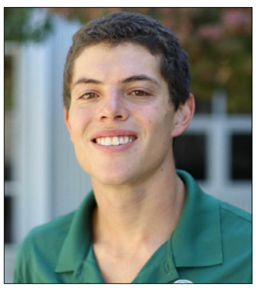
Characterization of Strawberry Host Plant Resistance to Powdery Mildew caused by *Podosphaera aphanis*

Principal Investigators
Dr. Gerald Holmes
Director, Strawberry Center
California State Polytechnic University, San Luis Obispo
(805) 756-2120
gjholmes@calpoly.edu



Michael Palmer
M.S. Student
Horticulture and Crop Science Department
California State Polytechnic University, San Luis Obispo
(847) 814-1010
michaelpalmer1010@gmail.com

Summary

Host plant resistance is an essential tool in plant disease management worldwide. Evaluations of strawberry (*Fragaria* x *annanassa*) cultivar resistance to powdery mildew (*Podosphaera aphanis*) were done previously in California, but many new cultivars have been released since the last evaluation in 1996 and merit evaluation for today's growers. Two studies were conducted over the winter and summer of 2020 evaluating 12 and 16 commonly grown cultivars, respectively. Powdery mildew-free plants were established in three-liter pots under high plastic tunnels and after three weeks (four to five-leaf stage) moved into a greenhouse where an active powdery mildew epidemic was present. Disease incidence and severity ratings were taken weekly beginning at the first sign of disease. Ratings taken at 40 days (winter trial) and 41 days (summer trial) after transfer to the greenhouse were used to make comparisons among cultivars. Significant differences were found in foliar disease severity among cultivars, but none were totally free of disease. Moderately resistant cultivars were 'Fronteras', 'San Andreas', and 'Sweet Ann'. Highly susceptible cultivars were 'BG 3.324', 'Royal Royce', and 'Warrior'. Two field evaluations of the ten cultivars common to both greenhouse trials confirmed the observed relative differences in host resistance under field conditions. This information is valuable to California strawberry growers who select cultivars based partially on their susceptibility to economically important diseases such as powdery mildew.

Introduction

Strawberry powdery mildew (PM) is caused by *Podosphaera aphanis Wallr*. (syn. *Sphaerotheca macularis* f. sp. *fragariae*). *P. aphanis* affects all above-ground parts of the plant including leaves, fruit, flowers, petioles, and stolons. Infected leaves have reduced photosynthetic capacity and can lead to yield loss (Bolda and Koike, 2015). Infected fruit is unmarketable (Horn et al., 1972). Powdery Mildew is favored by moderate temperatures (15-25°C) and high relative humidity (>35%) (Miller et al., 2003). These conditions occur throughout the strawberry growing season in every major fruit production region in California (Bolda and Koike, 2015). Powdery mildew is particularly severe in plants grown under high plastic tunnels due to the light filtering properties of the plastic and reduced exposure to direct sunlight.

Yield losses from PM can be mitigated by cultural and chemical control measures. Repeated application of fungicides with site-specific modes of action creates high selection pressure for resistant populations and leads to reduced efficacy (Palmer and Holmes, 2020, Pertot et al., 2007, Sombardier et al., 2009). Integrated pest management practices recommend using cultural control measures prior to chemical control (Koike et al., 2018). Perhaps the most important cultural control is the selection of a resistant cultivar. Host plant resistance is a key quality for nursery production as well, since producing disease-free transplants is crucial to establishing healthy plants for fruit production (Bolda and Koike, 2015).

Host plant resistance to any powdery mildew is multigenic/horizontal and conferred by mutations in the *Mildew* Locus O (MLO) protein (Jørgensen, 1992). This protein is found in the plasma cell membrane of the plant and is essential for mildew infection to occur. Mutations in this protein in grapevine have been reported to confer resistance to infection by *Erysiphe necator* (Pessina et al., 2016). MLO genes in strawberry have been identified (Cockerton et al., 2018; Tapia et al., 2020) but specific mutations conferring disease resistance have not. Since these genes were identified so recently, they are not yet used as genetic markers by breeding programs and were not specifically bred for in currently grown strawberry cultivars.

Evaluation of PM resistance can be informed by data gathered from replicated evaluations under conditions representative of commercial production. Previous reports from Florida (Kennedy et al., 2013) and California (Nelson et al., 1996) have indicated that strawberry cultivars can range from entirely resistant to highly susceptible. In California strawberry production, cultivars are typically replaced every five to 10 years by cultivars with improved traits. Presently grown cultivars in California have not been evaluated in replicated studies for susceptibility to PM.

In this study, we evaluate host plant resistance to PM in several of the most popular cultivars currently grown in California. Evaluations were done under greenhouse and field environments to establish a robust measure of susceptibility

Materials and Methods

Greenhouse trials. Strawberry cultivars were evaluated for their susceptibility to PM in a greenhouse environment at the California Polytechnic State University (Cal Poly) Crops Unit in San Luis Obispo, California. The initial evaluation (12 cultivars) took place in January and February 2020 (winter) and the repeat evaluation (16 cultivars) in May and June 2020 (summer) (Table 1).

Plants were established in 6-inch pots in a planting medium composed of equal parts peat, perlite, and coconut coir. The mix was amended with a nutrient mix of lime (0.37 oz/gal), potassium nitrate (0.11 oz/gal) and triple phosphate (0.08 oz/gal). Each pot was amended with 0.35 oz Osmocote Plus (Scotts Miracle-Gro Company, Marysville, OH) two weeks after planting. Plants were grown under high plastic tunnels and overhead irrigated daily. At the four- to five-leaf stage, plants were transferred into a greenhouse where an active PM epidemic was established on mature plants. Prior to transferring into the greenhouse, each plant was inspected to ensure that no visible symptoms or signs of PM were present.

Plants of the same cultivar were arranged into plots of four plants; each plot was replicated four times in a randomized complete block design. A single diseased plant was placed between each plot as a source of inoculum. A highly susceptible cultivar (‘BG 3.324’) was used as the inoculum source plants. These plants were started in the greenhouse and exposed to PM continuously for four weeks prior to transferring the disease-free cultivars. Each source plant exhibited high levels of PM infection on the day all other plants were transferred. All plants were irrigated four times per day (0.03 gal water) via stake emitters (Netafim USA, Fresno, CA).

Disease severity ratings began at observation of first symptoms (14 days after transfer into the greenhouse) and were done weekly for the following eight weeks. Each trifoliate in a plot was rated for total leaf surface area colonized by *P. aphanis*. The ratings for all trifoliates in a plot were averaged to get disease severity for that plot. The single ratings that best illustrated differences in cultivar susceptibility were determined to be at 40 and 41 days after transfer into the greenhouse in the winter and summer trials, respectively. Mean disease severity for each cultivar was statistically evaluated in JMP 14 (SAS Institute Inc. Cary, NC) using one-way ANOVA and Tukey HSD separation of means.

Table 1. List of cultivars used in greenhouse and field trials.

Cultivar	Breeding program	Greenhouse trials		Field trials
		Winter	Summer	
‘Albion’	UC	✓	✓	✓
‘BG 3.324’	Berry Genetics	✓	✓	✓
‘BG 4.367’	Berry Genetics	✓	✓	✓
‘Cabrillo’	UC	✓	✓	✓
‘Driscoll’s 1’	Driscoll’s	✓		
‘Driscoll’s 2’	Driscoll’s	✓		
‘Fronteras’	UC		✓	
‘Monterey’	UC	✓	✓	✓
‘Petaluma’	UC	✓	✓	✓
‘R858’	Lassen Canyon		✓	
‘Royal Royce’	UC	✓	✓	✓
‘Ruby June’	Lassen Canyon	✓	✓	✓
‘San Andreas’	UC	✓	✓	✓
‘Sangria’	Lassen Canyon		✓	
‘Sweet Ann’	Lassen Canyon	✓	✓	✓
‘Valiant’	UC		✓	
‘Victor’	UC		✓	
‘Warrior’	UC		✓	

Field evaluations. Ten strawberry cultivars were evaluated in each of two fields located at Cal Poly. These cultivars were included in both the first and second greenhouse trials and were part of a larger trial assessing host resistance to Macrophomina crown rot (Field A) and Verticillium wilt (Field B). Both fields were planted on October 23, 2019. The Macrophomina field had four inoculated replications and the Verticillium field had four naturally infected replications. All PM inoculum was naturally occurring in these fields. No fungicides were applied to control PM in either field. The results from each field are reported separately.

Five trifoliates from mature plants were rated from each of the ten plots and selected with a preference toward mature leaves showing symptoms of PM. Each leaf was scored for disease severity as described above. Evaluations for both fields were done on July 5, 2020.

Results

Winter greenhouse trial. Disease severity ranged widely among the cultivars evaluated (Figure 1). Both 'BG 3.324' and 'Royal Royce' were highly susceptible to PM with disease severity of 19.4% and 12.2%, respectively. 'San Andreas' and 'Sweet Ann' were the least susceptible cultivars with disease severity of 1.5% and 1.9%, respectively. 'San Andreas' and 'Sweet Ann' were the only two cultivars significantly more resistant to PM than 'BG 3.324' and 'Royal Royce' according to Tukey HSD separation of means. All other cultivars were only significantly different from 'BG 3.324' and are considered moderately susceptible.

Summer greenhouse trial. Disease levels were lower overall compared to the winter greenhouse trial. There were significant differences in disease severity among the cultivars evaluated (Figure 2). 'BG 3.324' and 'Warrior' were the most susceptible cultivars with disease severity of 10.0% and 8.9%, respectively. 'Fronteras' and 'Valiant' were the least susceptible cultivars with disease severity of 1.4% and 2.2%, respectively. The severity scores of the ten shared cultivars in the winter and summer greenhouse trials were significantly correlated (Table 2).

Field evaluations. The cultivar with the highest average disease severity in both Field A and Field B was 'BG 3.324' at 4.8% and 7.5%, respectively. The cultivar with the lowest disease severity in both Field A and Field B was 'Sweet Ann' at 0.8% and 1.8%, respectively (Figure 3). Disease severity from both fields were significantly correlated with a Pearson coefficient of 0.597 ($P < 0.0001$). Disease severity in both fields was also significantly correlated with the disease severity of the ten shared cultivars from the winter and summer greenhouse trials except for the Field A – summer greenhouse pairing (Table 2).

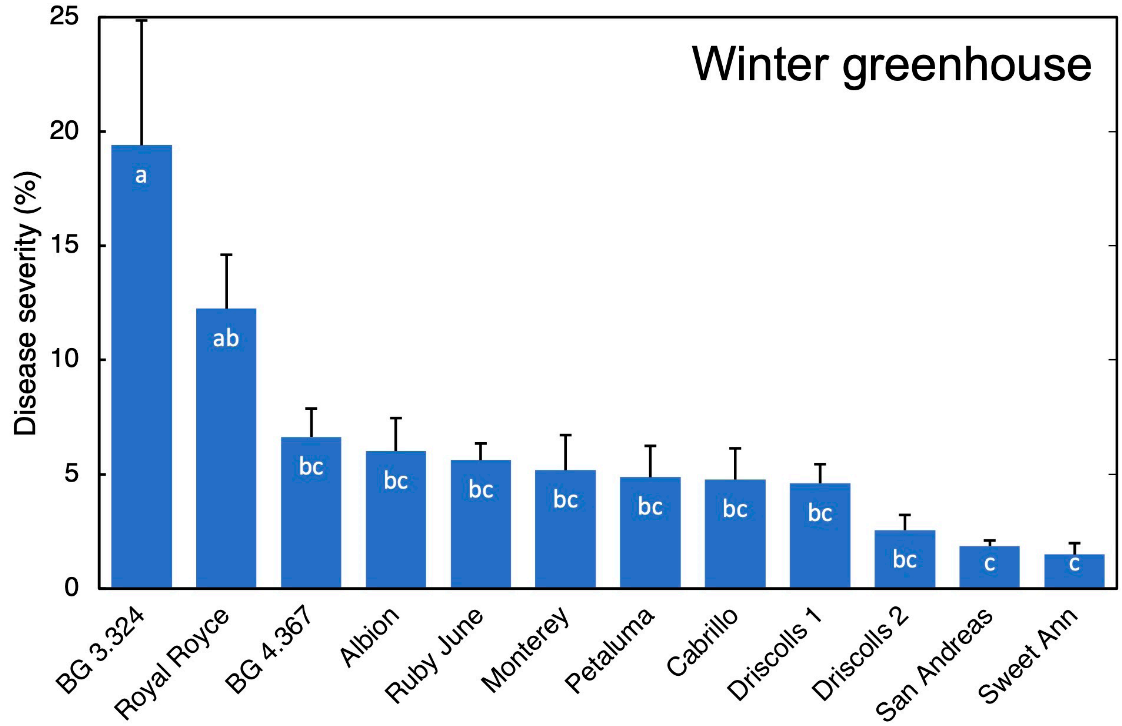


Figure 1. Mean disease severity for each cultivar evaluated in the winter greenhouse trial. Cultivars that do not share the same letter are significantly different according to Tukey HSD separation of means. Error bars represent the standard error of the mean.

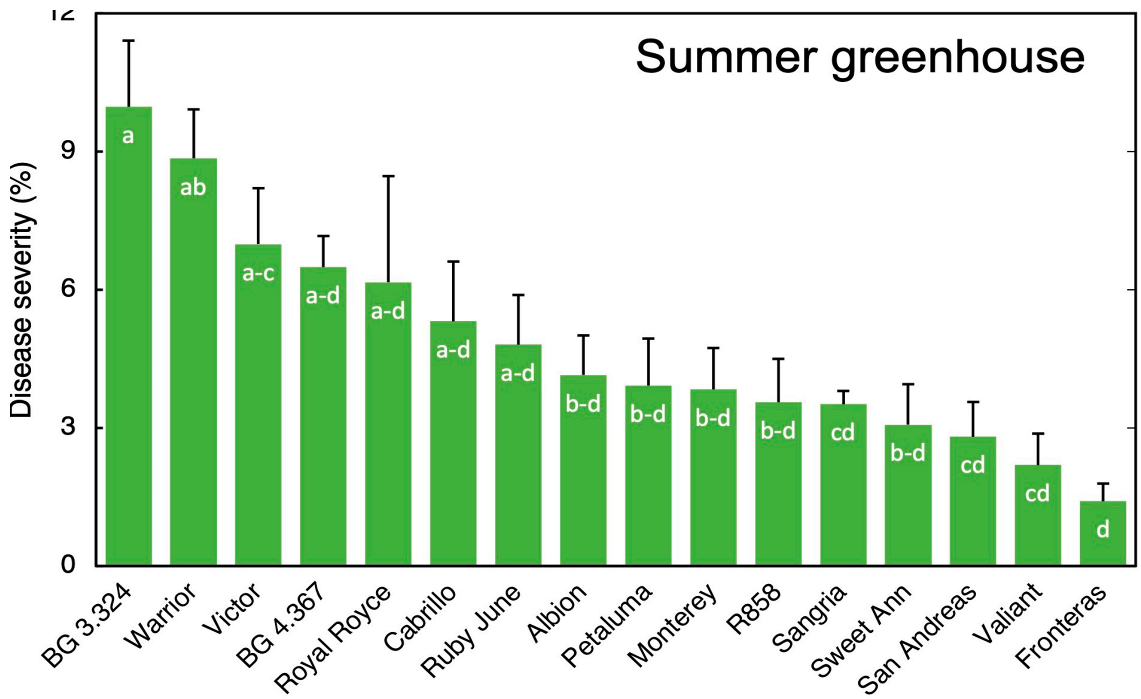


Figure 2. Mean disease severity for each cultivar evaluated in the summer greenhouse trial. Cultivars that do not share the same letter are significantly different according to Tukey HSD separation of means. Error bars represent the standard error of the mean.

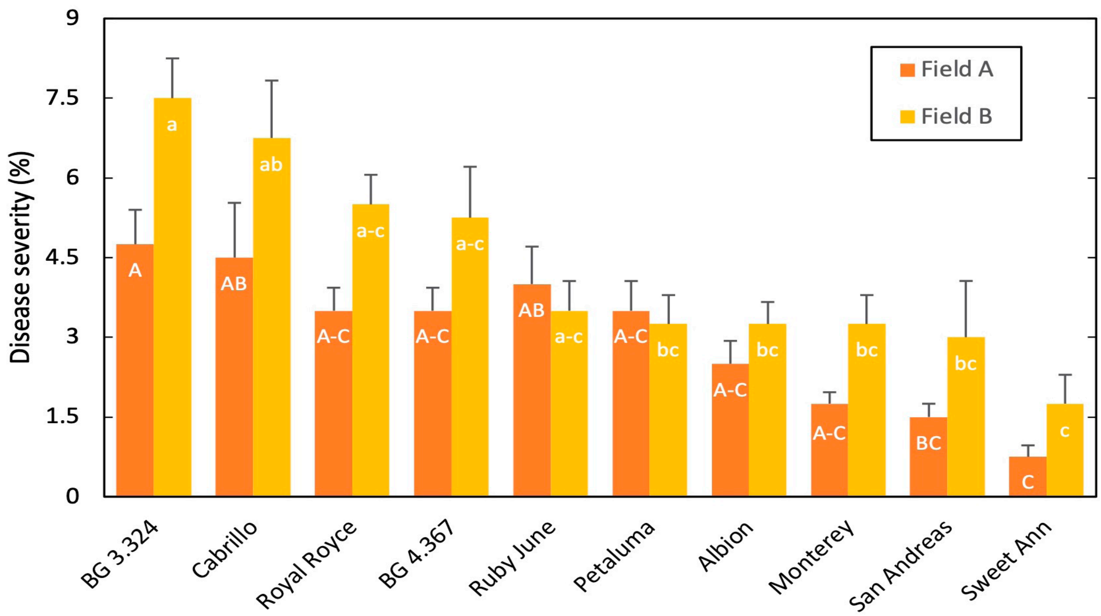


Figure 3. Mean disease severity for each cultivar evaluated in two field evaluations. Cultivars that do not share the same letter within a field are significantly different according to Tukey HSD separation of means. Error bars represent the standard error of the mean.

Table 2. Pearson correlation coefficients and subsequent P values generated by comparing each pair of disease severity scores in four separate experiments. Pairs that have a P value less than the established alpha of 0.05 are significantly correlated.

Experiment	By experiment	Pearson coefficient	*P* value
Winter greenhouse	Summer greenhouse	0.669	< 0.0001
Field A	Field B	0.597	< 0.0001
Winter greenhouse	Field B	0.565	0.0001
Summer greenhouse	Field B	0.488	0.0014
Winter greenhouse	Field A	0.388	0.0135
Summer greenhouse	Field A	0.281	0.0794

Discussion

The cultivars in the greenhouse trials and the field evaluations all showed variation in susceptibility to PM on a continuous scale. This was expected since previously published work has put host plant resistance to powdery mildew on a continuous spectrum rather than on a binary scale (Kennedy et al., 2013, Nelson et al., 1996). Our results are consistent with those of Cockerton et al., (2018) who showed that host plant resistance to powdery mildew in strawberry is controlled by multiple genes.

No cultivars were found to be entirely free of PM in any of the evaluations. Our findings are in contrast with those from Kennedy et al., (2013) where some completely resistant cultivars were characterized but confirms findings of other host resistance evaluations of PM where no disease-free cultivars were observed (Darrow et al., 1954; Peries, 1962; Nelson et al., 1996). The difference between our findings and those of Kennedy et al. (2013) are most likely attributed to a discrepancy in sampling methods. In this 2013 study, five mature leaves were randomly selected from each plot under field and high plastic tunnel environments. Random sampling of leaves raises the likelihood of selecting leaves without disease, especially in less susceptible cultivars.

The significant correlation among the results from greenhouse and field trials indicates that measuring host resistance in a greenhouse using artificial inoculation is comparable to host resistance in a field setting with natural inoculum. The correlation also confirms the findings of Nelson et al., (1995) which reported a similar relationship. Since field conditions are not always conducive to PM development, greenhouse evaluations are a more reliable means of obtaining results. Greenhouse evaluations are also typically done on a smaller scale and are less expensive to establish. The ease of establishing and moving disease-free plants into the greenhouse also allows for multiple evaluations in a single year.

These results are valuable to California strawberry growers as no PM host resistance study has been done in California for over 20 years (Nelson et al., 1996). Aside from evaluating some proprietary and newer varieties, this study evaluated six cultivars that make up at least 58% of the state's planted acreage: 'Monterey' (31.7%), 'San Andreas' (13.1%), 'Fronteras' (6.1%), 'Cabrillo' (4.6%), 'Petaluma' (1.2%), and 'Sweet Ann' (1.2%) (CSC 2018). Though host plant resistance to powdery mildew is not often the first consideration for growers, it is still a valuable trait that is now documented and will contribute to a more informed cultivar selection process.

REFERENCES

- Asalf, B., D.M. Gadoury, A.M. Tronsmo, R.C. Seem, and A. Stensvand. 2016. Effects of development of ontogenic resistance in strawberry leaves upon pre- and postgermination growth and sporulation of *Podosphaera aphanis*. Plant Dis. 100:72-78.
- Bolda, M., and S.T. Koike. 2015. Powdery mildew of strawberry. Online publication. California Strawberry Commission. https://www.calstrawberry.com/Portals/2/Reports/Research%20Reports/Production%20Guidelines/English/Powdery%20Mildew%20of%20Strawberry%20-%202015.pdf?ver=2018-01-12-155446-510
- Cockerton, H.M., R.J.Vickerstaff, A. Karlstorm, F. Wilson, M. Sobczyk, J.Q. He, D.J. Sargent, A.J. Passey, K.J. McLeary, K. Pakozdi, N. Harrison, M. Lumbreras-Martinez, L. Antanaviciute, D.W. Simpson, and R.J. Harrison. 2018. Identification of powdery mildew resistance QTL in strawberry (*Fragaria* x *ananassa*). Theoretical and Applied Genetics 131:1995-2007.
- CSC. 2018. 2018 California strawberry acreage survey. Online publication. California Strawberry Commission. https://www.calstrawberry.com/Portals/2/Reports/Industry%20Reports/Acreage%20Survey/2018%20Acreage%20Survey.pdf
- Darrow, G.M., D.H. Scott, and A.C. Goheen. 1954. Relative resistance of strawberry varieties to powdery mildew at Beltsville, MD. Plant Dis. Rep. 38:864-866.
- Horn, N.L., K.R. Burnside, and R.B. Carver. 1972. Powdery mildew of strawberry. Plant Dis. Rep. 4:368.
- Jørgensen, J.H. 1992. Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. Euphytica 63:141-152.
- Kennedy,C., T.N. Hasing, N.A. Peres, and V.M. Whitaker. 2013. Evaluation of strawberry species and cultivars for powdery mildew resistance in open-field and high tunnel production systems. HortScience 48:1125-1129.
- Koike, S.T., G.T. Browne, T.R. Gordon, and M.P. Bolda. 2018. Agriculture: pest management strategies: strawberry. Online publication. University of California Agriculture and Natural Resources. https://www2.ipm.ucanr.edu/agriculture/strawberry/.
- Miller, T.C., W.D. Gubler, S. Geng, and D.M. Rizzo. 2003. Effects of temperature and water vapour pressure on conidial gemination and lesion expansion of Sphaerotheca macularis f. sp. fragariae. Plant Dis. 87:484-492.
- Nelson, M.D., W.D. Gubler, and D.V. Shaw. 1995. Inheritance of powdery mildew resistance in greenhouse-grown versus field-grown California strawberry progenies. Phytopathology 85:421-424.
- Nelson, M.D., W.D. Gubler, and D.V. Shaw. 1996. Relative resistance of 47 strawberry cultivars to powdery mildew in California greenhouse and field environments. Plant Dis. 80:326-328.
- Palmer, M.G., and G.J. Holmes. 2021. Fungicide sensitivity in strawberry powdery mildew caused by *Podosphaera aphanis* in California. Plant Dis. 105: In Press
- Peries, O.S. 1962. Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr. Ex Fries). Ann. Appl. Biol. 50:225-233.

REFERENCES

- Pertot, I., F. Fiaming, L. Amsalem, M. Maymon, S. Freeman, D. Gobbin, and Y. Elad. 2007. Sensitivity of two *Podosphaera aphanis* isolates to disease control agents. J. Plant Pathol. 89:85-96.
- Pessina, S., L. Lenzi, M. Perazzolli, M. Campa, L. Della Costa, S. Urso, G. Valè, F. Salamani, R. Velasco, and M. Malnoy. 2016. Knockdown of MLO genes reduces susceptibility to powdery mildew in grapevine. Hort. Research 3:16016.
- Sombardier, A., M. Dufour, D. Blanchard, and M. Corio-Corset. 2009. Sensitivity of *Podosphaera aphanis* isolates to DMI fungicides: distribution and reduced cross-sensitivity. Pest Manag. Sci. 66:35-43. DOI 10.1002/ps.1827
- Tapia, R.R., C.R. Barbey, S. Chandra, K.M. Folta, V.M. Whitaker, and S. Lee. 2020. Genome-wide identification and chracterization of MLO gene family in octoploid strawberry (*Fragaria* x *ananassa*). BioRxiv preprint.