

U.P. HEDRICK STUDENT PAPER AWARD: SECOND PLACE WINNER 2009

Inheritance of the *Cr* Gene in *Ribes nigrum*DANIEL T. DALTON¹ AND KIM E. HUMMER²**Abstract**

Resistance to white pine blister rust (WPBR) disease, caused by *Cronartium ribicola* J.C. Fischer, is a critical objective for plant breeders seeking to release new black currant (*Ribes nigrum* L.) cultivars in North America. Genetic immunity to the disease was discovered in the Asiatic species, *R. ussuriense* Jancz. in the 1930s. 'Consort,' an immune F₁ genotype with the pedigree *R. nigrum* L. 'Kerry' × *R. ussuriense*, was released in 1952, and has developed neither uredinia nor telia in field or greenhouse inoculation trials. The objective of this study was to determine whether resistance in F₂ progeny of *R. nigrum* 'Ben Lomond' × 'Consort' segregates in a 1:1 ratio. Following artificial inoculation of single leaf cuttings in a controlled environment, 40 of the 86 F₂ genotypes were susceptible to WPBR; 46 exhibited no signs of the disease. Chi-square analysis failed to reject the H₀ that segregation of the resistance trait occurred 1:1. The *Cr* gene was inherited as a simple dominant allele in the F₂ generation. 'Consort' is heterozygous for the dominant *Cr* gene.

Genetic resistance to destructive pathogens is a vital characteristic for horticultural crops. Black currant production in North America was limited throughout the 20th century because the primary commercial species, *R. nigrum* L., was highly susceptible to *C. ribicola*, the causal agent of white pine blister rust (WPBR) (6). The *Ribes Cr* gene for immunity to *C. ribicola* has been characterized as a homozygous dominant trait from the Asiatic black currant species *R. ussuriense* Jancz. Controlled crosses of *R. ussuriense* with *R. nigrum* 'Kerry' produced non-segregating progeny for WPBR resistance (4). From these crosses, rust-immune cultivars 'Crusader' and 'Coronet' were released in 1948, followed by 'Consort' in 1952 (1). These cultivars have maintained resistance for over 60 years and are believed to be heterozygous carriers of the *Cr* gene (2). A test cross with a homozygous recessive individual should produce progeny segregating for immunity in a 1:1 ratio. The objective of this study was to determine whether immunity conferred by the *Cr* gene was inherited as a simple dominant trait in an F₂ population of *R. nigrum* 'Ben Lomond' × 'Consort.'

Materials and Methods

A population of 86 *R. nigrum* 'Ben Lomond' × 'Consort' F₂ seedlings was produced from a cross made in 2001, by Kim Hummer at the USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon. The seedlings were field-planted in 2005 and clonally propagated in 2007. Rooted cuttings were maintained in shade houses at the NCGR for the duration of the study.

Preliminary inoculation trials took place in 2007. Treatments were postponed until late in the season due to an early-season outbreak of powdery mildew. Because genotypes received few treatments, the 2007 data were excluded from statistical analysis. In 2008, the abaxial leaf surfaces of single-leaf softwood cuttings were inoculated on 11 dates. Each genotype was inoculated at least six times during the growing season. Susceptible cultivar 'Seabrook's Black' (PI 556176) and immune cultivar 'Coronet' (PI 617906) were included in disease screenings as controls (2, 3).

Aeciospores were collected from diseased seedlings of *Pinus monticola* Dougl. ex D. Don. Aeciospores were added to cold water-agarose solution (0.07% agarose concentra-

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tion) at a rate of 250 mg 1000 mL⁻¹. The spore solution was applied with a hand-held spray bottle. To generate urediniospores for late-season inoculation, plants of susceptible *Ribes* cultivars were inoculated with aeciospore solution on 8 August and 2 September 2008. Heavily infected leaves from these plants were harvested as needed to prepare urediniospore-water-agarose solution. Urediniospores were collected by gently scraping sporulating uredinia with the plastic tip of a disposable transfer pipette and washing the dislodged spores into a beaker with sterile water-agarose solution. Spore density was adjusted to 5×10^4 spores mL⁻¹. Urediniospore solution for late-season inoculation was not produced in sufficient volume for spray inoculation and was instead dabbed onto the leaf surfaces with a transfer pipette. Fresh batches of spore solution were prepared and applied on each treatment date except for 27 September 2008. On that date, a previously prepared solution was used.

By late June 2008, aeciospores collected from the USDA National Forest Service Dorena Genetic Resource Center during the previous spring had become moldy during storage at 4° C. To complete a scheduled aeciospore treatment, cryogenically preserved aeciospores from Miller Lake (ML), Klamath County, Oregon, were used. To acquire a bulk volume of viable aeciospores, a third population of WPBR was sampled from Tombstone Pass (TP), Linn County, Oregon, and stored at -20° C for the duration of the experiment. Both the ML and TP aeciospores were assayed on 2% agarose gel to assure viability prior to use. The TP aeciospores were used to generate urediniospores for late-season treatments. Small polypropylene inoculation chambers were constructed through modification of the design used by McDonald and colleagues (7). Treated plantlets were placed individually in the chambers and were kept inside an illuminated growth chamber programmed to provide a 16 h photoperiod on a diurnal cycle. Plantlets receiving aeciospore treatment were incubated for three days at 16° C, after which time the temperature was increased to 20°

C. Urediniospore-treated plantlets were kept at 20° C for the entirety of the period within the growth chamber. These temperatures are within the optimal range for spore germination and infection (5). The airtight lids of the inoculation chambers were replaced with tissue paper after two days to allow the interior of the chambers to dry and to discourage secondary infection.

Plantlets were examined weekly with a magnifying lamp (10x) for signs of *C. ribicola*. Genotypes were scored as susceptible if uredinia or telia were observed following any of the inoculation trials. If all plantlets of a given genotype failed to develop disease, the genotype was scored as resistant. A chi-square statistical procedure was applied to test the inheritance of resistance following artificial inoculation with the null hypothesis predicting that resistance should be expressed in 50% of the F₂ population (8).

Results and Discussion

Over the course of the experiment, 40 of the 86 F₂ genotypes (46.5%) showed susceptibility to one or both spore types (Table 1). The remaining 46 genotypes exhibited no signs of disease following artificial inoculation. Two additional genotypes were susceptible following exposure in the field to elevated disease pressure (data not shown). If *Cr* gene resistance is dominant, and the 'Consort' parent is heterozygous for this allele, then half of the F₂ progeny should show signs of the disease following artificial inoculation. These results

Table 1. Contingency table comparing *Cronartium ribicola* J. C. Fisher aeciospore and urediniospore inoculations on *R. nigrum* L. 'Ben Lomond' × 'Consort' F₂ progeny^z.

| | Aeciospores | | | |
|----------------|-------------|----|----|-------|
| | | + | - | total |
| Urediniospores | + | 28 | 8 | 36 |
| | - | 4 | 46 | 50 |
| | total | 32 | 54 | 86 |

^z Chi-square analysis: $\chi^2 = 0.419$; $p = 0.517$

failed to reject the null hypothesis that *Cr* gene resistance is inherited as a simple dominant trait ($\chi^2 = 0.419$, $p = 0.517$).

We conclude that the *Cr* gene, originally obtained from a homozygous resistant *R. ussuriense* parent, is inherited in a simple dominant Mendelian fashion in black currants. ‘Consort’ contains the dominant *Cr* gene in a heterozygous condition.

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ON THE BIOACCESSIBILITY OF PHENOLIC COMPOUNDS FROM APPLE

Phenolic compounds are widely reported to play an important role against reactive oxygen species, and are believed to be associated with reducing the risk of certain diseases. Not yet answered fully are question such as, “How much is good for you?” or “How much gets absorbed from different foods?” A recent article aimed at studying the transformations undergone by apple polyphenols during digestion. An in vitro model with dialysate membranes simulating the human alimentary tract was used to evaluate the composition and antioxidant properties of fresh apples, and products of their digestion. Epicatechin, chlorogenic acid, and procyanidins were the main antioxidant compounds in the whole fruits and flesh, and quercetin glycosides were present in the peel. As a result of in vitro digestion, both the concentration and the antioxidant activity of the polyphenolics in the dialysates increased as compared to the raw materials (from 35% to 95% and from 50% to 236%, respectively). In the simulated alimentary tract, procyanidins disintegrated to (+) catechin, which is well absorbed from the small intestine, while chlorogenic acid and quercetin glycosides had low bioavailability. Paraphrased from Tarko et al. 2009. Simulation of phenolic compounds transformations and interactions in an in vitro model of the human alimentary tract. Food Science and Technology International 15(3):235-241.