PROJECT SUMMARY

Instructions:
The summary is limited to 250 words. The names and affiliated organizations of all Project Directors/Principal Investigators (PD/PI) should be listed in addition to the title of the project. The summary should be a self-contained, specific description of the activity to be undertaken and should focus on: overall project goal(s) and supporting objectives; plans to accomplish project goal(s); and relevance of the project to the goals of the program. The importance of a concise, informative Project Summary cannot be overemphasized.

Title: **Direct and indirect effects of conserved and lineage-specific volatile organic compounds among eudicots for control of *Botrytis cinerea***

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CO-PD: PD/PI 7 Name (Last, First, MI)	**Institution:**

This postdoctoral project addresses the **plant health and production and plant products priority area** for two years under Dr. Daniel Kliebenstein at the University of California, Davis. The proposed project will empower the PD for a faculty position in ecology and evolutionary biology focused on plant phytochemical diversity's direct and indirect effects on plant-biotic interactions. In addition, this fellowship will provide extensive opportunities to build on their foundation of molecular and biochemistry skills by training in plant-fungal co-transcriptomics, fungal comparative genomics, and toxicology.

A recent focus of plant-biotic interactions comprises the utility of volatile organic compounds (VOCs) as multi-functional molecules, which can act as context-specific signaling molecules within and among species, have direct toxic effects on pathogens and herbivores, and can overcome vascular constraints in signaling among organs. In this proposal, we plan to assess the disease responses of VOC-biosynthetic enzyme expression in an association mapping panel of the generalist pathogen *Botrytis cinerea*, and a collection of eurosid and euasterid crops. We will identify and test lineage-specific and conserved VOCs for their ability to alter the growth and transmission potential of *B. cinerea* and identify genes associated with susceptibility towards VOCs in *B. cinerea*. Finally, we will assess the efficacy of VOC treatments pre- and post-infection with *B. cinerea* across the same collection of crops, providing fumigation guidance for VOC use in controlling *B. cinerea*.

Project Narrative

Introduction: In light of expected increases in pest and pathogen pressure and decreases in pollinator availability under climate change, we must harness plants' natural abilities to manipulate biotic interactions to increase sustainability and maintain high yields in our crop systems.[1–4] One system that plants have evolved to manipulate their biome is by creating volatile organic compounds (VOCs), specialized multifunctional metabolites capable of relaying dynamic and complex information across trophic levels.[5–7] Plant VOCs serve as direct signals of infection and damage, relaying complex cues across species boundaries, providing information to neighboring plants concerning pest and pathogen outbreaks.[6,8–12] From these chemical cues, neighboring plants enter a 'primed' state in which they can elicit a faster and more potent response when inevitably challenged with a biotic stressor (e.g., systemic acquired resistance(SAR)).[5,9,10,13] **In addition to volatile signals, VOCs can act directly by inhibiting pathogen growth or indirectly by altering the pathogen's transmission and infection success on new tissues.**[14–17] For example**,** cis-3-hexenol, linalool, and allo-ocimene(common VOCs found across plant tissues and species) reduce growth and spore viability in a generalist necrotrophic fungal pathogen *Botrytis cinerea* while also inducing SAR in several wild and domesticated plant species.[14–19] Thus, initiatives based on VOC use in agricultural practices are a promising avenue for sustainable agricultural research to reduce yield loss due to plant pathogens.[5,20] However, few studies have assessed variation in VOC efficacy as a biocide against genetically diverse pathogen isolates on various host crops.[18,21,22] As with any chemical defense or biocide, standing genetic variation in a pathogen combined with variation among hosts may create a traditional evolutionary chemical arms race.[10,23–27] In addition, current research often focuses on specialist pathogens with the common assumption of simple co-evolutionary models explaining the evolution of host-pathogen interactions. However, simple co-evolution is not comparable with generalist pathogens such as *B. cinerea*, which can infect over 1000 different plant species and has near-zero genetic indicators of host specialization.[28–30] Based on the ubiquity of VOC use across plants in pathogen resistance and defense signaling, the PD positions that they may play an integral role in generalist pathogen resistance.

In addition to VOCs, plant resistance consists of multiple layers of defense mechanisms, ranging from innate immune responses activated by classes or species of pathogens to defense strategies effective against single pathogen isolates. Resistance mechanisms can be present across multiple plant species or be lineage-specific even for conserved defenses such as non-host resistance.[31–33] Traditionally, the production of specialized metabolites and other resistance mechanisms are viewed as inherently metabolically costly, diverting resources from yield towards their production.[34–36]. Current efforts concerning SAR and induced defenses have inherent metabolic costs that are high in the short term and low over the plant's lifespan, as they include multiple defense mechanisms(metabolites, proteins, structural defenses).[37–39] VOC production may bypass these limitations as several studies indicate no significant reduction in yield from high VOC production.[7,40–42] Thus, VOCs may be harnessed as attractant or deterrent mechanisms at a low metabolic cost, with further potential to be exploited reactively via inducible SAR, providing a holistic plant-derived biocide.[5]

Preliminary Data: To test the potential of VOCs as novel mediators of plant-pathogen interactions, the PD will rely on a large body of published and unpublished integrative genomics, transcriptomics, metabolomics, and phenomics describing the interactions of *B. cinerea* across eudicots(Fig 1) to develop their research program centering, the use of VOCs in agriculture.

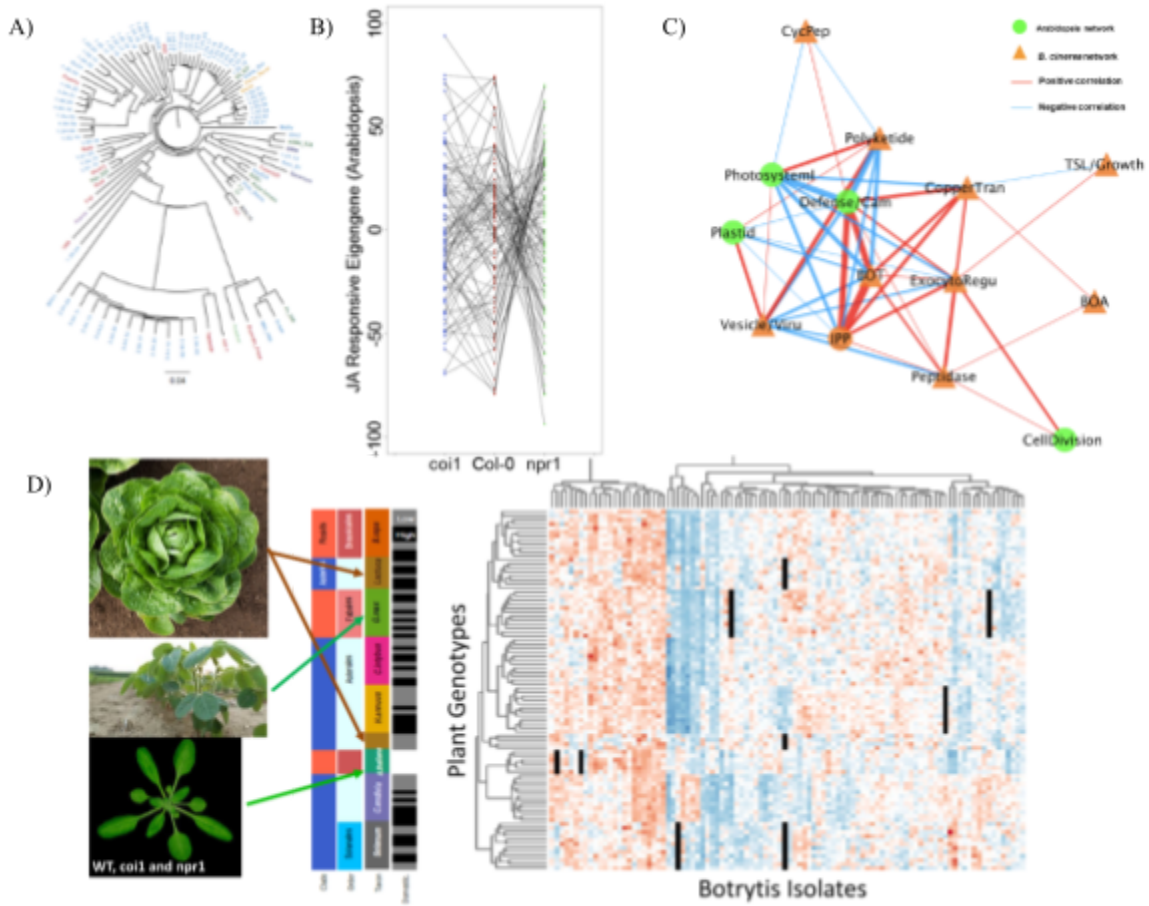


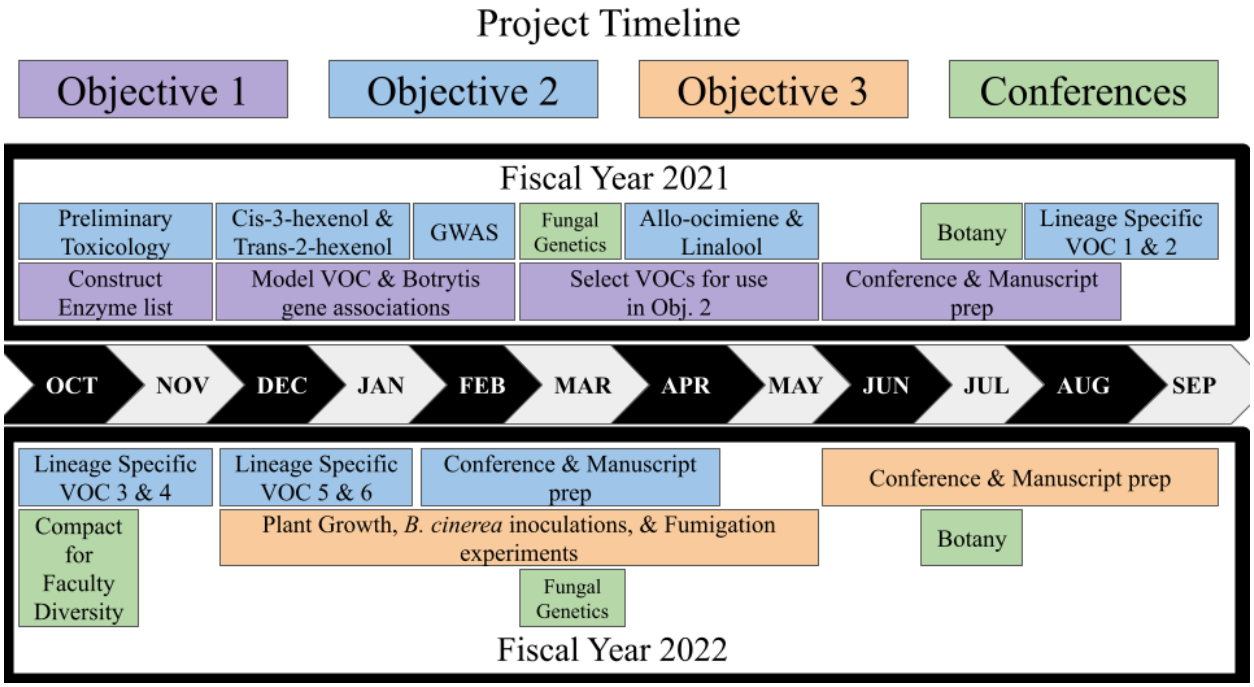
Figure 1. Summary of recent Kliebenstein lab efforts to untangle the evolutionary ecology of *B. cinerea* virulence across eudicots.

Figure 1A: The vast majority of branches in a Bayesian phylogeny of 96 isolates *B. cinerea* are not significant, supporting a high level of recombination in the species.[43,44] *B. cinerea* has extensive genomic variation and recombination supporting a wide range of virulence mechanisms.[43–48] Klibenstein has previously whole-genome sequenced all isolates with high coverage.[43–48] The diversity among isolates has multiple functional haplotypes at each locus and extensive recombination with linkage disequilibrium decaying within the distance of a gene.[29,43,44] The lab has since successfully mapped several traits such as host specificity and virulence with this population.[43,49,50] The lab's current genetic information consists of 271,749 SNPs with MAF greater than 0.20 and less than 20% of missing calls across the 42.9Mb B05.10 reference genome.[49] Among SNPs, half are in intergenic regions, with many in coding regions.[49]
Figure 1B: Kliebenstein conducted a co-transcriptomic study, where expression of a Jasmonic acid-responsive eigengene in response to infection by 96 diverse *B. cinerea* isolates indicated *A. thalania* has an isolate-specific and not a general *B. cinerea* response, contrary to assumptions.[45,51,52] The 72 most JA-linked genes were combined to create a single eigengene whose value was estimated in Arabidopsis WT Col-0, JA mutant *coi1*, and the SA mutant *npr1* indicating genomic variation in *B. cinerea* can shift JA's roles from inducive to repressive, and that plant defense pathways may interact with specific isolates to elicit an array of responses.[45]
Figure 1C: A co-transcriptomic meta-network of *A. thalania* host and *B. cinerea,* by Kliebenstein, implicates the manipulation of photosystem I genes during infection.[52]

Photosystem 1 links with the lipoxygenase pathway producing C6-aldehydes like cis-3-hexenol and other green leaf volatiles, pointing toward a mechanism of *B. cinerea* infections altering plant VOC profiles similar to observations in other plants.[17,41,53,54] **Figure 1D**: The lab conducted a meta-analysis of 96 *B. cinerea* isolates virulence on eight eudicots(six domestic and six wild genotypes across seven eudicots and Arabidopsis WT, JA, and SA mutants).[49] Domestication status did not affect lesion size; however, similar lesion patterns across isolates within a host species indicate host specificity without clear phylogenetic patterns.[49] Further, these data lay the groundwork for using *B. cinerea* as a model system to explore mechanistic relationships between plant defense and generalist pathogen diversity with extension across eudicots.

Rationale and Significance: **This project addresses the AFRI area of 'Plant Health and Production and Plant Products' as fungal infections, specifically by the generalist pathogen *B. cinerea,* pose a significant challenge.** *B. cinerea* can infect leaves, flowers, and fruits, severely affecting yield across hundreds of food, cash, plantation, and horticulture crops globally at every step of production, storage, and distribution**.** According to a 2012 study, *B. cinerea* was the second most important plant fungal pathogen with a global control cost of over $1,000,000,000 annually.[28] **Mycelial growth and spore viability are the primary targets of reducing disease severity and transmission in fungal infections, and because of the lack of large effect resistance genes in the host, this predominantly revolves around the identification of fungicides.**[1,3,28,55] However, *B. cinerea* has standing genetic variation enabling populations to adapt to fungicides rapidly. As such, there is a need to extend to find new sources of resistance. **The PD aims to harness VOCs to develop novel and innovative technology for sustainable control of major fungal pathogens using existing omics data to identify candidate VOCs.** Growing evidence shows VOCs can inhibit *B. cinerea* mycelial growth, spore viability and induce SAR. **The half-life of many VOCs in the atmosphere ranges from a few hours to a day, decreasing the risk of off-target effects due to environmental persistence.**[5,6,56] The PD's application of VOCs as natural plant-derived compounds with limited side effects has the potential to transform fumigation and plant breeding applications. This work has short-term goals of providing fungicides and SAR-inducing VOCs, which will further the PD's long-term goals of breeding plants for specific VOCs, which confer resistance at limited to no negative effects on yield and developing complex VOC mixtures based on pathogen populations.[5,6,14,16,16,18,40]

By studying a wide range of eudicots, the PD will identify a set of VOCs that may enable the formation of blends that could counter the tendency for pathogens to develop resistance. In addition to using multiple eudicots to identify different VOCs, it will be necessary to conduct dose-response and standing genetic variation assays on these compounds. The proposed work will allow the identification of VOCs effective at both low doses and have ubiquitous effects across all the genotypes of a pathogen. The extensive collection of specific *B. cinerea* isolates maintained within the lab provides a unique opportunity to assay both of these parameters to optimize any downstream application. These results combined lay the groundwork for the PD's future goals of VOC mixture modeling for fungicidal application in phytopathology, modeling potential resistance and susceptibility dynamics of future population genomic scenarios of *B.cinerea* and crops based on VOCs, and modeling the toxicity of plant organs based on VOC constituents for plant breeding initiatives.



Approach: The following approaches and objectives will allow the PD to specifically test and explore mechanisms related to their independent research concerning VOC-mediated plant-biotic interactions leveraging the resources and mentorship of Dr. Daniel Kliebenstein.

Objective 1: **Assess variation in conserved and lineage-specific expression of VOC biosynthetic enzymes across a diverse set of eudicots infected with diverse *B. cinerea* strains.**

Objective 2: **Identify the genetic architecture of *B. cinerea* variation in susceptibility to general and lineage-specific VOCs.**

Objective 3: **Validate the fumigation effects of select plant VOCs on reducing *B. cinerea* pathogenicity across eudicots.**

Objective 1: An understanding of the diversity of expression patterns of VOC biosynthetic enzymes elicited during B. cinerea infections across eudicots will help determine conserved and lineage-specific VOC biosynthetic enzyme expression patterns among *B. cinerea* infections. The PD will complete Obj. 1 entirely computationally, leveraging a co-expression dataset of 16 species (2 genotypes per species) of eudicots exposed to 72 strains of *B. cinerea* generated from NSF-IOS-1915886; these selected individuals are a subset used to assess quantitative virulence variation across eudicots among *B. cinerea* isolates (Table 1, Figure 1).

To survey relationships between potential VOC production during *B. cinerea* infection across the eudicots, the PD will explore associations between the expression of *B. cinerea* genes and plant VOC biosynthetic enzymes. For each species-specific expression data set, the PD will leverage open-source databases (e.g., Kyoto Encyclopedia of Genes and Genomes) to develop a list of enzymes responsible for producing 150 VOCs across six chemical classes (aliphatics, aromatics, fatty acid-derivatives, monoterpenes, diterpenes, and sesquiterpenes). We expect ~100-300 unique enzymes stemming from major pathways such as the MEP (monoterpenes, diterpenes), mevalonate (sesquiterpenes), shikimate (aromatics), lipoxygenase (fatty acid-derivatives), and the leucine and isoleucine biosynthetic pathways(aliphatics).[10,57] Narrowing down the analysis to predicted Plant VOC enzymes allows using a Bayesian approach to search for VOC gene expression to pathogen response. We will quantify linkages between plant VOC biosynthetic enzyme expression and *B. cinerea* gene

expression patterns through a Bayesian mixed linear model approach with one covariate, one random intercept, and one random slope. The complete model exists as Plant VOC enzyme Y expression ~ (1 | *B. cinerea* isolate) + (*B. cinerea* Gene X expression) + (*B. cinerea* Gene X expression | Isolate). Modeling *B. cinerea* isolates as a random intercept supply an estimate of the effect pathogen identity has on Plant VOC enzyme Y expression. *B. cinerea* Gene X is expressed as a fixed effect to estimate the effect of B. cinerea Gene X expression on Plant VOC enzyme Y expression. Finally, *B. cinerea* Gene X expression given *B. cinerea* isolate as a random slope provides an estimate of the effect of transcriptomic context of *B. cinerea* Gene X expression on Plant VOC enzyme Y expression. Models will be constructed and assessed in a combinatory fashion and compared in a Bayesian information criteria framework with Bayes factor analyses to examine model differentiation and selection evidence. We will also run the model using the virulence of the *B. cinerea* isolates in place of gene expression to identify plant VOC enzymes linked to *B. cinerea* virulence. Previous analyses demonstrate that simplification of random to fixed effects does not influence the effect size or significance of the estimates and dramatically decreases the computational requirements, so it will help us prioritize models for Bayesian analysis.[48–50] The *B. cinerea* genome consists of ~11,000 protein-coding genes, making pairwise and pathway comparisons of 100-300 VOC enzymes among each of the 16 plant species a tangible effort given the HPC computing resources at UC Davis. [58]An alternative to the proposed modeling efforts will use correlation networks between *B. cinerea* gene and Plant VOC enzyme expression similar to previous work (Fig 1).[45,52,59]

From the resulting modeling efforts, we expect, for each plant species, a list of VOC enzymes with expression patterns associated with the expression of specific *B. cinerea* genes, specific *B. cinerea* isolates, and/or *B. cinerea* virulence. We will compare results among plant species and select eight VOCs enzymes that have known or predicted products for use in Obj. 2. VOCs will consist of four with primarily positive coefficients of *B. cinerea* gene expression and four negative. Candidate VOC selection criteria will further be based on the VOC enzyme's distance to a terminal biosynthetic step, the number of *B. cinerea* genes with evidence as predictors of VOC enzyme expression, and ease of candidate gene biochemical validation using *in vitro* assays in *E. coli* and other suitable methods. As we have already identified a few general compounds, we will prioritize VOCs found in single plant species as they provide lineage-specific information. **Thus, the expected results of Obj. 1 are (1) a list of differentially expressed plant VOC biosynthetic enzymes across 16 plant species infected with 96 *B. cinerea* isolates of variable host-specificity and virulence patterns, and (2) a ranked list of plant VOCs for potential use as control agents against *B. cinerea*.**

Table 1. Plant Selection.

Family	Species
Cucurbitales	Cucumber
	Squash
Fabales	Soybean
	Common Bean
Brassicales	*A. thaliana*
	Brassica napus
Malvales	Cotton
	Hollyhock
Caryophyllales	Spinach
	Amaranth or Chard
Asterales	Sunflower
	Lettuce
	Chicory endiva
	Chicory intybus
Apiales	Parsley
	Celery
Solanales	Tomato
	Pepper

Objective 2: **To examine the range of VOC effects, we will compare the effects of plant VOCs on the direct growth, spore number, and viability of *B. cinerea* in a toxicology-based genome-wide association study (GWAS).** VOCs in Obj. 2 will consist of four known Plant VOCs that are major constituents across organs and species with observed positive (trans-2-hexenol) and negative (cis-3-hexenol, linalool, and allo-ocimene) effects on *B. cinerea* and eight VOCs selected from Obj. 1.[14–16,18,60] **The expected results for Obj. 2 are (1) dose-response curves**

for each VOC for each isolate and (2) a list of gene variant associations explaining effects of VOCs on aspects of *B. cinerea* pathogenicity.

The 96 isolates of the association mapping panel will be grown in the lab and assayed for VOC responses in 8-fold replication per isolate x VOC concentration. [43,44,50] This replication allows for the estimation of responses if heritability for VOC tolerance is ~25%.[58,59,61] *B. cinerea* mycelial agar plugs will be taken from the periphery of a growing colony and inserted into the center of a 9 cm petri dish containing PDA. Each dish will have a sterile paper disc attached to the interior lid of the dish containing 30 µl of the VOC solution. Treatment solutions will initially comprise four concentrations for each VOC, one control without treatment solution and one solvent control. Concentration ranges will be based on previously reported minimum inhibitory concentrations (MICs) for *B. cinerea* and other microbes when available.

To quantify variation in mycelial growth and VOC sensitivity, we will use an automated pipeline. The PD will re-purpose an automated leaf-lesion image analysis pipeline to measure mycelial growth at increments of 1, 2, 3, 4, and 5-days post-inoculation and treatment compared to positive and negative controls. [50] We selected time points based on observed recoveries of disease and lesion diameter incidence after three days relative to controls in a linalool fumigation study of strawberries.[18] The pipeline in its current form enabled the analysis of over 100,000 lesions across diverse plant species.[49,50] In addition to size, the pipeline also measures other traits such as eccentricity to provide unique insights into pathogen progression.[48] The PD has an extensive computer science background, and repurposing the pipeline poses little difficulty. If necessary, we will measure the radial growth of mycelia in ImageJ.[62] If we do not observe variation in mycelium growth among particular isolates, the experiment will be repeated as necessary with higher or lower concentrations of each compound. The MIC of each isolate will be modeled using logistic regression, developing dose-response curves for use as a function-valued trait in GWA.

Certain fungi compensate for hazardous conditions by investing energy into higher spore production, potentially increasing the transmission potential at the cost of decreased mycelia growth.[55,63,64] To assess post-treatment compensatory investment in spore production, we will determine spore count after five days. We will use sterile water to release spores from each plate. The resulting spore suspension will be filtered through glass wool to remove mycelial fragments and diluted counted with a hemocytometer. The same spores will be tested for viability using the protein stain sulforhodamine B (SRB) via a microplate reader according to the methods of Slawecki et al.[65]

To test the potential for VOC fumigation to influence pathogen transmission by reducing germination success directly, we will measure the dose-response effects of VOCs on spores. Healthy spores will be isolated from all 96 *B. cinerea* isolates grown for five days in unadulterated Potato dextrose agar(PDA) in this experiment. We will add 100ul of different VOC concentrations to the spore suspensions based on the observed MIC of mycelia and reports from the literature to each spore suspension. Spore viability and germination rates will be measured as described above. The toxicology experiments serve as a significant labor-intensive portion of this proposed work. However, we have allocated sufficient funds to support undergraduate researchers (URs) to help. **Obj. 2 will provide many direct, indirect, and function-valued phenotypes for a genome-wide association of direct and indirect effects of general and lineage-specific VOCs on *B. cinerea* pathogenicity and transmission potential**. GWA of these traits will allow us to assess if similar mechanisms underly susceptibility to VOCs across isolates and life-history stages, such as oxidative stress, impairment of cell membrane integrity,

mitochondrial dysfunction, altered nutrient transport, and metabolism suggested from RNAseq data of *B. cinerea* mycelia treated with linalool.[18] To assess the underlying genetic architecture of these traits, we will implore a Bayesian sparse linear mixed model (BSLMM) approach.[66] We selected this framework for ease of polygenic modeling and low dependency on population structure given the lack of population structure within this association mapping panel.[43,44,66] For all traits, the PD will construct a pipeline to produce sufficient priors, estimate hyperparameters, conduct the analysis, and produce publication-quality figures and tables. The PD and the Kliebenstein lab have constructed several GWAS pipelines, so repurposing poses limited pitfalls, specifically surrounding hyperparameter and prior estimation.[43,49,50,67] However, hyperparameter and prior specification are robust given extensive sampling.[66]

Objective 3: ***In vitro*** **and** ***in silico*** **estimates of biocontrols are helpful, but the validation of proposed methods and the production of application guidance is essential.** To test the ability of the VOCs to inhibit fungal virulence *in planta*, we will directly test the effect of the 8 VOCs from above on *B. cinerea* growth on all 16 plant species. **The expected results of Obj. 3 are (1) estimates of fumigation efficacy of select plant VOCs on inhibiting virulent *B. cinerea* growth on the leaves from 16 plant species inoculated with their, respectively, most virulent isolates of *B. cinerea*, and (2) a test if this inhibition is acting directly on the fungus or may involve inducing plant SAR, (3) fumigation application guidance for each VOC based on efficacy and timing.** For 16 plant species (Table 1), we will use the *B. cinerea* isolate with the highest virulence per species as a proxy for the most aggressive infection that would necessitate pesticide application to prevent yield loss.[49] For each plant species x *B. cinerea* isolate combination, we will identify fumigation effects using the predetermined mycelial MIC of each plant compound from Obj. 2 in one preventative and reactionary treatment. A second reactionary treatment will be included based on the MIC of spore viability. Replication and controls for Obj. 3 will be the same as Obj. 2, and more isolates may be added depending on logistics. We will grow all 16 species for both experiments, collect leaves, and complete the experiment using a detached leaf assay similar to Caseys et al.[49,50] Detached leaf assays have over 2500 google scholar citations, and lesion size is highly correlated with pathogen growth.[49,50,59] We will place detached leaves in individual Phyto-agar-lined polystyrene containers with holes covered in surgical tape to allow the gas exchange of O_2 and CO_2. **In the preventative experiment, we will place a disc containing a VOC treatment in the chamber with the leaf for 24 hours, at which point the disc will be removed, and the leaf will be inoculated.**[49,50] The preventative treatment assesses the ability of the VOC to induce SAR and SAR's magnitude of resistance across species. **In reactionary experiment A, the detached leaf will be inoculated with *B. cinerea,* and after 24 hours, the VOC treatment will be added for a subsequent 24 hours**. Reactionary experiment A assesses the efficacy of VOC treatment on active lesions, as all species have been observed to produce visible lesions after 24 hours of inoculation.[49,50] **In reactionary experiment B, the detached leaf will be inoculated with *B. cinerea* and coincidently receive a VOC treatment for 24 hours based on the MIC of spore viability.** Reactionary experiment B assesses the ability of VOC treatment to control initial spore dispersal onto new tissues. Lesions will be visualized every 12 hours for 72 hours post-inoculation and lesion development digitally measured as described above. **Larger reductions in growth than expected based on observations from Obj. 2 and controls in Obj. 3 indicate synergistic effects and potentially the induction of SAR by specific VOCs. In contrast, antagonist effects are indicated by a more minor reduction in growth than expected, whereby the VOC increases the plant's susceptibility to *B. cinerea.***

Training/Career Development Plan. ***The PD's career goals*** include developing a research program that advances our understanding of the ecology and evolution of plant-biotic interactions and the integral role chemical diversity plays in mediating these interactions. Further, the research program will focus on providing solutions to sustainable intensification and climate adaptation, integrating interdisciplinary research in computer science, chemistry, and biology. The PD aims to further develop their research program as a research faculty member at a research-focused university. In accentuating their research program, the PD strives to facilitate policy changes to improve recruitment and retention of historically excluded groups while taking active leadership roles in academia (college dean, department chair, etc.). The PD will be the first African American Ph.D. graduate from the University of Central Florida in Biology; their views on teaching, mentorship, retention, and recruitment have been shaped by their personal experiences and tempered with evidence on policy and strategy impacts explored across the literature. A fellowship from the USDA NIFA program would be the catalyst necessary to achieve their career goals by providing an opportunity to conduct cutting-edge research in the laboratory of Dr. Daniel Kliebenstein at the University of California, Davis. This fellowship will also allow the PD their first opportunity to focus on developing technical skills and expand their professional network at a University with multiple plant departments creating one of the most extensive collections of world-leading plant biology experts in the world. The proposed work will build off the PD's experience with comparative genomics, biochemistry, and chemical ecology and, by interacting with the Kliebenstein lab, will expand to encompass the new areas of host-pathogen interactions, toxicology, and fungal biology.

Through this fellowship, The PD will leverage agriculturally relevant eudicot and *B. cinerea* systems to accentuate their developing research program focused on identifying new ways to benefit U.S. agriculture through the emerging field of Plant VOC-mediated interactions impacting plant health and fitness. As per Dr. Kliebenstein, the outcomes of this work will be completely portable with the PD to foster their research career development. Future independent research grant applications will include: complementing the validation of fumigation effects in Obj. 3 with an investigation into comparative VOC-induced SAR, examining cross pathogen induction of SAR, and further developing mixture models of VOC toxicity from the data generated in Obj. 2. Exploring the variation in how *B. cinerea* modulates VOC biosynthetic enzymes across Eudicots (Obj. 1) will be the basis of work on plant-plant interactions providing context to understanding how disease pressures may influence the chemical information landscape of agro- and natural ecosystems. The products of this research will contribute toward breeding efforts focused on reducing *B. cinerea* and generalist pathogen spread by prioritizing the list of hundreds of potential VOC compounds produced across Eudicots for potential biocontrol of *B. cinerea*.

***Technical skills*:** The PD has extensive research experience in plant chemical ecology and comparative biochemistry. Their core dissertation research centered on the genetics and evolutionary ecology of specialized metabolites among cultivated and wild sunflowers (*Helianthus*). They have conducted independent research concerning quantitative genetics at the intra- and interspecific level incorporating GWAS & phylogenetic comparative analyses. During their Ph.D., they have been integral in starting both the Mason and Goolsby labs at the University of Central Florida by buying equipment, pioneering analytical chemistry methods, and creating data science infrastructure. Given the extra responsibilities of starting two new labs and successfully generating and securing funding for university-wide programs to combat systemic racial inequality and promote inclusive mentorship, the PD's publication record reflects

this contribution to UCF. The PD's main focus with the USDA NIFA fellowship is to develop new technical skills and refine those gained during their graduate education. Obj. 1 will provide them with comparative transcriptomics skills, specifically analyzing co-transcriptomic data. Objectives 2 & 3 will help refine comparative genomics skills, specifically applying GWAS to function-valued traits. They will develop microbial culturing skills for fungal pathogens and *in-planta*/*in-vitro* assessments of host-pathogen interactions in all objectives. These will expand the PD's ability to conduct controlled, manipulative experiments on pathogens on a broader range of plant systems. Finally, this will provide training in the efficient, high-quality publication efforts for which the Kliebenstein lab is known.

***Mentorship skills*:** To increase representation in science and public policy, the PD has demonstrated that successful mentorship of others is a priority. In their mentorship capacity, they rely on published works in biology education. Works from Thompson and Jensen-Ryan have informed their views on cultural capital disparities amongst undergraduate mentees.[68] As a first-generation college student and openly queer individual, the PD has a first-hand understanding of cultural capital disparities between visible and hidden identities. At an individual level, they have used the strategies put forth by Le et al. to foster science identity development and help students name aspects of science that align with their developing science identities.[69] Learning to navigate the academy's hidden curriculum and communicate scientific findings within a community that has historically been actively hostile towards people of color is a major challenge. In supporting a science identity and fostering positive development, the PD believes it is essential to be aware of mentee responsibilities outside of the lab. Therefore, they develop individual projects for students that work with their schedules, supplying a unique undergraduate research experience that, in their experience, is rarely observed. As of 2021, the PD has mentored over 50 undergraduates in varying capacities, most of whom (>90%) are from marginalized and/or underrepresented groups. Many of these students have continued in science at various levels (e.g., graduate school, private industry, government, and nonprofit work) focused on diversifying the face of science while increasing representation. As a postdoc in the Kliebenstein lab, the PD plans to provide in-depth, culturally competent mentorship and learn skills to mentor graduate students and postdocs. Like the PD's previous mentorship record, they will recruit and train several undergraduates in skills pertinent to this project and have mentees present and publish aspects of their contributions to the overall project while fostering the mentee's ability to create and pursue novel research ideas.

Teaching skills: The PD has extensive teaching experience both in and outside the classroom. The PD recently completed a faculty training course to develop independent classes where they developed two graduate and one senior-level undergraduate class on comparative biochemistry and chemical ecology. In addition, the PD has six semesters of teaching introductory biology as a TA at UCF and UNLV. In the fall of 2020, The PD developed and taught a research-intensive course on Plant genomics and biochemistry. To further their teaching and course development skills, the PD will apply and enroll in UC Davis's Professors for the Future program. A year-long program aimed at developing evidence-based teaching practices, providing interdisciplinary discussions on mentorship and teaching, and developing evidence-based projects centered on providing lateral training to enhance graduate student experiences. Also, Dr. Kliebenstein will help the PD seek opportunities for classroom guest lectures to enhance and expand their classroom communication skills.

Outreach and communication skills: Scientists need to interact with the public and groups their research may directly benefit. The PD has developed many skills aimed at science

communication with the general public in their professional capacity. The PD previously directed the education arm of a human anatomy and physiology museum in Las Vegas, NV (Bodies: The Exhibition), where the responsibilities included developing educational materials, programs, and events aimed at groups from kindergarten to medical professionals. The PD has also worked in the nonprofit sector (Great Basin Institute), producing programs and events to educate the populace on our nation's state and national parks. Another form of communication and outreach equally important is providing opportunities within academia to reflect and address systemic inequality. As a postdoc in the Kliebenstein lab, The PD plans to work with Dr. Kliebenstein to find opportunities to allow the PD to work towards equity at the department, college, and university level. The PD worked alongside Dr. Laurie Von Kalm at UCF to establish programs to foster diverse mentorship and reflect on systemic inequality within STEM. The previous projects initiated by the PD incorporated interdisciplinary collaboration across ten departments incorporating input from over 50 faculty members. The PD will take their experience and apply it to UC Davis and learn more about the administrative workings within the university and the Department of Plant Biology. This type of communications training will be invaluable in preparing the PD for working within a department at a top research university.

Mentoring Plan: Dr. Kliebenstein has expertise in functional, comparative, and evolutionary genomics, especially involving pathogens and their relationships to plant comparative biochemistry and chemical ecology. Dr. Kliebenstein's extensive experience with sequencing technologies and intimate knowledge of the *B. cinerea-Arabidopsis* pathosystem will be critical for all objectives and help improve the PD's technical skills. Further, Dr. Kliebenstein has an open door policy encouraging discussions whenever needed without notification. While in the Kliebenstein lab, the PD will present at lab meetings, at ongoing intergroup meetings, within the University, and at international meetings. Dr. Kliebenstein will coordinate with and assist the PD in recruiting and mentoring URs, specifically incorporating the lab's first-generation and transfer student program which has produced 10 award-winning URs that have all advanced to graduate school or biotech careers. As part of the PD's training to become an independent investigator with an extramurally funded research program, Dr. Kliebenstein will include the PD in preparing other grant proposals for the USDA, National Institute of Health (NIH), National Science Foundation (NSF). In addition to developing positive administrative practices and skillsets to help facilitate the PD's eventual transition to a faculty position.

Evaluation Plan. During weekly meetings throughout the fellowship period, Dr. Kliebensteinwill monitor the PD's progress towards the timely completion of the objectives outlined in the project timeline and professional development. Specific milestones related to the proposed research include identification of VOC biosynthetic enzymes that are affected by *B. cinerea* infection, assessment of toxicological effects of Plant VOCs on *B. cinerea* isolates, *in planta* assessments of fumigation efficacy, comparative genomic and transcriptomic analyses, presentation of findings, and preparation of at least three manuscripts for publication. Progress towards milestones will be assessed monthly to allow for alternative solutions to be created if problems arise. Dr. Kliebenstein will also guide the PD's progress on mentoring URs, navigating administration, and career development. Throughout the PD's fellowship, they will receive regular feedback on preliminary analyses, draft manuscripts, and presentations from members of the Kliebenstein lab. Together, these evaluations will provide the PD with the necessary resources and support network to achieve the proposed research objectives and attain their career goals.

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Equipment

The Kliebenstein laboratory is well equipped for plant quantitative genetic and molecular genetic research, including all aspects of recombinant DNA manipulation and large-scale marker genotyping. The lab has approximately 1200 ft^2 of modern laboratory space. This space includes three double-sided work benches, two sinks, a chemical hood, radioactive work area, and electrophoresis work area. The equipment located in the Kliebenstein laboratory includes: freezers (-20^o C and -80^o C), refrigerators (4^o C), laminar flow transfer hood, refrigerated low-speed floor model centrifuge, microplate readers, microcentrifuges, digital balances, water baths, pH meter, incubators, orbital shakers, electrophoresis gel rigs, power units, four 96-well thermal cyclers with minimal ramp time. The lab also has six quad-core computers equipped with assorted software (e.g., R, SAS, Excel, primer design programs, Mapmaker, Joinmap, QTLCartographer). Additionally there is a high-throughput extraction shaker that can handle several thousand samples per day. Finally, the Kliebenstein laboratory has two Agilent 1100 HPLCs equipped for high-throughput biochemistry that can facilitate metabolite profiling measurements.

Shared Plant Science department equipment and facilities include a gel documentation system run by Scion Image 1.57 software for data recording, a automatic autoradiograph developer, controlled growth rooms (25^o C and 15^o C), spectrophotometer, ultracentrifuges, liquid scintillation counter, transfer hoods, a photography darkroom, and a centralized dishwashing and autoclaving facility. We also have access to a Molecular Dynamics Storm FluorImager. **There is significant plant growth space available with greenhouses** Approximately 0.2 miles away that has space available for rent on demand. The PI has multiple growth chambers and growth rooms available in the UC Davis Controlled Environment Facility for the duration of the project.

Facilities & Other Resources

Genomics Facilities: The UC Davis Genome Center (Director R.W. Michelmore) consists of 17 genomics faculty (wet-lab biologists and bioinformaticists), and houses four state-of-the-art, technology core facilities that are available for use by all faculty on campus. These core facilities include bioinformatics, high-throughput genotyping, proteomics and metabolomics. The metabolomics core is established on a per sample recharge basis. This core facility is established such that it has the capacity to handle experiments ranging from a small collection of samples up to sample numbers in the tens of thousands. The metabolomics core facility handles the identification of peaks, quantification and generation of ArMet-compliant datasets for each project as a part of the per sample recharge. This process is facilitated by an automated sample handling and analysis pipeline.

The UC Davis Genome Center DNA Technologies and Expression Analysis cores perform high throughput sequencing using the Illumina Genome Analyzer platform. They currently operate three GAIIx sequencers and two paired end modules as well as two MiSeq's. They have been operating this platform since August 2007 and have had success in a variety of projects including mRNA-seq, ChIP-seq,de novo sequencing of simple genomes, resequencing, mutation discovery, and RNA tagging. The presence of multiple sequencers and local computational servers for pipeline analysis ensures rapid turnaround times on projects. In conjunction with the Genome Center bioinformatics core, they maintain a LIMS that allow users fast access to initial sequence data immediately following analysis. In addition, the Core maintains a range of instruments available for use by members of the UC Davis research community and others following training. These include a Nanodrop spectrophotometer, an Agilent Bioanalyzer, a Diagenode Bioruptor, a Molecular Devices 4000B fluorescent scanner, an Agilent fluorescent scanner, a BioRad VersArray Colony Picking Robot, a Molecular Devices plate reader, a BioMicro MAUI hybridization chamber, and several robotic liquid handlers.

The UC Davis College of Environmental & Agricultural Sciences Genomics Facility has the capacity for medium-sized sequencing projects (800 reads a day), and computer and software resources for routine sequence analysis, including PHRED, PHRAP and Consed. Other supported analyses include vector trimming, clustering via MegaBLAST and CAP3, preparation of batch submissions to NCBI, and automated annotation via BLAST. Genomics instrumentation is also available for use by researchers, including equipment for 96- and 384-plate applications (thermal cyclers, centrifuges, speed vac, colony growth, robotics), real-time PCR, and a CHEF gel system. Recharges are made for material costs.

Computer and Bioinformatics Facilities: The Department of Plant Sciences is connected through a LAN to facilitate intradepartmental communication. This network is used to access electronic mail, and provides cloud storage such that users can access their data on any connected computer and there is a daily automated backup storage service. All raw and processed data are stored on these cloud drives to provide instant access and backup service. When data analysis exceeds the capacity of our own lab computers, data can be directly uploaded to several larger clusters located in the Colleges of Ag/Env Sciences or Biological Sciences as well as the Genome center. The mentor has direct access to these computational clusters that are continually being upgraded and will extend privileges to the PD.

The mentor has access to the Genome Center Bioinformatics Core which provides expertise and infrastructure for the acquisition, curation, analysis, and distribution of large, complex datasets as well as develops and performs computations, analyses and simulations addressing a wide variety of biological questions from genomics to network biology. The Bioinformatics Core has seven staff members with overlapping expertise in computing infrastructure,

web/database, scientific programming, biological annotation and statistics. The core provides bioinformatics support for the wetlab service cores as well as for researchers with independent bioinformatics needs. The Bioinformatics core is located on the first floor of the Genome Center, where SB is housed. Computing infrastructure includes access to a 110-node cluster of dual socket, dual core AMD Opteron machines with at least 4GB of RAM (32 nodes have 8 GB of RAM); a mixed cluster consisting of 57 nodes of Intel and AMD Opteron machines; two SUN x4600 M2 large-memory machines with 128 and 512 of GB RAM; a cluster of 8 nodes, each with 32 GB of RAM; 8 dual-processor Itanium2 machines; and a Windows server setup for remote desktop access;. There is currently over 150 TB of storage spread across several file servers (including three Sun X4500 arrays), a 5.5 TB backup file server, and a Sun/StorageTek backup server with a 2TB disk array and a 30-slot LTO3 tape jukebox.
We will double our storage capacity in the near future to over 300 TB to accommodate the data from the Illumina GAII sequencers.

Plant Growth Facilities: Ample facilities are available for growing and maintaining large populations of plants, and for conducting greenhouse and growth chamber experiments. The growth chambers at the UC Davis Controlled Environment Facility utilized for the transcriptomics analysis are available for rent and are currently reserved for this project. Approximately 4500 ft^2 of greenhouse space is available to the mentor from the department. A temperature- and humidity-controlled seed storage vault is located at the greenhouse facility.

Clerical and Office: The Department provides secretarial, purchasing, and accounting services. Furnished office space adjacent to the laboratories is provided to the mentor and laboratory members.

Situation

Postdoctoral training in techniques and knowledge needed to develop an independent research program studying the direct and indirect effects of plant volatile metabolites on plant-biotic interactions

Inputs

- Mentoring:
  - Time
  - Mentor
- Professional Development:
  - Data
  - Travel funding
  - Institutional support
- Project:
  - Knowledge
  - Materials/Funding
  - Laboratory space
  - Sequencing
  - Growth Space
  - Fungal Isolates
  - Standards of Plant Volatile metabolites

Activities

- Mentoring:
  - Lab meetings
  - 1-on-1 meetings
  - Network building
  - Teaching opportunities
- Professional Development:
  - Conference presentations
  - Manuscript preparations
  - Undergraduate/graduate mentoring
- Research
  - Co-transcriptomics
  - Toxicology assays
  - Genome-wide association

Outputs

- Identification of co-transcriptomic patterns between *B. cinera* and core Eudicots
- Identification of the genetic architecture of resistance to plant volatile metabolites and
- Development of guidance for fumigation techniques among eudicot crops to control *B. cinera* with plant VOCs
- 3-4 scientific publications
- Increased network of colleagues both domestic and international
- Increased technique repertoire
- Scientific training of undergraduate and graduate researchers

Outcomes

- Personal:
  - Increased ability for interdisciplinary investigation
  - Improved scientific communication skills
  - Improved leadership, mentoring, and collaboratory abilities
- Public:
  - Increased knowledge of Plant-fungal interactions
  - New avenue for sustainable agricultural research, biocontrol, and plant breeding
  - A trained agricultural scientist advancing in academia and diversifying the workforce
  - Trained undergraduates in plant-fungal interactions and bioinformatics

Assumptions

- Plant volatile metabolites are a selective force on *B. cinerea* and variation in toxicity exists among isolates selected
- Variation exists among *B. cinerea* isolates in modulating expression patterns of volatile metabolite biosynthetic enzymes among eudicots and that variation indicates an evolutionary relationship centered around susceptibility and resistance

External factors

- Scientific conferences and other professional development opportunities occurring during time frame of project
- recruiting undergraduates for paid positions
-

Management Plan

The PD (Jordan Dowell) will oversee 3-5 undergraduate researchers throughout the project period. The Mentor (Dr. Daniel Kliebenstein) and the PD will seek out and department and university level committee opportunities for the PD as possible to provide diversity, equity, and inclusion experience and expertise on one or two important committees throughout the project period. The PD will be responsible for experimental design and execution and data analysis, with consultation with Dr. Kliebenstein through biweekly meetings. These meetings will help evaluate the progress of the project and the PD's training. When necessary or helpful, the PD will reach out to others at the University of California, Davis, and abroad to request training or resources and establish collaborations if beneficial to the project outcomes. The PD may also invite additional contacts to review prepared manuscripts for critical feedback or act in an advisory capacity as needed.

Attendance at scientific meetings and workshops proposed in the training plan will be done by the PD biannually. The proposed extra-mural activities will allow for input on the proposed work from the scientific community at large, focusing on gaining input from both botanists and mycologists. Publications will be co-written by Dowell and Kliebenstein and published in peer-reviewed journals to assess their quality and impact. In addition, the PD shall record the research project and techniques used in an electronic laboratory notebook with copies kept by both Dowell and Kliebenstein. Dowell plans to continue working on these systems and questions after this project period ends and will then move duplicates of isolates to their new lab location, as necessary.

The proposed work involves several undergraduate researcher's (UR) contributions, and their professional development is a major priority of both the PD and the mentor. All undergraduates will be expected to participate in the PD's project-centric lab meetings and encouraged to participate in full lab meetings. The PD will develop individualized training plans for each UR depending on their research and career interests. The PD and mentor will identify potential funding sources for URs to participate and accompany the PD at national conferences like Botany, Fungal Genetics Society meetings, the Compact for Faculty Diversity's Institute on Teaching and Mentoring, and the Society for Advancement of Chicanos/Hispanics and Native Americans in Science conference. URs will apply for resources to present their contributions to the overall project as a poster or talk, depending on their preferences. The PD will supply critical feedback and incorporate applying for resources like the PLANTS program (Botanical Society of America) and The UC-Davis conference travel award into each UR's training plan. The PD will be responsible for mentoring students through all stages of the process. Suppose students cannot secure funding for travel to conferences. In that case, the URs will present their research at the UC-Davis undergraduate research symposium and the Department of Plant Biology's Plant Science symposium. Alongside professional development resources provided by the PD, with consultation by the mentor, URs will be encouraged to take advantage of several undergraduate research programs that UC-Davis facilitates(NSF California Louis Stokes Alliance for Minority Participation, McNair Scholars Program, Biology Undergraduate Scholars program, Mentorships for Undergraduate Research in Agriculture, Letters and Science and UC leadership Excellence Through Advanced Degrees) to widen their experiences and professional development

Key Personnel

Project Director (PD)

Dr. Jordan Dowell (PD) will conduct all aspects of the proposed research, including running experiments, molecular laboratory work, data collection and statistical analysis, bioinformatic analysis, writing, and mentoring. The specific objectives of the proposed work involve culturing and assays of *B. cinera* and several other eudicots, molecular techniques, and computational analysis of toxicological results and RNA sequences; all of these will be conducted by the PD. The PD will summarize research findings and prepare and submit manuscripts for publication. They will also lead efforts focused on diversity equity and inclusion, mentoring, training of undergraduate students, and dissemination of results at conferences and in publication.

Mentor

Dr. Daniel Kliebenstein will provide mentorship of the PD (Dr. Dowell) with guidance and training throughout the project. Dr. Kliebenstein will supply feedback on project development through regular meetings at which progress toward completion of objectives will be evaluated. Dr. Kliebenstein will supply input on the interpretation and synthesis of data and preparation of manuscripts for publication. They will also facilitate access to equipment and other resources and help Dr. Dowell develop communication and leadership skills and meet his career goals.

Budget Justification (Total Budget = $224,982, FY2021 = $100,312,FY2022 = $124,670)

1. **PD Salary and Benefits. ($138,096.00, FY2021 = $67,834, FY2022 = $70,262)**
    a. The majority of the fellowship funds (61%) will be used to cover the salary and associated health benefits for Dr. Jordan Dowell at the University of California, Davis (UC-Davis). The total cost of two years of employment is $138,096.00 (FY2021; salary = $54,540.00, benefits = $13,294.00 and FY2022; salary = $56,176, benefits = $14,086.00). Wages and benefits follow standard postdoctoral rates at UC-Davis, which are commensurate with Experience. The PD will begin the USDA fellowship with <1 year of postdoctoral experience.
2. **Research personnel ($40,686, FY2021 = $15,978 , FY2022 = $24,708)**
    a. A total budget of $40,686 (FY2021 = $15,978 , FY2022 = $24,708) is requested to support the training and participation of at least three work-study undergraduate part-time researchers(WSUR) over the two-year period. In FY 2021 we request $15,660 to support two WSURs (salary = 7,830, benefits = $159 per person) at $15 per hour for a max of 20 hours per week to work on toxicology assays in botrytis. These workers will preferentially continue to FY2022, or new students will be trained, requesting another $16,472.00 (salary = $8,065, benefits = $171 per person) for more toxicology assays. In addition in FY 2022, we also request $8236 (salary = $8,065, benefits = $171 per person) to fund a third WSUR to work on fumigation efficacy assays across eudicot crops.
3. **Laboratory Materials and Supplies. ($30,000, FY2021 = $15,000, FY2022 = $15,000)**
    a. A total research budget of $30,000 is requested for the two-year period of the fellowship (FY2021 = $ 15,000, FY2022 = $15,000), to cover the cost of fungal rearing, laboratory consumables, analytical standards, and an absorbance microplate reader. The first-year budget included funds for toxicologic assessment of cis-3-hexanol, allo-ocimene, and two lineage-specific volatile organic compounds (VOC) among botrytis isolates. These consumables include the costs of consumables such as reagents, culture media, Petri dishes, and standards. The second-year budget funds are requested to cover the costs of fumigation efficacy and timing experiments for Objective 3 and continued toxicological assessment of lineage-specific VOCs. Further materials for Objective 3 include plant and fungal rearing supplies, analytical standards, and reagents.
4. **Travel. ($10,200, FY2021 = $3500, FY2022 = $6,700)**
    a. The PD requests a total travel budget of $10,200 (FY2021 = $3500, FY2022 = $6,700) to enable participation in 4 academic conferences, one required NIFA AFRI meeting, and one professional development workshop/meeting for underrepresented minorities in higher education. In March of FY2021, Dr. Dowell will present partial findings of Objective 2(GWAS of cis-3-hexanol in *B. cinerea*) at the Genetics Society of America's Fungal Conference in Paradise Grove, California ($500). The PD will present the findings of Objective 1 at Botanical Society of America's Botany conference in Anchorage, AK ($1,500) in July of FY2021. In FY2022, the PD will begin with attendance at the Compact for Faculty Diversity's Institute on teaching and mentoring, the largest collection of PhDs of color in the U.S. This professional development opportunity will serve as an opportunity to explore academic positions, meet with recruiters for 100+

universities, and foster opportunities for diverse mentorship. In March of FY2022 the PD will attend the European Conference on Fungal Genetics at Innsbruck, Austria ($2500) to present further findings of objectives 2 and 3. This will allow the PD opportunities to generate international collaborations. The last conference for FY2022 will be in July for Botanical Society of America's Botany conference to present the cumulative findings of objectives 1, 2, and 3 (location TBD; $1,200). An additional $3000(FY2021 = $1500, FY2022 = $1500) is requested to cover the costs for the PD to attend the NIFA EWD PD meeting in FY2021 and FY2022.

5. **Institutional allowance(total =$6000, FY2021 = $3,000, FY2022 - $3,000)**
    a. The PD requests $6000 (FY2021 = $3,000, FY2022 - $3,000) for Institutional allowance.
6. Indirect Costs.
    a. Per the guidelines of this proposal, indirect costs are fully excluded.
7. Cost-sharing.
    a. The proposed project is of national scope and is not commodity specific. Therefore, cost-sharing is not required

Data Management Plan

Expected Data Type. As described in the Project Narrative, the proposed work will generate 'primary data' consisting of dose response trait data in *B. cinerea* and 'metadata', consisting of detailed protocols of the experimental procedures and materials used to generate all datasets.

Data Format. Primary data will consist primarily of: results from *B. cinera* toxicological assays, co-expression analyzes of VOC biosynthetic enzymes and *B. cinerea* genes among several Eudicots. Metadata will consist of experimental and molecular protocols, scripts used for bioinformatic and statistical analyses, and detailed information about the sources and composition of materials (e.g., culture media, reagents, *B. cinerea* isolates, Eudicot GRIN identifiers, and analytical standards) used.

Data Storage and Preservation. Primary data will be recorded in electronic laboratory notebooks maintained by the PD; notebooks will be backed up daily to local laboratory servers, which themselves have separate backup storage.

Data Sharing and Public Access. The PD will ensure that all primary data and metadata are accessible to the research community and the broader public. In addition to details of experimental protocols and materials, metadata files will include shell, R, and python scripts that can reproduce all processing, analyses, and generate figures using the primary data. The bioinformatic and statistical metadata will be stored on the PD's GitHub account. Trait data will be deposited Dryad Digital Repository, as appropriate. These resources will be made available through prompt preparation and submission of manuscripts to peer-reviewed scientific journals. Access to publications and embargo periods of articles in press will follow the guidelines of the journals to which manuscripts are submitted. Citation with DOI will be provided by the journal or data repositories (e.g. Figshare, Dryad Digital Repository) used.

Management of Intellectual Property. Any intellectual property that results from the proposed research in the Kliebenstein lab will be governed by the University of California, Davis policies.

United States Department of Agriculture
National Institute of Food and Agriculture
Agriculture and Food Research Initiative
Pests and Beneficial Species in Agricultural Production Systems

Proposal 2021-08366 submitted by Dowell

Direct and indirect effects of conserved and lineage-specific volatile organic compounds among eudicots for control of Botrytis cinerea

The review panel grouped proposals into one of the relative categories below. The percentage indicates the final distribution of proposals in each category.

Outstanding %	25
High Priority %	42
Medium Priority %	33
Low Priority %	0
Do Not Fund %	0

This proposal was placed in : Outstanding

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Pests and Beneficial Species in Agricultural Production Systems - PANEL SUMMARY

The panel decision regarding your proposal is based on the input provided by the reviews and the collected expertise and judgment of the individual panel members. This panel summary reflects the consensus opinion of the panel regarding your proposal.

Proposal Number: 2021-08366 Project Director: Dowell

Proposal Title: Direct and indirect effects of conserved and lineage-specific volatile organic compounds among eudicots for control of Botrytis cinerea

Positive Aspects of the Proposal
The proposed work is relevant to the NIFA Pest and Beneficial Species in Agricultural Production Systems program. The reviewers considered the research concept novel, and felt that it had a good potential to advance our understanding of pathogenesis. The proposal is well-conceived and well-written. The focus on VOC biosynthetic pathways is exciting, the experimental approach is well-described and the methods are appropriate.

The PD and mentor are a good fit and they have complementary expertise. Dr. Dowell has a strong publication record, great communication skills and several noteworthy awards. The PD has outlined a good management plan for different aspects of proposal. PD has already had extensive mentoring experience and will become an even stronger mentor by taking advantage

of the UC Davis professional development programs. The outreach experience was also identified as a strength.

Negative Aspects of the Proposal
The reviewers found no substantive weaknesses. However, the proposal would have been strengthened by greater background information on environmental existence and fate of VOC?s.

Synthesis Comments
The review panel were very enthusiastic in support of this proposal and felt it has an excellent probability of success. The concept and rationale of the research question are strong and the expected results have a good likelihood of launching the PD?s independent career.

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The following reviews were submitted for your proposal, the names of the reviewers have been removed to maintain confidentiality.
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The focus of the project is to study volatile organic compounds involved in protecting plants from Botrytis cineria infections.
Scientific Merit of the Application
Merit of application for science research, education, and/or extension
Strengths: The proposal is innovative and makes use of several large, published datasets rather than proposing extensive sequencing of some kind. The focus on VOC biosynthetic pathways is exciting and the inclusion of four already identified VOCs, in addition to more that will be discovered improves the feasibility of the proposal. The background information clearly supports the proposed research, and the chosen pathogen is well known.
The specific discussion of co-evolution, host specialization, and generalist pathogens was especially interesting and well thought out. The decision in the proposal to prioritize VOCs found in single plant species was also a nice touch.
The objectives are clear, and the probability of success is high. The preliminary data included and the use of already published datasets and lab-specific collections increased the likelihood of success for the proposed experiments.
Weaknesses: The section describing objective 1 and the approach to examine species-specific expression of biosynthetic pathway enzymes was a little confusing; specifically, the Bayesian mixed linear model approach. I appreciate the skill of the PD in explaining most of the concepts included in the proposal, but this specific section was the weakest and possibly the most complicated. This could have been improved with the inclusion of a figure or graph illustrating the model.
It was unclear why the most virulent isolate of B. cinerea was the best choice for evaluating fumigation efficacy. Perhaps more information on the progression of disease or the effects of B. cinerea would have helped clarify this choice.
Qualifications of Project Personnel, Adequacy of Facilities, and Project Management

Strengths: Dr. Dowell has a strong publication record, an impressive list of invited lectures and presentations, and has received several noteworthy awards. The letters of recommendation are incredible. The proposal clearly demonstrates the communication skills of Dr. Dowell as PD.
The training/Career development plan are well written and demonstrate a clear demarcation of responsibilities and mentorship. The inclusion of an evaluation plan and weekly progress meetings is especially appreciated. The commitment to mentorship and the emphasis on increasing representation strengthens the proposal and the value of this fellowship.
The facilities and instrumentation are ideal for the proposed research.
Weaknesses: None.
Project Relevance
The proposed project specifically addresses biotic factors affecting the abundance of agriculturally important plant pests. The described research fits well within NIFA's goals and is deemed to be relevant.

Review Post-Doctoral proposal: 2021-08366
Direct and indirect effects of conserved and lineage-specific volatile organic compounds among eudicots for control of Botrytis cinerea
The proposed work will assess variation in expression of VOC biosynthetic enzymes in plants, identify the genetic variation in B. cinerea in VOCs and select and evaluate plant VOCs ability to inhibit B. cinerea in detached leaf assays. Data from an ongoing project (NSF-IOS-1915886) will be leveraged to complete the first objective and allow the PD to identify VOCs for study in objectives 2 and 3.
The approach is novel and addresses an understudied area of pathogenesis. It brings together plant chemistry, genetics and pathology in a multidisciplinary approach. Greater information on the role of VOCs in inducing SAR and subsequently suppressing disease onset will advance our understanding of pathogenesis.
One area that the proposal lacked was a strong description of the link between the current work and previous work and a conceptual description of how the results could be translated to sustainable management. For example, the proposal did not clearly link the objectives to previous work to justify that VOCs had potential for success in reducing disease. Questions regarding the temporal longevity of VOCs and whether VOCs have been successful in any known pathosystems were not addressed. A brief review of literature on VOC induction of SAR would have strengthened the proposal.
The approach to identify the eight VOCs associated with B. cinerea suppression was novel and seemed appropriate. It built on the strength of the PD and their background PhD research. It would have been beneficial to know how the concentrations that were used in the assays related to environmental concentrations or concentrations used in other research.
Appropriately, the PD will test whether the inhibition of mycelial growth results in increased spore production. The detached leaf assays are an important part of the proposal as they directly the VOCs to disease inhibition. Additional background on whether the length of time the leaves were exposed to the VOCs was realistic to a natural setting would have been helpful.

The postdoctoral fellow has documented achievement and received strong reference letters. Two faculty mentioned that he had received a highly competitive award, the UCF Pegasus Award. The references also stressed both the PDs leadership and mentorship achievements, which seem exemplary. The PD will apply to the UC Davis's Professors for the Future program, which has good potential to strengthen their leadership and professional skills further.
The mentor, Dr. Kliebenstein appears to be a strong mentor who will provide numerous opportunities for the PD's development. Kr. Kliebenstein described that the proposal was the PD's original work developed with appropriate consultation with his lab to also be a true collaboration.

Intellectual Merit
The PD plans to study the effect of plant volatiles as pesticides against Botrytis cinereal, a generalist necrotrophic fungus that attacks a large number of plant species and has significant impact in agriculture. The proposal will take advantage of the large amount of genomic and transcriptomic data generated by the host laboratory to identify in silico a set of plant genes predicted to contribute to the production of VOC in response to fungal infection, by analyzing plant and fungal coexpression from a panel of 16 plant species infected with different fungal isolates. The PD will also carry out in vitro toxicity assays for VOCs and genome wide association studies to identify fungal processes affected by plant VOCs, and finally test the VOC potential as fungal-control agents in planta.
While significant efforts have been dedicated to the understanding and use of VOCs in plant-insect interactions, much less is known about their usefulness as antimicrobial compounds. Thus, this proposal is innovative and could serve as a launch point for their independent career. The rationale of the proposal is solid, and the combined expertise of the PD and the host laboratory suggest a high probability of success.

Weaknesses
While both the PD and the host lab have significant experience in GWAS, more information on these studies would allow a better evaluation of this analysis. For example, it is not clear in the proposal if the GWAS will be done using SNP or other DNA traits, or if it will be done using transcriptome data. It is also not clear what the expected results are, or how they will be used to inform other portions of the proposal.

Postdoctoral plan
The proposal includes a significant plan for professional development and career building activities, and the PD seems to already have a strong mentoring, teaching, and management foundation. The postdoctoral advisor also has a good mentoring track record. More important, it is clear that the research program developed through this project will "belong" to the PD and serve as the focus of their independent career. Perhaps a formal evaluation tool such as IDP could be helpful, but all the pieces for a fruitful postdoctoral experience are in place.

Relevance

This is an innovative and solid proposal that can launch the independent career of the PD. The results from this project could also have an impact on the management strategies against a significant agricultural pest and provide new knowledge on the molecular basis of plant-pathogen interactions.