“CAREER: Rapid evolution of an invasive plant: the role of microbial interactions”

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CAREER PROPOSAL
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PROJECT SUMMARY

Overview: Microbiomes are now known to have large effects on the phenotype and fitness of many organisms. As a result, macroorganisms that are introduced to new regions of the globe may experience large changes in fitness and patterns of selection as a result of novel microbial interactions. If microbial interactions are overall less pathogenic in an introduced range, selection is predicted to favor evolution of investment away from defense and into other components of fitness, potentially facilitating invasive expansion of introduced species. Studies of plant-soil feedbacks reveal that introduced plants often escape negative microbial interactions, but how changes in microbial community interactions shape selection on plant genomes, particularly the evolution of defense and fitness traits, remains largely unknown.

The European plant yellow starthistle (*Centaurea solstitialis*) is highly invasive in grasslands of the Americas. The PI has described the evolution of increased growth and reproductive ability in its severe invasion of the western US and identified candidate regions of the genome associated with these rapid evolutionary changes. In this invasion, yellow starthistle experiences more favorable microbial interactions with the soil, and the PI has recently identified both i) reduced diversity of bacteria and ii) an apparent reduction in plant investment in aspects of immune function in these populations. The proposed project will identify microbial taxa involved in these interactions and their effect on plant trait evolution through 1) sequencing of the microbiome across multiple generations of plant-soil feedback experiments, 2) selection experiments with the microbial community to identify the impacts of microbial evolution on plant traits, 3) selection on plant genotypes to identify the genetic basis of fitness differences and to quantify trait evolution under different microbial environments, and 4) gene expression studies to identify plastic and genetic responses of genes to microbial interactions. Undergraduate students from groups underrepresented in STEM will lead studies of individual microbial taxa in culture and their effects on plant immune responses. These activities will establish a foundation for understanding how the evolution of plant-microbe interactions can influence plant traits and contribute to invasions, while promoting retention of high-achieving students.

Intellectual Merit: The mechanisms by which microbial community interactions shape plant traits are still largely unknown, and this project will leverage major shifts in plant-microbial interactions in natural populations to shed light on their role in plant evolution and ecology. In particular, the research will address the long-standing question of how plant traits and functions trade off against one another under different environmental contexts. Past work in this area has progressed largely through studies of model and crop plants and their interactions with specific pathogen strains. The proposed work contributes to advancing our understanding of the trade-offs underlying phenotypic and evolutionary consequences of variation in the microbial community in natural systems.

Broader Impacts: The research examines factors shaping fitness in a serious economic pest of rangelands, with potential opportunities for improved management. Through multiple types of selection experiments and establishment of a culture collection, the project will lay a foundation for further experimental studies of evolution and targeted studies of plant-pathogen interactions. The project will support the training of postdoctoral fellows, graduate, and undergraduate students in the growing areas of microbial biology, plant-microbe interactions, population genomics, and bioinformatics. A key component of the proposed work is the creation of an independent research experience aimed at improving confidence and STEM learning for underrepresented and underprivileged undergraduate students who desire to complete a degree in biology. Recent research and press have highlighted the poor graduation rate of disadvantaged students, and the strong potential for targeted experiences to reverse this pattern.
RESULTS FROM PRIOR NSF SUPPORT  Dlugosch is currently supported to conduct the project “EAGER-NEON: Genomic plasticity in response to variable environments” (#EF1550838; $300,000; 2016-2017) w/ PI MS Barker. This project investigates plasticity in gene expression and its relationship to plant performance under environmental variation at the Harvard Forest NEON site. Intellectual Merit: A major goal is to establish a framework to connect gene expression patterns to plant community change across the NEON network, with a particular focus on groups hypothesized to benefit from greater plasticity, including invasive species. Results to date have revealed a fundamental new insight into the nature of plasticity in gene expression: species tend to show either large changes in expression (high plasticity) at a small number of loci, or a smaller magnitude of expression plasticity across most of the genome (Marx et al., in prep). The award has facilitated 2 related publications to date [Maitner et al., in review] [1]. Broader Impacts: Training of 1 female postdoc, 4 graduate students (2 female), and 4 undergraduates (1 female). Products include plant vouchers, tissue samples, RNA, and sequence data.

OTHER FEDERAL SUPPORT  Dlugosch is also supported by the USDA for a project that is foundational to this CAREER proposal: “Genetic contributions to control of yellow starthistle (Centaurea solstitialis)” (USDA #2015-67013-23000 to PI Dlugosch, coPIs DA Baltrus and SM Swope; $500,000; 2015-2018). The goals of this project are to compare plant-associated bacteria in the native and invaded ranges of a major weed of rangelands, to examine the fitness and potential demographic impacts of bacterial infection on these plants, to map the regions of the plant genome associated with immune response, and to quantify the distribution of plant genetic diversity that may facilitate evolutionary changes in plant resistance to bacteria. We are on track to meet all of these objectives during the project period, with 8 publications submitted to date (see below). Intellectual Merit: This project is one of the first comparisons of microbiomes in the native and introduced ranges of an invasive plant, finding striking evidence of highly divergent bacterial communities, and a lower diversity of bacterial taxa (overall and specifically for pathogenic groups) in invaders [2]. Using a new implementation of a high-throughput assay of immune function, we find evidence for reduced immune response and a potential trade off between growth and defence in invading genotypes [3]. Population genomic work has quantified the distribution of genetic diversity in this plant, resolved the source regions for the US introductions, and identified key evolutionary transitions in plant traits [Braasch et al., in review] [4, 5]. This project has also supported broader analyses and perspectives on the evolution of invasive species [Maitner et al., in review; Welles & Dlugosch, in press] [6]. A renewal has been submitted (see Current & Pending) with a focus on quantifying variation in the yellow starthistle microbiome across space and time and to experimentally and observationally associate this variation with site characteristics and plant performance. Broader Impacts: The project has contributed to the research of 4 female postdocs, 5 graduate students (3 female), 1 female technician, and 7 undergraduate students (5 of which are part of underrepresented groups in STEM, including those that are female, minority, or both).

OVERVIEW & CAREER MOTIVATION  Fundamental trade offs between growth and defenses against enemies are well known in plants, arising because plants must allocate limited resources to these functions and because the biochemical pathways involved can suppress one another directly [7–9]. Plants are expected to experience strong selection to optimize their strategies under the growth-defense trade off, because enemies such as herbivores and pathogens can generate large losses of fitness, but at the same time defenses against these enemies come at a high cost to growth [10]. Importantly, defenses impose both direct biochemical costs and indirect ecological costs when responses to one enemy interfere with responses to other enemies or mutualists [11, 12]. These multi-species interactions are likely to generate complex fitness landscapes for plant defense [13, 14]. A great deal of progress has been made in quantifying how specific enemy interactions can select for particular plant defense strategies, but a major challenge in the field remains understanding how plants optimize growth-defense strategies under selection imposed by the entire community of species interactions [10, 12, 15, 16].

Defense interactions are likely to be especially complex between plants and microbial communities. We now know that microbial communities, including plant microbiomes, contain thousands of taxa, and the composition of these communities and their variation are only beginning to be described [17–19]. It is clear that microbial communities contain both plant pathogens and beneficial taxa, with the potential for both negative and positive effects on plant growth and fitness [20–22]. Plants can in turn
influence the composition of their microbial communities, and these interactions can be dynamic over time and across space [23–25]. Some of the best evidence for natural variation in plant-microbial interactions comes from plant-soil feedback studies of the native and non-native ranges of introduced plants [25–27]. These systems offer outstanding opportunities to quantify the evolutionary responses of plants to naturally occurring variation in interactions with complex microbial communities.

I have focused my career on the rapid evolution of plants that have been introduced to new regions of the globe. These introductions create novel interactions between plants and all aspects of their environment, altering selection on plant traits and potentially influencing the establishment and spread of these species [e.g. 28–33]. To disentangle the many ecological and genetic factors that may shape the rapid evolution of ecology in these situations, I have been establishing a central study system in my lab, focused on the evolutionary ecology and ecological genomics of the highly invasive plant yellow starthistle (Centaurea solstitialis, Asteraceae; hereafter ‘YST’). We have found that YST has evolved larger size and reproductive potential in its severe invasion of western North America [4, 34], and we have been identifying loci that are candidates for response to selection during invasion. Loci related to defense functions dominate these candidates, and our most recent work has revealed a major shift in microbial associations in this invasion [2]. Thus this system is ideal for studying the influence of microbial communities on plant evolution, particularly evolution along the growth-defense trade off.

Here I propose to develop a major new career focus on the evolution of microbial interactions in introduced plants. I propose to establish multiple long-term experiments involving feedback between YST plants and their microbes and selection on both microbial communities and on plant genotypes. These experiments will quantify how changes in YST-microbial interactions can contribute to the evolution of plant growth, will identify plant loci involved in microbial community interactions, and will lay a foundation for a career studying evolution along the growth-defense trade off and its potential impact on plant ecology.

SPECIFIC RESEARCH & EDUCATION OBJECTIVES

**AIM 1: Plant-soil feedbacks** Native and invading genotypes of YST will each be exposed to native and invaded range soil microbiomes over multiple generations to quantify the growth and fitness impacts of these interactions, their variation over generations, and the microbial taxa involved.

**AIM 2. Selection on the microbial community** The plant-soil feedback experiment in AIM1 will be leveraged to generate soil feedback lines for selection on the microbial community to maximize the growth of native YST genotypes, allowing the quantification of the potential plastic changes in plant traits arising from shifts in the microbial community.

**AIM 3. Selection on plant genotypes** Recombinant YST populations will be selected for increased growth under both native and invaded range microbial communities, allowing the identification of loci involved in rapid evolution of growth that are either dependent on the microbial context (differing between selection lines) or independent of microbial interactions (parallel between selection lines) and their relationship to known candidate loci.

**AIM 4. Plant gene expression** Gene expression will be used to quantify the plastic and evolutionary response of functional plant genes, including candidate loci, to differences in the microbial community in AIM1 and AIM3.

**AIM 5. Educational Development** A new Undergraduate Scholar Scientist program will be developed to enhance STEM engagement, academic success, and retention of at-risk students through our critical biology courses. The program will bring a targeted group of students from my Ecology course together to engage directly with independent research related to the proposed project, and linked to our set of core courses in Ecology, Evolution, and Genetics. The students will culture microbial strains from the above experiments and assay plant immune response to these taxa, building a valuable culture collection for further manipulative experiments.
INTRODUCTION Microbial communities are increasingly recognized for their potential to affect the functioning and fitness of macro-organisms across the tree of life [19]. Microbiome communities contain both pathogens and beneficial species in a complex network of interactions with each other and their hosts [20, 35–37]. Against these complexities, pathogens have long stood out for their ability to generate large fitness costs to their hosts, and strong selection on host defenses as a result [38]. In turn, defense functions are known to be among the most diverse and rapidly evolving components of multicellular genomes (e.g. [39–41]).

In plants, defense traits are also known to involve significant direct costs. Defenses against both pathogens and herbivores can have large direct trade offs with growth and future reproduction [reviewed in 7–12, 38, 42]. Defense functions involve recognition of both wounding and pathogens, with subsequent signalling and transcriptional alterations by multiple defense pathways (including salicylic acid, jasmonic acid, ethylene, cytokinins, and others). These signals can initiate a variety of defense functions that use plant resources (production of reactive oxygen species, cell wall fortification, secondary compounds), directly suppress growth through hormone crosstalk (via auxin, brassinosteroids, and gibberellins), and lead to loss of growth investments through tissue sacrificed to block the progress of pathogens (e.g. hypersensitive response, root die-off). Pathogens can in turn produce effectors that suppress plant responses and in some cases up-regulate growth, and plants can evolve counter-defenses to circumvent these effectors. Plant responses can therefore involve many pathways and can be inherited as single locus or quantitative traits [43].

There can also be indirect ecological costs of defense to plants. These can occur when increased resistance to one attacker raises vulnerability to other types of attack [11], or when defense systems deter favorable species interactions, such as beneficial microbial endophytes [12, 44]. As a result, interactions across multiple members of the community are likely to generate conflicting patterns of selection on plants and present one of the main challenges to understanding the evolution of plant defenses [13, 45–47]. For plant-microbial interactions in particular, interactions with individual components of these communities are still an active area of study, and the nature of selection imposed by the entire complex community is only beginning to be tackled in model basic and agricultural systems [10, 12, 15, 16, 48].

Introduced plants may present particularly good opportunities to study natural variation in plant-microbial community interactions and their effects on the evolution of plant traits. Through translocations to new biogeographic regions, non-native species will be presented with novel microbial environments, and plant-soil interaction experiments have repeatedly demonstrated evidence that plants respond differently to these different communities [25–27, 49–52]. Many species appear to have more favorable interactions with invaded-range soil microbial communities [53–59], suggesting opportunities for the evolution of reduced defenses (i.e. the ‘Enemy Release’ hypothesis; [60–62]). Introduced plants also frequently show evidence of having evolved larger size [63–65], consistent with a shift toward increased growth at the expense of defenses along the growth-defense trade off (this idea forms the basis of the ‘Evolution of Increased Competitive Ability’ hypothesis; [66–68]). Strong evidence for evolution along this trade off as a result of reduced enemy interactions is lacking [60, 69], however, as few studies have attempted to connect growth evolution to net changes in the enemy community and microbial studies of introduced plants are in general just beginning. Further, any contribution of growth-defense trade offs to the evolution of increased invasiveness is still controversial and will require explicit connections between the evolution of plant traits and increased population growth and spread in the field [26, 70].

Introduced plants may also experience ecological changes other than enemy escape that could select for evolution along the growth-defense trade off. If introduced species have access to greater resources (e.g. as a result of unique acquisition strategies, or a priori superior competitive abilities), this might favor the evolution of reduced defenses and greater tolerance of attack [71–73]. Studies have suggested that invasive plants as a group tend to produce more tissue with lower resource investment, which could reflect reduced defenses [74–76]. In this case, invaded range microbial communities would not generate more favorable growth than native microbial communities for native plant genotypes, but invaders would still be selected to reduce investment in defense. Alternatively, it has also been suggested that introduced species might experience simplified species interactions as naive members of their new communities, resulting in a reduction in antagonist selection from different species and increasing adaptation to the simplified interactions [77]. In this case, native plant genotypes would experience no
immediate benefit from novel interactions, but invading genotypes could be expected to be better defended against microbial communities in their introduced ranges, potentially with simultaneous increases in growth due to reduced indirect costs of defense. Similar increases in efficiency might also be expected if invading genotypes have evolved a novel innovation in defense (i.e. an innovation not present as standing variation in the native range [78–80]). Experiments that evaluate the growth response of native and invading genotypes to native and invaded range microbial communities therefore allow critical tests of these alternative possibilities of enemy escape, increased tolerance, or increased efficiency of defense (Fig 1). Studies of the genetic basis of these change can then reveal how such new strategies can be achieved and the sources of their underlying variation.

STUDY SYSTEM
YST is an annual and obligately outcrossing herbaceous species [81], native to parts of Eurasia [4]. Reproduction is via seed only, with germination typically in the fall or winter, growth as a rosette until bolting in the spring, and flowering throughout the summer. Individual plants can produce hundreds of flowers and thousands of seeds, and seed production is positively correlated with plant size [82–84]. Above and belowground biomass are also positively correlated [34].

The species was introduced accidentally in large numbers as a contaminant of alfalfa from the Old World brought to the Americas during the last few hundred years, and it has proliferated in rangelands on both American continents [85, 86]. Introduced populations have been observed to reach adult plant densities that are 25x greater than found in the native range [87–89]. As of 2006, the state of California alone had over 14 million acres infested, spending approximately $12.5 million annually on direct control efforts for rangelands [90, 91], and the invasion has only expanded since [92]. The effects of climate change are expected to enhance its fitness and spread [93, 94]. Six biocontrol insects and a fungus have been deliberately introduced in an effort to increase enemies of this plant, though with little success in controlling the invasion. There is evidence of poor synergy or even negative interactions among these biocontrol agents [90, 95–97].

YST is highly amenable to large scale genomic and phenotypic studies. It is easily reared in large numbers and manually pollinated [e.g. 34, 98, 99]. Germination rates for most populations are typically very high (>80%; Dlugosch, pers. obs. [100]). It has a moderately-sized genome of 850 MB [101], including eight chromosomes, and is diploid throughout its range [98, 102, 103]. Current genomic resources include 41 normalized transcriptomes [104], a microarray [105] and associated gene expression information in four environments (Dlugosch et al, in prep), draft genomes of native and invading accessions (Dlugosch et al. in prep), genome-wide Restriction site Associated DNA Sequence (RADseq) markers which have been used to create a genetic map (Dlugosch et al, in prep) and to survey population genomic variation across the worldwide range [4].

PRELIMINARY DATA
Plant evolution When YST genotypes from the native and invaded ranges are experimentally grown in the same environment, genotypes from the invaded range grow larger both above and below ground, under a variety of experimental conditions (Fig 2A) [34, 98, 106–108]. Larger size is associated with
earlier flowering and increased reproduction (Fig 2B), implying a fitness benefit and opportunities for increased population growth and spread [34, 106]. My lab and I have been studying these patterns of trait evolution in YST, their origins, and the ecological changes that have facilitated increased resource acquisition during the invasion [2–4, 34, 104].

An important product of this work is our recent resolution of the sources of invasive YST in the western United States. Using a large population genomic dataset, we identified several regions of the native range with divergent YST populations, and genotypes in the severe invasion of California were found to be derived almost exclusively from native genotypes in western Europe [4]. This information now allows us to infer specific evolutionary changes in the invaders relative to their progenitors. It has revealed that there have been least two transitions to larger size in the severe invasion of California. We have mapped a major locus responsible for this size increase in the plant (Dlugosch et al, in prep) and are currently mapping loci responsible for variation in immune function.

If larger size has evolved in YST because it confers an advantage in the invaded range, then screens for genomic signatures of natural selection could identify potential pathways and functions involved. We are identifying loci with evidence of exceptional losses of diversity due to putative selective sweeps (Fig 2C) and loci with significant shifts in gene expression in the invasions relative to western Europe (Dlugosch et al, in prep). Loci with putative defense functions are the most consistently differentially expressed group in our analyses, and candidates for selective sweeps include multiple loci with putative functions in growth-defense crosstalk (e.g. these include a cytochrome P450 [109], MAP kinase [110], auxin response factor [111], and an MLP-like protein [112]). One of our candidates aligns closely with the gene UGGT (UDP-glucose glycoprotein glucosyl transferase) in Arabidopsis thaliana. UGGT has been characterized as part of the pattern recognition receptor machinery that identifies bacterial infections as part of plant innate immunity [113, 114].

![Fig 2. YST shows rapid evolution in (A) size and (B) fecundity, and (C) we have been identifying loci with evidence of selective sweeps (blue).](image)

**Geographical variation in the YST-associated microbiome** Previous work by Dr. K. Andonian (a postdoctoral participant on this project) has indicated that invading YST has more favorable ecological interactions with its microbial community. His experimental tests of plant-soil interactions showed that 1) YST experiences net fitness reductions when grown in its local soil communities, 2) these negative effects are weaker (more favorable) for invaders in the invasion of California, USA, and 3) negative interactions with the soil community increase over generations of YST growth in the soil [88, 89, 115, 116]. Further experiments with fungicide treatments have revealed that fungal interactions are not likely to be the component of the microbial community that is driving more favorable interactions in this invasion [88].

My lab has now sampled bacterial communities associated with leaves and roots of YST in both the California invasion and its source region in Europe, using high-throughput sequencing of prokaryotic ribosomal 16S sequences. This is one of the first comparisons of plant microbiomes between invading populations and their native source region (see also[117]). We found clear differences in bacteria associated with native and invading YST (Fig 3A) [2]. Within plant compartments, bacterial diversity...
ranged from 18-40% lower in invaded range populations in the community overall, and specifically in pathogen-containing clades in the root endosphere (Fig 3B). This variation in diversity is similar in scale to other studies that have sampled distant geographic locations (e.g. across regions of North America [118, 119]). A variety of factors may explain this pattern, including environmental differences across sites [18, 21, 120, 121]. Soil type appears to have a particularly strong influence on microbial communities [24, 122], and is known to differ broadly across YST’s range [88].

We also observed an effect of plant genotype on the microbiome, such that for populations within each range, bulk microbial diversity in the roots was predicted by plant diversity. Such within-species plant genotype effects have been observed in other studies and may interact with the effect of environment to shape microbial communities [119, 123]. Our pending renewal to the USDA focus on quantifying population-scale variation in the YST microbiome and its potential relationship with factors including soil, climate, and plant diversity.

![Image](image-url)

**Fig 3.** (A) NMDS plots of bacterial OTU composition in phyllosphere (green), rhizosphere (light blue), and root endosphere (dark blue) samples for native (open symbols) and invading (closed symbols) populations. (B) OTU diversity of pathogenic clades in the root endosphere. (C) Evidence of trade offs between size and immune function.

**Variation in plant immune response** We have begun to assay immune responses in YST by examining the ‘oxidative burst’ that is created during plant triggered immunity and hypersensitive responses in reaction to wounding and bacterial infection [124, 125]. This response is a localized production of reactive oxygen species (ROS) used to hinder pathogen invasion [126]. The oxidative burst consists of superoxide radical (\(\cdot O_2^-\)), hydrogen peroxide (H\(_2\)O\(_2\)) and hydroxyl radical (\(\cdot OH\)) which are released from NADPH oxidase and the apoplastic peroxidase enzymes (cite). The apoplastic peroxidase enzymes have previously been used to measure plant immune response to pathogens in *A. thaliana*, using a high-throughput assay of peroxidase activity in leaf disks, which results in a quantifiable color change [127]. We recruited this assay to compare the oxidative burst of native and invading YST genotypes in response to pathogens cultured from YST leaves. We found consistent differences in the influence of both plant genotype and bacterial strain on this aspect of immune response [3]. In particular, genotypes from the native European range had a higher peroxidase response to both wounding and bacterial infection than genotypes found in the invaded range. These patterns were consistent with a potential negative trade off between immune response and growth in YST (Fig 3C).

**PROPOSED RESEARCH ACTIVITIES**

**AIM 1: Plant-soil feedbacks** YST has already been observed to experience negative plant-soil feedbacks across its range, with weaker negative interactions in the first generation between invaded range plant genotypes and invaded range micro-organisms [88, 89, 115, 116]. Here these experiments will be extended to include plant growth and fecundity responses to reciprocal combinations of native and invaded range plant genotypes and microbial communities, followed over four generations. The root microbiome will be sampled at each generation to reveal the composition of interacting microbial taxa, its response to plant genotype, and its change over generations of feedback with native and invading YST.
Collections: Soil microbial communities will be collected from both the native and invaded ranges for use in these experiments. Four sites in western Europe (the source region for the invasion) where we have previously surveyed the YST microbiome in detail will be revisited (SAL, GRA, CAZ, SAZ; [2]). Four invaded sites from the previous survey that span much of the California invasion will also be revisited (DIA, CLV, TRI, RB). At each site, we will collect 15L soil from the top 15cm (as in e.g. [116]), from at least five grassland locations that appear not to have been recently disturbed (avoiding sampling directly under YST plants, which is possible because YST is patchy on a fine scale, and requires disturbance to germinate [82, 88]). Soils will be collected in the summer (June) and allow to air dry before shipping to UA. Air drying mimics the natural conditions during summer drought in these Mediterranean-type climates (when YST completes reproduction and senescence before germination begins with fall rains), and air drying has similarly been employed in previous YST-soil interaction studies [89, 115, 116]. Samples from across all collections in each range will then be bulked and well-mixed to form representative soil mixes for experiments. Soil microbiomes from these collections will be sequenced (see below) and plant microbiomes from each of these field sites will be included for reference alongside sequencing of microbiomes in experiments.

Experimental design: Native and invading YST genotypes will be germinated into soil microbial community treatments in 400ml deepots (Stuewe and Sons). Soil composition will be a 3:2 mixture of soil:sand, using soil from the invaded range, sterilized by triple autoclaving; [89, 115, 116]. Microbial communities will be added as an inoculum treatment. To create the inocula, live (unsterilized) soil will be mixed to a slurry with sterile water in a 1:5 ratio and filtered through bridal veil mesh to remove large soil particles. Each pot will receive either 15 mL of inoculum or 15mL of sterile water slurry with sterile soil. Inocula will come from either the invaded range bulk collection, the native range bulk collection, or a 50:50 combination of native and invaded range soils to form a microbial treatment that includes taxa from both ranges, with a control treatment receiving only sterile soil and inoculum. Pots will be watered by fine spray from above and a layer of sterile sand will be applied to the top of each cone to prevent splashing of soil among cones [89, 115, 116]. Each soil treatment will be planted with native and invading YST genotypes, initially from a single exemplar source population in each range, produced by crossing parental plants in a common greenhouse environment to minimize maternal effects (GRA and CLV, progeny already available in the Dlugosch lab). Additional pots will include plantings of a native bunchgrass that co-occurs with YST in North America, Stipa pulchra, whose interactions with the microbial community are known to differ from YST [128]. Grass pots will be used to provide a non-sterile reference soil feedback treatment for comparison to non-sterile YST feedback soils and sterile control soils in each year of the experiment. This will result in blocks of 11 plant-soil treatments (4 soil inocula [native/invaded/combined/sterile] x 2 YST sources [native/invading], plus grass in 3 non-sterile inocula), which will be replicated 30 times (330 total) in the first year (Fig 4).

![Fig 4. Design for plant-soil feedback experiment block, with native (N), invading (I) and combined (C) treatments.](image-url)
To sample the root microbiome, half of the YST plants in each treatment will be harvested at eight weeks of age, when root and shoot growth are active and plants have not yet maximized size in this container volume [34] (Dlugosch per obs). Aboveground biomass will be harvested and dried to quantify plant performance. Roots will be collected for both rhizosphere and endosphere fractions (as in our previous studies [2] for microbial sequencing as described below. The remaining plants will be maintained through flowering, and the production of flowering heads counted and final biomass harvested. Soil in these pots will be allow to dry, and then will be bulked by plant source [native YST / invading YST / grass] for use as an inoculum in the next generation.

Feedback experiments will proceed by growing YST from the same seed lots (i.e. with no change in plant genotype frequencies) in soil inoculated with their 3 non-sterile soil treatments from the previous generation, as well as in sterile soil, and soil grown with grass in the previous generation. Grass will be grown in its own feedback lines with the three starting non-sterile soil inocula. This will result in blocks of 14 plant-soil treatments (4 soil inocula [sterile, and inocula from YST feedbacks with 3 non-sterile inocula] x 2 YST sources, plus grass in soil from grass feedbacks from 3 non-sterile inocula, plus 3 grass feedback inocula x 2 YST sources), replicated 30 times (420 total) in the second and third generations (Fig 4). In the final generation, three additional populations from each range (total includes all four populations providing the original soil collections) will be grown in feedback soils, to test for population variation in response to feedback soil communities. This will result in blocks of 56 plant-soil treatments (4 soil inocula [sterile, and inocula from YST feedbacks with 3 non-sterile inocula] x 8 YST sources, plus 3 grass feedback inocula x 8 YST sources), replicated 15 times (840 total).

**Microbial amplicon sequencing:** DNA will be extracted from root rhizosphere washes and root endosphere tissue for sequencing of the microbial community, as I have done previously [2] using modified versions of protocols by [129] and [44]. Briefly, this involves collecting the upper 2-5 cm of the taproot, together with accompanying lateral roots. Tissue will be shaken in sterile wash solution to obtain the rhizosphere compartment, and root tissue surface sterilized for extraction of the endosphere. DNA will be extracted using standard MOBio PowerSoil kits.

Each collection of YST-soil combinations will include 15 replicates. These will be bulked by groups of five, to obtain 3 replicate bulks of each treatment in each year. In addition, three replicate samples of the bulk soil treatment inoculum at the start of the experiment and the start of each feedback year will be included. These collections will yield a total of 57 DNA pools for sequencing in the first generation (3 replicates x 3 soil inocula, plus 3 reps x [8 YST-inoculum combinations x 2 plant compartments]), and 87 in the remaining years (3 reps x 7 soil inocula bulks, plus 3 reps x [11 regional YST-inoculum combinations x 2 compartments]).

To identify microbial taxa, the v4 region of 16S bacterial rDNA will be amplified to identify the bacterial community, as in my previous work [2], and the ITS1 region of fungal DNA will also be amplified to identify the fungal community [as in 117, 130, 131]. All DNA will be archived for any potential future investigations of other amplicons or metagenomics sampling (and included in this study if continued improvements in sequencing costs make this feasible). Reads will be quality filtered and clustered (97% threshold) using tools from seqtk, USEARCH, and QIIME [132–134]. Samples will be rarefied by plant compartment (rhizosphere and endosphere), subsampling to the minimum number of reads necessary to include all samples, excluding any that are outliers for low read count.

**Analyses and predictions:** This experiment will identify the microbial taxa that accumulate when grown with native and invading genotypes of YST, the effects of microbial inocula on YST growth and reproduction, and variation in these microbial community dynamics and fitness effects over three generations of feedback. Root-associated microbiomes in each treatment and plant compartment will be compared overall using NMDS ordination, testing for a linear effect of generation using the envfit approach in R/vegan [135]. Composition will be analyzed both for the total community and for predicted pathogens specifically (based on FAPROTAX assignments [136]). Non-parametric tests will be used to compare metrics of OTU diversity among treatments and plant compartments. Significant differences in the representation of reads for individual OTU will be analyzed using linear models, recruiting the powerful analytical machinery of RNoSeq tools, which have been formulated precisely to test for significant differences in a the representation of a sequence given a finite sequencing effort of each DNA.
pool. Specifically, the Tuxedo pipeline [137] will be used to align reads to the reference set of OTU to obtain recounts per sequence effort (rpkm) and calculate differential representation (see additional detail in AIM4 below). The counts will be imported into R/limma [138, 139] for linear model analyses of OTU representation as a function of fixed effects of inoculum source, plant source, generation of feedback, and their interactions. Similarly, plant biomass will be compared among treatments using a linear model, with fixed effects of soil inoculum, plant source, generation of feedback, and their interactions.

Given past work demonstrating significant differences in the effects of native and invading soil inocula on YST growth, and my recent discovery of strong differences in native and invading bacterial communities, I expect to recover differences between native and invading range microbial communities, their accumulation of taxa during feedback with YST, and their effects on plant traits. The design will allow identification the major taxa involved and tests to determine whether the same taxa accumulate in each treatment over time, or whether the composition of the community is dynamic. The use of a combined native+invaded range soil inoculum will allow for tests of the effects of plant genotype on microbial recruitment across all microbial taxa present in both ranges. Sterile vs. non-sterile soil treatments will reveal the net impact of interacting with a microbial community, and grass vs. YST feedback treatments will provide a comparison of YST vs. non-YST feedback effects over time. YST plants are predicted to have lower biomass and reproduction in non-sterile soil, and in YST feedback soil vs. grass feedback soil. The relative performance of native and invading YST genotypes with native and invaded YST soil inocula will allow for tests of alternative hypotheses of enemy escape, increased tolerance, or increased efficiency (Fig 1).

**AIM 2. Selection on the microbial community to favor YST growth** The plant-soil feedback experiment in AIM1 will be leveraged to generate lines for selection on the microbial community to maximize growth in YST native genotypes, with no change in YST genotype composition. The microbial taxa contributing to this microbial selection line will be identified, and growth benefits to YST compared to the effects of selection on the plant genotypes (see AIM3) to disentangle the relative potential contributions of changes in the microbial community and changes in plant genotype to the invaded range YST-microbial interaction.

**Experimental design:** In the first year of the AIM1 experiment, the three fastest growing native plants in non-sterile soil will be identified for use in a selection line. These microbiome samples will be sequenced separately from the remainder of the AIM1 experiment and half of the soil from these plants used to make a separate soil inoculum pool for the AIM2 selection line. In the subsequent four generations, 30 native genotypes (same seed stock) will be grown in feedback with this community, each generation sequencing the root microbiome of half (15) of the plants (as in AIM1), but selecting the soil from only the top three growers for use as inoculum in the next year. Microbial composition of the soil from selected plants will be analyzed separately, and compared to three pools of non-selected soil each generation. This experiment will result in 6 additional individual rhizosphere and endosphere samples in the first year, and 12 samples per generation in generations 2-4 (3 non-selected soil pools, 3 selected soil samples, plus 3 pools of roots samples x 2 plant compartments).

**Analyses and predictions:** Microbial composition and plant traits will be quantified and analyzed as in AIM1. Specific comparisons will include changes in the microbial composition of the selection inocula over time, taxa with significant increases in representation, and changes in plant growth and reproduction over time. Plant traits will be compared with native YST genotypes in the AIM1 treatments (and with selected plants in the AIM3 experiment) to quantify the extent of increase in YST performance possible under microbial selection in this experiment.

**AIM 3. Selection on plant genotypes to favor YST growth** Parallel selection experiments with a recombinant F2 YST population will be conducted under both native and invaded range soil communities. Allele frequency differences in the plants with the highest growth rates, relative to the entire population, will be used to identify loci involved in the rapid evolution of increase plant growth, with a particular interest in the representation of loci already identified as candidates underlying growth evolution and with
evidence of selection in the invasion. Selected loci that differ between the two experiments will be candidates for growth-related loci that are involved in response to microbial interactions.

Selection experiment: A large F2 stock of seed derived from a cross between an outbred invading and an outbred native genotype of YST is already available in the Dlugosch lab. These seeds comprise a large panel of combinations of native and invading alleles within individuals plants, on which selection can be imposed to identify evolutionary response of plant traits and the changes in allele frequency associated with trait changes. For each generation of selection, 300 F2 plants will be grown (methods as in A1Ms1-2) with native soil inoculum and 300 with invaded range soil inoculum. The 10% of plants with the highest growth rate in each treatment will be crossed to form the next generation of 300 plants, for a total of four generations in the present study. (Long term propagation of this selection line is anticipated). Soil inocula will not be evolved: all inocula will come from the same initial (dried) bulk collection, and sequenced each year to monitor any changes over time in storage. Leaves from each plant in each year will be dried and stored for genotyping. Root collections for future microbiome sampling will also be saved from all plants not selected for crossing each year (as these can be sampled destructively).

Plant genotyping: Plants will be genotyped for genome-wide markers using double-digest Restriction Site Associated DNA sequencing [RADseq; 140, 141], which Dlugosch has already optimized in the YST system [4]. In this method, DNA from each individual is fragmented using restriction enzymes (here Psfl and MseI), individual DNA barcodes and priming regions are ligated onto the restriction sites, the fragments are amplified, and the fragments are sequenced starting from the restriction sites. Because all individuals will generally share the same restriction enzyme recognition sites, the resulting sequences will be derived from the same location across individuals and will align among samples. The single nucleotide polymorphisms (SNPs) in the sequences shared across individuals can then be used as genetic markers for further analyses [142, 143].

To maximize the sequencing of informative loci and the number of individuals that can be sequenced, baits will be used to capture a subset of polymorphic loci across individuals [RADcap; 144]. Dlugosch has already developed MYbaits capture baits (MYcroarray, Ann Arbor, MI) to target 10,000 variable sequence fragments, allowing multiplexing of 300 YST individuals on a lane of Illumina Next-Seq 2x150bp (Lu-Irving & Dlugosch, unpubl data). With this approach, two lanes of sequencing will be required each year. Sequences will be filtered for quality and adapter sequences using our rigorous SnoWhite cleaning pipeline [104]. SNPs at each RADseq locus will be identified using a combination of the software STACKS [145] and our own existing scripts.

Analyses and predictions: Differences in allele frequencies in the high growth cohort (selected for breeding) in the first year, relative to the entire population, will be used to quantify the strength of selection at each locus (cite/method). The distribution of selection estimates will be used to identify outliers showing evidence of strong selection relative to the rest of the genome. The same analysis will be applied to the final year of selection in this study, when power to detect these allele frequency shifts relative to the starting population should be greater after three generations of selection. Regions under selection that are related to growth alone and are not influenced by microbial community treatments are expected to respond similarly in both treatments. Candidate regions related to differential response to microbial interactions, with consequence for growth, are expected to differ between the treatments, and treatment differences will be tested directly using linear models of response to selection as a function of treatment, with correction for multiple tests to maintain an overall error rate under 5%. Of particular interest will be the percent variance explained by loci putatively related to microbial interactions vs. those with effects on growth independent of microbial interactions, and the concordance between candidate loci from this experiment and candidate loci already identified in our screens for selection in invading YST.

The distribution of plant growth traits will be analyzed each year for response to selection and heritability under both soil inocula treatments. Under the hypothesis of enemy escape (Fig 1A), interactions with the native microbial community should oppose increases in growth more strongly than those with the invaded range community, due to poor performance of poorly-defended genotypes, though this may not be apparent in the experiment if selection is via effects of pathogens on fecundity (a null result which will be informative in conjunction with growth and reproduction data from AIM1). Under
alternative hypotheses (Fig 1B,C), there should be few consequences of microbial community variation for response to selection under constant experimental conditions.

**AIM 4. Plant gene expression** The plastic and evolutionary response of individual plant genes to microbial interactions in AIM1 and AIM3 will be quantified through gene expression. Both root and shoot gene expression will be surveyed to capture difference in soil interaction and growth related functions.

**Sampling:** In AIMs1, plants harvested for their root microbiomes will have leaf and root tissue flash frozen in liquid nitrogen for RNA extraction. RNAseq analysis will focus on differences between native and invading genotypes growing in natural native and invaded soil inocula in the first year. In each treatment, samples will be combined into three pools with even representation of five plants each, for a total of 24 samples (3 reps x 2 plant compartments x 4 treatments). In AIM3, among the 270 destructively sampled plants in each treatment, root and shoot material will be flash frozen and combined into bulks of even quantities of five plants each by size. Among the plants not sampled and saved for crosses to select the next generation, only leaf samples only will be collected. RNAseq analysis will focus on the first and final generations in this study, for each inoculum treatment. In each treatment and year, three bulks of slowest growing plants (root and leaf), will be compared to three bulks of the selected plants (leaf only) and three bulks of the fastest growing plants among those that were not selected (root and leaf), for a total of 30 samples.

**Gene expression:** RNA will be extracted from all samples (Spectrum Plant Total RNA Kit, Sigma-Aldrich), quantified (Qubit, Invitrogen) and submitted for library preparation and sequencing on the Illumina NextSeq500 2 x 150bp at Arizona State University’s CLAS Genomics Core facility. I have found this workflow to be extremely reliable for RNAseq of YST and many other species in the ongoing EAGER-NEON project. Raw sequence will be filtered for adapter sequences and base quality using the SnoWhite pipeline [104]. Reference de novo transcriptomes are already assembled for YST and available to guide differential expression analyses [104], which will use the Tuxedo pipeline [137]. This pipeline indexes the reference de novo transcriptome using Bowtie2 (bowtie2-build) [146], aligns reads in each treatment separately to the bowtie-indexed transcriptome with TopHat2 [147], and then merges reads from treatments into a single transcript annotation in Cufflinks [137], providing a basis to compare fragment alignments output from TopHat2 and calculate differential expression [148]. Significantly differentially expressed transcripts (quantified as log$_2$(FoldChange) between treatments) will be identified with Cuffdiff2 [148, 149].

**Analyses and predictions:** Analyses of AIM1 plants will focus on identifying evolutionary and plastic differences in native and invading plant genotype responses to microbial communities. Evolutionary differences will be tested by identifying significantly differentially expressed genes between native and invading plant genotypes in the same soil microbial environment. Plastic responses will be identified as differentially expressed genes within a plant genotype in response to native and invaded range soil communities. Overall plasticity in these responses can be compared between native and invading genotypes, quantified as the fraction of differentially expressed genes and as the magnitude of log$_2$(FoldChange) absolute values [150]. Plasticity differences at specific loci will be assessed with linear models corrected for multiple comparisons in edgeR [151]. Of special interest will be evidence of differential expression in candidate loci from our ongoing analyses of evolution in the YST invasion.

**AIM5: Bacterial culture and immune response assays by Undergraduate Scholar Scientists (USS)** Any future experiments to identify the detailed mechanisms of plant-microbe interactions, or quantify their impacts on plant fitness independently and as parts of larger microbial communities, will require propagation of strains in culture. While only a small fraction of microbial taxa have been grown in culture, many of the major bacterial pathogen groups observed in our studies of YST to date have been propagated [17], and it will be of particular interest to establish which of the common YST-associated strains in our AIM1&2 experiments are able to be cultured. These activities are ideal for undergraduate projects, and students involved in the Scholar Scientist program (detailed further below) will take the lead on these activities as independent research.
Cultures: Students will culture and identify individual strains of bacteria using protocols that we have previously developed [3]. Students will collect five different 1 cm sections of root from a plant already being harvested from one of the treatments above, homogenize these in MgSO4 buffer, and plate the resulting solutions in a dilution series onto KB agar. They will re-plate the resulting colonies separately to propagate individual taxa, and photograph their morphology. Approximately 10 individual cultures per student will be identified to strain using Sanger sequencing of the 16S v4 region as well loci diagnostic for strains within larger taxonomic groupings and which are commonly used in multi locus sequence analysis (based on information in the Plant Associated and Environmental Microbes Database; e.g. [3, 152]). The students will be assisted to align their DNA for identification and research what is known about the taxa indicated. The 16S sequences of cultured strains will also be aligned with OTUs recovered from the AIM1&2 experiments to identify their abundance in these source microbiomes.

Experiment and predictions: The students will assay the interactions of YST genotypes with their cultured bacteria. They will germinate plants at the beginning of the semester, and collect leaf disks for assays of the oxidative burst in response to their strains, as described above in preliminary data [3]. The students will have opportunities to test plant genotypes from our large panel of collections (now 51 source populations in the western US, 10 sources in Argentina, and 20 sources in Eurasia). They will be assisted to analyze differences in response among plant populations and strains using linear models that predict absorbance (peroxidase activity) as a function of fixed effects of source population, bacterial strain, and their interaction, with a random effect of plant individual. In general, invading genotypes are predicted to respond more weakly to bacteria than native strains, but plant-microbial interactions can be highly variable among individual plant genotypes and strains, and students will examine their data for evidence of divergent interactions. The students will interpret their data in terms of evidence of plant immune response (PTI) versus bacterial avoidance of immune response (ETI) from the assay, with additional context about the abundance of that strain in plant rhizosphere and endosphere samples from the AIM1/2 experiments.

This research is an outstanding microcosm of the concepts that the students will be exploring throughout their core course requirements in ecology, genetics, and evolution, allowing discussion of connections with course material throughout the year. The collective work from the USS scholars across five years and beyond will in turn contribute directly to this research program by building a culture collection for YST-associated taxa, as well as associated information about potential immune system interactions between plants and strains. These assays will generate opportunities for future targeted experimentation with YST-strain combinations to investigate the distribution of standing genetic variation for immune response.

SIGNIFICANCE & INTELLECTUAL MERIT Some of the best evidence to date that microbial community interactions can naturally vary among populations, generating significant impacts on plant growth, has come from the study of introduced plant species in their native and invaded ranges [27, 53–59]. The proposed research will be among the first to characterize the microbial community responsible for natural variation in microbial interactions and feedbacks with plant genotypes, to identify the plant loci involved in plastic and evolutionary response to this community variation, and to test alternative hypotheses regarding how microbiome variation can contribute to evolution along growth-defense trade offs. These contributions will fundamentally advance our understanding of the nature of selection on growth and defense generated by interactions with a complex microbial community.

FUTURE DIRECTIONS – RESEARCH The proposed research creates a foundation for a career of investigation into the evolution of plant-microbial interactions and their ecological importance in the YST system. Long term goals include elucidating the mechanisms of evolution along growth-defense trade offs (through the identification of specific genetic variants involved in YST response to microbial interactions, and detailed functional and evolutionary characterization of these loci), the ecological costs and benefits of evolution of growth-defense traits (through laboratory and field experiments, particularly to test for the mechanisms hypothesized to generate the alternative predictions in Fig 1), and the consequences of these evolutionary changes for population growth and spread (through field experiments and
PROPOSED EDUCATIONAL DEVELOPMENT  Popular press in recent years has highlighted the growing graduation gap between high and low-income undergraduate students, the increasing financial cost of those failed efforts to gain a degree, and the worsening consequences of lacking a college degree in today's economy [153–158]. These issues are a central concern for me as an educator, as I have recently begun co-teaching our large (~200 person) core course in Ecology, required for all of our biological science majors. Students with poor grades in my course are likely to be prevented from pursuing the biology degree that they had hoped for. For students transferring from community colleges (~30% of my class each year), my course may be among their first at a large research university, and so has the strong potential to become a stumbling block in their career. The reasons for poor completion rates among disadvantaged college students are undoubtedly complex, but research in educational psychology has suggested ways in which targeted experiences that support empowerment and resilience in students can have long-lasting positive impacts on educational achievement [e.g. 159–162]. A key path to student engagement at a research university can and should be through exceptional research opportunities, which I propose here.

I propose to invite applicants from my Fall semester core Ecology course (ECOL302) for participation in a small group (up to 12 per year) of Undergraduate Scholar Scientists (USS). The goals for the USS experience will be 1) to support student success in pursuing independent projects in which they can place personal investment and derive confidence, 2) to connect those projects to general concepts in ecology, evolution, and genetics, and 3) to give the students the opportunity to contribute to genuine advancement of science in an active research laboratory. To reach these goals, the students will complete individual projects described in AIM5 above as a cohort during Fall semester, and present their completed projects at our Spring undergraduate poster session. Execution of their research would be facilitated by a dedicated graduate Research Assistant, and they would meet bi-weekly with the RA and myself to discuss primary literature that is related to their projects and to the material that they are studying concurrently in the core courses. The students would receive course credit for Independent Research throughout the academic year.

Selection of the USS would be based on the potential for the experience to impact their advancement in any area of biological sciences (including medicine) or biology education. The target group will be underrepresented and/or underprivileged students who have a strong academic history and interest in biology, but little experience with independent research. Students newly transferred to UA will be given particular preference, as will low-income students already identified by our Arizona Assurance program. We do not currently offer any other experience like this proposed program. There are no independent research experiences of any kind linked to the core Ecology course (or any other core course, to my knowledge). We do offer a supplemental paper discussion group for Honors students, to fulfill Honors credit for the course. This option is not available to all students, and does not target underprivileged or underrepresented students. The proposed USS program would be a new development, which will evolve a leadership component as more hands-on research is integrated in the core course itself into the future (see Future Directions - Education below).

EDUCATIONAL ASSESSMENT  The goals of assessment in the USS program will be both formative, to aid the students and myself in improving student confidence and success with core course material during the progress of each USS year-long program, and summative to assess the contribution of the program to student degree completion in the biological sciences. These assessments will take the following forms:

Formative Assessments

- FA1) Open discussion of questions and concerns with both research and core course material during the bi-weekly group meetings with the RA and myself. While I find that students are often initially reluctant to enter into dialog, even at the graduate level, this is essential in engaging with
their own learning and allowing me to assess their state of knowledge and uncertainty. I will use open ended questions and encouragement of active participation and discussion to draw students out and assess their progress.

- **FA2) Self assessment.** I will ask the students to fill out a written questionnaire about their goals, challenges, and objectives (sensu [163]) for both their research project and core biology courses at three times during each semester (beginning, midterm, and end). I will meet with each student individually to discuss strategies for meeting their goals after each evaluation.

- **FA3) Peer assessment.** USS students will present their research finding to the group at the end of Fall semester, their poster content to the group early in Spring semester, and their practice presentation of their posters before the final public poster session. At each of these meetings, the other students will be asked to provide both verbal and written feedback for their peers.

**Summative Assessments**

- **SA1) I will evaluate the performance of the USS relative to their peers in the Ecology core course.** We currently evaluate summative performance in the course with four exams, a scientific writing assignment, and a final course grade. These metrics for the USS will be compared with the distribution of the class as a whole, as well as a distribution derived from a statistical re-sampling (bootstrapping) of scores from other applicants to the USS program itself (which should provide the most comparable group).

- **SA2) Core course completion and UA graduation rates will also be compared against distributions from all biology majors, individually for each cohort year.**

- **SA3) For a final assessment of learning from the USS research itself, I will complete a written evaluation of their final poster projects in terms of completion, understanding of content, and presentation.**

- **SA4) Students will complete standard UA summative evaluations for the independent study course, to which will be added questions about their perceived value of the USS experience and its impacts on their future educational plans.**

**FUTURE DIRECTIONS - EDUCATION** The USS program will continue beyond the duration of this CAREER award as the students assume a leadership role in conducting these projects with the rest of the Ecology class as a whole. The USS students will continue have responsibility for handling the cultured strains and learning laboratory techniques and analyses ahead of the rest of the class, and they will meet as a group throughout the year to discuss and present their work. Each laboratory section (max 14 students) of the remainder of the class will plate root sections, analyze sequence data for identification, rear plants and collect tissue for the peroxidase assay. The USS students will visit lab sections to discuss the experiments and to assist the lab groups in methods and data analysis. A small increase in course fees (approx. $5 per student) will support the associated costs into the future. The expanded number of groups conducting research in the course will also expand the potential projects that can be tackled, for example culturing and microbial sequencing from future YST microbiome field collections and from microbiomes of other plant species in these communities.

**INTEGRATION OF EDUCATION & RESEARCH** The goal of my proposed career development is to identify genetic and evolutionary mechanisms that contribute to changes in plant traits and ecology. This goal meshes closely with the content of our three biology core courses of Ecology, Evolution, and Genetics. Since taking over co-teaching our Ecology core course, I have been working to bring strong evolutionary connections into my course content, reinforcing learning across the core areas. My goal with the proposed Scholar Scientist program is to develop experiences that will strengthen the success of underrepresented students that come into our program already academically strong and excited about biology, and enrich active learning for the class as a whole. These experiences will involve rigorous
independent research on my own study system at the interface of ecology, genetics, and evolution, advancing the research goals of my lab group while feeding back into my development as an educator.

**BROADER IMPACTS OF THE PROPOSED WORK** This research examines factors shaping fitness in a serious economic pest of rangelands, with potential opportunities for improved management. Through multiple types of selection experiments and establishment of a culture collection, the project will generate community resources for further experimental studies of evolution and targeted studies of plant-pathogen interactions. The project will also support the training of postdoctoral fellows, graduate, and undergraduate students in the growing areas of microbial biology, plant-microbe interactions, population genomics, and bioinformatics, to which I have consistently attracted female and minority trainees. A key component of the proposed work is the creation of an independent research experience aimed at improving confidence and STEM learning for underrepresented and underprivileged undergraduate students who desire to complete a degree in biology. Recent research has highlighted the poor graduation rate of such disadvantaged students, and the strong potential for targeted experiences to reverse this pattern. The proposed educational development will involve students in independent scientific achievement while also advancing the larger research aims. Lessons learned from these activities will inform future development of educational experiences in core courses required for the biology degree. Finally, this project will also support the career development of a female early career investigator.

**TIMELINE** The first year plant-microbial interaction experiment (AIM1) will be led by incoming student [personal details removed], with later years of feedback for AIM1 led by incoming student [personal details removed]. The postdoctoral associates will lead AIMs2-4 throughout all years.

![Timeline Diagram]

**RESPONSE TO PREVIOUS REVIEW** This is the third and final CAREER submission for this PI. All submissions have focused on leveraging aspects of the YST system to advance fundamental understanding of the evolution and genetics of ecological strategies. Previous reviews (each year by both the Evolutionary Ecology and the Population & Community Ecology panels) have been unanimously positive about the potential for the system to support novel and exciting research, and about its suitability for a CAREER proposal. In addition, the Scholar Scientist educational program specifically and Broader Impacts generally received unanimously positive reviews and no additional suggestions for improvement in the second submission. In response, these elements remain cornerstones of the current proposal.

Research in both previous proposals focused on the relationship between rapid evolution in plant growth and aspects of competition and ‘invasiveness’ in YST. Across both panel reviews, there was some debate about whether the research was sufficiently transformational, and there were several recommendations for expansion of the work (particularly into plant physiology by the PCE panel) and justification of the relevance of plant growth to invasiveness per se. Recognizing the concerns of previous reviews, the current proposal leverages new insights from my recent work to develop an entirely new career focus in plant-microbial interactions, that will be transformational not just to invasion biology but also to the broader understanding of growth-defense trade offs in plant responses to microbial interactions.