J Plant Growth Regul (2000) 19:217–231
DOI: 10.1007/s003440000024

J P G R
Journal of Plant Growth Regulation

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Signaling Organogenesis in Parasitic Angiosperms: Xenognosin Generation, Perception, and Response

W. John Keyes, Ronan C. O'Malley, Dongjin Kim, and David G. Lynn*

Searle and Jones Chemistry Laboratory, 5735 Ellis Avenue, The University of Chicago, Chicago, IL 60637, USA

Abstract

Parasitic strategies within the angiosperms generally succeed by tightly coupling developmental transitions with host recognition signals in a process referred to as xenognosis. Within the Scrophulariaceae, *Striga asiatica* is among the most studied and best understood parasitic member with respect to the processes of host recognition. Specific xenognosins regulate seed germination, the development of the host attachment organ, the haustorium, and several later stages of host-parasite integration. Here we discuss the signals regulating the development of the haustorium, the critical vegetative/parasitic transition in the life cycle of this obligate parasite. We provide evidence for the localized production of H_2O_2 at the *Striga* root tip and suggest how this oxidant is used to exploit host peroxidases and cell wall pectins to generate a simple benzoquinone signal. This benzoquinone xenognosin proves to be both necessary and sufficient for haustorial induction in cultured seedlings. Furthermore, evidence is provided that benzoquinone binding to a redox active site completes a "redox circuit" to mediate signal perception. This redox reaction regulates the time-dependent expression of specific marker genes critical for the development of the mature host attachment organ. These studies extend the emerging series of events necessary for the molecular regulation of organogenesis within the parasitic plants and suggest novel signaling features and molecular mechanisms that may be common across higher plants.

Key words: *Striga asiatica*; Parasitic angiosperms; Signal transduction; Organogenesis; Haustorial development; Benzoquinones; Xenognosin

Introduction

All organisms must acquire and process information about their environment. Even the simplest unicellular organisms must detect nutrients, mediate dormancy, and navigate physical and biologic dangers. The *Escherichia coli* genome contains as many as 50 so-called two-component signal transmitter/response regulator systems (Chang and Steward 1998) through which environmental cues may be monitored. Because of the simplicity of these systems, the molecular mechanisms of perception and response are likely to be first understood in bacteria.

Higher eukaryotes require further cellular coordination. Probably the most dramatic example is found in the sessile higher plants, which alter form throughout their life cycle to exploit their environmental niche. This ability to sculpt one's architec-

*Corresponding author; e-mail: dlynn2@emory.edu

ture to a particular physical, chemical, and biologic niche is powerful and has obviously been successful but requires mechanisms for coupling external signals with coordinated cell growth and cellular differentiation. In animals the body plan is typically set in the embryo, but plants continue to grow and change their form by exploiting the specialized, perpetually embryonic regions known as meristems. Both the presence of a cell wall, which prevents cellular migration, and the absence of efficient circulation require the plant to maintain a greater cellular autonomy than seen in animals. The meristems therefore empower the plant with the ability to respond to diverse environmental stimuli and do so both through enhanced cellular plasticity and a greater response pleiotropy. Although this plasticity has enabled agricultural manipulation of plant growth, the increased response pleiotropy has greatly complicated our understanding of the nature of the signals perceived, the mechanisms of signal perception, and the signal-transducing cascades that mediate the diversity of known cellular responses.

In the most obvious and best-characterized systems, for example, the light-mediated responses controlled by phytochrome, the actual molecules involved in signal transduction, other than the photoreceptor itself, remain poorly characterized (Nagy and Schafer 1999). Even more striking, the modes of action of plant hormones, at least those controlling crucial events in growth and development, even those whose structures have been known for more than half a century, are virtually unknown (Arteca 1996; Brault and Maldiney 1999; Coenen and Lomax 1997). Although it is clear that appropriate plant tissues respond to either light or hormones by altering the transcription of genes within minutes (Guilfoyle and others 1989; Guilfoyle and others 1993; Nagy and Schafer 1999; Silverthorne and Tobin 1987), the signaling cascades that mediate hormonal induction of gene expression are just now being explained (Abel and Theologis 1996; Brault and Maldiney 1999; Hooley 1999; Jones 1994). Even though the characterization of these pathways is critical, their definition remains only the first critical step in understanding the hormones' mechanisms of action. For example, rapid responses to both the plant hormones auxin and cytokinin have been documented (Fowler and others 1999), but the first division is seen in isolated tobacco pith tissue after 3–5 days of continual culture.

It is a combination of this confusion about the required time for signal exposure, the apparent pleiotropic effects of the known signals, and the lack of a simple and rapid developmental system where the initiating signal is well understood that has so stymied our efforts to define the molecular controls regulating plant growth. Here we discuss the parasitic plants as a model system to dissect the events necessary for plant organogenesis. In these plants, the signals controlling the development of the host attachment organ, the haustorium, originate in the host plant. The obligate parasites have a limited lifetime without attachment, and the parasite meristematic tissue must respond immediately to the signal. Such an immediate homogeneous commitment, and across multiple individuals, synchronizes the response to allow the time dependence of gene expression to be analyzed during organogenesis. Here we discuss the specific model for signal perception and response that has emerged from studies on the African witchweed, *Striga asiatica.*

The Parasitic Plants

One percent of all flowering plants are not solitary but search out and form parasitic associations with other plants (Cronquist 1988; Kuijt 1969; Kuijt 1977; Musselman 1980). Parasitism occurs broadly across the angiosperms, being found in at least 16 families, in the form of trees, shrubs, vines, or herbs, and in habitats that range from the poles to the dry and humid tropics (Molau 1995). This broad distribution is a manifestation of the repeated appearance of parasitism throughout angiosperm evolution (DePamphilis 1995; Musselman and Press 1995).

Searcy was the first to seriously consider the molecular evolution of the parasitic plants and proposed three general phases (Searcy 1970; Searcy and MacInnis 1970). The first phase consisted of developing the specialized organ that forms the physiologic bridge to the host, the haustorium. After this phase, the second and third transitions were ones of specialization; the second phase being the loss of both biochemical pathways and morphologic structures that become redundant with host attachment, and the third phase being the accrual of more complex adaptations specific to an obligate parasite, including host specialization and mechanisms to overcome host defenses.

Over the last 10 years, several groups have been able to test predictions made by Searcy's proposal (DePamphilis 1995). Analyses of the nonphotosynthetic holoparasite *Epifagus virginiana* provided striking support for evolutionary reduction. The plastid DNA (ptDNA) of this organism was approximately a third the size of a nonparasitic relative (*Nicotiana tobacum*). Essentially all the plastid-encoded photosynthetic genes—the NADPH dehydrogenase genes, all four RNA polymerase genes, 13 of the 30 plastid encoded tRNAs, and 6 of the 22 plastid-encoded ri-

bosomal protein genes—were either missing completely or existed as pseudogenes (DePamphilis and Palmer 1990; Wolfe and others 1992). Accelerated rates of molecular evolution of both the remaining plastid genes and the rDNA of these parasites have also been documented and explained by molecular population genetic models of mutation, selection, and drift (DePamphilis 1995; Nickrent and Starr 1994). In addition, significant biochemical evidence now exists for highly specialized processes in host selection (Boone and others 1995; Lynn and Chang 1990), distance regulation (Chang and others 1986; Fate and Lynn 1996; Fate and others 1990), and overcoming host resistance (Olivier and others 1991; Riopel and Timko 1995).

By Searcy's proposal, the critical transition to the parasitic mode would be phase one. Histologic studies of mature haustoria are available, and although clear differences in anatomic features are present across the parasitic plants, the early initiation events appear similar (Atsatt 1983; Kuijt 1969; Riopel and Timko 1995). The development of lateral haustoria in the rhinanthoid Scrophulariaceae consist of early rounds of cell division and radial swelling by the pericycle and inner cortex to give rise to the haustorial primordia. Lateral roots and root nodules are also absorptive tissues, and the early stages of their formation are quite similar to those of the haustoria (DeKlerk and others 1999). Therefore, a simplifying hypothesis would maintain that parasitism arose from the loss of some signaling function that was complemented externally by another plant. It would then follow that the events responsible for inducing these organs have similar biochemical and mechanistic origins. In the case of the haustorium, both the inducing signal and some understanding of signal perception are available (Lynn and Chang 1990) and can be exploited to dissect the signaling pathway. At the very least, biochemical dissection of the signaling pathway for haustorial induction could provide insight into both the induction of plant organogenesis and the early steps in parasite evolution.

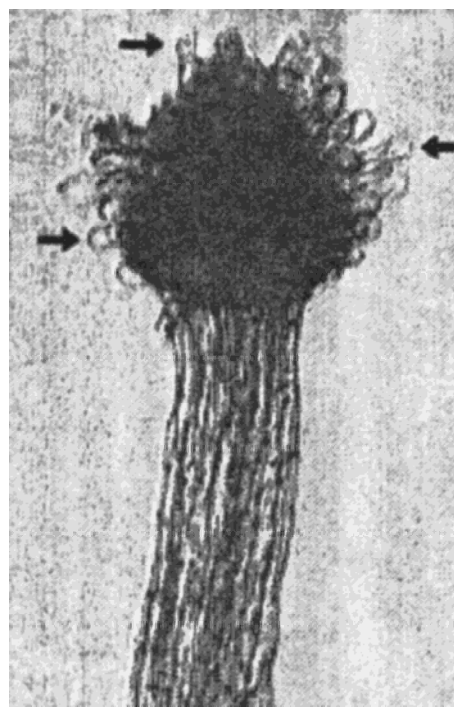

Figure 1. Two-day-old *S. asiatica* seedling was incubated at 30°C in 1 mL of H_2O containing 10 μM DMBQ. The photograph shows a typical swollen root tip with haustorial hair formation at the site of the arrows, after 24 h under these conditions.

Haustorial Development

Early rounds of cell division by the pericycle and inner cortex give rise to the lateral haustorial primordia in many of the Scrophulariaceae parasites (Kuijt 1977; Riopel and Musselman 1979). This postembryonic origin is similar in both lateral root (Malamy and Benfey 1997) and legume nodulation formation (Crespi and Galvez 2000; Mylona and others 1995) where coordinated cell expansion and divisions occur along the primary root proximal to the vascular tissue in the pericycle layer. The developmental programs of all three are induced by environmental signals and respond to hormones. Haustoria-like structures (Riopel and Baird 1987) and the early stages of nodule development (Crespi and Galvez 2000; Mylona and others 1995) are induced by cytokinins, whereas IAA appears to be important for initiation, morphogenesis, and continued viability of lateral roots (DeKlerk and others 1999; Malamy and Benfey 1997). Compounds that inhibit auxin transport (Hirsch and others 1989) also induce nodulelike structures.

In the terminal haustoria formed in *S. asiatica,* organogenesis is manifested primarily in the redirection of cellular swelling events. The cells distal to the meristem switch from longitudinal to radial growth, and the circumscribed pre-epidermal cells form haustorial hairs as shown in Figure 1. The extent of swelling required for the new organ can be significant, with an increase in diameter from twofold to fourfold, and the rate dramatic, development being complete within 24 h of induction. The swollen cells create minimally a larger surface area likely to be critical for host attachment, and it is within this swollen tip that the haustorial primordia form, giving rise to the infection peg and ultimately the ma-

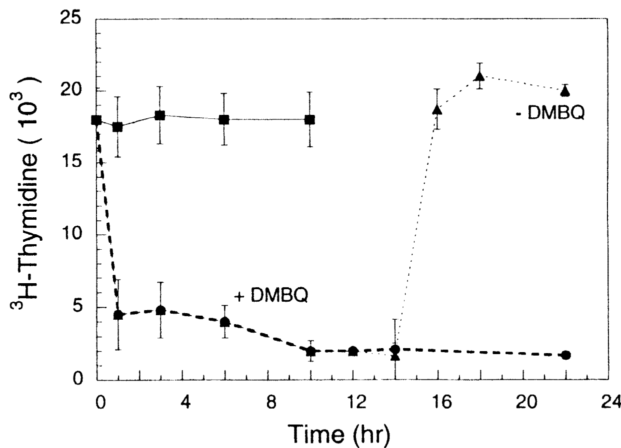

Figure 2. Two-day-old *S. asiatica seedlings* (100) were labeled at 30°C in 1 h in 200 µL media containing 1 µCi of [^{3}H]-thymidine after each indicated incubation time with (●) or without (■) 2 µM DMBQ. At 12 h, a portion of the seedlings incubated with DMBQ were washed (3×) and further incubated in water with the indicated time points used for [^{3}H]-thymidine incorporation (▲). After the [^{3}H]-thymidine incubation, the seedlings were ground into a fine powder in liquid nitrogen and transferred to a tube with 500 µL of the extraction buffer (100 mM EDTA, 250 mM NaCl, 100 µg proteinase K) and the mixture was incubated at 55°C for 1 h. After centrifugation at 12,000 × *g* for 5 min, the insoluble pellets were removed, and the same volume of 10% TCA was added to the supernatant. After incubation on ice for 10 min, the suspension was filtered through a Whatman GF/C glass filter, and the filter was washed with 3 mL of a 5% TCA solution. The radioactivity of the filters was determined by scintillation counting. Data were acquired in triplicate and expressed as ± SD.

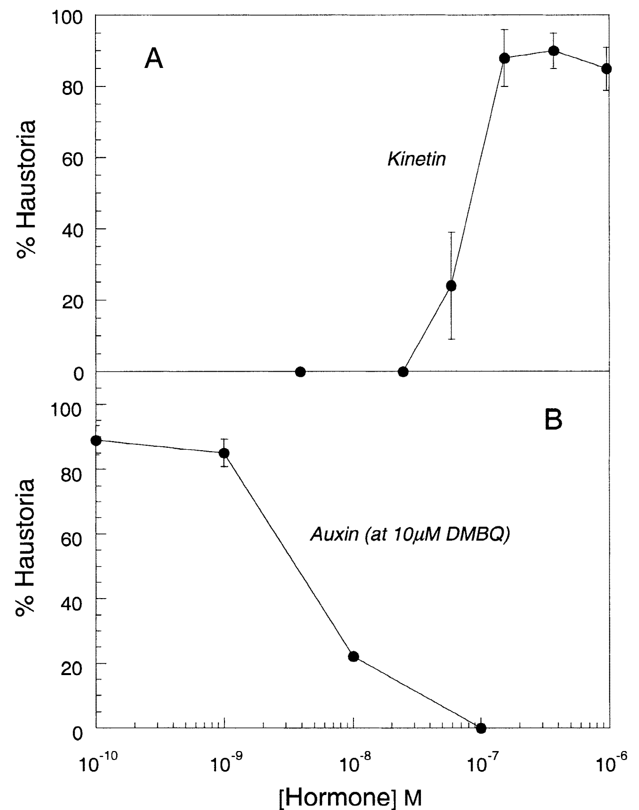

Figure 3. Two-day-old *S. asiatica* seedlings (20–30 per well) were incubated at 30°C in 1 mL of H_2O containing either **(A)** kinetin or **(B)** 10 µM DMBQ with α-IAA. Seedlings were scored at 24 h. The induction percentage was determined in triplicate and expressed as ± SD.

ture host interface (Kuijt 1977; Riopel and Baird 1987; Riopel and Timko 1995).

Results from radioactive thymidine incorporation, as shown in Figure 2, have established that haustorial development in *S. asiatica* is the result of a dominant, if not exclusive, redirection of cellular expansion and is independent of new cell division. Moreover, incorporation is arrested immediately after exposure to the xenognostic quinone, with little significant uptake detectable for at least 12 h. Immediately after removal of the inducing signal, 2,6-dimethoxy-1,4-benzoquinone (DMBQ), DNA synthesis recovers to the steady-state level seen for normal root growth. The role of the existing meristem initials in this recommitment is not yet clear.

Kinetin has been shown to induce haustorial development in parasitic Schrophulariceae (Riopel and Musselman 1979), and as shown in Figure 3A, micromolar concentrations are sufficient for induction in *S. asiatica.* However, haustorial hair growth is more exaggerated and the radial swelling not as pronounced as with DMBQ induction. Later stages of development also appear somewhat compromised by continual kinetin exposure, giving morphologically distorted haustoria, but the induction process appears normal. In contrast, auxins are very potent inhibitors of haustorial induction. Figure 3B establishes that nanomolar concentrations of α-naphthol acetic acid or indole acetic acid (data not shown) are sufficient to inhibit xenognosin induction, but the structurally similar β-naphthol acetic acid, which has no auxinlike activity, has no effect on haustorial organogenesis. The cells of the root meristem of *S. asiatica* therefore appear poised for the transition to haustorial organogenesis and are generally responsive to plant hormones.

Xenognostic Signaling

The angiosperm parasitic strategy generally succeeds by tightly coordinating early developmental stages with chemical signals from the host (Smith and oth-

ers 1990). *S. asiatica,* probably the best studied in terms of its signaling chemistry, is chlorophyllous as an adult but requires host-derived signal compounds for both germination (Boone and others 1995; Lynn and Chang 1990; Worsham 1987) and the development of the haustorium (Albrecht and others 1999; Chang and Lynn 1986; Lynn 1985; Riopel and Timko 1995). Consistent with Searcy's general evolutionary model of structural and biochemical minimization, the young seedlings have no lateral roots or root hairs. With the initiation of the haustorium, the vegetative phase of *Striga*'s life cycle ceases, and the parasitic phase begins. In the absence of viable host attachment, the apical meristem never develops, and because of its limited seed reserves, the seedling survives for less than 5 days.

The first structural characterization of specific host-derived molecules that cued the vegetative to parasitic growth transition came from work with host exudates. Xenognosin A (Lynn and others 1981) and B (Steffens and others 1982) provided both the origin for the term xenognosin, or host recognition signal, and established that specific phenols could play a necessary, and possibly sufficient, role in haustorial induction. Later work with *S. asiatica* uncovered evidence that the activity was associated with phenolics of the host cell wall, leading to the actual finding that specific quinones oxidatively released from the wall pectins constituted the active signal (Chang and Lynn 1986; Lynn 1985). These findings led to the hypothesis that oxidative enzymes released from the parasite generated a sufficient xenognostic quinone concentration only at a viable host surface rich in wall pectins (Lynn and Chang 1990). This mechanism was appealing in that it regulated parasitic commitment only in close proximity with a viable host so as to increase the likelihood of successful attachment.

PoxA and PoxB were identified as the only apoplastic phenol oxidases present in *S. asiatica* seedlings that were capable of oxidizing the more than 60 known inducing phenols into active quinones (Kim and others 1998). However, these enzymes were not unique to the parasite, because peroxidase homologs were far more abundant in the walls of *Striga*'s Graminae hosts. More critically, these enzymes require H_2O_2 as a co-substrate. H_2O_2 was shown to be the limiting substrate by incubating *S. asiatica* seedlings in the presence of catalase, an enzyme that disproportionates H_2O_2 to water and molecular oxygen during induction. In the presence of quinones, a range of 10^{-2} to greater than 10^3 u/mL of catalase had no effect on haustorial induction (Figure 4). At approximately 10^2 u/mL, however, catalase half-maximally inhibited haustorial induction by phenylpropanoids, likely cell wall substrates. The data for 10^{-4} M syringic acid are shown in Figure 4. The inhibition gradually diminished with lower catalase concentrations, such that below 10^{-2} u/mL normal haustorial induction was possible. These data established that with phenols the constitutive production of H_2O_2 by the parasite was essential; however, quinones alone were necessary and sufficient for haustorial induction.

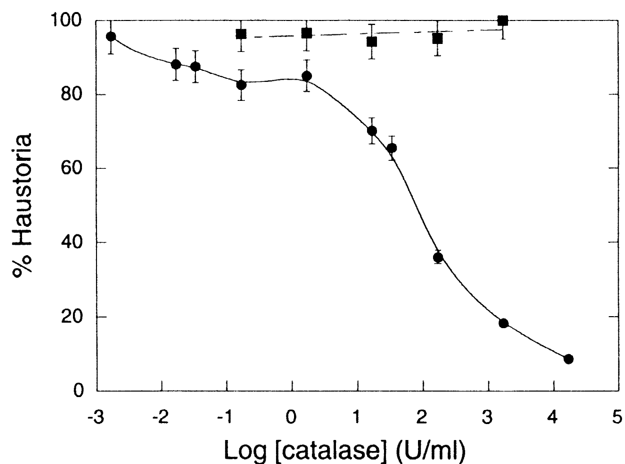

Figure 4. Two-day-old *S. asiatica* seedlings were incubated at 30°C for 24 h with (●) 100 µM syringic acid or (■) 10 µM DMBQ at the indicated catalase concentration before scoring for haustoria. The induction percentage was determined in triplicate and expressed as ±SD.

Visualization of H_2O_2 production was investigated with the redox dyes pyrogallol and syringaldazine, and the confocal image of dichlorofluorescein oxidation at the *Striga* root meristem is shown in Figure 5. Dichlorofluorescein diacetate taken up by the *Striga* seedlings can be cytoplasmically saponified, and the released dye oxidized in an H_2O_2-dependent manner to the fluorescent derivative. As with the other dyes, only the cells at the root tip contained sufficient oxidative potential to be detected under these conditions. This localization of H_2O_2 to the root tips taken together with the relatively low level of oxidative enzymes in these cells, requires the development of a new model for xenognosis in *S. asiatica.*

At the host-parasite interface, as outlined in Figure 6, the constitutive production of H_2O_2 is most critical. Although the appropriate peroxidase enzymes are present in the parasite wall, explaining why high phenol concentrations are xenognostic, the presence of these enzymes is likely not to be essential or even critical given the abundance of related host wall enzymes. Sufficient quantities of available phenols, most likely in the form of phenyl-

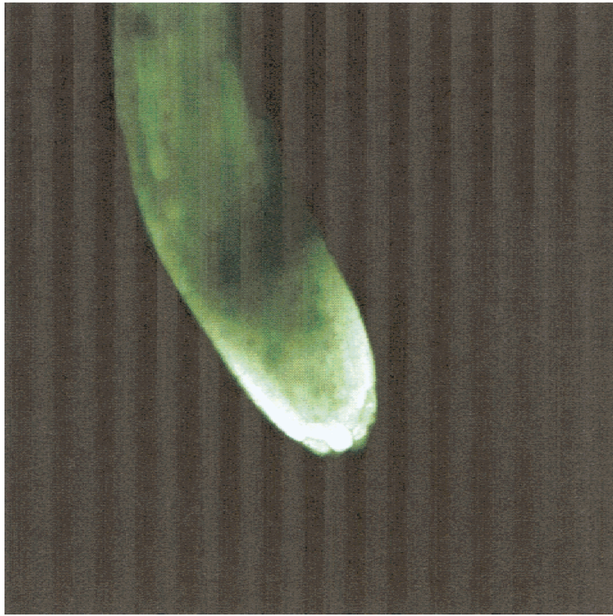

Figure 5. One microliter of 100 mM dichlorofluorescein-diacetate in DMSO was added to *S. asiatica* seedlings in 1 mL of water buffered at pH 5.5. After 15 min, the staining of the tissue was detected with a Zeiss LSM 510 confocal microscope. A green argon-ion laser (488 nm) was used for excitation, and fluorescence was detected at 525 nm. No staining was detected along the root axis or around the seed coat.

propanoid esters decorating the pectins (*insert A*), provide the co-substrate for the peroxidases. As shown in insert B, oxidative cleavage of model phenols generates the quinone. A sustained quinone concentration is critical, because the quinone must be present for many hours for terminal commitment to organogenesis (see later). This time restriction places a critical threshold on the necessary H_2O_2 and phenol concentrations. Most critically, *Striga* cell walls are low in phenols and probably devoid of phenols at the root tip. In this way, the parasite is able to provide H_2O_2 to the host wall as the limiting reagent for sustained quinone production.

How general might this mechanism for signal generation be among the parasitic plants? The model requires the acquisition of reaction pathways capable of continually producing low levels of hydrogen peroxide. H_2O_2 is produced ubiquitously among both plants and animals as a first line of biotic defense, and several known pathways exist for its generation (Kim and others 1998; Lamb and Dixon 1997; Mehdy 1994). Each one, in principle, should be capable of constitutive H_2O_2 generation at the root apex, and the possibilities are not easily narrowed by this restriction. The cells involved in this localized production of the reactive oxygen species (ROS) must also avoid general oxidative toxicity, as well as the ability of ROS to activate other defense pathways (Baker and Orlandi 1995; Lamb and Dixon 1997). *S. asiatica* is somewhat unusual among the parasitic plants in that it is a dicot parasitizing monocots and may be capitalizing on differences in sensitivities and/or responses to the ROS in its monocot hosts. The responses to the generated quinones may also be very different in monocots and dicots; the same quinone distribution should be readily produced in most plant tissues by simple wounding events. Finally, age differences, given the 5-day window for *Striga* seedling viability, are almost certainly critical for this obligate parasite, but such age differences are not likely to be as pronounced in the facultative parasites.

Modifications to this mechanism, or completely different strategies for xenognosin generation, are therefore likely to exist across the parasitic angiosperms, but an important principle of parasitism may have emerged from the work on *Striga*. The "offensive" xenognosis pathway is minimally using components of reaction pathways necessary for protection of the plant from biotic stresses. Clearly elements of self/non-self recognition are essential to the establishment of the haustorial interface (Atsatt 1983) and are apparently functionally connected to recognition. Further definition of the mechanisms of xenognosis in this and other parasites should provide a unique opportunity to uncover genes critical to the early steps in cell-cell recognition and non-self resistance.

Xenognosin Perception

The quinones expected to be generated from the oxidation of the common cell wall phenolics include benzo-1, 4-quinone (BQ), methoxybenzo-1,4-quinone (MBQ), and DMBQ, all three of which induce haustorial formation in both New and Old World parasitic angiosperms (Albrecht and others 1999; Lynn and Chang 1990). Attempts in *S. asiatica* to establish a functional correlation among synthetic variants of these quinones and haustorial induction revealed a dependence on electromotive potential (Smith and others 1996), Em, the energy required to add a single electron reductively to the quinone nucleus. As shown in Figure 7 (Smith and others 1996), no quinone whose Em potential lay outside of a defined redox range, −250 to 0 mV relative to SCE, was active, and the quinones at the redox boundaries show only partial induction of haustorial development. The heavily substituted benzoquinones, all trisubstituted and most disubstituted, were inactive regardless of redox potential.

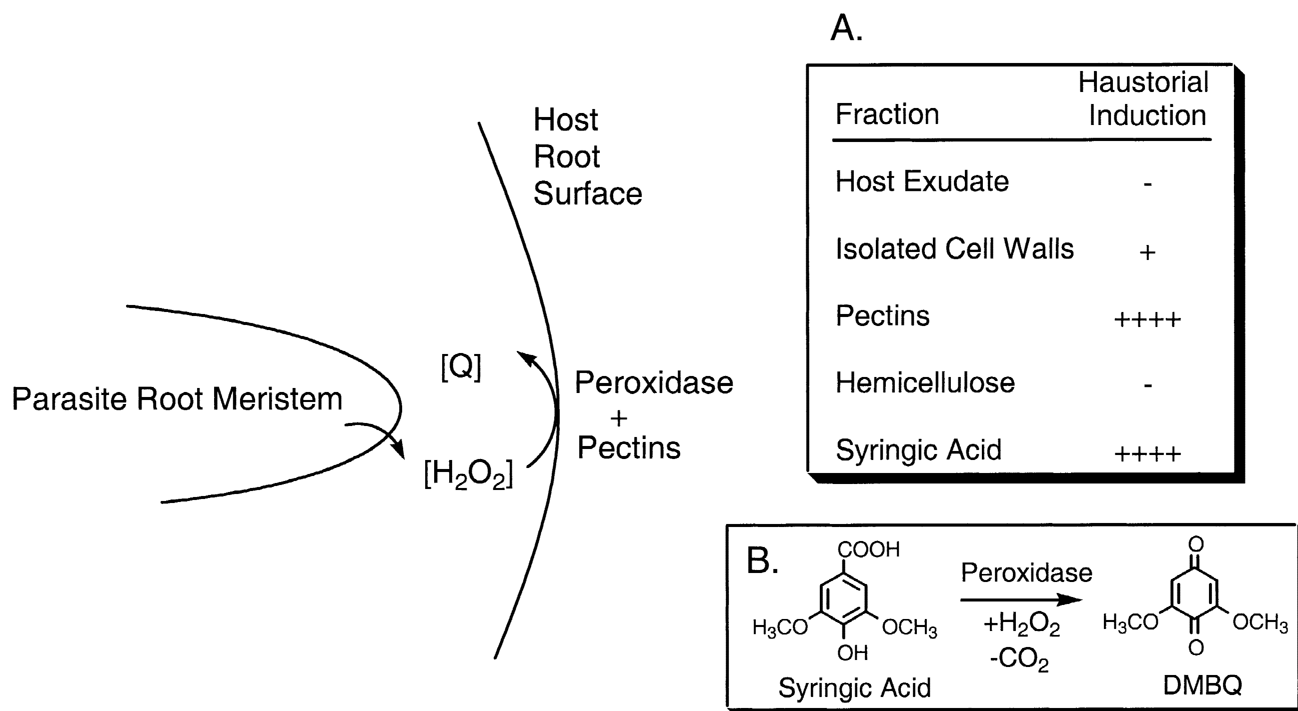

Figure 6. The model for the generation of the haustorial inducing quinones envisions H_2O_2 generated at the parasitic seedling root tip accumulating at the host interface. The H_2O_2 is used as an oxidation cosubstrate, together with wall pectins, by the host cell wall peroxidases to generate the xenognostic quinones. *Insert* **A** shows sorghum wall fractionations and establishes that the pectins contain the peroxidase substrate as measured by haustorial inducing activity. *Insert* **B** shows the oxidative cleavage of the model substrate, syringic acid.

The observation that the active range is defined by the first half-volt potential and bounded by both oxidative and reductive extremes is consistent with the quinones serving as single electron carriers. This finding was interesting in light of earlier studies that had established a precise time dependence for the induction process (Smith and others 1990). These observations were incorporated into a model in which the inducing quinone signal was perceived by means of an electron transport chain. This putative redox circuit would require quinone binding to be completed, and the time dependence of depolarization resulting from the electron flow between two redox pools could be functionally coupled with the time dependence of the commitment to haustorial development (Smith and others 1996). Several physical tests of this model for signal perception now exist.

Based on other more well-characterized biologic redox circuits, including oxidative phosphorylation and photosynthesis where quinones function as electron carriers, the reactive one-electron reduced semiquinone is expected to be bound tightly within a redox binding site. Structural analogs distributed at the extremes of the redox window were used to confirm this prediction. Tetrafluorobenzo-1, 4-quinone (TFBQ) is of similar steric size to BQ, and because it is strongly electropositive, should be readily reduced within the defined redox window to the semiquinone (Figure 8). Reoxidation, however, would be thermodynamically restricted as a result of the oxidative limits placed on the active window. Therefore, if TFBQ were bound at a site within the window, further reaction would be arrested at the one-electron reduced semiquinone. Consistent with the prediction, TFBQ inhibited induction and was very potent, half-maximally inhibiting 10^{-5} M DMBQ induction at 10^{-7} M TFBQ (Smith and others 1996). Two important observations can be derived from the TFBQ experiments. First, both one-electron oxidative and reductive reactions are important for induction, that is, if either are blocked, haustorial induction does not continue. Second, the inhibition by TFBQ is freely reversible and not the result of general toxicity resulting from the generation of a reactive TFBQ semiquinone. Therefore, the TFBQ semiquinone most probably binds tightly within an active site, blocking the second required electron transfer step, and by doing so, inhibits haustorial induction.

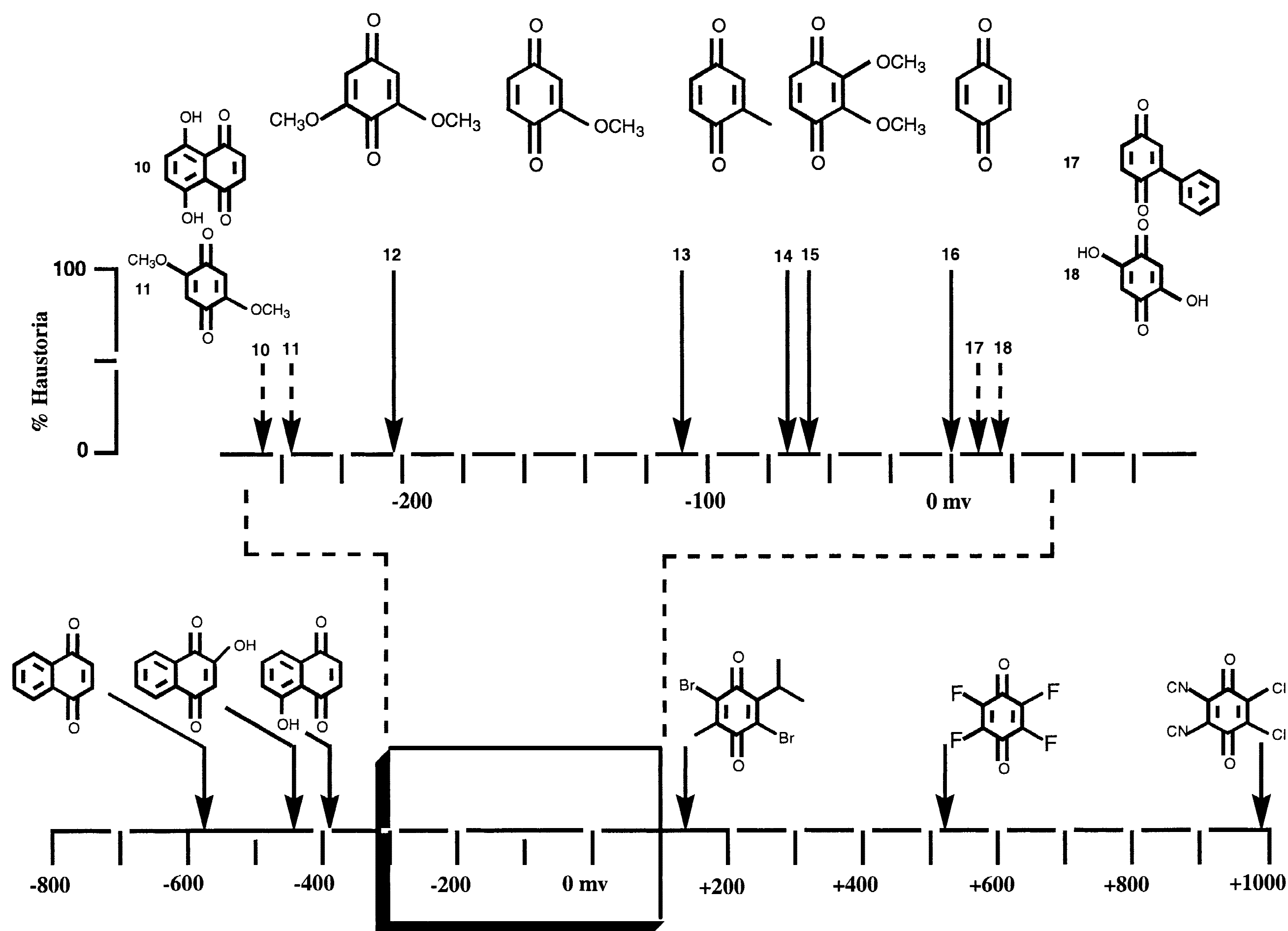

Figure 7. The quinone analogs are plotted as a function of their Em potential (*lower scale*). The upper scale is an expansion of the redox range in the active window and contains the active haustorial inducers (9–17). The half-wave reductive potential of the quinones was measured with a saturated Calomel reference electrode, and the relative potentials are compared with that of benzoquinone, which was set at zero (Smith and others 1996). The abscissa represents the haustorial induction percentage referenced to DMBQ at the maximal inducing concentration. The solid arrows indicate 100% of control (85% with DMBQ). Dashed arrows indicate partial haustoria induction between 40 and 50% of the DMBQ controls.

Further support for the existence of a semiquinone intermediate was derived from the development of quinone analogs containing specific benzylic leaving groups. Several compounds, at least conceptually derived from the naturally occurring bioreductively activated antitumor antibiotics (Zeng and others 1996), were developed and shown to inhibit haustorial induction specifically (Smith and others 1996). The most specific inhibitors were the simple oxirane and cyclopropane benzoquinone analogs. Cyclopropyl benzoquinone (CPBQ) is unique in that the cyclopropane ring opens readily by radical delocalization from the semiquinone state (Figure 8). The lifetime of the ring-opened cyclopropane was shown to be sufficient for bond rotation and racemization (Zeng and others 1996), generating a quinone methide ring-opened intermediate. Quinone methides are strong alkylating agents, and it was proposed that CPBQ alkylated the binding site. Consistent with the prediction, and in contrast with TFBQ inhibition, CPBQ inhibition was irreversible (Smith and others 1996).

Accepting that the limiting redox range is important and that the semiquinone intermediate exists, what is this redox reaction? The redox range in haustorial induction is similar to that seen for the quinone cofactors in both photosynthesis and oxidative phosphorylation. In these processes, the quinone is reduced to a semiquinone intermediate by an electron that is generated at a donor site by either photochemical excitation or NADH oxidation. The resulting semiquinone is re-oxidized when the

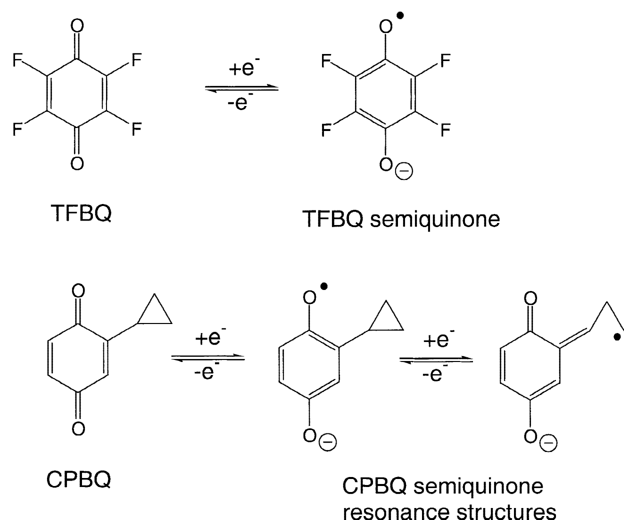

Figure 8. One-electron redox process between TFBQ, CPBQ, and their corresponding semiquinones. Notice the ring-opened cyclopropane structure of the CPBQ semiquinone.

electron passes to the next acceptor in the electron carrier chain. As in haustorial induction, both reductive and oxidative steps are required for electron flow, and only quinones within a specific redox window serve as carriers. The similarities between these organellar pathways and the observed events in haustorial induction are striking and suggest that cellular organelles might be involved in the process. At high concentrations, benzoquinones are metabolic toxins that can cause oxidative cellular damage, but there is no evidence that unsubstituted benzoquinones interfere in the microsomal electron transport chains at lower concentrations. Nevertheless, the plastid genomes of parasitic plants have evolved under very different pressures (DePamphilis 1995), and further evaluation of the functional role these organelles may play in haustorial development will be important.

More generally, there is a long history of animal and plant cell redox processes in addition to those of the cellular organelles, and models have been presented suggesting that altered electron flow through different pathways directs cell growth (Crane and others 1988). Moreover, transcriptional factors from viral, bacterial, and mammalian sources are known to be under redox control (Abate and others 1990; Allen 1993; McBride and others 1992; Staal and others 1990; Storz and others 1990; Tagaya and others 1994), and in plants, chloroplast genome expression is regulated by redox reactions (Allen 1994; Buchanan 1991; Danon and Mayfield 1994). Therefore, a mechanistic involvement of redox reactions with the more complex cellular processes of growth and development has been recognized.

Early studies detected NAD(P)H oxido-reductases in all plant membranes investigated (Moller and Lin 1986). The plasma membrane (PM)–associated oxido-reductases have been associated with an electron transport system in roots (Rubinstein and others 1984), leaves (Marre and others 1998), individual cells (Barr and others 1985; Misra and others 1984), protoplasts (Lin 1982; Lin and others 1984; Thom and Maretzki 1985), isolated membranes (Misra and others 1984), and purified plasma membrane fractions (Barr and others 1985b; Buckhout and Hrubec 1986; Qui and others 1985). These PM-localized electron transport systems have been studied for many years with impermeable electron acceptors. In fact, the initial studies associated redox events with the control of cell growth (Baron and Hoffman 1929; Brooks 1947). Impermeable oxidants like ferricyanide and diferric transferrin promote growth in animal cells (Crane and others 1985) and generally inhibit growth in plants (Barr and others 1985a), presumably in both cases by some alteration of the transmembrane redox state (Boss and Morre 1988; Crane and Barr 1989; Crane and others 1988).

Some of the enzymes involved in these redox processes have been identified. NAD(P)H-$K_3Fe(CN)_6$ and NAD(P)H-duroquinone reductases were found in the plasma membranes of several plants (Asard and others 1987; Askerlund and others 1988; Buckfout and Hrubec 1986; De Luca and others 1984) and have been purified from zucchini microsomes (Guerini and others 1987; Valenti and others 1990) and corn root purified PMs (Luster and Buckhout 1988). In the corn PMs, two enzymes were found, a ferricyanide reductase and a 28-kDa protein, which has both duroquinone and ferricyanide reductase activity. The 28-kDa enzyme was reported to have a flavin requirement (Luster and Buckhout 1989), and monoclonal antibodies directed to this protein were prepared (Buckhout and Luster 1988).

More recently, NADPH oxidoreductase in plants has been immunologically correlated with the better-characterized human neutrophil machinery responsible for the defensive oxidative burst Desikan and others 1996; Dwyer and others 1996; Vera-Estrella and others 1994; Xing and others 1997). This oxidative complex consists of a heterodimeric flavocytochrome *b*-558 of subunit mass approximately 22 and 91 kDa (p22-phox and gp91-phox) (Segal 1989), a Rac GTPase of ~22 kDa (Knaus and others 1991; Kwong and others 1993), and two novel polypeptides of approximately 47 and 67 kDa

(p47-phox and p67-phox) (Nunoi and others 1988; Volpp and others 1989). This complex is predicted to participate in defensive oxidative burst reactions in plant cells (Lamb and Dixon 1997; Mehdy 1994), as it does in animals.

Earlier studies had identified a soybean NADH oxidase that is stimulated by auxin and inhibited by actinomycin D, mimicking the effects seen on growth in vivo, and the activity was isolated from hypocotyl plasma membranes (Brightman and others 1988). The enzyme appeared to be localized in the regions of most rapid elongation (Qui and others 1985), and the auxin response was not detected in the mature portions of the hypocotyl (Brightman and others 1988). Three proteins purify with the activity, 36, 52, and 72 kDa, but whether one or all of these components are required for activity is not known. The connection between the NADH and NAD(P)H activities, the identified proteins, and the relationship between growth and defensive pathways needs to be clarified.

Earlier models involving two separate pathways have been presented (Boettger and Hilgendorf 1988; Crane and Barr 1989) where the e^- flow is channeled either through the NADH oxidase to O_2 or through the ferricyanide/duroquinone oxidoreductase. Auxin stimulates NADH oxidase and cell elongation, whereas ferricyanide or duroquinone drain e^-flow through the reductase and inhibit cell elongation. At this point it is not clear how the pathways would be antagonistic given that the normal terminal acceptor, O_2, is apparently the same. The possibility that an NADH oxidase is a site of auxin action (Brightman and others 1988) further argues that the oxidoreductases may provide a molecular linkage between cellular defense (Lamb and Dixon 1997; Mehdy 1994) and the control of cell growth and differentiation (Crane and Barr 1989).

No current evidence correlates these enzymatic activities, which occur widely in plants, with the activities required for haustorial induction in the parasitic members. However, the information available on the specific signals regulating haustorial induction and the insight gained from these chemical and mechanistic studies provide the necessary tools to begin to define the genetic loci involved.

Signal Response

The developmental program mediating organogenesis of the haustorium in *S. asiatica* is certainly streamlined, and apparently the inherent controls normally placed on developmental commitments are simplified. Nevertheless, precision in the host response of these obligate parasites is critical, and for that reason, it is important that the functional reasons for redox reactions being selected for xenognosis remain a critical part of our thinking. In this context, the response to different concentrations of the xenognostic quinones is most remarkable. Figure 9 shows a series of exposure/removal experiments and the corresponding response time for haustorial induction with both 2 and 10 µM DMBQ. Both concentrations quantitatively induce the transition to the parasitic mode, an unusual circumstance for simple signal/receptor activation. Equally unusual, the exposure times required for both inducers are very long, and the lower concentration requires 4 additional hours.

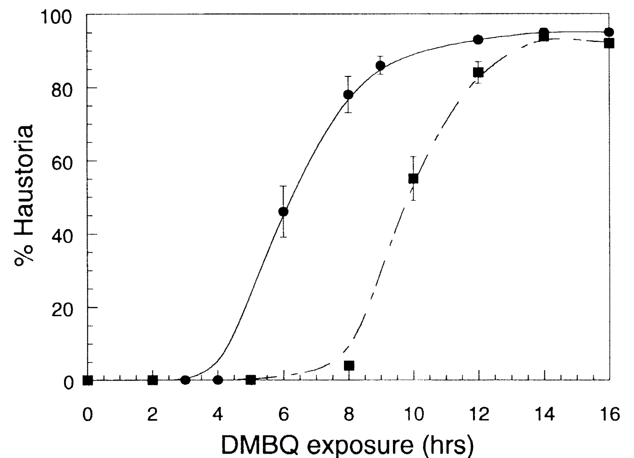

Figure 9. Two-day-old *S. asiatica* seedlings (20–30 per well) were incubated at 30°C in 1 mL of H_2O containing either 2 µM (■) or 10 µM (●) DMBQ. At the indicated time points the seedlings were washed and incubated with H_2O. At 24 h the seedlings were scored for haustorial induction, with each time point taken in triplicate and expressed as ± SD.

The exposure/removal experiments in Figure 9 evaluated the terminal irreversible commitment to haustorial development. With premature removal of the xenognostic signal, haustorial development is aborted, and meristematic growth is re-established (Smith and others 1990; 1996). From the perspective of the parasite, a weaker signal provides a less reliable marker of a viable host, and the ability to respond more slowly to a lower signal concentration could enhance the precision in the commitment to the host. Seedling resources not committed to haustorial development are available for further root elongation, continuing the search for a viable host.

The first insight into the response to this apparent "internal clock" regulating haustorial commitment came from observations of the morphologic changes inherent in haustorial development. The initial re-

sponse of *Striga* to xenognosin exposure is a radial swelling of the cells just distal to the meristem. The extent of swelling was found to be linearly dependent on both the concentration of the signal and the length of time of its exposure (Smith and others 1996). This notion of cell size has been a critical part of our understanding of cell cycle control and differentiation for many years (Fowler and others 1999). The recent discovery of the expansins (Cosgrove 1999), a set of highly conserved plant proteins widely involved in plant cell swelling, suggested a genetic locus whose expression may be an early response to haustorial induction. Of the almost one dozen expansins found in *S. asiatica,* three, SaExp1, SaExp2 and SaExp3, were regulated during haustorial induction (O'Malley and Lynn 2000). For SaExp3, the sequence most similar to the known seedling expansins (Cho and Kende 1997), a steady-state message level that is maintained during vegetative growth was very rapidly depleted. The other two RNAs gradually accumulated over the xenognosin exposure time. Curiously, the rate of accumulation of both SaExp1 and SaExp2 was dependent on the xenognosin concentration; higher DMBQ concentrations gave larger rates. The relative concentration-dependent rate of root tip swelling was the same as that observed for SaExp1 and SaExp2 message accumulation (Smith and others 1996).

With regard to specificity, SaExp message accumulation is regulated by the same xenognostic signals that regulate haustorial induction. Kinetin at 10 μM requires the same time dependence for haustorial induction as 1 μM DMBQ and induces both SaExp1 and SaExp2 accumulation and SaExp3 depletion at the same rate and to the same level as the quinone (O'Malley and Lynn 2000). Heavily substituted quinones, even those that fall within the active redox window such as *t*-butyl benzoquinone (tBuBQ), are not haustorial inducers (Smith and others 1996) and do not stimulate the accumulation of SaExp1 and SaExp2. DMBQ (10 μM)-induced depletion of SaExp3 and accumulation of SaExp1 and SaExp2 transcripts were completely blocked by co-incubation of the seedlings with either TFBQ (1 μM) or auxin (0.1 μM). Therefore, the chemical signals that mediate haustorial development also regulate the expression of these transcripts.

A further series of timed exposure/removal, re-exposure/removal experiments were developed to evaluate the additivity of multiple exposures (Smith and others 1990). If seedlings were exposed to DMBQ for 4 h, washed, and then re-exposed to DMBQ, this subsequent re-exposure required an additional 2 h (Figure 10). Therefore the total 6-h exposure time necessary to reach the threshold was necessary and was independent of the intervening signal removal. However, if the re-exposure was delayed for up to 6 h before re-exposure, the 6-h clock is reset, and a full exposure time was required for commitment to haustoria. A gradual increase in the required re-exposure time is seen as the delay increases between 2 h and 6 h.

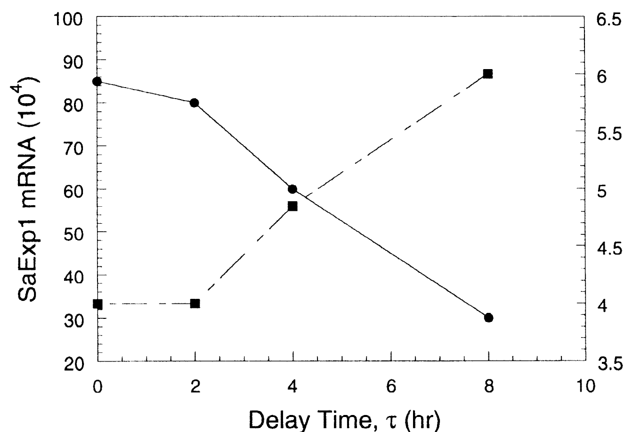

Figure 10. Two-day-old *S. asiatica* seedlings were incubated with DMBQ for 4 h. The DMBQ solution was removed and the seedlings washed three times and incubated in water for a variable delay time, τ, before being either re-exposed to DMBQ (■) or having their mRNA extracted and SaExp1 transcripts quantified by RT-PCR (●) (O'Malley and Lynn 2000). The time τ on the abscissa represents the delay after the first 4-h exposure.

A corresponding time dependence is seen in expansin SaExp1 and SaExp2 accumulation (Figure 10). A 4-h exposure to DMBQ leads to a significant increase in SaExp1 and SaExp2 message accumulation. After signal removal, there was an approximate 2-h delay or overshoot in which little change occurs in the message level. By 6 h, the SaExp1 and SaExp2 message had returned to the basal level seen before signal exposure. The response time of SaExp1 and SaExp2 message accumulation is slow, but on the same order as the time for commitment to haustorial development. These expansins mark the accumulation toward a threshold that must be reached before commitment to haustorial development. In addition, message instability correlates with the loss of memory of previous xenognosin exposures. Therefore, expansin message provides a reliable molecular marker of the accumulating threshold that must be reached before terminal commitment to haustorial development and the parasitic phase of *Striga*'s life cycle.

Conclusions

At this point, it is not clear how generally the signaling events described for *Striga asiatica* apply to the

diverse members of the parasitic angiosperms. Certainly, the importance of the plant cell wall, the outermost boundary of the cell, provides the critical reservoir of signal molecules in *Striga* parasitism. The use of localized H_2O_2 generation by *Striga* to liberate the xenognostic quinones would cleverly exploit both the existing defensive host peroxidases and aromatic components of the pectins, both critical determinants of a viable host. Is it a robust and general strategy for xenognosin generation and host detection? It may be a difficult strategy for the host to avoid given the importance of ROS to defense.

Given the common release of H_2O_2 from the plant cell as a first line of defense, these same benzoquinones must be commonly released from the wall, certainly during cell wounding. With the redox perception mechanisms of *Striga* and the abundance of redox machinery already known to be present in all plants, it will be important to determine the function of benzoquinone detection both to host and parasite. Clearly both rapid and long-term responses to these xenognositic quinones occur in *Striga*. The inherent physical instability of quinones in plant tissues may further complicate these experiments and may have masked general responses to quinones already. As with the parasites, the precursor phenols could be important, and in that context, specific cell wall phenols capable of being oxidatively converted into benzoquinones have already been shown to mediate cell growth in tobacco (Lynn and Chang 1990; Tamagnone and others 1998).

The time dependence and additive effects of multiple exposures to the quinones are certainly the most interesting and novel aspects of this signaling process. Haustorial induction has been likened to a leaky molecular capacitor (O'Malley and Lynn 2000). As long as the quinone is present, the cells continue to build up the SaExp1 and SaExp2 charge. When this charge reaches a defined threshold, the "discharge" is manifested in the terminal commitment to haustorial organogenesis. If the circuit is broken, by premature removal of the xenognostic quinone, the SaExp1 and SaExp2 charge slowly dissipates and seedling growth resumes. As shown in Figure 10, the time dependence for re-charging is a function of the extent of this dissipation. Such time-dependent responses, and the way in which they control the terminal commitment to organogenesis, are likely to be important for all facets of plant growth and development, particularly those events regulated by external environmental factors. The models developed will be important to our understanding of topics as diverse as plant cell-cell signals, the mechanisms of their generation and perception, commitment to organogenesis, strategies of pathogenesis, and angiosperm evolution. The acquired mechanistic and biochemical understanding should not only enrich our understanding of the form and function of higher plants but could also contribute to the worldwide constraints these parasites place on agricultural productivity.

ACKNOWLEDGMENTS

We are indebted to Rebecca Norris at the USDA laboratory in Oxford, NC, for providing seeds of *Striga asiatica,* and we are grateful to the US Department of Energy ER20024, NIH GM47369, and the Rockefeller Foundation for financial support.

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