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Phoebe Young April 13, 2012

Semagenesis in Nonparasitic Plants

by

Phoebe Young

David G. Lynn, Ph.D
Adviser

Department of Chemistry

Professor David G. Lynn, Ph.D

Adviser

Assistant Professor Jacobus de Roode, Ph.D

Committee Member

Assistant Professor Nicole Gerardo, Ph.D

Committee Member

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Abstract

Semagenesis in Nonparasitic Plants
By Phoebe Young

The parasitic plant Striga asiatica *detects monocot host roots by semagenesis:* Striga's *root tip exudes reactive oxygen species which oxidize phenols on nearby hosts' cell walls, releasing quinones, which trigger parasitism in* Striga*. In another plant-plant detection phenomenon, many nonparasitic plants adjust their root architecture so that their roots avoid or grow toward competitor roots. This density-dependent phenotype is not fully explained by the detection of limited resources around another root or by the toxicity of allelochemicals. Semagenesis may be used by nonparasitic plants as a third mechanism of detecting nearby competitors. This study considers the two unconfirmed steps of semagenesis in nonparasitic dicots: 1) do reactive oxygen species (e.g. H_2O_2) result in the release of quinones from nonparasites' roots? and 2) is the response to semagenesis signals the same as the response to high population densities? When ten day old* Arabidopsis thaliana *seedlings were treated with 0, 10, 25, or 50 µM H_2O_2, dimethoxy-*p*-benzoquinone (DMBQ) and methoxy-quinone were not detected in an ethyl acetate extraction of the growth medium, possibly because this dicot plant does not have enough phenols in its cell walls to produce high levels of quinones. The same extraction of the growth media showed that 48 h treatment with 25 or 50 µM H_2O_2 causes an increase in exudation of both camalexin and indole-3-carboxylic acid, indicating that such treatments elicit a stress response. In the second step of semagenesis, elicited quinones should trigger a morphological and physiological change consistent with density-dependent morphology. Ten day old* Arabidopsis *seedlings treated with DMBQ had shorter roots and more lateral roots than untreated seedlings. With* Arabidopsis *seedlings grown at varying densities, no consistent density-dependent morphology was observed. Semagenesis is more likely to occur in 20 d old* Arabidopsis*, as these older plants have more phenols and a known root exudate-dependent root architecture. Semagenesis may serve as a detection mechanism for stressed dicots, which release phenols in quantity, or as a mechanism for dicots to detect monocots, which have more phenols in their walls than do dicots.*

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Introduction

Chemical signaling between organisms

Chemical signaling is the most universal type of communication between organisms, occurring across kingdoms and in all environments. This exchange of information mediates inter-organism relationships of all types and can drive biological equilibria, as organisms use chemical information to optimize their performance in a competitive world. Air-borne examples of chemical signaling include the benzyl acetate released by flowers to attract pollinators for the optimal period of time before using nicotine to repulse them and send them to other flowers (Kessler et al. 2008) and the waxy pheromones released by queen honeybees to coordinate the timing of worker bee fertility relative to queen fertility (Brunner et al. 2011). In a marine environment, L-tryptophan from red abalone (*Haliotis rufescens*) is responsible for attracting sperm of the same species for fertilization (Himes et al. 2011); in freshwater, arginine signals the predation of adult California newts (*Taricha torosa*) on worms (*Eisenia rosea*), calming the predator avoidance behavior of newt larvae, which otherwise avoid the tetrodoxin (TTX) released by cannibalistic newt adults (Ferrer and Zimmer 2007). Sometimes these signaling events are tritrophic: for example, herbivore-wounded *Nicotiana attenuata* release terpenoids and green leaf volatiles (GLVs) to attract predators of the herbivores (Halitschke et al. 2008).

Within species, chemical signaling can drive social behavior. In bacterial quorum sensing, chemical signals from bacteria coordinate processes among populations of bacteria. Their function depends not only on concentrated signal from higher density bacteria, but also on the local environment and spatial arrangement of the bacteria (Williams 2007). In the first case of quorum sensing discovered, *N*-(β-ketocaproyl)homoserine lactone, an acyl homoserine lactone

(AHL), causes bioluminescence of *Vibrio fischeri*, but only when bacteria reach a high enough density to accumulate enough of the AHL (Eberhard et al. 1981). The same AHL coordinates virulence in *Erwinia caratovora* when populations accumulate enough of it—exoenzymes are produced to digest cell walls, and antibiotics are synthesized to deter potential competitors (Bainton et al. 1992, Jones et al. 1993). This type of signal-mediated population level behavior is not limited to bacteria. In mountain pine beetles, pheromones and kairomones are used to coordinate attacks on individual pine trees: attacks composed of too few beetles will fail to overcome the tree's resistance, whereas attacks composed of too many beetles will overstimulate the tree's sap response and result in overcrowding on the tree. Volatile chemical signals allow beetles to attack trees at the optimal number (Logan et al. 1998). Thus, both in the water and in the air, chemical signals are used by organisms from several kingdoms to mediate intertrophic interactions and even to orchestrate complex behaviors requiring entire populations of a species to exchange information and coordinate in a density-dependent fashion.

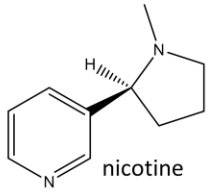
While chemical signaling processes are more difficult to test in the soil matrix, a few well-studied examples indicate that chemical signaling may also be a common method of information transfer between organisms in the rhizosphere. The parasitic bacterium *Agrobacterium tumefaciens* requires chemical stimuli to detect suitable host dicot cells for transformation. Phenolic compounds, which are likely to be released from wounded, susceptible host cells, activate *Agrobacterium's* virulence (*vir*) genes (Stachel et al. 1985), and sugars, possibly also from broken host cell walls, increase the virulence response (Ankenbauer and Nester 1990). In another bacterium-plant interaction, rhizobium species form nodules inside legume roots and fix nitrogen in exchange for nutrients from the plant. Originally, the legume-rhizobium signaling sequence was thought to be a simple two-step sequence to initiate

symbiosis: a flavonoid or isoflavonoid from the legume triggers the release of a Nod factor from the bacteria (Peters et al. 1986, Fisher and Long 1992, Cooper 2007). More recent results indicate that both legume-rhizobium and Agrobacterium-host interactions may be interconnected with the bacteria's quorum sensing (Gurich and Gonzalez 2009, White and Finan 2009). Arbuscular mycorrhizal fungi (AMF) also play a key role in plant nutrition; these fungi are thought to colonize 70-90% of the world's plants, receiving carbohydrates in return for increasing the plants' efficiency in phosphate uptake (George et al. 1995, Solaiman and Saito 1997, Parniske 2008). The fungi respond to strigolactones from nearby plant roots by beginning extensive branching (Akiyama et al. 2005), preparing them for more successful penetration of plant roots. These strigolactones were originally discovered as possible germination stimulants in witchweed (*Striga lutea*) (Cook et al. 1966).

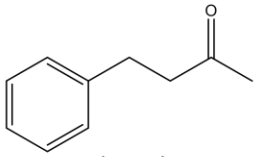
One of the few well-documented instances of an underground plant-plant signaling interaction is the scouting of the parasitic Scrophulariaceae species for host plant roots. *Striga asiatica,* an obligate parasite from Africa, uses two signaling sequences to locate its monocot hosts' roots; the successful completion of each sequence unlocks the next stage in *Striga*'s stepwise development. 2-hydroxy-5-methoxy-3-[(8'Z,11'Z)-8',11',14'-pentadecatriene]-*p*-hydroquinone (sorghum xenognosin, or SXSg), a small molecule originally discovered in the organic exudate of *Sorghum bicolor* roots, releases the first stage of *Striga* growth: germination (Chang et al. 1986). When isolated, SXSg readily autooxidizes, depleting the steady-state concentration of SXSg below the concentration required for *Striga* germination, but the methylated analog of SXSg (4,6-dimethoxy-2-[(8'Z,11'Z)-8',11',-14'-pentadecatriene] resorcinol) serves to stabilize SXSg. These two species combine to set up a sufficient spatial-temporal gradient of SXSg to account for *Striga* germination on agar plates (Fate et al. 1990,

Fate and Lynn 1996). The second signaling sequence, semagenesis, initiates the next stage of *Striga* growth in two steps. The parasitic plant initiates by releasing reactive oxygen species (ROS) from its radicle tip into the surrounding area (Kim et al. 1998). Then, if a host plant is close enough to the root tip, the ROS will find peroxidases in the host plant cell walls to oxidize phenols found in the pectin of the cell walls, releasing quinones. Upon reaching the parasitic plant, the quinones are necessary and sufficient to trigger the development of the haustorium, the parasitic organ (Smith et al. 1990).

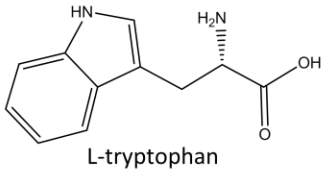
Although chemical signaling plays central roles in many intraspecific relationships (quorum sensing, fertilization) and interspecific relationships (predation, parasitism, mutualism), the majority of the cases studied involve interspecific relationships. Many interspecific relationships have simple phenotypes—disease, predation, and mutualistic morphology (e.g. nodules). When the identity of the chemical signals is entirely unknown, extracts and fractions of extracts can be assayed for bioactivity by looking for a scorable phenotype. In cases where intraspecific signaling has been shown, the phenotypes were also simple to score: abalone fertilization results in viable embryos and quorum sensing in *Vibrio fischerii* results in bioluminescence. However, some interactions among populations and communities result in complex tradeoffs that are just as likely to be governed by chemical equilibria, but are harder to score. With quorum sensing, once a few compounds were found active in vibrios, many papers followed, showing the activity of similar compounds in a variety of bacteria. Thus the type of compound, rather than the simple phenotype, can become the tool for elucidating inter-organism signaling.



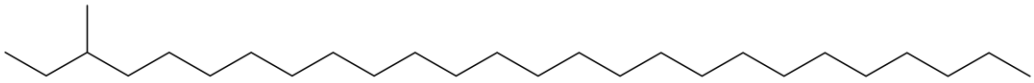
nicotine

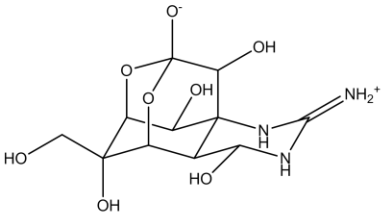


benzyl acetone

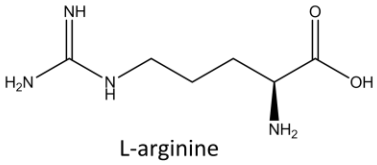


L-tryptophan

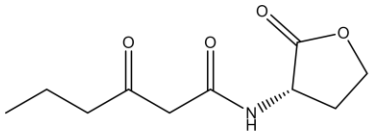

3-methylhexacosane



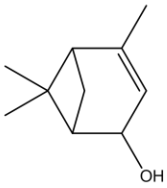
tetrodoxin



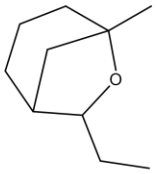
L-arginine



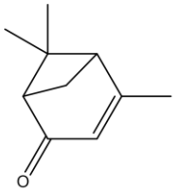
N-(β-ketocaproyl)homoserine lactone



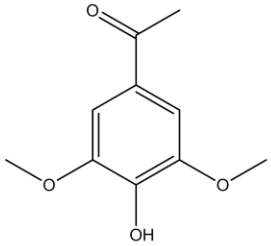
trans-verbenone



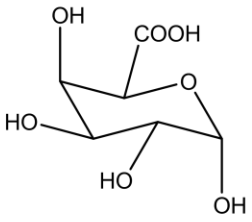
exo-brevicomin



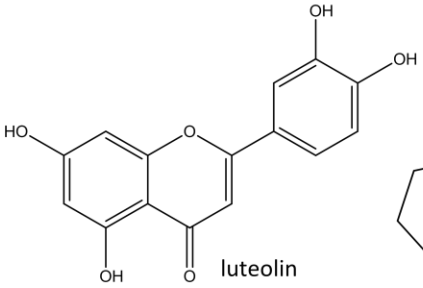
verbenone



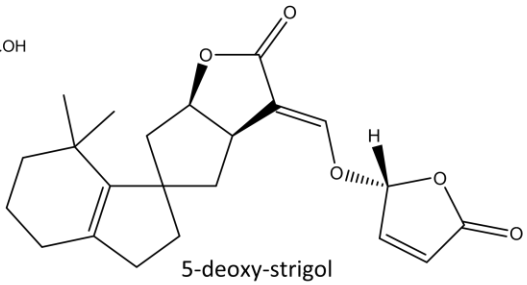
acetosyringone



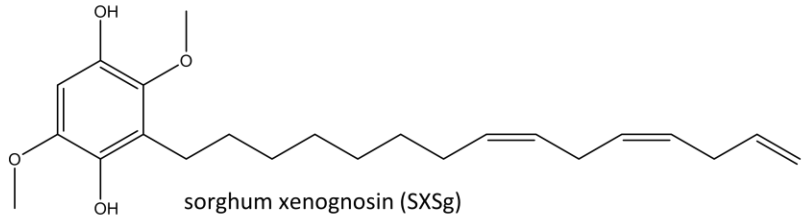
D-galacturonic acid



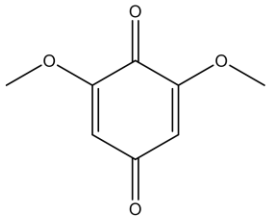
luteolin



5-deoxy-strigol



sorghum xenognosin (SXSg)



DMBQ

Figure 1 Compounds used for signaling between organisms. Nicotine and benzyl acetone control pollination in tobacco flowers, L-tryptophan guides fertilization in red abalone, 3-methylhexacosane is one of several pheromones that coordinate bee fertility, tetrodoxin and L-arginine drive cannibal-avoidance behavior in newts, *N*-(β-ketocaproyl)homoserine lactone is a quorum sensing molecule, trans-verbenone, exo-brevicomin, and verbenone coordinate mountain pine beetle attacks, acetosyringone (AS) and D-galacturonic acid attract pathogenic *Agrobacterium tumefaciens*, luteolin attracts rhizobia, 5-deoxy-strigol is one of several strigolactones that encourage branching in arbuscular mycorrhizal fungi, and SXSg and 2,6-dimethoxy-*p*-benzoquinone (DMBQ) regulate the development of *Striga asiatica.*

Competition in plants

Chemical signaling may also affect competition in plants. Studies of root architecture indicate that when plants are grown close together, the root systems do not grow directly underneath the crowns, but rather minimize root overlap or contact (Brisson and Reynolds 1994, Mou et al. 1995, Caldwell et al. 1996, Pechackova et al. 1999). In a split root experiment where half of a pea plant's root system was planted in an empty pot and the other half in a pot with other plants, the split plant's total root mass was constant, but the root mass in the pot with competitors was inversely related to the number of other plants in that pot (Gersani et al. 1998). This fits the idea of ideal free distribution, that conspecific individuals should settle a heterogeneous habitat so that no individual grows more than the others (Fretwell 1972). Mapping of the root systems of creosote bushes indicates that creosote root systems can be approximated by polygons with minimal overlap rather than circles directly underneath the crown (Brisson and Reynolds 1994). Thus, plants alter their root growth in the presence of other plants.

This detection of other potentially competing plants has often been attributed to resource competition or interference competition. Competition may take both direct and indirect forms and is typically categorized as (1) exploitative, or resource competition, the depletion of resources required by both parties, (2) interference competition, direct attack on the competitor to weaken that competitor's ability to compete, and (3) apparent competition, competition in which success of one species increases predation or parasitism of both species by a common predator or parasite. Resource competition might drive density-dependent plant root architecture if a plant root recognized the nutrient-depleted zone around another plant and changed its root growth to avoid a nutrient-depleted area. Interference competition (allelopathy) might influence density-dependent root architecture if a root's growth were inhibited by an allelochemical from another plant.

Spatial heterogeneity of limited resources makes proliferation of roots more advantageous. In particular, limited availability of nutrients that do not readily diffuse (e.g. P) or spatial variability of nutrients can make lateral root production essential for competitiveness and fitness (Robinson et al. 1999, Fitter et al. 2002). Since the rhizosphere around another root may be nutrient deficient, roots may proliferate in other directions, resulting in apparent avoidance of competitors. Interference competition, or allelopathy, has also been implicated as a mechanism of competition in plants, especially invasive plants. Well-known examples include growth inhibition of heterospecifics by juglone from black walnut (*Juglans nigra*) (Davis 1928), by ailanthone from tree of heaven (*Ailanthus altissima*) (Heisey 1996), and by SXSg from sorghum (*Sorghum bicolor*) (Einhellig and Souza 1992). Autotoxicity is prevented by limiting production to the root hairs (sorghum) (Czarnota et al. 2001) or transporting in the non-toxic hydroquinone

form (walnut) (Lee 1969). In all these cases, the allelochemical is highly toxic to other plants and almost completely deters the growth of susceptible plants within the vicinity.

However, allelopathy and resource competition are not sufficient to explain density-dependent root architecture. In some cases, avoidance is not removed when nutrients are added externally (Caldwell et al. 1996). In *Larrea tridentata* and *Ambrosia dumosa*, avoidance is species-specific, with *Larrea* inhibiting both plants, but *Ambrosia* only inhibiting *Larrea*. The addition of activated carbon was sufficient to stop *Larrea's* inhibition of the other plants, indicating that the *Larrea* inhibition was likely caused not by nutrient deficiency alone, but by some exuded compound (Mahall and Callaway 1992). Similarly, the negative effect of the invasive English ivy (*Hedera helix*) on germination of the endemic *Coreopsis lancelota*, in spite of abundant nutrients and light, suggests a competition mechanism other than resource depletion (Biggerstaff and Beck 2007). Many plant roots grow successfully within 1 cm of each other, and their nutrient-independent avoidance mechanisms and root architecture occur on this scale (Mahall and Callaway 1992, Pechackova et al. 1999, Caffaro et al. 2011). That the roots can grow this close together precludes avoidance because of a toxic allelochemical, and that the mechanism is nutrient-independent indicates that it is not caused by avoidance of nutrient-depleted areas.

One possible explanation for this avoidance is a much less toxic chemical signaling mechanism mediated by the root exudate. In their study on *Arabidopsis* root exudates, Caffaro et al. showed decreased lateral root development and increased contacts between roots in *Arabidopsis* when activated carbon removed root exudate small molecules from solution (2011). When root exudates were added back into the medium, this phenomenon was reversed, and plants grew more lateral roots and had fewer root-root contacts. If an *Arabidopsis* root tip is

growing in a certain direction, and it detects an active compound exuded by a nearby root, it might stop elongating toward that root and begin branching to explore another area. Thus root exudates may direct roots to avoid each other. In this study, we propose that the root exudate-based mechanism for detecting nearby plants is the root-root signaling mechanism called semagenesis.

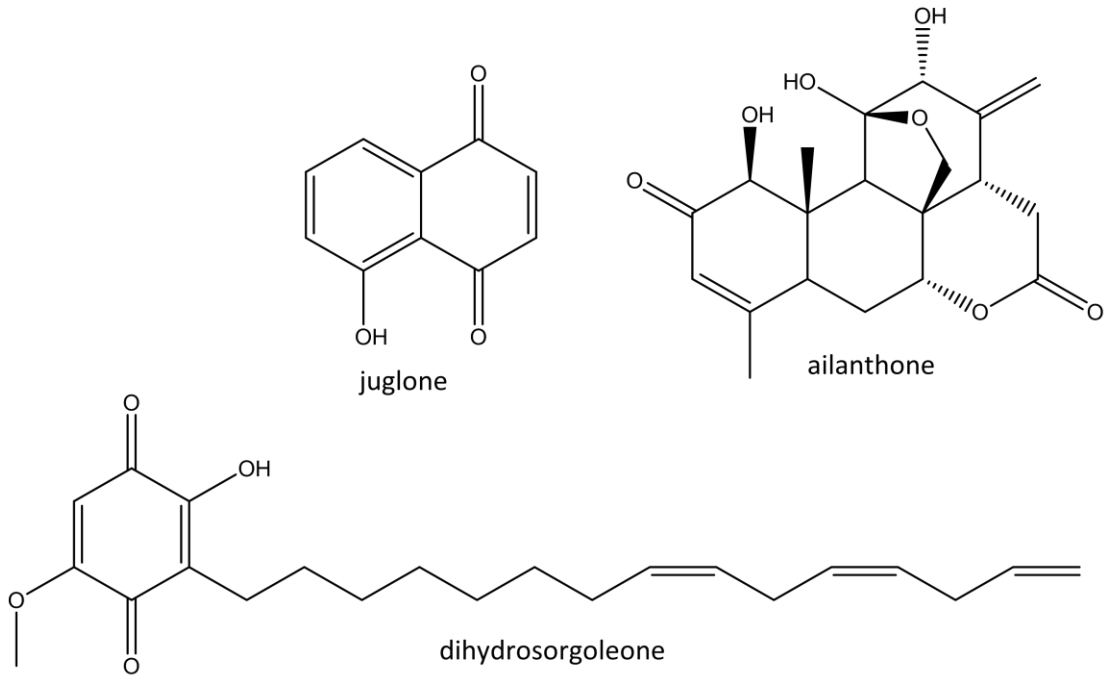


Figure 2 Examples of allelopathic compounds

Semagenesis in nonparasitic plants—a method of detecting competition?

If avoidance of competition among plants can involve species-specific sensing and is not always triggered by simple resource depletion, plants must have an alternative method of perceiving competition. One possibility is that, just as bacteria recognize density using quorum sensing and mountain pine beetles optimize the amount of intraspecific competition by detecting quorums, plants also detect each other by chemical signals and modify their root systems in some

way that optimizes their fitness. Although aboveground signals can affect below ground morphology (Kessler et al. 2008), since root system morphology is not well correlated with above ground morphology, it seems more likely that a root sensing process would occur entirely below ground. The most well-characterized belowground plant-plant sensing processes occur between *Striga asiatica* and its host as the parasitic plant tries to find its host.

In their review on quorum sensing, Miller and Bassler point out that after the discovery of quorum sensing in a few bacterial species, "these bacterial communications systems were believed to be anomalous, and in general, bacteria as a whole were not believed to use cell-cell communication." (Miller and Bassler 2001) Later, researchers discovered that the same class of quorum-sensing compounds is used among Gram-negative bacteria for many different ecological functions. With this in mind, it is worth asking if underground signaling between *Striga asiatica* and allied species and their hosts is indeed anomalous or if it serves a sort of general quorum sensing function in other plant species. In this study, we test the possibility that one of these signaling processes, semagenesis, is more general and occurs in nonparasitic plant roots to detect their competitors. After using semagenesis to locate their competition, these plants would use this information to strategize root architecture for maximal fitness. Semagenesis was chosen as a possible signaling process not only because the steps are specifically known in *Striga asiatica*, but because the required components exist in typical nonparasitic plant roots. (Figure 3)

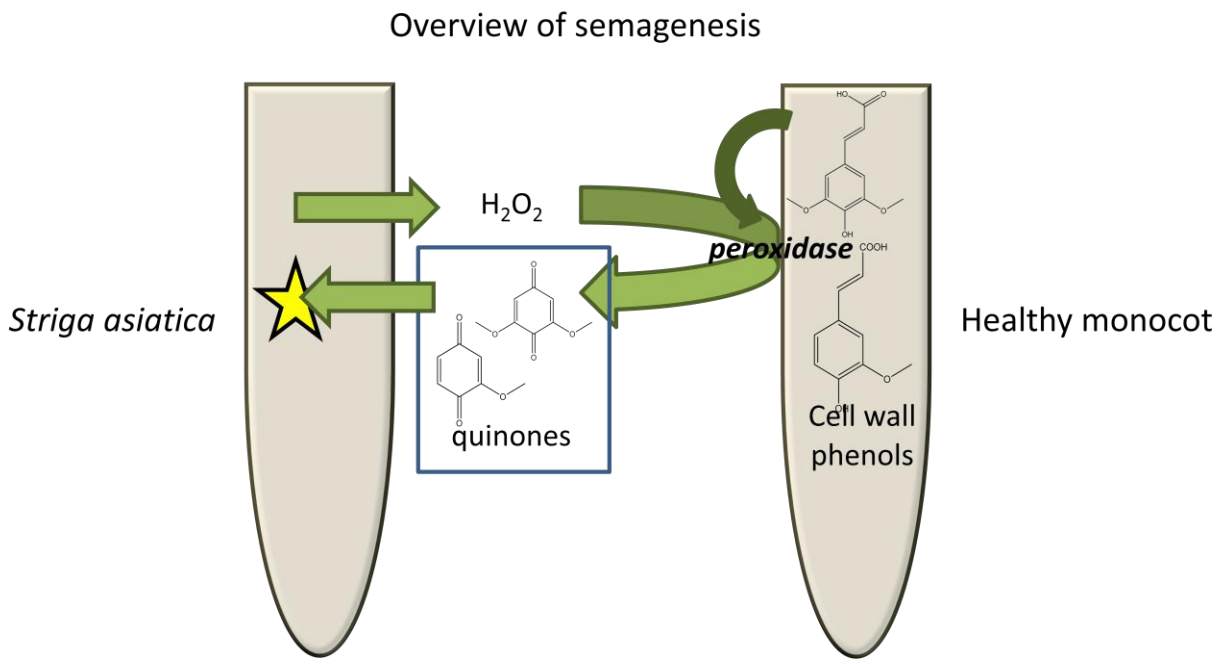


Figure 3 An overview of semagenesis in *Striga asiatica.* The *Striga* seedling radicle releases ROS, e.g. H_2O_2, which oxidizes cell wall phenols on host monocot roots, with the help of a peroxidase. The resulting quinones trigger parasitism in *Striga.*

In the first step of semagenesis, reactive oxygen species (ROS) travel from *Striga*'s radicle to the host plant root. Reactive oxygen species include superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl ($\cdot OH$) and are used in plants for root and root hair elongation (Foreman et al. 2003), response to mechanical and pathogenic stress (Wojtaszek 1997), and lignification. Reactive oxygen species are also produced during metabolism and homeostasis. General restructuring of root morphology has also been attributed to ROS (De Tullio et al. 2010). A steady state concentration of H_2O_2 is maintained both in the apoplast and in the area surrounding the root in soybeans (*Glycine max*), peas (*Pisum sativum*), sunflowers (*Helianthus annuus*), and maize (*Zea mays*) (Frahry and Schopfer 1998). This occurs over the surface of the

root, but not at the meristem (Frahry and Schopfer 1998). Another reactive oxygen species, superoxide, was stained by nitroblue tetrazolium (NBT) in the meristem, elongation zone, and stele of *Arabidopsis thaliana* seedlings, but not in the cortical cells of the differentiation zone. In the same study, hydroxyphenyl fluorescein (HPF) stained H_2O_2 in the end of the elongation zone and beginning of differentiation zone, especially in expanding root hair cells and elongating lateral roots (Dunand et al. 2007). Thus the first signaling molecule for semagenesis is readily available in nonparasitic plant roots and in the area around them.

ROS from *Striga* oxidizes the phenols on the surface of the host plant, probably using the peroxidases in the host plant root. In general, plant cell walls consist of four types of polymer matrices: cellulose, hemicellulose, pectin, and lignin. Embedded in the matrix of cellulose and hemicellulose is pectin, which is composed mainly of sugars but also includes some phenylpropanoids. Autofluorescence of the seedlings indicates presence of the phenolic substrates (Palmer 2008), and another dicot, soybean, has been shown to have ether- and ester-linked phenylpropanoids in pectin (Lozovaya et al. 1999). In the presence of enough oxidant, the linking between the pectin sugars and phenols could feasibly be reversed. Staining with *o*-dianisidine in the presence of H_2O_2 revealed peroxidase along the entire length of *Arabidopsis thaliana* seedling roots (Dunand et al. 2007). Thus, the components exist in *Arabidopsis* for ROS from one root to oxidize phenols in another root to quinones.

Finally, the quinones from the host plant reach *Striga*, triggering a major developmental change and a change in internal ROS levels. If semagenesis does occur in nonparasitic plants as a method of detecting potential competitors, the final quinone signal should trigger not haustorial development, but a root restructuring characteristic of plants grown at higher density. Preliminary tests indicate that both *Tabacum nicotiana* and *Arabidopsis thaliana* continually

exposed to 50 µM 2,6-dimethoxy-*p*-benzoquinone (DMBQ) develop shorter roots and fewer root hairs (Palmer et al. 2009). Stunting caused by exogenous addition of monolignols was rescued by ROS scavengers, indicating that the quinone species were required for the phenotype. The growth-limiting effect was concentration-dependent and could be stimulated by a number of the quinones. *N. tabacum* seedlings responded to quinone treatment by increasing NTB staining, indicating increased internal $\cdot O_2^-$. While these morphological and biochemical responses did not match *Striga*'s drop in NTB staining and haustorial development, the mechanism-based inhibitor CPBQ blocks the effect of DMBQ in both types of plants, possibly indicating a similar mechanism for detecting benzoquinones. Finally, activated carbon, which can adsorb phenols and quinones, has been shown to cause longer root growth and fewer lateral roots in *Arabidopsis thaliana,* suggesting that a compound like DMBQ normally results in shorter root growth and the development of lateral roots (Caffaro et al. 2011).

Testing the semagenesis model in nonparasites

The components for semagenesis in nonparasites are in place: ROS, phenols, peroxidases, and a quinone response. In principle, the two chemical signaling events in semagenesis must occur at least at a minimal level in nonparasitic plants, but the question remains whether or not this signaling process is a significant player in nonparasites' detection of other plants. In order to address this question, this study considers two as-yet unconfirmed steps in nonparasite semagenesis: 1) do reactive oxygen species result in the release of quinones from nonparasites' roots? and 2) is the response to semagenesis signals the same as the response to high population densities?

Methods

Germination of seeds

Wild-type *Arabidopsis thaliana* seeds were surface-sterilized with full-strength bleach (The Clorox Company, Oakland, CA) by adding seeds to an Eppendorf tube and suspending in bleach. Seeds were pelleted and resuspended in ddH_2O to rinse for a total of three rinses. Seeds were resuspended in ddH_2O for plating and plated on Petri dishes with sterile solid agar medium using a 200 µL pipette.

Treatment with H_2O_2

100x100x15 mm square plates were prepared with 3% sucrose, full strength Murashige & Skoog (MS) medium, supplemented with 9g agar per liter medium, and adjusted with 1 M KOH to pH 5.8. Seeds were plated at about 60 seeds/plate and placed vertically in an incubator to germinate at 25°C with light for 7 days. After 7 days, individual healthy plants were transferred to 6 well plates, with 1 plant and 5 mL of liquid MS 1% sucrose medium at pH 5.8 in each well. These were placed on a shaker-style growth rack under a 16 h light/8 h dark cycle for three days. 10 or 18 day old seedlings were treated with different concentrations of H_2O_2 by removing the existing medium and replacing with aqueous dilutions of H_2O_2 (0, 10, 25, and 50 µM). These concentrations were chosen because the semagenesis effect of quinones plateaus around 20 µM. (Kim et al. 1998) Plates were closed, parafilmed, and returned to shaker racks for the requisite duration of treatment (6, 24, or 48 h). In experiments where organic extracts were taken, the plants were grown from seed under 16 h light/8 h dark cycles on a fixed growth rack.

Sample extraction of DMBQ

A stock solution of 1 mM dimethoxy-*p*-benzoquinone (Sigma-Aldrich, St. Louis, MO) in DMSO was diluted to 1 mM, 100 µM, or 10 µM and run on reverse phase LC-MS, using a 10-90% MeOH:H_2O gradient over 40 min. The chromatography system included a C18 column (Dionex, Sunnyvale, CA), two P-680 columns from Dionex, and a UV-vis detector. Masses were detected by a single quadrupole mass spectrometer using both positive and negative modes, alternately.

Ethyl acetate and chloroform were each used to extract DMBQ using a known procedure for benzoquinone extraction. (Tomoskozi-Farkas and Daood 2004) 15 mL of 0.025 µM DMBQ solution (aq) was extracted 3 times with the organic solvent. The organic fractions were combined, washed twice with ddH_2O, dried with excess $NaSO_4$, vacuum filtered, and left overnight to dry. After drying, the ethyl acetate extract had yellow spots remaining and nothing was visible from the chloroform extract. Samples were redissolved in 500 µL of EtOAc, and 10 µL of each was injected into the HPLC. Both ethyl acetate and chloroform extractions were successful in extracting DMBQ, but ethyl acetate was chosen for future exudate extractions because it extracts fewer lipids.

Aqueous extraction of root exudate

The following exudate extraction procedure was modified from the one used by Badri et al. (2010) The aqueous medium for each treatment group was combined, filter sterilized, frozen at -80°C, and lyophilized completely. After resuspending each sample in 5.0 mL H_2O and 5.0 mL EtOAc, the samples were vortexed, allowed to stand for 10 min, and centrifuged for 10 min at 5000 rpm. The EtOAc fraction was pipetted out into a glass centrifuge tube. This extraction was repeated for a total of 10 mL of EtOAc extract. The extract was air dried, dissolved in 1 mL

EtOAc, vortexed, dried, redissolved in 100 µL EtOAc, vortexed, and transferred to vials for the HPLC autosampler. As positive and negative controls, 5mL of 45 µM DMBQ or 5mL ddH_2O was extracted using the same EtOAc extraction procedure. 45 µM DMBQ was chosen based on a maximum estimate of 25 µM DMBQ in 18 5mL samples. HPLC injection volumes were 30 µL for each extracts and 10 µL for each control. All samples were run in 10-90% MeOH:H_2O over 40 minutes and the MS traces searched for the molecular ions of both 2,6-dimethoxy-*p*-benzoquinone and methoxy-benzoquinone (Figure 4).

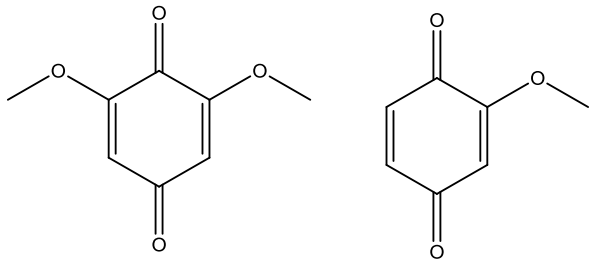


Figure 4 2,6-dimethoxy-*p*-benzoquinone and methoxy-benzoquinone

Organic extraction of root exudate

Roots of H_2O or H_2O_2 treated plants were immersed in 50 mL of dichloromethane (DCM) in a glass Petri dish for 1 minute to obtain an exudate extract. The extract was dried at 25°C and stored at -20°C. Crude extract was analyzed by HPLC, with 10-90% MeCN:H_2O with 0.1% TFA using a Waters Delta 660 pump, Waters 996 Photodiode Array Detector, and Atlantis dC18 5µM column.

Effect of population on root morphology and on $\cdot O_2^-$ accumulation in the root tip

In this experiment, seeds were sterilized by vapor sterilization. Open Petri dishes of dry seeds and an open container of bleach were placed together in a closed container for 3 hours. Dry seeds were placed in a sterile water bath with sterile ddH_2O and placed under a 16 h light/8 h dark cycle. On day 3, healthy seedlings were selected and transferred to 96 well plates, at different densities: 20, 40, or 60 seedlings per well. Wells were then filled with water, and the water was replenished periodically. At day 5, eight randomly selected seedlings per treatment were imaged with bright-field microscopy before and after staining with 50 µM nitro-blue tetrazolium for 15 min. All images were taken with a Leica DM IRB microscope.

Effect of DMBQ on morphology

Sterile seeds were grown for 10 days on 16 h light/8 h dark light cycles on half-strength MS medium with 9 g agar/L with or without 50 µM DMBQ. This medium was chosen because it was used by Palmer, et al. to test the effect of DMBQ (2009). The effect of DMBQ on *Arabidopsis thaliana* increases with concentration, but germination decreases past 50 µM (Palmer et al. 2009); thus 50 µM was chosen to have the maximum effect. Seeds were arranged at a set density of about 60 seeds per plate and 6-7 rows per plate, with about 12 mm between seeds on the same row and about 14 mm between seeds on different rows. After 10 days, seedlings were removed and the radicle length, hypocotyl length, and width from tip to tip of the seed leaves (cotyledon width) were measured. Lateral root count was observed by bright field microscopy. The whole length of the root was photographed by bright field microscopy, and a root hair count was found by counting the root hairs found in these photographs. Root hair counts are estimates since not all root hairs were in focus, some root hairs were out of sight

behind the root, and in some areas, root hairs were too dense to count exactly. Some effort was made to take multiple photographs per section of root in order to show as many root hairs in focus as possible.

Effect of population density on morphology

Sterile seeds were grown for 10 days on the same medium, but without DMBQ. Each plate had exactly 19 seeds with two concentric hexagons and one seed in the center, so that each plant was the same distance from the others. (Figure 5B) To avoid an edge effect, only plants on the inner hexagon (shown in green in Figure 5B) were measured. At 10 days, these seedlings were removed and their radical length, hypocotyl length, and cotyledon width were measured and lateral roots were counted at 20X magnification using a Leica DM 4500B microscope.

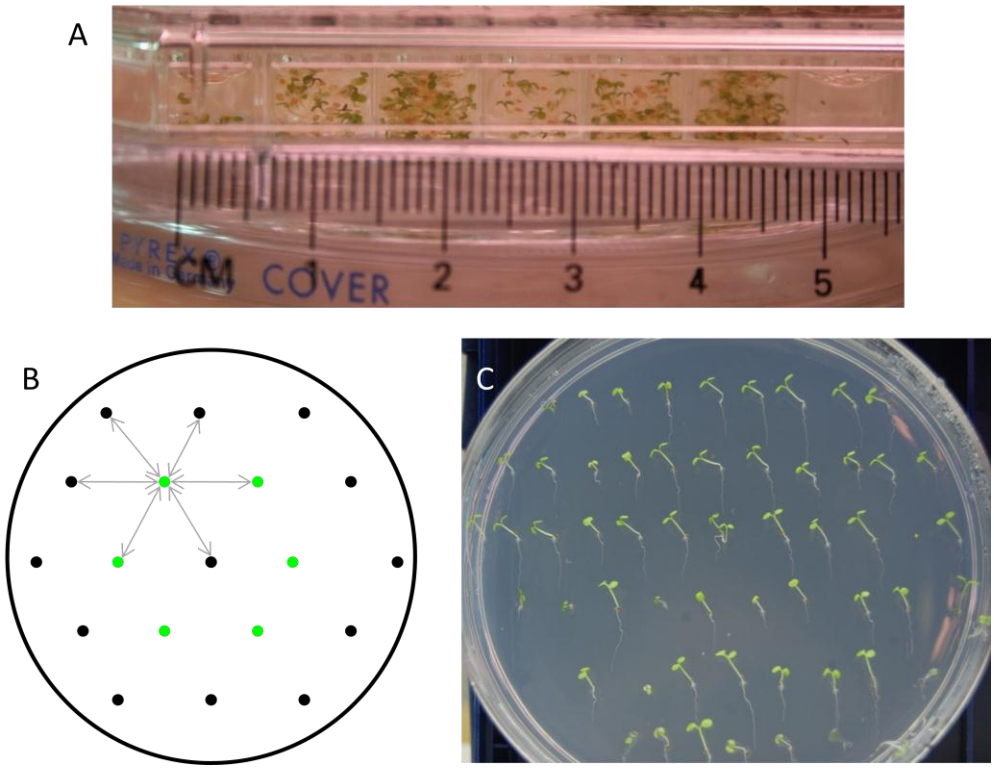


Figure 5 Growth of seedlings with 20, 40, or 60 seedlings per well in a 96 well plate (above),

Data analysis

Fisher's exact test was used to compare staining effects. Radicle length, hypocotyl length, cotyledon width, and root hair density were compared in plants grown with or without DMBQ using t-tests. In the same experiments, Fisher's exact test was used to compare lateral root counts. In experiments to test the effect of population density on root morphology, I used a linear mixed effects model using restricted maximum likelihood, with plate as a random effect. All data analysis was performed using the statistical program R (version 2.13.1).

Results

DMBQ extraction

Based on UV-vis, mass spectra, and comparison with DMBQ before extraction (Figure 6-7), DMBQ can be extracted by both ethyl acetate and chloroform (figure 8).

Treatment with H_2O_2

Of the plants treated at 10 d for 6h, 24 h, or 48 h, or at 18 d for 48 h, only 10 d old plants grown at 48 h showed a clear change in exudate with treatment. 10 d old plants treated for 6 h showed very little exudate at all; most peaks were also found in the blank injection. (Figure 9) One explanation for the low signal-to-noise is that that injection combined exudate extracts from only 18 plants. Plants treated for 24 h showed a few clear exudate peaks at 29.8, 31.1, and 40.4 min; in this case, instead of preparing 2 18-plant exudate extractions, 1 36-plant exudate extract was prepared for each treatment, giving clearer signal-to-noise. (Figure 10) However, the 24 h treatment still appears to have no effect on composition or quantity of the components of the exudate.

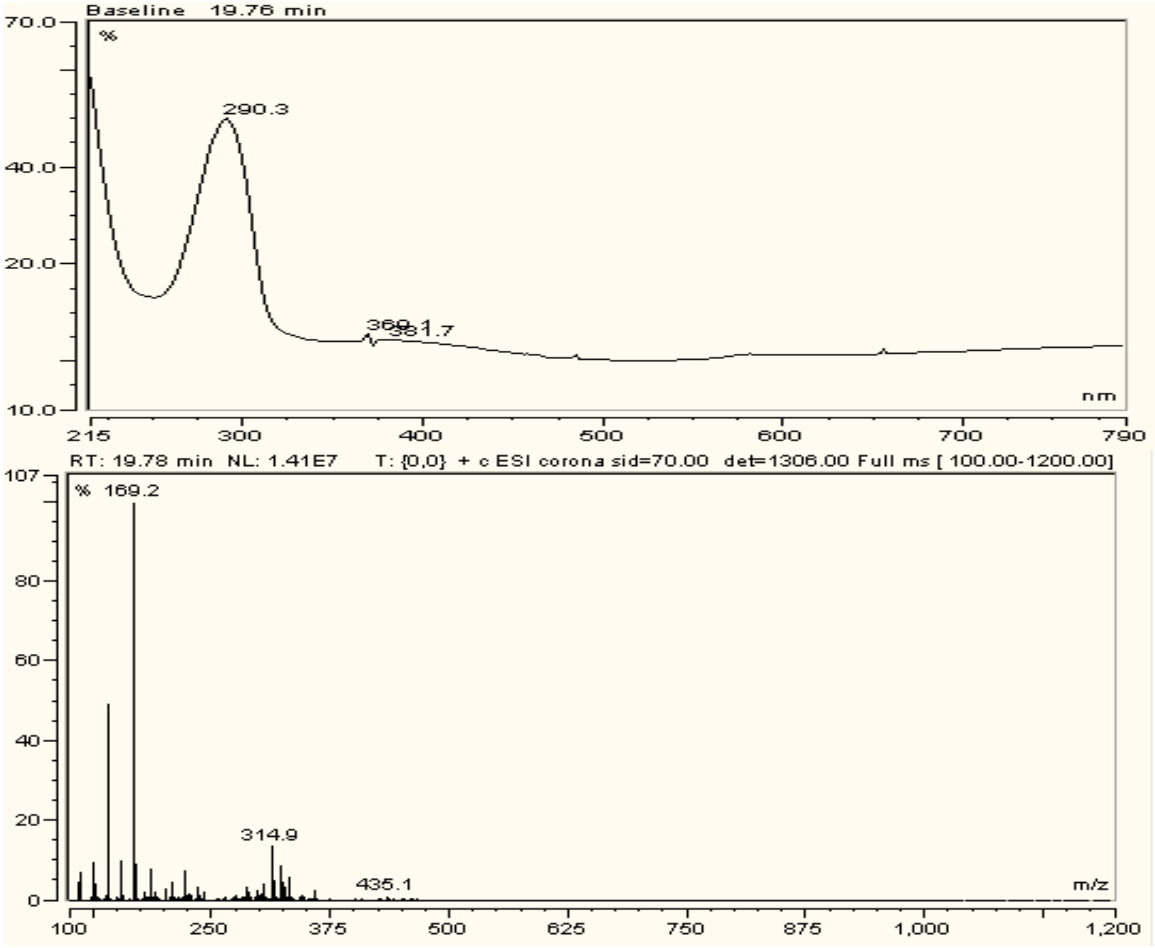


Figure 6 The UV-vis (above) and MS spectra (below) for the DMBQ standard. UV-vis spectrum displays the expected absorption at 290 nm, and the MS spectrum shows the molecular ion at 169.2 m/z.

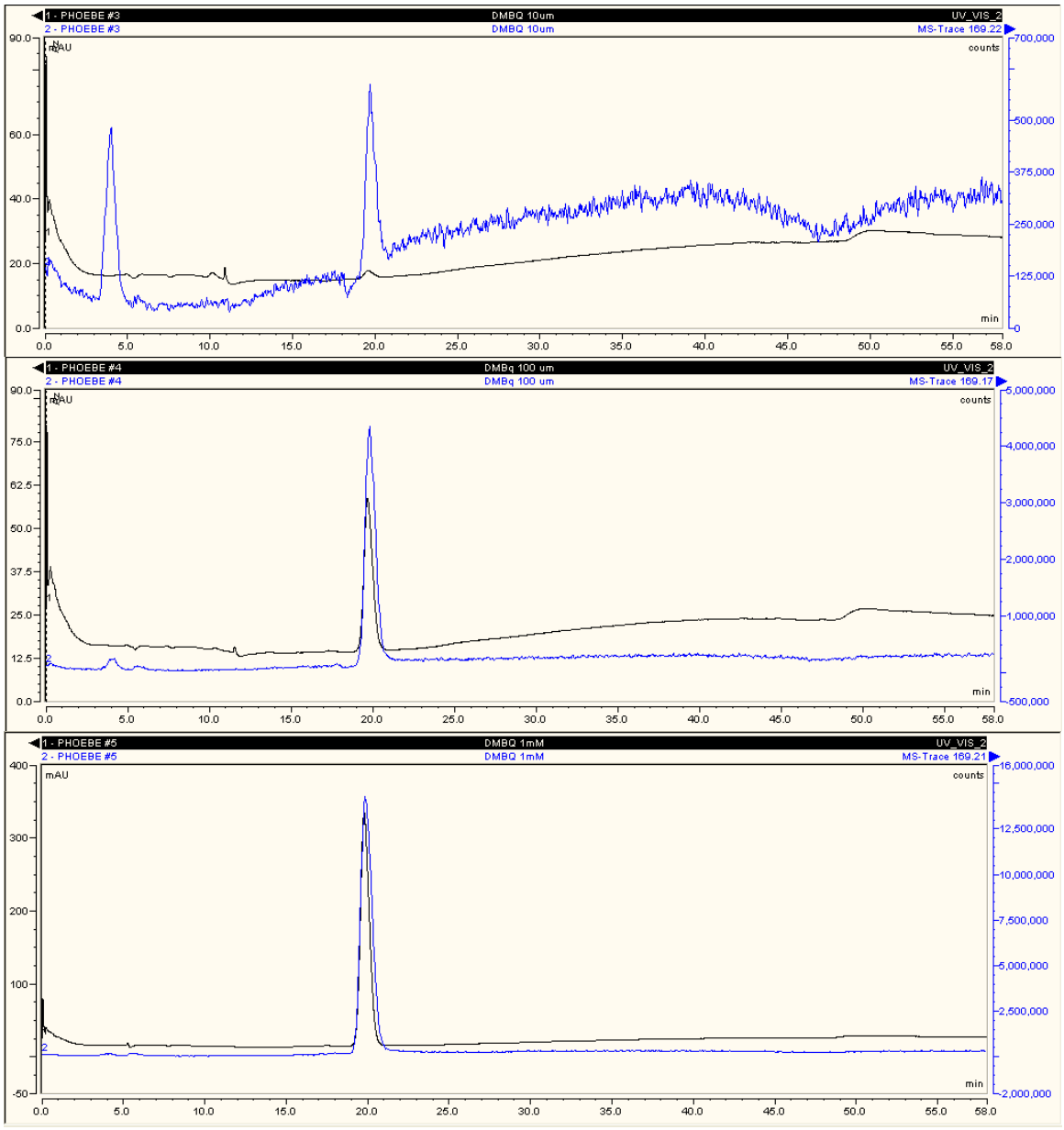


Figure 7 LC-MS traces for 10.00 µL injections of DMBQ standard at different concentrations: 10 µM, 100 µM, and 1mM. Retention times were 19.6, 19.68, and 19.78 respectively, and integrations were 1.545, 27.7, and 205.2 mAU·min. Blue lines show the MS trace at 169.22, 169.17, and 169.21 m/z, and black lines follow the UV trace at 280 nm.

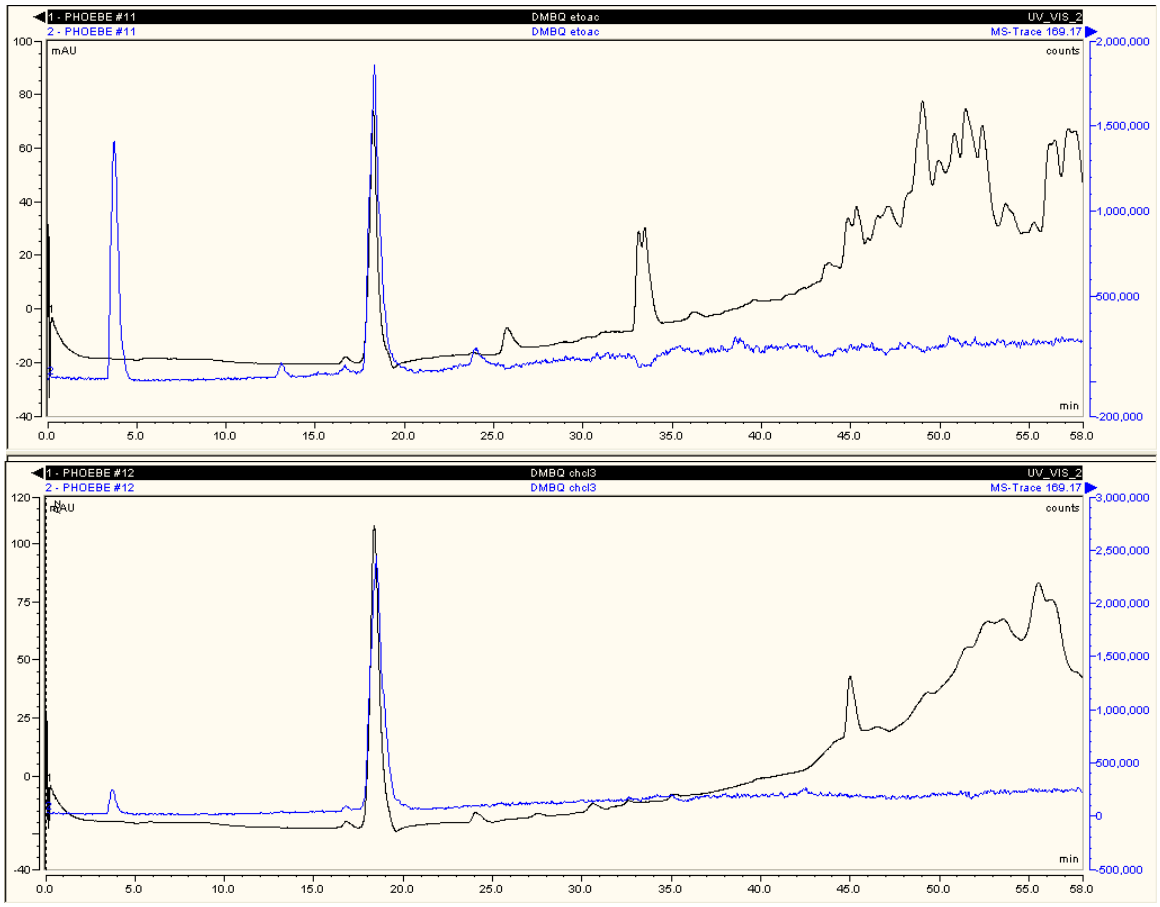


Figure 8 LC-MS results for 10.00 µL injections of EtOAc (above) and $CHCl_3$. Blue lines follow the MS trace at 169.17 m/z, and the black lines show the UV-vis trace at 280 nm. Elution times are 18.2 and 18.4 min, with integrations of 52.7 and 81.0 mAU·min.

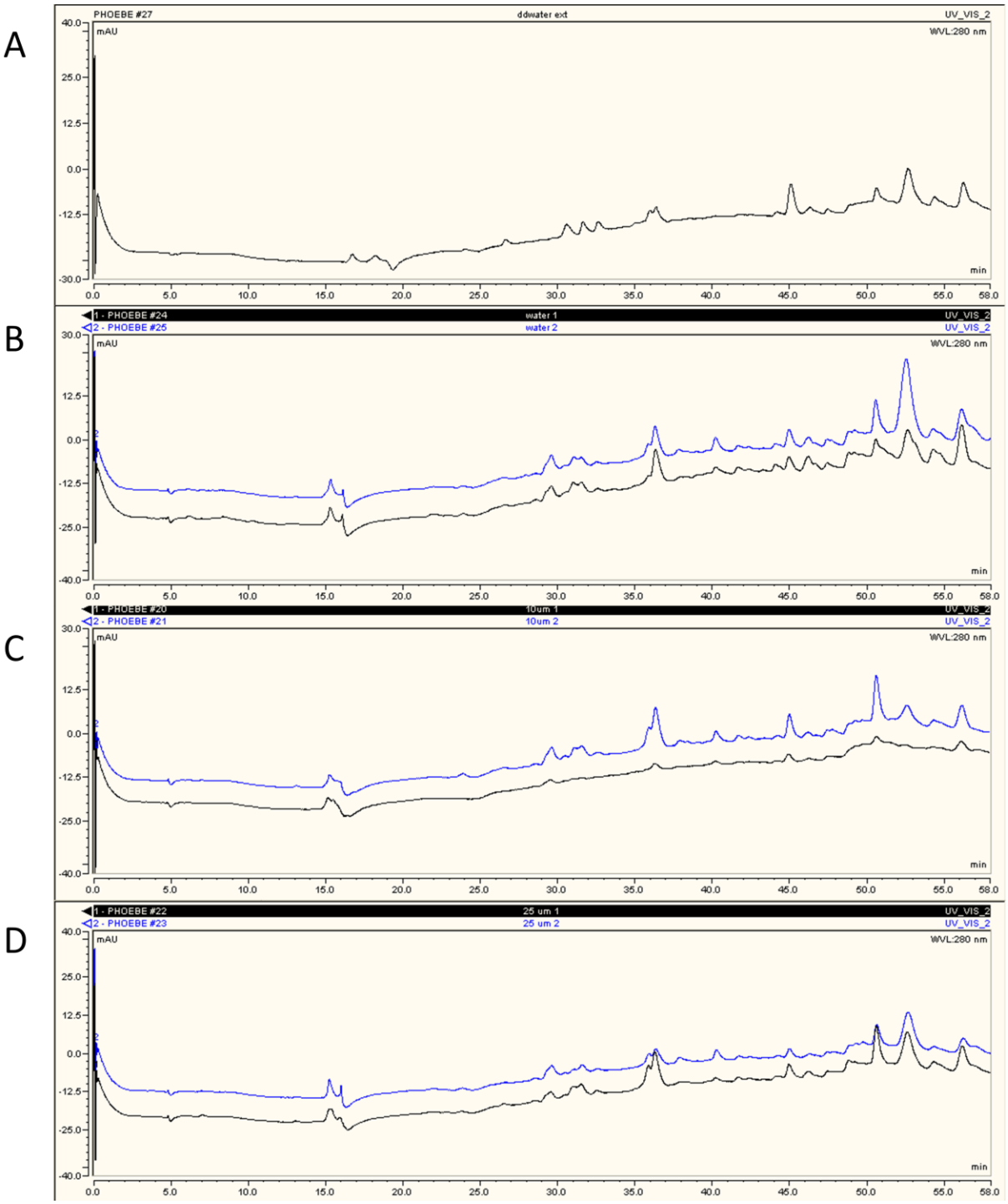


Figure 9 HPLC traces for blank injection (A) and for exudates from 10 day old plants treated for 6 h with H_2O (B), 10 µM H_2O_2 (C), and 25 µM H_2O_2 (D). Each chromatogram shows the overlay of two replicates of the same treatment.

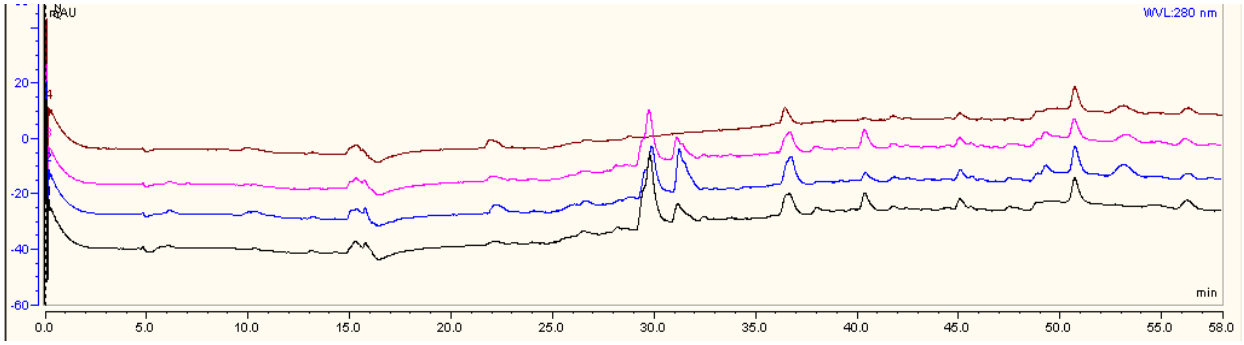


Figure 10 LC-MS traces for blank injection (brown) and for exudates from 10 day old plants treated for 24 h with H_2O (pink), 10 µM H_2O_2, and 25 µM H_2O_2. Each trace represents the combined exudate of 36 plants.

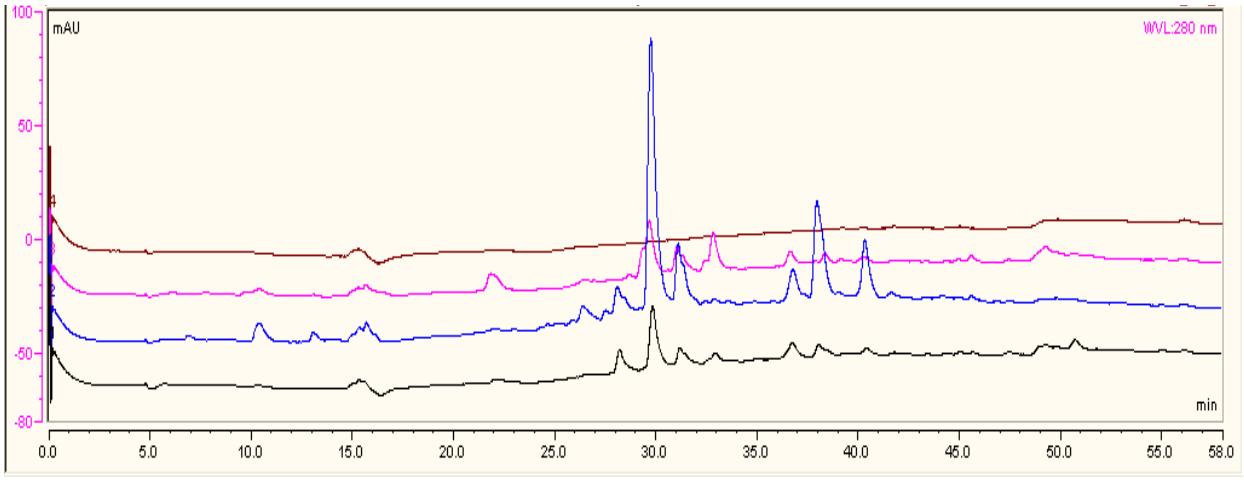


Figure 11 HPLC traces for blank injection (brown) and for exudates from 10 day old plants treated for 48 h with H_2O (pink), 25 µM H_2O_2 (black), and 50 µM H_2O_3 (blue). Each trace represents the combined exudate of 36 plants, except the 25 µM H_2O_2 trace, which represents 22 plants.

10 d old plants treated for 48 hours did show changes in the exudate. (Figure 11) Keeping in mind that the H_2O trace represents exudate from 36 plants and the 25 µM H_2O_2 trace represents exudate from 22 plants, the main differences between these traces are the

disappearance of the H_2O cultured peak at 21.85 min in the 25 µM H_2O_2 trace and the appearance of the peak at 28.2 min in the 25 µM H_2O_2 trace. Comparing the 25 µM H_2O_2 trace and the 50 µM H_2O_2 trace, which combines the exudate from 36 plants, the primary differences are the appearance of a peak at 26.4 min in the 50 µM H_2O_2 exudate, the larger peak area at 29.8 min with 50 µM H_2O_2, the disappearance of a peak around 32.2 min, and the appearance of two large peaks at 37.9 and 40.3 minutes. The 29.8 min peak, which was found in all three traces, but increases significantly with the highest concentration of H_2O_2, corresponds to the mass and UV of indole-3-carboxylic acid (Davis et al. 1976). (Figures 12-14) The 31.1 min peak, found in similar quantity, in all three traces matches the mass spectrum and UV spectrum for kaempferitrin, a yellow flavonoid (Merck Index, 2006, Matsuda F 2011). (Figures 15-17) In the 50 µM H_2O_2 spectrum, the 40.3 min peak has the same molecular ion and UV spectrum as camalexin. (Figure 18-20) Camalexin is a phytoalexin, a compound used for defense against microbes, and, like indole-3-carboxylic acid, is a member of the tryptophan pathway, which includes indole-3-acetic acid (auxin). Both indole-3-carboxylic acid and camalexin were found in all three traces, but their concentration increased with H_2O_2 concentration. Finally, although 48 h treatment with H_2O_2 at the 10 d time point affected the exudate composition, the MS traces for 139 m/z and 169 m/z show no MBQ (molecular ion: 139 m/z) or DMBQ (molecular ion: 169 m/z). (Figure 21)

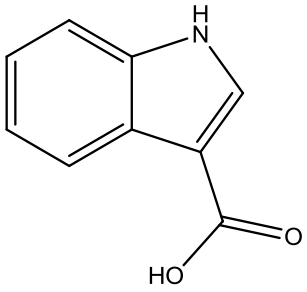


Figure 12 Indole-3-carboxylic acid.

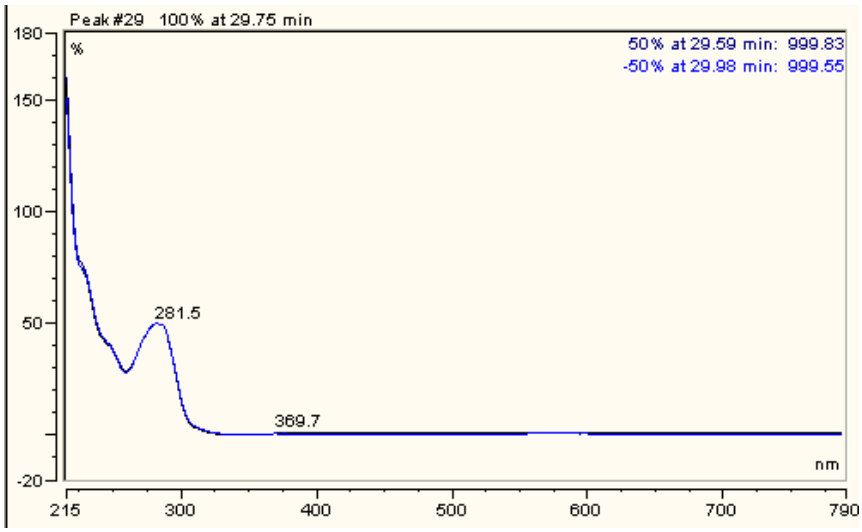


Figure 13 UV spectrum for 29.75 min for the 50 µM H_2O_2 exudate. The 281.5 nm absorption matches the known absorption maximum for the indole chromophore in indole-3-carboxylic acid.

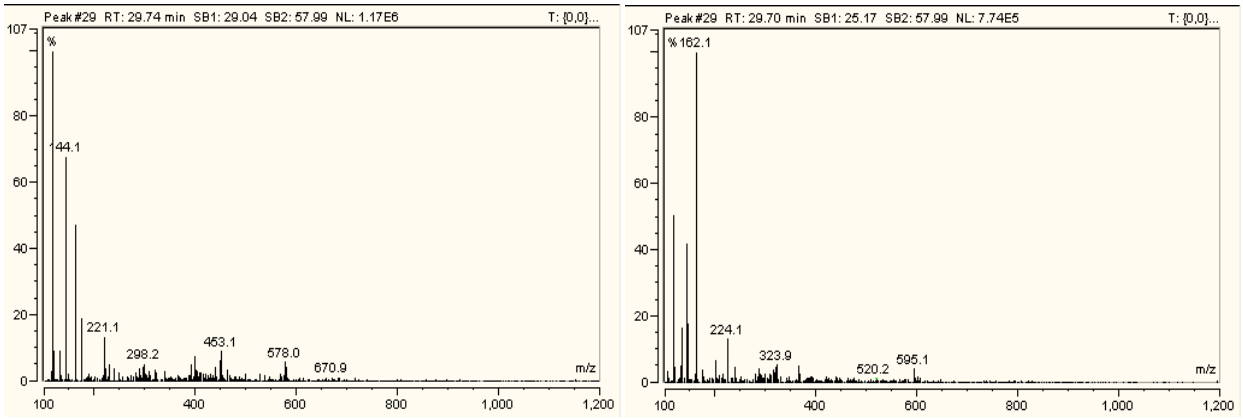


Figure 14 162.1 is the molecular ion, 144.1 m/z corresponds to the loss of a hydroxyl, and a possible 118 m/z peak in both spectra would correspond to the indole ring alone.

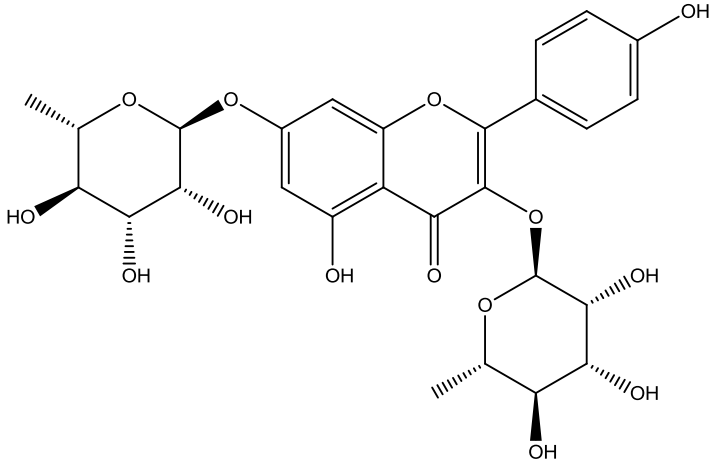


Figure 15 Kaempferitrin, or kaempferol 3,7-dirhamnoside.

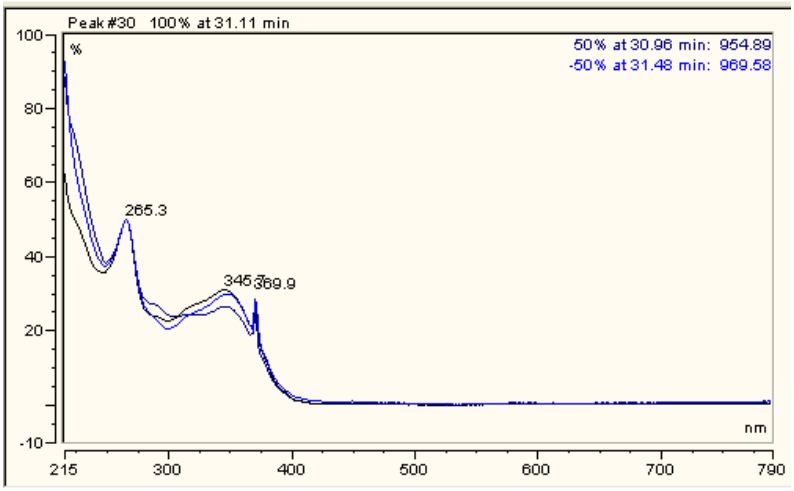


Figure 16 UV spectrum of peak at 31.11 min from the 48 h treated 50 µM H_2O_2 exudate. The violet trace indicates the UV spectrum at 30.96 min, and the blue trace shows the UV spectrum at 31.48 min. The absorption maxima at 265 nm and 369.9 nm correspond to the known absorption maxima from kaempferol (265, 365 nm). (Merck Index, 2006)

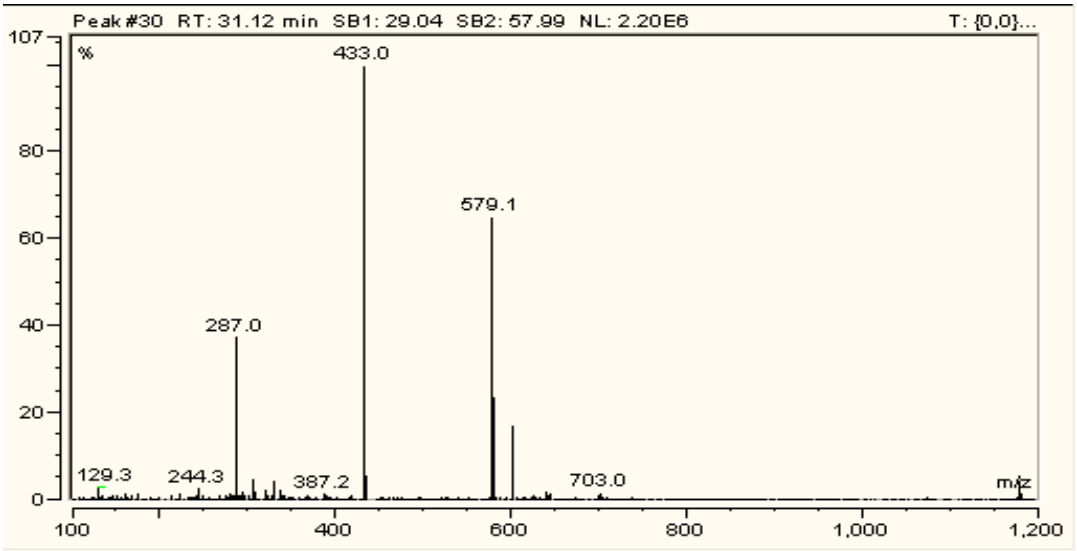


Figure 17 MS for peak at 31.12 min from the 48 h treated 50 µM H_2O_2 exudate. 579.1 is the molecular ion, 433.0 m/z is the kaempferol with one rhamnose, and 287.0 m/z peak corresponds to kaempferol.

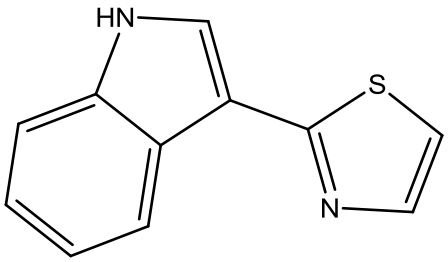


Figure 18 Camalexin, a phytoalexin and member of the indole-3-acetonitrile (IAN) pathway.

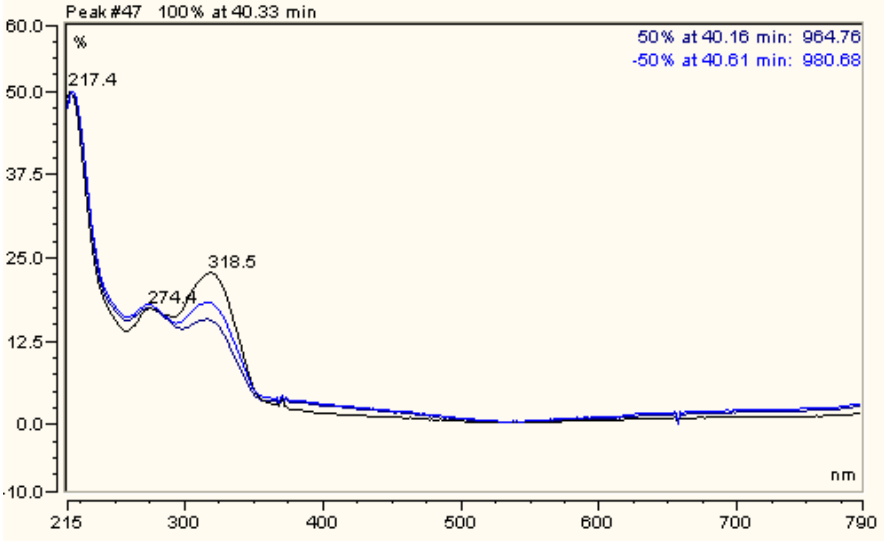


Figure 19 The UV spectrum for the peak at 40.33 min in the 50 µM H_2O_2 exudate. The violet trace corresponds to the UV spectrum at 40.16 min, and the blue trace corresponds to the spectrum at 40.61 min. The three absorption maxima (217.4, 274.4, 318.5 nm) match the reported maxima for camalexin. (Ayer et al. 1992)

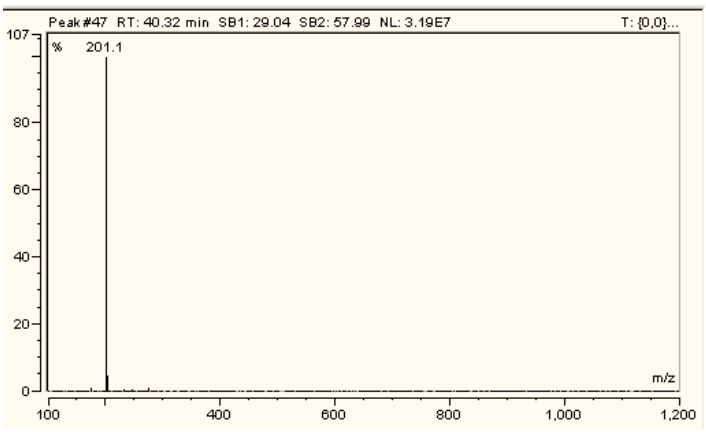


Figure 20 MS for peak at 40.32 min for 50 µM H_2O_2. 201.1 m/z is the molecular ion for camalexin.

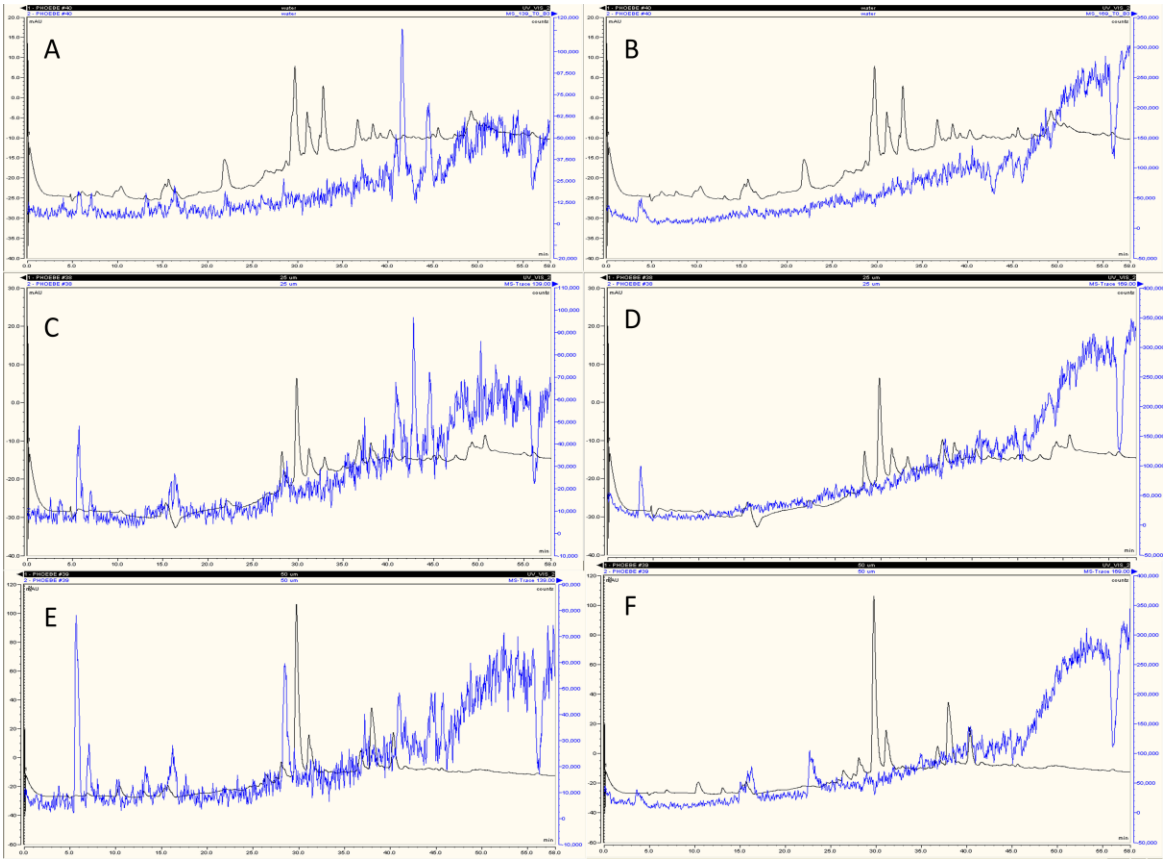


Figure 21 LC-MS traces for exudates from 10 day old plants treated for 48 h with H_2O (A, B), 25 µM H_2O_2 (C,D), and 50 µM H_2O_2 (E,F). Black lines represent UV traces at 280 nm, and blue lines represent MS traces at 139 m/z (left column) and 169 m/z (right column).

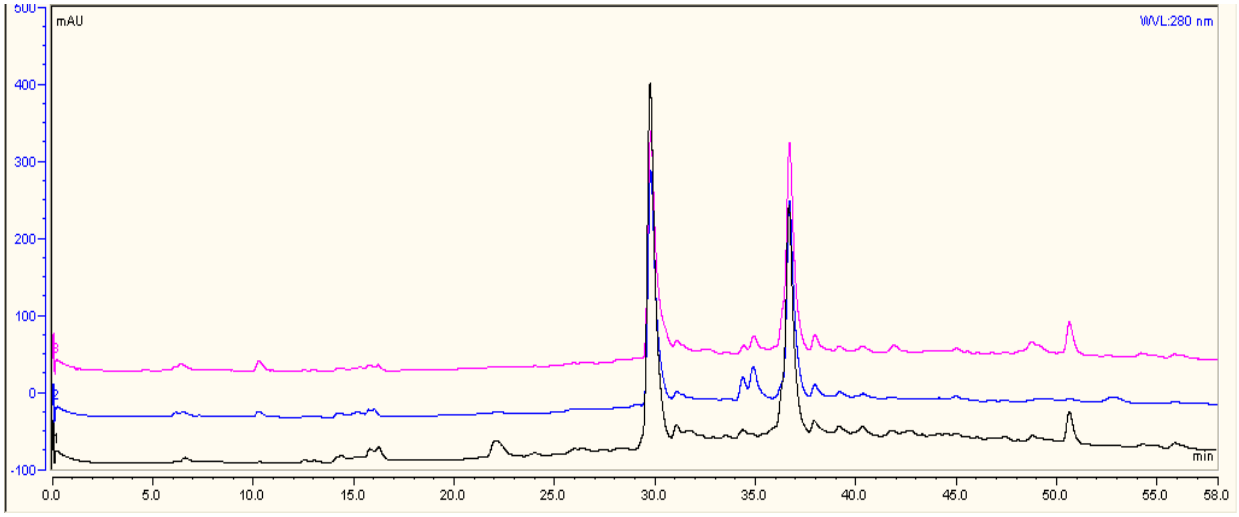


Figure 22 HPLC traces for exudates from *Arabidopsis* seedlings at 18 d treated for 48 h with H_2O (black), 25 µM H_2O_2 (blue), 50 µM H_2O_2 (pink). UV detected at 280 nm.

LC-MS results for H_2O_2 treatment of plants at 18 d show only minor differences, even after 48 h treatment with 50 µM H_2O_2. (Figure 22)

Organic extraction of root exudate

HPLC results for DCM root exudate extracts show no dominant components when compared with the aqueous extract on the same HPLC system. (Figure 24) Unlike organic extracts of root exudates from other plants (Chang et al. 1986, Czarnota et al. 2001), the organic extract of *Arabidopsis* seedling root exudate has no clear major component.

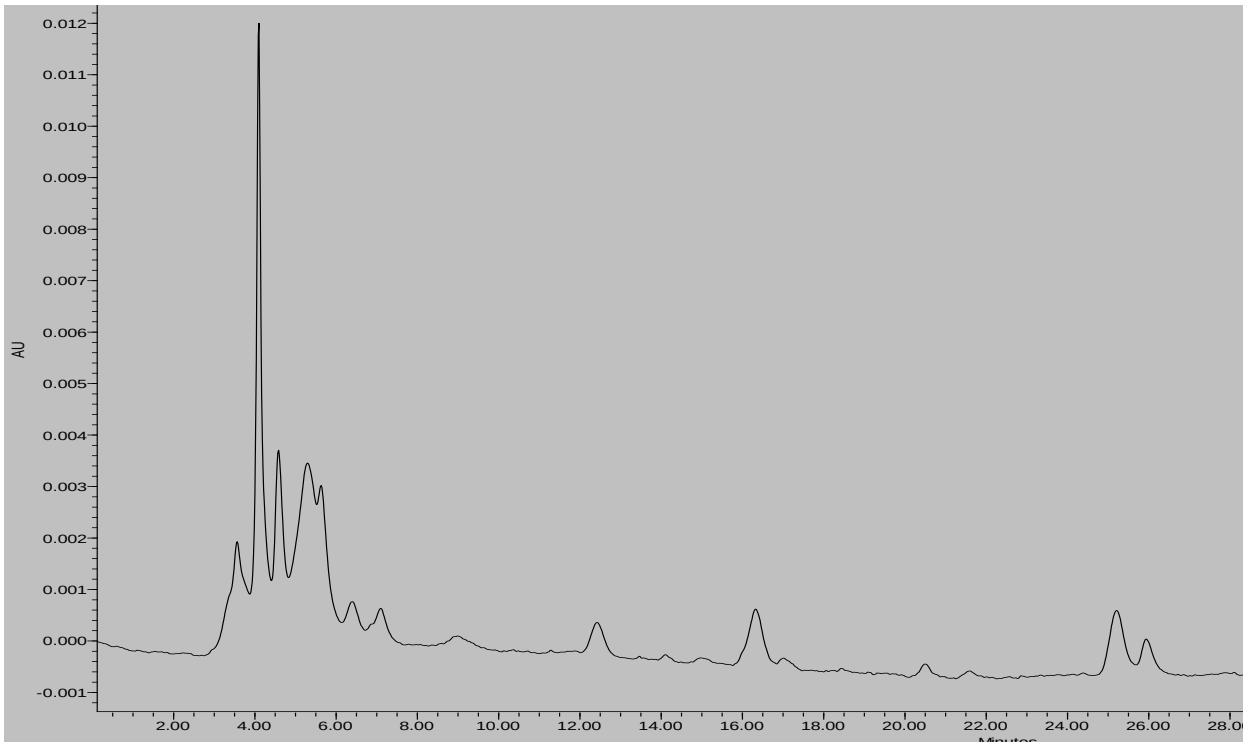


Figure 23 HPLC chromatogram for the organic extract of the root exudate from *Arabidopsis* seedlings treated with H_2O at 10 days. 0-40 min: 15-60% MeCN in H_2O with 0.1% TFA.

Effect of population density on root morphology and $\cdot O_2^-$ accumulation in the root tip

NTB staining increased with increasing plant density: 1 out of 7 seedlings from 20 seedlings wells stained, 4 out of 7 from 40 seedling wells, and 5 out of 8 from 60 seedling wells. (Figures 24-25) However, this increase in $\cdot O_2^-$ accumulation in the root tip was not significant by a Fisher's exact test ($p=0.18$). With 4 out of 8 seedlings from 20 seedlings wells had lateral roots, 5 out of 8 from 40 seedling wells, and 2 out of 8 seedlings from 60 seedling wells, there was a decrease in lateral root number with increased density; however, this was not significant by one-way ANOVA ($F=2.8$, $df=10$, $p=0.11$).

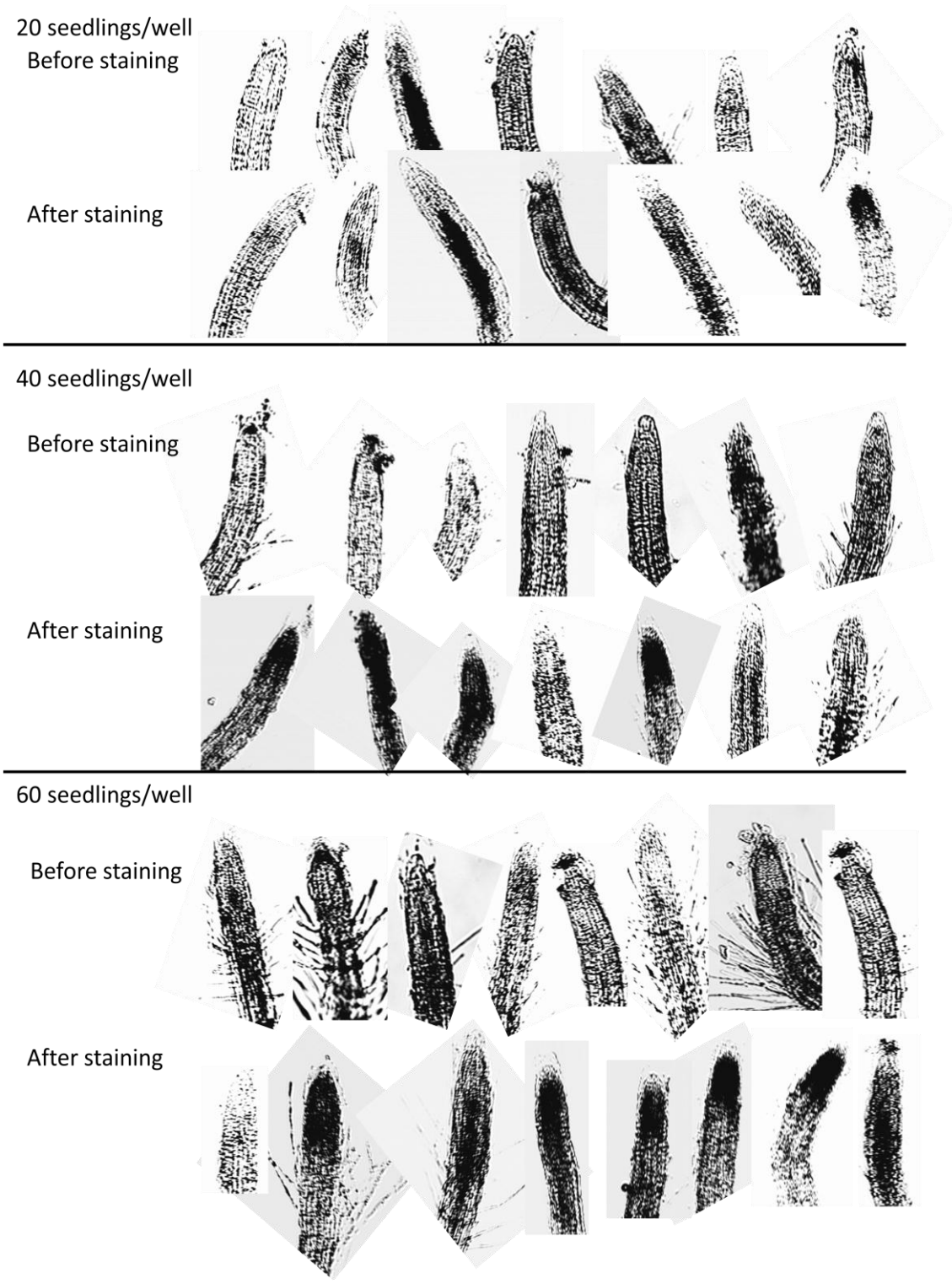


Figure 24 Root tips from seedlings grown with 20, 40, or 60 *Arabidopsis* seedlings per well in a 96 well plate, before and after staining for 15 min with nitro-blue tetrazolium (NBT).

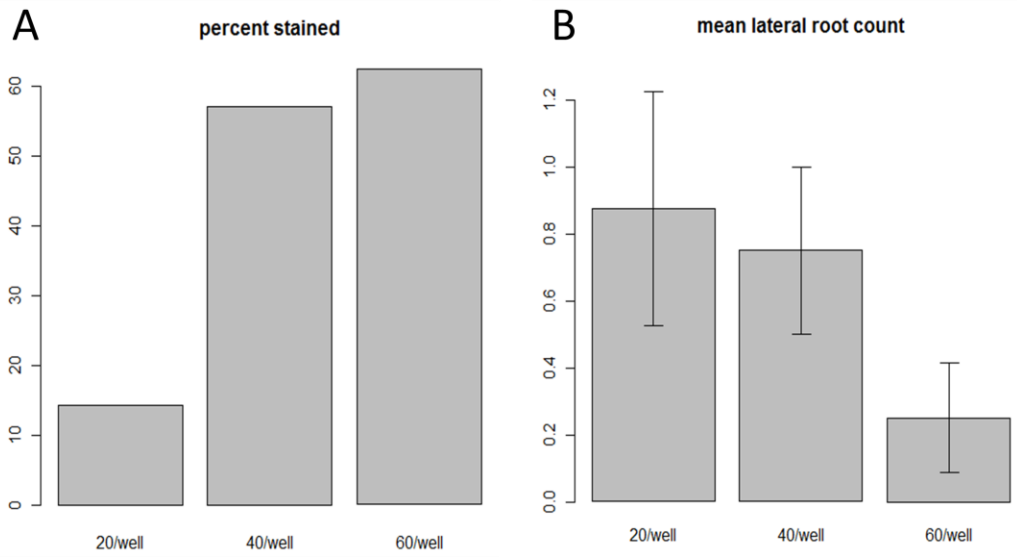


Figure 25 Percent of seedlings stained (A) and mean lateral root count (B) for plants in 20 seedling, 40 seedling, and 60 seedling wells. Error bars in B correspond to standard errors.

Effect of DMBQ on morphology

Plants grown with DMBQ had shorter roots (Trial 1: t=4.8, df=31, p<1E-4, Trial 2: t=2.6, df=42, p=0.01) and more lateral roots (Trial 1: p<1E-10, Trial 2: p<1E-11) than plants grown without DMBQ in both trials. (Figures 26 and 27) In trial 2 only, plants without DMBQ had slightly wider leaves (Trial 2: t=2.4, df=49, p=0.02). Otherwise, there was no effect of DMBQ on hypocotyl length (Trial 1: t=-0.63, df=39, p=0.53, Trial 2: t=0.05, df=59, p=0.96), cotyledon width (Trial 1: t=1.7, df=35, p=0.09), or root hair density (Trial 1: t=0.78, df=44, p=0.44).

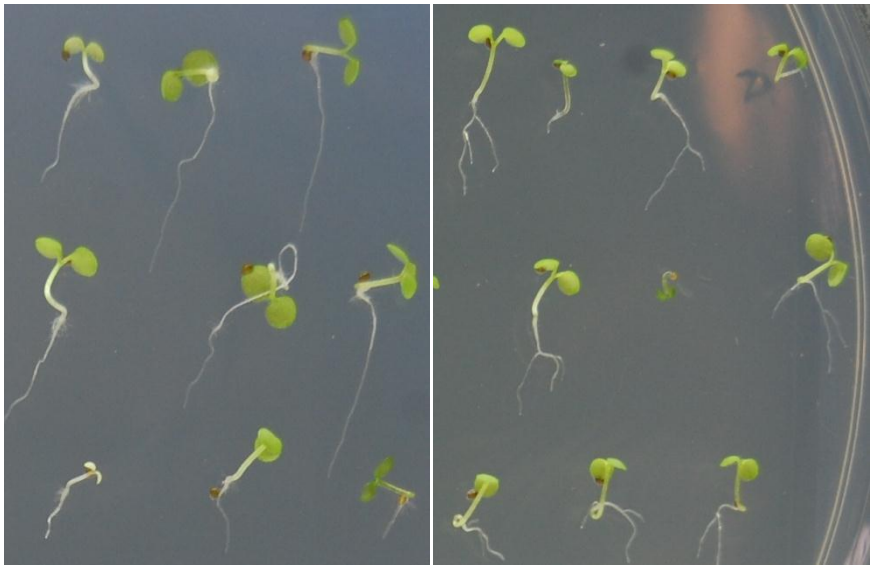


Figure 26 Plants grown without DMBQ (left) and with 50 µM DMBQ (right). Plants in DMBQ have more lateral roots.

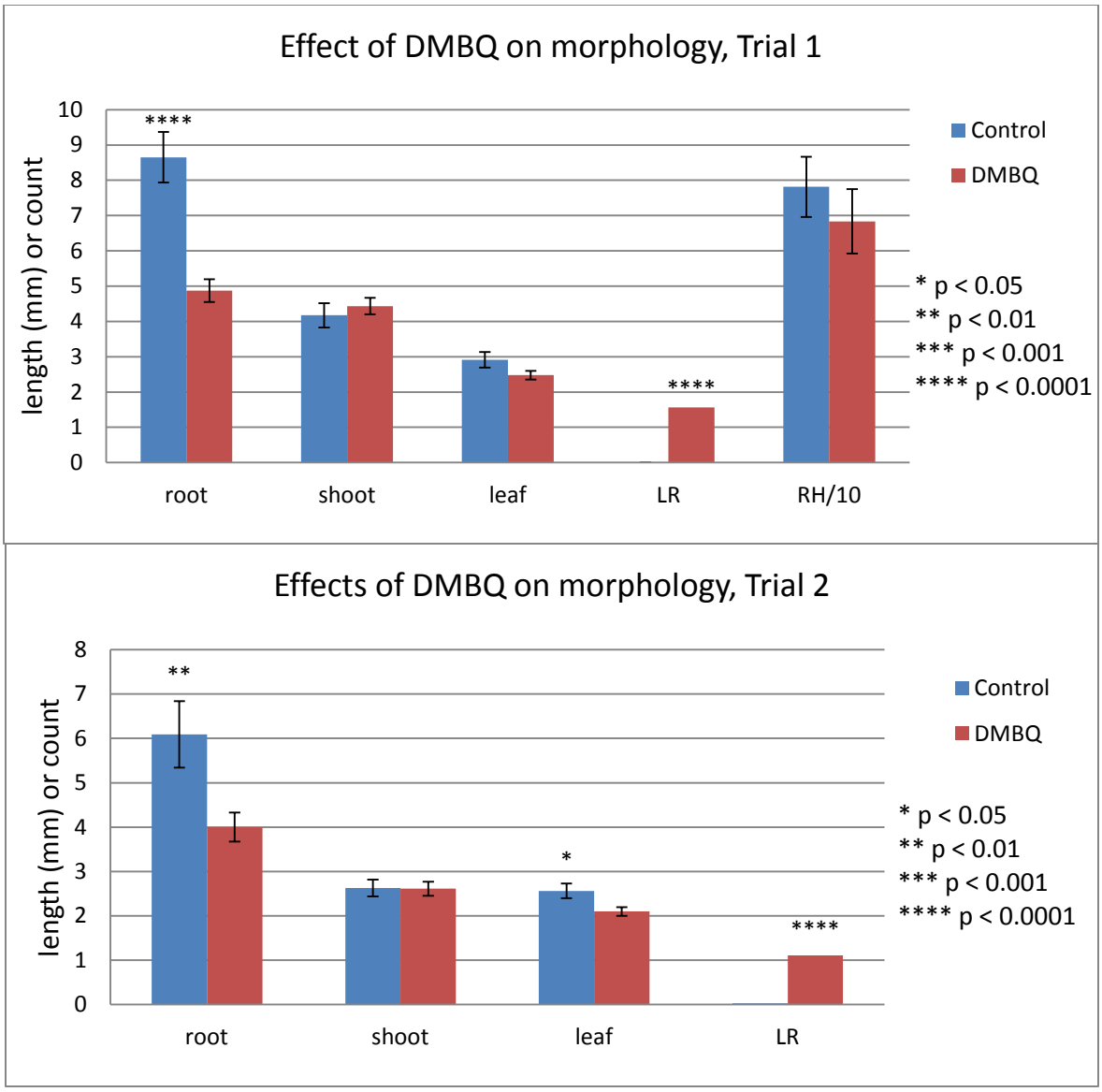


Figure 27 Change in root length, shoot length, leaf length, lateral root count (LR), or root hair count divided by 10 (RH/10) in 10 d old *Arabidopsis* seedlings grown with (blue) or without (red) 50 µM DMBQ. Error bars show the standard errors.

Effect of population density on morphology

Arabidopsis thaliana seedlings were grown at 3 or 5 different densities, with several plates per density treatment. In two separate trials, plate identity had little effect as a random effect in the

mixed effects model. Very few lateral roots were found in either trial, so no obvious phenotype emerged from these data. In trial 1, five different densities were tested (Figure 28), and no root morphology traits were affected by distance between plants (df=114, radicle: slope=0.32, p=0.28, hypocotyl: slope=-0.12, p=0.20, and cotyledon: slope=0.32, p=0.28). (Figure 29) Based on the intercepts calculated from the linear mixed effects model, typical root length was 6.3 mm ($p<1E-4$), shoot length was 3.2 mm ($p<1E-4$), and width across leaves was 1.8 mm ($p<1E-4$). In contrast, in the second trial, both root length and width across leaves increased with increasing distance between plants (df=98, radicle: slope=0.89, p=0.02, cotyledon: slope=0.26, p=0.003). (Figure 30) As in trial 1, shoot length was not affected by density (slope=-0.15, p=0.22). Since the only difference in experimental setup between the two trials was that trial 2 only included density groups 1-3, the same linear mixed effects models were used to test the effect of density on morphology in just groups 1-3 in trial 1. These were also not significant, indicating that plant morphology can vary significantly between trials.

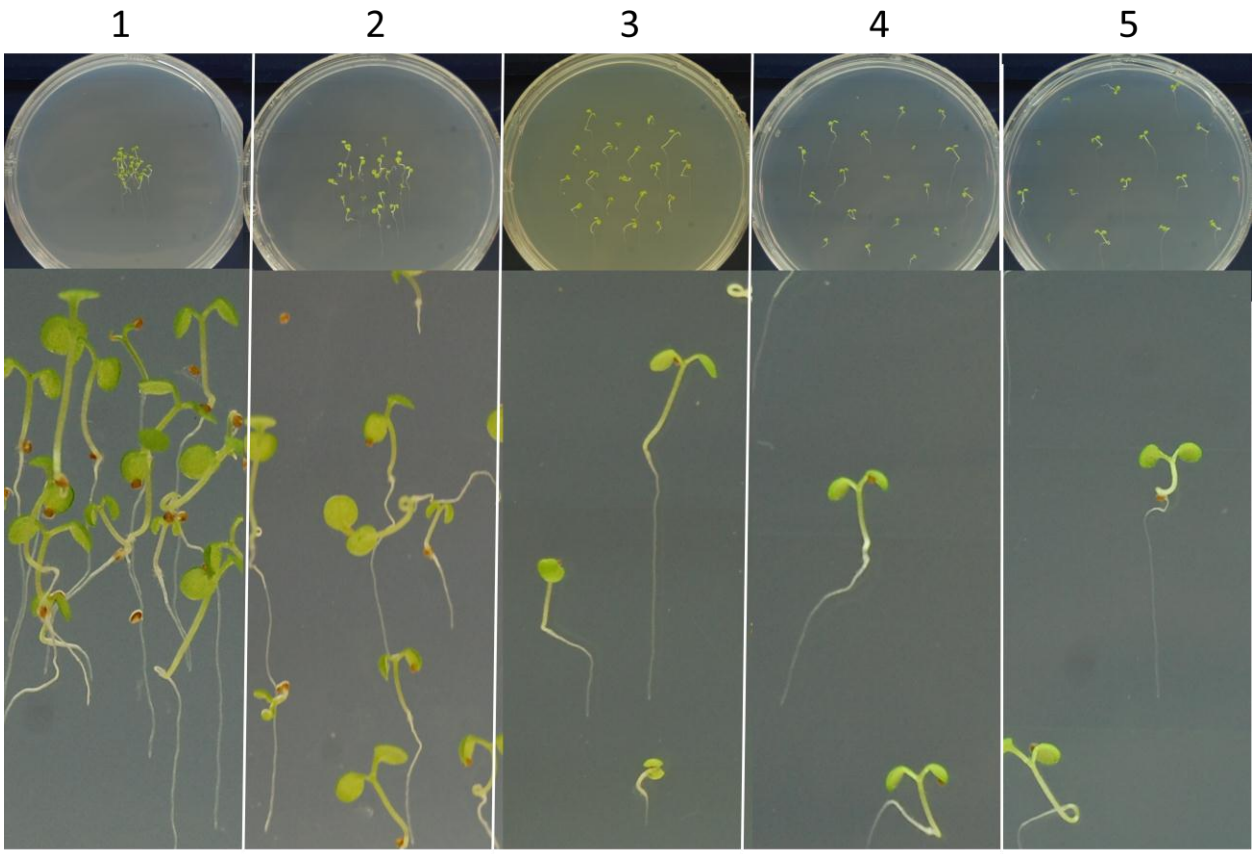


Figure 28 *Arabidopsis* seedlings grown in hexagonal pattern (see Figure 4) with increasing distance between plants. Views of whole Petri dishes (above) and expanded views of the same plates (below).

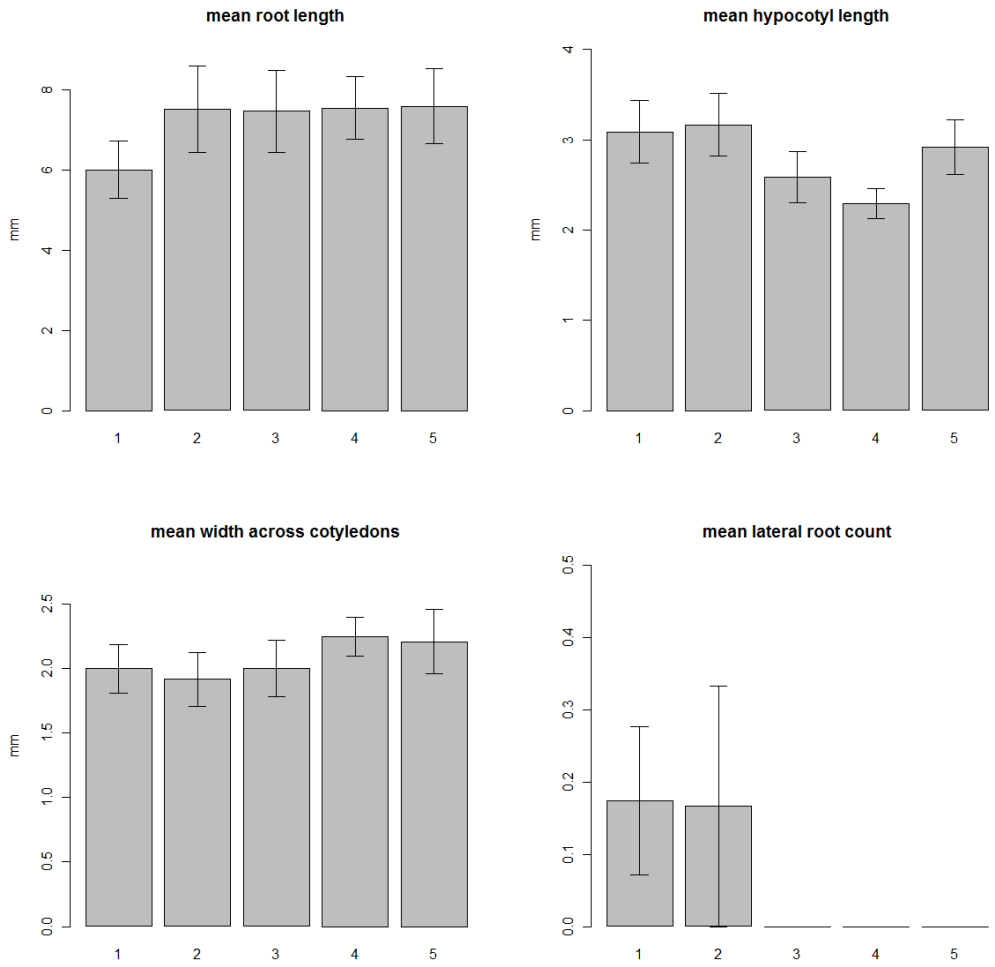


Figure 29 Plant morphology at different population densities, trial 1. Groups 1-5 represent increasing distance between plants (see Figure 24). Error bars correspond to standard errors. Distance between plants had no significant effect on root length, hypocotyl length, and width across cotyledons. Statistical significance was not tested for lateral root count data.

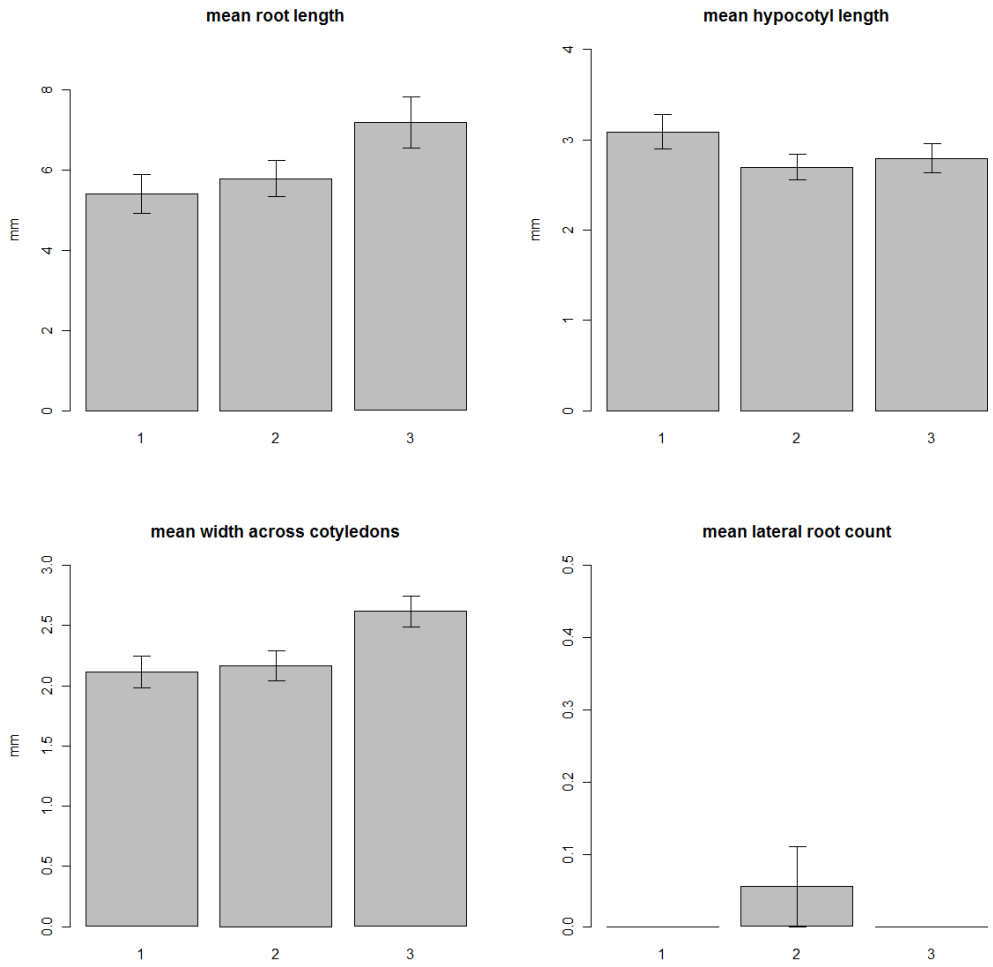


Figure 30 Plant morphology at different population densities, trial 2. Groups 1-3 represent increasing distance between plants (see Figure 24). Error bars correspond to standard errors. Root length and width across cotyledons increased with distance between plants. Distance between plants had no effect on hypocotyl length. Only one plant had lateral roots.

Discussion

In this study, the two steps of semagenesis in nonparasitic plants were considered: (1) the use of external reactive oxygen species (ROS) and plant surface peroxidases to oxidize phenols in the pectin in the plant cell wall and (2) the response of plant roots to quinones. In the first step, treatment with H_2O_2 affected the root exudate of *Arabidopsis* seedlings, but only after 48 hours of treatment. Neither 2,6-dimethoxy-*p*-quinone (DMBQ) nor methoxy-benzoquinone (MBQ) were found to be induced by H_2O_2 treatment at any of the concentrations or time points. Any simple oxidation of cell wall phenols to quinones should have occurred quickly, possibly by the 6 h timepoint. Based on HPLC traces of dilutions a DMBQ standard and accounting for the concentration of exudate during extraction, this procedure's minimum detection is 0.01 µM DMBQ in each 5 mL well for 18 plants. While this seems a low concentration compared to the minimum effective treatment of DMBQ, ~10 µM (Palmer et al. 2009), since semagenesis only occurs in a small area around the plant, any release of DMBQ that would accumulate in the area around the root would be diluted over 5 mL in this experiment. Thus, it is possible that quinone release was under the detection limit for this experiment. Moreover, the degree of methoxylation of the phenols in *Arabidopsis* cell walls is not clear; some quinone other than DMBQ or MBQ might have been in the exudate and the molecular ion of this quinone missed in the analysis of the LC-MS data.

However, it is also possible that the necessary oxidation of phenols does not occur at a relevant scale in dicots, given the concentration of phenols in dicot walls compared to *Striga* host grass walls (around 0.1% in dicots compared to 1-5% in grasses) (Lozovaya et al. 1999, Vogel 2008). Small stresses in the soil may be required to trigger release of a relevant amount of phenols from dicots. Wounded dicots release large amounts of phenols, which are picked up by

the pathogenic bacterium *Agrobacterium tumefaciens* (Stachel et al. 1985). A lesser stress, perhaps nutrient deficiency or drought, might trigger the release of enough phenols to trigger semagenesis. In both cases, resource competition might be an issue, and it would be advantageous for the plant to avoid another plant. Studies where nutrients were provided in excess argue against a nutrient deficiency-dependent mechanism (Caldwell et al. 1996, Caffaro et al. 2011).

The change in exudate in the 48 h treated plants may be a stress response to H_2O_2. The wound response in tobacco can take 2 days or more (Liu 2012), so this is a reasonable timescale for a stress response. The increase in some components and decrease in others is consistent with a complex stress response, and the two up-regulated compounds, camalexin and 3-indole-carboxylic acid, match the current understanding of *Arabidopsis* stress responses. Camalexin is released from *Arabidopsis* in response to pathogens or abiotic oxidative stress (Zhao et al. 1998, Hagemeier et al. 2001, Bottcher et al. 2009). Compounds like 3-indole-carboxylic acid have been found at the same time in the root exudate, and are from the same indole-3-acetonitrile (IAN) pathway (Bottcher et al. 2009). Interestingly, another study found that, of 10 main extract components of *Pseudomonas* infected *Arabidopsis* leaves, 2 were kaempferols with sugar moieties and 5 were tryptophan derivatives, including indole-3-carboxylic acid and camalexin (Hagemeier et al. 2001). The kaempferol concentrations were not affected by the infection, whereas all five tryptophan derivatives were strongly induced. In this study, kaempferitrin was also found in all 48 h treatments of 10 d old plants, and, given the peaks eluting at the same time in all the other HPLC traces, kaempferitrin may also have been in the 6 h treatment and 24 treatment exudates. That the clear stress response to 48 h treatment with H_2O_2 was not repeated in the 18 d old plants may reflect an altered defense strategy or a change in the chemical

environment around the roots. The 18 d old untreated root exudate is markedly different from the 10 d old untreated exudate (Figure 22), and the 18 d exudate may not have the precursors for the induced compounds found in the 10 d exudate.

To test the second step of semagenesis, *Arabidopsis'* physiological and morphological response to density was compared to its response to DMBQ. In the physiological experiment, the increase in NTB staining with increased density matched the increased staining with DMBQ treatment; however the change with density was not significant. The number of lateral roots decreased, but this was also not significant. In this experiment, the lowest tested density was relatively high, and the lack of a solid medium to grow on may also have caused some stress. Thus, no density-dependence was observed on the physiological level, either because *Arabidopsis* has no significant increase in $\cdot O_2^-$ concentration in the root tip or change in lateral root number or because the baseline level of stress was too high.

In the morphological experiment, DMBQ treatment resulted in clear increases in lateral root count and decreases in root length, which is consistent with the decrease in root length reported by Palmer et al. (2009) In this case, the decrease in root hair density was not significant, however. This is consistent with the root exudate removal phenotype seen by Caffaro et al (2011), who observed that the addition of activated carbon to adsorb small molecules like DMBQ caused a decrease in lateral root growth and an increase in root length, resulting in more contacts between plants. In their experiment, untreated plants were able to avoid each other by growing more lateral roots and shortening root length. This similar phenotype with DMBQ treatment is consistent with a quinone-dependent process like semagenesis controlling the avoidance phenotype.

Despite the broad range of densities tested in the morphological test, density-dependent morphology was not consistent between trials. In the second trial, where density was a significant factor, increased density was correlated with shorter roots, which is consistent with the DMBQ phenotype. Plants appeared to have contact with each other at the higher densities (Figure 28); however, the avoidance phenotype seen by Caffaro et al was not apparent. When there was a density-dependent phenotype, the *Arabidopsis* morphological and physiological phenotype matched the DMBQ-treated phenotype, however the inconsistent results mean it is not clear that *Arabidopsis* has density-dependent morphology or ROS expression at this age.

Given the recent results published by Caffaro et al, the most promising future experiment is a repeat of the same experiment with a 20 day growing period. In the Caffaro experiment, 20 day old, almost mature *Arabidopsis* had the time to grow significantly different root architecture with or without activated carbon. In this experiment, the *Arabidopsis* only had 10 days to grow; a difference in lateral root growth might have been apparent after 10 more days. Also, given the different root exudate in *Arabidopsis* at 20 d (Narasimhan et al. 2003), a different set of compounds, including a larger number of phenols, are available later in the plants' life, to drive the avoidance phenotype.

Once a morphological and physiological phenotype is established, the mechanism of semagenesis can be tested by applying an inhibitor for one of the two steps of semagenesis. ROS scavengers such as potassium iodide (KI) or bovine catalase should be able to remove oxidizing agents and prevent the oxidation of phenols to quinones. Quinone perception could be prevented by adding cyclopropyl-benzoquinone (CPBQ) to the growth medium. CPBQ was designed as an irreversible inhibitor for DMBQ in *Striga* semagenesis (Smith et al. 1996, Zeng et al. 1996) and has also been shown to inhibit the effect of DMBQ on *Arabidopsis thaliana* (Palmer et al. 2009).

If semagenesis is indeed responsible for some of the avoidance phenotype, adding one of these inhibitors should have the same effect as the addition of activated carbon or growth at lower density. The exudate-dependent phenotype observed by Caffaro et al. was seen with individual plants, meaning that, in their experiments, individual *Arabidopsis* are able to detect their own exudate. In this type of agar-based experiment, one plant's exudate may build up in the medium and saturate the plant's ability to recognize exudate. In this case, increasing the population density will not establish a density-dependent phenotype. Rather, mechanism-based inhibitors will be required to understand a plant's detection of itself.

A final possibility is that semagenesis occurs primarily between dicots and monocots. As illustrated by *Striga asiatica's* successful detection of its monocot hosts, monocots can respond to a ROS signal by producing sufficient quinones to induce a semagenesis response. Dicots are less likely to be able to produce quinones because their base level of pectin phenols is much lower (Lozovaya et al. 1999, Vogel 2008), and, in this study, dicot *Arabidopsis* seedlings did not produce a detectable amount of quinones. While they respond to the first signal of semagenesis, monocots have only a minimal response to quinones. The three dicots *Nicotiana tabacum* (tobacco), *Ocimum basilicum* (basil), and *Arabidopsis thaliana* all respond to quinone treatment, but *Striga*'s two monocot hosts, *Zea mays* (maize) and *Sorghum bicolor* (sorghum) have very little response to DMBQ (Palmer et al. 2009). Since monocots respond more strongly to the first signal of semagenesis (ROS), and dicots respond more strongly to the second signal (quinones), this signaling process may occur primarily as a way for dicots to detect monocots, either as a method for a parasitic plant to detect its monocot hosts, or as a way for nonparasitic dicots to avoid their monocot neighbors.

Whether semagenesis may be relevant among dicots when they are stressed or at a different stage in their development is unclear. Given the lower pectin phenylpropanoid percentage in dicots, semagenesis between dicots may rely on an additional stimulus to trigger the release of additional phenols from the cytoplasm. Semagenesis may serve a complex function in dicots, detecting monocots under normal conditions and dicots under stressful conditions or at certain times in development. Thus, just as density- and environment-dependent quorum sensing molecules can dictate the behavior of populations of bacteria, a signaling mechanism such as semagenesis may coordinate the growth of an entire population, in the context of biotic or abiotic stresses in the environment.

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