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FACTORS AFFECTING THE DISEASE SEVERITY OF ALTERNARIA BLACKSPOT IN NATURAL *BRASSICA RAPA* POPULATIONS ON THE CALIFORNIA AND OREGON COASTS

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ABSTRACT

Fungal disease has important effects in natural and agricultural plant populations; however, we are still uncovering factors that influence severity in these systems. Understanding these factors is especially important because it has been predicted that climate change will increase fungal disease widely as a result of changes in precipitation. Here, we investigate the role of water availability and other ecological variables in determining disease severity of a foliar fungal disease. For this study, we focused on natural populations of an important annual, herbaceous plant, *Brassica rapa* and its fungal disease, Alternaria blackspot. We explored three hypotheses: (1) The factors that drive disease severity differ early in the growing season compared to late in the growing season. (2) Disease severity patterns for this fungus are driven by water availability to a greater extent than other ecological variables. (3) Disease severity increases with plant density. To address these hypotheses, data were collected in a spatially structured manner at two time points during the summer of 2011 from four *B. rapa* plant populations along the California and Oregon coasts. We found no clear factors drove disease severity early in the growing season, while factors such as host density, herbivory, sun exposure, and host developmental stage influenced disease severity later in the season. We found that soil moisture did not have a clear relationship with disease severity, and that greater host density supported greater disease severity. Our findings suggest that there are many factors influencing fungal disease, and the effects of these factors vary over the course of the growing season. These results have important implications for monitoring and predicting the effects of climate change on plant disease.

Key Words: Alternaria blackspot, *Brassica rapa*, disease, natural populations, water availability.

Plant pathogens are detrimental to plants, substantially reducing crop yield in agricultural systems, as well as influencing species distributions, population dynamics, community structure and evolution in natural systems (Burdon et al. 2006). The disease severity of foliar fungal pathogens is thought to be driven largely by water availability. However, disease dynamics in natural populations are exceedingly complex and factors such as host density, host developmental stage, and other microclimatic variables such as sunlight have been cited (Agrios 2005). Resolving the factors that drive disease severity by conducting multifactorial studies is an ongoing and high priority endeavor for both agricultural and natural plant systems (Pautasso et al. 2012; Thompson et al. 2014).

Since the environment plays a pivotal role in plant disease, recent research has focused on how climate change, including increases in temperature and altered precipitation patterns (IPCC 2014), will affect plant disease dynamics. Generally, climate change is expected to increase infectious disease in many plant ecosystems (Ayres 1984; Paul and Ayres 1987; Coakley et al. 1999; Garrett et al. 2014). Warmer temperatures or increased rainfall, which is predicted in some regions, might increase rates of pathogen growth and the size of vector populations (Patz et al. 2005), and water or temperature stress, which is predicted in other regions, might weaken host immunity (Desprez-Loustau et al. 2006).

In this study, we utilized natural plant populations, growing along a water availability gradient down the West Coast of the United States, to determine the relative importance across the growing season of water availability versus other host and ecological factors (i.e., host density, host developmental stage, herbivory, and sunlight) in determining disease severity of a foliar fungal pathogen. We focused on an economically important plant species, *Brassica rapa* L. (field mustard, Brassicaceae) and its

common foliar fungal disease Alternaria blackspot, which is caused by *Alternaria brassicae* (Berk.) Sacc. (1880) (Conn et al. 1990). This plant-pathogen system was chosen because *B. rapa* is an important crop species cultivated as seven diverse varietals ranging from leaf vegetables to oilseed crops (e.g., bok choi, napa cabbage, oilseed, turnip) (Rakow 2004). *Brassica rapa* is thought to have been brought to the U.S. from Europe for crop cultivation, and has since also formed feral populations with considerable genetic and phenotypic variation including variation in phenology. These populations inhabit a wide variety of environmental conditions, exhibit local adaptation, and are extremely evolvable (Franks et al. 2007). The pathogen, *A. brassicae*, is a common pathogenic sac fungus, which mainly spreads through rain splash, and causes damping off, leaf spots, and defoliation, and infects most cruciferous crops worldwide, severely decreasing crop yield (Rotem 1994; Meena et al. 2010). Many ecological factors affect Alternaria blackspot disease. Host density has been shown to have a positive relationship with disease levels, as has host developmental stage, with older plants being more diseased. In addition, rainfall/moisture has been shown to have large effects, with the highest levels of disease reported in areas of high rainfall (Humpherson-Jones and Phelps 1989; Rotem 1994; Agrios 2005; Meena et al. 2010; Nowicki et al. 2012).

In this study, we used natural populations of *B. rapa* to investigate the influence of multiple factors on disease severity, including time in the growing season, water availability, and spatial factors. Based on field observations and the literature, we formulated three main hypotheses: (1) The factors that drive disease severity differ early in the growing season compared to late in the growing season. (2) Disease severity patterns for this fungus are driven by water availability to a greater extent than other ecological variables (e.g., herbivory and sunlight). This would be supported at a regional scale by greater disease severity in areas with more rain, and at a finer scale by greater disease severity in quadratic plots with greater soil moisture. (3) Disease severity increases with plant density, with larger, denser patches that are closer together supporting greater disease severity. To explore these hypotheses, we collected data on ecological factors and measured Alternaria blackspot disease severity in four populations of *B. rapa* at two time points during the growing season of 2011.

Material And Methods

Field Locations

Field locations were chosen for this study along the California and Oregon coasts to represent a range of environmental variables, including rainfall. Two locations in central California (Muir Beach and Bodega Bay), and two locations in Oregon (Newport and Cape Perpetua) were chosen. All field locations were on the coast and ranged from relatively dry in central CA (~100 cm/yr), to wetter in central Oregon (~200 cm/yr) based on precipitation data from 1961 to 1990 from NOAA Cooperative stations and USDA-NRCS SNOTEL stations (Daly et al. 1994) (Table 1). All locations also had considerable coastal fog and well-drained soil (USDA web soil survey 22 Feb 2012).

Sampling

Sampling was conducted towards the beginning (May) and end (July) of the 2011 *B. rapa* growing season, in a spatially structured manner (as described below), at all field locations, referred to by location codes CA1 for Muir Beach, CA2 for Bodega Bay, OR1 for Newport, and OR2 for Cape Perpetua (Table 1). Each population was mapped using an engineering compass and meter tape. The maps were then digitized and size of host patches and populations were determined in ImageJ (Schneider et al. 2012). To facilitate the analysis of patch size data, which had a few extreme outliers, patches were categorized into four size bins. Population size (total number of individuals) was estimated by multiplying density (see below) by patch size.

To explore the variation in Alternaria blackspot disease severity, we collected disease severity data from at least 10 quadrats in each population (see Table 2 for sample sizes), selected at random, from within host patches. Disease severity was measured as the percent of a quadrat with host tissue displaying symptoms, and was assessed visually by two independent researchers and averaged. Alternaria blackspot symptoms were clearly identifiable in the field, characterized by brown necrotic spots surrounded by chlorosis (Fig. 1). To verify that these symptoms were due to infection with *A. brassicae*, plant tissue was collected from all field locations for identification by spore morphology by the Plant Pathology Center at Oregon State University. Total pathogen load for a population was estimated by multiplying disease severity by patch size and summing across all patches.

To verify that the visual disease assessment was reliable, we conducted a greenhouse study ($n = 576$) in which we inoculated *B. rapa* accessions with field-collected *A. brassicae* under controlled conditions and measured resulting disease severity (O'Hara et al. in review). For this study, fresh spores were generated from field collected single spores by growing on carrot dextrose agar plates for a week, followed by carrot agar plates for another week (work permitted under APHIS license #P526P-11-00130). Fresh spores were strained through gauze to remove hyphae, and adjusted to a concentration of 1×10^6 spores/mL in distilled water and 0.05% Tween. Ten μL of a fresh spore solution were applied to 2-wk-old leaves that had been wounded with a pipette tip. Control plants were wounded and treated with 10

TABLE 1. Characterization of field locations including precipitation and soil type. Precipitation data averaged over 10 yr was acquired from NOAA Cooperative stations and USDA-NRCS SNOTEL stations. Soil data was obtained from the USDA (USDA web soil survey Feb 22, 2012).

Location	Location info	ID	Region	Precipitation (cm/yr)	Soil type	Soil drainage
Muir Beach	37.863824 N, −122.573960 W	CA1	Central CA	101	Cronkhite (40%) and Barnabe (30%)	Moderately well-drained
Bodega Bay	38.313507 N, −123.061042 W	CA2	Central CA	101	Baywood loamy sand (85%)	Very well-drained
Newport	44.625044 N, −124.062710 W	OR1	Central OR	203	Neskowin-Salander silt loams (90%)	Well-drained
Cape Perpetua	44.282632 N, −124.109385 W	OR2	Central OR	203	Neskowin-Salander silt loams (90%)	Well-drained

FIG. 1. Characteristic Alternaria blackspot lesions on a *B. rapa* leaf. Photo by N. O'Hara.

μL of 0.05% Tween. Following inoculation, plants were kept at 90% humidity for 3 d and then placed at ambient humidity. The disease severities of the inoculated leaves for a subset of randomly selected plants (n = 277), including both control and infected, were scored 21 d post inoculation, using a visual index which ranged from 1 to 10 based on the amount of chlorosis and necrosis (Buchwald and Green 1992). Disease severity scores were independently verified by two researchers. Infected leaves displayed a highly significant increase in disease severity (one-way ANOVA comparing inoculated versus control plants: inoculated mean = 4.62 (±0.20), non-inoculated mean = 3.64 (±0.16), $F_{1,117} = 41.34$, $P < 0.0001$), demonstrating that the spores that were isolated from the field were also causing the disease under greenhouse conditions.

We also quantitatively validated our visual index with a detached leaf assay (n = 50). Prior to inoculation, fully expanded leaves were detached from plants and placed in petri dishes on filter paper pre-moistened with distilled water and inoculated (as described above). Four days after inoculation, leaves were cleared, stained, and visualized through a microscope. Leaves were cleared by shaking overnight in a 1:3 acetic acid to ethanol solution, followed by a 1:5:1 acetic acid, ethanol, and glycerol solution. Leaves were rinsed in water, boiled in a solution of 5% Parker black ink and distilled white vinegar, and destained in vinegar acidified water, followed by a 5% vinegar wash (Vierheilig et al. 1998). Invading spores were counted at 100× magnification. Infected, stained leaves had an average of 9.5 (±8.7) spores per wound, while uninfected plants were free of symptoms and spores. We found that spore counts were correlated with the disease severity scores (Pearson correlation: r = 0.784, P = 0.0002).

To explore how ecological variables affect disease severity, we also collected the following field-data from the same patches for which disease severity data

TABLE 2. Characterization of populations by area. Area of population determined by mapping population, scanning and measuring in ImageJ. Host stems per m^2 and disease severity were calculated by averaging across quadrats at each location at the late time point. Standard deviations (SD) are given in parentheses.

Location	# of quadrats sampled	Total area of *B. rapa* (m^2)	# of host stems per m^2 (±SD)	Disease severity at late time (±SD)
CA1	18 early; 12 late	2,502.40	13.5 (±7.4)	35.2% (±32.7)
CA2	12 early; 11 late	1,415.90	20.6 (±15.3)	21.9% (±21.1)
OR1	13 early; 13 late	8,081.80	7.8 (±4.7)	26.5% (±21.1)
OR2	10 early; 10 late	139.4	4.3 (±2.1)	28.0% (±13.9)

was collected: host density (percent cover in each quadrat assessed visually), height of host (measured and averaged height in each quadrat), developmental stage of host (most common developmental stage in each quadrat classified as either young, flowering, fruiting, or senescing assessed visually), herbivory, (damage visually assessed), level of sun (visual assessment of amount of shade), and soil moisture in each quadrat (measured by TDR and verified gravimetrically as described below). Visual assessments were independently collected by two researchers and averaged. Distance between host patches was a spatial factor that was used in the analysis, and was determined by measuring the area of neighboring patches that fell within 3 m of the edge of each patch. We chose a 3 m distance because *Alternaria brassicicola*, a closely related fungus with similar means of dispersal, spreads by water splash primarily within that distance (Chen et al. 2003). Similar to patch size, distance between host patches had a few extreme outliers, so we grouped into five bins to facilitate our analysis: less than 12 m^2 of neighboring patches fell within 3 m of the patch of interest, 12–15 m^2, 15–18 m^2, 18–20 m^2, and >20 m^2.

To obtain measurements of soil moisture in each quadrat, we used a Field Scout TDR 100 Soil Moisture Meter (Spectrum Technologies, Inc.) with 10 cm probes. TDR readings were verified gravimetrically using a 25 cm soil core (Black 1965). There was a highly significant positive correlation between soil probe and gravimetric measurements so the soil probe, for which we had a more complete dataset, was used in all analyses (Pearson correlation: $r = 0.63$, $P < 0.0001$). All field data and soil moisture data were collected on the same day at each location. Because the time since the last rainfall varied between field locations, soil moisture measurements were not used to directly compare between populations, instead these data were analyzed using a nested design (see Analysis).

Analysis

To determine how disease severity was influenced by water availability and other factors over the growing season, two generalized linear mixed effects models (GLMMs) with location as a random effect were constructed, one for the early season collection, and one for the late. We chose to analyze our data using a mixed model because our experimental design was hierarchically nested by location, with multiple predictor variables collected from each location, so they were not entirely independent from each other. Separate early and late models were constructed because disease severity patterns and explanatory variables varied greatly between time points, and we were interested in exploring these patterns individually. Explanatory variables in the models included host density, soil moisture, host height, herbivory, and level of sun. Models were built to only include variables with strong literature support as being important factors in driving disease severity. For variables, such as host coverage and number of host stems per meter, which were highly correlated, we only included the variable that had the most biological support for being a possible explanatory variable; in this way did not include variables that were highly correlated. We used likelihood ratio tests to determine significance and degrees of freedom, by comparing models with and without each factor (Winter 2013). An FDR correction was conducted and uncorrected and corrected P values are reported. All analyses were conducted using R 3.0.1 (R Core Team 2013). Preceding analysis, data were transformed to meet model assumptions (Supp. Table 1). A Shapiro-Wilk Normality test was performed on all data following transformation to verify a normal distribution.

After fitting the full nested models, we were interested in further exploring the patterns of disease severity within each site at each time point. To determine how disease severity varied over time across locations (Hypotheses 1, 2), we conducted a 2-way ANOVA with location and time as fixed effects, and transformed disease severity data (Supp. Table 1) as the independent variable. A Tukey HSD post-hoc analysis was used to determine which locations were significantly different from each other. To determine if disease severity varied with soil moisture within locations (Hypothesis 2), we regressed disease severity on soil moisture for each location using transformed TDR values (Supp. Table 1), with early and late time points analyzed separately. An FDR correction was conducted and uncorrected and corrected P values are reported. To determine if disease severity varied with host density within locations (Hypothesis 3), we regressed disease severity on host density (coverage) for each location using transformed values (Supp. Table 1), with early and late time points analyzed separately, followed by an

FDR correction (both uncorrected and corrected P values are reported). We chose to focus on host coverage over patch size and distance between neighbors as a measure of host density in this analysis because coverage was at an appropriate scale for dispersion of the fungus (Humpherson-Jones and Phelps 1989; Rotem 1994; Nowicki et al. 2012).

Results

Overall we found that Alternaria blackspot symptoms were widespread (Table 2), with 22.2 % of *B. rapa* individuals showing symptoms across the season. Disease severity varied widely and ranged from 21.9% of plants infected at Bodega Bay (CA2) to 35.2% infected at Muir Beach (CA1) at the late time point. Newport (OR1), which had by far the largest population of *B. rapa* (~8,082 m^2), also had by far the largest pathogen load with ~1,681 m^2 of host vegetation showing disease symptoms. We found that multiple ecological factors are statistically associated with increased disease severity of *A. brassicae* infection.

Different Factors Influence Disease Early and Late in the Growing Season

Addressing Hypothesis 1, we found that the factors influencing disease early versus late in the season differed. The early time point model explained 29.6% of the variation in disease severity, although no individual factors in the model were significantly correlated with disease severity. The late season model explained 58.3% of the disease severity variation observed. For this model we found that host density (% cover of host) significantly affected disease severity, with denser areas supporting greater disease severity (Beta = 0.009 ± 0.004; Table 3). We also found that herbivory and disease severity were negatively correlated and plants with greater herbivory damage displaying lower disease severity (Beta = –0.598 ± 0.161; Table 3). We found that size of host patch had a significant effect on disease severity with the largest patches having the greatest disease severity (Beta = 0.341 ± 0.225; Table 3). Level of sun had a significant effect on disease severity with quadrats with the lowest and highest levels of sun having the greatest disease severity (Beta lowest = 0.526 ± 0.206; Beta highest = 0.506 ± 0.109; Table 3). Distance between patches had a significant effect on disease severity with the very isolated patches having the greatest disease severity (Beta = 0.769 ± 0.281; Table 3). We also found that host developmental stage and disease severity were positively correlated with older plants displaying greater severity (Beta = 5.410 ± 1.991; Table 3).

We also found considerable variation in disease severity between the early and late time points. Disease severity was significantly higher at the later time point (2-way ANOVA and a post hoc Tukey's HSD test: $F_{1,91} = 9.8$, $P = 0.003$), with an increase in average disease severity from 17.1% tissue infected at the early time point to 28.0% at the later time point (Fig. 2A).

TABLE 3. Results of GLMM models for early and late season exploring the factors affecting disease severity. Df and P values were calculated based on likelihood ratio tests (see text). Disease effect sizes (Beta estimates + SE) reported in text for significant variables. AIC early model = 52.7, late model = 21.7. Significant results are bolded.

	DF	Chisq	P value	FDR corrected P value
Early model				
Host coverage	1	0.388	0.534	0.712
Soil moisture (TDR)	1	0.012	0.914	0.983
Height	1	0.636	0.425	0.68
Herbivory	1	0.786	0.375	0.68
Patch size	3	4.831	0.185	0.68
Level sun	3	3.279	0.351	0.68
Distance between patches	3	7.034	0.071	0.568
Stage	1	0	0.983	0.983
Late Model				
Host coverage	**1**	**6.069**	**0.014**	**0.028**
Soil moisture (TDR)	1	0.353	0.553	0.553
Height	1	1.714	0.191	0.218
Herbivory	**1**	**11.671**	**0.0006**	**0.002**
Patch size	**3**	**7.502**	**0.024**	**0.032**
Level sun	**3**	**17.846**	**0.0001**	**0.0001**
Distance between patches	**3**	**7.808**	**0.02**	**0.032**
Stage	**1**	**6.247**	**0.012**	**0.028**
Error	19			

Soil Moisture, Host Coverage and Disease Severity

To address Hypothesis 2, that moisture availability affects disease severity, we examined these variables at a regional scale by comparing the sites, which had different amounts of rainfall, and at a local scale, by regressing disease severity on soil moisture within locations. While there was some variation among locations in rainfall, soil moisture, and disease severity (Tables 1 and 2), location and the interaction between time and location did not have a significant effect on disease severity (2-way ANOVA; Fig. 2B). At the local scale, the relationship between soil moisture and disease severity varied across field locations and time points and was not significant for any of these locations following an FDR correction. However, we saw a trend for the early Newport (OR1) time point and a significant relationship for the early Cape Perpetua (OR2) time point before the multiple test correction (Table 4). For Newport (OR1), soil moisture explained 24.4% of the variation and the relationship was negative, while for Cape Perpetua (OR2), soil moisture explained 54.7% of disease variation and the relationship was positive.

To address Hypothesis 3, that host cover affects disease severity, host cover was included as an explanatory variable in the GLMM model in

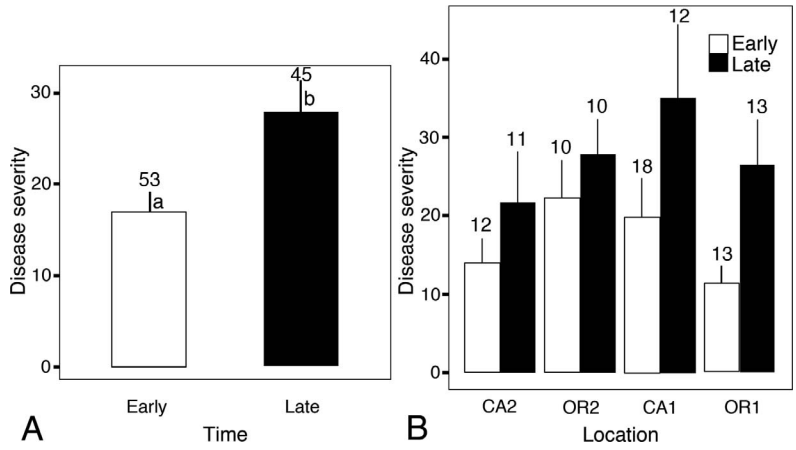

FIG. 2. Results of 2-way ANOVA with disease severity as independent variable and time and location as factors, followed by a post hoc Tukey's HSD test. Analysis conducted on log transformed disease severity data with untransformed data shown with (±SEM). A. Disease severity was significantly higher at the later time point, as indicated by the letters over the bars. B. Location at both early and late time points had no significant effect on disease severity. Sample sizes are shown above each bar.

addition to being explored within each site by regressing disease severity on coverage at each time point separately. In our GLMM analysis, we found that host cover had a significant effect on disease severity, with more dense areas supporting greater severity (reported above). According to the results from our within-location regressions, this relationship varied across field locations and time points and was significant for the CA2 location late in the season before a multiple test correction, but was only a trend after the correction (Table 5). Coverage was not significant for other locations at either time point (Table 5).

DISCUSSION

In this study, we found that disease severity varied widely with time, and several environmental and host factors. The models we created from data on these factors could explain approximately one-third to two-thirds of the variation in disease severity at two time points, respectively. Although this study was observational and does not definitively establish causation, the results provide important information on how a number of ecological factors vary with the severity of plant fungal disease.

Disease Severity Over Time

In testing Hypothesis 1, the effect of time on disease severity, we found that disease severity was significantly worse later in the season (Fig. 2), and that host developmental stage was significantly correlated with disease severity at the later time point (Table 3), a pattern common in disease studies (Madden and Hughes 1995). Studies on *Alternaria* fungal infection in *Brassica* species show that older plant tissue exhibits higher disease severity (Mridha and Wheeler 1993) and studies have shown that increases in disease severity occur later in the season due to weakened defenses at later developmental stages of the host plant (Rotem 1994). An alternative explanation is that the increase in severity over time is due to the prolonged length of time that the fungus has to establish and spread. While we cannot distinguish between these two explanations, we did find support for the role of developmental stage.

TABLE 4. Disease severity regressed on soil moisture taken by TDR. Regression was run on transformed data (Supplemental Table 1). Significant results (according to uncorrected or corrected P value) are bolded.

Location	Time	Sample size	Beta (SE)	T value	P value	FDR corr. P value	Multiple R^2
CA1	Early	18	−0.02 (0.02)	−1.08	0.297	0.594	0.067
	Late	12	−0.02 (0.02)	−1.26	0.237	0.594	0.137
CA2	Early	12	0.01 (0.01)	0.78	0.452	0.723	0.058
	Late	11	−0.01 (0.02)	−0.35	0.734	0.839	0.013
OR1	Early	13	−0.02 (0.01)	−1.89	0.086	0.344	0.244
	Late	13	0.002 (0.01)	0.17	0.866	0.866	0.003
OR2	**Early**	**10**	**0.02 (0.01)**	**3.11**	**0.015**	**0.12**	**0.547**
	Late	10	0.01 (0.02)	0.47	0.652	0.839	0.027

TABLE 5. Disease severity regressed on host coverage. Regression was run on transformed data (Supp. Table 1). Significant results (according to uncorrected or corrected P value) are bolded.

Location	Time	Sample size	Beta (SE)	T value	P value	FDR corr. P value	Multiple R^2
CA1	Early	18	−0.0003 (0.004)	−0.07	0.948	0.948	0.0003
	Late	12	−0.02 (0.01)	−1.83	0.097	0.194	0.251
CA2	Early	12	0.003 (0.01)	0.51	0.622	0.829	0.025
	Late	**11**	**0.02 (0.01)**	**3.36**	**0.008**	**0.064**	**0.556**
OR1	Early	13	0.01 (0.004)	1.9	0.084	0.194	0.248
	Late	13	0.002 (0.01)	0.26	0.797	0.911	0.006
OR2	Early	10	0.01 (0.01)	1.39	0.201	0.322	0.195
	Late	10	0.02 (0.01)	2.15	0.064	0.194	0.37

Our two largest field locations, Muir Beach (CA1) and Newport (OR1), had the greatest area of infected tissue, particularly late in the season (Table 2). This suggests that a larger host population might support a greater amount of infection, however we would need many more locations included in the study to assess this systematically. This could be important when considering that feral crop plants, such as *B. rapa*, can act as a disease reservoir for closely related crops, such as canola (Burdon and Thrall 2008).

Although we cannot generalize across years because our data represents a single year, we can infer differences between early versus late season for the year we sampled. We found considerable differences in our early versus late season models. For the early time point we did not find a significant effect of any variables on disease severity. It is possible that during establishment of the fungus the factors measured do not have a major influence, or that the variation in disease was so low that we did not have enough power to detect associations. Our late season model had a much better goodness of fit and was able to explain about twice the variation in disease severity as the early model (~60%). Using this model we found that host density, host developmental stage, herbivory and level of sun all had significant effects on disease.

Overall, we found support for Hypothesis 1, which states that disease varies across the season and different variables mediate disease early versus late in the growing season. Timing could be an important consideration in conducting plant pathogen studies in natural populations.

Soil Moisture and Disease Severity

In exploring Hypothesis 2, we expected to see a positive correlation between disease severity and soil moisture in agreement with many disease studies, due to the fact that moisture is needed for the dispersal and growth of these fungal spores. Alternaria species have been shown to need high relative humidity in order to germinate, as well as water splash to disperse (Humpherson-Jones and Phelps 1989; Rotem 1994; Nowicki et al. 2012). Interestingly, soil moisture had no effect on disease severity either early or late in the full models, however we did see a positive association using linear regression early in the growing season for one Oregon location (OR2), before a multiple test correction. This suggests that the relationship between moisture and disease depends on factors that vary among locations, and that water availability might not be driving disease severity in this plant pathogen system in many locations. Additionally, at the regional scale, we did not find greater disease in the wetter Oregon locations than in the drier Californian locations (Fig 2), although we would need to study more locations to test this.

Overall, we did not find support for Hypothesis 2. Instead, we found that other ecological variables (discussed below) played a greater role in influencing disease severity than water availability.

Host Density and Disease Severity

In exploring Hypothesis 3, we found a significant positive correlation between disease severity and host density (% cover) (Tables 3 and 5), consistent with literature reports that increased host density contributes to pathogen transmission (Burdon and Chilvers 1982). The density of the host did not have a significant effect in the early GLMM model, which suggests that density plays a greater role once infection has been established and is spreading, as one would expect later in the season.

Distance between patches also had a significant effect on disease severity in the late model, with the greatest disease severity found in patches that were the most isolated (Table 3), which is contrary to our expectations. We do not have a good explanation for this pattern, but it is possible that other factors correlated to patch distance, such as sun exposure or herbivory, could play a role.

Overall, we found support for Hypothesis 3, that host density played a role in disease severity with greater density supporting greater severity.

Other Ecological Factors and Disease Severity

The level of sun exposure had a significant effect on disease severity at the later time point (Table 3), and severity was highest for plants that received extreme amounts of sunlight (either very shaded or very high levels of sun). It is possible that the increase

in disease severity under low sun exposure is due to the inverse relationship between moisture and sun exposure, with a high level of moisture at the microclimate level supporting spore germination and spread. It is also possible that the increase in disease severity under high levels of sun exposure is due to an excess of light, which can stress plants and weaken plant defenses (Agrios 2005), however this would need to be tested experimentally in this system.

We found that herbivory had a highly significant and negative correlation with disease severity at the later time point (Table 3). This is contrary to many studies that have found that disease severity and herbivory are positively correlated (Kennedy and Barbour 1992; Simms and Rausher 1993). However, it is important to note that the relationship between disease severity and herbivore damage is complex and is mediated by many factors, including defensive compounds produced by the plant and food preference of the herbivores (Taiz and Zeiger 2006). In agreement with our finding, another study in *B. rapa*, exploring the relationship between herbivory by the gall midge and Alternaria fungal infection found that plants that had the highest level of fungal infection were preyed upon the least, which might have been due to food choice (Nakamura et al. 1995).

Conclusions

Over the course of a growing season the factors that drove disease severity of this foliar fungal pathogen varied, with no clear variables driving disease earlier in the season and host density, host developmental stage, level of sun exposure, and herbivory playing a role later in the season, when disease severity increased. This study illustrates that factors influencing disease severity in this natural plant pathogen system are complex, and that the timing of data collection and the study of multiple variables can be of great importance in understanding disease dynamics. Such complexity will have to be accounted for when developing models to predict the effects of environmental changes, such as climate change.

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