

Communication between Two *Lactuca* Species

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Abstract

There is increasing evidence of plant communication and behavior. We examine how two *Lactuca* species, *L. sativa* and *L. serriola*, communicate with themselves and one another via root exudates. We exposed both species to their own, to the same species, and to the other species root exudates. We then measured the length of their primary root as a proxy for competitive effort. *L. serriola* produced longer roots when exposed to its own exudates relative to when exposed to *L. sativa*'s. In contrast, *L. sativa* produced shorter roots when exposed to its own root exudates. These results indicate kin recognition in these species. Further, the results show that *L. sativa*, a domesticated species, shares resources well with conspecifics. In contrast, *L. serriola*, a sparsely distributed species, is more competitive with conspecifics than with other species. We argue that artificial selection and domestication of *L. sativa*, from its progenitor *L. serriola*, modify how the species interprets and respond to exudate cues from neighboring plants.

Keywords

Plant Communication, *Lactuca*, Root Exudate

1. Introduction

Emerging research shows that plants have complex systems of communication and behavior. A mounting number of studies show plants are able to self-identify and recognize closely related kin [1]-[9]. Early evidence of self-recognition in plants was demonstrated with the desert shrub *Ambrosia dumosa*. When in contact with another individual's roots, *Ambrosia* showed a precipitous decline in root growth. In contrast, there was no such decline when it was in contact with its own roots. Further, *Ambrosia* can distinguish individuals from its own population from that of *Ambrosia* plants from a different region. When in contact with its local neighbors, it responded with a decline in root growth. However, when in contact with plants from another region, its root growth remained robust [1]. It is believed that this behavior is a mechanism to limit

competition with closely related kin. Other examples of self- and kin-recognition in plants reveal alternate plant behaviors. *Impatiens palladida* grown in the presence of siblings plants, had greater root growth than that of plants grown with strangers [10]. As evidence mounts, it is clear that plant species signal to one another, can determine the identity of the plants sending the signals and in some cases, can even distinguish between the sexes [11]. Following the receipt and identification of signals, plants then respond accordingly. It seems it is time to accept plants as autonomous creatures with communication and behavior.

As root communication studies emerged, they were criticized for not controlling variables such as pot volume, nutrient effects, and inherent size inequalities between different populations [6] [7] [12]. Furthermore, there are complex underground microbial communities that influence plant behavior. Different plant species, and even ecotypes, generate unique microbial communities in the soil, and these communities feedback on plant growth [13]-[19]. Therefore, it is difficult to determine if plant behaviors are a result of direct interaction, or mediated by soil organisms. Biedrzycki *et al.* 2010 [5] addressed these issues by growing *Arabidopsis* in liquid media and exposing them to the root exudates of siblings, and of strangers. By not including the soil, they eliminated any influence of soil microbial communities. They found that plants exposed to the root exudates of strangers had greater root growth than those exposed to sibling exudates, showing that plants recognized and responded to direct communication from their neighbors [5].

While root interactions have been implicated in intra- and interspecific competition, we are not aware of any studies that consider root interaction as a potential mechanism for plant dispersion. Plant-soil feedbacks, competition, herbivory, and seed dispersal have been implicated in plant dispersion and rarity [15]-[21]. We suggest that underground root communication is also in-part driving plant rarity and likewise abundance in the wild. We also believe that plant-to-plant interactions are equally important agriculturally. How plants respond to their neighbors can determine the viability of a given cultivar for either monocropping or intercropping. Different combinations of species can translate to a range of agricultural yields [8] [22]. For example, when the maize variety GZI was grown with soybean HX3, the roots tended to avoid each other. However, when maize variety NE1 was grown with soybean HX3, roots showed an affinity for one another [8].

We examined *Lactuca sativa*, a cultivated lettuce, in comparison with *Lactuca serriola*, its wild progenitor, to see whether artificial selection for agricultural use, changes the way of species communication and behavior. These two species are so genetically close that some argue they can be considered conspecific [23]. However, one of their key differences is their dispersion; *L. sativa* grows well in monoculture, and *L. serriola* is found distributed sparsely in the wild.

Work done to look at growth and competition between the two species has revealed *L. sativa* to be a stronger competitor. However, *L. serriola* generates a more negative plant-soil feedback than *L. sativa*. This soil community feedback negatively on *L. serri-*

ola growth, but also gives the *L. serriola* host increased competitive ability (Aguilera *et al.*, in review). This work suggests that *L. serriola*'s sparseness in the field is in-part due to the negative feedback of its soil community. Likewise it implies that *L. sativa*'s success as a monoculture crop is again in-part, due to a release from these negative feedbacks. However to understand the complete picture we must first understand the individual effects of microbial feedback and direct communication.

In this paper, asked whether *L. sativa* and *L. serriola* can distinguish self, from the same species, or from another species. We grew each species in liquid media and exposed them to their own exudates, the same species exudates, or the other species exudates. We expected that *L. sativa*, the cultivated species would reduce root growth when exposed to its own species, relative to when exposed to *L. serriola*, as a means of reducing intraspecific competition. In contrast, with the same design using *L. serriola*, we expected to see that opposite. Due to *L. serriola*'s reluctance to grow in monoculture in the wild we predicted that it will show reduced root growth with *L. sativa*, relative to itself: thereby showing a preference for growing next to someone other than a conspecific.

2. Methods

We sterilized all seed in a 50% bleach solution for 5 minutes, followed by three 3-minute rinses with DI water. Following seed sterilization we germinated all seeds in sterilized liquid media (Murashige and Skoog Basal Medium). Once the seeds germinated (approximately 10 days), we placed seedlings into 3.5 ml wells in experimental well culture plates. In trial 1, we exposed *L. sativa* individuals to their own exudates (OWN), so other *L. sativa* exudates (SAME), and to *L. serriola* exudates (OTHER). In a procedure modified from Biedrzycki *et al.* (2010) [5], every day for seven days individuals in the OWN treatment were lifted out of their well using forceps then and placed them back into their original well. For the SAME treatment, individuals were rotated from their own well into a neighboring *L. sativa* well. Finally for the OTHER treatment, individuals were paired with with a *L. serriola* seedling and each day individuals were switched from one well to the other (Figure 1). We replicated this design 6 times. All experimental well plates were kept in a growth chamber used to simulate a typical New England environment with 15 hours of daylight at 25°C. Inside the growth chamber well plates were on a rotary shaker to maintain a constant mixing of the media solution and to prevent a state of hypoxia. Seven days after the start of the experimental treatments we removed the seedlings and measured the length of their primary root using calipers. We used root growth as a proxy for competitive effort, with increased root growth indicating increased below-ground competitive effort. For trial 2, we used an identical experimental design with *L. serriola* plants for the focal species and *L. sativa* plants for the OTHER treatment. All data were analyzed with a one-way ANOVA and pairwise comparisons were performed using the Holm-Sidak method in SigmaPlot.

3. Results

We found significant differences in primary root length in both trial 1 with *L. sativa*

(ANOVA, $F_{2,15} = 4.930$, $P = 0.023$) (Figure 2) and in trial 2 with *L. serriola* (ANOVA, $F_{2,15} = 26.465$, $P < 0.001$) (Figure 3). Pairwise comparisons show that there was no significant difference in root length for either species when exposed to their OWN or SAME species root exudates, indicating no difference in competitive effort when faced with their own versus the same species exudates. However, when *L. sativa* was exposed to OTHER root exudates the primary roots grew significantly longer than when exposed to its OWN or SAME exudates (Holm-Sidak, OWN-OTHER, $P = 0.039$; SAME-OTHER, $P = 0.038$) (Figure 2). In contrast when *L. serriola* was exposed to OTHER

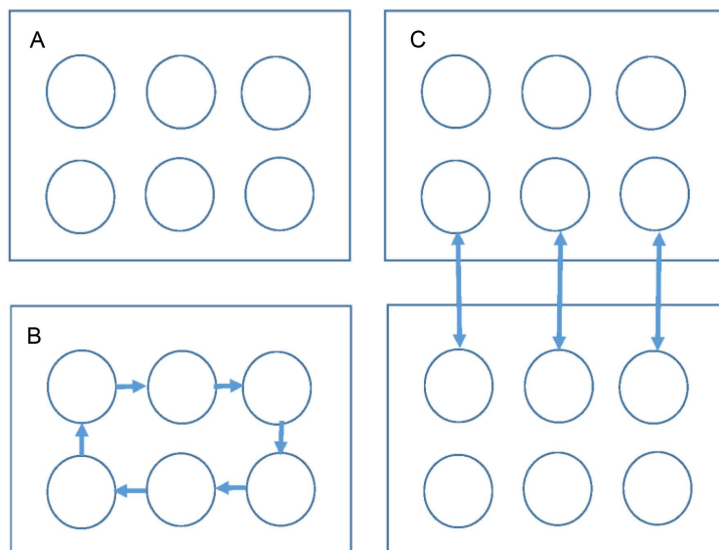


Figure 1. Schematic diagram of the experimental design for both trial 1 and trial 2.

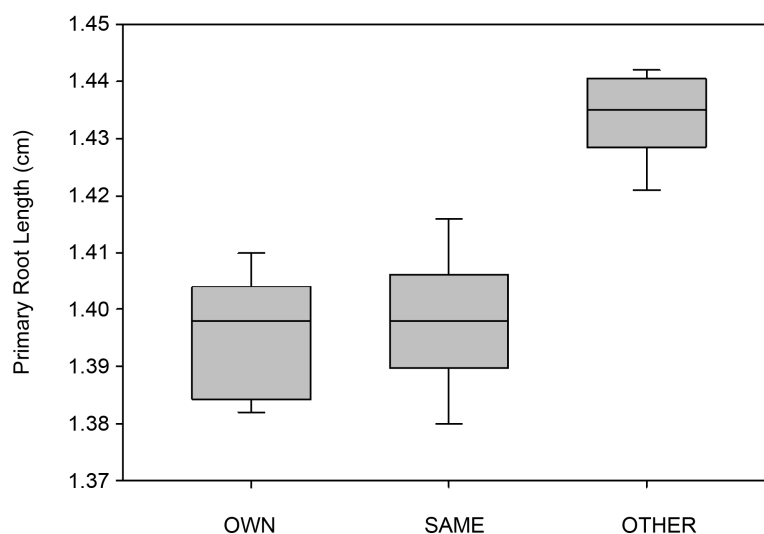


Figure 2. Box and whisker plot showing the spread of the data for trial 1 with *Lactuca sativa*. Exudate treatment is listed on the x axis and root length is on the y axis. For each treatment, the lines inside the boxes show the median, the first and third quartiles are shown the ends of the boxes, and the maximum and minimum values are shown at the ends of the whiskers.

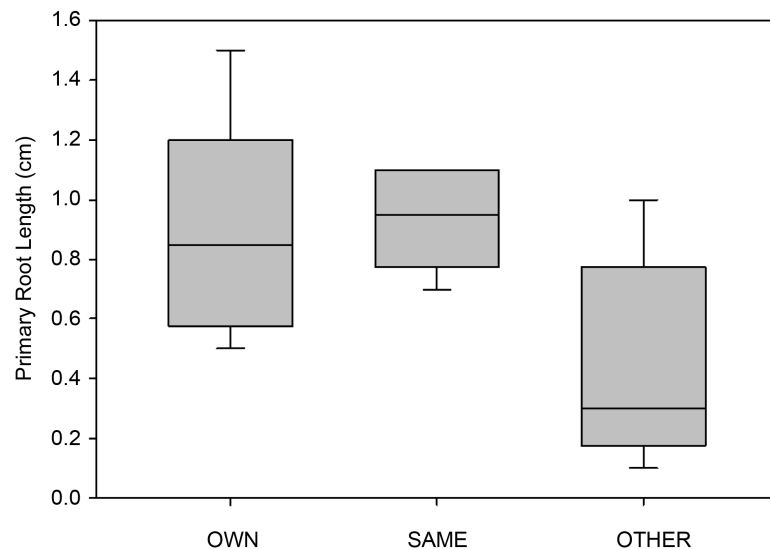


Figure 3. Box and whisker plot showing the spread of the data for trial 2 with *Lactuca serriola*. Exudate treatment is listed on the x axis and root length is on the y axis. For each treatment, the lines inside the boxes show the median, the first and third quartiles are shown the ends of the boxes, and the maximum and minimum values are shown at the ends of the whiskers.

root exudates the primary roots were significantly shorter (Holm-Sidak, OWN-OTHER, $P < 0.001$; SAME-OTHER, $P < 0.001$) (Figure 3). When considered as a proxy for competitive effort this root data shows that *L. sativa* exerts less competitive effort when exposed to the same species root exudates. In contrast, *L. serriola* showed increased competitive effort when exposed to the same species root exudates.

4. Discussion

Our experiment shows clear evidence for kin recognition in *Lactuca* species. Both *L. sativa* and *L. serriola* react to conspecific root exudates as if they were their own (Figure 2 & Figure 3). It is important to distinguish kin-recognition from self-recognition. In true self-recognition, plants are able to distinguish genetically identical clones from themselves. For example, when *Ambrosia* roots are in contact with still connected sister-roots, there is no change in root elongation rates. However, when in contact with physically separate, genetically identical clones, *Ambrosia* plants respond with reduced root elongation [1]. Kin recognition is in contrast when plants are able to distinguish kin presumably through genetic similarity [24]. For example the grass, *Distichlis spicata*, can distinguish sibling plants (from the same mother plant) from those of the same species but not siblings [10]. Our results indicate that while *Lactuca* root exudates may mediate kin-recognition, we see no evidence for exudate mediated self-recognition.

Most interesting, is that these two species show different behaviors when exposed to their OWN or SAME exudates versus OTHER root exudates. As we predict, the domesticated lettuce cultivar, *L. sativa*, has reduced primary root production when exposed to its own species root exudates. Further, *L. serriola* shows the opposite pattern; increases root production when exposed to the same species root exudates. These results imply

that *L. sativa* reduces its below competitive effort when grown with its own species. Conversely, *L. serriola* increases its competitive effort when grown with conspecifics.

While many studies implicate competition, plant-soil feedbacks, and herbivory, in plant distribution and abundance [16] [17] [18] [19] [20] [25], we know of none that implicate direct plant-to-plant communication. Clearly, distributions in the field are the net result of all of these interactions (and more) occurring simultaneously [26]. It is argued that plant-soil feedbacks and competitive interactions should never be considered separately [27]. We argue that this should be extended to include direct plant communication as well. The soil microbial communities of *L. sativa* and *L. serriola* show that *L. serriola* has a less diverse microbial community in its rhizosphere soil than *L. sativa* [28]. Further, the microbial community of *L. serriola* creates a more negative soil feedback that influences both *L. serriola* growth and competition. Interestingly, *L. serriola* grows larger in the presence of *L. sativa*'s soil microbial community than it does in the face of its own soil community. In competition experiments in sterilized and non-sterilized soil, the microbial community of *L. serriola* makes it a stronger competitor (Aguilera *et al.*, in review). In summary, when compared with *L. sativa*, *L. serriola* has a less diverse soil community that creates a more negative plant-soil feedback for itself and its neighboring competitor plants. Our results indicate that in addition to this, *L. serriola* is also sending direct plant-to-plant signals that discourage growth next to conspecifics. Taken together, it is a picture of how *L. serriola* carves out its own "personal space" for each individual. Conversely, this implies that in selecting for agriculturally beneficial traits, we have altered the message which *L. sativa* plants send to each other. In essence, agriculture has been a selective force driving the evolution of the *L. sativa* towards a more social behavior, allowing them to grow well with conspecifics.

Our work supports the assertion that the environment requires plants to make decision and act on them. Furthermore, those plants may be better understood once it is accepted that they are endowed with some degree of autonomy [4] [29]. Indeed, it is not just below ground where plants are directly communicating. Crepy and Casal (2015) [30] showed that *Arabidopsis* plants recognized neighboring kin and subsequently reoriented their leaves to minimize mutual shading of neighboring kin. Other examples of cooperative behavior in plants involve the release of volatile organic compounds (VOCs) following herbivore attack that cues neighboring plants to increase herbivore defenses and avoid damage. Response to VOCs can also be population specific where receiver plants respond only to VOCs sent from individuals within their population [31]. Clearly plants are complex creatures that receive information in a variety of ways, process that information, and act accordingly.

Using plant-plant communication to understand plant dispersion and abundance has major implications for agriculture, invasive species, and conservation of rare species. Understanding the messages plants send to each other will aide in determining the best varieties for intercropping versus monoculture. Indeed, it has been shown that crops in mixtures produce more roots. Further, greater roots in these systems are correlated with higher yields [23]. Recognizing what allows invasive plants to thrive in dense mo-

nocultures can be an important tool for invasive plant control. Finally, when protecting rare species, it is vital to understand which species share resources in the most efficient way. In short, to understand and manage our vital plant resources, we need to listen to what plants are saying to each other, and understand how it affects their behavior.

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