THESIS

INTRASPECIFIC VARIATION IN INVASION TRAITS OF THE EXOTIC GRASS *AEGILOPS TRIUNCIALIS*

Submitted by

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ABSTRACT

INTRASPECIFIC VARIATION IN INVASION TRAITS OF THE EXOTIC GRASS *AEGILOPS TRIUNCIALIS*

Intraspecific variation is critical for adaptation to novel environments, but is often ignored in studies of invasive species. In cases where intraspecific variation is considered, it is most often to compare differences between populations in the home versus invaded ranges, rather than within the invaded range itself. In my thesis I investigated whether the two most invasive lineages of the exotic grass, *Aegilops triuncialis*, differ in traits important to invasion-ability. Invasive species can influence nutrient cycling and availability for subsequent generations. Soil nutrient availability is mediated by activity of the soil microbial community, which is influenced by nearby roots. Thus, I tested whether lineages differ in rhizosphere activity that leads to nutrient mineralization in the soil. Additionally, as invasive species are well known to out-compete native species, I tested whether invasive lineages differ in their response to competition by three common grassland species. I found that two lineages ('East' and 'West') differed in rhizosphere community activity, in reproductive traits, as well as in competitive abilities. The East lineage showed increases in rhizospheric enzyme activity throughout and after the growing season, and produced fewer, but larger, seeds. The West lineage showed no change in rhizospheric enzyme activity throughout the growing season, and produced more, but smaller seeds. Additionally, we found a significant negative correlation between rhizospheric enzyme activity early in growth and average seed weight produced, showcasing that plant-soil microbe relationships have the potential to evolve via natural or artificial selection. In terms of response to competition, the East was most resilient in its competitive response to common grassland species by flowering at the same time and producing the same number of spikelets, regardless of whether it was in competition or grown alone. The West flowered later and produced fewer spikelets with decreased biomass production when in heavy competition with the same grassland species.

The results of my work not only highlight the need to incorporate intraspecific variation into studies on invasive species within the invaded range, but demonstrate that these widely distributed lineages can have different impacts on their communities and interact with neighbor species differently. Variation in the activity of rhizospheric microbial communities that lead to nutrient mineralization means lineages may differentially affect available nutrients in the soils they invade, and pose different problems for restoration efforts. The variation in response to competition by common grassland species may mean that alternative mechanisms for control and eradication of *Ae. triuncialis* are warranted depending on the genetic lineage present. I underline the need to include intraspecific variation in future studies, particularly concerning the impact of invasive species in the novel range.

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INTRODUCTION

Invasive species cause major ecological and economic impacts in ecosystems worldwide. These species alter fire and nutrient cycling dynamics, reduce native species diversity, impact crop yields and cost billions of dollars annually through control and eradication efforts in the areas they invade. The magnitude of these impacts merits in-depth research on the causes, processes, and consequences of invasion. Yet, despite decades of focused research, effective management is hampered by a lack of both specific and broadly-applicable knowledge on mechanisms of invasion.

Invasive species inherently face novel biotic and abiotic conditions when introduced to a new range. Adaptation to such novel environments requires genetic variation in the introduced species. Invasive species, however, often experience genetic bottlenecks during the invasion process. Consequently, the explosive success experienced by invasive species is counterintuitive. This may be explained by additional introduction events that add genetic diversity. If an original bottleneck is followed by multiple introductions, this would lead to genetic variation in the novel range upon which selection can act. Consequently, successful lineages from separate introduction events will likely have different traits that confer invasive ability, such as those linked to reproductive success or competitive abilities. Despite the potential for this genetic variation among populations to influence traits in the invaded range, most work often investigates distinctions between the native and invaded ranges, rather than genetic variation within and among invading populations.

In order to test questions of intraspecific variation within the novel environment I employed the exotic grass, *Aegilops triuncialis*. *Ae. triuncialis* is a federally-listed noxious weed in both California and southern Oregon, where it invades arid and semi-arid grassland communities. Meimberg et al (2010) found that all sampled populations within the invaded range identified with one of three invasive lineages, which is likely the result of three separate invasion events. In these subsequent works I will focus on the two most common lineages in the invaded range, referred to as ‘East’ and ‘West.’ In addition to being a

successful and tenacious invader, the genetic structuring of invasive populations of *Ae. triuncialis* offers the ideal opportunity to study the role of intraspecific variation in colonizing novel environments.

Invasive species can have large impacts on nutrient cycling and availability of resources. In addition to affecting nutrient cycling through litter quality and phenology, plants may also influence nutrient availability through the activity of the soil microbial community in their rhizospheres. The activity of the soil microbial community mediates the abundance of plant-available nutrients in the soil. The question of how invasive species affect resident soil communities has been previously investigated with regard to taxonomic differences in soil microbial communities between invaded and uninvaded plots. Batten et al. (2006) found that the genetic composition of soil microbial communities from stands of *Ae. triuncialis* differed from nearby uninvaded plots. Whether such differences in community composition also lead to differences in community activity remains unclear as there is much functional redundancy among taxa in soil microbial communities. Furthermore, as only a single lineage is present at the study site, this experiment did not consider intraspecific variation within *Ae. triuncialis* nor if invasive lineages differ in their impacts on the soil microbial community. In Chapter 1, I use multiple populations of the East and West lineages of *Ae. triuncialis* to determine if the functions of soil microbial communities differ between lineages. I investigate whether function changes over time, and if the stimulation of soil microbial activity by the resident plant impacts fitness later in growth.

In Chapter 2, I extend my research of intraspecific variation in invasion-related traits to investigate differences among lineages for competitive ability. Invasive species surpass comparable native species in various metrics of fitness, and can consequently use superior competitive abilities as a mechanism of invasion. Competitive abilities are known to differ among individuals of the same species, and many studies have looked at differences in competitive abilities between home and invaded ranges. However, whether invasive lineages differ in their competitive ability within the invaded range remains relatively unexplored. I tested whether populations of the East and West lineages of *Ae. triuncialis* differed in their response to competition with three common and co-occurring grassland species. To supplement this study

on competitive abilities, I used herbaria specimens representing initial introductions into counties throughout northern California to investigate whether current distributions of lineages are representative of initial introductions. If additional lineages were introduced into California throughout the invasion process of *Ae. triuncialis* but are not currently widely distributed, this would indicate that the current three lineages have greater fitness than other introduced lineages in the invaded range.

The investigation of intraspecific variation within the invaded range and the impact on important invasion-related traits has been largely ignored in invasive species literature. Our results indicate that understanding the impact of genetic lineages on invasion traits may lead to more targeted and effective eradication and restoration efforts for a given area. My results act as a stepping stone towards understanding that genetic lineage, rather than species identity, may play a more important role in invasive species impacts in the invaded range than previously thought.

CHAPTER 1: IMPACTS OF GENETIC LINEAGE ON SOIL MICROBIAL COMMUNITY FUNCTION

INTRODUCTION

Plant-soil relationships are recognized as integral to plant health, and interactions between plants and their soil microbial communities can directly impact plant growth and fitness. Recent work has illuminated the importance of these interactions (Berendsen et al. 2012, van der Putten et al. 2013) but also that we have just scratched the surface, which highlights the importance of deeper investigation into this field. The abundance of plant soil interactions occur in the rhizosphere, long defined as the area of the soil that is influenced by plant roots (Hiltner 1904, Hartmann et al. 2008). Composition and function of rhizosphere communities can differ among plant species growing in the same soil (Ibekwe and Kennedy 1998, Kourtev et al. 2002), and differences in genetic composition of rhizosphere communities are seen among plant genotypes (Micallef et al. 2009, Weinert et al. 2011, Velmala et al. 2013). Consequently, there is now great interest to extend the relationship of plant taxonomy and rhizosphere community function to the genotype level. Recent work has begun to tackle the role of genotype in plant associations with their rhizosphere communities, with Peiffer et al. (2013) demonstrating the importance of plant genotype on genetic composition of the rhizosphere community during flowering of maize plants. However, as there is considerable functional redundancy among soil microbial taxa, this raises the question of whether rhizosphere communities differ in their function, as well as genetic composition, among genotypes. Furthermore, genetic composition of soil microbial communities can act as drivers of plant reproductive timing and output (Lau and Lennon 2012, Wagner et al. 2014), but whether plant-soil microbe relationships assemble neutrally or are under selection remains relatively unexplored.

The availability of nitrogen (N) – the element which most often limits terrestrial primary productivity (Chapin et al. 2002, LeBauer and Treseder 2008) – is mediated by soil microbial activity (Richardson et al. 2009). While most soil N is contained in complex organic polymers that are unavailable to plants,

extracellular enzymes produced by soil microbes depolymerize this organic matter into monomers that can be mineralized into inorganic N for plant uptake (Schimel and Bennett 2004). Plants may be able to influence microbial production of N-degrading enzymes and subsequent mineralization rates through root exudation and other mechanisms (Phillips et al. 2011). If genotypes differ in the ability to stimulate enzyme production by their root-associated microbial communities, then these differences may influence their ability to compete for resources with neighboring individuals. From this perspective, plant impacts on their microbial communities can be viewed as a functional trait that influences plant fitness. Therefore, this trait is under selection and may play a role in adaptation and range expansion when this trait is heritable.

We investigated how plant genetic identity and development interact to affect the potential activity of soil extracellular enzymes that degrade N-rich chitin and peptides. We take a novel approach to directly assay variation in functional ability of the rhizosphere caused by both plant genotype and plant development. This interaction of plant genotype and development stage remains relatively unexplored despite that both factors influence rhizosphere communities (Micallef et al. 2009, Zancarini et al. 2013). We use *Aegilops triuncialis*, a selfing invasive annual grass which is characterized by most populations in the invaded range identifying with one of three invasive genotypes. This genetic structuring of invasive populations provides a unique opportunity to investigate genetic variation in plant-rhizosphere interactions through the plant functional trait of microbial enzyme stimulation, and the impact of these strategies on reproductive output and fitness.

MATERIALS AND METHODS

Study species and seed source

Aegilops triuncialis is a selfing, annual grass native to Eurasia, which invades arid and semi-arid grasslands throughout northern California and southern Oregon (USDA 2014). Meimberg et al. (2010) sampled populations throughout California and identified three multi-locus genotypes (East, West and

South): the East and West genotypes occupy much larger areas than the South (Meimberg et al. 2010). We used offspring from a common garden experiment (Espeland and Rice 2012) which included 3 East and 3 West populations (defined as populations 101, 104, 106, 123, 124 and 125 in (Meimberg et al. 2010)). Full-siblings of individuals (known as 'families') were used as replicates at each destructive harvest (see details below).

Experimental design

Soil was collected from a previously uninvaded, but suitable habitat from the University of California-Davis Donald and Sylvia McLaughlin Natural Reserve in Lower Lake, California, USA. These soils are derived from Great Valley Sequence parent material and are classified as thermic Typic Haploxeralfs. Soil was ground to break up large pieces of clay-loam, then sieved through a 3 mm sieve and homogenized using a cement mixer. In soil was poured into 164 mL cone-tainers (Stuewe and Sons, Tangent, OR, USA) until two-thirds full. The remainder of the soil for the cone-tainer was filtered through a 1.5 mm sieve to ensure proper soil characteristics for germination. Two seeds from the same seed family (i.e. full-sibling individuals) within a population of a particular genotype were seeded into 360 cone-tainers in May 2012 (two genotypes, each with three populations, and 10 families per population that each had two full-sib individuals to act as replicates at each of three harvest stages). An additional 18 pots were filled with soil but remained unplanted to account for changes in the enzymatic activity of the soil community in the absence of plants. Pots were placed on a mist bench at planting and were thinned down to a single individual per pot after germination (removing the smallest).We moved the pots to a growth chamber after germination in order to simulate the day length and temperature conditions found in their invasive range in California (Appendix 1, Table A1.1).

We destructively harvested individuals during the course of development in order to measure rhizosphere enzyme activity with fluorometric enzyme assays. Plants were harvested during the tillering stage when production is mostly vegetative (Day 150 after planting, n=65), during the flowering stage when spikelets

were fully formed but still green (Day 200, n=81) as well as post senescence (Day 260, n=102). Post-senescence harvests allowed all individuals to senesce (i.e. spikelets easily released from stalks with perturbation) at which point all pots were watered to soil saturation then left with standing biomass for three weeks in the growth chamber before plants were harvested. This post-senescence harvest was performed in order to test persistent effects of each genotype on the soil communities. Simultaneous enzyme assays of the control soils were performed during each harvest to determine natural variations in enzyme activity in the soil (n = 3, 3 and 12, respectively). For all harvest periods aboveground plant biomass was clipped and oven dried at 60° C for 48 hours and weighed. After soil was collected from the rhizosphere of the roots and assayed (see below), the roots were cleaned, oven dried and weighed (considerable root decomposition prevented collection from post-senescence soils).

Enzyme assays

Soils harvested from the rhizosphere of individual plants and from control pots with no plants were collected and assayed within 24 hours of harvest. Two hydrolytic soil enzymes involved in the degradation of protein (leucine aminopeptidase, 'LAP') and chitin (N-acetyl-β-Glucosaminidase, 'NAG') were assayed using standard fluorometric techniques (Steinweg et al. 2012, Bell et al. 2013). A soil slurry was made by blending 91 mL of 50 mM Tris Buffer and 2.75 g of soil for 1 minute. An aliquot of 800 µL of slurry was added to wells in each of three 96 deep well (2 mL) plates. Two-hundred microliters of 200 µM fluorometric substrate for each enzyme, mentioned above, was added to the wells in Plate 1 with one substrate per well. Plates 2 and 3 had dilutions of 4-methylumbellfferone or 7-amino-4-methylcoumarin added, respectively, to each well to create a standard curve (for NAG and LAP, respectively) to be used for analysis. The final row in each column served as negative controls with no liquid in the well. Plates were sealed, inverted and incubated in the dark for 3 hours at 25° C. Plates were then centrifuged and 250 µL of the solution was pipetted onto a black, flat-bottomed 96-well plate and fluorescence was measured with a Tecan Infinite M200 plate reader using excitation wavelength 365 and emission wavelength 450.

Standard curves were used to convert the fluorescent readings to sample $nmols^{-1}$ g dry $soil^{-1}$ $hour^{-1}$, which we define as the activity of each enzyme in each soil sample.

An index of total N-degrading enzyme activity in invaded soil was calculated by the sum of the enzyme activities of LAP and NAG. The stimulation of N-degrading enzyme activities by each plant ('plant-stimulated enzyme activity') was determined as the total N enzyme activity of control soil (see above) subtracted from the total N enzyme activity of rhizosphere soil for each sample. Relative total ability of the soil microbial community (activity of plant rhizosphere soil divided by control soil) was investigated and showed similar patterns to plant-stimulated enzyme activity.

Carbon and Nitrogen Leaf Tissue concentration

Dried leaf material from the tillering harvest (n = 11) was placed into individual 2 mL micro-centrifuge tubes with three 1.99 mm ball bearings and ground in a modified paint shaker until a fine powder was reached. Around 2 mg of ground tissue was placed into tin capsules and combusted in a Carlo-Erba NA 1500 elemental analyzer (Milano, IT) to measure C and N content of the tissue.

Statistical analysis

Plant-stimulated enzyme activity was analyzed as a function of harvest time, genotype and their interaction to assess the functional ability of the soil through time relative to each genotype. Population was included as a random effect.

In order to test the relationship between seed number and weight among genotypes, we analyzed mean seed weight as a function of total seed number produced for each genotype. Additionally, we used a Pearson correlation to test the relationship of enzyme activity at the tillering stage on seed production to assess the extent activity at early developmental stages relates to later reproductive output. In order to test whether differences in root biomass were associated with differences in rhizosphere enzyme activity we

used a Pearson correlation to test the relationship of root mass fraction (root biomass/total biomass) and plant-stimulated enzyme activity at tillering and flowering.

To quantify variation in vegetative output among genotypes, differences in belowground biomass, aboveground biomass and root-to-shoot ratios were separately analyzed at each harvest stage as a function of genotype. The same model was used to test differences in percent nitrogen in the leaf tissue at the tillering stage.

RESULTS

We grew multiple populations of the two most invasive genotypes of *Ae. triuncialis* (referred to as 'East' and 'West') in a greenhouse and harvested cohorts of three developmental stages to assess potential soil enzyme activities. At each stage we quantified the ability of the rhizosphere soil community to break down complex N-compounds, and studied the phenotypic status of the plant including biomass, flowering time and reproductive output. We assessed the rhizosphere soil three weeks post-senescence to determine potential differences among genotypes in their soil community persisting after the growing season.

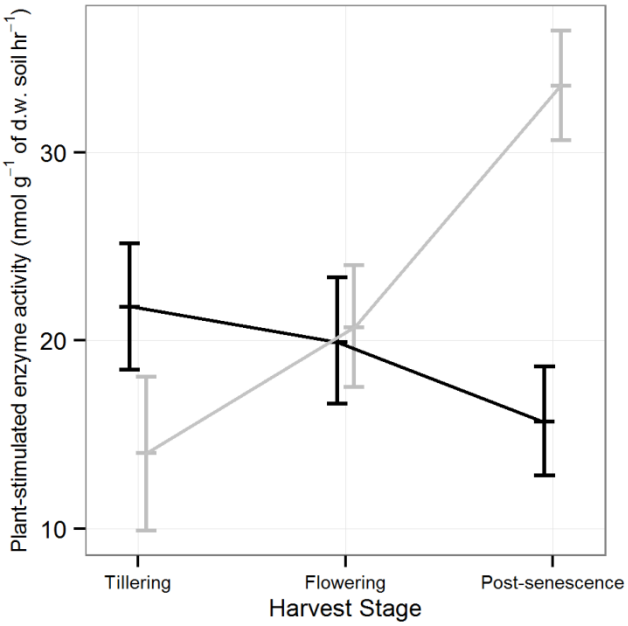

Figure 1.1: Average plant-stimulated enzyme activity (enzyme activity of control soil subtracted from enzyme activity of rhizosphere soil) for both genotypes across all harvest stages with center bars representing means (vertical bars are ± SEM). The East (grey bars) shows a significant increase over time while the West (black bars) is not significantly different at any stage.

N Activity in the soil

Potential N-degrading enzyme activity in the rhizosphere ('plant-stimulated enzyme activity' measured in nmol per gram of dry soil per hour) varied with time, but the relationship was

dependent on genotype (Appendix 1, Table A1.2). The East increased in activity from tillering to post-senescence and the West remained relatively constant throughout harvests (Figure 1.1).

Plant Biomass

Throughout the course of development the East genotype maintained on average >30 percent more biomass than the West (Appendix 1, Table A1.3). Root biomass (mg) and root to shoot ratio for the East were consistently >40% and >10%, respectively, than West at both tillering and flowering (Appendix 1, Table A1.3).

Plants can enhance belowground resource acquisition by exploring larger volumes of soil through increased root growth. As plant-stimulated enzyme activity may be a complementary route for acquiring more resources, we investigated whether there was a relationship between root mass fraction (root biomass/total biomass) and enzyme activity. We found no significant correlation between these traits at either tillering (Pearson's correlation coefficient = -0.05, $p < 0.6$) or flowering (Pearson's correlation coefficient = 0.03; $p < 0.75$).

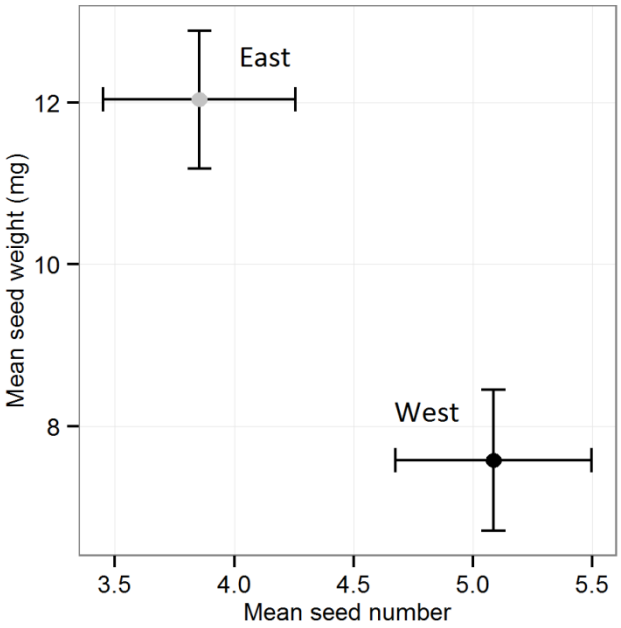

Figure 1.2: Means of average seed weight produced and seed number (bars represent 95% confidence intervals) for each genotype. The East produces fewer, but larger seeds, and the West produces more, but smaller seeds.

Percentage nitrogen in leaf tissue

Individuals from the East genotype had significantly greater percentage of N (g of N per g of tissue) in the leaf tissue at tillering than those from the West (Appendix 1, Table A1.3). As East individuals had greater biomass at this stage (see 'Plant Biomass'), this resulted in greater total grams of N in aboveground biomass for the East.

Plant Reproductive Fitness

The West genotype flowered 12 days earlier than the East genotype ($p < 0.0001$) and produced 30 percent more seeds per individual than the East (Appendix 1, Table A1.3). However, the total weight of seeds produced (mg), and the mean weight of individual seeds (mg) was 18 and 62 percent greater, respectively, for the East genotype than for individuals of the West genotype (Appendix 1, Table A1.3). This resulted in the East producing fewer, larger seeds and the West producing more, smaller seeds (Figure 1.2). Across genotypes, planted seed weight was positively correlated with more biomass (Pearson's correlation coefficient = 0.054, $p < 0.05$) as well as with producing greater mean seed weight (Pearson's correlation coefficient = 0.21, $p < 0.05$).

The ability of a plant to produce seeds and the quality of the seeds produced are influenced by resource acquisition throughout the growing period. Consequently we investigated whether there was a relationship between plant-stimulated enzyme activity (which leads to mobilization of nutrients) early in the growing season and quality of seeds produced (based on seed weight) at the final harvest. As individuals at the tillering stage were destructively harvested, we used family means for each trait. Across families there was a significant negative correlation (Pearson's correlation coefficient = -0.53 $p < 0.0005$) between plant-stimulated enzyme activity at the tillering stage and average seed weight at final harvest (Figure 1.3).

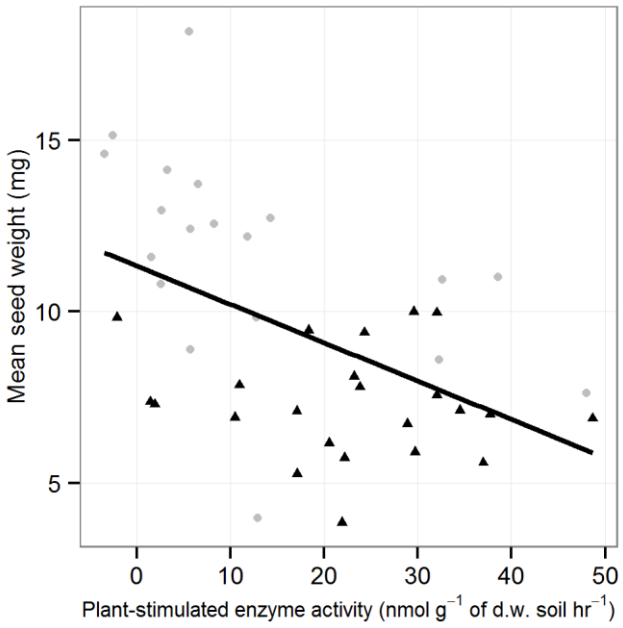

Figure 1.3: Plant-stimulated enzyme activity at tillering and mean seed weight produced per family for East (grey circle) and West (black triangles). The line represents a significant negative Pearson's Correlation between enzyme activity and mean seed weight.

DISCUSSION

We compared two invasive genotypes of *Ae. triuncialis* to understand their interactions with the rhizosphere and how that may influence plant fitness. Despite the key role of intraspecific variation in evolution and ecology (Darwin 1859) studies of plant-soil relationships often fail to consider the consequence of genotypic variation. We show that plant genotype not only determines resource acquisition and seed allocation strategies but also defines temporal dynamics of interactions with the soil microbial community.

While it is well known that plant genotypes differ in their ability to acquire, utilize and compete for available inorganic N (Tillman et al. 1991, Ahmad et al. 2008, Harrison et al. 2008, Barraclough et al. 2014), little is known about how they may differ in their influence on microbial depolymerization of N-rich organic compounds, the rate-limiting step in N mineralization (Schimel and Bennett 2004). We found that the activity of the rhizosphere communities differed between the two genotypes in the timing and amount of N-degrading extracellular enzymes produced. Specifically, the East showed a steady increase in enzyme activity over time and the West showed no significant change (Figure 1.1). Nitrogen bound in chitins and proteins is inaccessible for plants, thus the degradation of these compounds releases N that can become available for plant uptake. The East and West genotypes also differed in their acquisition of N from the soil, with the East having a greater percentage of nitrogen in leaf tissue at the tillering stage. Coupled with greater aboveground biomass, this resulted in greater total grams of N in East compared to West individuals, despite having lower N enzyme activity at this stage (Figure 1.1). Additionally, proportionately more biomass was allocated belowground for the East. As N acquisition is a function of plant root traits, the greater allotment of biomass to roots may explain the greater N acquisition. Our finding of differences in resource acquisition and microbial activity between the East and West is consistent with Zancarini et al. (2013) who showed that the taxonomic composition of rhizosphere bacterial communities was specific to plant genotypes that differed in nutrient acquisition and use.

In addition to biomass production, the genotypes differed in both the quantity and size of seeds produced at the end of the growing season (Figure 1.2). Although the East had greater biomass at all harvest stages, the West produced significantly more seeds, which may be a result of flowering earlier. However, there was a tradeoff of quantity versus size- the West produced more, but smaller seeds, and the East produced fewer, but larger seeds (Figure 1.2). Although we did not test germination of seeds produced from this experiment, the initial planted East seeds were heavier and more likely to germinate than West seeds (data not shown) and initial seed weight was positively correlated with biomass as well as average seed weight. These factors result in the East producing seeds better equipped for the next growing season in this environment.

The traits associated with N uptake could affect the relative fitness and success of the genotypes if they affect survival or fecundity in the next generation. We observed a negative correlation between enzyme activity at tillering and average seed weight produced (Figure 1.3). This relationship shows that plant-soil microbe stimulation is a trait that has the potential to be selected upon in this environment. Microbial activity has been positively correlated with plant productivity (Broughton and Gross 2000), mostly through plant-soil feedbacks, but we are unaware of any studies that demonstrate a relationship of microbial activity and reproductive fitness in the same generation. Differences in seed weight associated with enzyme activity levels create legacy effects that will influence plant population dynamics simply by altering the fitness of the subsequent generation. Additionally, selection for different seed allocation strategies based on environment (Sadras 2007, Muller-Landau 2010) could consequently influence enzyme activity, and thus the ability for N mineralization, in future generations.

Differences in the ability of the East and West genotypes to acquire N and influence N-mineralization demonstrates that multiple positive plant-soil linkages throughout the growing season lead to a feedback. Plant-stimulated enzyme activity was correlated with seed size, and thus differences in enzyme activity directly influence the next generation and have the potential to be selected upon. Although we do see strong evidence for soil microbes to impact the success of subsequent plant generations, more work needs

to be done to define the direct mechanism of the plant-soil interaction, for example if this is due to differences in root architecture or root exudate production. Plant-stimulated enzyme activity was not correlated with root mass fraction at tillering or flowering, demonstrating that factors other than root biomass must be influencing this trait. *Ae. triuncialis* has the ability to invade soils ranging from nutrient-limited serpentine communities to more resource-rich loam soils, thus further studies should be performed in the field and on multiple soil types to determine the consistency of these results. Additionally, as we do see differences in reproductive output between genotypes, the findings of this study would be enhanced if the relative success of the offspring in different environments were investigated to determine the impact of these differences on subsequent generations.

We show that intraspecific variation determines plant-soil relationships and we also highlight a novel relationship between plant-stimulated enzyme activity and fitness components that has the potential to influence population evolution. Our results emphasize the importance of studying the interactions of individual genotypes with their soil microbial communities (lending insights into the mechanisms promoting genotypic distributions within species' ranges) and the role of soil microbial communities on plant success in variable environments. Further research on these interactions could provide a greater understanding of adaptive potential of populations in the face of changing climactic conditions and shifting range margins. By studying ecosystem-relevant plant traits and the functional consequences of differences in microbial community activity, we may relate plant-soil microbe traits on the small scale to ecosystem processes and species distributions on a large scale.

CHAPTER 2: IMPACTS OF GENETIC LINEAGE ON COMPETITIVE ABILITIES

INTRODUCTION

Competition for resources between individuals of different species plays an important role in determining the composition of resident plant communities (Booth and Grime 2003, Gibson et al. 2012). Consequently, understanding the outcomes of competitive interactions is critical to understanding ecosystem assembly and is of interest to a wide variety of scientists and resource managers. Invasive species can surpass comparable native species in fitness, size and growth rates (van Kleunen et al. 2010), and thus competitive superiority is employed as a mechanism of invasion (Levine et al. 2003, Vila and Weiner 2004). If invasive species successfully outcompete natives, competition could result in reduced native species abundance and diversity.

The success of an invader in a novel environment is dependent on key traits of that invader (Smith and Knapp 2001, Prinzing et al. 2002) as well as traits of the resident community (Levine et al. 2004). Invasive species may outcompete natives (Levine et al. 2003, Vila and Weiner 2004), but a resilient native community can resist invasion via the exclusion of exotics (Keane and Crawley 2002). Therefore, the outcome of competitive interactions between natives and invaders are the result of differences in competitive abilities. The competitive abilities of an individual can be broken down into two traits: their competitive effect, which is the impact an individual has on their neighbor's growth; and their competitive response, which is the impact on the individual's growth due to the lower resource levels resulting from neighbor presence (Goldberg and Landa 1991). For invasive species, competitive effect is more important for the initial stages of invasion leading to establishment, while competitive response is more important for persistence in a given community (Muller-Scharer et al. 2004, Bossdorf et al. 2005). Competition dynamics between individuals are driven by competitive abilities and competitive responses. These dynamics are further mediated by intraspecific variation (Turkington 1996).

Intraspecific variation in invasive species is determined by the amount of genetic variation that accompanies colonization in the novel environment. Invasion events are frequently characterized by multiple introductions (Durka et al. 2005). The introduced populations face novel environmental conditions that result in unique selection pressures compared to the native range (Mooney and Cleland 2001). If multiple introductions of invaders result in genetic variation in the introduced range, this could lead to different suites of traits that are selected for in the novel environment, and thus different competitive abilities among invasive populations. Accordingly, invasive lineages of exotic species can differ in important reproductive and phenotypic traits (Meimberg et al. 2010). Most work to date has been based on the assumption that competitive effect or response is a uniform species trait (Mariotte et al. 2012, Bennett et al. 2013). If intraspecific variation is considered in competitive abilities, it is usually to compare native versus introduced populations of an invasive species (Bossdorf et al. 2005, Kumschick et al. 2013). A better approach is to consider the role of intraspecific variation within the invaded range to account for potential trait differences resulting from introduction and selection dynamics.

To test if invasive lineages of a single species differ in their competitive ability we used two genetic lineages of the invasive grass, *Aegilops triuncialis*. *Ae. triuncialis* is native to Eurasia and currently invading grasslands throughout California and southern Oregon where it has subsequently reduced native plant numbers and extent (Batten et al. 2006). All sampled invasive populations identified with one of three lineages which differ in their range sizes as well as in reproductive traits (Meimberg et al. 2010). We investigated whether established populations of the two most invasive lineages of *Ae. triuncialis* differed in their competitive response to three common grassland species known to persist after *Ae. triuncialis* invasion. Additionally, we used herbarium records to study the historical distribution of genotypes, and to test the hypothesis that the most competitive genotype introduced to a county is the genotype currently found at this location.

METHODS

Species selection and seed source

Aegilops triuncialis is a selfing, annual grass native to Eurasia, which invades arid and semi-arid grasslands throughout northern California. Meimberg et al. (2010) sampled invasive populations throughout California and identified three lineages (East, West and South) with the East and West lineages occupying much larger areas than the South (Meimberg et al. 2010). We used offspring from a common garden experiment (Espeland and Rice 2012), which included 11 populations (8 East and 3 West, defined as populations 102, 103, 105, 106, 107, 115, 117, 119, 122, 124 and 125 in (Meimberg et al. 2010)).

In order to test the competitive response of these 11 populations of *Ae. triuncialis*, we chose three competitor species that persist in California grasslands following *Ae. triuncialis* invasion (Paul Aigner, pers. com.): 1) *Poa secunda*, a native perennial bunchgrass found throughout California and the western United States; 2) *Festuca microstachys*, an annual grass of short stature native to California, and 3) *Festuca perennis*, an exotic annual/biennial grass that was introduced throughout California as a forage species and is larger than *P. secunda* and *F. microstachys*. Seeds for *P. secunda* and *F. microstachys* were obtained from Hedgerow Farms (Winters, CA USA) and originally collected from Yolo County, CA. Seeds for *F. perennis* were collected from University of California- Davis McLaughlin Natural Reserve (Lake County, CA USA). *Ae. triuncialis* is currently invading both Yolo and Lake Counties.

Perennial Competition

We used the native grass *P. secunda* to test the competitive response of *Ae. triuncialis* to perennial grass competition. *Ae. triuncialis* is more likely to encounter *P. secunda* as a plant than as a seed, and thus we established plants of *P. secunda* prior to planting *Ae. triuncialis*. Two seeds of *P. secunda* were planted each into 120 pots (five cm in diameter) in a greenhouse in March 2012 using 4-P Mix, Professional Formula potting soil (Fafard, Agawam, MA, USA). Pots were placed on a greenhouse bench and thinned

to a single (larger) individual after germination. Plants were kept well watered and experienced the equivalent of a one year life cycle after which they went dormant. In December 2012 dormant plants were removed from pots and split down the middle. Both halves of each plant were then repotted into the center of a 12.7 cm diameter pot, leaving 3 cm space between each half. We used this approach to ensure *Ae. triuncialis*, when planted, interacted with *P. secunda*. Pots were then watered for two weeks allowing all *P. secunda* individuals to come out of dormancy. Two *Ae. triuncialis* seeds from the same seed family (i.e. full-sibling individuals) within a population of a particular genotype were then sown into each *P. secunda* pot, 60 pots per genotype. *Ae. triuncialis* was thinned to a single (larger) individual after germination. Three weeks after planting nitrogen additions began and were applied weekly to half of all pots (n=60 pots, n=30 per genotype) at the application rate of 10 g/m^2 over the growing season. Two months after planting diameters of individual *P. secunda* did not exceed 3.3 cm at the base and could be as small as 0.3 cm (mean= 1.7 cm SE 0.6). The small stature of *P. secunda*, in addition to the rapid rate at which *Ae. triuncialis* seedlings surpassed the height of *P. secunda* individuals after germination (Gomola, personal observation), suggests that initial above ground competition was negligible between *P. secunda* and *Ae. triuncialis*.

Annual Competition

In January 2013 two *Aegilops triuncialis* seeds (equivalent in family/population origins to *Perennial Competition* above) were sown into the center of 12.7 cm diameter pots filled with 4-P Mix, Professional Formula potting soil (Fafard, Agawam, MA, USA). Eight *Festuca microstachys* or *Festuca perennis* seeds were then planted around the *Ae. triuncialis* seed (2-3 cm radius from seed) to create maximum competition for *Ae. triuncialis*. This resulted in 120 pots per competitor (*F. microstachys* or *F. multiflorum)* with 60 pots sown with East lineage seeds and 60 with West lineage seeds per competitor. Plants were thinned down to a single *Ae. triuncialis* individual in the center of the pot surrounded by four competitors (the smallest individuals were removed). Plants were allowed to grow for three weeks prior

to N addition, after which half of the pots received weekly N additions at the same application rate as the *P. secunda* pots (see *Perennial Competition)*.

Data and biomass collection

Dates of germination, first flower and death were recorded for all individuals (*Ae. triuncialis* and competitors). Flowering stalks that senesced were collected weekly to avoid loss of biomass or release of seeds. The remaining aboveground biomass of all individuals was harvested on the date of final *Ae. triuncialis* senescence. All individuals who had not senesced 210 days after planting were destructively harvested. Biomass was oven dried at 60C for 48 hours and weighed.

Statistical Analysis

For *Ae. triuncialis* the response variables days to flower, total biomass and total flowers were analyzed as a function of genotype, competitor species and their interaction to test for the effect of competitor species on phenotypic traits of each genotype. Total flowers were square-root transformed to meet assumptions of normality. Total competitor biomass was retained as a random variable but never accounted for greater than 3% of variation. At the end of the experiment both control and N addition pots indicated non-limiting levels of N in the soil and so we averaged our results over N treatments.

Herbaria samples

We investigated differences between current and historical distributions of lineages with herbaria samples to test if competitive response explains current patterns of genotype abundance within counties. Meimberg et al. (2010) sampled populations in 14 counties in 2007 and identified populations in each county as belonging to a single lineage (East, West or South). Of the 14 counties sampled, we were able to obtain plant material for the first recorded introduction into 10 of those counties (n=11 samples: one from each county except for Sacramento) from herbaria throughout northern California (Appendix 1, Table A2.1). We were not able to amplify DNA from herbaria samples representing initial introductions

into Colusa, Sierra, Tuolumne or Yolo counties. Seeds from previously genotyped individuals belonging to all three invasive lineages were grown in a greenhouse to act as representatives of current invasive populations for lineage comparison. Leaf tissue from these samples was collected and freeze-dried for 48 hours immediately after collection. Dried leaf tissue from herbaria samples and controls was placed individually into 2 mL micro-centrifuge tubes with three 1.99 mm ball bearings and ground in a modified paint shaker until a fine powder was reached. DNA was then amplified from each ground sample using the CTAB technique as described by Doyle and Doyle (1987). Measurements for microsatellite polymorphisms in each sample were performed according to the protocol Meimberg et al. (2010) used to originally identify the three invasive lineages. We ran each herbaria sample two times with reference samples for each run, and merged the results for simplicity. Any contrasting alleles between technical replicates of the same primer were deleted and the genotype at that marker was considered missing data. Additionally, if we had data for less than 5 marker genotypes for a single sample we did not consider the sample usable. Microsatellite data from herbaria samples were compared to samples of current invasive populations in order to determine whether they identified with previously classified lineages or represented a novel lineage/introduction. We then compared initial introduction identity (lineage) with the lineage occupying the same county in 2007 (as per Meimberg et al 2010).

RESULTS

Flowering and Reproductive Output

The West flowered significantly earlier than the East when grown alone ($p < 0.02$) as well as when grown in competition with *F. microstachys* and *F. perennis* ($p < 0.0001$ for both, Figure 2.1). When grown in competition with *P. secunda*, lineages did not differ in days to flower ($p > 0.5$, Figure 2.1). Within the East lineage, days to flower did not differ from the control for any competitor treatments (Appendix 2, Table A2.2). For the West, days to flower when grown in competition with *F. microstachys* or *F.*

perennis did not differ from the control (Appendix 2, Table A2.2), but the West flowered significantly later when grown in competition with *P. secunda* ($p < 0.03$, Figure 2.1).

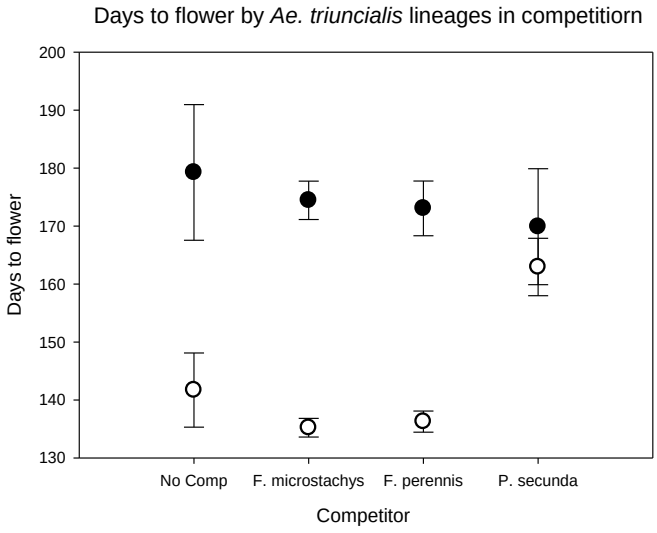

Figure 2.1: Mean days to flower per genotype in all treatments, the East lineage with filled circles and West lineage with open circles (standard error bars). The West always flowers significantly earlier than the East in all treatments except *P. secunda*. Mean days to flower for the West is no different when in competition with *F. microstachys* or *F. perennis* compared to the control, but is later when in competition with *P. secunda*. Mean days to flower for the East is no different in any treatment compared to the control.

The West produced significantly more spikelets than the East when grown alone, as well as when grown in competition with *F. microstachys* and *F. perennis* ($p < 0.0001$ for all, Figure 2.2). When grown in competition with *P. secunda*, genotypes did not significantly differ in spikelet production ($p > 0.2$, Figure 2.2). The East lineage produced significantly more spikelets when grown with *F. microstachys* compared to the control ($p < 0.05$). Spikelet production did not differ for the East when grown with *F. perennis* or *P. secunda* compared to the control (Appendix 2, Table A2.2). When the West was grown in competition with *F. microstachys* or *F. perennis*, spikelet production did not differ from the control (Appendix 2, Table A2.2), but the West produced significantly fewer spikelets when grown in competition with *P. secunda* compared to the control ($p < 0.0001$).

Biomass

Ae. triuncialis lineages did not significantly differ in biomass production across treatments ($p>0.15$). Biomass production across lineages did not differ when grown in competition with *F. microstachys* compared to the control (Appendix 2, Table A2.2). Biomass production across lineages was lower when

Ae. triuncialis was grown in competition with *F. perennis* and *P. secunda* compared to when grown alone ($p < 0.01$ and $p < 0.0001$, respectively, Figure 2.3).

Herbaria Results

Of the 11 herbaria samples that were tested, six of them had the same multi-locus genotype as is currently found in the county in Meimberg et al. (2010) (Table 2.1). Introductions in three counties represented novel multi-locus genotypes; these were Amador, Calaveras, and Sacramento counties (Table 2.1). The Amador sample was most similar to the South lineage, but contained one private allele, as well as two alleles that are found in the East and West but not the South. The Calaveras sample contained five private alleles and was consequently the sample that was most divergent from current lineages. The Sacramento sample was most similar to the East lineage, and contained two private alleles although they only differed by a single base pair.

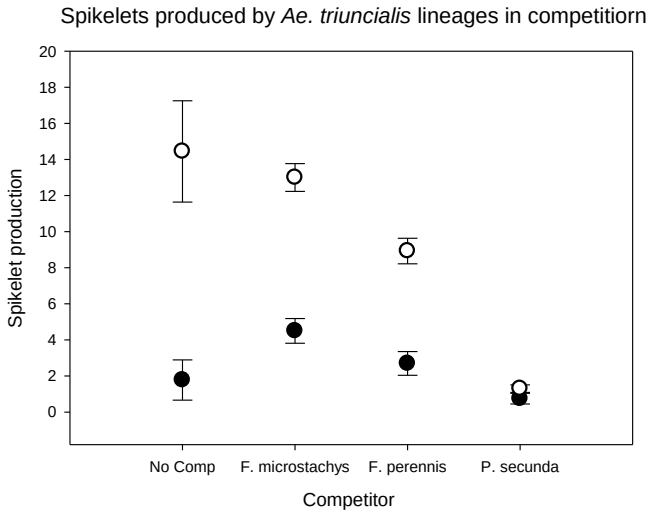

Figure 2.2: Mean number of spikelets produced per genotype in all treatments, the East lineage with filled circles and West lineage with open circles (standard error bars). Spikelet production was square-root transformed for analysis, but data presented represent untransformed values. Number of spikelets produced by the West is significantly higher than the East in all treatments except *P. secunda.* Number of spikelets produced by the West is no different when in competition with *F. microstachys* or *F. perennis* compared to the control, but is lower when in competition with *P. secunda*. Number of spikelets produced by the East is higher when grown in competition with *F. microstachys* compared to the control, and does not differ when grown with *F. perennis* or *P. secunda* compared to the control.

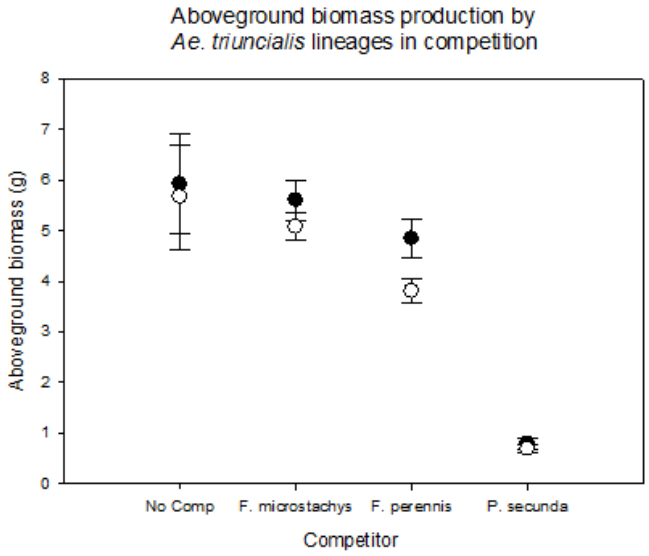

Figure 2.3: Mean aboveground biomass production produced per genotype in all treatments, the East lineage with filled circles and West lineage with open circles (standard error bars). Aboveground biomass production is not significantly different when grown with *F. microstachys* or *F. perennis* compared to the control, and was significantly lower when grown with *P. secunda* compared to the control.

Table 2.1: Comparisons of microsatellite results of herbaria samples ('first recorded introduction') to lineage occupying the county in 2007 ('2007 lineage...'). '*' indicate that the first recorded introduction represents a different lineage than was identified in the same county in 2007. Three herbaria samples did not align with previously identified lineages, five herbaria samples identified with different lineages at the first recorded introduction than were identified in 2007.

County	First Recorded Introduction	Lineage identity of first introduction	2007 Lineage according to Meimberg et al (2010)
Amador	1959	**Novel ***	East
Butte	1967	East	East
Calaveras	1928	**Novel ***	South
Colusa	2007	West	West
El Dorado	1939	East	East
Lake	2001	**West ***	East
Mendocino	1953	**East ***	West
Napa	1970	West	West
Sacramento	1932	**East and Novel ***	East
Solano	1951	West	West
Yuba	1951	East	East

DISCUSSION

Our findings demonstrate that genetic lineage of the invasive grass *Ae. triuncialis* is a better predictor of competitive response than identity of neighbor species. In other words, genetic variation within *Ae. triuncialis* contributed more to competitive outcomes than interspecific variation among competitors. Furthermore, we find an interaction between lineage and competition such that the East exhibits no significant response to competition in flowering phenology or spikelet production, while the West can be affected by competition in both metrics (Figure 2.1, 2.2). This result supports those of previous work demonstrating genetic lineages respond differently to competition (Latta et al. 2004, Cahill et al. 2005, Muller and Bartelheimer 2013), and that competitive tolerance between invasive plant lineages differ from the home and invaded ranges (Bossdorf et al. 2008, Kumschick et al. 2013). We add to this body of work by showing that genetic lineages within the invaded range respond differently to competition. In addition, we find the degree of competitive response is dependent on the interaction between lineage and competitor species.

Lineages responded similarly to competition treatments in biomass production, but their reproductive trait responses differed. For aboveground biomass production, *P. secunda* exerted the strongest competitive effect among competitor species on *Ae. triuncialis* (Figure 2.3). Although both lineages exhibited this reduction in biomass, reproductive traits differed between lineages in response to the same competitor. The West flowered later and produced fewer spikelets when in competition with *P. secunda*, while the East acted no differently than when grown alone for both of these traits (Figures 2.1, 2.2). Furthermore, the East was consistent in its lack of competitive response across all treatments: flowering phenology did not change and spikelet production never decreased. These reproductive traits of the West lineage were unaffected by competition with the annual grass *F. microstachys*. Reproductive response of West to competition had parallel trends when *F. perennis* and *P. secunda* were the competitors. Differences in competitive response between East and West are likely due to different resource uptake and allocation strategies that may in turn reflect differences in invasion mechanisms between the two lineages.

Our results demonstrate two different competitive strategies among lineages, with the East showing no response to competition while the West does. When the West lineage produced less biomass under competition it also produced fewer spikelets (Figures 2.1, 2.3), indicating that reproductive output may be biomass dependent. These results are consistent with well-established research showing positive relationships between vegetative and reproductive output (Weiner et al. 2009). The East, however, exhibits a contrasting pattern of consistently flowering at the same time and producing similar numbers of spikelets, regardless of biomass accumulation. The consistency in seed output by the East despite competition may serve as a successful mechanism of invasion for this lineage.

Our herbaria analysis indicates that both lineages have successfully persisted after being introduced, but there is also evidence that each lineage is capable of replacing the other (Table 2.1). The East was found in more sampling locations in 2007 than the West (Meimberg et al. 2010), but the East also appeared in our herbaria comparisons 20 years earlier than the West (Table 2.1). Thus, the East being the most abundant of the three California lineages may be a result of an earlier introduction, its lack of response to competition, or a combination of the two. This idea was originally based on its larger range but is further substantiated by the results of our competition study. The success of the West lineage may be due to colonization of sites where there is minimal competition, such as recently disturbed areas.

However, the West lineage also occupies one county where the East was originally introduced (Table 2.1), indicating the potential for the West to outcompete or replace the East. Other than in Lake County, the East and West occupy discrete areas (Meimberg et al. 2010). The results of our herbaria analysis indicate that although the East is the most abundant lineage when lineage distributions are discrete, if distributions overlap the West may become the more abundant of the two.

Understanding intraspecific variation in invasive species may inform more effective management. Both lineages are able to sustain reproductive output regardless of the competitor, but the West is able to produce greater numbers of spikelets if it is able to acquire more biomass. Therefore, management

strategies such as introducing native species to effect higher competition and lower biomass acquisition may be more successful with West lineages. Efforts to reduce inflorescence development such as mowing may be more useful in populations of the East as reproductive output will likely be more consistent in this genotype regardless of competition.

Our study underlines the need to examine intraspecific variation in invasive species response to common competitors in the invaded range, as the two lineages used in our study showed important distinctions in their responses to the same competitor. Furthermore, we demonstrate that genetic identity of the target individual is more important to competitive response than presence of a neighbor. Although intraspecific variation in competition has been recognized as an important factor in competitive response and interactions (Gustafson et al. 2004, Latta et al. 2004, Cahill et al. 2005), possible mechanisms of invasion tend to be thought of as a uniform trait of a given species. Here we show evidence that lineages can differ in their response to competition, and that one lineage may use its resilience to competition as a mechanism of invasion, while the other appears more vulnerable to competition and may depend on disturbance or other factors that limit direct plant-plant interactions for its success. Multi-species approaches conducted in the field and at multiple sites are rarely performed, but are needed to determine the consistency of competitive response in the face of biotic and abiotic variability. Additionally, our results show that genetic variation within a species can determine competitive outcomes more than interspecific variation among competitors. This has profound implications for community assembly rules as well for mechanisms of invasion and effective control of invasive species.

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APPENDIX 1

Table A1.1: Climatic conditions mimicked in the growth chamber to stimulate environmental conditions in the invaded range. Numbers are based on mean temperatures and day lengths for Sacramento, CA obtained from Climate-Charts.com (© 2007) and were rounded to the nearest half unit.

Month	**Mean High Temp (^{o}C)**	**Mean Low Temp (^{o}C)**	**Mean Day Length (hr)**
December	13	4.5	9.5
January	12.5	4	9.5
February	15.5	5.5	11.00
March	18	7	12
April	22.5	8.5	13
May	26	11	14.5
June	30.5	12.5	15
July	33.5	16	14.5

Table A1.2: Genotype by harvest stage effects on plant-stimulated enzyme activity (nmols^{-1} g dry soil^{-1} hour^{-1}) means (± SEM). The interaction is significant at $p < 0.0005$.

	East	West
Tillering	13.99 (3.6)	21.80 (2.5)
Flowering	20.76 (2.9)	20.00 (2.2)
Post-Senescence	33.56 (4.4)	15.74 (2.6)

Table A1.3: Effect of genotype on plant trait means (± SEM).

Plant Aboveground Biomass (mg)			
	East	West	P-value
Tillering	138.29 (9.15)	102.25 (4.26)	<0.05
Flowering	327.73 (11.05)	253.06 (8.21)	<0.0005
Final Harvest	349.71 (8.69)	258.05 (9.13)	<0.005
Plant Root Biomass (mg)			
	East	West	P-value
Tillering	181.60 (11.55)	117.79 (5.66)	<0.05
Flowering	139.24 (6.49)	98.29 (4.52)	<0.0001
Root: Shoot Biomass			
	East	West	P-value
Tillering	1.492 (0.11)	1.172 (0.042)	<0.005
Flowering	0.421 (0.01)	0.381 (0.01)	<0.005
Percentage N in leaf tissue			
	East	West	P-value
Tillering	2.585 (0.29)	1.729 (0.09)	<0.01
Number of Seeds			
	East	West	P-value
Final Harvest	3.78 (0.18)	4.92 (0.27)	<0.001

APPENDIX 2

Table A2.1: Table of Herbaria Collection Material by Site

County of Introduction	Year of First Recorded Introduction	Herbarium	Location
Amador	1959	University of California-Davis	UC Davis Center for Plant Diversity 1026 Sciences Laboratory Building Davis, CA 95616, USA
Butte	1967	University of California-Davis	UC Davis Center for Plant Diversity 1026 Sciences Laboratory Building Davis, CA 95616, USA
Calaveras	1928	University of California-Berkeley	1001 Valley Life Sciences Building MC2465 University of California- Berekely Berkeley, CA 94720, USA
El Dorado	1939	University of California-Davis	UC Davis Center for Plant Diversity 1026 Sciences Laboratory Building Davis, CA 95616, USA
Lake	2001	California Department of Food and Agriculture	Plant Pest Diagnostic Center- CA Dept of Food and Agriculture 3294 Meadowview Road Sacramento, CA 95832, USA
Mendocino	1960	University of California-Davis	UC Davis Center for Plant Diversity 1026 Sciences Laboratory Building Davis, CA 95616, USA
Napa	1970	California Academy of Sciences	55 Music Concourse Drive Golden Gate Park San Francisco, CA 94118, USA
Sacramento	1932	University of California-Davis	UC Davis Center for Plant Diversity 1026 Sciences Laboratory Building Davis, CA 95616, USA
Solano	1951	University of California-Davis	UC Davis Center for Plant Diversity 1026 Sciences Laboratory Building Davis, CA 95616, USA
Yuba	1951	University of California-Berkeley	1001 Valley Life Sciences Building MC2465 University of California- Berekely Berkeley, CA 94720, USA

Table A2.2: Table showing means, standard errors and p-values from ANOVAs performed for response variables. 'P-value control' shows a test of whether response in competitor treatment was significantly different from control, 'P-value lineage' shows a test of whether responses of lineages were significantly different in that treatment. * indicates a significant difference. Note: Spikelet production is given in untransformed means and standard errors, p-values are based on square-root transformed data.

Days to Flower							
Competitor:	East Mean	St Err	P-value control	West Mean	St Err	P-value control	P-value Lineages
Control	179.25	11.71	N/A	141.71	6.39	N/A	0.0128*
F. microstachys	174.44	3.31	0.9771	135.22	1.61	0.7985	<0.0001*
F. perennis	173.05	4.72	0.9579	136.27	1.83	0.8745	<0.0001*
P. secunda	169.89	10.00	0.9005	162.94	4.96	0.0231*	0.5246
Spikelet production							
Competitor:	East Mean	St Err	P-value control	West Mean	St Err	P-value control	P-value Lineages
Control	1.60	1.01	N/A	13.00	2.89	N/A	<0.0001*
F. microstachys	4.50	0.68	0.0132*	13.00	0.77	0.9362	<0.0001*
F. perennis	2.70	0.66	0.5136	8.72	0.72	0.3255	<0.0001*
P. secunda	0.67	0.27	0.6013	1.02	0.18	<0.0001*	0.2054
Aboveground biomass (g)							
Competitor:	Mean	St Err	P-value control				
Control	5.79	0.70	N/A				
F. microstachys	5.34	0.24	0.7627				
F. perennis	4.25	0.23	0.0083*				
P. secunda	0.74	0.07	<0.0001*				