

EFFECTS OF PLANT-PLANT AIRBORNE INTERACTIONS ON PERFORMANCE OF
NEIGHBORING PLANTS USING WILD TYPES AND GENETICALLY MODIFIED LINES
OF *ARABIDOPSIS THALIANA*

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ABSTRACT

M. Gabriela Bidart, Advisor

Understanding plant-plant communication further elucidates how plants interact with their environment, and how this communication can be manipulated for agricultural and ecological purposes. Part of understanding plant-plant communication is discovering the mechanisms behind plant-plant recognition, and whether plants can distinguish between genetically like and unlike neighbors. It has been previously shown that plants can “communicate” with neighboring plants through airborne volatile organic compounds (VOCs), which can act as signals related to different environmental stressors.

This study focused on the interaction among different genotypes of the annual plant *Arabidopsis thaliana*. Specifically, a growth chamber experiment was performed to compare how different genotypes of neighboring plants impacted a focal plant’s fitness-related phenotypes and developmental stages. The focal plant genotype was wild type Col-0, and the neighboring genotypes included the wild type Landsberg (Ler-0), and the genetically modified (GM) genotypes: *Etr1-1* and *Jar1-1*. These GM lines have a single point-mutation that impacts their ability to produce a particular VOC. This allows for the evaluation of a particular role that a VOC may have on plant-plant airborne communication. Plants were grown in separate pots to eliminate potential belowground interactions through the roots, and distantly positioned to avoid aboveground physical contact between plants. In addition, to avoid potential VOC cross-contamination between different treatments (genotypes), each neighboring plant treatment occurred in separate, sealed growth chambers.

Results showed that when *A. thaliana* Col-0 plants were grown alongside neighbors of different genotypes, they exhibited some significant differences in fitness-related traits, such as increased rosette width, stem height, aboveground biomass, and total fruit number. However, these results differed with neighbor identity, and when the experiment was repeated.

Arabidopsis thaliana also experienced developmental delays in bolting and flowering time, when exposed to neighbors having a mutation in their ethylene receptors (*Etr1-1*), but not from any other genotypes.

These results indicate that *Arabidopsis thaliana* is capable of differentiating neighbor identities through airborne VOCs. Since all mutations caused some significant changes to *A. thaliana*'s growth, it is likely that *A. thaliana* is sensitive to multiple changes in VOC signatures. However, there was high variability between replications, and some phenotypes did not experience expected changes based on previous studies. Therefore, more studies should be performed to discover the effects of different VOCs on plant-plant communication via airborne volatiles.

To my parents, for teaching me to look for the beauty in the world.

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INTRODUCTION

Plants are known to communicate through volatile organic compounds, or VOCs (Frost et al., 2008). VOCs are secondary metabolites, which may not be directly required for survival and reproduction, but they serve diverse roles in competition, defense, and transporting agents of other metabolites (Das et al., 2013; Demain & Fang, 2000; Šimpraga et al., 2016). Plants emit these compounds, which are received by neighboring plants. For example, during the uptake of CO₂, these chemicals can be absorbed and be used to “prime” neighboring plants. Priming can be defined as the plants’ ability to induce the production of physiological defenses or mechanisms that would enhance future responses against an environmental stress or predator (Frost et al., 2008; Scala et al., 2013). For example, green leaf volatiles (GLVs) released through the stomata have been proven to be involved in plant-plant communication; however, many of these VOCs are ephemeral in nature and readily react with other molecules in the air such as ozone, nitrate radicals, and hydroxyl radicals, which make them difficult to identify and measure in the field (Atkinson & Arey, 2003; Pinto et al., 2007).

Plant-plant communication has been previously shown to be genotype-specific, in both wild plants as well as in crop species; with neighboring plants responding differently to focal plants that vary in their genotypes (Ninkovic et al., 2016). Also, factors in the environment can influence plant production of VOCs. The presence of insect herbivores has been shown to modify interplant communication by interfering with how plants communicate with each other. A plant can detect the presence of chewing insects by the vibrations caused by feeding, which may lead them to release a volatile compound (Body et al., 2019). The surrounding plants of the same species receive these chemical signals, and may respond in a way that would enhance growth or the production of defensive chemicals (Dicke, 2009; Dicke & Hilker, 2003; Frost et al., 2008). A previous study has

shown that sagebrush plants could prime their neighbors over half a meter away, depending on the presence of proper airflow (Karban et al., 2006). In addition, insects may avoid “detection” by their plant hosts, so those plants will be less likely to send out VOC distress signals to their neighbors (Kim et al., 2013). Some insects, such as the pea aphid, can even inhibit a plant’s ability to produce VOCs in the presence of multiple predator species (Schwartzberg et al., 2011).

While the mechanisms related to plant-plant communication are still being studied across many species, recent studies hold promising results that could be used in future applications for humans. Priming could lead to plants with improved defense against insects, and higher growth rates (Ninkovic et al., 2016), which can be highly valuable to modern agriculture, because it may serve as another alternative pest management strategy. For example, inducing chemical defense mechanisms in plants can improve their resistance against a particular species of mite, which could potentially reduce farmers’ dependence on pesticides (Agut et al., 2018). Most of the previously mentioned studies have been performed using model plants, such as *A. thaliana*, but plants used for agricultural purposes appear to have similar responses. Known plant-plant interactions via volatiles occur in agriculturally significant plants and can potentially be used to supplement traditional pesticides (Giron-Calva et al., 2017). Using plant-plant communication could also decrease dependence on fertilizers, enhancing plant growth. One study found that lima beans under attack from herbivorous insects grew faster when primed by VOCs from neighboring plants, or from other parts of the plants themselves, when compared to undamaged plants (Heil & Bueno, 2007). By tapping into VOC production, farmers could not only protect plants against pests, but also enhance the growth of their crops (Tahir et al., 2017). These studies are relatively new, and their implications are yet to be deciphered. One recent study found that cabbage plants sprayed with monoterpene passively absorbed the VOC and re-released it, resulting in fewer diamondback

moth eggs ovipositing on their leaves (Mofikoya et al., 2017). Some methods of artificial VOC application exist, although to use them effectively, scientists must accurately decipher the complex VOC bouquets that crops use (Pickett & Khan, 2016).

In the field, there are constant biotic and abiotic environmental pressures acting on wild plants and crops. This pressure may influence communication within a plant, as well as between neighboring plants. However, previous research has rarely focused on how plant-plant communication may mediate responses to an important environmental factor such as competition. Until recently, very little was known regarding whether plants could use plant-plant communication for kin recognition and selection and have different competitive types of responses to neighbors that are genetically similar or distinct. Producing volatiles is costly to a plant, and if a plant is competing with a neighbor for limited resources (e.g., space, water, sunlight), they may allocate different resources to defense and growth depending on neighbor genetic identity (Shimola, 2018). Several plant-plant competition studies have been done involving root systems. *Cakile edentula* lengthen their root systems to compete for water when grown in the same pot as neighbors of different genotypes (Dudley & File, 2007). *Impatiens pallida* are capable of kin recognition through physical contact through their root systems and can allocate their resources to increase stem elongation and aboveground biomass allocation, which would increase their chances of accessing sunlight (Murphy & Dudley, 2009). *Tagetes patula* produce thiophenes through their roots in the presence of neighbors, kin and non-kin alike. However, while neighbor genotype had little to no effect on the amount of thiophenes released, non-kin neighbors had significantly smaller biomass than kin controls, showing that root thiophenes can inhibit the growth of non-kin neighbors (Weidenhamer et al., 2019). These plants produce thiophenes without touching their neighbors, which implies they can also identify signals from these neighbors (Weidenhamer et al., 2019). In

other studies, using the plant species *Ambrosia dumosa* and *Artemisia tridata*, researchers found that there was no change in the growth of plants when there were root interactions among the same clones. However, when they touched roots of other plant species they secreted exudates to inhibit their neighbor's growth (Karban & Shiojiri, 2009; Karban et al., 2013).

More examples exist in the literature regarding the effects of plant species or genotype-specific responses in terms of both growth and defense. Lima bean plants (*Phaseolus lunatus*) produce defensive chemicals in response to signals from nearby damaged lima bean plants, but only if the damaged plant belonged to the same population as the neighboring plants (Moreira et al., 2016). This implies that *P. lunatus* is highly sensitive to differences in the genetic makeup of its neighbors, even without physical contact. Rice cultivars 'Huagan' and 'Lingyou' can distinguish between the genetic differences of eighteen other cultivars through secretion in their roots, and will grow longer, more complex root systems when planted near distantly related cultivars (X.-F. Yang et al., 2018). This led to fewer resources being allocated to flowering and fruiting, resulting in lower rice yields. Curiously, when these cultivars were planted near kin lines (genetically similar lines, but not the same), they had higher grain yields than when they were grown in monocultures (X.-F. Yang et al., 2018).

A previous study by Shimola and Bidart (2019) corroborated previous findings related to the importance of neighbor genotype identity, in terms of plant fitness-related responses, which are likely driven by genotype-specific airborne volatiles (since plants did not have any physical contact). Using the annual plant *Arabidopsis thaliana*, this study showed that if a focal plant was of a different genotype (i.e., Landsberg erecta, *Ler-0*) than neighboring plants (Columbia genotype, *Col-0*), the neighboring plants would respond in a "competitive-like" way and grow larger. However, this type of response did not occur if the focal and neighboring plants had the

same Col-0 genotype (Shimola and Bidart 2019). These results are supported by another study with barley (*Hordeum vulgare*), which found that when a barley cultivar known as ‘Kara’ was exposed to volatiles from another cultivar known as ‘Alvar,’ it responded with higher root biomass and specific leaf area, even though their total biomass was the same when exposed to clean air or ‘Kara’ volatiles (Ninkovic, 2003). Once again, a plant was able to recognize intraspecific genetic differences of their neighbors and react in a ‘competitive’ way.

The main goal of this research project was to focus on identifying the role that VOCs may play in genotype-specific plant-plant competitive-like interactions in *A. thaliana*. One way to evaluate this goal is by using genetically modified (GM) lines of *A. thaliana*, which share the same genetic background as the wild type Columbia (Col-0), but differ in single genes coding for different VOCs. The annual plant *Arabidopsis thaliana* was selected for this study because it is a well-studied model organism with a fully sequenced genome and hundreds of mutant lines available through The Arabidopsis Information Resource (TAIR). This annual plant commonly known as thale cress or mouse-ear cress, is a member of the Brassicaceae family. This species is also short-lived and can be grown in large numbers. The Columbia (Col-0) wild type has a lifespan of six to twelve weeks, it germinates within five to ten days, and flowers within thirty to fifty days from germination time (Boyce et al., 2001). The plasticity of fitness-related traits in this plant makes it ideal to study responses to environmental factors and genotypic differences in these responses.

For this study, four different genotypes were selected. Two wild types: Columbia (Col-0) and Landsberg erecta (*Ler-0*), and two Col-0 mutants (*Jar1-1* and *Etr1-1*). The genotypes Col-0 and *Ler-0* are polymorphic at a gene on the fourth chromosome called CYP74B2, which codes for hydroperoxide lyase (HPL), which has a downstream effect on C-6 volatiles, jasmonic acids, and

aliphatic glucosinolates (Duan et al., 2005). Columbia plants have a single-nucleotide deletion in the first exon, leading to fewer transcripts of CYP74B2. However, Landsberg has a complete first exon, leading it to have more transcripts of this gene; and therefore, higher levels of GLVs (Duan et al., 2005; Snoeren et al., 2010). These are all important secondary chemicals involved in the response to herbivores and other environmental factors (Jander et al., 2001; Snoeren et al., 2010). These genotypes were also chosen to extend the work of a past BGSU graduate student (Jennifer Shimola) in Bidart's lab. In her research, she found that Col-0 grown alongside *Ler-0* was significantly larger, and produced more fruits than when grown with like neighbors (Shimola & Bidart, 2019). These results suggested that *A. thaliana* may be able to distinguish between genetically similar or distinct neighbors, and respond in a “competitive-like” way in the presence of a different genotype (*Ler-0*). However, these two genotypes have other phenotypic differences, in addition to distinct VOC profiles.

One of the Col-0 mutants included in this study, *Etr1-1*, has a dominant mutation of the *ETR1* gene, which makes it insensitive to ethylene (see figure 1a). Etr1 is a receptor in plant cells that interacts with ethylene and induces several cascades involving defense and development (Klee, 2004). When mutated, Etr1 cannot bind to ethylene and induce those cascades. In other words, this mutant's phenotype shows a repression of ethylene-mediated responses, along with suppressed metabolism of other important plant hormones, such as auxin (which promotes stem elongation) and cytokinin (which promotes stem growth, as well as senescence) (Bleeker et al., 1988; Vandenbussche et al., 2007). Ethylene is an important VOC produced by plants, which is involved in multiple functions related to growth, development, and responses to pathogen attacks. Ethylene has been documented in several cases to impact the growth of plants, when applied exogenously (Gunderson & Taylor, 1991; He & Davies, 2012). When a plant absorbs exogenous

ethylene, that ethylene induces shade avoidance traits to outcompete its neighbors for sun (Pierik et al., 2003). These traits include early flowering, upward bending of leaves (known as hyponasty), and longer shoot length. Transgenic tobacco plants insensitive to ethylene (due to the addition of the mutated *Etr1-1* gene from *A. thaliana*) exhibited delayed shade-avoidance phenotypes when in the presence of wild-type neighbors, but not when grown alone or alongside other transgenic plants. showing that ethylene, and the *Etr1-1* gene in particular, is an important factor in above-ground competition with other plants (Pierik et al., 2003). If Etr1 receptors are insensitive to ethylene, it might not be processed by the plant, and ethylene could accumulate within the chambers in this experiment, leading to higher than normal levels of ethylene in those chambers.

The other Col-0 mutant used in this study was *Jar1-1*, which has a mutation in the *JAR1* gene. This mutation makes this seed line largely insensitive to jasmonic acid (or JA), which is a signaling molecule involved in several plant functions, such as the productions of GLVs (Staswick & Tiryaki, 2004). *Jar1-1* plants therefore would be expected to have lower emissions of GLVs such as E-2-hexanal and Z-3-hexanol, though direct measurements have not been documented. Upon wounding, α -linolenic acid (a precursor to JA) is released from plastid membranes. JAR1 converts jasmonic acid methyl ester (JA-Me) to Ja-Ile, JA's isoleucine conjugate, which triggers gene expression in the nucleus to synthesize JA production, and regulates resistance to herbivores (Staswick & Tiryaki, 2004; Wasternack & Feussner, 2018). Jasmonic acids are a type of oxylipin (Wasternack & Feussner, 2018), which is nearly ubiquitous in land plants; therefore, if *A. thaliana* is sensitive to changes in JA, other species could be, too (Meyer et al., 1984).

Previous research has shown that plants have the ability to communicate in genotype-specific ways. They can emit signals through the leaves when wounded, and roots can behave competitively by secreting exudates through the roots to bolster their growth and inhibit the growth

of others. However, little research has been done regarding airborne plant-plant communication effects on plant growth. Shimola and Bidart (2019) found Col-0 plants had a “competitive” like response (grew larger) in the presence of a different genotype (*Ler-0*) compared to when grown with other Col-0 plants (with no physical contact in any case). For this study, the focus was on identifying specific VOCs that could be involved in plant-plant interactions, and affect growth responses of neighboring plants. It was hypothesized that since Col-0 and *Ler-0* produce GLVs in different amounts, those differences may influence plant growth in Col-0 when exposed to *Ler-0*. In addition, the mutants *Jar1-1* and *Etr1-1* were used to test the prediction that jasmonate and ethylene airborne signaling may be important in plant-plant communication. Since these compounds are present in the majority of plant families, results from this study may have implications for other plant species as well.

EXPERIMENTAL DESIGN

Study Organism and Growing Conditions

To test the previous hypothesis, the following experiment was performed, which manipulated the genotype of “neighboring” plants. All “focal” plants were wild type Col-0 genotype. Four different neighboring plant genotypes were used: 1) wild type Col-0 (CS70000); 2) *Ler-0* genotype (CS20); 3) Col-0 mutant, *Etr1-1* (CS237); and 4) Col-0 mutant, *Jar1-1* (CS8072). As previously mentioned, the Col-0 mutant *Etr1-1* has dysfunctional ethylene receptors, which suppress their ability to react to ethylene signals. In addition, the Col-0 mutant *Jar1-1* has a deficiency in jasmonic acid production; therefore, it has a suppressed production of E-2-Hexenal and other important GLVs used in signaling.

This experiment was performed using four sealed environmental chambers (Percival Scientific model AR36L3C8, Perry, IA, USA), which prevent volatiles from travelling between treatments. Each chamber was set to a 14:10 hour day:night cycle at a temperature of 21°C. Chambers were lit using 32-Watt 4 ft. Alto Linear T8 Fluorescent Tube Light Bulbs, Daylight (6500K), spaced on all shelves to emit uniform light intensity in all chambers. To prevent volatile contamination between chambers, the testing room was allowed eighty minutes to ventilate between accessing each chamber. An equation by Atkinson et al was used to calculate how much time was needed for the volatiles in the room to reach a low enough concentration (2009). The equation used the concentration of volatiles inside the growth chambers, the volume of the chamber room, and the exhaust rate of the room’s ventilation system, which was calculated by Shimola and Bidart for previous experiments involving volatiles (2019).

Test plants were seeded in 5-cm pots and filled with moistened ProMix Micorrhizae General Purpose Soil Seeds, covered to retain soil moisture and reduce exposure to light, and were

placed in cold treatment for five days at 4°C to simulate cold stratification to break dormancy. After cold treatment, focal and neighboring plants were arranged on trays as seen in Figure 2 and placed in the growth chambers. Plants were watered with 15ml of deionized water every forty-eight hours until germination, then 10ml every forty-eight hours until senescence.

The experimental setting consists of two central focal plants in separate pots (all wild type Col-0 genotype), and six neighboring plants surrounding the focal plants, each in their own pots, and arranged equidistantly from each other, as seen in Figure 2. Each neighboring treatment (i.e., wild type Col-0, *Etr1-1*, *Ler-0*, or *Jar1-1*) was randomly assigned to each of the four environmental chambers. Since focal treatments were confounded with chamber, the experiment was replicated in time to avoid pseudo-replication. The whole experiment was replicated twice, randomizing treatments to chambers each time. Each growth chamber contained a total of 36 neighboring pots per trial (experimental replication) and 12 focal pots, or a total of 144 neighboring plants per experimental replication and 48 focal plants per replication.

Plant Fitness-related Traits

At senescence, the following variables were measured in focal plants: apical meristem height (cm), measured from the base of the rosette to the tallest stem; rosette width (cm), measured across the rosette leaves at the widest point; aboveground biomass (g), primary branch number, and fruit number (to measure reproductive output). The lengths of time from plant germination until the appearance of their apical meristem (bolting time), and the opening of the first flower (flowering time) were also measured. Rosette width was measured after all test plants put up their apical meristem. The rest of the measurements were taken after all plants had senesced (~17 weeks). To determine plant biomass (as dry weight), harvested plants were placed in envelopes

and dried in an oven at 70°C for five days before weight was recorded. All these variables were measured on all neighboring plants as well, although that data was not included here.

Statistical Analysis

Analyses of variance were performed to test for the effects of genotype and experimental repetition (two sets) on plant height, fruit number, branch number, change in rosette diameter, aboveground biomass, bolting time, and flowering time. When assumptions of normality and heterogeneity of variance were not met, data were transformed using a logarithmic or rank transformation. Pairwise comparisons among treatment means were performed using Fisher's Least Significant Difference tests when ANOVA models were significant (protected LSD). Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Results from analyses of variance showed significant effects of neighboring genotypes and repetition of the experiment on the measured variables (Table 1). Specifically, when focal Col-0 plants grew alongside *Ler-0* plants, they had 74.87% and 32.27% larger aboveground biomass in the first and second experimental sets, respectively, compared to when grown with Col-0 plants as neighbors (“control” genotype treatment). In addition, focal Col-0 plants with *Ler-0* neighbors were also significantly taller (29.77%), had a larger rosette width (7.25%), and higher fruit number (32.20%) than the control treatment. However, these trends differed between the two experimental sets (see Figures 3 a-d, and Figure 4 a). In addition, Col-0 plants grown surrounded by *Jar1-1* neighbors showed significant effects only for larger rosette width in the second experimental set (8.32%). All the other Col-0 variables were not altered by the presence of *Jar1-1* (Figures 3 and 4).

When focal Col-0 plants had *Etr1-1* plants as neighbors, they displayed significantly higher height (3.4%) in the first set (Figure 3 a), and larger aboveground biomass (52.37% and 44.68%, in sets 1 and 2, respectively) (Figure 3 d) than the control Col-0/Col-0 plants. The presence of *Etr1-1* neighbors also induced lower fruit number (43.78% and 4.83%) (Figure 3 b), and lower primary branch number in the second set (19.05%) (Figure 3 c) in focal Col-0 plants. However, again, results differed between the two experimental sets, with the exception of aboveground biomass, which was larger in both sets (Figure 3 d). Col-0 plants also had significantly larger rosette widths (21.95%) (Figure 4 a), and significantly longer periods to start bolting (26.03%) (Figure 4 b) and flowering (24.47%) (Figure 4 c) when grown surrounded by *Etr1-1* neighbors. It is worth noting that focal Col-0 plants showed significant changes in bolting and flowering time only when exposed to *Etr1-1* plant neighbors. Focal Col-0 plants growing in the presence of the other three genotypes bolted within an average of 32.22 ± 0.84 , 32.92 ± 1.104 ,

and 32.08 ± 0.85 days after germination, respectively; and flowered within an average of 35.08 ± 1.094 , 35.00 ± 1.130 , and 35.00 ± 1.094 days, respectively. Meanwhile, Col-0 plants with *Etr1-1* neighbors bolted on average 40.75 ± 1.081 days after germination and flowered about 43.67 ± 0.5656 days after germination (Figures 4 a b). These results denote a role of ethylene, as an airborne signal, influencing plant-plant communication.

DISCUSSION

The results of this study showed that neighbor genotype identity has a significant impact on fitness-related traits of Col-0 *A. thaliana* plants. A previous study by Shimola and Bidart (2019), found a “competitive like” effect in Col-0 plants growing in the presence of *Ler*-0 plants (with no physical contact). These Col-0 plants grew larger in size having significantly higher stem height, branch number, aboveground biomass, and fruit number. This current study corroborated previous trends, but they were not statistically significant in both experimental rounds, with the exception of aboveground biomass of Col-0 focal plants, which consistently grew larger in the presence of Landsberg (*Ler*-0). Other factors that could have influenced some differences between the two experimental rounds could have been related to significant microenvironmental differences in light or humidity between chamber or shelves within chambers (even though these were controlled as much as possible). In addition, it was interesting that there were significant effects of the presence of the mutant *Etr1-1* on growth (rosette width) and phenology (bolting and flowering time) of focal Col-0 plants.

Focal Col-0 plants expressed significantly delayed bolting and flowering time when exposed to neighboring *Etr1-1* plants. The *ETR1* gene is responsible for multiple functions, including development and growth (Gray, 2004; Schaller, 2012). The absorption of exogenous ethylene by plants induces shade avoidance traits to outcompete its neighbors for sun (Pierik et al., 2003). These traits include early flowering, upward bending of leaves (known as hyponasty), and longer shoot length. The *Etr1-1* mutant is insensitive to ethylene, because the Etr1 receptor cannot bind to ethylene, and have reduced metabolism of auxin and cytokinin, all of which are responsible for plant development. Previous studies have shown that high ethylene levels can slow plant growth (Gunderson & Taylor, 1991; He & Davies, 2012). It could have been possible

that high levels of ethylene in the growth chamber delayed development of focal Col-0 plants. However, ethylene exposure also negatively impacted aboveground biomass (He & Davies, 2012). Similarly, focal Col-0 plants had higher biomass when exposed to *Etr1-1* neighbors within the growth chambers. If the presence of *Etr1-1* plants induced a delay in the development of neighboring plants; then, it could be hypothesized that focal Col-0 plants might have allocated more energy into competition for light, which may explain the larger rosettes, and delayed bolting and flowering shown in these plants.

Results from this study were in accordance with trends observed by Shimola and Bidart (2019), which showed a “competitive” like response of Col-0 plants in the presence of Landsberg (*Ler-0*) plants. Focal Col-0 plants had higher average height, and aboveground biomass when exposed to *Ler-0* neighbors. I referred to “competitive” like effect, because, as previously mentioned, plants had no physical contact with each other; therefore, they were not competing for shared resources. Limited studies have observed plants changing their phenotypes in response to neighboring genotypes (with no physical contact), which can possibly be interpreted as “competitive” (Shimola & Bidart, 2019; Weidenhamer et al., 2019). Increased rosette size increases overall leaf surface area, which could be a competitive trait to out-shade non-kin neighbors and collect more sunlight for photosynthesis. One could make a similar assumption about stem height. In this study, a “competitive” phenotype may pertain to light acquisition and aboveground space, since the test plants were grown in individual pots, and therefore, they were not competing for belowground resources like water, nutrients, or belowground space. However, even though plants were kept at a distance to avoid contact between leaves from different plants, Col-0 plants might have still been induced by *Ler-0*, via airborne volatiles, to “compete” for light and aboveground space. The genotype Col-0 differs

from Landsberg through a single nucleotide deletion in the first exon of the CYP74B2 gene (Duan et al., 2005). This gene is involved in the production of hydroperoxide lyase (HPL), which has a downstream effect on Green Leaf Volatiles, jasmonic acids, and aliphatic glucosinolates (Duan et al., 2005; Snoeren et al., 2010). Therefore, Col-0 plants have fewer transcripts for the genes encoding the biosynthetic enzymes that produce these secondary chemicals than *Ler-0*. It could have been possible that Col-0 plants were “primed” by enhanced levels of airborne volatiles produced by *Ler-0*, and some of these chemicals could have induced shade avoidance phenotypes in Col-0 focal plants.

In contrast to *Ler-0*, the *Jar1-1* mutant had a significantly smaller impact on growth-related traits of Col-0 focal plants, which was expressed as significantly higher rosette width, and aboveground biomass, but only in the second experimental set. No other significant changes were observed, which suggested a small role of jasmonate levels (decreased in *Jar1-1* mutant) in plant-plant communication influencing each other’s growth and development. Since *Jar1-1* has lowered jasmonic acid levels; and therefore, lower production of green leaf volatiles (Staswick and Tiryaki, 2004), its VOC output would likely be similar to that of Col-0. With more similar volatile profiles, it could be expected that Col-0 would not show many differences in its phenotypic expression in the presence of *Jar1-1* plants.

Avenues that were not researched in this study include those of light reflection and microorganisms. Research has shown that *Arabidopsis* is capable of kin recognition by perceiving red/far red and blue light profiles from their neighbors (Crepy & Casal, 2015). Since plants of differing genotypes were grown together under shared light sources, differing far-red light reflection between genotypes could have impacted the results. Another avenue that was not explored in this project was that of microbial interaction with plants. Studies have found that

microbes in the roots can play a significant role in plant signaling (Mhlongo et al., 2018). Specifically, bacteria in the rhizome can prime their plant hosts for increased defense. In turn, plants exude metabolites, which regulate the types of microbes that can grow in the rhizosphere (Hacquard et al., 2017; Y. Yang et al., 2017; Zhang et al., 2014). However, the rhizospheres of the test plants in this study were not compared, so no potential effects on signaling by microbes were accounted for.

This study has further confirmed that *A. thaliana* is capable of differentiating neighbor identity, and that this process is possibly mediated by specific volatiles, or blends of VOCs. Focal Col-0 plants showed shade avoidance phenotypes in the presence of *Ler-0* neighboring plants. This result was in accordance with a previous study by Shimola and Bidart (2019). Even though, Col-0 and *Ler-0* do not share the same genetic background, there are some differences in the production of Green Leaf Volatiles between these two genotypes (increased production in *Ler-0*), which could be involved in the observed responses as GLVs have documented uses as signaling chemicals between plants for other purposes, such as defense (Scala et al., 2013). This hypothesis certainly warrants further testing. In contrast, responses of Col-0 to the presence of *Jar1-1* were minimal, suggesting little involvement of jasmonates in plant-plant communication, at least, in the absence of herbivores or other stressors. An interesting novel result was related to Col-0 plant responses to *Etr1-1* neighbors, as this genotype significantly influenced growth and developmental phenotypes of focal Col-0 plants. While previous studies have confirmed that exogenous ethylene can impact a plant's growth and development, this study showed that surrounding plants' ability to absorb ethylene may be able to impact these phenotypes. Even though this study had limitations in time and space, it provided useful information for future studies trying to elucidate which individual or blends of VOCs are most important in airborne

plant-plant communication. The use of genetically modified lines for specific VOCs sharing the same genetic background, such as *Jar1-1* and *Etr1-1* (both Col-0 mutants) is an invaluable tool to answer ecological relevant questions related to plant-plant communication.

LITERATURE CITED

- Agut, B., Pastor, V., Jaques, J. A., & Flors, V. (2018). Can Plant Defence Mechanisms Provide New Approaches for the Sustainable Control of the Two-Spotted Spider Mite *Tetranychus urticae*? *International Journal of Molecular Sciences*, *19*(2), 1–20.
<https://doi.org/10.3390/ijms19020614>
- Atkinson, J., Chartier, Y., Pessoa-Silva, C. L., Jensen, P., Li, Y., & Seto, W.-H. (Eds.). (2009). *Natural Ventilation for Infection Control in Health-Care Settings*. World Health Organization. <http://www.ncbi.nlm.nih.gov/books/NBK143284/>
- Atkinson, R., & Arey, J. (2003). Gas-phase tropospheric chemistry of biogenic volatile organic compounds: A review. *Atmospheric Environment*, *37*, 197–219.
[https://doi.org/10.1016/S1352-2310\(03\)00391-1](https://doi.org/10.1016/S1352-2310(03)00391-1)
- Bleeker, A. B., Estelle, M. A., Somerville, C., & Kende, H. (1988). Insensitivity to Ethylene Conferred by a Dominant Mutation in *Arabidopsis thaliana*. *Science*, *241*(4869), 1086–1089.
- Body, M. J. A., Neer, W. C., Vore, C., Lin, C.-H., Vu, D. C., Schultz, J. C., Cocroft, R. B., & Appel, H. M. (2019). Caterpillar Chewing Vibrations Cause Changes in Plant Hormones and Volatile Emissions in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *10*.
<https://doi.org/10.3389/fpls.2019.00810>
- Boyes, D. C., Zayed, A. M., Ascenzi, R., McCaskill, A. J., Hoffman, N. E., Davis, K. R., & Görlach, J. (2001). Growth Stage–Based Phenotypic Analysis of *Arabidopsis*: A Model for High Throughput Functional Genomics in Plants. *The Plant Cell*, *13*, 1499–1520.
- Crepy, M. A., & Casal, J. J. (2015). Photoreceptor-mediated kin recognition in plants. *New Phytologist*, *205*(1), 329–338. <https://doi.org/10.1111/nph.13040>

- Das, A., Lee, S.-H., Hyun, T. K., Kim, S.-W., & Kim, J.-Y. (2013). Plant volatiles as method of communication. *Plant Biotechnology Reports*, 7(1), 9–26.
<https://doi.org/10.1007/s11816-012-0236-1>
- Demain, A. L., & Fang, A. (2000). The Natural Functions of Secondary Metabolites. In A. Fiechter (Ed.), *History of Modern Biotechnology I* (pp. 1–39). Springer.
https://doi.org/10.1007/3-540-44964-7_1
- Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant, Cell & Environment*, 32(6), 654–665. <https://doi.org/10.1111/j.1365-3040.2008.01913.x>
- Dicke, M., & Hilker, M. (2003). Induced plant defences: From molecular biology to evolutionary ecology. *Basic and Applied Ecology*, 4, 3–14. <https://doi.org/10.1078/1439-1791-00129>
- Duan, H., Huang, M.-Y., Palacio, K., & Schuler, M. A. (2005). Variations in CYP74B2 (hydroperoxide lyase) gene expression differentially affect hexenal signaling in the Columbia and Landsberg erecta ecotypes of Arabidopsis. *Plant Physiology*, 139(3), 1529–1544. <https://doi.org/10.1104/pp.105.067249>
- Dudley, S. A., & File, A. L. (2007). Kin recognition in an annual plant. *Biology Letters*, 3(4), 435–438. <https://doi.org/10.1098/rsbl.2007.0232>
- Frost, C. J., Mescher, M. C., Carlson, J. E., & De Moraes, C. M. (2008). Plant Defense Priming against Herbivores: Getting Ready for a Different Battle. *Plant Physiology*, 146(3), 818–824. <https://doi.org/10.1104/pp.107.113027>
- Giron-Calva, P., Li, T., & Blande, J. (2017). Volatile-Mediated Interactions between Cabbage Plants in the Field and the Impact of Ozone Pollution. *Journal of Chemical Ecology*, 43(4), 339–350. <https://doi.org/10.1007/s10886-017-0836-x>

- Gray, W. M. (2004). Hormonal Regulation of Plant Growth and Development. *PLOS Biology*, 2(9), e311. <https://doi.org/10.1371/journal.pbio.0020311>
- Gunderson, C. A., & Taylor, G. E. (1991). Ethylene Directly Inhibits Foliar Gas Exchange in *Glycine max* L. *Plant Physiology*, 95(1), 337–339.
- Hacquard, S., Spaepen, S., Garrido-Oter, R., & Schulze-Lefert, P. (2017). Interplay Between Innate Immunity and the Plant Microbiota. *Annual Review of Phytopathology*, 55(1), 565–589. <https://doi.org/10.1146/annurev-phyto-080516-035623>
- He, C., & Davies, F. T. (2012). Ethylene reduces plant gas exchange and growth of lettuce grown from seed to harvest under hypobaric and ambient total pressure. *Journal of Plant Physiology*, 169(4), 369–378. <https://doi.org/10.1016/j.jplph.2011.11.002>
- Heil, M., & Bueno, J. C. S. (2007). Herbivore-Induced Volatiles as Rapid Signals in Systemic Plant Responses. *Plant Signaling & Behavior*, 2(3), 191–193.
- Jander, G., Cui, J., Nhan, B., Pierce, N. E., & Ausubel, F. M. (2001). The TASTY Locus on Chromosome 1 of *Arabidopsis* Affects Feeding of the Insect Herbivore *Trichoplusia ni*. *Plant Physiology*, 126(2), 890–898.
- Karban, R., Shiojiri, K., Huntzinger, M., & McCall, A. C. (2006). Damage-induced resistance in sagebrush: Volatiles are key to intra- and interplant communication. *Ecology*, 87(4), 922–930.
- Karban, R., Shiojiri, K., Ishizaki, S., Wetzal, W. C., & Evans, R. Y. (2013). Kin recognition affects plant communication and defence. *Proceedings. Biological Sciences*, 280(1756), 20123062. <https://doi.org/10.1098/rspb.2012.3062>
- Klee, H. J. (2004). Ethylene Signal Transduction. Moving beyond *Arabidopsis*. *Plant Physiology*, 135(2), 660–667. <https://doi.org/10.1104/pp.104.040998>

- Meyer, A., Miersch, O., Büttner, C., Dathe, W., & Sembdner, G. (1984). Occurrence of the plant growth regulator jasmonic acid in plants. *Journal of Plant Growth Regulation*, 3(1), 1. <https://doi.org/10.1007/BF02041987>
- Mhlongo, M. I., Piater, L. A., Madala, N. E., Labuschagne, N., & Dubery, I. A. (2018). The Chemistry of Plant–Microbe Interactions in the Rhizosphere and the Potential for Metabolomics to Reveal Signaling Related to Defense Priming and Induced Systemic Resistance. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.00112>
- Mofikoya, A. O., Kim, T. H., Abd El-Raheem, A. M., Blande, J. D., Kivimäenpää, M., & Holopainen, J. K. (2017). Passive Adsorption of Volatile Monoterpene in Pest Control: Aided by Proximity and Disrupted by Ozone. *Journal of Agricultural and Food Chemistry*, 65(44), 9579–9586. <https://doi.org/10.1021/acs.jafc.7b03251>
- Moreira, X., Petry, W. K., Hernández-Cumplido, J., Morelon, S., & Benrey, B. (2016). Plant defence responses to volatile alert signals are population-specific. *Oikos*, 125(7), 950–956. <https://doi.org/10.1111/oik.02891>
- Murphy, G. P., & Dudley, S. A. (2009). Kin recognition: Competition and cooperation in Impatiens (Balsaminaceae). *American Journal of Botany*, 96(11), 1990–1996. <https://doi.org/10.3732/ajb.0900006>
- Ninkovic, V. (2003). Volatile communication between barley plants affects biomass allocation. *Journal of Experimental Botany*, 54(389), 1931–1939. <https://doi.org/10.1093/jxb/erg192>
- Ninkovic, V., Markovic, D., & Dahlin, I. (2016). Decoding neighbour volatiles in preparation for future competition and implications for tritrophic interactions. *Perspectives in Plant Ecology, Evolution and Systematics*, 23, 11–17. <https://doi.org/10.1016/j.ppees.2016.09.005>

- Pickett, J. A., & Khan, Z. R. (2016). Plant volatile-mediated signalling and its application in agriculture: Successes and challenges. *New Phytologist*, 212(4), 856–870.
<https://doi.org/10.1111/nph.14274>
- Pierik, R., Visser, E. J. W., Kroon, H. D., & Voesenek, L. a. C. J. (2003). Ethylene is required in tobacco to successfully compete with proximate neighbours. *Plant, Cell & Environment*, 26(8), 1229–1234. <https://doi.org/10.1046/j.1365-3040.2003.01045.x>
- Pinto, D. M., Blande, J. D., Nykänen, R., Dong, W.-X., Nerg, A.-M., & Holopainen, J. K. (2007). Ozone Degrades Common Herbivore-Induced Plant Volatiles: Does This Affect Herbivore Prey Location by Predators and Parasitoids? *Journal of Chemical Ecology*, 33(4), 683–694. <https://doi.org/10.1007/s10886-007-9255-8>
- Scala, A., Allmann, S., Mirabella, R., Haring, M., & Schuurink, R. (2013). Green Leaf Volatiles: A Plant's Multifunctional Weapon against Herbivores and Pathogens. *International Journal of Molecular Sciences*, 14(9), 17781–17811.
<https://doi.org/10.3390/ijms140917781>
- Schaller, G. E. (2012). Ethylene and the regulation of plant development. *BMC Biology*, 10(1), 9.
<https://doi.org/10.1186/1741-7007-10-9>
- Schwartzberg, E., Böröczky, K., & Tumlinson, J. (2011). Pea Aphids, *Acyrtosiphon Pisum*, Suppress Induced Plant Volatiles in Broad Bean, *Vicia Faba*. *Journal of Chemical Ecology*, 37(10), 1055–1062. <https://doi.org/10.1007/s10886-011-0006-5>
- Shimola, J. (2018). *How do Volatile Cues Impact Plant-Herbivore Interactions in Arabidopsis thaliana?* Bowling Green State University.

- Shimola, J., & Bidart, M. G. (2019). Herbivory and Plant Genotype Influence Fitness-Related Responses of *Arabidopsis thaliana* to Indirect Plant-Plant Interactions. *American Journal of Plant Sciences*, 10(08), 1287. <https://doi.org/10.4236/ajps.2019.108093>
- Šimpraga, M., Takabayashi, J., & Holopainen, J. K. (2016). Language of plants: Where is the word? *Journal of Integrative Plant Biology*, 58(4), 343–349. <https://doi.org/10.1111/jipb.12447>
- Snoeren, T. A. L., Kappers, I. F., Broekgaarden, C., Mumm, R., Dicke, M., & Bouwmeester, H. J. (2010). Natural variation in herbivore-induced volatiles in *Arabidopsis thaliana*. *Journal of Experimental Botany*, 61(11), 3041–3056. <https://doi.org/10.1093/jxb/erq127>
- Staswick, P. E., & Tiryaki, I. (2004). The Oxylinin Signal Jasmonic Acid Is Activated by an Enzyme That Conjugates It to Isoleucine in *Arabidopsis*. *The Plant Cell*, 16(8), 2117–2127. <https://doi.org/10.1105/tpc.104.023549>
- Tahir, H. A. S., Qin Gu, Huijun Wu, Raza, W., Safdar, A., Ziyang Huang, Rajer, F. U., & Xuewen Gao. (2017). Effect of volatile compounds produced by *Ralstonia solanacearum* on plant growth promoting and systemic resistance inducing potential of *Bacillus* volatiles. *BMC Plant Biology*, 17, 1–16. <https://doi.org/10.1186/s12870-017-1083-6>
- Tania N. Kim, Nora Underwood, & Brian D. Inouye. (2013). Insect herbivores change the outcome of plant competition through both inter- and intraspecific processes. *Ecology*, 94(8), 1753–1763.
- Vandenbussche, F., Habricot, Y., Condiff, A. S., Maldiney, R., Straeten, D. V. D., & Ahmad, M. (2007). HY5 is a point of convergence between cryptochrome and cytokinin signalling pathways in *Arabidopsis thaliana*. *The Plant Journal*, 49(3), 428–441. <https://doi.org/10.1111/j.1365-313X.2006.02973.x>

- Wasternack, C., & Feussner, I. (2018). The Oxylipin Pathways: Biochemistry and Function. *Annual Review of Plant Biology*, 69(1), 363–386. <https://doi.org/10.1146/annurev-arplant-042817-040440>
- Weidenhamer, J. D., Montgomery, T. M., Cipollini, D. F., Weston, P. A., & Mohny, B. K. (2019). Plant Density and Rhizosphere Chemistry: Does Marigold Root Exudate Composition Respond to Intra- and Interspecific Competition? *Journal of Chemical Ecology*, 45(5), 525–533. <https://doi.org/10.1007/s10886-019-01073-5>
- Yang, X.-F., Li, L.-L., Xu, Y., & Kong, C.-H. (2018). Kin recognition in rice (*Oryza sativa*) lines. *New Phytologist*, 220(2), 567–578. <https://doi.org/10.1111/nph.15296>
- Yang, Y., Wang, N., Guo, X., Zhang, Y., & Ye, B. (2017). Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. *PLOS ONE*, 12(5), e0178425. <https://doi.org/10.1371/journal.pone.0178425>
- Zhang, N., Wang, D., Liu, Y., Li, S., Shen, Q., & Zhang, R. (2014). Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. *Plant and Soil*, 374(1), 689–700. <https://doi.org/10.1007/s11104-013-1915-6>

APPENDIX A. TABLES

Table 1. Analyses of variance evaluating effects of neighboring genotype (Col-0, *Etr1-1*, *Jar1-1*, or *Ler-0*), and experimental repetition (set 1 or 2) on focal Col-0 fitness-related traits of *Arabidopsis thaliana*.

	<i>df</i>	<i>ANOVA source of variation (F-values)</i>		
		Genotype	Repetition	Gen x Rep
<i>Rosette Width</i>	3, 82	8.88***	--	--
<i>Stem Height</i>	3, 82	4.94**	85.93***	3.76*
<i>Fruit Number</i>	3, 82	3.33*	44.08***	7.72***
<i>Branch Number</i>	3, 82	1.10	96.15***	4.27**
<i>Aboveground Biomass</i>	3, 82	15.15***	56.5***	3.53*
<i>Days until Bolting</i>	3, 44	13.22***	--	--
<i>Days until Flowering</i>	3, 44	11.85***	--	--

*P<0.05; **P<0.01; ***P<0.001.

APPENDIX B. FIGURES

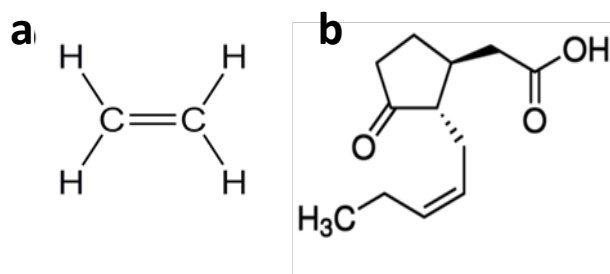


Figure 1. Chemical formulas for Ethylene (a), and Jasmonic acid, or JA (b) in *Arabidopsis thaliana*. Mutants of Col-0 *A. thaliana* plants related to these chemicals were used in this study. Ethylene is a simple alkene which serves as a growth hormone in plants. Ethylene regulates the growth and senescence of leaves, flowers, and fruits. Jasmonic acid, is found throughout most land plants. Over thirty uses have been observed, including the production of secondary metabolites, insect resistance, pathogen defense, stomata regulation, stress tolerance, root and shoot growth, senescence, and development of sex organs. Jasmonic acids are also a necessary component in the production of green leaf volatiles, such as E-2-Hexanal. Nearly all these functions have been observed in *Arabidopsis thaliana*.

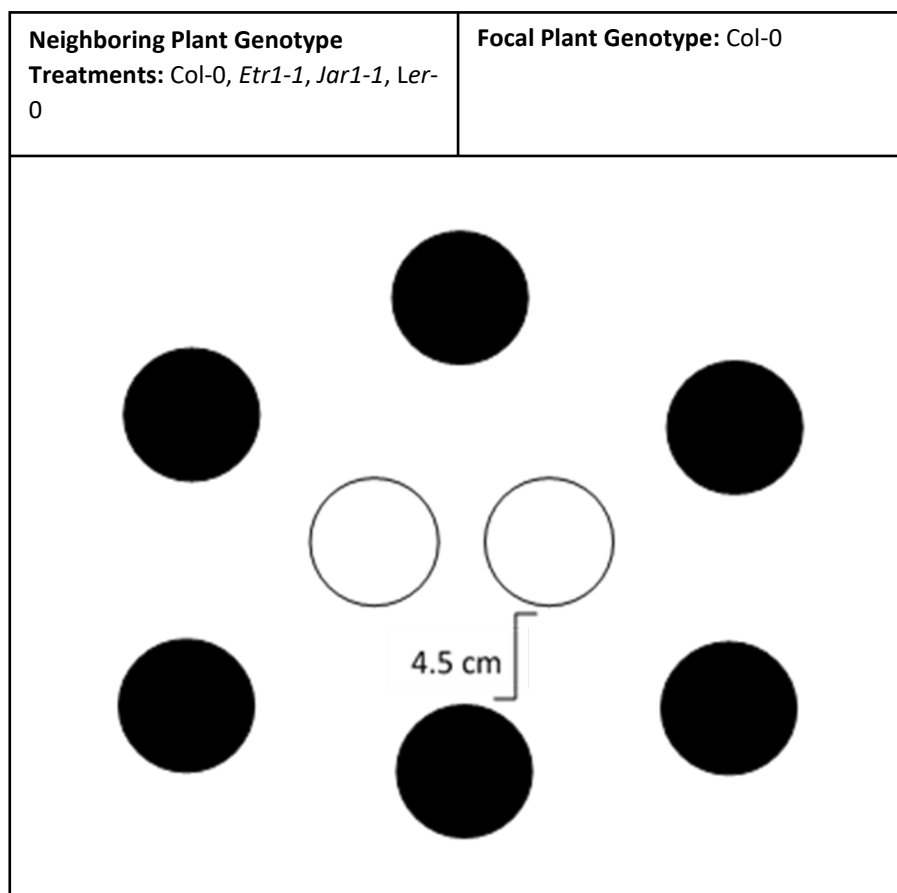


Figure 2. Schematic of experimental design. Two central focal plants (white circles) are both wild type Col-0 genotype. Neighboring plant treatments (black circles) included four different genotypes (i.e., wild type Col-0, *Etr1-1*, *Jar1-1*, and *Ler-0*).

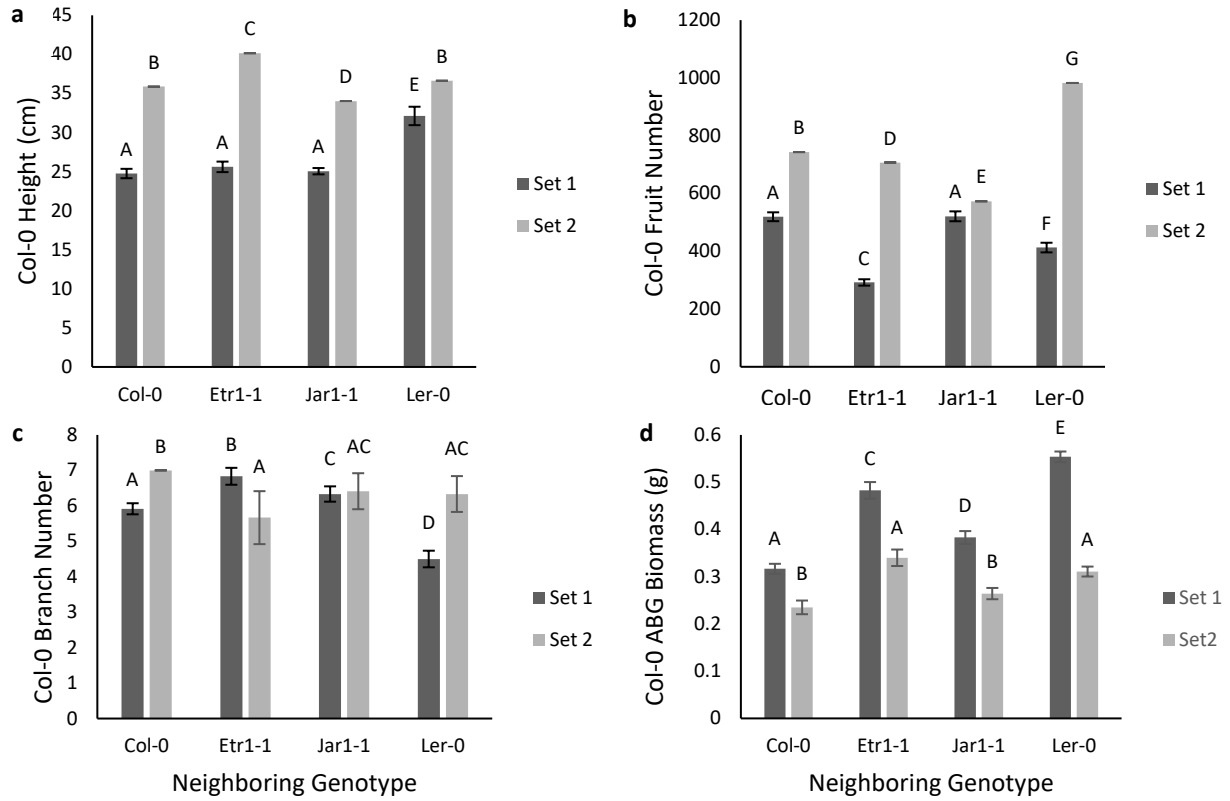


Figure 3. Effects of neighboring genotypes on Col-0 fitness related traits (means and standard errors): stem height (a), fruit number (b), branch number (c), aboveground (ABG) biomass (d). Letters above bars refer to significant differences ($P < 0.05$) between genotype treatment means (Set 1: $n=42$; Set 2: $n=48$).

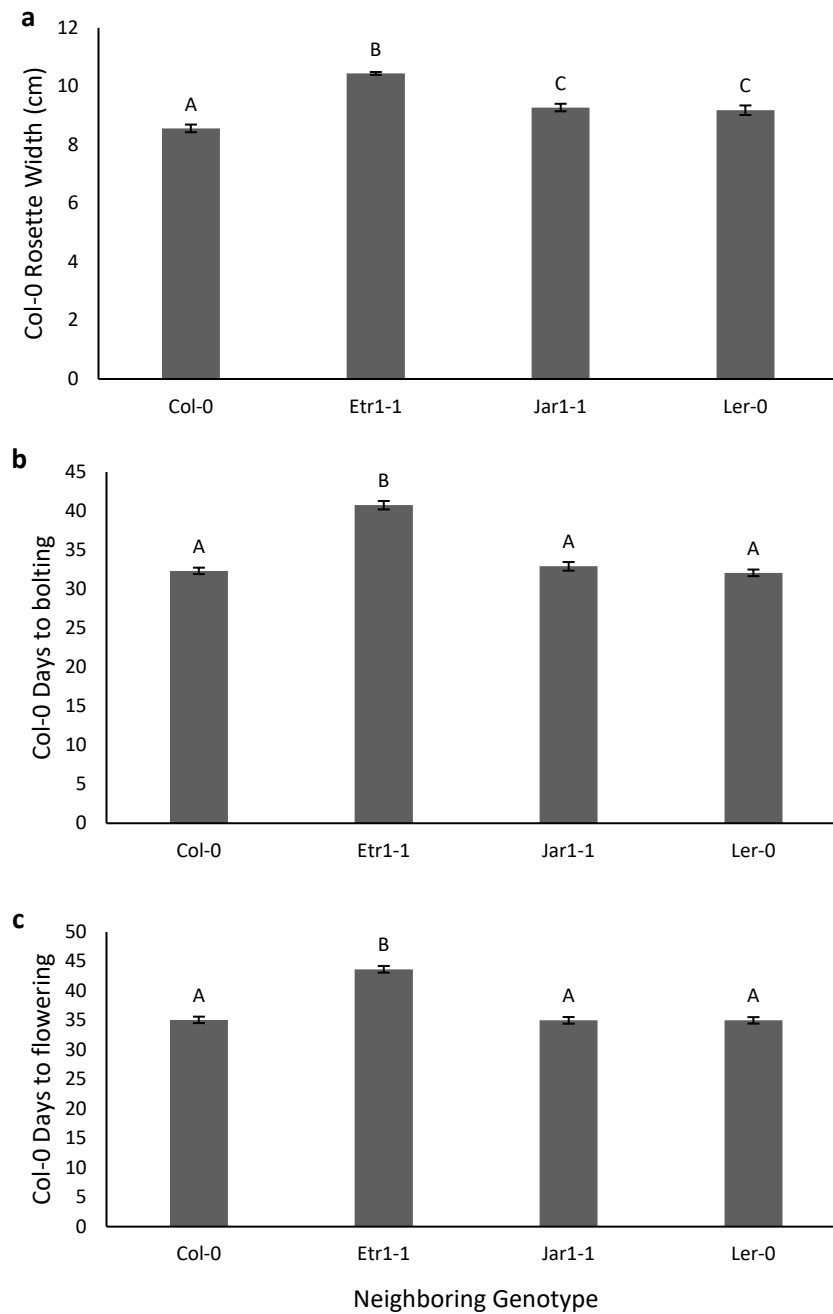


Figure 4. Effects of neighboring genotypes on focal Col-0 trait means measured only in the second experimental set: rosette width (a); number of days from germination to bolting (b); number of days from germination to first flowering. Error bars represent standard errors, and letters above bars refer to significant differences ($P < 0.05$) between genotype treatment means. (Set 2: $n=48$).