

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE:

A Virulent Strain of the Powdery Mildew Fungus Threatens the Oregon Hop Industry

RESEARCH LEADER:

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SUMMARY:

A three-year study was completed that quantified the prevalence and incidence of powdery mildew on cultivars possessing R6. There has been a general increase in powdery mildew levels on cultivars possessing R6 in both Washington and Oregon during 2012 to 2014. The greatest incidence of powdery mildew on leaves and cones has tended to be in yards on the Yakima Indian Reservation. Pesticide applications reported by growers indicated differences in the number of powdery mildew sprays made among regions and cultivars, both within and among years, and also a general increase in fungicide applications over time. Present evidence indicates differential susceptibility of cultivars, with the greatest susceptibility in Apollo, followed by Bravo, Millennium, and Nugget in Washington. In Oregon, Nugget appears to be more susceptible than in Washington. However, laboratory and field studies indicate plants with R6 possess some level of quantitative resistance as compared to plants lacking R6. There also appears to be a fitness penalty incurred to the pathogen for overcoming R6, manifested as a reduced ability to sporulate. On cultivars lacking R6, the frequency of strains of the powdery mildew fungus that can overcome R6 is correlated with regional disease pressure. Race characterization of R6-virulent strains of the fungus predicts that R1 and R2 are the only known major R genes that remain effective. Seedlings were selected in 2014 to combine both R1 and R6 to create pyramided resistance that should be more durable than R1 alone.

OBJECTIVES:

- (1) Identify the distribution of virulent strains in Oregon;
- (2) Assess the susceptibility of previously resistant varieties; and
- (3) Determine if the defeated resistance gene still possess any value in breeding efforts for disease resistance.

PROCEDURES:

Determine race of R6 virulent isolates and what, if any, resistance genes remain effective.

The capacity to conduct race characterization was developed during 2013. Cultivars Yeoman (Rb), Zenith (Rb, R1), Target (R2), Challenger (Rb, R3), Serebrianka (R4), Early Choice (R5), and Nugget (R6) were obtained from the National Clonal Germplasm Repository or from Dr. Cindy Ocamb. These plants served as differential cultivars to infer the virulence and race of isolates of *P. macularis*. The presence of

the resistance genes in these cultivars was confirmed in 2013 and 2014 by inoculating cultivars with *P. macularis* isolates known to possess or not possess the corresponding virulence.

Once the differential set of cultivars was validated, isolates of *P. macularis* were collected from various hop yards in Oregon and Washington and maintained on detached leaves of a susceptible cultivar. After two weeks of growth, conidia were transferred to cultivar Nugget to confirm the presence or absence of V6 in the isolate. Following confirmation of V6, conidia of the isolates were transferred to three detached leaves of each of the cultivars in the differential set. After 3 weeks of incubation at 18°C, the inoculated leaves were evaluated for presence of powdery mildew. Leaves with no mildew present were confirmed with the aid of a stereomicroscope.

Surveys to determine geographic extent of powdery mildew on cultivars possessing R6-based resistance. To develop information on the extent and severity of powdery mildew on cultivars thought to contain R6-based resistance, a survey was conducted in 21 yards in Washington and 14 yards in Oregon planted to cultivars with R6-based resistance to powdery mildew. Yards included in the survey were dispersed across the main production regions in Washington and Oregon (termed Yakima Indian Reservation, Moxee, Lower Valley, and Oregon). In Oregon, cultivars Nugget and Super Galena were included in the survey. Cultivars Apollo, Bravo, Millennium, and Nugget were included in the survey in Washington. When possible a subset of each cultivar was included in each region to minimize confounding effects of a particular cultivar only appearing in one region in Washington. However, it was not possible to identify Millennium in the Lower Valley region or Nugget and Apollo in the Yakima Indian Reservation region.

Disease assessments were conducted during late-June in Oregon and early-July in Washington using established sampling methods (Gent et al., 2006). In Washington, the incidence of cones with powdery mildew also was estimated at harvest by collecting 25 cones from each of 20 plants along a single transect per hop yard (Gent et al., 2005). These same yards also were surveyed again during late September (post-harvest) and the incidence of plants with powdery mildew was rated. Time constraints prevented assessment of powdery mildew on cones or post-harvest in Oregon.

To understand powdery mildew susceptibility of cultivars possessing R6 more clearly, plots also were established in two hop yards in Washington of the cultivar Nugget. Cooperating growers were requested to withhold powdery mildew fungicide sprays to non-treated strips throughout the season. Each non-treated strip was at least five rows wide and at least 1000 feet in length. Assessments of disease incidence were made regularly on 75 plants in the middle row of the non-treated plot and in a directly adjacent plot where commercial management procedures were applied for powdery mildew. Experimental plots of cultivar Nugget also were established at WSU-Prosser during 2012. Refer to Dr. Grove's report for results.

Quantify susceptibility of hop lines possessing or lacking R6 to determine fitness and aggressiveness of isolates capable of overcoming R6. The susceptibility of hop genotypes lacking the R6 resistance gene to R6-virulent isolates of powdery mildew was assessed in growth chamber studies. Progeny from a cross made between Perle and the male USDA19058M were previously screened for powdery mildew resistance and molecular markers associated with powdery mildew susceptibility were identified (Henning et al., 2011). In that research, Henning et al. (2011) identified four markers associated with susceptibility to powdery mildew. Twelve individuals that were rated as susceptible to powdery mildew and possessed all four susceptibility markers and 12 individuals that were rated as resistant and lacked all four susceptibility markers were selected at random from the Perle x USDA19058M population. These plants were propagated and increased in a greenhouse free of powdery mildew, and 12 young,

fully expanded leaves were removed from each genotype and placed into double Petri dishes with the petiole in water to maintain the leaf. A set of 6 six leaves of each genotype was then inoculated with a mixture of three clonal isolates of *P. macularis* with confirmed pathogenicity on Nugget (R6-virulent) and a field population of *P. macularis* from Oregon that was not able to infect Nugget (non R6-virulent). A suspension of conidia of each inoculum source was prepared, adjusted to 10,000 conidia per mL, and applied to the leaves. The leaves were incubated at 18°C for 10 days, and then rated to assess common correlates of quantitative resistance, specifically latent period duration, lesion size, the number of conidia produced per unit of leaf area, and number of lesions produced per unit of leaf area. The experiment had an incomplete factorial structure and was randomized temporally four times in a complete block design. Factors evaluated were (1) host resistance (R6 resistance or not) and (2) *P. macularis* virulence (V6-virulent or not). Each run of the experiment was considered a random factor.

Prevalence of R6-virulent isolates of P. macularis. Extensive collection of powdery mildew samples was conducted during 2012 to 2014 to determine the frequency of R6-virulence amongst *P. macularis* isolates on cultivars lacking R6. To do this, 4 to 5 leaves or cones with powdery mildew were collected from each of 5 to 6 hop yards in Oregon, the Yakima Indian Reservation, Moxee, and the Lower Valley regions. From these samples, conidia were transferred to a susceptible cultivar lacking R6. After two to three weeks of growth, conidia were subsequently transferred onto three detached leaves of Nugget. After 3 weeks of incubation, the inoculated leaves were evaluated for presence of powdery mildew using a stereomicroscope.

Selection of germplasm with multiple R genes. A strategy to make qualitative resistance more durable is to combine multiple resistance genes into a single cultivar. This is referred to as stacked or pyramided resistance. This requires the pathogen to overcome multiple genes to infect the plant, which generally enables resistance to remain effective for a longer period when deployed broadly. To ensure that multiple R genes are in an individual plant one needs to be able to detect the presence of the genes through either genetic markers or based on phenotypic traits, which makes it difficult to detect stacked resistance.

To generate plants with pyramided resistance, a cross of Zenith (which possess R1) × 19058M (R6) was made by Dr. John Henning in 2013. This cross resulted in 396 seedlings. To select for progeny that putatively contained both R genes, a series of three sequential inoculations were conducted. Firstly, progeny were inoculated with 5×10^4 spores/mL of a common race of the fungus (virulence Vb, V3, V5) originating from multiple Oregon fields. Three weeks later, individuals that were free of powdery mildew were inoculated again with a strain of the pathogen able to overcome R6 but not R1. Three weeks after this, individuals that were remained free of powdery mildew were inoculated under quarantine conditions with a race of the fungus able to overcome R1, but not R6. Individuals that did not develop powdery mildew following this final inoculation were presumed to possess both R1 and R6.

SIGNIFICANT ACCOMPLISHMENTS:

The occurrence, distribution, and prevalence of powdery on formerly resistant cultivars was clarified, providing strategic guidance to growers on the threat posed to crops in their production region.

The susceptibility of formerly resistant cultivars was clarified through field surveys over multiple seasons, which helped the industry tailor disease management intensity to the susceptibility of the different cultivars.

Characterization of pathogen race structure identified major R genes and R gene combinations which

remain effective against the disease. The value of R6 in ongoing breeding programs was clarified.

Hop germplasm with putatively combined powdery mildew resistance factors was identified. Our intention is that both a male and female with reasonable vigor and other desirable characteristics can be selected from these plants and released as germplasm for future breeding efforts.

BENEFITS & IMPACT:

The hop industry has widely adopted new management approaches for powdery mildew on formerly resistant cultivars. This has helped to reduce crop damage in now susceptible cultivars and avoid unnecessary treatments in fields planted to less susceptible cultivars. The impact of these accomplishments has not been measured, but is probably several million dollars per year throughout the Pacific Northwestern U.S. A future germplasm release is expected, which will help to mitigate the damage from virulent strains of the powdery mildew fungus.

ADDITIONAL FUNDING RECEIVED: \$90,846

FUTURE FUNDING: None known at this time.