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Assessing Cultivated Strawberries and the *Fragaria* Supercore for Resistance to Soilborne Pathogens

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Abstract

The soilborne pathogens *Verticillium dahliae*, *Macrophomina phaseolina*, and *Fusarium oxysporum* f. sp. *fragariae* are a challenge for strawberry (*Fragaria ×ananassa*) growers. The loss of methyl bromide and increasing restrictions on the use of other fumigants due to health and environmental concerns make the development of effective non-fumigant disease control options critical for the future economic survival of the industry. Genetic resistance can be an economical option to manage these diseases. Little is known about the genetics mediating resistance to these pathogens. Thus, there is a great need to identify sources of resistance for these pathogens to assist future breeding efforts. As such, 21 *F. ×ananassa* accessions and 30 individuals from the *Fragaria* Supercore were evaluated for *V. dahliae*, *M. phaseolina*, and *F. oxysporum* f. sp. *fragariae* resistance. Six plants of each accession were inoculated via root dips prior to planting and percent mortality was recorded. Accessions with less than 33.3% mortality were considered resistant. Of the accessions evaluated, 29 were resistant to *V. dahliae*, 20 were resistant to *M. phaseolina*, and 36 were resistant to *F. oxysporum* f. sp. *fragariae*. Future work is needed to identify the resistance genes, develop tools for DNA-informed breeding, and introgress resistance from the Supercore into *F. ×ananassa*.

Soilborne diseases are a common challenge for strawberry (*Fragaria ×ananassa* Duch. ex Rozier) growers. Verticillium wilt (*Verticillium dahliae* Kleb) and Fusarium wilt (*Fusarium oxysporum* f. sp. *fragariae* Winks & Y.N. Williams) have become greater problems in California, and charcoal rot [*Macrophomina phaseolina* (Tassi) Gold] is becoming an increasing problem in California and Florida (Gordon et al., 2006; Koike et al., 2009, 2016; Mertely et al., 2005; Pincot et al., 2018). Pre-plant fumigation is commonly used to control these diseases (De Cal et al., 2005; Gordon et al., 2006; Pincot et al., 2018). Fumigation is a non-specific, highly toxic soil treatment that is detrimental to microbial communities (De Cal et al., 2005; Pincot et al., 2018). Moreover, it is not a management option during the second year of production in biennial production systems (Samtani et al., 2019). The loss of methyl bromide and increasing restrictions on the use of other fumigants due to health and environmental concerns make development of effective non-fumigant disease control options critical for the future economic survival of the industry.

Genetic resistance is an economical and environmentally friendly option for disease management. Currently, little is known about which accessions are resistant to *M. phaseolina* or the genes underlying resistance. Antanaviciute et al. (2015) identified seven *V. dahliae* resistance quantitative trait loci

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(QTLs) that were stable over three years in a 'Redgauntlet' × 'Hapil' population. The breadth of resistance these QTLs provide is unknown and it is likely that *V. dahliae* races exist that are virulent on these QTLs. A selection of octoploid strawberry accessions have also been evaluated for resistance to a single isolate of *V. dahliae* (Vining et al., 2015). The dominant gene *Fw1* was identified in an association mapping study that was conducted for *F. oxysporum* f. sp. *fragariae* resistance in 565 *F. ×ananassa* accessions (Pincot et al., 2018). Similar to the *V. dahliae* QTLs, it is unknown if *F. oxysporum* f. sp. *fragariae* races exist that are virulent on *Fw1*. As such, additional resistance genes need to be identified that can be pyramided to create cultivars with durable resistance.

Germplasm repositories offer a logical starting point towards identifying sources of disease resistance. The USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, OR is home to the U.S. *Fragaria* collection. The collection includes 601 cultivated and 1,338 wild accessions from 41 countries. A portion of this collection includes 38 wild accessions collected from North and South America known as the *Fragaria* Supercore (Hancock et al., 2002). The *Fragaria* Supercore collection is composed of octoploid *F. virginiana* Mill and *F. chiloensis* (L.) Duchesne ex Weston accessions and one decaploid *Fragaria cascadensis* Hummer accession. *Fragaria virginiana* and *F. chiloensis* hybridized to form the cultivated strawberry *F. ×ananassa* and are in the primary gene pool (Hancock et al., 2010). The *F. cascadensis* accession was originally included in the Supercore as a representative of *F. virginiana* ssp. *platypetala*. Upon the discovery of *F. cascadensis* in 2012, the taxonomy of this accession was corrected (Hummer, 2012). *Fragaria cascadensis* is in the secondary gene pool for *F. ×ananassa*. There may be previously unknown resistance genes in these accessions that could be quickly incorporated into breeding programs. Therefore, 30 accessions from the NCGR *Fragaria* collection and 21 *F. ×ananassa* accessions from the Michigan State University (MSU) and USDA-ARS Horticultural Crops Research Unit (HCRU) strawberry breeding programs were evaluated for resistance to these three pathogens to inform future crosses and genetic analyses.

Materials and Methods

Germplasm. The NCGR in Corvallis, OR provided 30 accessions from the *Fragaria* Supercore including 15 *F. chiloensis* accessions, 14 *F. virginiana* accessions, and one *F. cascadensis* accession (Table 1). A total of 21 *F. ×ananassa* accessions were also evaluated (Table 2). These accessions consisted of 10 selections and cultivars from the MSU strawberry breeding program in East Lansing, MI and 11 from the HCRU breeding program in Corvallis, OR. The public cultivars Monterey, Portola, and Albion were used as susceptible controls for *Fusarium*, *Verticillium*, and *Macrophomina*, respectively. Plant propagation was conducted to produce daughter plants for replicated inoculation trials for the Supercore and *F. ×ananassa* accessions. Runners for the *F. ×ananassa* accessions were produced by the University of California, Davis in a high elevation nursery in Dorris, CA and sent to the California Polytechnic State University (Cal Poly) in San Luis Obispo, CA. Runners for the Supercore accessions were produced at Cal Poly. The Supercore mother plants were maintained in 7.6-L containers and fertilized with slow-release Osmocote Plus (15N-3.9P-4.8K). Plants were irrigated as needed throughout the trial using a shower-type sprayer nozzle directed at the base of the crown. To establish daughter plants for inoculation, runners of each accession were rooted in plug cells (L × W × D of 8.00 cm × 3.94 cm × 5.92 cm; Greenhouse Megastore, Sacramento, CA) containing a substrate consisting of 33% coconut coir, 33% sphagnum peat moss, and 33% perlite. The runners were kept under intermittent mist for seven to 10 days until roots colonized the substrate. Once rooted, plants were allowed to grow for an-

other four to six weeks before host resistance screens were initiated.

Phenotyping. Trials were conducted in the strawberry greenhouse at the Cal Poly Crops Unit. Host resistance screens were conducted for each of the three pathogens (*F. oxysporum* f. sp. *fragariae*, *V. dahliae*, and *M. phaseolina*). Seven plants of each selection were used per pathogen screen, inoculating six and leaving one as a non-inoculated control. Local isolates of each pathogen originating from diseased strawberry crowns were used for each inoculation. Inoculum for each trial consisted of 1×10^6 conidia per mL of water for *F. oxysporum* f. sp. *fragariae* (isolate GLI080), 5×10^6 conidia per mL of water for *V. dahliae* (isolates Vd1, Vd3, Vd7, and Vd20), or a slurry of *M. phaseolina* microsclerotia at 2,500 colony-forming-units per mL of slurry (isolates Mp8, Mp21, and Mp22). Plants were inoculated by soaking the roots in the inoculum for 5 min. The non-inoculated controls were soaked in water for 5 min. Plants were then transplanted into 0.5 L pots (10.2 cm diameter) with the same potting mix as previously described and arrayed randomly on a greenhouse bench. Plant mortality was assessed after the susceptible control cultivars had a mortality of greater than 83.3 % for their respective diseases. Susceptible control cultivars were evaluated only in trials in which they were the susceptible control. *Fusarium* and *Verticillium* trials were conducted twice for all accessions. *Macrophomina* trials were conducted once for Supercore accessions and twice for breeding accessions. Average percent mortality was calculated and accessions with less than 33.3 % mortality were considered resistant.

Results and Discussion

Fragaria Supercore accessions. Resistance was observed in the Supercore to each pathogen tested (Table 1). Many of the accessions displayed high levels of resistance to the pathogens evaluated. Unfortunately, not all accessions were evaluated for resistance to *V. dahliae* and *M. phaseolina*. For *F. oxysporum* f. sp. *fragariae,* 24 of 30 accessions evaluated were resistant. Sixteen of 21 Supercore accessions were resistant to *V. dahliae*. Eleven of these accessions were also evaluated by Vining et al. (2015). A direct comparison between the current study and Vining et al. (2015) is difficult due to differences in the rating methodology used. Four of these accessions (PI 612316, PI 612320, PI 612486, and PI 612570) had similar susceptible disease responses between studies. Different disease responses were observed for six accessions (PI 236579, PI 551445, PI 551527, PI 552091, PI 602570, and PI 612489). In Vining et al. (2015) all replicates of each of these accessions were dead or nearly dead, compared to a 0% mortality in the present study (Table 1). Different isolates of *V. dahliae* were used in this study and in Vining et al (2015) indicating the presence of different avirulence genes between the pathogen isolates. Unfortunately, in the present study multiple isolates were inoculated simultaneously, preventing a differential analysis. Finally, 12 of 15 accessions were resistant to *M. phaseolina.* Of the 15 accessions evaluated with all three pathogens, seven were resistant in all trials. Four of these accessions (PI 551736, PI 612487, PI 612489, and PI 612490) displayed 0 % mortality for each pathogen.

MSU and USDA breeding program accessions. Resistance and susceptibility were observed in the accessions from the MSU and HCRU breeding programs (Table 2). For *F. oxysporum* f. sp. *fragariae*, 12 of the 21 accessions were resistant. Of 21 accessions, 13 were resistant to *V. dahliae*. Interestingly all of the HCRU accessions evaluated were susceptible to *M. phaseolina*. Conversely, 8 of the 10 MSU accessions were resistant to *M. phaseolina*.

'Marys Peak' and ORUS 2427-1 are full siblings and had similar disease responses to *F. oxysporum* f. sp. *fragariae* and *M. phaseolina* but very different responses to *V. dahliae* (Table 2; Finn et al., 2018). The parent that provided the resistance to *V. dahliae* is un-

Table 1. Phenotypic data for the *Fragaria* Supercore. Two trials for *Fusarium* and *Verticillium* were conducted and average percent mortality was calculated. Only a single trial for *Macrophomina* was conducted. Accessions with less than 33.3% mortality are considered resistant.

Accession	Species	Origin	*Fusarium* % Mortality	*Verticillium* % Mortality	*Macrophomina* % Mortality
PI 551527	*F. cascadensis*	Oregon, U.S.A.	0	0	-
PI 236579	*F. chiloensis*	Chile	0	33.3	50
PI 551445	*F. chiloensis*	California, U.S.A.	0	16.7	0
PI 551453	*F. chiloensis*	Washington, U.S.A.	83.3	0	33.3
PI 551459	*F. chiloensis*	Oregon, U.S.A.	58.3	16.7	50
PI 551735	*F. chiloensis*	California, U.S.A.	0	0	-
PI 551736	*F. chiloensis*	Ecuador	0	0	0
PI 552091	*F. chiloensis*	Chile	0	0	-
PI 602568	*F. chiloensis*	Chile	0	0	-
PI 602570	*F. chiloensis*	Chile	0	0	-
PI 612316	*F. chiloensis*	Chile	25	66.7	75
PI 612317	*F. chiloensis*	Chile	25	16.7	0
PI 612487	*F. chiloensis*	British Columbia, Canada	0	0	0
PI 612488	*F. chiloensis*	British Columbia, Canada	0	-	-
PI 612489	*F. chiloensis*	Oregon, U.S.A.	0	0	0
PI 612490	*F. chiloensis*	California, U.S.A.	0	0	0
PI 612320	*F. virginiana*	Georgia, U.S.A.	0	50	-
PI 612323	*F. virginiana*	Alabama, U.S.A.	83.3	50	16.7
PI 612324	*F. virginiana*	South Carolina, U.S.A.	50	0	0
PI 612325	*F. virginiana*	North Carolina, U.S.A.	0	-	-
PI 612486	*F. virginiana*	Mississippi, U.S.A.	33.3	66.7	33.3
PI 612494	*F. virginiana*	South Dakota, U.S.A.	0	-	-
PI 612495	*F. virginiana*	Montana, U.S.A.	33.3	-	-
PI 612497	*F. virginiana*	Ontario, Canada	66.7	-	-
PI 612498	*F. virginiana*	Minnesota, U.S.A.	0	-	-
PI 612499	*F. virginiana*	Minnesota, U.S.A.	0	-	-
PI 612500	*F. virginiana*	Alberta, Canada	16.7	-	-
PI 612501	*F. virginiana*	Montana, U.S.A.	0	-	-
PI 612569	*F. virginiana*	Mississippi, U.S.A.	0	8.3	16.7
PI 612570	*F. virginiana*	Florida, U.S.A.	100	58.3	0

known. The accession FVC 11-58 was developed by recreating the initial hybridization event between *F. chiloensis* and *F. virginiana* that formed *F. ×ananassa* (Hancock et al., 2010). FVC 11-58 was resistant to *F. oxysporum* f. sp. *fragariae* and *M. phaseolina* and susceptible to *V. dahliae*. FVC 11-58 resulted from a cross between four Supercore accessions: *F. virginiana* (Frederick 9, PI 612493 × LH 50-4, PI 612495) × *F. chiloensis* (Scotts Creek, PI 612490 × 2 MAR 1A, PI 602567). Of the grandparental accessions evaluated in this study, one of the *F. chiloensis* parents PI 612490 was resistant to all three pathogens while one of the *F. virginiana* parents PI 612495 exhibited resistance to *F. oxysporum* f. sp. *fragariae* and was not evaluated for resistance to the other two pathogens. Of the *F. ×ananassa* accessions evaluated, only MI 10-24-52 was resistant to all three diseases.

Future breeding needs. Further research is needed to understand the genetics mediating

Table 2. Phenotypic data for the *F.* ×*ananassa* accessions from the USDA and Michigan State University (MSU) breeding programs. Two trials were conducted for all accessions and average % mortality was calculated. Accessions with less than 33.3% mortality are considered resistant.

Accession	Breeding Program	*Fusarium* % Mortality	*Verticillium* % Mortality	*Macrophomina* % Mortality
FVC 11-58	MSU	0	75	8.3
MI 10-11-33	MSU	33.3	66.6	16.7
MI 10-24-52	MSU	16.7	0	16.7
MI 8-27-73	MSU	58.3	8.3	8.3
MI 9-16-26	MSU	16.7	50	16.7
MI 9-16-5	MSU	16.7	50	16.7
MSU 25	MSU	91.7	0	8.3
MSU 59	MSU	50	16.7	33.3
MSU 60	MSU	16.7	8.3	41.7
MSU 61	MSU	50	33.3	91.7
Charm	USDA	16.7	58.3	100
Marys Peak	USDA	58.3	16.7	50
ORUS 2427-1	USDA	41.7	66.7	66.7
ORUS 2490-1	USDA	66.7	33.3	100
ORUS 2780-1	USDA	50	41.7	100
ORUS 3138-1	USDA	33.3	58.3	100
ORUS 3140-1	USDA	0	16.7	50
ORUS 3142-3	USDA	25	33.3	75
ORUS 3152-3	USDA	16.7	16.7	100
ORUS 3171-1	USDA	33.3	16.7	100
Sweet Bliss	USDA	83.3	33.3	100

resistance to *F. oxysporum* f. sp. *fragariae*, *V. dahliae*, and *M. phaseolina*. The identification of *Fw1* for *F. oxysporum* f. sp. *fragariae* and the *V. dahliae* resistance QTLs is a promising start, however little is known about the virulence diversity of pathogen populations in discreet geographic regions. *Fw1* may not provide resistance to *F. oxysporum* f. sp. *fragariae* isolates in other strawberry production regions (Pincot et al., 2018). The need to study pathogen virulence diversity as it relates to resistance gene deployment is accentuated by the different disease responses observed between the current study and Vining et al. (2015) for *V. dahliae*. Moreover, deploying a single resistance gene alone risks increased selection pressure for virulence on the deployed gene in pathogen populations. As such, incorporating multiple resistance sources for a disease into a single cultivar is important for resistance gene stewardship.

The accessions evaluated in this study from the Supercore and FVC 11-58 likely comprise a diverse set of resistance sources. However, pre-breeding will be needed when using these accessions as founders to mitigate the effects undesirable alleles have on other important traits. The most prudent course forward is to begin pre-breeding efforts while simultaneously developing biparental populations to identify and characterize the genes mediating resistance in these accessions. Efforts towards introgressing novel sources of resistance to *F. oxysporum* f. sp. *fragariae* and *M. phaseolina* from FVC 11-58 has begun and pedigree linked populations have been created to identify the genetic regions associated with resistance. The identification of these genes will allow for the development of DNA-based tools to as-

sist breeders select and combine these sources of disease resistance during and after the pre-breeding process.

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