogenic. We collected root exudates from hydroponically grown rice, bound small molecules to the non-ionic

absorbent Bio-Beads SM2, and eluted them with methanol. After the methanol was evaporated, the dried exudate material was dissolved in water and diluted to concentrations either more or less concentrated than the original exudate. The diluted exudates were then applied to roots of *Triphysaria* seedlings as described for the haustorium bioassays. Phytotoxicity was estimated after three days by visually examining the roots and noting the degree of browning. Additionally, cell viability was assayed by staining the roots with fluorescein diacetate (FDA) and monitoring the loss of fluorescence as the dye leaked from dead cells (Bais et al. 2003). Figure 4 summarizes the results (Shin and Yoder in prep.). Haustorium formation was maximal with about 90% of the roots forming haustoria at original, undiluted exudate concentration (1X). As exudate concentrations increased, haustorium formation decreased with a concomitant increase in cytotoxicity by both direct visualization and loss of FDA staining. At exudate concentrations six times that of the original, *Triphysaria* roots did not develop haustoria and were beginning to turn brown (Figure 4).

Rice exudates applied to *Triphysaria* roots

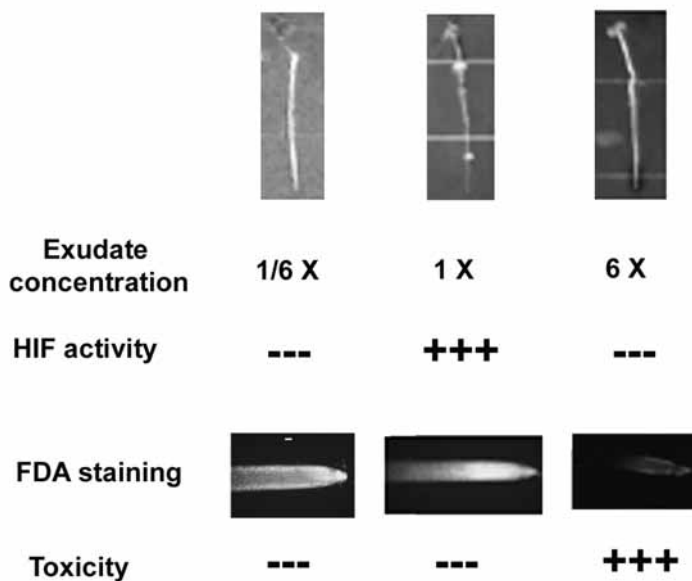


Figure 4. Rice root exudates have both HIF and phytotoxicity activities. *Triphysaria* seedlings were treated with different concentrations of rice root exudates and scored for haustorium formation and toxicity using FDA staining and root browning as indicators. The three photos at the top of the figure are representative of seedlings treated with 1/6X, 1X and 6X concentrations of exudate.

Purified haustorial inducing molecules are also phytotoxic at high concentrations. The frequently referenced HIF isolated from sorghum roots, 2,6 dimethoxybenzoquinone (DMBQ), is an illustrative example (Chang and Lynn 1986). DMBQ was originally characterized as a mammalian cell cytotoxin and later as a microbial antibiotic and DNA mutagen (Nishina et al. 1991; Brambilla et al. 1988; Jones et al. 1981). We showed that while DMBQ is an active inducer of *Triphysaria* haustoria at concentrations between one and thirty μM , at concentrations one hundred μM or higher it is phytotoxic and *Triphysaria* roots turn brown and die (Jamison and Yoder 2001). In conclusion, both complex root exudates and purified factors can have either haustorium-inducing or phytotoxic activities depending on their concentrations.

TRIPHYSARIA GENES REGULATED BY HOST CONTACT FUNCTION IN ALLELOCHEMICAL DETOXIFICATION

A second factor linking host-parasite recognition and allelotoxin defence is the overlap in transcripts differentially regulated in each system. This was discovered by analysing the sequences of cDNA libraries enriched for transcripts regulated in *Triphysaria* roots after contact with host roots or DMBQ (Tomilov, Tomilova and Yoder in prep. Matvienko et al. 2001a). In brief, host contact was realized by laying the roots of *Arabidopsis* seedlings across those of *Triphysaria* growing along the surface of agar plates. The *Arabidopsis* seedlings were removed at various times ranging from immediately after contact to up to five hours later. These times correlated with early haustorium development prior to host-root penetration. *Triphysaria* roots were then dissected, frozen in liquid nitrogen and subjected to mRNA isolation. PCR-based suppression subtractive hybridization (SSH) was used to prepare two cDNA libraries, one enriched for transcripts up-regulated (host forward, 'HF') and one enriched for transcripts down-regulated (host reverse, 'HR') by contact with *Arabidopsis* roots (Diatchenko et al. 1996). Approximately 3000 inserts of each library were sequenced and assembled into contigs representing over 1000 distinct transcripts in each class.

BLASTN analyses showed that approximately 80% of the cDNAs were specific to one or the other library. We assigned a tentative function to each cDNA by virtually translating the assembled transcripts and comparing the predicted proteins to those catalogued in the *Arabidopsis* protein database (ATH1.pep_cm_20040228) using BLASTX (Rhee et al. 2003). The corresponding GO annotations for each of the best hits was obtained through the Gene Ontology (GO) function at TAIR (TAIR 2005). GO annotations provide a uniform vocabulary to describe the roles of genes and gene products in all organisms and allowed us to categorize the putative functions of each translation product into one of nine general biological processes (Ashburner et al. 2000). The number of transcripts in each category for either the HF or HR libraries allowed us to determine which biological functions were over- or under-represented in each library. Three classes of transcripts were significantly ($p < 0.01$) more abundant in the HF than HR libraries; those involved in stress responses, electron transport or cellular transport (Table 1). As previously observed, many of

these transcripts function in xenobiotic detoxification and/or protection from reactive oxygen species (Matvienko et al. 2001a).

Table 1. Representation of biological functions in different SSH libraries

	HF ¹	HR ²	Chi ²	P
Total # annotated transcripts	702	910		
DNA or RNA metabolism	28	44	0.67	NS
cell organization and biogenesis	44	48	0.73	NS
electron transport or energy pathways	93	64	17.41	p < 0.001
protein metabolism	174	220	0.08	NS
signal transduction	28	35	0.02	NS
transcription	43	48	0.54	NS
transport	157	153	7.86	p < 0.01
response to abiotic or biotic stimulus	58	47	6.24	NS
response to stress	52	34	10.58	p < 0.001

¹ Host forward subtracted library

² Host reverse subtracted library

Chi² and P show significance values for the functional category being differentially represented in either the HF or HR libraries.

We are interested in genes predicted to function in allelochemical oxidoreduction because of their hypothesized roles in haustorium initiation and allelopathic phytotoxicity. Two distinct quinone oxidoreductases were selected from the SSH libraries and studied in detail (Wrobel et al. 2002; Matvienko et al. 2001b). TvQR2 encodes a 205 aa protein with significant homology to a quinone oxidoreductase in the wood-rotting fungus *Phanerochaete chrysosporium*. The *P. chrysosporium* quinone oxidoreductase functions to protect the fungus from the variety of toxic electrophiles produced during lignin degradation (Brock and Gold 1996). These enzymes are related to the carcinogen detoxification enzyme DT-diaphorase that reduces quinones to non-toxic hydroquinones by catalysing two-step hydride transfers from NAD(P)H to enzyme-bound FMN (or FAD), and then from FMNH₂ (or FADH₂) to the quinone. These detoxifying quinone reductases thereby reduce quinones to hydroquinones in a single-step reaction that avoids radical intermediates (Li et al. 1995).

TvQR1 encodes a 329 aa protein related to a family of NAD(P)H-dependent quinone oxidoreductases that produce semiquinone radicals through univalent quinone reductions. These enzymes catalyse the reduction of several natural quinones and have been identified in plants, animals and microbes (Babiychuk et al. 1995; Thorn et al. 1995). Electron paramagnetic resonance spectroscopy indicates that these enzymes catalyse single electron reductions that yield unstable semiquinone intermediates (Rao et al. 1992). The activated semiquinones are then readily detoxified by modifications with various chemical groups (Testa 1995).

We expressed and purified the TvQR1 protein from *E. coli* and the TvQR2

protein from *Pichia pastoris*. We spectrophotometrically monitored the reduction of quinone substrates and the oxidation of NADH to show that these enzymes catalyse NAD(P)H-dependent reductions of DMBQ, juglone and other allelopathic quinones (Wrobel et al. 2002, Petit and Yoder unpubl.). The biochemical analyses confirmed the homology predictions that these enzymes function in allelochemical detoxification.

Northern analyses showed that the steady-state transcript levels of TvQR1 and TvQR2 increased within 30 minutes of treatment with DMBQ, 2,6-dimethylbenzoquinone, menadione and, most strongly, juglone (Matvienko et al. 2001b). Steady-state levels reached a maximum 8-12 hours after treatment and returned to non-induced levels by 24 hours post-treatment, precisely corresponding to the times of haustorium ontogeny. The protein-synthesis inhibitor cycloheximide prevented haustorium development when applied to *Triphysaria* roots prior to host factors indicating that *de novo* protein synthesis is required for haustorium development. However, cycloheximide did not block transcriptional induction of TvQR1 or TvQR2 indicating that their transcriptional regulation is a rapid, primary response to both HIFs and allelochemical cytotoxins (Matvienko et al. 2001a).

Similar Northern blots were performed after exposing roots of three non-parasitic *Scrophulariaceae*, *Lindenbergia muraria*, the closest non-parasite to the parasitic clade of *Scrophulariaceae* (DePamphilis et al. 1997), *Mimulus aurantiacus* and *Antirrhinum majus*, to DMBQ. TvQR2 homologues were induced in all species. In contrast, TvQR1 was only up-regulated in roots of parasitic species. Moreover, TvQR1 was up-regulated in response to DMBQ application in inbred lines of *T. pusilla* that formed haustoria but not in those selected to be non-responsive to DMBQ (Jamison 2003). The correlation between the up-regulation of TvQR1 and haustorium development holds for intraspecific as well as intergeneric comparisons.

The correlation of TvQR1 transcript regulation with haustorium development together with its biochemical function suggests that this enzyme may play a role in haustorium formation. We hypothesize that semiquinone radicals produced by univalent quinone reductions catalysed by TvQR1 initiate the signal transduction pathway leading to haustorium development. Alternatively, semiquinone radicals and associated reactive oxygen intermediates may take a more direct role in early haustorium development. For example, cortical cell swelling and epidermal hair elongation may directly reflect the action of reactive radicals produced by over-expression of TvQR1. In either case, the induction of a univalent reducing quinone oxidoreductase by haustorium-inducing factors may be a critical developmental step that distinguishes parasitic plants from non-parasitic autotrophs. The development of a *Triphysaria* transient transformation system will allow us to test these hypotheses using inhibitory RNAs (Tomilov et al. 2004, Tomilov, Tomilova and Yoder in prep.).

CONCLUSIONS

Allelopathic plants release phytotoxic molecules into the soil as a means of limiting the growth of other plants. These can be thought of as molecules that defend

allelopathic plants against neighbouring plants that compete for limiting resources. The phytotoxicity of these molecules results primarily from reactive oxygen species generated during redox cycling between reduced and oxidized states of the allelochemical. Plants and other organisms encode enzymes that detoxify reactive oxygen species; these protein families originated early in evolutionary history in defence against damage associated with aerobic environments (Testa 1995). Parasitic plants seem to have recruited some of the enzymes that function in xenobiotic detoxification for use in host root identification. Conclusive evidence that the parasite host recognition system is derived from an allelochemical detoxification system awaits gene-silencing experiments in transgenic parasites. But in any case, host defence and host recognition are clearly associated in parasitic plants and Atsatt's analogies between parasitic plants and herbivorous insects have to date withstood molecular investigations.

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