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## Arabidopsis thaliana growth more than defense affects Myzus persicae populations

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# Arabidopsis thaliana growth more than defense affects Myzus persicae populations

## Peer Review

This work has undergone a double-blind review by a minimum of two faculty members from institutions of higher learning from around the world. The faculty reviewers have expertise in disciplines closely related to those represented by this work. If possible, the work was also reviewed by undergraduates in collaboration with the faculty reviewers.

## Abstract

Aphids and other herbivorous insects are able to travel between plants in their environment. While plants cannot directly choose their consumers, they may stop or mitigate the harmful effects of herbivory using secondary metabolites. A salicylic acid (SA) pathway facilitates defense from biotrophic fungi and some necrotrophic pathogens, while a jasmonic acid (JA) pathway is associated with defense from other necrotrophic pathogens and chewing insects. We investigated the effect of deficiency in SA or JA in *Arabidopsis thaliana* on plant growth and on herbivory by green peach aphids (*Myzus persicae*). Three ascensions of *A. thaliana* were placed in two blocks with three aphids on each individual. The genotypes consisted of a SA-deficient ascension (Sid2), a JA-deficient ascension (Lox2), and a wild type (Col). Wild-type *A. thaliana* showed significantly greater growth relative to the other ascensions, suggesting better performance, as well as dramatically higher aphid count. Aphid count showed a significant positive correlation with plant growth, suggesting that *M. persicae* is attracted to signs of growth in *A. thaliana*, or reproduces more on growing plants. It appears that wild-type plants showed greater growth throughout the experiment, and were thus targeted by the highly responsive *M. persicae*. This behaviour may be exploited for agricultural purposes; herbivorous insects may be diverted to fast-growing trap crops lacking agricultural value in order to protect primary crops.

## Keywords

aphid, Arabidopsis, Col, feeding, foraging, growth, herbivory, Lox2, Myzus, Sid2

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## INTRODUCTION

Understanding the interactions of plants and animals is vital for a number of applications, including pest control in agriculture. Including pathogens and weeds, herbivorous insects are arguably the main source of crop damage and loss and agriculturally valuable plants must be protected from them. A number of methodologies, other than pesticide use, have been employed to reduce herbivore damage. For example, trap crops are used to draw agricultural pests away from more valuable plants (Koul, Dhaliwal, & Cuperus, 2004). Individual pests are drawn to the trap crop, and these individuals consume the trap crop instead of the more valuable, commodity crop. This has been effective in soybean (*Glycine max*) systems where green beans (*Phaseolus vulgaris*) are planted prior to the soybeans and used as a trap to protect soybeans from the Mexican bean beetle (*Epilachna varivestis*) (Rust, 1977). Improved understanding for the interactions of pest species and particular crop species (or cultivars) may allow for the identification of superior trap crops.

Many agro-ecological interactions may be assessed in smaller-scale models that can be extended to field scales. One such commonly assessed model interaction occurs between the mustard green, *Arabidopsis thaliana* (Brassicaceae), and the green peach aphid, *Myzus persicae* (Heteroptera: Aphididae) (De Vos & Jander, 2009; Hunt et al., 2006).

*A. thaliana* is a small member of the Brassicaceae family native to Eurasia (Hoffmann, 2002) and has a typical life cycle of only six weeks, from germination to maturation (Meinke, 1998). Its small, fully sequenced genome, relatively short generation time, small size, high reproductive capabilities, and ability to thrive in various environments make *A.*

*thaliana* well-suited to many experimental designs (Meinke, 1998; The Arabidopsis Genome Initiative, 2000).

Like most flowering plants, *A. thaliana* primarily uses two defense pathways in response to herbivory or fungal attack. (1) A salicylic acid (SA) pathway induces the expression of pathogenesis-related genes for defense from biotrophic fungi and some necrotrophic pathogens. (2) A jasmonic acid (JA) pathway is associated with enhanced expression of several defense genes and facilitates defense from other necrotrophic pathogens and chewing insects (Ellis, Karafyllidis, & Turner, 2002). In this experiment we studied three accessions of *A. thaliana*: a wild type (Columbia; Col-0, henceforth Col), an accession deficient in SA (Sid2), and an accession deficient in JA (Lox2).

*Myzus persicae*, the green peach aphid, is one of the most significant herbivorous pests of cultivated crops (Kettles, Durey, Schoonbeek, Maule, & Hogenhout, 2013). *M. persicae* are found in all parts of the world, including North America, and show a preference for cold and wet environments (Capinera, 2005). Like other aphids, *M. persicae* possess specialized stylet mouth parts that pierce plant tissue to access phloem (Kettles et al., 2013). Aphid feeding, like biotrophic pathogens, induces the SA pathway. Although the JA response is effective against aphids and other piercing-sucking insects, it is not induced because their stylets do little mechanical damage to plant tissue (Ellis et al., 2002). Further, effector proteins in aphid saliva modify the response of the host. Injected saliva modulates cellular processes and suppresses plant defenses in order to promote infestation (Bos et al., 2010).

These feeding characters coupled with the near completion of its genome sequence, small body size, its ability to

thrive in a diverse set of environments, and its short generation time make *M. persicae* well-suited to many experimental designs (Capinera, 2005; Louis & Shah, 2013).

In this study, we assessed the effects of plant accession, as a proxy for plant defense pathways, on two aspects of this important plant animal interaction. First, does an impaired defense signalling pathway affect aphid population growth and distribution? Second, does plant accession have an effect on plant growth? With these two questions, key highlights into the potential cost-benefits of the defense and counter-defense interaction may be better understood.

As the typical response of *A. thaliana* to aphid attack involves increased production of salicylic acid (Clarke, 2000), we expected Sid2 variants to attract more aphids and have relatively less growth. As both JA and SA pathways are effective for defense in *A. thaliana* (Ellis et al., 2002; van Poecke, 2002; van Wees, de Swart, van Pelt, van Loon, & Pieterse, 2000), we also expected Col plants to have greater overall defense, and thus growth, compared to Lox2 plants.

## MATERIALS AND METHODS

All plants and aphids were grown in laboratory conditions (16L:8D) for four weeks prior to start of the experiment. 15 pots of each *A. thaliana* accession were separated into two blocks of 22 and 23 randomly arranged plants, within each respective block. Each plant was separated enough to avoid shading and light competition.

All Col plants were bolting on day zero, while none of the other accessions had bolts. To make data more comparable, bolts were cut off of all bolting plants. It was also noted that trichomes were not visible on Lox2 plants.

The most central leaf on each plant was marked with a Sharpie™ permanent marker, and each pot was inoculated with three *M. persicae* adults. Many pots contained more than one plant ( $1.98 \pm 0.14$  plants per pot; mean  $\pm$  1S.E.). The number of plants in each pot was recorded.

The experiment was run for 12 days. The number of living aphids in each pot was counted on days five and 12.

Throughout the experiment, four aphids were found mummified by a parasitoid wasp (*Diaeretella* sp.; Hymenoptera: Braconidae). Once detected, mummies were removed from the experiment and were not counted as living aphids. As there were only four mummies we do not suspect parasitism played a meaningful role in overall results.

Plants were watered on the morning of day one. Soil moisture was checked regularly, but soil remained moist, and the plants were not watered again.

On day five and 12, plant growth in each pot was assessed by counting the number of leaves observed to be smaller and more central than the leaf marked on day zero. Relativized plant growth was calculated for each pot by dividing growth (number of new leaves) by the number of plants in the pot.

## Statistical Methods

Statistical analysis was performed using R (R v.3.0.3; R Foundation for Statistical Computing, 2014).

The effect of time and accession on relativized growth was assessed using repeated measures analysis of variance (ANOVA). *Post hoc* comparisons were made using the Tukey method.

The effect of time and accession on  $\log(n + 1)$  transformed aphid count was assessed using repeated measures analysis of covariance (ANCOVA), with relativized

growth as a covariate. *Post hoc* comparisons were made using the Tukey method.

To ensure that results did not differ between blocks, relativized growth in blocks A and B was compared using repeated measures ANOVA.

## RESULTS

There was a significant interaction on relativized plant growth between time and accession ( $F_{4,84} = 14.29$ ,  $p < 0.0001$ ). All accessions grew significantly during the experiment, but wild-type (Col) plants produced significantly more new leaves than non-wild-type (Sid2 and Lox2) plants (Figure 1).

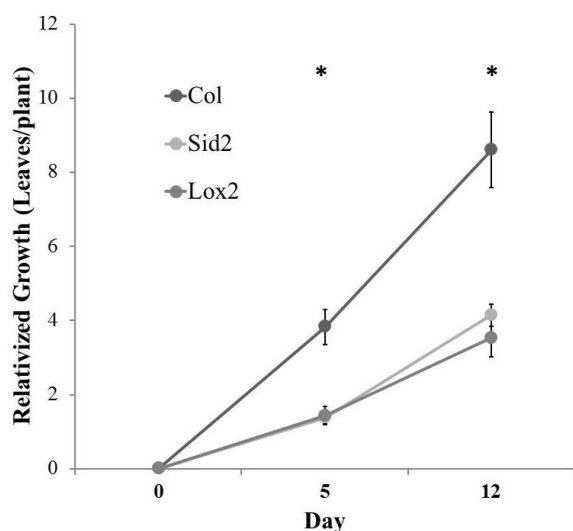


Figure 1: Relativized growth for each accession on each of the three recording days. Error bars represent  $\pm 1$  SEM. All genotypes grew significantly over the observation period ( $p < 0.0001$ ), but wild-type plants grew significantly more than Sid2 and Lox2 plants ( $p < 0.0001$ ).

There was also a significant interaction on log-transformed aphid count between time and accession ( $F_{4,83} = 15.18$ ,  $p < 0.0001$ ). Aphid count was highly positively correlated with relativized growth ( $F_{1,83} = 60.26$ ,  $p < 0.0001$ ), and was dramatically higher on wild-type plants

compared to non-wild-type plants (Figure 2).

It appears that aphids moved easily between plants, as aphid count varied greatly between plants and across time periods.

There was no significant difference in relativized growth between block A and block B at any time point ( $F_{2,86} = 0.68$ ,  $p > 0.5$ ) and no difference overall ( $F_{1,43} = 0.70$ ,  $p > 0.05$ ).

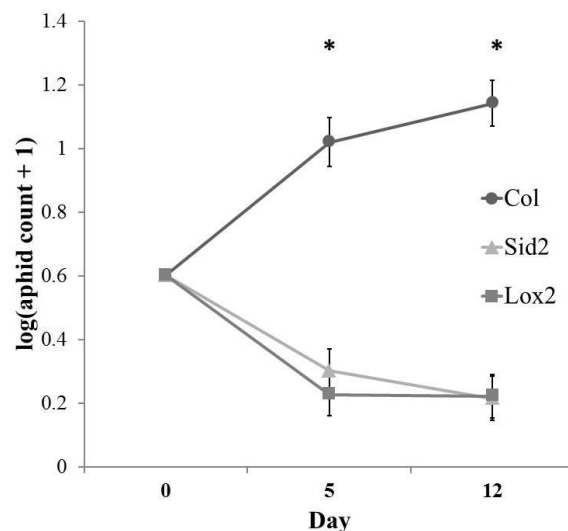


Figure 2: Log-transformed aphid count for each accession on each of the three recording days. Error bars represent  $\pm 1$  SEM. There were significantly more aphids on wild-type plants at both the fifth and 12<sup>th</sup> days ( $p < 0.0001$  on both days).

## DISCUSSION

Wild-type plants grew considerably more than other accessions, suggesting that the wild-type plants in our study performed better than the non-wild plants. It should be noted that Lox2 and Sid2 pots contained slightly more plants (on average) than wild-type pots. This may have led to intraspecific competition, particularly for soil resources within the pot, and thus may have impacted individual plant growth. Intraspecific competition among *A. thaliana* has been

shown to increase costs for fitness due to resource allocation (Cipollini, 2002).

Differences were significant in both relativized growth and the number of aphids between the wild-type and non-wild pots.

Consistent with our hypothesis, wild-type pots performed best in terms of relativized growth. However, wild-type pots also housed significantly more aphids compared to Sid2 and Lox2 pots. Since herbivory can be expected to decrease plant performance (Weigel & Glazebrook, 2002), these qualities appear to contradict one another. This suggests that the density of aphids ( $6.0 \pm 1.4$  aphids per pot) was not enough to substantially impair plant growth.

The presence of aphids feeding on *A. thaliana* invokes an SA response but not a JA response (Ellis et al., 2002; Moran, 2001). However, if an SA response were the only factor affecting aphid presence, Lox2 and wild-type accessions might be expected to perform similarly, which was not the case in our experiment. Aphid-induced resistance in *A. thaliana* is local and is thus only effective when the region in which the aphid may feed is limited (Ellis et al., 2002). As our plants were close together and the accessions were distributed uniformly, *M. persicae* were likely able to move between pots of various accessions in order to evade plant defenses. However, this does not sufficiently explain *M. persicae* behaviour in our experiment, as the plants with the most capable defenses (wild-type) also housed the greatest number of aphids, and no difference was seen between Sid2 and Lox2 plants, which use different defense pathways.

Wild-type plants grew significantly more leaves and appeared to have larger under-leaf surface area than mutant plants. This suggests that wild-type plants presented a more attractive feeding environment for *M. persicae*, which often feed on the underside of leaves (De Vos & Jander, 2009). *M. persicae* seem to prefer high-

performing plants, suggesting that *M. persicae* were attracted to a sign of growth in the plant. Previous studies suggest that aphids respond to volatile cues released in plant-plant communication (Glinwood, Ninkovic, Pettersson, & Ahmed, 2004; Ninkovic, Olsson, & Pettersson, 2002). We hypothesize that *M. persicae* behaviour may be affected by volatile cues related to growth in *A. thaliana*.

Since flowering organs and growing leaves are strong nutrition sinks (Imlau, 1999), bolting and growing stems likely have a higher rate of nutrient flow. These would provide greater nutrition to *M. persicae*. *M. persicae* may have evolved a preference for new leaves, as the nutritional benefit would give a selective advantage.

Aphids often cluster at the base of an inflorescence (Weigel & Glazebrook, 2002). As only wild-type plants were bolting at the start of the experiment, aphids may have experienced greater attraction to wild-type plants because of the presence of bolting stems, which are associated with inflorescence, and to the presence of newly growing leaves. Our data indicate that there is a positive relationship between relativized plant growth and aphid count (Figure 2).

*A. thaliana* leaves develop different epicuticular wax, trichomes and other characteristics depending upon the stage of the plant's life cycle (Kerstetter & Poethig, 1998). As wild-type *A. thaliana* entered the experiment at a different life stage from the other accessions (wild-type plants were bolting), they may have produced leaves with different characteristics, which may have been more attractive to *M. persicae*. Lox2 plants in the experiment did not have any visible trichomes, but this seems to have had negligible effect as the aphids generally migrated away from Lox2 individuals.

We were unable to determine if deficiency in SA response or in JA response contributed to plant growth as there was no

significant difference in relativized growth between Sid2 and Lox2 plants. Previous literature suggests that JA plays a role in promoting plant growth, especially after wounding (Creelman & Mullet, 1997), while SA plays a role in inhibiting growth (Bosund, Erichsen, & Molin, 1960). However, Sid2 and Lox2 mutants grew at approximately the same rate throughout the experiment, despite similar levels of damage from herbivory. Thus, it appears that JA and SA did not significantly influence the relative growth rates of plants in this experiment.

The attraction of *M. persicae* to plants showing signs of growth, as discussed in this paper (Figure 1), may be applied in the selection of trap crops to divert aphid pests from attacking crops. Less valuable plants may be planted prior to crop plants, enticing aphids to infest the less valuable plants. Integrated pest management including using pesticides, predators, or other strategies may then be used to eradicate the aphids before the desired crop can be infested (Shelton & Badenes-Perez, 2006). Trap crops can be an effective method of reducing pest aphid populations (Hussein & Samad, 1993; Kloen & Altieri, 1990). Our study suggests that fast-growing plants such as Wisconsin Fast Plants™ (*Brassica rapa*; Williams and Hill, 1986) may be useful as trap crops for controlling aphid pests.

Future research should attempt replication of the experiment, exploring additional variables in order to identify the specific volatile factors affecting aphid foraging behaviour. Such research may have agricultural applications in selecting superior trap crops.

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