EVIDENCE FOR A GROWTH-DEFENSE TRADE-OFF IN *CENTAUREA SOLSTITIALIS*

By

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As members of the Master's Committee, we certify that we have read the thesis prepared by Elizabeth Marjorie Carpenter, titled Evidence for a Growth-Defense Trade-off in *Centaurea solstitialis* and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

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Abstract

An important question in invasion ecology is why certain introduced species become invasive. It is thought that some invasive plants escape their natural enemies, and therefore might adapt to devote more resources to growth under a general trade-off between growth and defense. Yellow starthistle (*Centaurea solstitialis*) has previously been shown to grow larger in its invaded range. This study sought to test whether yellow starthistle is evolving differences in immune activity along a growth-defense trade-off or through local adaptation to its pathogen communities. This study used a high-throughput peroxidase assay to quantify immune activity after leaves were exposed to bacteria from native and invaded ranges. Immune activity was measured using the light absorbance of a substrate for peroxidase enzymes. Plant genotypes from the invaded range showed a lower immune response when exposed to bacteria from all ranges. Three bacteria strains from the invaded range elicited a lower immune response when exposed to plant genotypes from both ranges. These results support the hypothesis that yellow starthistle is evolving to invest less in defense, and future work should investigate the connections between these changes and investment in growth.

Introduction

A primary question in the field of invasion ecology is how certain introduced species become invasive (Elton 1958). These are introduced species that establish and increase to become one of the most abundant species in the community (Rejmánek et al. 2005; Richardson and Pyšek 2006). Plant-pathogen interactions may play a role in facilitating the success of recently established species within their non-native environment (Gilbert and Parker 2010). There are two related hypotheses for how interactions between pathogens and plants could explain invasion: Enemy Release (ERH) and Evolution of Increased Competitive Ability (EICA). The ERH states that invaders have increased success due to the absence of natural enemies in their introduced environment (Keane and Crawley 2002; Mitchell and Power 2003; Inderjit, Cadotte, and Colautti 2005). EICA states that the absence of specialized enemies will select for the evolution of investment along a trade-off between defense and growth, such that invaders will have decreased investment in specialist defenses and increased investment in biomass relative to their native counterparts. This is possible because plants are known to have biochemical pathways that allocate resources between growth and defenses (Coley, Bryant, and Chapin 1985; Herms and Mattson 1992; Huot et al. 2014). An increase in biomass will then, in turn, allow the invading species to better compete for resources (Blossey and Notzold 1995; Bossdorf et al. 2004). There is evidence from other species that invaded range genotypes can differ genetically from their native genotype due to the escape of their natural predators (Siemann and Rogers 2001). While this scenario could explain how species become invasive, whether invasive plants gain an advantage by evolving along a growth-defense trade-off is still unclear, and has been tested with mixed results (Ridenour et al. 2008; Felker-Quinn, Schweitzer, and Bailey 2013; Gruntman, Zieger, and Tielbörger 2016). An alternative hypothesis to explain these patterns is that invaders have evolved to be locally adapted to their enemies in the introduced range, and are no longer well adapted for defense against native range enemies (Belotte et al. 2003; Kraemer and Boynton 2017).

This study sought to test whether there is evidence for evolution along a growth-defense trade-off within the invasive plant *Centaurea solstitialis* (yellow starthistle, Asteraceae). *Centaurea solstitialis* is an annual plant that was introduced to South America as early as the 1600s from western Europe (John D. Gerlach 1997; Barker et al. 2017). It was introduced to North America in the California San Francisco Bay area in the late 1800s, and spread throughout the state (J. D. Gerlach Jr 1997; John D. Gerlach 1997)). Since its introduction, it has become highly invasive in grasslands in western United States (particularly in California) and Argentina (Maddox, Mayfield, and Poritz 1985; Pitcairn et al. 2006). It has been shown that invasive *C. solstitialis* genotypes have evolved larger growth and increased reproduction than genotypes from the native range (Widmer et al. 2007; Dlugosch et al. 2015). This is consistent with the possibility that invaders have evolved to allocate resources to growth at the cost of other functions such as defense.

A previous study tested for evidence of reduced investment in defense in *C. solstitialis* (Kaczowka et al. 2017). The study focused on the production of reactive oxygen species as an

indicator of immune activity in the plant. One of the most important and primary immune systems in plants is the hypersensitivity response (HR) (Chisholm et al. 2006). Receptors on the plant's cell surface are recognized by pathogen-associated molecular patterns (PAMP). This starts PAMP-triggered immunity (PTI) or effector-triggered immunity (ETI) (Jones and Dangl 2006). Both PTI and ETI trigger HR, which is associated with the release of reactive oxygen species (ROS) (Tenhaken et al. 1995). The production of ROS compounds hinders the spread of pathogens by cell wall hardening, cell death, and the production of apoplastic peroxidase enzymes. It is known that ETI triggers a stronger HR response in plants (Camejo, Guzmán-Cedeño, and Moreno 2016). This would occur if these pathogens have evolved a more specialized antagonistic relationship with their host plant (Wojtaszek 1997). Previous studies have been able to use apoplastic peroxidase enzymes as a measurement of immune activity in plants (Wojtaszek 1997; Daudi et al. 2012; Camejo, Guzmán-Cedeño, and Moreno 2016). Peroxidase enzymes are involved in the breakdown of reactive oxygen species and that the crosstalk between peroxidase enzymes and ROS are known to regulate growth during early development (Černý et al. 2018). The study by Kaczowka et al (2017) found that invading *C. solstitialis* showed lower activity of peroxidase enzymes than native genotypes when wounded or exposed to several different strains of bacteria from the native range, supporting the hypothesis of the evolution of reduced defenses.

Here I tested the alternative hypotheses that invading *C. solstitialis* are evolving immune function A) along a growth-defense trade-off versus B) through local adaptation. I repeated the assay of immune activity with native and invading plant genotypes exposed to both native and invaded range bacteria strains. If a growth-defense trade-off best explains patterns of immune function, I predict that invaded genotypes will have a lower peroxidase activity when exposed to all strains of bacteria. If instead immune activity in invading genotypes is locally adapted, I predict that invading genotypes will have a lower peroxidase activity when exposed to bacteria not from their local range, and higher activity in response to their local microbes (Fig. 1).

Materials & Methods

Obtaining Bacteria

Bacteria strains were collected from basal leaves of plants from three native sites in western Europe and from three invaded range sites in California (Table 2, Fig. 2). Leaves that showed disease symptoms were collected in sterile 50 mL falcon tubes on ice and later refrigerated at 4 °C. Each leaf sample was removed with sterile forceps bleached in a 200-ppm solution for 2 minutes and rinsed with sterile distilled water. A 1 cm^2 slice of the leaf was cut using a sterilized scalpel and placed within a 1.5 mL tube filled with 400 µL sterile 10nM $MgCl^2$. Leaves were then ground up using a sterilized plastic pellet pestle for 45s. An additional 600 µL10nM $MgCl^2$ was added to each tube, bringing the solution to 1 mL. 150 µL of each leaf solution was pipetted and spread onto a malt extract agar plate using a sterile plastic cell spreader. Bacteria plates were incubated under low light for 2 days at approximately 27 °C. Bacteria culture was further diluted by combining 1 µL of leaf solution with 9 uL sterile distilled water. The resulting 10 uL solution was pipetting onto a second agar plate.

Single colonies from the resulting cultures were picked and cultured in 2 mL lysogeny broth (LB) media for 2 days. 750 µL of sterile 80% glycerol was mixed with 750 µL liquid culture and samples were stored at -80 °C. Samples were plated again for sequencing. A single colony was picked and added to 25ul of sterile water. Native bacteria were sequenced using the 16S rRNA and primers 149R (5'AGAGTTTGATCMTGGCTCAG3') and 27F (5'TACGGYTACCTTGTTACGACTT3') were used (Wilson, Blitchington, and Greene 1990). Amplification used Phusion Flash master mix (Thermo Scientific) in a 50 µL reaction with 1 µL single colony dilution, 2.5 µL of each primer at 10 µm, 0.625 µl Taq (Thermo Scientific), 1.25 µL of 5mM dNTPs, and 5µL of 10x PCR buffer (Fisher Scientific). PCR conditions included 5 minutes at 95 °C and 35 cycles of 15 seconds at 94 °C, 30 seconds at 50 °C, and 1 minute 30 seconds at 72 °C. PCR products were sequenced using Sanger by Genoscreen (Lille, France). BLAST was used to compare results to existing sequences in GenBank for identification. Invaded range 16S rRNA sequences were cloned using a TOPO2.1 vector and then sequenced using 149R (5'AGAGTTTGATCMTGGCTCAG3') 27F(5' TACGGYTACCTTGTTACGACTT3') tailed end primers (TOPO TA Cloning Kit Invitrogen Thermo Fisher Scientific). Amplification used the same protocol as native bacteria strains. Transformations were performed using 100 µL top10 chemically competent cells (Fisher Scientific). They were then heat shocked for 40 seconds at 42°C. Bacteria strains were incubated at 37 °C for 4 hours in 300 µL LB media then picked and cultured on antibiotic LB plates consisting of 30-50 µg/ml kanamycin and 40 µg/ml of x-gal. Cultures grew overnight and plasmids were isolated. PCR products were sequenced using Sanger by EtonBio Sciences (San Diego, USA).

Thirty-three strains were available from this work, and ten strains of bacteria were selected for this experiment (Table 1). Five of the strains were isolated from native range plants: *Pseudomonas viridiflava* (1031), *Curtobacterium flaccumfaciens* (1038), *Pantoea agglomerans* (1034, 1039, 1041), including 1031 and 1038 used in the previous work by Kaczowka et al. 2017, and 3 previously unexamined strains. The following five strains were selected haphazardly from invaded range strains: *Curtobacterium sp.* (1233), *Bacillus aryabhattai (*1234), *Pseudomonas sp* (1235), *Pantoea sp*. (1236), bacteria culture (1238). These strains were identified after the analysis of the data, therefore strain 1238 is included in the results, however noted that it is not pure culture.

To obtain cultures for use in the assay, a 2 ul aliquot of frozen bacteria culture was streaked onto LB plates and incubated at 27 °C for 36 hours. Each bacterial culture was picked and cultivated in 3 mL liquid LB recipe. Each tube was agitated using a shaker (New Brunswick Scientific Excella E24 series) at 27 °C for 46 hours. Cultures were diluted by 50% by adding liquid LB. The optical density of each sample was taken and diluted until each sample reached a concentration of OD_{550} to standardize the concentration of bacteria across strains.

Plant Genotypes

The invading plant genotypes were acquired from three sites in California: GIL, TRI, LEB (Table 2, Fig. 2). The native genotypes were obtained from two western Europe sites in

Spain: GRA, SAZ (Table 2, Fig. 2). Seeds were collected along a 30 meter transect at 1-meter intervals. Each genotype was collected from a separate mother plant.

Seeds were planted in May 2017 and grown in a soil mixture containing a 3:2:1 mixture of Sunshine Mix#3 soil (SunGo Horticulture), vermiculite, and 20 grit silica sand respectively. At least five seeds from the maternal plant were reared at 20 °C for four weeks. Plants were then transferred into 410 ml Deepots (Stuewe & Sons) and grown in a greenhouse. Greenhouse conditions were kept at 14-hour daylight with a daily minimum temperature between 16-26 °C and daily high temperature between 20-30 °C throughout the summer and autumn. These included plants from original seed collections from the field and plants from the first generation of seeds produced from parents reared in a common environment and manually pollinated within populations. A total of 19 mother plants were selected for the assay, 4 were from GIL, 4 from LEB, 4 from TRI, 4 from GRA, and 3 from SAZ.

Leaves were collected in October 2017 after 24 weeks of growth. Healthy basal leaves were selected to be at similar developmental stages when available. Leaf disks were collected by punching holes using a 4.8 mm diameter cork borer. Leaf disks were positioned away from major veins and the edge of the leaf. Leaves and cork borer were sterilized between collections by wiping with 70% ethanol. Each disk was placed in a 2 ml microfuge tube filled with 1 ml 1X Murashige & Skoog basal salt mixture (MS) (Sigma-Aldrich). Disks were then agitated in a shaker for 1 hour at 27 °C. Disks were placed within a 96 well suspension culture microliter plate (Cellstar, Greiner Bio-One), prepared with 1.2 g MS w/v 250 mL MS solution within the inner 60 wells. The outer edged wells were filled with 300uL millipore water.

Three leaf disks of each of the 19 plant genotypes were exposed to each of the 10 bacterial strains in these wells (30 disks per plant). Each well was inoculated with 1uL of bacteria culture. Plates were sealed with parafilm and agitated on a shaker at 27 °C for 16-24 hours. Leaf disks were removed and 50uL of 1mg/mL 5-aminosalicyclic acid (5-AS), 0.01% hydrogen peroxide solution was added to each well to provide a substrate for the peroxidase enzyme. After 1 minute, 20 uL of 2 N NaOH solution was added to stop the reaction. Plates were placed into a Synergy H1 Hybrid Multi-Mode Microplate Reader (Bio Tek) to record the absorbance for each plate at 550 nm.

Nineteen wells were used for each plant replicate with one additional well used as a control (‘No Disk’). Replicates of nine of the bacteria were arranged across 10 plates. The 10th plate contained strain 1235 in all wells.

Statistical analysis

A linear fixed effects model was used to test for a difference in absorbance between No Disk wells and wells containing leaf disks. For wells containing disks, a linear fixed effects model was used to test for the effects of plant region, plant genotype nested within region, and bacterial strain on absorbance. The effect of bacterial strain crossed with plant region was also included to determine if there was an interaction. A Tukey’s HSD post hoc test was used to

determine which bacterial strains were significantly different when compared to each other. All statistical analysis was done in JMP v. 14.0.1 (SAS Institute 2018).

Results

There was a significant difference between 'No Disk' and wells containing leaf disks ($F_{1,598}$=102.5604 P<0.0001). The 'No Disk' wells showed a low absorbance response (Fig. 3). Absorbance across all wells with leaves averaged approximately four times higher than these controls.

There was no significant interaction between strain and plant region (interaction term: $F_{9,529}$=1.3462, P=0.2101). This interaction term was dropped in the final reduced model. There was a significant difference between strains ($F_{9,529}$=62.7991 P<0.0001), and between plant regions, where the invaded range genotypes had a lower absorbance ($F_{1,529}$=22.1578, P<0.0001). Native bacterial strains elicited a higher absorbance response of leaf disks overall (Fig. 6). There was also a significant nested effect of plant genotype within plant region ($F_{1,17}$=5.8652, P<0.0001).

A post-hoc comparison using Tukey's HSD showed that there were three groups of strains that were significantly different from one another (Fig. 4). Strains 1235 and 1031 were not significantly different from one another, and these also had the highest absorbance. Strains 1233, 1234, and 1238 had absorbance that was significantly lower than the other groups (Fig. 4).

Discussion

This study sought to test alternative hypotheses of a growth-defense trade-off or local adaptation in *C. solstitialis* immune activity. I found that invaded range plant genotypes had a significantly lower immune response when exposed to bacteria from either range. This result supports the predictions of the growth-defense hypothesis. I also found evidence that bacteria strains differed in the immune response that they induced across plants from either region, with three invaded range strains eliciting particularly low responses from plants. These results indicate that plant-bacteria interactions vary in this system, and could differ between ranges.

The peroxidase assay showed that eight out of ten of the strains resulted in mean absorbance that was lower in invaders. There was also no significant interaction between plant range and bacteria strain, indicating that there was no statistical support that the remaining two strains (1031 and 1039) trended in a different direction, and that there was an overall consistent support for a lower immune response in invaders. It is unknown how pathogenic these bacteria are on these plants. These interactions are potentially complex as there is evidence that microbes in this study may have a positive or negative fitness effect on invaders (Thorpe et al. 2009; Li et al. 2019). *Pantoea* (1236) is known to have species that are pathogenic and others that form a mutualistic relationship within plant species (Walterson and Stavrinides 2015). *Pantoea*

agglomerans (1034, 1039, 1041) strains are a known pathogen of leaf blight and gall formation within beet cultivars (Manulis and Barash 2003; Lu, Jia, and Gao 2015; Dutkiewicz et al. 2016). *Pantoea agglomerans* has also been known to be saprophytic for certain plant species and has been shown to induce resistance within a number of plant cultivars, including inducing the oxidative burst reaction and promoting root shoot growth (Egamberdiyeva and Höflich 2003; Ortmann and Moerschbacher 2006). *Curtobacterium flaccumfaciens* (1038) is another bacteria with a number of strains that respond differently in different plant families (Harveson et al. 2015). It is known to cause bacteria wilt within Fabaceae cultivars. It is also known to promote the growth of root and shoots and will induce an immune response within the rhizosphere in cucumber plants (Raupach and Kloepper 2000; Osdaghi et al. 2018). *Pseudomonas* (1235) is a known pathogen genus for a number of plant species, including plants within the same family as *C. solstitialis* (Kearing, Nowierski, and Grey 1997) Previous studies with *Arabidopsis* suggests that genetic variation within plant species may determine whether the interaction of a *Pseudomonas sp.* is pathogenic or saprophytic (Bartoli, Berge, and Monteil 2014; Haney et al. 2015).

The bacterial strains were statistically grouped into three categories based on highest to lowest absorbance across both native and invading plant genotypes). The highest absorbance response belonged to bacteria strains that belonged to the *Pseudomonas* genus. *Pseudomonas viridiflava* (1031, from the native range) a variant of the *Pseudomonas syringae* complex, is widely known for causing potato soft rot in a number of plant cultivars. This genus is known to include many pathogenic strains including *Pseudomonas aeruginosa*, a strain that native range *C. solstitialis* is known to produce defensive secondary compounds against (Carev et al. 2017). Strain 1235 from the invaded range could not be identified to species but is also a member of the *Pseudomonas* genus. The plants may be adapted to recognize these strains that are often pathogenic, and to respond with a strong oxidative burst.

The strains with the lowest response were all from the invaded range. *Curtobacterium spp.* (1233) is a widely distributed bacteria genus that is known to have both pathogenic and saprophytic activities. *Bacillus aryabhattai* (1234) is a commonly observed bacteria in soils and studies support that the plant-microbe interaction is often non-pathogenic with these strains (Lee, Ka, and Song 2012; Bhattacharyya et al. 2017; Ghosh et al. 2018; Shin et al. 2019). *Bacillus aryabhattai* has been found to promote growth, to provide resistance to heat stress, alleviate toxicity from other pathogenic and abiotic compounds within soil, and allocate resources towards growth. As a result, *C. solstitialis* plants might have evolved a beneficial relationship with these bacteria and do not defend against them.

It is also possible that some bacteria are playing an active role in suppressing immune function in the plants. This interaction may have evolved to be mutualistic or pathogenic. Mutualistic bacteria could be suppressing immune function if they are able to modify immune signaling or resource recruitment (Lahrmann et al. 2015). Beneficial species might quickly establish a symbiotic relationship with a newly established plant species, as seen in some plant-rhizobia interactions (Lohar et al. 2006). Pathogenic microbes might also have evolved to suppress defenses more quickly than the plant has been able to evolve recognition of these pathogens. These recognition pathways between microbe and plant will evolve dynamically over

time and it is difficult to predict how these interactions have developed with a newly established plant (He, Shan, and Sheen 2007). Microbes can also suppress immune response if they are acting as the defensive regulator of pathogenic microbes. There are a number of endophytic root colonizing fungi that are thought to aid in the activation of HR including ROS production and the promotion of regenerative growth within damaged plant tissues (Akum et al. 2015; Johnson et al. 2018).

The mechanism behind the defense against pathogens and the trade-off with growth may vary depending on a number of immune responses connected to pathogen defense. The growth-defense trade-off can result from hormonal crosstalk between PTI and salicylic acid regulation. Salicylic acid is induced during PAMP as a major hormone commonly used for immune regulation. There is further cross talk between salicylic acid and other important plant hormones auxin, brassinosteroids and gibberellins, three hormones responsible for plant growth. These hormones also interact with hydrogen peroxide, an important ROS molecule that is broken down by peroxidase enzymes during PAMP (Černý et al. 2018). It's been shown that these hormones are responsible for regulating and suppressing growth during times of stress or pathogen presence (Kempel et al. 2011; Zhang et al. 2013). Some of these functions directly affect the trade-off between growth and immunity including photosynthesis, seedling production, and auxin signaling responsible for root-shoot growth (Huot et al. 2014). Mutant *A. thaliana* displaying an overexpression of salicylic acid accumulation consistently suppressed growth. (Wang et al. 2007; Ye et al. 2019)). A genetic basis for growth and defense regulation of auxin was identified in maize, where the expression of the gene ZmAuxRP1 was responsible for the regulation of defensive compound production and synthesis of auxin compounds (Ye et al. 2019). Brassinosteroids are thought to be regulated via the brassinosteroids-ethylene pathway using a transcription factor known as OsBIHD1(Luo et al. 2005). This factor has been found to bind to promoters that suppress growth, and the upregulation of OsBIHD1 increases stress resistance during an immune response (Liu et al. 2017). Gibberellins regulate growth at multiple stages of plant development and are linked to the breakdown of growth inhibitor DELLA proteins, responsible for several HR signaling responses. There is evidence to suggest that DELLA proteins aid in degrading ROS, thus preventing the spread of cell death within plants and reinforcing cell wall structure (De Bruyne, Höfte, and De Vleesschauwer 2014).

This study found evidence consistent with a lower investment in immune defense in invading *C. solstitialis.* Previous studies in this system have shown that the microbial environment is different in the invaded range and that invaded range soil is less pathogenic (Andonian and Hierro 2011; Lu-Irving et al. 2019). It has also been shown that there is a significant difference between the diversity of the plant microbiomes between each range, where plant in the invaded range had an overall lower microbial diversity in their leaves and roots (Lu-Irving et al. 2019). These differences could have favored the evolution of a lower immune response due to the lack of microbial pathogens, a less pathogenic microbial community, or an increase in beneficial interactions between the plant and the invaded range microbiome (Andonian and Hierro 2011). Plants that have favorable genotypes for increased growth are more likely to be selected if the antagonistic pressure from pathogenic microbes were relaxed in their new range (Kulmatiski et al. 2008; Van der Putten et al. 2013; Faillace, Lorusso, and Duffy

2017). This is significant in that it is known that *C. solstitialis* grows larger in the invaded range than the native region (Dlugosch et al. 2015). In a previous study that examined peroxidase responses of *C. solstitialis* genotypes exposed to only native range bacteria, there was a negative linear relationship between plant size and absorbance, where genotypes from the invaded range had larger plant size and the lowest immune response (Kaczowka et al. 2017).

The peroxidase assay has been informative for assessing immune response across varied genotypes, but many additional studies will be important for understanding how growth-defense trade-offs might explain how invasive plants evolve to be more successful in their invaded ranges. Future studies should determine what fitness effects individual strains have on *C. solstitialis.* In addition, this study looked only at bacteria collected from the phyllosphere. There is mounting evidence that the rhizosphere plant microbiome is especially critical for plant success (Chaparro, Badri, and Vivanco 2014; Wingfield et al. 2017; Levy et al. 2018). Studies looking into growth-defense trade-offs should focus additional attention upon root or rhizosphere interactions and their effects on plant fitness. Finally, further investigation is needed to understand how investments in defense functions are directly related to investments in growth. My future research will test whether the phenotypic traits related to growth and immune defense are located within the same genomic region, connecting growth and immune defense on the genetic level within *C.solstitialis.*

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Figures and Tables

Table 1: Bacteria taxonomic identification and field site information. Site details are given in Table 2.

Bacteria Code	Order	Taxonomic Name	Field Site
		Native Range	
1031	Pseudomonadales	Pseudomonas viridiflava	CAN-Spain
1034	Enterobacteriales	Pantoea agglomerans	GRA-Spain
1038	Actinomycetales	Curtobacterium flaccumfaciens	SAZ-Spain
1039	Enterobacteriales	Pantoea agglomerans	SAZ-Spain
1041	Enterobacteriales	Pantoea agglomerans	SAZ-Spain
		Invaded Range	
1233	Actinomycetales	Curtobacterium spp.	CLV-CA
1234	Bacillales	Bacillus aryabhattai	GIL-CA
1235	Pseudomonadales	Pseudomonas sp	CLV-CA
1236	Enterobacteriales	Pantoea sp.	RB-CA
1238	N/A	Bacteria culture	GIL-CA

Table 2: Plant genotype and bacteria source locality information. Native plant collections occurred in 2008. Native bacteria collections occurred in 2015. Invasive plant collections and bacteria collections occurred in 2016.

Site Code	Nearby City	Range	Country	Longitude	Latitude	Plant Collected	Bacteria Collected
GIL	Gilroy	Invaded	USA(CA)	-121.53674	37.03373	Y	Y
TRI	Triangle	Invaded	USA(CA)	-119.79218	37.46178	Y	N
LEB	Lebec	Invaded	USA(CA)	-118.8705634	34.8243437	Y	N
RB	Red Bluff	Invaded	USA(CA)	-122.27103	40.27085	N	Y
CLV	Clovis	Invaded	USA(CA)	-119.79341	36.91603	N	Y
SAZ	Villares de Saz	Native	Spain	-3.77061	43.74842	Y	Y
GRA	Granada	Native	Spain	-3.66497	37.26836	Y	Y
CAN	Canales	Native	Spain	-3.66488	41.00085	N	Y

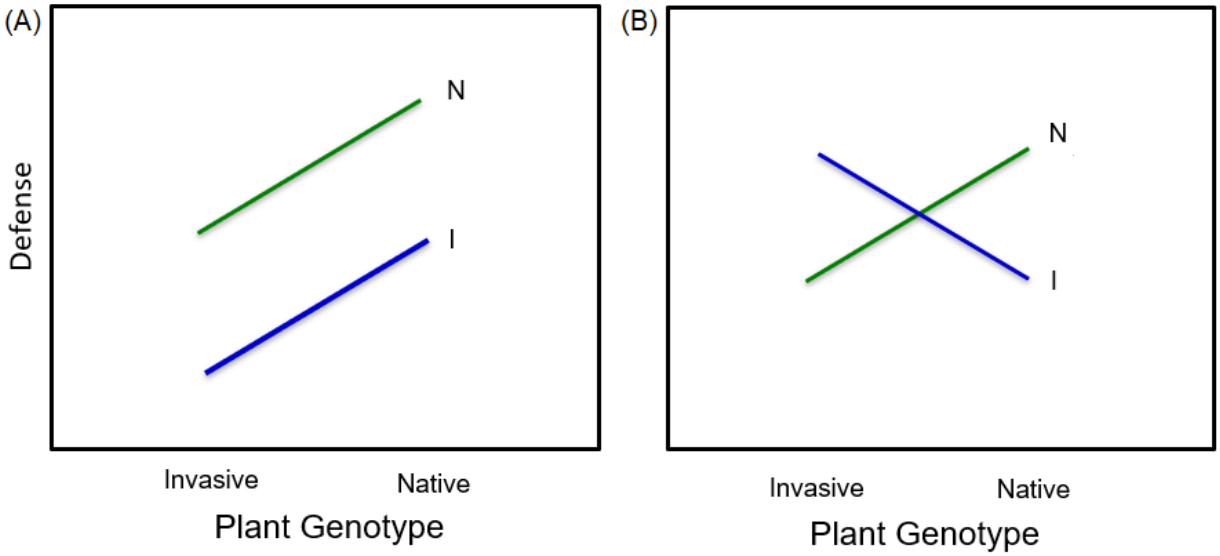

Figure 1. Hypothesis of how native and invaded range plants respond to native range bacteria (N) and invaded range bacteria (I) as predicted by the growth-defense trade-off hypothesis or local adaptation. (A) Growth-defense trade-off, where plants exposed to invaded ranged bacteria should show a lowered immune response because they grow larger. (B) Local adaptation, where plants have a higher immune response to their own local bacteria.

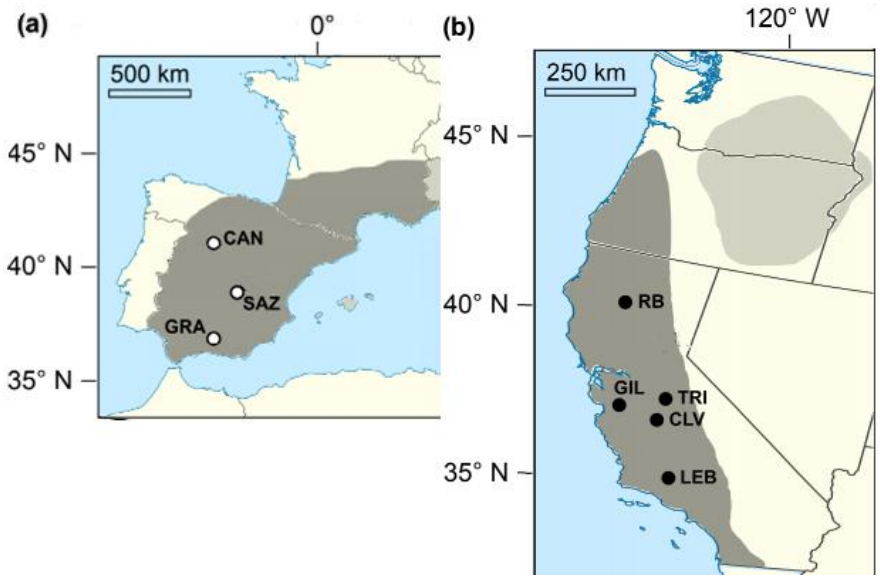

Figure 2. Source locality map for plant and bacteria genotypes. (a) The native range sites in Spain. (b) Invaded range sites in the western United States. Plant genotypes were collected from three sites in California and two sites in Spain (Table 2). Bacteria strains were collected across three sites in the invaded range and three sites in the native range (Table 2). The western European distribution is the source population for the California invasion.

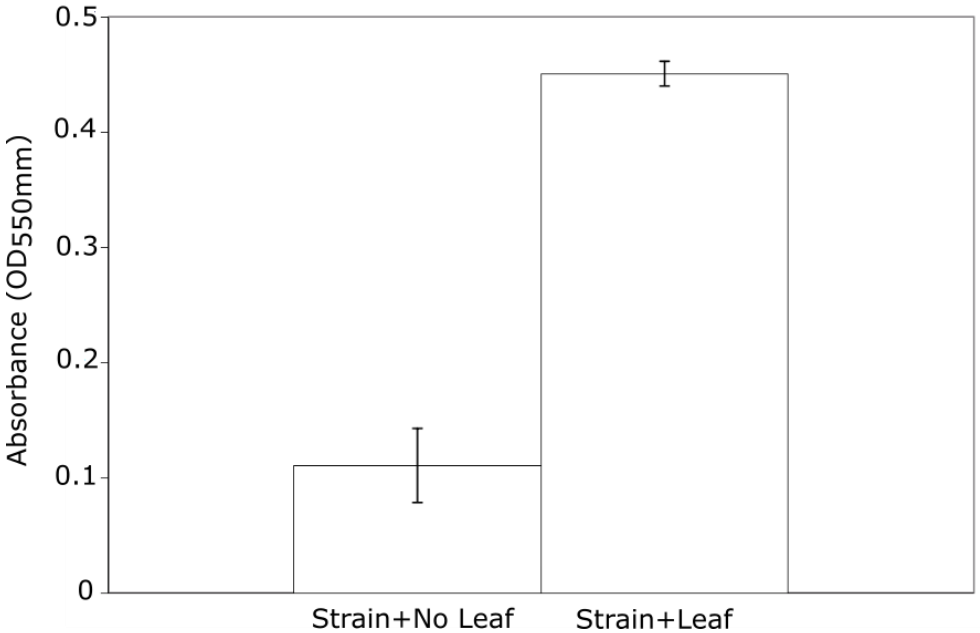

Figure 3. The absorbance of no leaf versus leaf-containing wells (all strains combined). Shown are the least squared means +/- S.E. from the linear model predicting absorbance as a function of leaf disk presence/absence ($P < 0.0001$). All wells contained bacteria.

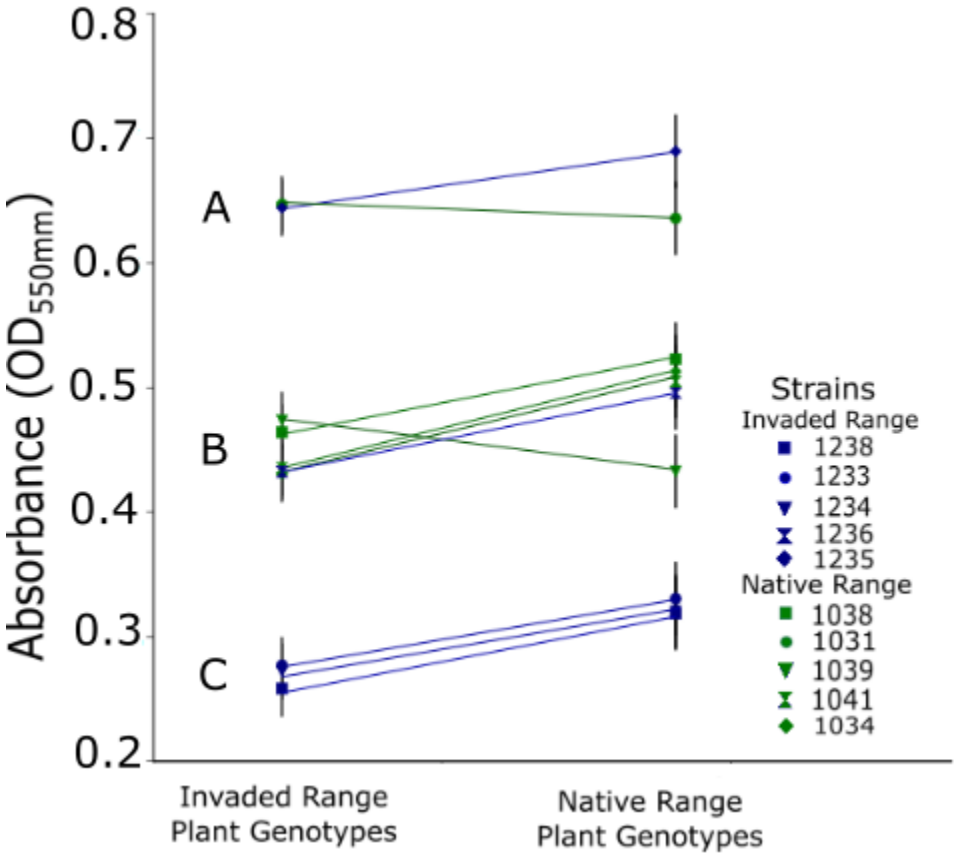

Figure 4. The interaction between bacterial strain and plant genotype source region. The y-axis is the absorbance from peroxidase activity based on optical density (550mm). Plotted are the least squared means +/- S. E. from the full linear fixed effects model including the interaction between strain and plant region. The green color indicates the native range strains. The blue color indicates the invaded range strains. The symbols indicate different strains as identified in the legend. Letters A, B, and C represent significant differences among groups of strains according to the post-hoc Tukey's HSD test.

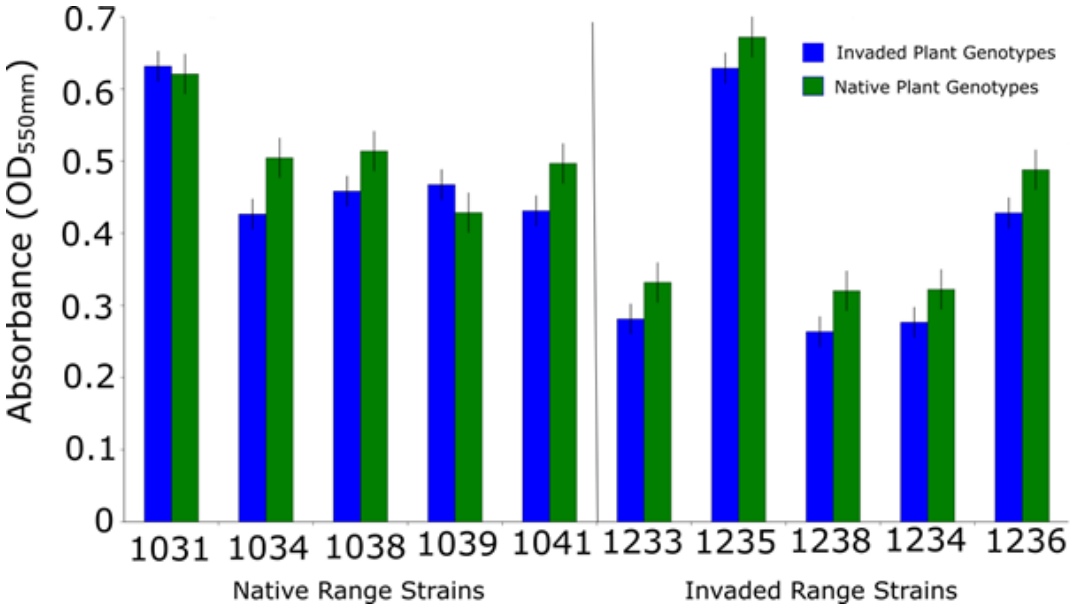

Figure 5. The individual breakdown of the interaction of strain and plant genotype, results as in figure 4. The y-axis is the absorbance during peroxidase activity adjusted optical density (550mm). Shown are the least squared means of a linear fixed effect model with effects of strain and plant region.

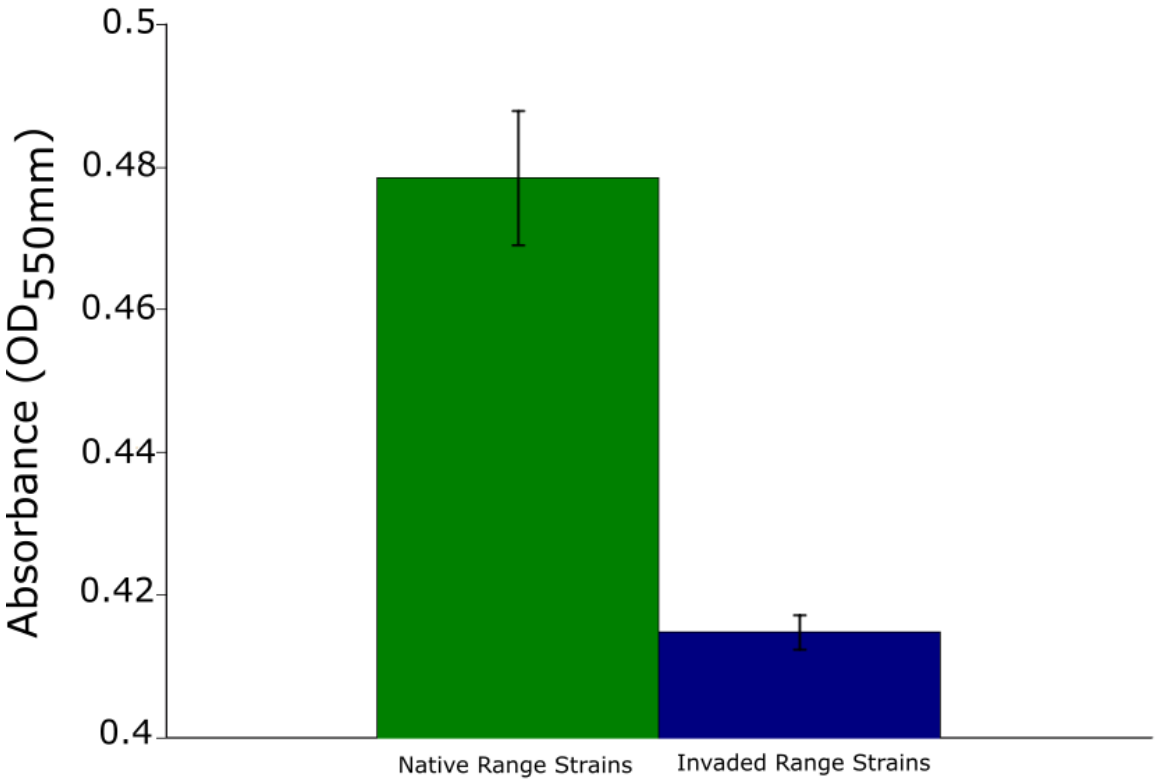

Figure 6. The absorbance of native versus invaded ranged bacteria strains. The native range strains had an overall higher absorbance response than invaded range strains (P <0.0001). Plotted are the least squared means +/- S. E. from the reduced linear fixed effects model.