ENVIRONMENTAL EFFECTS ON SEEDLING IDENTITY RECOGNITION

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By

SINAH K. LEE, B.Sc.

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AUTHOR: Sinah K. Lee, B.Sc. (McMaster University)

SUPERVISOR: Dr. Susan A. Dudley

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Abstract

Adaptive plasticity is important for plants. For example, plants can respond to light by elongating their stem or to low nutrients by increasing root proliferation. Kin recognition in plants is a type of phenotypic plasticity, but to relatives versus non-relatives. Recently kin recognition has been demonstrated for the first time in a plant species using the annual plant *Cakile edentula*. Plants responded to kin recognition only when sharing a pot where the roots could interact. This indicates that plants were responding to the identity of their neighbours through touch, a volatile chemical, or a soluble chemical.

Dr. Harsh Bais has developed a methodology to test whether kin recognition occurs through plant exudates without physical contact. Arabidopsis seedlings responded differentially to an exudate source belonging to kin or strangers. Using this methodology we proposed to determine if *Chenopodium album* seedlings and *Arabidopsis thaliana* seedlings respond to kin recognition from exudates sources under different environmental conditions. For Chenopodium seedlings we manipulated the nutrient treatments, as per a previous experiment where juvenile Chenopodium demonstrated kin recognition under differing nutrient treatments. For Arabidopsis seedlings we changed the irradiance and quality of light for seedlings exposed to different exudate sources to see if it changed the response. The goal was to find a repeatable, reliable assay for kin recognition.

Chenopodium album and *Arabidopsis thaliana* both demonstrated kin recognition. Chenopodium responses were consistent with juvenile plants, where seedlings in scarce nutrients displayed an increased root:shoot ratio compared to those in regular nutrients

which had a low root:shoot ratio and were overall larger seedlings. Seedlings placed in exudates belonging to kin in regular nutrient conditions had a higher root:shoot ratio than those exposed to stranger exudates, indicating that those in stranger exudates had more aboveground growth. Arabidopsis displayed inconsistent kin recognition, with responses varying between different environmental conditions and among families. There was significant genetic variability among families which resulted in a change in response to exudate source within trials and between trials.

Both *Chenopodium album* and *Arabidopsis thaliana* respond to an exudate source and demonstrate kin recognition. However, more work needs to be done to find a reliable, repeatable assay for Arabidopsis seedlings.

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Introduction

The seedling stage is an important period of development, because the decisions made can determine the outcome of a plants' overall fitness (Leck 2008). A seedling emerging from the seed is bombarded with information on its new environment. It makes development decisions on the basis not only of its current conditions but also anticipated future conditions (Novoplansky 2009). Environmental cues provide the seedling with several kinds of information; is it shaded (Smith 1982, Smith et al. 1990)? Are there any aboveground neighbours nearby (Ballaré et al. 1987, Ballaré et al. 1990)? Are there nutrients and water available (Casper and Jackson 1997, Lamb et al. 2007)? Are there neighbouring roots belowground that may deplete resources (Maina et al. 2002, Murphy and Dudley 2007, Gersani et al. 2001)? Whose roots are they (Mahall and Callaway 1991, Falik et al. 2003, Dudley and File 2007)? Seedlings make developmental changes based upon these environmental cues. The seedling will also make decisions that affect morphology; does it elongate the stem? Produce more roots? Grow towards a limiting resource such as nutrients or light? All of these different choices based on the surrounding environment can lead to success or failure. Novoplansky (2009) concisely summarized these potential issues that "plants need to know how to pick their battles wisely".

Phenotypic Plasticity

Phenotypic plasticity is the ability of a plant of one genotype to produce several different phenotypes in different environments. This ability to change phenotypes can be adaptive to spatial variations within the environment (Bradshaw 1965, Schlichting 1986, Dudley and Schmitt 1996). Seeds dispersing from a maternal plant do not always land in the same environment as the parent, favouring the ability to survive in multiple varying environments. For example, shade avoidance is an adaptive phenotype in response to aboveground competition for light that allows a plant to avoid shading from another plant by elongating the stem (Dudley and Schmitt 1996). Phenotypic manipulation can be used to measure the fitness consequences of different phenotypes under different environments; for example, plants with elongated stems did better in high density stands whereas those suppressed with shorter stems did better in low density stands (Dudley and Schmitt 1996). Belowground, if nutrient availability is low, many species increase biomass allocation to roots, and decrease allocation to leaves and stem (McConnaughay and Coleman 1999). These examples of adaptive plasticity demonstrate how plants can survive in differing environmental conditions

However, there may be costs and limits to phenotypic plasticity (DeWitt et al. 1998); otherwise, why not have one infinitely plastic organism (Whitlock 1996)? Limits to plasticity were demonstrated with *Plantago lanceolata*. This species is found in both hayfields and pastures of Europe, where they exhibit different phenotypes in each environment both because of adaptive plasticity and because of genetic differentiation

resulting in local adaptation (Van Tienderen 1990). The hayfield plants have tall erect thin leaves in order to compete with the tall hay, whereas the pasture plants are shorter, with thicker leaves and have more reproductive structures. If plants from the two populations are transplanted into each other's environment, they are able to change phenotypes; however, they are not as fit as in their home environments as no genotype could change sufficiently to match the other environment (Van Tienderen 1990). The optimal responses for an environment may not be able to be achieved by some genotypes.

There are costs associated to being plastic, and having the ability to adapt to several environments. One such cost is that a plant may not be as successful as one adapted to a particular environment, "a jack of all trades is master of none"(Whitlock 1996, DeWitt et al. 1998). Plasticity is selected for in a spatially variable environment, where a plant is likely to find itself in differing possible environmental conditions (Via and Lande 1985). Plasticity is not selected in a stable environment as there is no advantage to having differing phenotypes; either the environment doesn't vary or it fluctuates too quickly for any change in development to be advantageous (Whitlock 1996, DeWitt et al. 1998). Phenotypic plasticity is selected in environments that are prone to change and being plastic increases the likelihood that the plant will survive and reproduce regardless of the environment it finds itself in (Whitlock 1996, DeWitt et al. 1998, Schlichting and Smith 2002).

Plants have mechanisms in place to acquire information; plants respond to different cues that will cause a signal transduction cascade that causes changes in metabolic rates and development accordingly (Aphalo and Ballaré 1995, Aphalo et al. 1999). This

information that is acquired from these cues are very important in the acquisition of resources and competition (Aphalo and Ballaré 1995).

Light and aboveground competition

Plants use light not only for photosynthesis but also to provide them with information on their surrounding environment (Aphalo and Ballaré 1995, Smith 1995, Ballaré 1999, 2009). Light sensing in plants is a complex system, with multiple signals and multiple sensory systems acting in tandem (Ballaré 1999). A newly emerged seedling can assess its environment through light cues and react quickly in response. Light is needed for photosynthesis which uses CO_2 to create photosynthate, sugars, etc. (Taiz and Zeiger 2002). Sensing the light environment, especially whether there are any neighbouring plants, can allow the seedling to adapt to that environment (Smith et al. 1990). Plants can react to neighbours within as three days of being placed near other plants, before they even become shaded by another plant (Ballaré et al. 1990). Within the hour of being shaded by another plant there are changes in gene expression occurring (Schlichting and Smith 2002). They do so by sensing a reduction in the red:far-red ratio of light (R:FR), which is caused by the chlorophyll present in neighbouring plants absorbing red light (Ballaré et al. 1987, Ballaré et al. 1988).

Plants have photoreceptors, which are proteins, containing pigments, that absorb light from specific parts of the light spectrum, which triggers a biochemical cascade (Raven 1999). Photoreceptors absorb light at: red (660 nm) (R) and far red (730) (FR)

light and blue light (300-500nm) (Smith 1982). Phytochromes are photoreceptors that absorb red and far red light. There are two forms of phytocromes; Pr, which absorbs red light and is converted to Pfr, which absorbs far red light, which is then converted back to Pr (Smith 1995). In Arabidopsis there are 5 known phytochromes (phy A-E). PhyA and PhyB react to differing light conditions as PhyA is light-labile and PhyB is light-stable. In PhyA Pfr is unstable and so when there is a lot of red light and little far red light (which would convert Pfr back to Pr), Pfr becomes degraded. This does not occur in PhyB and Pfr is not degraded (Smith 1995). The amounts of Pfr and Pr give the plant critical information on the environment. PhyA and PhyB are involved in control of seed germination, de-etiolation, regulation of stem elongation and flowering time (Smith 1995, Whitelam and Devlin 1997). PhyA is more sensitive and responds to FR light and some R light while PhyB is more sensitive and responds to R light and changes in the R:FR ratio. PhyC, D and E are also light-stable and have control over some of the same mechanisms as PhyA and PhyB providing some redundancy if those phytochromes are inactive. The plant can survive successfully with only PhyC-PhyE (Smith 1995, Whitelam and Devlin 1997, Ballaré 2009).

The ratio of R:FR in the light spectrum provides the plant with important information. Chlorophyll absorbs light strongly in red, but not in far-red part of the spectrum. Therefore light low in red light indicate that there are other leaves nearby. Plants are able to sense low R:FR and so use it as a cue for early detection of competition (Ballaré et al. 1990, Smith et al. 1990, Smith 1995). This results in a shade avoidance response, including stem elongation, thereby increasing the plant height to obtain light

above other plants (Dudley and Schmitt 1995). Plants in a dense stand respond by elongating their stems and allocating more biomass to stems than leaves (this response occurs before actual shading (Ballaré et al. 1987, Ballaré et al. 1988, Dudley and Schmitt 1996).

Much experimental work has been done to observe plants' response to changes in R:FR by artificially raising and lowering the R:FR using shades. CuSO4 solution can be used to change the light quality. It increases the R:FR ratio as it passes through the solution by absorbing FR light (Ballaré et al. 1990, Smith 1995). CuSO4 has been used to 'fool' a plant into thinking it has no neighbours and suppressing the shade avoidance response (Ballaré et al. 1990, Dudley and Schmitt 1996). Similarly, a simulated vegetation shade made with one part Solvaperm Yellow G and four parts Hostaperm Violet RL 02 (both from Hoeschst) (Dudley and Schmitt 1995), mimics the light reflected from other leaves, absorbing the red light to lower the R:FR ratio, cueing a shade avoidance response (Lee 1988, Dudley and Schmitt 1995, Sleeman and Dudley 2001).

Blue light also gives the plant information that cues responses. Blue light photoreceptors in plants, the cryptochromes and phototropins, absorb blue and ultraviolet-A light (Cashmore et al. 1999, Briggs and Christie 2002). Cryptochromes, which are found in both plants and mammals, are involved in circadian rhythms and are involved in stem elongation in low light (Cashmore et al. 1999, Ballaré 2009). Phototropins on the other hand are only found in plants, they respond to directional light and are involved in phototropism, chloroplast movement and stomatal opening (Briggs and Christie 2002,

Ballaré 2009). Phototropins allow plants to sense directional light, and grow towards higher light, such as gaps in the canopy (Ballaré 2009).

Belowground competition; nutrients, water and kin recognition

Belowground, plants compete for nutrients and water. Herbaceous plants are made up of 80-95% water, and it is essential for transpiration and photosynthesis. Nitrogen is one of several crucial mineral nutrients that are used in the formation of many different essential carbon compounds used by the plant (Taiz and Zeiger 2002). The "balanced growth" hypothesis suggests that plants allocate more resources to the organs that acquire the most limiting resource. For example, plants produce more roots when nutrients are the limiting resource (Shipley and Meziane 2002). When plants are in competition, other plants have the potential to reduce the available water and nutrients (Casper and Jackson 1997). When nutrients are depleted by neighbours, plants will increase their root proliferation in order to seek out more nutrients thereby competing for nutrients with their neighbours (Schenk 2006). However, plants in a shared pot, allocated more to belowground biomass than plants who were alone in a pot, even though the nutrient availability per plant was kept constant (Maina et al. 2002, Murphy and Dudley 2007, Gersani et al. 2001). These results suggest that plants can anticipate future belowground competition.

There are several different tactics a plant can use in response to sensing a potential competitor; competitive avoidance, confrontation and tolerance (Novoplansky 2009). Competitive avoidance is seen in seeds with increased dormancy and even shade avoidance, which can also be competitive competition, but in this case, plants elongating stems to escape the competition for light (Novoplansky 2009). For example, *Portulaca oleraea* grows away from neighbouring plants and towards high R:FR (Novoplansky et al. 1990). Competitive confrontation is head on competition with another plant, increasing shoot allocation to aboveground competition and root allocation in response to belowground competition. And lastly, competitive tolerance is where plants do the best with the resources available to them without making direct competitive overtures (Novoplansky 2009).

A plant can sense roots belonging to a neighbour, differentiating them from their own roots (self/non-self recognition) (Mahall and Callaway 1991, Falik et al. 2003), and even potentially distinguishing the identities of their neighbours. Desert shrubs react differently to roots depending on the species of shrub (Mahall and Callaway 1991, 1992). It has been demonstrated in three species of plants that they can recognize their own kin from strangers (Dudley and File 2007; Murphy and Dudley 2009; Dudley, Lee et al. unpublished data). Kin recognition can be viewed as another form of phenotypic plasticity, in this case, to relatedness; the identity of the neighbour can change the phenotype expressed. Kin selection should favour reduced competition, and some species of plants have been shown to have higher fitness when sharing space with their siblings (Donohue 2003). With reduced competition, all of the plants have a higher fitness from

the reduced costs of competition. However, a stand of genetically similar plants may not be able to access resources as efficiently as a stand with more genetic variability (Waldman 1988, Cheplick and Kane 2004). On the other hand, some plants may show no kin recognition or species recognition. Rametes (clonal plantlets attached by a stolon) of *G. Hederacea* and *F. Vesca* were grown with either rametes attached or disconnected, and with different genotypes or different species (Semchenko et al. 2007). *G. Hederacea*r displayed an avoidance pattern, growing away from roots regardless of the identity of the neighbouring plant or if they were connected or not. *F. Vesca* was unresponsive, growing equally towards and away from any other roots. This response may be species specific, as plants with rametes, depending on the length of the stolon, will either grow very close or further away from a connected plant and may have evolved a strategy to match (Semchenko et al. 2007).

Why have kin recognition? Hamilton's Law shows that genetically related individuals will behave altruistically when the benefit to the recipient outweighs the cost to the donor (Hamilton 1964, Waldman 1988, West et al. 2002). There are various benefits to recognizing kin. In *Cakile edentula*, plants growing in a stand of siblings had a higher fitness than plants growing in a stand of stranger (Donohue 2003). Plants who are grown with siblings may decrease competition for shared resources and avoid a tragedy of the commons situation, where plants sharing resources increase competition to the detriment of all (Hardin 1968, Gersani et al. 2001). Species recognition can also be beneficial, as plants who are adapted to growing with specific species can compete successfully (Weinig 2000).

Exudates

Plants were only able to recognize their kin if the roots were within the same pot (Dudley and File 2007, Murphy and Dudley 2009). Exudates are a plausible mechanism for identity recognition. Plants interact with their environment by exuding chemicals from the roots into the surrounding soil, thereby affecting the abiotic environment and surrounding organisms, such as insects, microbes and other roots. They can exude compounds such as amino acids, organic acids, sugars, phenolics and other secondary metabolites as well as mucilage and proteins (Bais et al. 2004, Bais et al. 2006). These exudates can play a large part in the interactions with other plants. Allelopathy is an example of a negative interaction due to plant exudates. Some plants can exude chemicals, such as phytotoxins, as a defensive mechanism to compete with other plants, by reducing competitor growth and survival (Bais et al. 2004, Bais et al. 2006).

Seedlings have been found to exude as much as 40% of their carbon from their roots into the soil (Badri and Vivanco 2009). Environmental effects, such as light, can affect plant exudates. Plants can sense and respond to microbes leading to a change in the soluble chemicals that plants exuded. It is possible that plants could sense neighbouring roots, possibly even identity of neighbouring roots, and change the chemical suite being exuded (Badri and Vivanco 2009).

Previous experiments

Biedrzycki, Jilany et al (in review) showed that *Arabidopsis thaliana* seedlings responded to the relatedness of another seedling's exudates, and demonstrated kin recognition. These seedlings were only exposed to the exudates of another seedling, but they never physically came in contact, showing that it was the exudates that caused the kin recognition response, not a physical contact response (Biedrzycki et al. in review). Seedlings exposed to kin and stranger exudates were significantly different from each other. Seedlings exposed to stranger exudates had more lateral roots than those exposed to kin exudates. A shift from aboveground to belowground growth was seen, with a larger belowground competitive trait in the stranger treatment, which is consistent with other experiments showing shifts in biomass from aboveground to belowground in response to competition (Murphy and Dudley 2007, Gersani et al. 2001). An exudate inhibitor treatment, sodium orthovanadate (Na_3VO_4) was combined with the above experiment. Sodium orthovanadate alters the exudate profile by inhibiting membranal ATPases and ABC transporters (Loyola-Vargas et al. 2007). Seedlings exposed to the exudate inhibitor did not increase their lateral roots when exposed to strangers. The result was that it reduced the difference in the number of lateral roots the seedling produced in response to being exposed to exudates of either kin or strangers. Seedlings exposed to stranger or kin exudates had shorter roots than those only exposed to their own exudates, however this

result was unaffected by the sodium orthovanadate treatment (Biedrzycki et al. in review). This shows that there is a response to own exudates that differs from kin or strangers.

Dudley, Lee et al (unpublished data) looked at juvenile *Chenopodium album* plants and how they responded to neighbours, nutrients and kin. The greenhouse study consisted of two nutrient conditions (regular nutrients and scarce nutrients – $1/10^{th}$ of the regular nutrients), and within each nutrient condition there were kin/stranger and neighbour/solitary interactions. In the absence of belowground neighbours, nutrient availability did not affect morphology. When plants were with belowground neighbours, they allocated resources to leaves if belowground resources were not limiting, and to roots if belowground resources were limited. When nutrients were readily available, plants would increase their aboveground competitive ability when they were with strangers, but did not change their morphology with kin. When nutrients were scarce, plants increase their belowground competitive ability with both kin and strangers. This experiment demonstrated that *C. album* was able to recognize its kin, but that when resources were limited, the identity of the neighbour was not more important than obtaining the limiting resource (Dudley, Lee et al. unpublished data).

Now three studies have looked at kin recognition in juvenile plants, but only one has looked at kin recognition in other life stages. Biedrzycki, Jilany et al. (in review) looked at kin recognition in the seedling stage of Arabidopsis and found it does exhibit kin

recognition. However, I have found that those results depend on many unknown environmental effects, especially light. In this paper I look at different environmental conditions and how it affects kin recognition. We looked at kin recognition in Chenopodium seedlings and Arabidopsis seedlings. With Chenopodium we changed the nutrient treatments to mirror those of Dudley, Lee et al. (unpublished data), however with Arabidopsis we changed the light treatments. We asked the following questions: Do *Chenopodium album* seedlings respond to an exudate source (kin, own or stranger)? How does the response to an exudate source change under different nutrient conditions? Do these results parallel those found in juvenile Chenopodium? Do *Arabidopsis thaliana* seedlings respond to an exudate source (kin, own or stranger)? How do seedlings respond to changes in light irradiance and quality? Does the response to an exudate source change under these different light treatments? Are these results similar to those found by Biedrzycki, Jilany et al (in review)? Can we find an assay that is reliable and repeatable?

Materials and Methods

Study species

Arabidopsis thaliana is a weed from the mustard family and is found across North America, Europe and Asia. It is a small plant with a short generation time of only 6 weeks from germination to seed (Meinke et al. 1998). It was first studied by Laibach, and later by George Rédei. The accession, Landsberg, was obtained by Rédei from Laibach in Germany. Columbia (Col-0) is an accession started by Rédei from a non-irradiated Landsberg strain. The other lab accession Wassilewskija (Ws-0) came from Belarus (Passardi et al. 2007). It is the genetic model used for all plants as it has been sequenced. It has a small genome, only having five chromosomes and more than 20000 genes. There is a large collection of mutants available, as well as over 750 natural accessions (Meinke et al. 1998, Passardi et al. 2007).

Chenopodium album is a well studied invasive weed. It is often found in cultivated soils, such as fields and gardens, and in areas of high nutrients, especially nitrogen, which is why it is so often found flourishing in crop fields (Williams 1963, Li and Watkinson 2000). It is often found in patches and has a low dispersal rate, with the seeds falling around the maternal plant and therefore having a high probability of growing with siblings (Williams 1963). It is a strong aboveground competitor and much work has been done on its marked red to far red response (Causin and Wulff 2003). Chenopodium has been shown to tolerate excess nutrients and can respond quickly to an increase in nutrient

availability and can use carbon resources rapidly, which may be why it is such a successful weed (Li and Watkinson 2000)

Seed sterilization and plating

Seeds were placed in microfuge tubes for sterilization. First, 1mL of 70% Ethanol was placed into the tubes, which were inverted several times over 2 minutes. The Ethanol was removed and 1mL of the seed sterilizing solution (6.99mL sterile distilled water, 3mL bleach (store bought) and 10µl Tween 20) was placed into the microfuge tube. The seeds were left in the sterilizing solution for 10 min, being shaken occasionally. The solution was discarded and the seeds were rinsed, in sterile conditions, five times with sterile distilled water. 1mL of phytagar (0.1%) (Phytagel, Sigma), for Arabidopsis, and 1mL of sterile distilled water, for Chenopodium, was added to the microfuge tube and then placed in the refrigerator at 4°C for a set amount of time (overnight for Chenopodium, up to a week for Arabidopsis).

Seeds were later plated on solid Murashige and Skoog (MS) (Murashige and Skoog 1962) plates. For Chenopodium, plates were made up of 4.3g/L Murashige and Skoog Macro and Micronutrients (Caisson Laboratories) for regular nutrients plates, 0.43g/L for scarce nutrient plates, 0.103g/L Murashige and Skoog vitamin powder 1000x (Sigma), 10g/L sucrose (Bioshop) and for regular nutrient plates 8.5g/L phytoblend (Caisson Laboratories) and for scarce nutrient plates 6.5g/L phytoblend. For Arabidopsis, plates

were made up of 4.3g/L Murashige and Skoog basal salt mixture (Sigma), 0.103g/L MS vitamins, 10g/L sucrose and 7.5g/L phytagel (Sigma).

The seeds were plated in a sterile hood or a flow bench. With a sterile pipette, the seeds were evenly distributed over the plate. The plates were then sealed using Micropore surgical tape (3M). The plates were then placed under 24 hour lights for germination.

Methodology

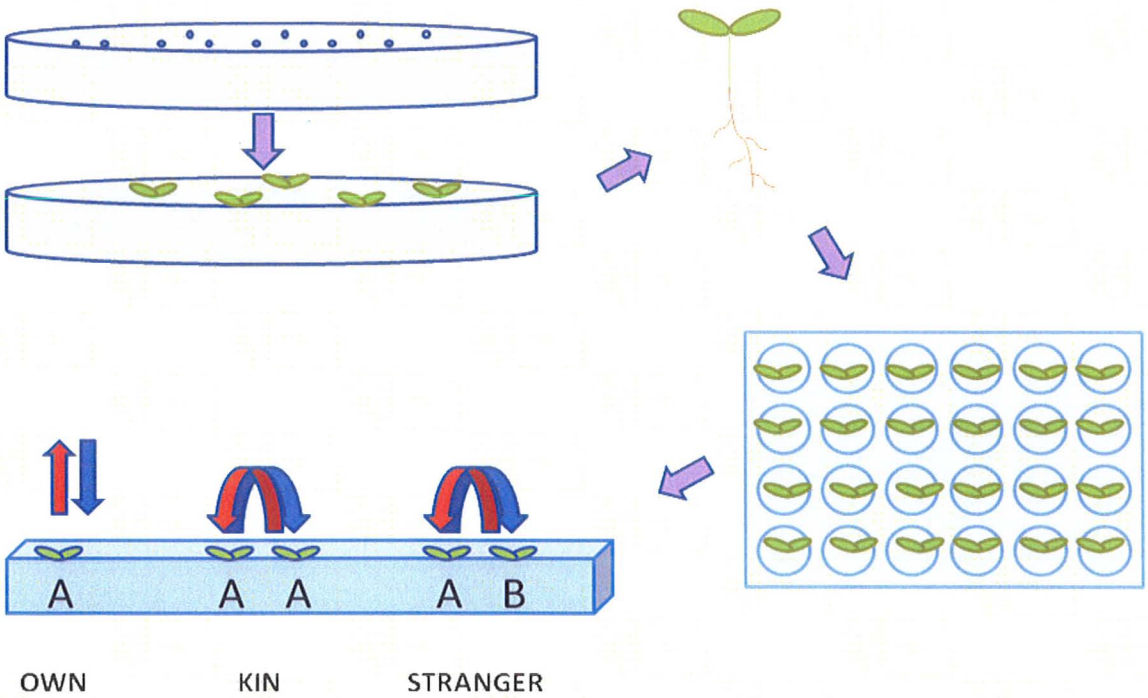
Dr. Harsh Bais, from the University of Delaware, has developed a methodology for testing root exudates on plant interactions. Tafari Jilany, an undergraduate thesis student in the Dudley lab, has adapted this methodology in order to test for kin recognition. The following is a procedure developed by these two.

Liquid MS media (same as MS plates not including agar) was placed into wells of a 24 well tissue culture plate (Falcon). 1mL of the media was placed into each well using a sterile pipette; the procedure was performed in a sterile flow bench. Seedlings, 4 days after being plated on solid media, were transferred from the plates into the wells using sterilized forceps. One seedling was placed in each well. The well plates were then placed under 24 hour lights on an orbital shaker at 50rpm. Every day for 3 days, the seedlings would be transferred from their well, using sterile forceps, to an adjacent well and the other seedlings taking its place in the newly vacated well. In this manner, the seedlings would be placed in media where another seedling has sat for approximately 24 hours

presumably exuding a suite of chemicals. Each time a seeding is transferred between wells the forceps were sterilized with ethanol to avoid contamination (Figure 1).

Each day the seedlings were switched with a known partner who was either a sibling (kin treatment) or a non-sibling (stranger treatment). A control treatment was used, called "own" where the seedling lifted (as if to be switched) was not switched with another, and therefore was only exposed to its own exudates. On the fifth day the seedlings were harvested, measurements being taken of root length, hypocotyl length and number of laterals. Chenopodium seedlings were measured by hand and Arabidopsis seedlings were photographed and measured on a computer.

Figure 1. Methodology for seedlings grown in well plates. First, seeds were plated on solid MS plates, then after germination transferred to 24 well plates with liquid MS. Seedlings were switched daily with another seedling of known identity; either a kin or a stranger seedling and therefore were exposed to known exudates. Some seedlings were not switched with another, but used as a control, only experiencing their own exudates.


A
A A
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OWN
KIN
STRANGER

Chenopodium experiment

To test for kin recognition in Chenopodium we used the methodology described above. *Chenopodium album* seeds used for this experiment were collected from the Royal Botanical Gardens (Burlington, Ontario) in the fall of 2006. Of the 12 families collected, the five used for this experiment were chosen because of their past germination success and viability, and were used in previous Chenopodium experiments.

There were three trials, each trial consisting of six trays of twenty-four wells for a total of 144 seedlings per trial. Three of the trays were in regular nutrient treatment (4.3g/L of MS nutrients), the remaining three trays were in a scarce nutrient treatment, $1/10^{th}$ of the regular nutrient treatment (0.43g/L MS nutrients). Of each of the three trays per nutrient treatment, one was a 'Kin' tray (all siblings), one a 'Stranger' tray and the last one an 'Own' tray.

Seeds were sterilized and stored in the refrigerator overnight then plated and placed under lights. Four days later the seedlings were transferred into the well plates with liquid media and placed under the lights on an orbital shaker at 0.5 rpm. The seedlings were switched with their designated partner daily and were harvest on the 8^{th} day after being plated and measurements taken by hand, including leaf number, hypocotyl length, number of laterals, length of the longest lateral and root length.

Arabidopsis experiments:

Arabidopsis seeds (Col-0 and Ws-0) were donated by Dr. Robin Cameron, McMaster University; Dr Kathleen Donohue, Duke University provided us with the CHA families from a wild population near the Charles River in Massachusetts, and I collected seeds from the Royal Botanical Gardens (RBG) in Burlington Ontario. All the seeds with the exception of the RBG seeds were of the same accessions used by Biedrzycki, Jilany et al (in review).

High Artificial Light

We used high artificial light, four fluorescent light bulbs and two plant bulbs; with an irradiance of 93μmol photons/m^2/s. We tested for kin recognition in *Arabidopsis thaliana* using the methodology described above. Trials were run between December 2008- April 2009. Each trial consisted of a tray of Kin, Own and Stranger with families consisting of Col-0, Ws-0 and varying CHA (25, 28, 8, 31) and RBG (4) families. Seeds were sterilized and placed in the refrigerator for one week prior to being plated. Seeds were plated in a sterile flow bench and then placed under lights for germination. Four days after plating the seedlings were transferred into liquid MS media in well plates and placed on an orbital shaker set at 0.5 rpm under lights. The seedlings were switched daily in sterile conditions and on the fourth day after being transferred the seedlings were harvested. Pictures were taken of each of the seedlings and were measured on a computer,

measurements being taken of the hypocotyl, number of laterals and root length. There were 7 trials analysed of this experiment, for a total of 720 seedlings.

Low Artificial Light

The seedlings had not responded as we had predicted based on previous results, so we tested how some environmental factors affected the responses to the kin treatment, the first of which was lower light levels. In this experiment we only used two plant bulbs, minus the additional four fluorescent; previously one small plant light had been used. The same experiment as above was performed with three trays: Kin, Own and Stranger. Only two families were used in this experiment, Ws-0 and Col-0.

Seedlings in a shared well

This experiment was a preliminary exploration into an unexplained result obtained in another experiment. Using rectangles of nylon net filters (filter pore size 100μm, Millipore) we glued them to the sides of each well, of a 12 well plate, using agar to create a divider within a well. After allowing the construction to dry overnight in the flow bench 2mL of liquid MS media was placed into each well and seedlings placed on either side of the divider. This allows the exudates in the media to flow between the divider while not allowing the roots to penetrate it. A second tray was set up without the dividers and two seedlings placed into each well. This was only a trial test to see if the construction held up

through the duration of an experiment. All interactions were kin as only one family (CHA 31) was used for this test.

Changes in light irradiance and quality under high artificial lights

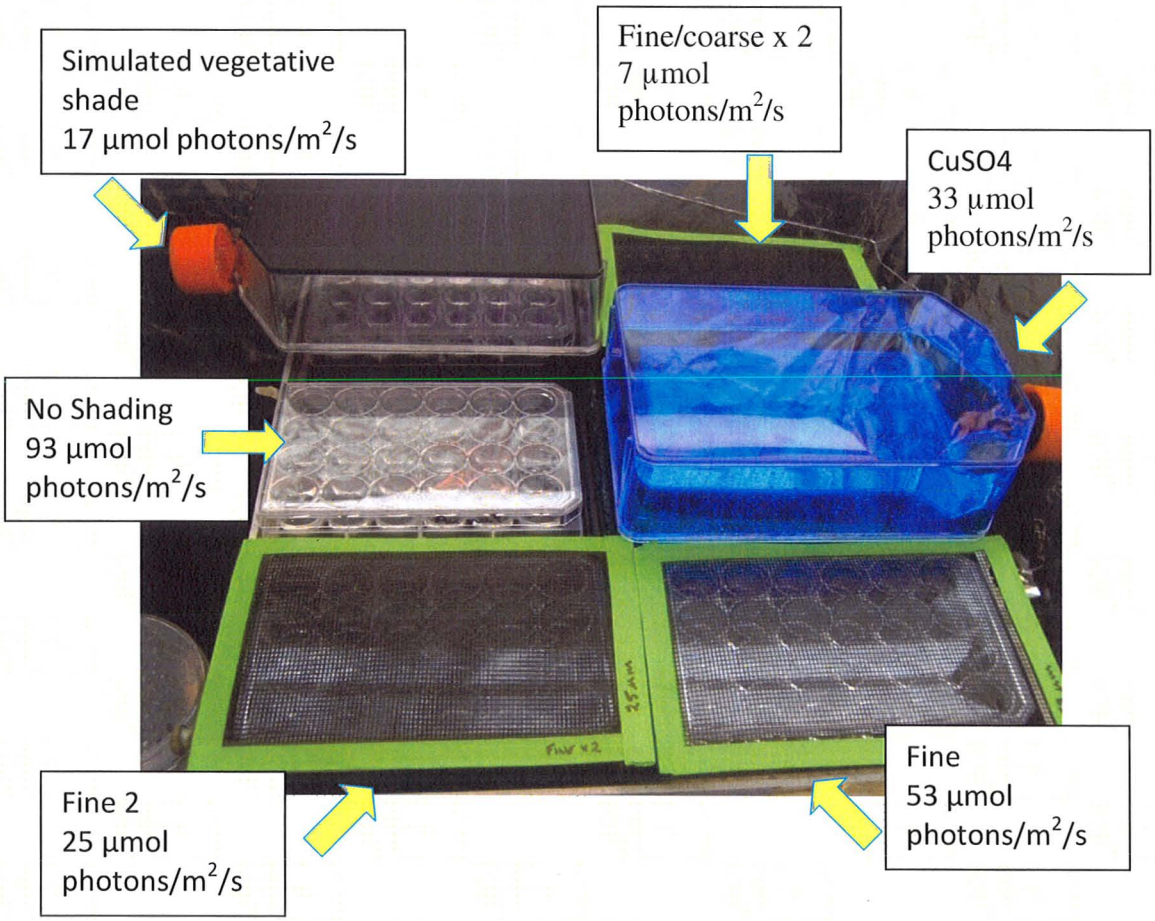
Using different shades placed over the well plates we have tested to see how the seedlings respond to different irradiance levels and different R:FR levels. I used a Copper Sulfate solution (100g $CuSO_4$ to 1000mL distilled water) to increase the R:FR and reduce irradiance. The solution was placed in a cell culture flask which was placed on top of the well plate, so that light passing through the top of the well plate first had to pass through the $CuSO_4$, thereby changing the light quality the seedlings will experience. The second shade was a vegetative shade, to simulate another plant shading the seedlings, reducing the R:FR and reducing irradiance. This shade was made by mixing: 5.6g purple pigments (Hostaperm Violet RL 02, Hoeschst), 1.4g yellow pigments (Solvaperm Yellow G, Hoeschst) and 90 mL clear varnish. This was mixed until smooth and then painted thinly onto the cell culture flasks. There were 3 sets of neutral shades used, compromising of combinations of layers of coarse and fine black meshes. Irradiance under each of the shades was measured using a light meter (Table 1; Figure 2).

Table 1. List of shades and corresponding light levels

Shade	**Light levels (µmol photons/m^2/s)**
No shade	93
$CuSO_4$	33
Vegetative Shade	17
Fine mesh	53
Fine x 2	25
Fine/coarse x 2	7

There were six well plates per trial, each placed under one of the shades. Within each well plate there were both stranger and kin interactions. The families used for this experiment were Col-0, Ws-0, and CHA 38. The trial was performed twice for a total of 288 seedlings.

Figure 2. One trial Arabidopsis seedlings in differing light quality and irradiance. The different shades were used to create different levels of irradiance (μmol photons/m^2/s). Simulated vegetative shading lowers R:FR, CuSO4 solution raises R:FR.


Simulated vegetative shade
17 µmol photons/m²/s
Fine/coarse x 2
7 µmol photons/m²/s
CuSO4
33 µmol photons/m²/s
No Shading
93 µmol photons/m²/s
Fine 2
25 µmol photons/m²/s
Fine
53 µmol photons/m²/s

Low natural light supplemented with low artificial light

This experiment is another trial to test a theory that light quality and irradiance affected results and to see if we can replicate a previous experiment. The original experiment, Biedrzycki, Jilany et al (in review), was performed in a north facing window with a supplemental plant bulb, but subsequent experiments I did were performed away from the window in completely artificial light. The lights were again reduced, this time using only one plant bulb and one fluorescent bulb reducing the light to 83μmol photons/m^2/s. The experiment consisted of five trays. Three of the trays were under the lights as per normal; the trays were Kin, Own or Str. The last two trays were placed under a shade mesh (fine2) to lower the light level to 25 μmol photons/m^2/s, one of the trays were Kin the other Str. Families used in this experiment were Col-0, Ws-0 and CHA 25.

Increased exudate concentration

We tested to see if the strength of the exudates present makes a difference. Under normal circumstances the seedling is not just competing with one other seedling. In this experiment three seedlings were placed into a well together. Those three seedlings were either all of one family or of mixed families. After one day a single seedling is placed into these exudates and the three moved to a new tray. The experiment otherwise is as above. There were three trays in this experiment; one was kin and the other two stranger. However, in one of the stranger trays the single seedling was placed into exudates of all

one stranger family and in the other tray a seedling was placed in exudates of a mixture of two stranger seedlings and one kin seedling. Only Col-0 and Ws-0 seedlings were used.

Chenopodium seedlings were all measured by hand, however Arabidopsis seedlings were measured on the computer using Meazure™2.0 (C Thing Software, http://www.cthing.com/Meazure.asp 21/09/09). Measurements taken for Chenopodium seedlings were: hypocotyl length, root length, number of laterals, length of longest lateral and length of leaves. Measurements taken of Arabidopsis seedlings were: hypocotyl length, root length and length of the longest lateral.

All statistical analyses were performed using SAS 9.2. PROC GLM was used for all analyses of variance and covariance (ANOVA, ANCOVA) with LSMEANS option. PROC PRINCOM procedure was used for Principal Component Analysis (PCA). LSMEANS are unobtainable if the design is not balanced, and for some of the following analyses, specific treatments have been omitted.

Results Part 1- Chenopodium experiments

We looked to see if Chenopodium seedlings responded to an exudate source and if the response changed under different nutrient conditions. Using the methodology outlined previously, we exposed Chenopodium seedlings to exudates belonging to kin, strangers or their own exudates. Seedlings were either placed in a scarce nutrient treatment or a regular nutrient treatment.

Several seedling traits responded to an exudate source (hypocotyl length, root length and number of laterals) indicating an overall kin recognition response (Table 2). There was genotypic variance found , indicated by the significant family effect, for hypocotyl length, root length, number of laterals, and length of the longest lateral (Table 2; Figure 3). However, there is no family × exudate interaction showing that the kin recognition response did not change with genotypic variance. A trial × nutrient × exudate interaction was seen for root length and length of the longest lateral indicating the response to exudates varied between trials.

Changes in nutrient levels had significant effects on hypocotyl length, root length and number of laterals, but had no effect on the length of the longest lateral (Table 2; Figure 3). The effects of nutrients did change with genotypic variance indicated by a significant nutrient × family effect for hypocotyl length, root length, number of laterals and length of the longest lateral.

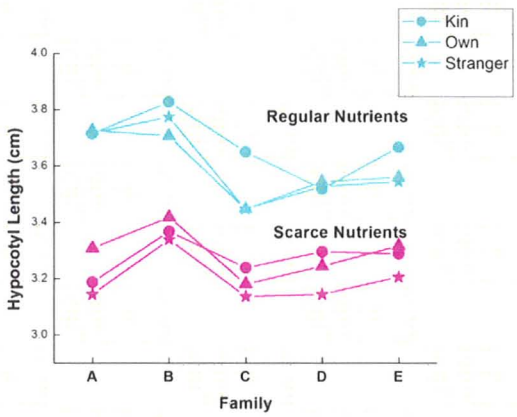
There was significant variability in the response for all traits between trials (Table 2). Trial × nutrients effects for all traits indicates there was variation in the response to nutrients among trials.

Table 2. Analyses of Variance for Chenopodium seedlings under regular and scarce nutrient conditions.

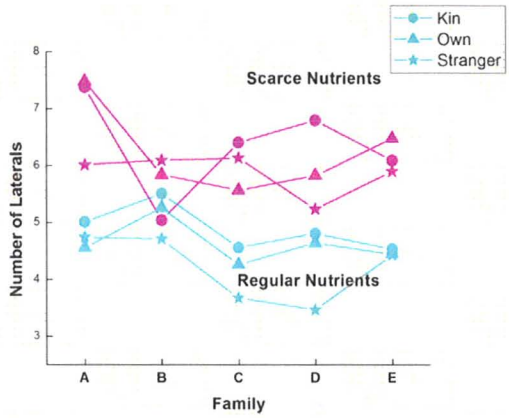
		Hypocotyl Length		Root Length		Number of Laterals		Length of the Longest Lateral	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Trial	2	70.64	**<.0001**	6.39	**0.0019**	16.70	**<.0001**	25.17	**<.0001**
Nutrient	1	290.08	**<.0001**	18.06	**<.0001**	110.89	**<.0001**	1.19	0.2764
Trial × Nutrient	2	8.95	**0.0002**	144.18	**<.0001**	41.46	**<.0001**	13.88	**<.0001**
Exudate	2	4.46	**0.0122**	11.28	**<.0001**	5.60	**0.0040**	0.01	0.9853
Trial × Exudate	4	0.42	0.7914	1.85	0.1190	0.40	0.8061	0.44	0.7803
Nutrient × Exudate	2	2.49	0.0841	1.69	0.1860	0.77	0.4641	1.13	0.3255
Trial × Nutrient × Exudate	4	1.17	0.3235	3.51	**0.0079**	0.85	0.4931	2.46	**0.0451**
Family	4	10.40	**<.0001**	8.92	**<.0001**	3.31	**0.0110**	2.46	**0.0452**
Nutrient × Family	4	3.03	**0.0175**	9.07	**<.0001**	3.20	**0.0133**	12.19	**<.0001**
Family × Exudate	8	0.75	0.6436	0.93	0.4909	1.22	0.2852	0.24	0.9828
Nutrient × Family × Exudate	8	0.43	0.8999	1.50	0.1546	1.38	0.2052	0.33	0.9527

Note - Degrees of freedom for the error terms were 388 for hypocotyl length, root length, number of laterals, length of longest laterals. Bold numbers indicate significance.

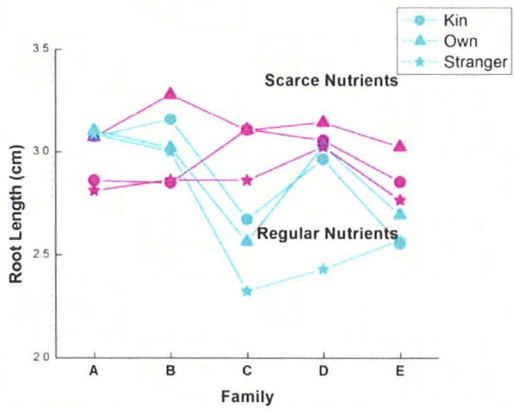
Figure 3. Reaction norms (change in a response by genotypes under different environmental conditions) for Chenopodium seedlings in two nutrient treatments (regular and scarce) and their response different exudate sources (kin, own and stranger) for different traits; a) hypocotyl length, b) number of laterals and c) root length.


Kin
Own
Stranger
Regular Nutrients
Scarce Nutrients
Hypocotyl Length (cm)
4.0
3.8
3.6
3.4
3.2
3.0
A
B
C
D
E
Family

a)


Kin
Own
Stranger
Scarce Nutrients
Regular Nutrients
Number of Laterals
8
7
6
5
4
3
A
B
C
D
E
Family

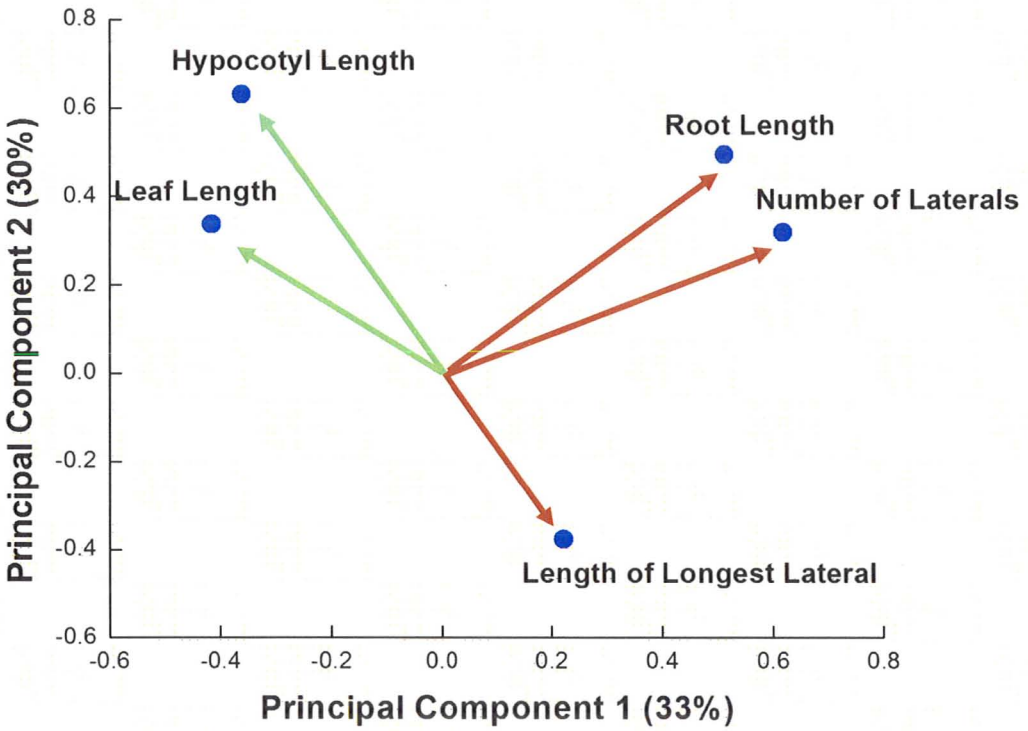
b)


Kin
Own
Stranger
Scarce Nutrients
Regular Nutrients
Root Length (cm)
3.5
3.0
2.5
2.0
A
B
C
D
E
Family

c)

Principal Component Analysis (PCA) was used on the above data for five morphological traits (Figure 4). The first principal component 1 (PC1) describes 33% of the variance and the second principal component 2 (PC2) describes 30% of the variance. The vector loadings from PC1 and PC2 show a clear separation between the different traits. Vector loadings for PC1 show the change between aboveground and belowground traits, when belowground traits (root length, number of laterals and length of the longest lateral) are large, the aboveground traits (leaf length, hypocotyl length) are small. The relationship between aboveground and belowground traits are demonstrating a classic root to shoot ratio. PC2 describes overall size; when all the other traits are large, the length of the longest lateral is smaller, showing that there are not fewer lateral roots, but that they are smaller in general.

Figure 4. Chenopodium seedling traits for the vector loadings for Principal Component 1 (PC1) and Principal Component 2 (PC2). A trade-off between aboveground and belowground traits (PC1) and change in the size of the traits (PC2) can be observed. This PCA allows us to examine the structuring of the principal components with regard to the different traits so that we can interpret them in later analyses.


Hypocotyl Length
Root Length
Leaf Length
Number of Laterals
Length of Longest Lateral
Principal Component 2 (30%)
Principal Component 1 (33%)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8

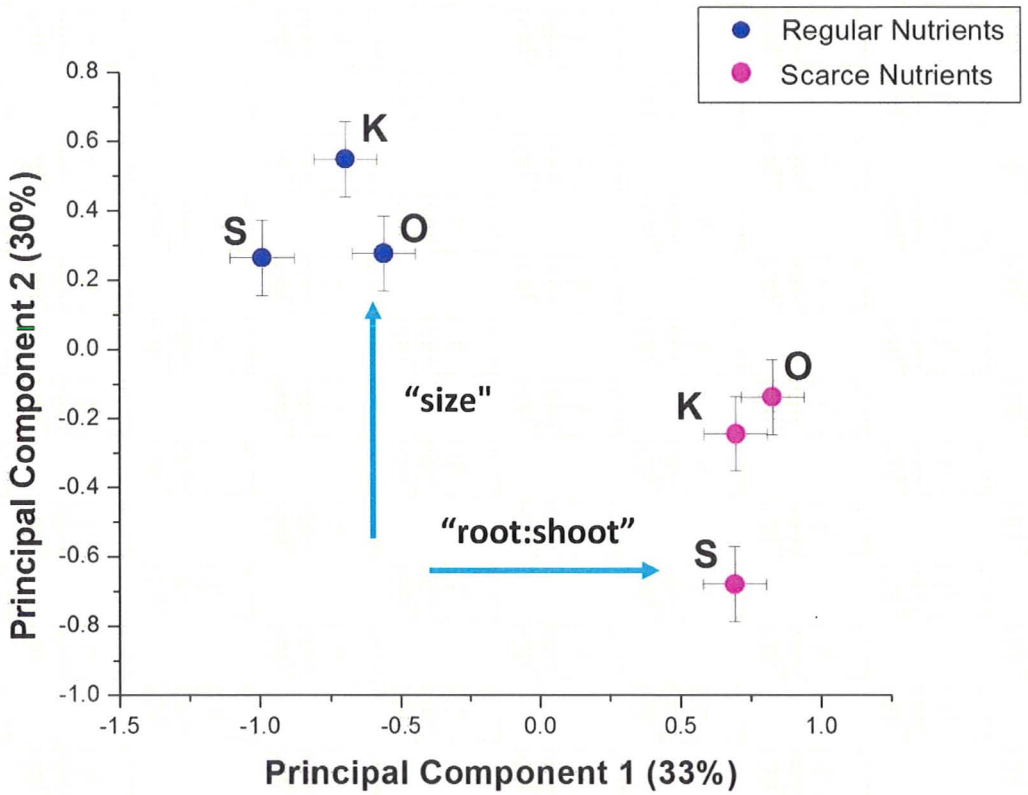
An ANOVA of the PCA data gives significant results for kin and nutrients (Figure 5), showing a clustering of regular nutrients and scarce nutrients along the two principal components. As we saw in Figure 4, PC1 describes a root to shoot ratio, and PC2 describes size. The seedlings from the scarce nutrient treatment are clustered where the root to shoot ratio is high, showing that these seedlings have more roots than the regular nutrient seedlings. Within the scarce nutrient cluster you can see that the seedlings exposed to stranger exudates are smaller than those exposed to kin exudates and those only exposed to their own exudates, as seen along PC2. The seedlings from the regular nutrient treatment cluster together and show that they are larger than the scarce nutrient seedlings and have more shoots (a low root:shoot ratio). Within the cluster you can see that the seedlings exposed to kin exudates have more roots than the seedlings with strangers and own exudates, who have more shoots in this treatment. Also, similarly to the scarce nutrient cluster, seedlings exposed to kin exudates are overall larger than those exposed to stranger exudates.

Table 3..Analysis of Variance for Principal Components 1 (PC1), Principal Component 2 (PC2) and Principal Component 3 (PC3) of the Chenopodium seedlings.

		PC1		PC2		PC3	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Trial	2	6.43	**0.0018**	31.36	**<.0001**	28.34	**<.0001**
Nutrient	1	282.82	**<.0001**	22.96	**<.0001**	75.49	**<.0001**
Trial × Nutrient	2	18069	**<.0001**	83.11	**<.0001**	18.91	**<.0001**
Exudate	2	2.37	0.0945	7.83	**0.0005**	0.26	0.7677
Trial × Exudate	4	0.92	0.4500	0.46	0.7637	0.77	0.5427
Nutrient × Exudate	2	1.08	0.3422	3.29	**0.0379**	1.91	0.1493
Trial × Nutrient × Exudate	4	2.85	**0.0238**	2.36	**0.0528**	1.25	0.2900

Note - degrees of freedom for the error terms were: PC1 412; PC2 412; PC3 412. Bold numbers indicate significance.

Figure 5. PC1 and PC2 from the ANOVA on the Chenopodium PCA data set for two nutrient treatments (scarce and regular) and for different exudate sources (Kin, Own and Stranger). Changes in size and a root:shoot ratio are seen for seedlings in the different nutrient treatments and when exposed to different exudate sources.


Regular Nutrients
Scarce Nutrients
K
S
O
"size"
"root:shoot"
O
K
S
Principal Component 2 (30%)
Principal Component 1 (33%)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0

Results Part 2 - Arabidopsis experiments

I carried out several series of experiments to explore the responses of Arabidopsis to exudates under different experimental conditions (Table 4). For each experiment or set of experiments we ask whether there is any indication of kin recognition, indicated by an exudate source effect, which would indicate an overall kin recognition response, or a family × exudate interaction, which would indicate genetic variation in a kin recognition response, or trial × exudate interaction, which would indicate among trial differences in a kin recognition response. A trial × exudate × family indicates that the genetic variation in a kin recognition response differed among the experiment dates.

A significant family effect indicates genetic variability in a trait. A significant trial effect indicates an effect of experiment date on a trait. A significant family × trial indicates genetic variation in the response to experiment date.

Table 4. Multiple experiments using Arabidopsis thaliana seedlings organized into treatment, family, trials and size of the experiment.

Experiment	Families	Trials (dates)	Trays per trial
High artificial light	Col-0, Ws-0, CHA25, CHA28, CHA8	3 (Dec 5, Dec 11, Jan 23)	3
High artificial light	Col-0, Ws-0, CHA31, RBG4	4 (Mar 6, Mar 13, Mar 20, April 3)	5 (Mar 20 – 6 trays)
Low artificial light	Col-0, Ws-0	1 (May1)	3
Shared wells	CHA 31	1 (May 8)	2
Changes in light quality and quantity	Col-0, Ws-0, CHA 38	2 (June 5, June 12)	6
Low natural light supplemented with low artificial light	Col-0, Ws-0, CHA25	1 (June 19)	5
Increased exudate concentration	Col-0, Ws-0	1 (June 26)	3

High artificial light

These experiments were performed under high artificial light, which consisted of four fluorescent light bulbs and two plant bulbs. There was no indication of kin recognition in either set of trials (Table 5, 6). There was variability among families for hypocotyl length, root length and number of laterals (Table 5, 6; Figure 6). For all traits, there were significant differences among trials. For root length Dec 5 – Jan 23 (Table 4) and hypocotyl length March 6 – April 3 (Table 5) there was significant genetic variation in responses in the different trials.

Table 5. Analyses of Variance on Arabidopsis seedlings in High artificial light for trials: Dec 5, Dec 11, Jan 23; Families: Col-0, Ws-0, CHA25, CHA28, CHA8.

		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Exudate	2	1.81	0.1682	0.46	0.6304	1.64	0.1971
Trial	2	9.89	**<.0001**	15.68	**<.0001**	30.93	**<.0001**
Exudate × Trial	4	1.53	0.1960	0.93	0.4500	0.24	0.9128
Family	4	13.06	**<.0001**	18.61	**<.0001**	12.41	**<.0001**
Family × Exudate	8	1.01	.04337	0.34	0.9472	0.95	0.4790
Family × Trial	8	1.96	0.0557	2.06	**0.0430**	2.17	**0.0332**
Family × Exudate × Trial	16	1.12	0.3384	0.88	0.5915	1.40	0.1480

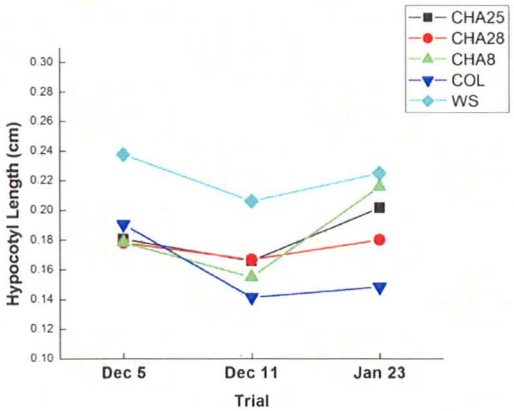
Note - degrees of freedom for the error terms were: hypocotyl length 139, root length 150 and number of laterals 150. Bold numbers indicate significance.

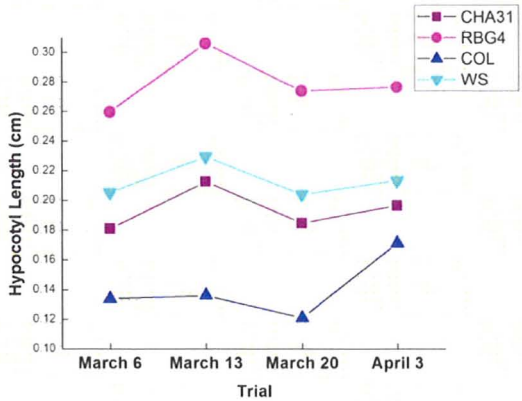
Table 6. Analyses of Variance on Arabidopsis seedlings in High artificial light for trials: March 6, March 13, March 20 and April 3; Families: Col-0, Ws-0, CHA31, RBG4.

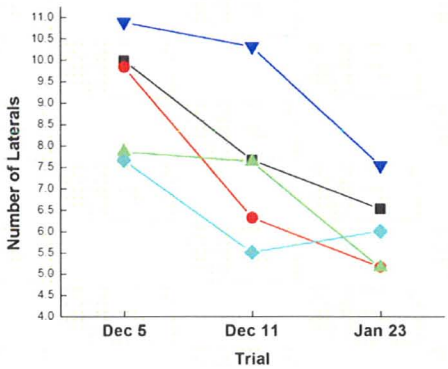
		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Exudate	2	2.75	0.0655	0.25	0.7817	0.60	0.5481
Trial	3	6.10	**0.0005**	3.42	**0.0175**	9.03	**<.0001**
Exudate ×Trial	6	1.98	0.0671	1.37	0.2233	1.98	0.0670
Family	5	83.00	**<.0001**	21.34	**<.0001**	17.61	**<.0001**
Family ×Exudate	10	1.36	0.1984	0.61	0.8090	0.51	0.8807
Family ×Trial	8	2.15	**0.0305**	0.80	0.5994	1.46	0.1683
Family × Exudate × Trial	16	0.98	0.4792	0.87	0.6047	0.92	0.5437

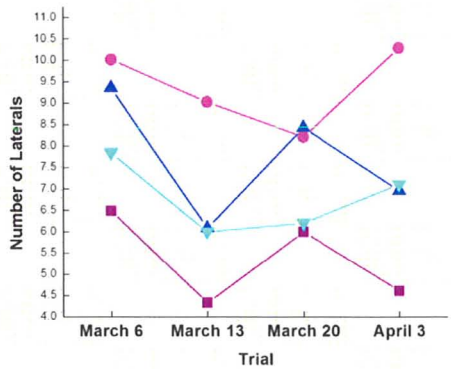
Note - degrees of freedom for the error terms were: hypocotyl length 371, root length 409 and number of laterals 409. Bold numbers indicate significance.

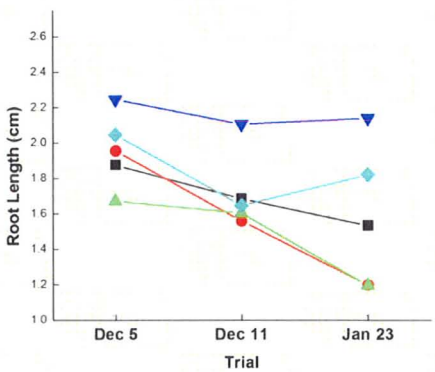
Figure 6. Reaction norms for Arabidopsis seedlings in high artificial light for hypocotyl length, number of laterals and root length across trials (Dec 5 – April 3). Families used from Dec 5 – Jan 23 were: CHA25, CHA28, CHA 8, Col-0, Ws-0. Families used from March 6 – April 3 were: CHA31, RBG4, Col-0, Ws-0.


CHA25
CHA28
CHA8
COL
WS
Hypocotyl Length (cm)
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
Dec 5
Dec 11
Jan 23
Trial


CHA31
RBG4
COL
WS
Hypocotyl Length (cm)
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
March 6
March 13
March 20
April 3
Trial


Number of Laterals
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Dec 5
Dec 11
Jan 23
Trial


Number of Laterals
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
March 6
March 13
March 20
April 3
Trial


Root Length (cm)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Dec 5
Dec 11
Jan 23
Trial

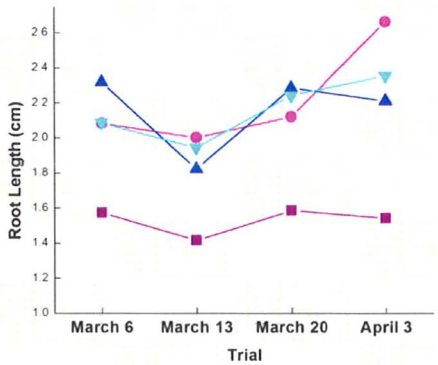

Root Length (cm)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
March 6
March 13
March 20
April 3
Trial

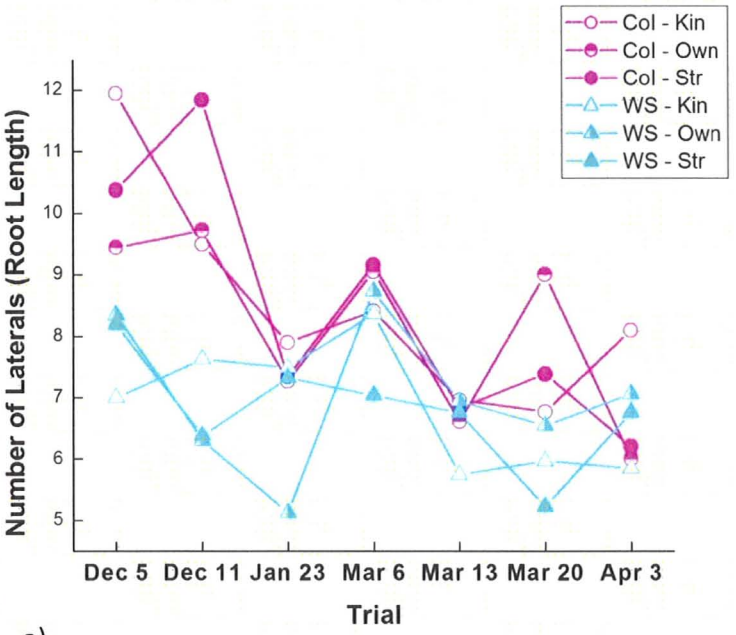
Table 7. Analyses of Covariance for Arabidopsis seedlings in high artificial light using root length as the covariate to control for size. Analyzed using only Ws-0 and Col-0 as they were represented in all of the seven trials.

		Hypocotyl Length		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*
Root Length	1	4.16	**0.0424**	97.18	**<.0001**
Exudate	2	3.02	0.0506	0.05	0.9536
Trial	6	5.24	**<.0001**	9.73	**<.0001**
Exudate × Trial	12	1.13	0.3382	0.77	0.6778
Family	1	188.75	**<.0001**	29.98	**<.0001**
Family × Exudate	2	0.18	0.8330	0.31	0.7319
Family × Trial	6	2.17	**0.0457**	3.16	**0.0050**
Family × Exudate × Trial	12	1.12	0.3423	0.93	**0.0305**

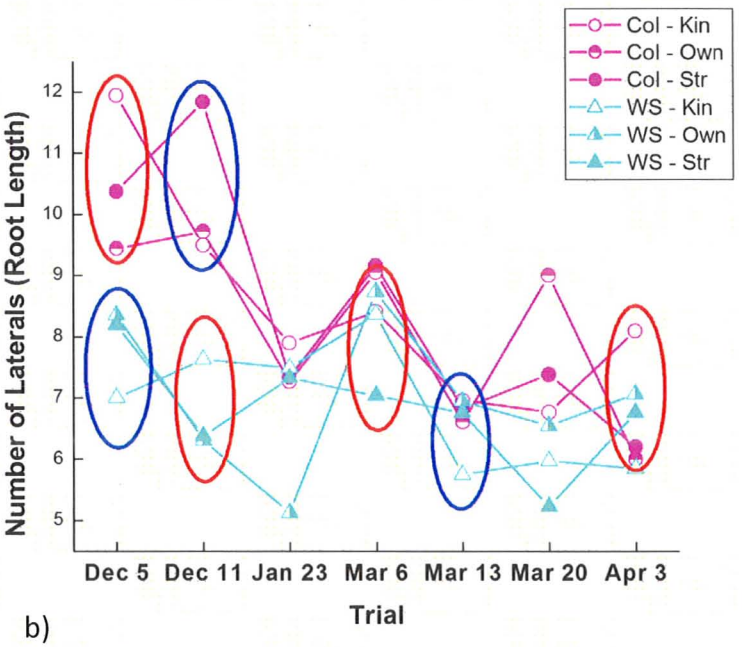
Note - degrees of freedom for the error terms were: hypocotyl length 285 and number of laterals 327. Bold numbers indicate significance.

To control for plant size, I did an Analysis of Covariance on hypocotyl length and number of laterals, using root length as a covariate (Table 7). Only Ws-0 and Col-0 seedlings were included, and all trials. In this analysis many interactions were significant. There is still the family variation that was seen in the previous analysis and also between trials. There is also a Family × Exudate interaction that varies between trials for number of lateral roots. For Dec 5 and Dec 11 there is significant differentiation between Col-0 and Ws-0, which diminishes in the later trials (Figure 7a). There are responses to exudate source seed throughout the trials, although this response is not consistent between trials. For example, Dec 5, Col-0 seedlings exposed to kin exudates had more lateral roots than seedlings exposed to stranger exudates. But, on Dec 11 seedlings exposed to stranger exudates had more lateral roots than those exposed to kin, which is the result discovered by Biedrzycki, Jilany et al (in review) (Figure 7b).

Figure 7. Reaction norms from an ANCOVA on high artificial light for number of laterals (with root length as the covariate) across trials looking at reactions to exudate source specifically in the two lab grown accessions, Ws-0 and Col-0. **Blue circles** those results found by Biedrzycki, Jilany et al (in review), **red circles** those results showing the opposite reaction.

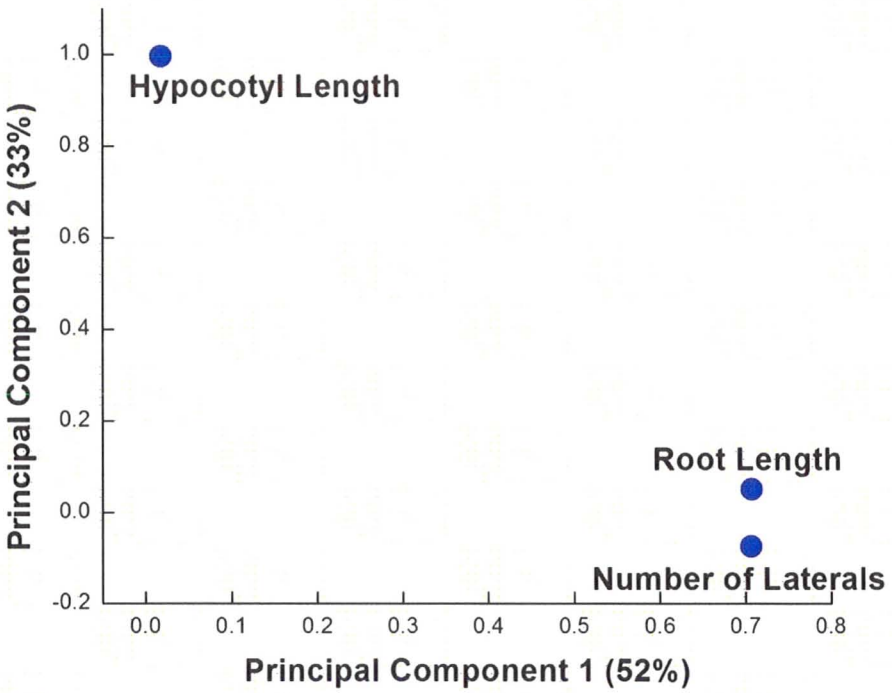

Col - Kin
Col - Own
Col - Str
WS - Kin
WS - Own
WS - Str
Number of Laterals (Root Length)
12
11
10
9
8
7
6
5
Dec 5
Dec 11
Jan 23
Mar 6
Mar 13
Mar 20
Apr 3
Trial

a)


Col - Kin
Col - Own
Col - Str
WS - Kin
WS - Own
WS - Str
Number of Laterals (Root Length)
12
11
10
9
8
7
6
5
Dec 5
Dec 11
Jan 23
Mar 6
Mar 13
Mar 20
Apr 3
Trial

b)

Figure 8. Principal Component Analysis for Arabidopsis seedlings in High artificial light demonstrating that belowground traits are correlated, However aboveground and belowground traits are uncorrelated.


Hypocotyl Length
Root Length
Number of Laterals
Principal Component 2 (33%)
Principal Component 1 (52%)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8

The principal component analysis (PCA) was done on the combined high artificial light set of data, for the three morphological traits allowing us to look at correlations for multiple traits. The first principal component (PC1) described 52% of the variance and the second principal component (PC2) described 33%. PC1 showed high loadings for root length and number of laterals, both belowground traits, while PC2 showed a high loading for hypocotyl length, an aboveground trait (Figure 8). For PC1, there was no influence of hypocotyl length, whereas for PC2, root traits had no influence. This suggests aboveground and belowground growth were uncorrelated overall.

Low artificial light

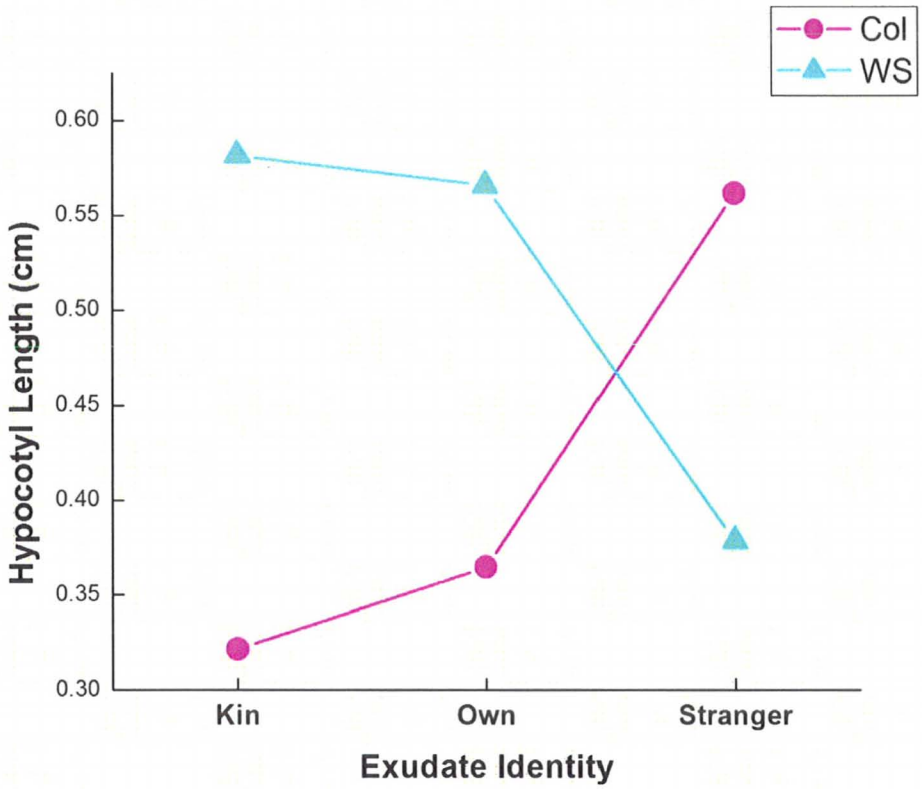
Low artificial light consisted of two plant bulbs, same as the high artificial light experiment, but without the four fluorescent bulbs. Seedlings did not show an overall response to exudate source in low light conditions. However, there was significant genetic differentiation in responses to exudates for hypocotyl length and root length (Table 8; Figure 9). Interestingly, we see Col-0 and Ws-0 responding in opposite directions to stranger exudates, indicating genetic variation between the two accessions.

Table 8. Analyses of Variance for Arabidopsis seedlings in low artificial light, families include Ws-0 and Col-0.

		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Exudate	2	0.14	0.8654	0.00	0.9990	1.04	0.3578
Family	1	10.19	**0.0022**	0.30	0.5858	0.83	0.3645
Exudate × Family	2	23.19	**<.0001**	4.30	**0.0176**	1.73	0.1849

Note - degrees of freedom for the error terms were: hypocotyl length 65, root length 65 and number of laterals 65. Bold numbers indicate significance.

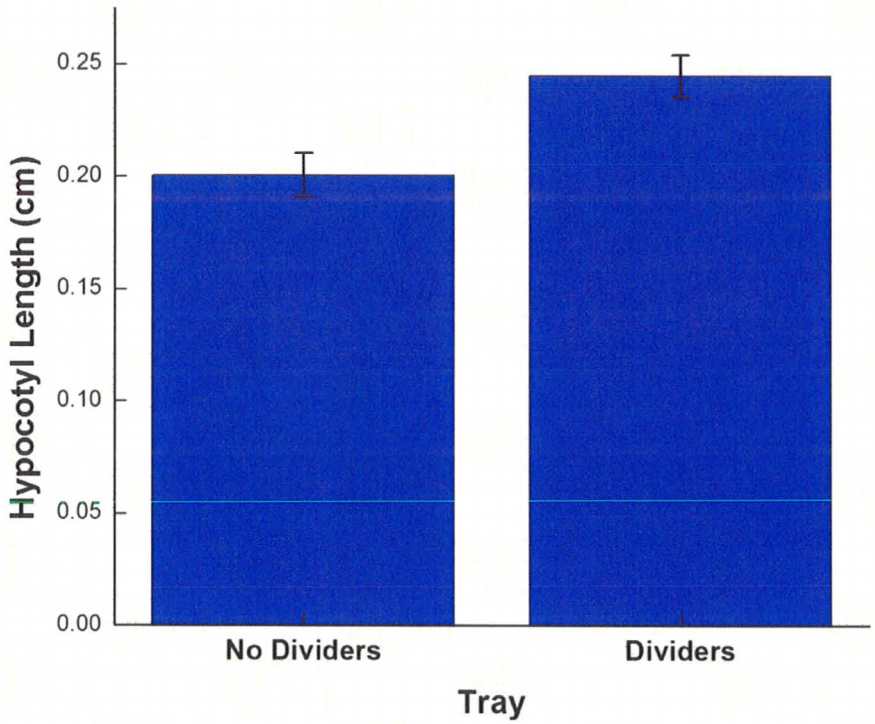
Figure 9. Norms of reaction for Arabidopsis seedlings in low artificial light responding to exudate source for hypocotyl length. Only Ws-0 and Col-0 used in this analysis, note the different responses from the two different genotypes.


Col
WS
Hypocotyl Length (cm)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
Kin
Own
Stranger
Exudate Identity

Seedlings in a shared well

Kin recognition was not tested in this experiment as only one family was used. Under high artificial light, seedlings responded to being placed together with or without dividers separating them from touching (Figure 10). We predicted that seedlings in trays without dividers would have longer hypocotyls due to a change in R:FR from the other seedling. However, we found the opposite to occur in that seedlings in divided wells had longer hypocotyls. This was a small sample experiment to test the dividers, but poses some interesting results and is worth looking into more in the future

Figure 10. Changes in hypocotyl length between Arabidopsis seedlings exposed to the exudates of a neighbour sharing a well that is either divided or not divided. Only one family is used here (CHA31), so there is no exudates interaction.


0.25
0.20
0.15
0.10
0.05
0.00
Hypocotyl Length (cm)
No Dividers
Dividers
Tray

Changes in light quality and quantity under high artificial lights

These experiments were performed under high artificial light (four fluorescent bulbs and two plant bulbs), with different shades used to manipulate the irradiance and quality of light. There is no overall response to exudate source (Table 9). However, there was a family × exudate response for hypocotyl length indicating genetic variation in kin recognition (Figure 11). There was also a family × exudate × trial interaction for hypocotyl length demonstrating that the genetic variation in a kin recognition response differed between the two trials (Table 7). There are significant family effects for hypocotyl length, root length and number or laterals, indicating that there is genetic variability amongst families (Table 9). Ws-0 did not respond to increasing irradiance by producing more lateral roots as the other families did.

The different shade treatments had a significant effect on hypocotyl length, root length and number of laterals (Table 9; Figure 12). In lower irradiance, seedlings had longer hypocotyls. Hypocotyl length increased under the simulated vegetative shading (low R:FR) compared with neutral shading, as predicted (Figure 12). Seedlings under the CuS04 solution (high R:FR) responded similarly to seedlings receiving neutral shade.

There were significant trial effects for hypocotyl length, root length and number of laterals indicating variation between the trials. There were also significant shade × trial effects for hypocotyl length and number of laterals showing the response to shades varied between trials. The family × exudate effect also varied between trials for hypocotyl length (Table 9).

Table 9. Analyses of Variance for Arabidopsis seedlings in differing light quality and quantity for Col-0, Ws-0 and CHA38.

		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Exudate	1	0.50	0.4785	0.03	0.8620	1.52	0.2187
Family	2	59.34	**<.0001**	15.16	**<.0001**	22.50	**<.0001**
Family × Exudate	2	3.25	**0.0409**	1.02	0.3637	0.27	0.7642
Shade	5	136.49	**<.0001**	3.62	**0.0036**	11.60	**<.0001**
Exudate × Shade	5	0.58	0.7120	0.43	0.8247	0.33	0.8955
Family × Shade	10	4.24	**<.0001**	1.09	0.3735	3.01	**0.0014**
Family × Exudate × Shade	10	0.46	0.9133	1.71	0.0791	1.39	0.1881
Trial	1	3.96	**0.0480**	3.38	0.0674	22.92	**<.0001**
Exudate × Trial	1	0.09	0.7583	1.72	0.1915	0.02	0.9010
Family × Trial	2	1.86	0.1579	2.40	0.0932	1.61	0.2024
Family × Exudate × Trial	2	3.32	**0.0380**	1.68	0.1884	0.52	0.5955
Shade × Trial	5	1.56	0.1734	2.87	**0.0157**	0.78	0.5622
Exudate × Shade × Trial	5	1.76	0.1231	0.45	0.8112	0.53	0.7505
Family × Shade × Trial	10	1.14	0.3360	0.82	0.6140	0.82	0.6141
Family × Exudate × Shade × Trial	10	1.71	0.0811	1.17	0.3147	1.33	0.2175

Note - degrees of freedom for the error terms were: hypocotyl length 202, root length 214 and number of laterals 213. Bold numbers indicate significance.

Figure 11. Reaction norms for Arabidopsis seedlings averaged over differing light quality and irradiance for hypocotyl length in response to different exudate sources. Families used were CHA38, Col-0 and Ws-0.

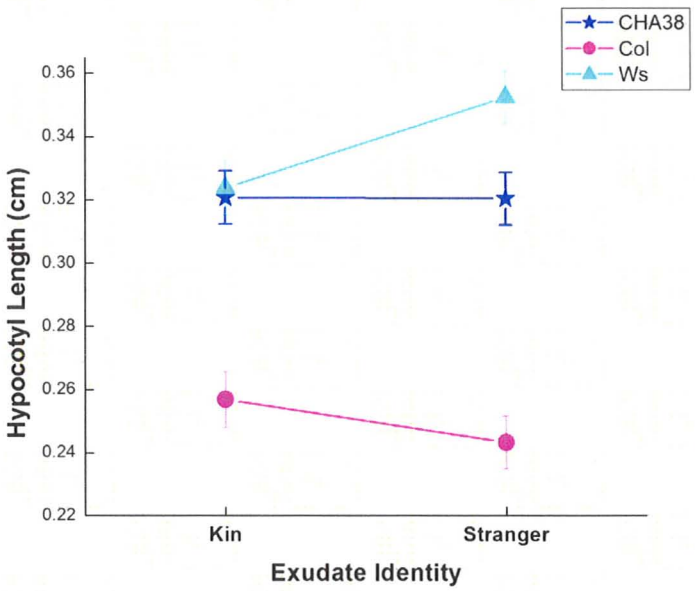
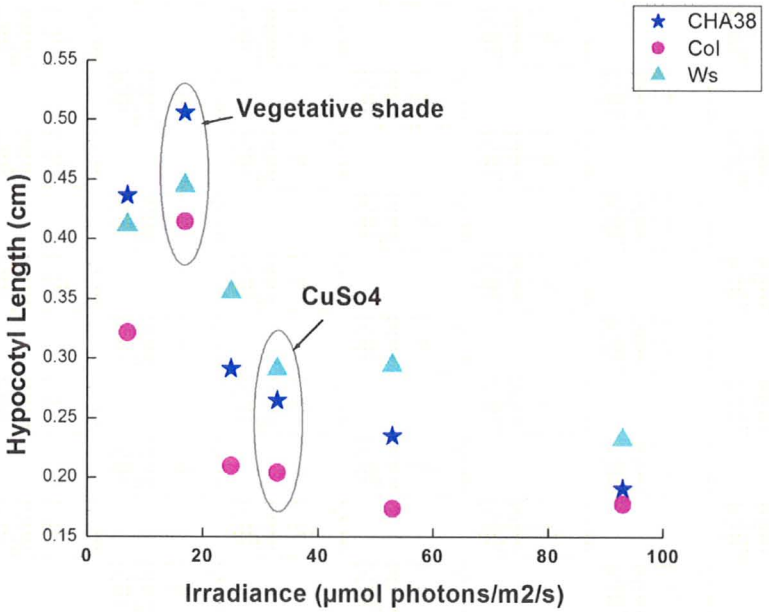
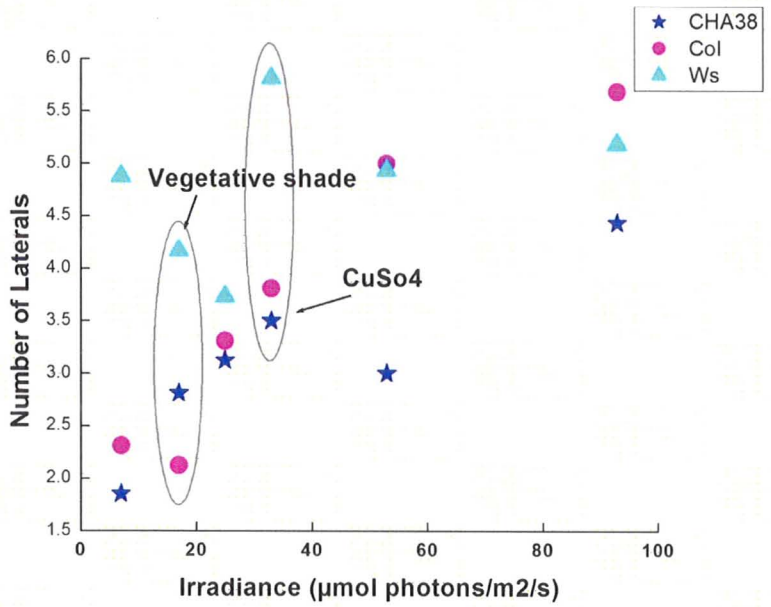

CHA38
Col
Ws
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
Hypocotyl Length (cm)
Kin
Stranger
Exudate Identity

Figure 12. Effect between families to changes in light quality and irradiance. Vegetative shade (low R:FR) and CuS04 (high R:FR) were used to change the light quality. Different shades caused a change in irradiance. With increasing irradiance we can wee decreasing hypocotyl length, for all families and increasing number of laterals for Col-0 and CHA38.


CHA38
Col
Ws
Vegetative shade
CuSo4
Hypocotyl Length (cm)
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0
20
40
60
80
100
Irradiance (µmol photons/m2/s)


CHA38
Col
Ws
Vegetative shade
CuSo4
Number of Laterals
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0
20
40
60
80
100
Irradiance (µmol photons/m2/s)

Low natural light supplemented with low artificial light

The light conditions for this experiment were low natural light (diffused light from a north facing window) and low artificial light (one fluorescent bulb and one plant bulb). Because of insufficient seeds the trials under the two light levels are not equal and the results have been shown in two ways. The first analysis looks at a subset of the data as a whole, omitting Ws-0 and own treatments as they do not appear in both shade treatments, and the other is an analysis of the two shade treatments independent of each other where all of the data can be used.

The first analysis examines a subset of all the data (no own or Ws-0). Seedlings responded to an exudate source in both hypocotyl and root length indicating kin recognition (Table 9). Genetic variation to kin recognition was shown through a family × exudate interaction in hypocotyl length, root length and number of laterals (Figure 13). A significant family effect indicates that there is genetic variability amongst all the traits (Table 10).

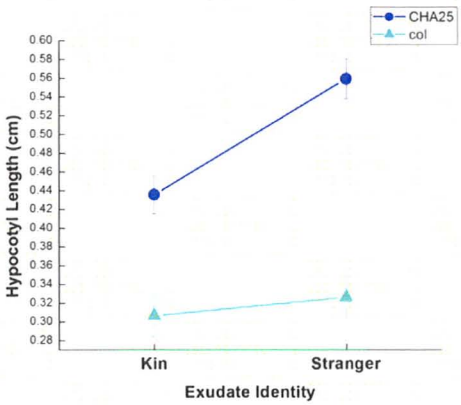
There was a significant effect produced by the two different levels of light for hypocotyl length and root length (Table 10). There was also a shade × exudate interaction indicating that kin recognition response changed between light levels.

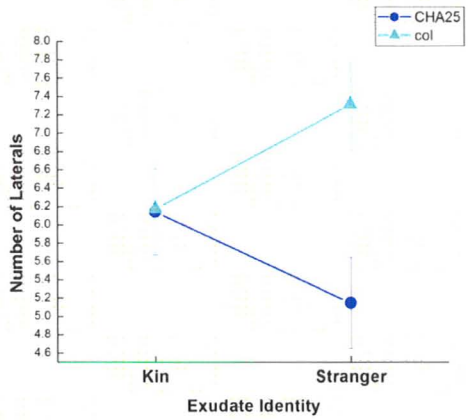
Table 10. Analyses of Variance for Arabidopsis seedlings in low natural light supplemented with low artificial light, under two different levels of irradiance for Col-0 and CHA25. Analysis does not include Ws-0 or Own due to an unbalanced experiment.

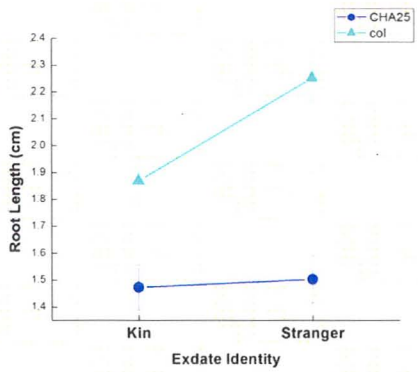
		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Exudate	1	11.14	**0.0013**	5.51	**0.0216**	0.02	0.8847
Family	1	71.14	**<.0001**	42.29	**<.0001**	4.93	**0.0295**
Family × Exudate	1	5.86	**0.0180**	4.02	**0.0488**	4.66	**0.0342**
Shade	1	67.75	**<.0001**	7.04	**0.0098**	2.63	0.1089
Exudate × Shade	1	0.01	0.9405	4.57	**0.0358**	0.98	0.3265
Family × Shade	1	0.79	0.3784	0.52	0.4752	0.00	0.9751
Family × Exudate × Shade	1	0.03	0.8672	2.96	0.0897	0.14	0.7138

Note - degrees of freedom for the error terms were: hypocotyl length 73, root length 73 and number of laterals 73. Bold numbers indicate significance.

Figure 13. Reaction norms for Arabidopsis seedlings in low natural light supplemented by low artificial light. These graphs represent overall data under different light levels. Data not used in this analysis were Ws-0 and own treatments as they do not appear in all trays (unbalanced design).


CHA25
col
Hypocotyl Length (cm)
0.60
0.58
0.56
0.54
0.52
0.50
0.48
0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
Kin
Stranger
Exudate Identity


CHA25
col
Number of Laterals
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
Kin
Stranger
Exudate Identity


CHA25
col
Root Length (cm)
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
Kin
Stranger
Exdate Identity

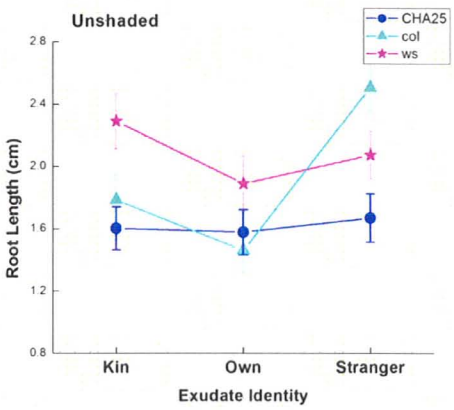
The second analysis looks at the shade treatments independently. There was an exudate source effect in both light treatments, similar to that seen in the previous analysis; for hypocotyl length in fine2 shade and for both hypocotyl length and root length when seedlings were not shaded (Figure 14). It is important to note that the effect of exudate source is much more significant in non-shaded than shaded (Table 11). The genetic variability seen amongst families is similar to the previous analysis as well. For seedlings under no shade there was an exudate × family interaction indicating genetic variation in the kin recognition response (Figure 14).

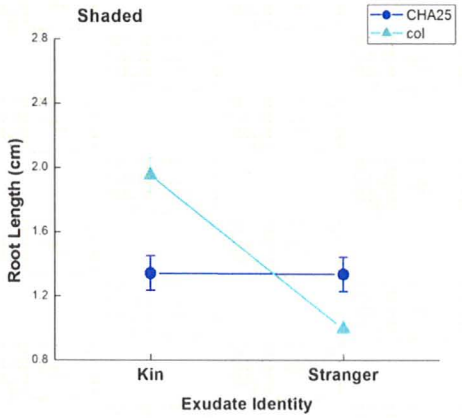
Table 11. Analyses of Variance for Arabidopsis seedlings in low natural light supplemented by low artificial light under differing light levels for Col-0, CHA25 and Ws-0 (no shade only). Shade treatments were separated due to the experiment being unbalanced.

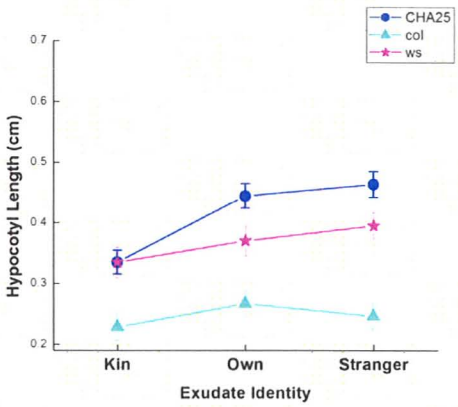
		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Shaded							
Exudate	1	4.82	**0.0334**	0.03	0.8650	0.37	0.5439
Family	1	39.38	**<.0001**	35.08	**<.0001**	2.53	0.1189
Exudate × Family	1	2.30	0.1366	0.05	0.8167	3.37	0.0733
Not shaded							
Exudate	1	8.56	**0.0005**	6.03	**0.0041**	1.07	0.3479
Family	1	48.94	**<.0001**	6.94	**0.0019**	0.68	0.5093
Exudate × Family	1	2.02	0.1021	3.81	**0.0078**	6.38	**0.0002**

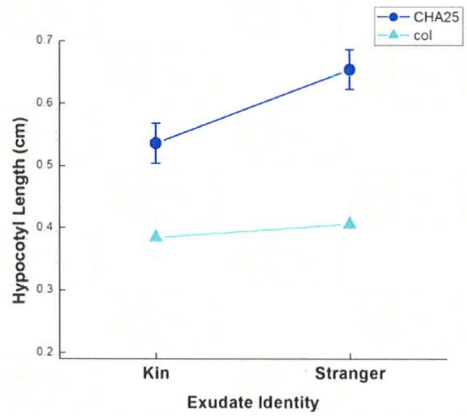
Note - degrees of freedom for the error terms for fine2 were: hypocotyl length 44, root length 44 and number of laterals 44. Degrees of freedom for the error terms for no shade were: hypocotyl length 62, root length 62 and number of laterals 62. Bold numbers indicate significance.

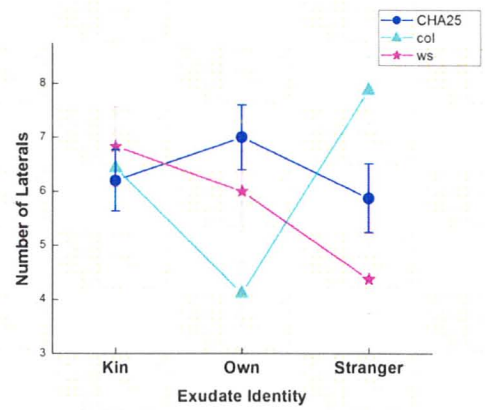
Figure 14. Norms of reaction for Arabidopsis seedlings in low natural light supplemented by low artificial light for two different light treatments in response to exudate source. The two light irradiances used were: no shade (83 μmol photons/m^2/s) and shaded (25 μmol photons/m^2/s).

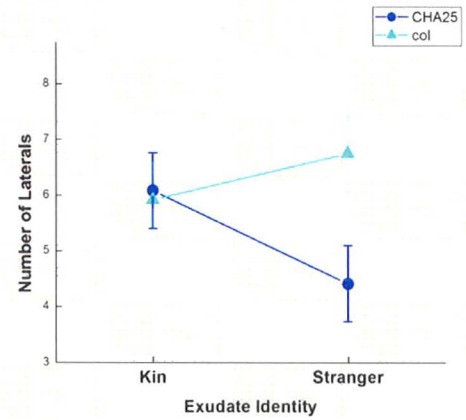

Unshaded
CHA25
col
ws
Root Length (cm)
2.8
2.4
2.0
1.6
1.2
0.8
Kin
Own
Stranger
Exudate Identity


Shaded
CHA25
col
Root Length (cm)
2.8
2.4
2.0
1.6
1.2
0.8
Kin
Stranger
Exudate Identity


CHA25
col
ws
Hypocotyl Length (cm)
0.7
0.6
0.5
0.4
0.3
0.2
Kin
Own
Stranger
Exudate Identity


CHA25
col
Hypocotyl Length (cm)
0.7
0.6
0.5
0.4
0.3
0.2
Kin
Stranger
Exudate Identity


CHA25
col
ws
Number of Laterals
8
7
6
5
4
3
Kin
Own
Stranger
Exudate Identity


CHA25
col
Number of Laterals
8
7
6
5
4
3
Kin
Stranger
Exudate Identity

Increased exudate concentration

Exudate concentration was increased in this experiment by placing three seedlings, instead of one, together in a well, and using the media from the three seedlings as the source exudate. This experiment was performed under high artificial light. There is a significant kin recognition effect to exudate source for number of laterals (Table 12; Figure 15). There was a family effect for hypocotyl length indicating genetic variability between families (Figure 16). This was a small experiment and used only as an investigative tool; further experiments would be needed to look into this result.

Table 12. Analyses of variance for Arabidopsis seedlings placed in increased exudate concentration for Ws-0 and Col-0.

		Hypocotyl Length		**Root Length**		**Number of Laterals**	
Source	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Exudate	2	0.24	0.7893	0.47	0.6280	3.21	**0.0468**
Family	1	81.43	**<.0001**	0.77	0.3844	2.10	0.1525
Exudate × Family	2	0.61	0.5470	0.44	0.6445	0.29	0.7489

Note - degrees of freedom for the error terms were: hypocotyl length 65, root length 65 and number of laterals 65. Bold numbers indicate significance.

Figure 15. Effect of exudate source on number of laterals for Arabidopsis seedlings placed in increased exudate concentration. "Stranger mixed" consisted of two stranger seedlings and one kin seedling while "stranger same" consisted all of stranger seedlings. A significant increased in number of laterals are seen when seedlings were exposed to exudates belonging to stranger.

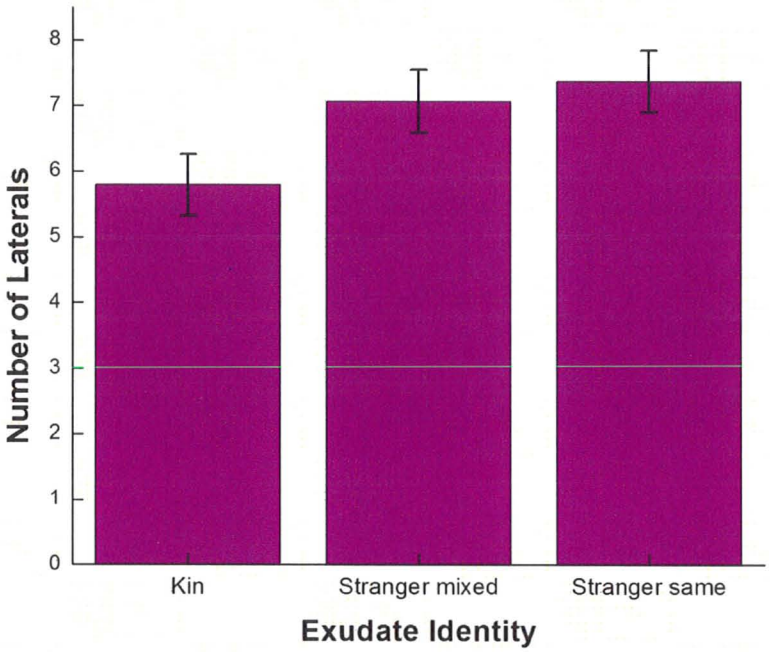
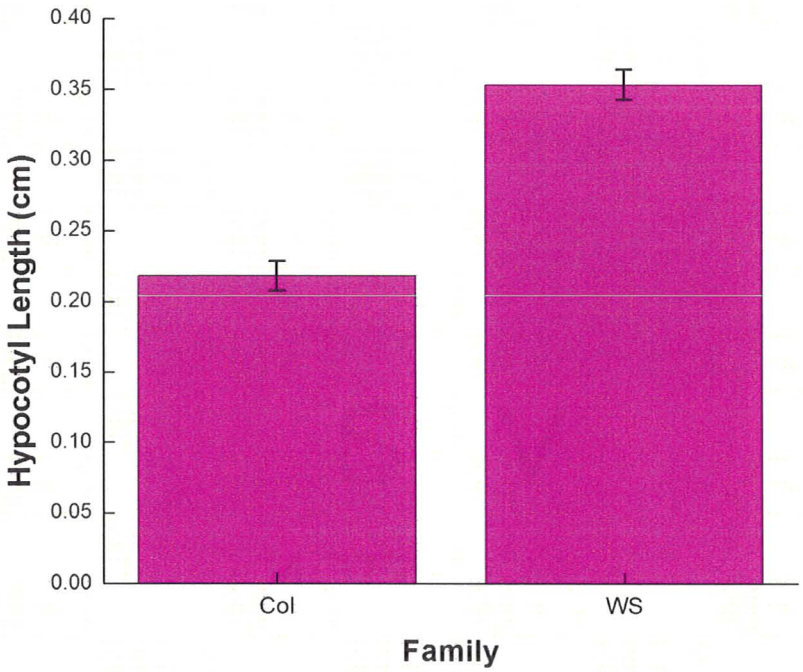

Number of Laterals
0
1
2
3
4
5
6
7
8
Kin
Stranger mixed
Stranger same
Exudate Identity

Figure 16. Genotype variance between families for hypocotyl length in Arabidopsis seedlings in increased exudate concentration.


0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Hypocotyl Length (cm)
Col
WS
Family

Overall grouped findings

When all the different experiments were plotted together there was an interesting relationship between number of laterals and hypocotyl length (Figures 17), though root length showed no correlation with either of these traits. With an increasing hypocotyl length, number of lateral roots decrease. In Figure 17 there were two lines, with the different shades having fewer numbers of laterals than window nat and window fine2, which were experiments that included natural light. Experiments ranged from Dec5 to Apr3 were all in a vertical column, having the same hypocotyl length, with only number of laterals varying, which may be due to trial variation. The differing shade levels show the trend of increasing hypocotyl length with decreasing light (Figure 12).

When the different experiments were looked at with different exudates treatments plotted the trend remains much the same (Figure 18). However, when the different families from each experiment were plotted with hypocotyl against number of laterals the split disappears with one family, Ws-0, filling in the space (Figure 19). CHA and Col-0 show the same trend as in the other graphs, however Ws-0 shows to be less responsive to the different experiments and does not show as large a variance.

Figure 17. Plot of all experiments for hypocotyl length by number of laterals. Dec 5 – April 3 are all from high artificial light experiment. Lights Nat, Fine, CuS04, Fine, Fine2, Finecoarse, Vegetative shade are all different irradiances and qualities of light. Not shaded and shaded are the two different shade treatments for seedlings in low natural light.

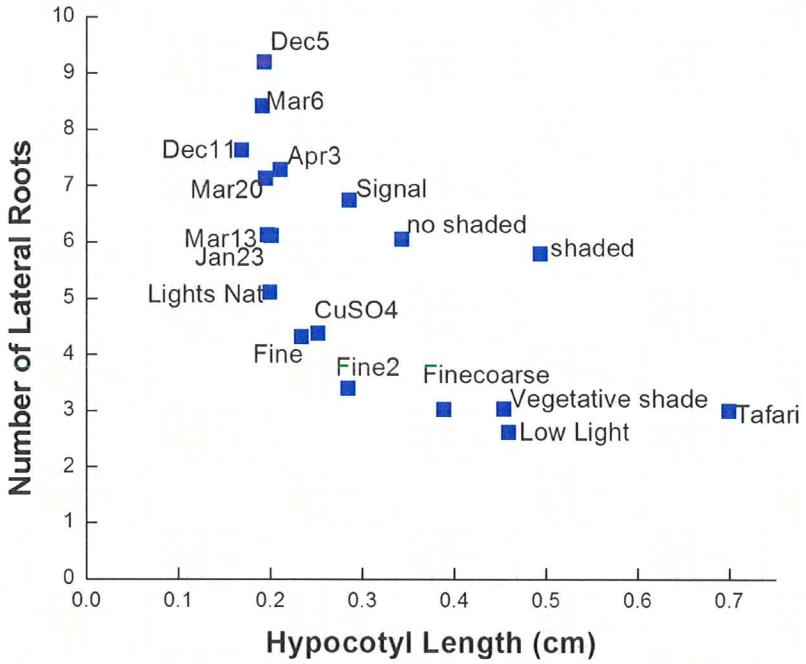
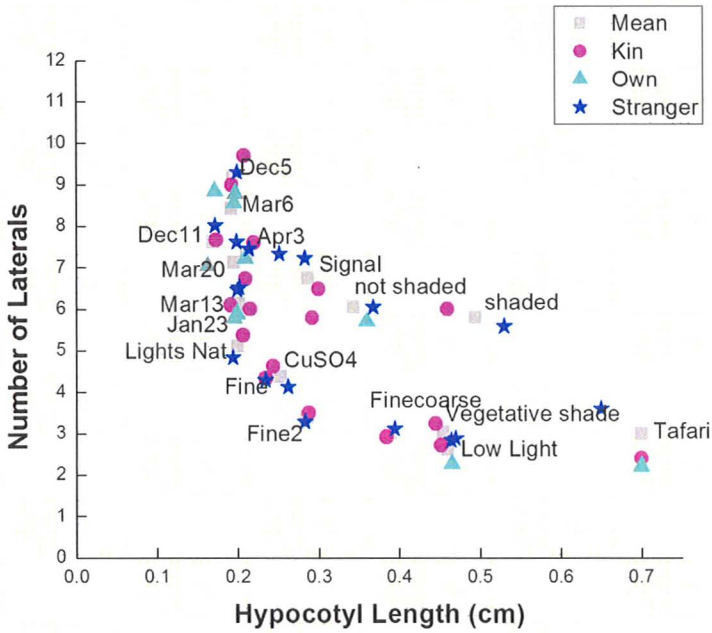
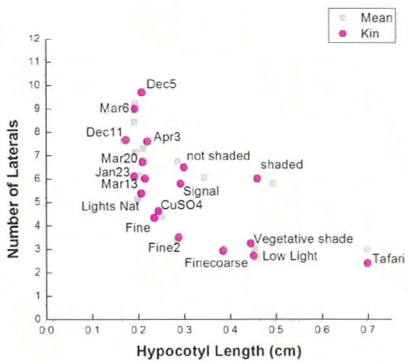
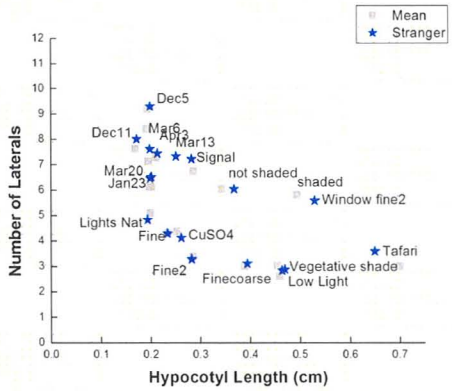

Number of Lateral Roots
Hypocotyl Length (cm)
0
1
2
3
4
5
6
7
8
9
10
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Dec5
Mar6
Dec11
Apr3
Mar20
Signal
no shaded
Mar13
Jan23
shaded
Lights Nat
CuSO4
Fine
Fine2
Finecoarse
Vegetative shade
Tafari
Low Light

Figure 18. Plot of all experiments for hypocotyl length by number of laterals, separated by **exudate source**. Dec 5 – April 3 are all from high artificial light experiment. Lights Nat, Fine, CuS04, Fine, Fine2, Finecoarse, Vegetative shade are all different irradiances and qualities of light. Not shaded and shaded are the two different shade treatments for seedlings in low natural light.


Mean
Kin
Own
Stranger
Number of Laterals
Hypocotyl Length (cm)
Dec5
Mar6
Dec11
Apr3
Mar20
Signal
not shaded
shaded
Mar13
Jan23
Lights Nat
CuSO4
Fine
Finecoarse
Vegetative shade
Fine2
Low Light
Tafari


Mean
Kin
Number of Laterals
Hypocotyl Length (cm)
Dec5
Mar6
Dec11
Apr3
Mar20
not shaded
shaded
Jan23
Mar13
Signal
Lights Nat
CuSO4
Fine
Vegetative shade
Fine2
Low Light
Finecoarse
Tafari


Mean
Stranger
Number of Laterals
Hypocotyl Length (cm)
Dec5
Dec11
Mar6
Apr3
Mar13
Signal
Mar20
not shaded
shaded
Jan23
Window fine2
Lights Nat
Fine
CuSO4
Tafari
Fine2
Finecoarse
Vegetative shade
Low Light

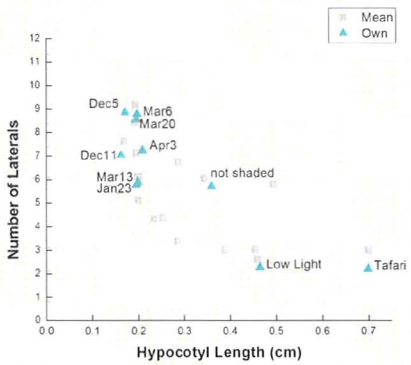
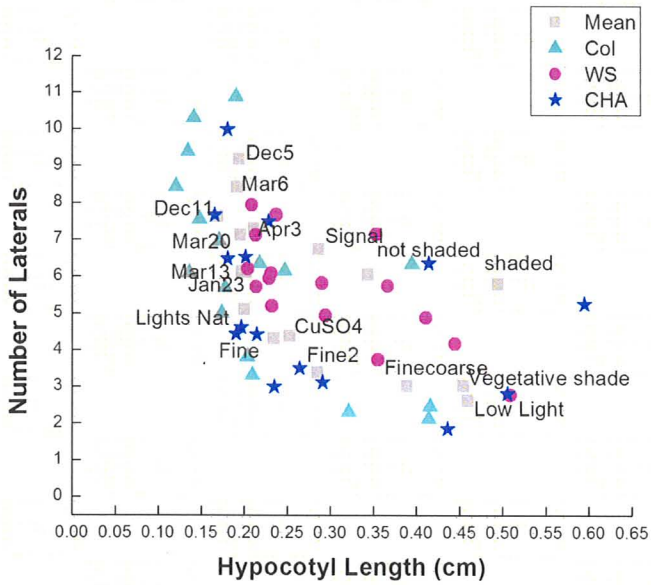
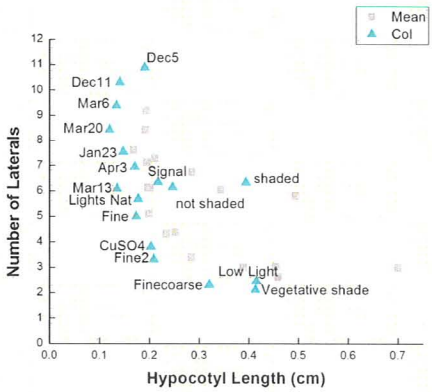
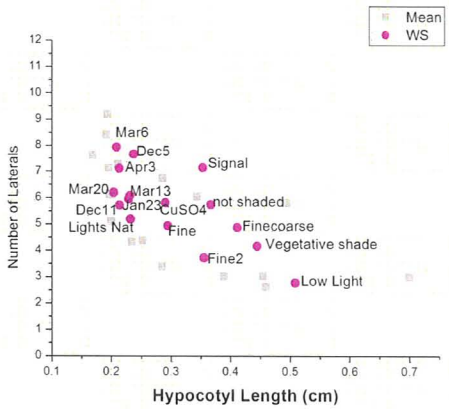
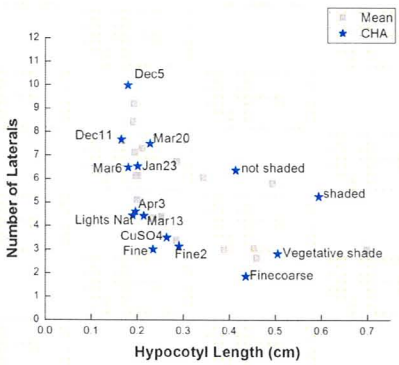

Mean
Own
Number of Laterals
Hypocotyl Length (cm)
Dec5
Mar6
Mar20
Apr3
Dec11
not shaded
Mar13
Jan23
Low Light
Tafari

Figure 19. Plot of all experiments for hypocotyl length by number of laterals, separated by **family**. Dec 5 – April 3 are all from high artificial light experiment. Lights Nat, Fine, CuS04, Fine, Fine2, Finecoarse, Vegetative shade are all different irradiances and qualities of light. Not shaded and shaded are the two different shade treatments for seedlings in low natural light.


Mean
Col
WS
CHA
Number of Laterals
Hypocotyl Length (cm)
Dec5
Mar6
Dec11
Apr3
Signal
Mar20
not shaded
shaded
Mar13
Jan23
Lights Nat
CuSO4
Fine
Fine2
Finecoarse
Vegetative shade
Low Light


Mean
Col
Number of Laterals
Hypocotyl Length (cm)
Dec5
Dec11
Mar6
Mar20
Jan23
Apr3
Signal
shaded
Mar13
Lights Nat
not shaded
Fine
CuSO4
Fine2
Low Light
Finecoarse
Vegetative shade


Mean
WS
Number of Laterals
Hypocotyl Length (cm)
Mar6
Dec5
Apr3
Signal
Mar20
Mar13
Dec11
Jan23
CuSO4
not shaded
Lights Nat
Fine
Finecoarse
Vegetative shade
Fine2
Low Light


Mean
CHA
Number of Laterals
Hypocotyl Length (cm)
Dec5
Dec11
Mar20
Mar6
Jan23
not shaded
shaded
Apr3
Lights Nat
Mar13
CuSO4
Fine
Fine2
Vegetative shade
Finecoarse

Discussion

The studies on *Chenopodium album* and *Arabidopsis thaliana* were designed to determine if kin recognition involved soluble chemicals, by determining if seedlings responded to exudates from kin, own or stranger. In Chenopodium I also manipulated the nutrient treatment to see how they responded and how the response to exudate sources changed in different nutrient conditions. These results can be compared with those previously found in juvenile Chenopodium. In Arabidopsis we changed the quantity and quality of the light to see how the seedlings respond, and if it affected their response to an exudate source. These results can be compared to those by Biedrzycki, Jilany et al (in review) to see how they differ, with the goal of finding a repeatable, reliable assay for kin recognition. Kin recognition was found in both *Chenopodium album* and *Arabidopsis thaliana*. Chenopodium seedlings responded in a similar manner as was found in juvenile plants by Dudley, Lee et al (unpublished data). In Arabidopsis the responses did not consistently match those by Biedrzycki, Jilany et al (in review). The response was dependent on the environmental conditions; different quantity and quality of light caused a range of reactions and differences in kin recognition. Genotypic variance played a large role in the range of reactions exhibited.

Dudley, Lee et al (unpublished data) showed that juvenile Chenopodium seedlings were able to differentiate between kin and strangers when they have belowground neighbours. However, they responded differently depending on the nutrient availability.

When nutrients were readily available, plants would increase their leaf:root ratio when they shared a pot with strangers, competing aboveground for light. However, when they shared a pot with kin there was no marked increase in aboveground growth compared to those who didn't share a pot with neighbours. When nutrients were scarce, plants increased their root:leaf ratio independently of the identity of the neighbour they shared a pot with. Chenopodium did show kin recognition; however, where nutrients were limiting, the identity of the neighbour was less important than obtaining the limiting resource.

In the seedling Chenopodium experiment, I found that seedlings responded most strongly to nutrients. A common response to nutrients is an increased root:shoot ratio in low nutrients compared to high nutrients (Wulff et al. 1999). The Chenopodium seedlings had more lateral roots and longer roots, and shorter hypocotyls in scarce nutrients, indicating more belowground growth, similar to the juveniles (Dudley, Lee et al. unpublished data) which had more belowground mass in scarce nutrients. The seedlings in regular nutrients had longer hypocotyls and shorter roots and less lateral roots, indicated more growth aboveground. This again parallels the juvenile study (Dudley, Lee et al. unpublished data), where plants in regular nutrients had more aboveground growth. I found little among-family variance in reaction norms, unlike Wulff, Causin et al. (1999), but that may be due to the fact that the families were artificially seleccted from a larger group based on seed viability and germination time.

Chenopodium seedlings responded differentially to exudates belonging to kin, own and strangers, thereby showing that they responded to a soluble chemical cue rather than a physical interaction between roots. Seedlings exposed to kin exudates in both regular and scarce nutrients were larger than those exposed to stranger exudates. However, the interaction between the kin treatments and nutrients treatments is less distinct than in the juvenile plants. Those in regular nutrients exposed to kin exudates had a higher root:shoot ratio, which is similarly displayed in the response by the juvenile Chenopodium plants.

Arabidopsis did respond to exudates belonging to kin, own and stranger in some of the trials. However, the responses were variable, and tended to involve hypocotyl and root length changes than changes in number of laterals. Biedrzycki, Jilany et al (in review) observed rather that seedlings in the presence of stranger exudates had more lateral roots. We did find this response in some of the high artificial light trials (Figure 7) and Ws-0 in natural light (Figure 13, 14) and in increased concentration of exudates (Figure 15). Biedrzycki, Jilany et al (in review) grew their seedlings in diffused natural and artificial low light, When experiments were done with mixed natural and artificial light, similar to the work by Biedrzycki, Jilany et al (in review), there was a significant increase in the response to exudate source for all traits. A response to exudates source was seen in low artificial light, but it was variable between families (Table 7). The reaction to natural light may be due to the change in the light spectrum. Hypocotyl length has been shown to be inhibited by sunlight, high blue light and high red light while it increases in high far-red light and low R:FR light (Yanovsky et al. 1995).

Differences in light quality and irradiance in artificial light did affect the seedling morphology. With increasing irradiance seedlings produce shorter hypocotyls and more lateral roots (Figure 11). As predicted the seedlings under the simulated vegetative shade (low R:FR) had elongated hypocotyls (shade avoidance response). There were no exudate × shade interactions, which indicated that differences in light quality and irradiance did not affect a response to source exudates. However, in this experiment Ws-0 did increase hypocotyl length in the presence of stranger exudates (Table 8; Figure 10).

Organisms tend to have genetic variation in plasticity, where genotypes vary in the response to differing environmental conditions, e.g. *Prunella vulgaris* L (Winn and Evans 1991), *Danthonia spicata* (Scheiner and Goodnight 1984), *Impatiens capensis* (Schmitt 1993) and *Arabidopsis thaliana* (Pigliucci et al. 1995). This genotype by environment effect was demonstrated clearly with the different seedling families and their variation in responses to differing conditions. Plasticity to exudate source in Arabidopsis was dependent on the environmental conditions experienced by the seedlings as well as family.

Two seed families that I used in every experiment were Ws-0 and Col-0. They are both lab accessions and come from different parts of the world, and have been maintained in lab conditions, potentially resulting in artificial selection. Ws-0 originated in Belarus, Col-0 originated in Germany and is an accession of Landsberg that was not irradiated (Passardi et al. 2007). In many of the experiments, treatments elicited contrasting phenotypes in Ws-0 and Col-0. In many cases Ws-0 would produce a longer hypocotyl and Col-0 a shorter hypocotyl whereas Ws-0 would produce shorter roots and Col-0

would produce longer roots. We saw examples of this in: high artificial light (Figure 6); low artificial light (Figure 4); changes in light quality and irradiance (Figure 11, 12); low natural and low artificial light (Figure 14); increased exudate concentration (Figure 16) and an overview of all the experiments (Figure 19). Passardi, Dobias et al. (2007) found that Col-0 had longer roots than Ws-0 and that Ws-0 had a faster growth rate, which was probably due to its earlier flowering time (Passardi et al. 2007). This might explain why Ws-0 always had a longer hypocotyl than Col-0. Col-0 and Ws-0 also vary in their response to short days versus long days and whether or not they require vernalization. Col-0 did not need vernalization and flowered quickly in long days whereas Ws-0 flowered faster after vernalization in short days and was one of the fastest ecotypes to flower in long days (Karlsson et al. 1993). The contrasting responses we observed for Ws-0 and Col-0 are supported by these reported differences in ecotypes.

Among the CHA families, there was less genetic variability as they were all from the same population, so reactions were fairly similar amongst those families. The RBG4 family was always larger as seedlings, with longer hypocotyls, longer roots and more lateral roots. This was probably due to RBG4 being a local population has a adapted to this environment.

We can conclude that both Chenopodium album and Arabidopsis thaliana can recognize kin from an exudate source. However, in Chenopodium the reaction is not as apparent as in juvenile seedlings and in Arabidopsis the reaction is dependent on environmental conditions. For Arabidopsis, more experiments need to be done to establish a solid assay.

I was not able to successfully find a consistent methodology. However, the results do suggest some direction for future experiments. Increasing the concentration of the exudates may allow for more consistent response with a stronger signal. Changes in light quality and irradiance may still play an important role, especially natural light. Seedlings sharing a well and providing a constant source of exudates may increase the response, similar to the increased concentration of exudate. Another experiment would be to examine the seedlings over a time course to see if leaving them in the wells longer may increase the chance of a response to exudate source to develop. Changes in temperature and relative humidity may alter the response to exudates, and may be why there were so many differences between trials. The experiments were performed over a long period, spanning several different seasons. This change in outside weather had an impact on the environment in the lab through heating or air conditioning, which would cause variation in the temperature and humidity. Genotype × environment × exudate source will be an important interaction to examine in future experiments.

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