

Cross-resistance Between the Parasites *Pseudomonas syringae* and *Scaptomyza nigrita* in
the Native Crucifer *Cardamine coridifolia*

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Introduction

Natural enemies of plants (e.g. herbivores, bacterial pathogens) have been widely studied when it comes to pairwise host-pathogen interactions; however, comparatively less research has been performed on the interactions between co-infecting parasites on the same host. *Scaptomyza nigrita* is a leaf mining drosophilid fly that specializes on its host plant *Cardamine cordifolia* (Collinge and Louda, 1989). *Cardamine cordifolia* is a mustard (crucifer) native to the Rocky Mountains and is found in montane, mesic areas, primarily in the shade of willows and spruces/firs (Louda 1995). The shade-restricted distribution of this plant is a textbook example of how a parasite can be a major factor in shaping its host plant's distribution (Louda and Rodman 1996). I decided to study interactions between this host-plant on of its most important natural enemies, *S. nigrita* and previously unstudied *Pseudomonas* spp. bacteria. *Pseudomonas* bacteria are Gram-negative bacteria commonly associated with plants and can cause disease (Hirano and Upper, 1990) in leaves. It is likely that *Pseudomonas syringae* and *P. viridiflava* infect *C. cordifolia* based on previous unpublished work from the Whiteman Laboratory. These two natural enemies likely directly interact because of the mode of feeding by *S. nigrita* adult females and larvae. Other researchers have shown that prior infection with *P. syringae* in the model plant *Arabidopsis thaliana* causes resistance or susceptibility to secondary herbivore attack, depending on the presence of single avirulence or virulence genes in *P. syringae* (Cui et al. 2002). This suggests that prior infection by *Pseudomonas* bacteria in *C. cordifolia* could have major effects on herbivore resistance, population dynamics and potentially, community structure.

Gravid females of *S. nigrita* cut a small incision into a leaf, called a feeding puncture or a stipple, using a special serration on the ovipositor. After making the incision it turns and feeds on the wound exudates, and in some cases oviposits into the stipple. The egg then hatches, the larvae create a mine and complete development in one or more leaves in roughly three weeks (Collinge and Louda, 1989). *Pseudomonas syringae* and *P. viridiflava* are biotrophic (an organism that receives nutrients from living tissue) intercellular bacteria of plants (Rico 2003). As generalist pathogens, they infect multiple species of plant, but pathovars typically show high degrees of host specificity. Although typically vectored abiotically, primarily through the water cycle, they are likely introduced into the plant apoplast by wounds made by adult female *S. flava* and mines created by larvae. Both forms of herbivory likely facilitate entry of these bacteria from the leaf surface, where they are typically found, to the interior of the leaf. After entry into the leaf *P. syringae* and *P. viridiflava* cause disease. Both of these natural enemies (bacteria and *S. flava*) are commonly found co-infecting the same *C. cordifolia* host individuals, but whether the presence of one affects the performance of the other is unknown.

Cardamine cordifolia, like all plants, has several major hormones involved in modulating defense responses to attack by natural enemies (Gupta 2000). Jasmonic acid (JA) is a hormone that triggers a cascade response to chewing herbivores and necrotrophic pathogens, resulting in the production of toxic glucosinolates (Feys 2000). Salicylic acid (SA) is another hormone produced in response to biotrophic pathogens (e.g. *P. syringae*), usually causing triggering localized cell death in the plant. These two pathways are tightly regulated by plants due to the costs of producing the

defenses at the bottom of these signaling transduction cascades. JA and SA are found to inhibit one another (are mutually antagonistic) at multiple points in the cascade response (Traw 2003). When JA is induced, *Arabidopsis thaliana* plants become more susceptible to bacterial pathogens. In the same way, when SA is induced *A. thaliana*, plants are more susceptible to necrotrophic pathogens (which feed on dead tissue) and herbivores (Vos 2006). However, certain strains of *P. syringae* have evolved the ability to produce a JA-mimic called coronatine. By mimicking JA, this phytotoxin causes the plant to inhibit SA production (Nuske 2003), allowing *P. syringae* to increase its virulence in the host, while only encountering minimal defenses. *Pseudomonas syringae* and *S. nigrita* could induce the SA and JA pathways in ways that have major effects on the success of each pathogen. The goal of this study was to test the understood antagonism between jasmonic acid pathways and salicylic acid pathways in a naturally occurring system.

Methods

Study System

Cardamine cordifolia is native mustard that grows the southern Rocky Mountains, Sierra Nevada Mountains, and Cascade Mountains (Harrington 1954). Bittercress grows clonally; large patches can be of the same rhizome, and therefore, genotype. This allows for large studies to be done using a single genotype. *Cardamine cordifolia* begins growing in the spring right after the snowmelt (Louda and Collinge 1992).

Scaptomyza nigrita, a leaf-mining fly, uses bittercress for oviposition, feeding and the larval portion of its lifecycle. It is thought to be the primary parasite of bittercress

(Louda and Collinge 1992) and is likely the most important herbivore in terms of plant fitness. The fly's range overlaps with that of *C. cordifolia*; it lives from California to Wyoming and up to Alaska. Adult flies are most active in mid-June. Females oviposit on lower or mid-stem leaves immediately after snowmelt, early in the plant's growth season (Collinge and Louda 1989a; 1989b). When the larvae hatch they begin to create mines in the leaf.

Location

The experiments were conducted in the laboratory at the Rocky Mountain Biological Laboratory in Gothic Colorado using field-collected samples. I collected plants, larvae and bacterial isolates from research sites along the Copper Creek trail near Gothic, Colorado.

Study Design and Methodology

Pseudomonas spp. isolates were collected by cutting leaves off of adult plants, surface sterilizing them for 30 seconds in 70% ethanol, followed by washes in sterile water and then plating two discs on solid Kings B media agar. Leaf discs in this experiment were taken using a cork borer that was seven millimeters in diameter. The plates were grown in the dark on plates at room temperature. Eighteen isolates were obtained, and their growth rate and disease-causing ability (virulence) were measured on "tester" *C. cordifolia* plants. Two lower (basal) leaves in each of 22 *C. cordifolia* were infiltrated with bacterial strains, two for each strain and two mock infiltrations with the same solution for use as controls. Infiltrations were performed using a blunt syringe to inject buffer containing bacteria into the leaf. Leaves were infiltrated until 0.3 mL was infiltrated or until 75% leaf area appeared wet. At the end of a four-day period the plants

were checked for symptoms including cell death, chlorosis, and leaf wilt. The growth rate of *P. syringae* was assayed by plating serial dilutions (1:10, 1:100, 1:1000) of two homogenized leaf discs on solid agar and counting the colonies that grew after 2 days at room temperature. Of these 18 strains, an isolate (called isolate 12) that was highly virulent and symptom causing was chosen for use in the experiment (fig 1).

For the first experiment I collected 80 plants from the field. Larvae were collected from the field by gathering leaves with mines and transporting them to the lab, I transplanted these larvae into the two lowest healthy leaves of test plants (Whiteman *et al.* 2010). The experiment was conducted in two blocks. In each block 20 plants were treated with larvae and 20 plants were wounded to simulate the transplantation, to serve as mock controls. Larvae were weighed on a microgram balance prior to being transplanted. The larvae were allowed to mine for four days, a sufficient time to allow plant defenses to activate (Whiteman *et al.* 2010). At the end of the four-day period, the plants were infiltrated with the bacteria selected in the previous assay (isolate 12). One day after the infiltration, the leaves that were transplanted with larvae were clipped, the larvae were weighed, and I photographed the leaves to measure leaf area mined. I measured leaf area mined using ImageJ (National Institute of Mental Health, Bethesda, MD). The leaves infected with bacteria were left to grow for four days. At the end of the four days the leaves were clipped off of the plant. The leaves were photographed, and leaf discs were taken. Bacterial growth was measured by plating the leaf discs and counting colony-forming units after serial dilutions.

For the second experiment, I collected 80 plants from the Copper Creek site. I infiltrated two leaves on 20 plants with bacteria in buffer and two leaves on 20 plants

with sterile 10 mM MgSO₄ buffer to serve as a mock control. The experiment was done in two blocks for a total of 40 treated plants and 40 mock plants. The bacteria were allowed to grow in plants for four days, after the four days, one larva was transplanted into each of two leaves above the leaves treated with bacteria. On the fifth day the leaves with bacteria were clipped off the plant. The larvae were left to feed for a total of four days. At the end of the feeding period, the leaves with larvae were clipped from the plant. The leaves were photographed, leaf area mined was calculated as above, and the larvae were weighed.

Data Analysis

T-tests were run in excel to compare average leaf area mined in the two treatments in experiment 1. I also ran t-tests on colony forming unit number between treatments for experiment 2.

Results

Isolates collected varied widely in growth rates and symptoms. Isolate 12 was chosen for the experiments due to its high virulence and recognizable symptoms in planta (Figure 1).

Pre-treatment in leaves with isolate 12 caused a reduction in the leaf area mined by larvae placed in the same rammet, but in systemic leaves (Figure 2, $P = 0.0027$). Plants that received a mock pre-treatment had 1.5x the leaf area mined compared to those pre-treated with bacteria.

Pre-treatment with larvae in systemic leaves had no effect on the growth rate of isolate 12 ($P = 0.389$).

Discussion

We found that infection by one natural enemy in *C. cordifolia* does influence the growth of a different natural enemy in planta. However, the interaction was not reciprocal, only pre-treatment with bacteria influenced larval growth and not vice versa. Specifically, pre-treatment with isolate 12 had a negative impact on larval fitness. The effect may be due to isolate 12 causing a jasmonic acid (JA) response in the plant, or some other unknown mechanism. This effect has been observed in other strains of *P. syringae* and is due to the production of coronatine, a JA mimic (source). The growth rate of isolate 12 remained unaffected when the plant was pre-treated with larva, this could be due to the virulent nature of isolate 12, which was shown in the prior bioassay of the isolates. In these assays, isolate 12 was able to grow many orders of magnitude faster than other strains. The chronology of infection seems to have major impacts on *S. nigrita*'s fitness, and on *C. cordifolia*'s ability to induce defenses.

Some experimentation needs to be done into the cause of these results. Real time PCR could be used to test for the expression of genes encoding the molecule coronatine, this would give insight into the mechanism (systemic signal) underlying the negative impact of bacteria on larval fitness. Measuring the jasmonic acid levels present in the plant before and after the infiltration with bacteria could also give insight into whether this bacterium was inducing JA.

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References Cited

- S. K. Collinge and S. M. Louda. " *Scaptomyza nigrita* Wheeler (Diptera: Drosophilidae), a Leaf Miner of the Native Crucifer, *Cardamine cordifolia* A. Gray (Bittercress)." *Journal of the Kansas Entomological Society* 62.1 (1989): 1-10.
- Cui, Jander, Racki, Kim, Pierce, Ausubel. "Signals involved in Arabidopsis resistance to *Trichoplusia ni* caterpillars induced by virulent and avirulent strains of the phytopathogen *Pseudomonas syringae*". *Plant Physiology*. 2.129 (2002). 551-564.
- B Feys. "Interplay of signaling pathways in plant disease resistance." *TIG*. 16.10 (2000): 449-456
- Vaijayanti Gupta, Michael Willits, Jane Glazebrook. "*Arabidopsis thaliana* EDS4 Contributes to Salicylic Acid (SA)-Dependent Expression of Defense Responses: Evidence for Inhibition of Jasmonic Acid Signaling by SA." *MPMI*. 13.5 (2000): 503-511
- Harrington, H.D. 1954. *Manual of the plants of Colorado*. Sage Books, Denver, Colorado, USA.
- Hirano, Upper. "Population Biology and Epidemiology of *Pseudomonas syringae*". *Annual Review of Phytopathology*. 28 (1990). 155-177.
- Y. Koda. "Similarities of Biological Activities of Coronatine and Coronafacic Acid to those of Jasmonic Acid." *Phytochemistry*. 41.1 (1996): 93-96
- Louda, S. M. and J. E. Rodman. 1996. Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). *Journal of Ecology* 84: 229 - 237.

Jorg Nuske, Friedmann Bublitz. "In vitro coronatine production by several *Pseudomonas syringae* pv. *glycinea* isolates." *Basic Microbiolgy*. 33 (1993) : 241-246

Arantza Rico, Sarah L McCraw and Gail M Preston. "The metabolic interface between *Pseudomonas syringae* and plant cells." *Current Opinion In Microbiology*. 14.1 (2011): 31-38

J. Rodman, S Louda. "Phenology of Glucosinolate Concentrations in Roots, Stems and Leaves of *Cardamine cordifolia*." *Biochemical Systematics and Ecology*. 12.1(1984): 37-46

Jyoti Shah. "The salicylic acid loop in plant defense." *Current Opinion in Plant Biology*. 6(2003) :365–371

M.B. Traw, J, Kim, S. Enright et al. " Negative cross-talk between salicylate- and jasmonate- mediated pathways in the Wassilewskija ecotype of *Arabidopsis thaliana*." *Molecular Ecology* 12 (2003): 1125-1135.

De Vos, M. "Herbivore-Induced Resistance against Microbial Pathogens in *Arabidopsis*." *Plant Physiology* 142.1 (2006): 352-63.

Figure captions.

Figure 1. Isolates of bacteria from leaves of *Cardamine cordifolia* (endophytes) sampled from Copper Creek and 401 trail sites and infiltrated at OD600 = 0.008 in three leaves of *C. cordifolia*. Bacteria were allowed to grow for three days and leaf samples were taken with a cork borer (7mm), discs were ground up in buffered water and plated on King's B media plates. Serial dilutions were made and colony forming units were quantified. Isolate 12 was selected for the bacterial treatments in this experiment and caused major symptoms.

Figure 2. Left: Results of pre-infection of *C. cordifolia* plants with larvae of *S. nigrita* or mock (needle prick) followed by infection with *Pseudomonas* isolate 12 (OD600=0.008) in systemic leaves four days after introducing larvae. Right: Results of pre-infection of *C. cordifolia* plants with *Pseudomonas* isolate 12 (OD600=0.008) or sterile buffered water controls followed by infection of systemic leaves with two larvae of *S. nigrita*. Leaf area mined is a proxy for larval performance and is positively correlated with weight gain.

