AN ABSTRACT OF THE THESIS OF

Megan M. Mathey for the degree of Master of Science in Horticulture presented on June 17, 2013

Title: Phenotyping Diverse Strawberry (*Fragaria* spp.) Germplasm for Aid in Marker-Assisted Breeding, and Marker-Trait Association for Red Stele (*Phytophthora fragariae*) Resistance Marker *Rpf1*

Abstract Approved:

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Chad E. Finn

Strawberry is one of four crops included in the USDA-NIFA Specialty Crop Research Initiative-funded RosBREED project along with apple, peach, and cherry. Phenotyping strawberry for specific horticultural and commercial traits is important to identify associations with genotypic marker(s). This process is the first step in translating genomic knowledge into enhanced breeding efficiency through marker-assisted breeding. Four trait categories were studied: phenology and flowering traits, plant characteristics, fruit characteristics, and fruit chemistry. RosBREED strawberry germplasm consisted of 947 individuals representing the breadth of diversity used in breeding the domesticated strawberry. These individuals were planted in replicated fields in Oregon, California, Michigan, New Hampshire and Florida and phenotyped in 2011 and 2012.

In addition, a simple sequence repeat (SSR) set composed of the *Rpf1* SSR marker and ARSFL007 was used to genotype the RosBREED strawberry germplasm for marker presence and parentage confirmation. A subset of 153 individuals from the RosBREED collection was inoculated in bench tests with two races of *Phytophthora fragariae* and disease response was compared to validate the *Rpf1* SSR association.

Ten progeny from 36 crosses of genotypes representing eastern and western North American and European short day and remontant strawberry cultivars were included as part of the RosBREED strawberry phenotypic collection. This set of germplasm was used to calculate genotype by environment interaction as well as determine general and specific combining ability variance components and effects for the populations among the RosBREED field sites (OR, CA, MI, NH and FL).

# Phenotyping Diverse Strawberry (*Fragaria* spp.) Germplasm for Aid in Marker-Assisted Breeding, and Marker-Trait Association for Red Stele (*Phytophthora fragariae*) Resistance Marker *Rpf1*

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Megan M. Mathey, Author

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## CONTRIBUTION OF AUTHORS

Chad Finn assisted with phenotype protocol development, germplasm selection, interpretation of statistical analyses and editing this thesis. Nahla Bassil assisted with using and interpreting the *Rpf1* SSR marker results along with editing this thesis. Jim Hancock assisted in phenotype protocol development, aided in data interpretation, and reviewed sections of this thesis. Umesh Rosyara assisted with statistical analysis and data interpretation in the genotype by environment and general and specific combining ability analyses. Andrew Jamieson oversaw the *Phytophthora fragariae* disease screening. Eric van de Weg provided the *Rpf1* SSR marker for the validation study and his immense expertise in the genetics of red stele resistance in strawberry. Sonali Mookerjee, Kazim Gündüz, Lise Mahoney, Phil Stewart and Vance Whitaker recorded phenotypic data for 2011 and 2012 in Michigan (Sonali and Kazim), New Hampshire (Lise), California (Phil) and Florida (Vance). Hanna DeVyldere and Madeline Abrams assisted me in phenotype collection for 2011 and 2012, respectively. Ted Mackey and Mary Peterson assisted in field maintenance and propagation. April Nyberg, Liz Alperin and Barb Gilmore trained and assisted me in DNA extraction, PCR, genotype submission and data analysis.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF TABLES (Continued)

Table Page

LIST OF TABLES (Continued)

Table Page

LIST OF APPENDIX TABLES

Table Page

FIGURES

Figure Page

# General Introduction

## Taxonomy and history

Strawberry (*Fragaria* L.) is a member of the Rosaceae, subfamily *Rosoideae*. This genus is native to North America, South America, Asia, and Europe (Hummer et al., 2011). It is the most widely distributed fruit crop in the world and is grown in every country with a temperate or subtropical climate (Chandler et al., 2012). The current *Fragaria* taxonomy includes 21 wild species, three described naturally occurring hybrid species, and the cultivated *F.* ×*ananassa* encompassing an array of ploidy levels from diploid to decaploid (Hummer et al., 2011, Hummer, 2012).

The first domesticated strawberry in the Old World, *F. vesca* L., was originally cultivated by the Greeks and Romans and by the 1300's had spread throughout Europe (Darrow, 1966). While *F. vesca* was popular from the 1500's to the 1600's, after the introduction of *F. virginiana* Mill., from Canada and Virginia, *F. vesca* was surpassed in popularity (Hummer and Hancock, 2009). In the early 1700's, a French spy, Captain Amédée Frézier, introduced a clone of *F. chiloensis* (L.) Mill, with a domestication history in Chile of about 1,000 years (Darrow, 1966; Hancock et al., 1999; Finn et al., 2013). After this introduction, unique seedlings began appearing in European gardens. In 1766, Antoine Nicolas Duchesne, a botanist, recognized that these unique plants were hybrids of *F. chiloensis* and *F. virginiana*. This is the origin of today's cultivated strawberry, *Fragaria* ×*ananassa* Duch. ex Rozier (Hummer and Hancock, 2009).

Formal strawberry breeding began in England in 1817 by Thomas A. Knight. He was one of the first systematic breeders of any crop, and integrated crosses of clones of *F.*

*chiloensis* and *F. virginiana* (Darrow, 1966, Hancock, 1999). Public breeding in the U.S. began at the New York Agricultural Experiment Station (Geneva, NY) in 1889 and by 1917, 12 cultivars were developed and released (Darrow, 1966).

The United States strawberry industry began in about 1800 with wild selections of *F. virginiana*. Species selections predominated in cultivation until about 1840 when a cultivar 'Hovey' replaced about one-tenth of the plantings (Darrow, 1966). A larger change took place in 1858 when the cultivar Wilson began replacing the native species (Darrow, 1966). From this pivotal point the strawberry industry has continued to advance via introductions of new cultivars with superior quality.

Traditionally, strawberry cultivars were classified into two distinct types of floral initiation categories based on response to photoperiod. June-bearing strawberry genotypes are defined as facultative short-day (SD) plants and everbearing were classified as long-day (LD) plants. A third type, photoperiod-insensitive plants or day-neutral (DN) was added in the 1970's after Bringhurst and Voth (1978) successfully integrated day-neutrality (Bradford et al., 2010). Temperature also has an influence on flower initiation and runnering in each category, complicating classification. Flower initiation of SD types can occur under any photoperiod, if temperatures are cool enough (<15 °C) (Bradford et al., 2010) and runnering of SD types is prompted by long days and temperatures >21 °C (Bernadine C. Strik, pers. comm.). Cultivars that are DN generate flower buds cyclically regardless of photoperiod when temperatures are moderate (<28 °C) (Serçe and Hancock, 2005a). Everbearing plants under high temperatures (>25 °C) respond as qualitative LD types, meaning plants rely on LD to initiate flowers (Bradford

et al., 2010). Everbearers growing under lower temperatures (10 to 25 °C) respond as quantitative LD types, meaning flower initiation is promoted by long days, but not necessarily dependent upon them for flower initiation (Bradford et al., 2010). These LD plants can also be considered DN at temperatures below 10 °C (Bradford et al., 2010). Runner production in general, is stimulated by high temperatures (>20 °C) and long days (>14 h). However, DN cultivars tend to have low runner production (Bradford et al., 2010, Darrow 1936; Durner et al., 1984; Heide and Sønsteby 2007).

The inheritance, potential native sources, and environmental influences of everbearing and day-neutral strawberry have been studied for almost a century (Sønsteby and Heide, 2007). Today all modern day-neutral cultivars trace back to the original Bringhurst and Voth (1978) cross. While that cross revolutionized the strawberry industry as we know it, integrating day-neutrality from that wild source into high quality commercial acceptable cultivars took many years. Therefore, current research efforts focus on finding the gene or genes for day-neutrality to implement in marker-assisted breeding.

Understanding the genetic control of day-neutrality has been the focus of many studies (Gaston et al., 2013; Serçe and Hancock, 2005b; Shaw and Famula, 2005; Weebadde et al., 2008). Eight quantitative trait loci (QTL) were found associated with day-neutrality (Weebadde et al., 2008). Recently, a single QTL was discovered that controls flower initiation and runnering in octoploid strawberry (Gaston et al., 2013). However, this locus was not orthologous to the loci affecting repeat flower initiation and runnering in *F. vesca*, therefore suggesting different genetic control among *Fragaria* spp.

(Gaston et al., 2013). This is consistent with observations of Serçe and Hancock (2005a) where DN was observed in *F. virginiana* that produced greater runners than *F.* ×*ananassa*. These discoveries could allow possible introduction of greater runnering to DN *F.* ×*ananassa* cultivars.

Production worldwide

Strawberries are the most economically significant soft fruit crop in the world (Hummer and Hancock, 2009). The United States has been the world's top producer of strawberries since at least 1961, which is as far back as the United Nations Food and Agriculture Organization Statistical Database (FAO) has records. In 2011, the United States produced 1,312,960 metric tons of fruit, followed by Spain, Turkey, Egypt and Mexico (FAO, 2013). In 2010, the Americas led the world in regional production with 39.13% of the world's strawberry fruit followed by Europe (32.62%), Asia (18.41%) Africa (9.03%) and Oceania (0.81%) (FAO, 2012). However, this is a recent lead, as in 2006 Europe led the Americas 39.20% to 35.96% (FAO, 2012).

The United States most important fresh strawberry export markets were Canada and Japan in 2011, with 248.3 and 8.5 million pounds respectively, and 22.5 million pounds of fresh fruit was exported to other countries (USDA-ERS, 2012). Canada and Japan received the majority of the United States frozen export in 2011, at 22.6 and 14.9 million pounds, respectively, with 7.6 million pounds going to other countries (USDA-ERS, 2012).

The major North American production regions were California and Florida, with 38,000 and 9,000 harvestable acres respectively in 2011 (USDA-ERS, 2012). Oregon had the third largest acreage in 2011 at 2000 acres. Following Oregon in production are New York, North Carolina and Washington, which each harvested 1,400 and 1,500 acres in 2011 (USDA-ERS, 2012).

Strawberry uses in the U.S.

The major focus of the Pacific Northwest (PNW) (Washington, Oregon and British Columbia) growing region is producing berries for processing and frozen products, and a smaller amount is consumed locally fresh. The PNW is second to California for production region of processed fruit in the US (Hokanson and Finn, 2000). Even though the PNW is second in production, it produces the world's highest quality processing strawberries (Hokanson and Finn, 2000). Specific traits that make this region the leader in quality standards include a cap (calyx) that is easily removed at time of harvest, intense flavor, high acid and sugar levels, red internal and external color, and low drip-loss or integrity of the fruit after being frozen (Hokanson and Finn, 2000). Even though California is the major producer of processing strawberries, the fruit for processing makes up roughly one-quarter of the total fruit produced in the state. In 2010 in California, 1,057,755 metric tons of fruit were sold fresh and 254,418 tonnes were processed (NASS, 2013).

The breeding of improved fresh market cultivars places high importance on appearance, uniformity, large-size, and firm fruit that can be shipped throughout the

world while maintaining quality (Hokanson and Finn, 2000). In North America, fresh market berries are produced largely in California, Florida and Mexico. With the advent of different cultural practices, including planting times, and different climates, fresh strawberry fruits can be found in the US market year round.

Strawberry plants are also used ornamentally. Some are noteworthy for their glossy, ground covering foliage, a trait of *F. chiloensis*, or pink flowers, which were the result of an intergeneric hybridization with *Potentilla palustris* L. Scop. (Mabberley, 2002). Others have variegated foliage and species material such as *F. vesca* can make a nice addition to a woodland landscape.

Main production systems

Strawberries in the wild are perennials, but for commercial production in the United States they can be grown either as a perennial or annual. The annual system is popular in California, and the mid-Atlantic and southeastern states and is modified to accommodate each region. Perennial production is more common in the Pacific Northwest, the upper Midwest, and the Northeast. Depending on the specific growing requirements of the region, the production systems are modified with changes in planting date, amount of chilling the nursery plants receive, mulch type on beds, bed height, irrigation, weed management, and renovation (Hancock, 1999).

Perennial production usually uses a matted row or the hill system. The matted row is the most common production system in the Pacific Northwest. This system requires a one-year establishment period and usually SD cultivars are used. Typically, dormant bare

root plants are planted 30-45 cm apart on raised beds with no plastic mulch. The mother plants produce runners that are encouraged to root and develop as daughter plants to fill out the bed. The first crop is harvested 12-14 months after planting. After harvest, the planting is renovated to encourage the production of vigorous daughter plants for subsequent crops. The plantings are harvested typically for 2-3 years, however isolated much older plantings are not uncommon.

The hill system is used mainly for annual production but has also been used in perennial systems to grow DN cultivars everywhere and SD types in areas that have warm winters and either hot or moderate summers (Hancock, 1999). The hills can be left as bare ground or covered in plastic. Runner plants are removed, and the plant then can divert more energy to branch crown formation and increasing crown diameter, thus increasing the sites for flower bud initiation.

In California there are two primary management systems, a summer planted system is used in northern and central California (e.g. Watsonville, CA) and the winter planted system in southern coastal California (e.g. Oxnard, CA). In the summer planted system, dormant plants are dug from a low elevation nursery in mid-winter then stored at -1.5 °C and planted in June-July on raised beds and harvested May through December (Bernadine C. Strik, pers. comm.). This production system uses day-neutral strawberry cultivars and the plantings are typically only fruited for one harvest season.

The winter production system is generally used in southern California. This system usually includes SD genotypes that were grown in high elevation nurseries in northern California or southern Oregon. Chilling temperature accumulation is monitored

carefully and when sufficient chilling has been accumulated, the plants are dug, trimmed, and quickly replanted in southern California. Typically digging takes place in September and October. Depending on genotype, some plants are stored for up to four weeks at 1.1 °C to achieve chilling requirements. Plants established in the fall will fruit from January to May in southern California. Plants in this system are usually only fruited for one year.

Florida is the second largest strawberry producing state in the US. Their system is somewhat similar to California's winter production system. Plants that have sufficient chilling are usually dug from nurseries in eastern Canada in fall. Unlike in California, the foliage is kept intact and not trimmed. The dug plants are quickly transported and replanted in Florida in September through October. Plants established using this approach will fruit in December through April. The plants are only fruited for one year.

The last major annual system modification is the North Carolina plasticulture system. Many of the practices are similar to those in California's winter production system, however instead of bare root plots, plants grown in plug trays produced in protected culture are used. Depending on the latitude, the plants are established in early fall further south and late summer further north. The goal is to get a healthy plant established before the plants go dormant. The fields are usually only harvested for one year. The main cultivar used in this system is the SD 'Chandler'. This is the commonly used system for neighboring states without harsh winters.

In Europe, while most production systems are comparable to one of the systems described above, there are other systems such as waiting bed production. With waiting bed production, mature plants are grown in a nursery and transplanted into the production

system so that the plants can be in production about eight weeks after planting. This system allows the grower to get into production very quickly, but the plant costs are much more significant.

Production challenges

Strawberries are subject to numerous biotic and abiotic stresses. Multiple diseases, viruses, insects and weather events threaten strawberries. Each one of these presents a challenge to the grower and puts pressure on the breeder to develop cultivars that are resistant to these stresses. While there are numerous fungal, bacterial, viral and insect pests of strawberry, they vary by location. A few common problems or problems typical of the Pacific Northwest discussed below.

Grey mold (*Botrytis cinerea*, Pers. ex Fr.) is a universal problem and produces a fuzzy grey mold encompassing the fruit (Hancock et al., 1996). Powdery mildew (*Podosphaera macularis* (Wallr.) U. Braun & S. Takam.) can colonize the fruit and leaf surface with a white fungal layer making the fruit bitter flavored (Hancock et al., 1996). Anthracnose fruit and crown rot (*Colletotrichum acutatum* Brooks, *C. gloeosporioides* Penz.) cause a sunken black spot on the fruit and can cause the crown to die (Nickerson, 1998). Phytophthora wilt, crown rot and red stele (*Phytophthora citricola* Sawada, *P. cactorum* [Leb. et Cohn] Schroet., and *P. fragariae* var. *fragariae* Hickman., respectively) all cause root damage, leading to a stressed plant and eventual death (Hancock et al, 1996) and will be discussed in more detail below. Leaf spot (*Mycosphaerella fragariae* [Tul.] Lindau) causes necrotic splotches on the leaves and is

problematic in perennial plantings, as the fungus can overwinter on past season leaves (Nickerson, 1998). Leaf scorch (*Diplocarpon earliana* Ell. and Everh.) gives the plant the appearance of being burned (Hancock et al., 1996). Verticillium wilt (*Verticillium albo-atrum* Reinke and Berth. and *V. dahlia* J.F.H. Beyma) affects the roots of the plants causing the plant to appear drought stressed eventually leading to collapse and death (Nickerson, 1998). The main bacterial disease problem is angular leaf spot (*Xanthomonas fragariae* Kennedy & King) (Nickerson, 1998).

Viruses are a significant threat to strawberries and are spread mainly by aphids, but can be transmitted by whitefly (major problem in Eastern US and California), and nematodes, as well as through the pollen, seed and vegetatively. More than 30 viruses affecting strawberry are known (Martin et al., 2001; Martin and Tzanetakis, 2006). The four most economically significant viruses vectored by aphids are *Strawberry crinkle virus*, *Strawberry mottle virus*, *Strawberry mild yellow edge virus*, and *Strawberry vein banding virus* (Martin and Tzanetakis, 2006). General symptoms of these viruses include: reduction in fruit yield and size, deformed leaves and petioles, leaflets with chlorotic spots, size reduction in leaves, and necrotic spots on runners, reduced vigor, petioles and petals (Martin and Tzanetakis, 2006). Viruses can be controlled using certified virus free transplants, as well as insecticides to control insect vectors where pressure on the crop is high.

Arthropods and nematodes can also have an impact on strawberry production. Nematodes, thrips and aphids, as mentioned previously, vector viruses. Mites feed by sucking leaf surfaces leaving mottled leaves, plants often look dusty as mites leave a fine

webbing over the plant. Lygus bugs feed on the fruit as well as flower buds which results in small, deformed and undeveloped fruit (Easterbrook, 2000). Root weevils have been a big problem in the PNW since the 1920's (Wilcox et al., 1934). Multiple species exist and the larvae cause the most damage as they feed on strawberry crowns and roots (Hancock, 1999) causing red leaves and undersized berries. Adult weevils feed on leaf margins during the night causing noticeable but not detrimental damage (Hancock, 1999). Cultivars have varying degrees of resistance. 'Stolo' is recent release with root weevil resistance (Kempler et al., 2011).

Abiotic stresses can have an impact on strawberry production and some can create the perfect environment for diseases. Frost can kill or damage flowers leading to less fruit production or non-uniform, monkey-faced fruit. Soil salinity can cause physiological changes leading to leaf burn, stunting and eventual death (Barroso and Alvarez, 1997). Nutritional deficiencies such as boron can lead to deformed fruit and zinc and copper deficiency can lead to poor fruit set and irregular development. An abundance of nitrogen can cause the plants to grow more vegetatively at the expense of fruit production or decrease fruit quality. High temperatures can sunburn fruit and cause remontant types of strawberry to cease flower initiation until moderate temperatures return. High moisture levels, leading to waterlogged soils, combined with cool conditions create the perfect environment for *P. fragariae* to infect and weaken or kill plants.

# BREEDING

## History

While there was a substantial history breeding strawberries in Europe, the first public breeding program in the U.S. began at the New York Agricultural Experiment Station (Geneva, NY) in 1889 and by 1917, 12 cultivars had been released (Darrow, 1966). However, the first conventional cross to be made in the U.S. was most likely by Charles Hovey of Cambridge, Massachusetts in 1834 (Hendrick, 1925). At that time strawberry cultivars had been imported from Europe, however, the hot summers and cold winters were hard on the plants. Hovey took advantage of the native *F. virginiana* and crossed it with a European berry (*F. ×ananassa*) with the hopes of getting large fruit and flavor from the *F. ×ananassa* but with the hardy nature of the *F. virginiana.* This cross resulted in the first important North American cultivar 'Hovey' (Hendrick, 1925).

Strawberry breeding in Oregon began in 1911 and continued to 1918 under the direction of V.R. Gardner. After a two-year break, the program resumed in 1920 under C.E. Schuster (Darrow, 1966). In 1928, the Oregon breeding program "was made cooperative" with the U.S. Department of Agriculture. The work was taken over by George M. Darrow from 1930 to 1932 and Schuster began nut research. During this time, George F. Waldo was working for the U.S. Department of Agriculture, at the station in Glenn Dale, Maryland, in conjunction with Darrow at the Corvallis, OR station until they swapped positions in 1932 (Finn, 2006). Over the years many breeding objectives were pursued. Gardner bred for superior shipping cultivars and Schuster for a canning cultivar. Later, the USDA-ARS expanded on the objectives of previous breeders and began to

focus specifically on freezing, preserving and shipping quality. Specific traits that make this region the leader in quality standards include a cap (calyx) that is easily removed at time of harvest, intense flavor, high acid and sugar levels, red internal and external color, and low drip-loss or integrity of the fruit after being frozen (Hokanson and Finn, 2000).

## Current breeding

Today, the largest public breeding programs in North America are at the University of California and the University of Florida. Driscoll Strawberry Associates (Watsonville, CA) is believed to be the largest private breeding initiative in the U.S. (Hancock et al., 2008). In addition to the public programs in California and Florida, there are seven other state supported programs in the U.S.: Washington State University, Michigan State University, Rutgers University, Cornell University, North Carolina State University, University of Wisconsin-River Falls, and University of Florida (Hancock et al., 2008). Until 2012, Many breeding programs existed in Canada, including: Agriculture and Agri-Food Canada in Kentville (Nova Scotia), University of Guelph, Ontario, Horticulture Research and Development Center in Quebec and the Agriculture and Agri-Foods Canada program in Agassiz, British Columbia. As of 2012, Agriculture and Agri-Food Canada decided to close down all of their cultivar development programs for all crops across Canada (C. Kempler, pers. comm.). The largest cultivated strawberry breeding locations in Europe are in France, Italy, the Netherlands, Spain, and the U.K. (Hancock et al., 2008). In Asia, Japan has had the most long term and significant breeding efforts. Though breeding efforts are specific to each region, general goals

include developing plants that are high yielding and disease resistant and that produce large fruit with high quality for whatever the most important market is for the crop (Hancock, et al., 2008).

Development of day-neutral flowering cultivars is a major accomplishment

Incorporating the day-neutrality flowering characteristic into high quality strawberry cultivars was one of the most significant developments in strawberry breeding. Day-neutrality was introduced from a cross made during the 1960's at the University of California (Bringhurst and Voth, 1978). The trait was found in a native *F. virginiana* ssp. *glauca* (Watson) Staudt clone from the Wasatch Mountains of Utah and integrated by backcrossing to *F. ×ananassa* (Bringhurst and Voth, 1978). Up until this discovery the flowering of the cultivated strawberry was strongly tied to day-length and therefore limited fruiting to a short time frame.

Today, SD and DN cultivars make up the majority of the plants grown for fruit production in the U.S. (Serçe and Hancock, 2005b). One of the main problems with the expression of DN is that flower initiation is inhibited where temperatures rise above 26 °C. This is not a problem in coastal growing regions where temperatures stay moderate (below 26 °C) such as in coastal California but becomes a problem in most of the growing regions in the other parts of the US. Developing cultivars that are not only day-neutral but also continue to flower and set fruit under warmer conditions is a major goal of breeding programs outside of California.

Breeding vegetatively propagated crops

Vegetatively propagated or asexual crops are propagated clonally for commercial production. Cultivars of clonally propagated crops represent a superior combination of genes and generally are highly heterozygous. Inbreeding, a popular method for developing homozygous lines of seed crops, leads to a decline in vigor and fertility in clonally propagated crops, therefore different breeding strategies need to be used (Mehlenbacher, 1995). The first step in a breeding program is identifying the trait or traits of interest and identifying the germplasm sources from which that trait can be integrated into the offspring. A method of "complementary hybridization" or phenotypic recurrent selection where weaknesses of one parent are matched by the strengths of the other parent in the hopes that some of their offspring have the strengths of both parents is a common method (Mehlenbacher, 1995). Evaluation after the initial cross is then stepwise; the first step being the identification of superior offspring; the second step is further evaluation; the third step replicates advanced selections and compares them against commercial standards. After the advanced trials, the best performing selection may be released as a new cultivar while other advanced selections may be used as parents (Mehlenbacher, 1995). This method of complementary hybridization is then repeated generation to generation. This is the typical approach in strawberry breeding programs.

Another major breeding method is the modified backcross method, which involves multiple crosses to transfer a single trait from a wild or non-cultivar quality plant. This involves using a "different recurrent" parent with each backcross to avoid inbreeding depression (Mehlenbacher, 1995). This was the method used for transferring day-neutrality into the cultivated strawberry (Bringhurst and Voth, 1978).

Different tools exist for predicting the best parent to use in crosses including estimating heritability and general and specific combining ability. Heritability is usually calculated in a broad or narrow-sense. Broad sense heritability ($H^2$) for a trait is an estimate of how much of the phenotypic variability for a trait is due to genetic versus non-genetic control (Bernardo, 2010a). Narrow-sense heritability ($h^2$) is an estimate of the proportion of the phenotypic variability that is due to additive genetic variance (Bernardo, 2010a). Additive variance is the total variance contributed by the additive effects of each gene (variance of breeding values). The amount of additive genetic variation reflects the amount of potential improvement that can be realized in response to selection pressure. A low narrow-sense heritability indicates that phenotypic value is a poor indicator of the breeding value (Bernardo, 2010a).

Estimating general and specific combining ability effects (GCA and SCA) respectfully have been useful tools in traditional breeding programs allowing for identification of the best parents and the best crosses. The GCA effects estimate the contribution a parent has to its progenies for a specific trait and is expressed as the deviation from the mean of all crosses. The SCA is a function of all non-additive effects of the genes for a particular trait (Bernardo, 2010b). The combining ability estimates can be considered as fixed or random in the linear model and the associated variance components can be estimated by ordinary least squares computations (ANOVA), Maximum likelihood estimation or restricted maximum likelihood (REML). REML is most versatile method of estimation (Little et al., 2006). Whether parents involved in combining ability estimates are considered as fixed or random depends upon which

inferences are to be made (Baker, 1978). In fixed effect models, the inference is made about the parents involved, while in random effect models the inference is made about the population they represent (Baker, 1978).

Marker-assisted breeding

Marker-assisted breeding is based on using molecular tests to look for specific valuable trait alleles in either parent material (marker-assisted parent selection MAPS) or to screen seedlings for specific alleles (marker-assisted seedling selection MASS). Marker-assisted breeding has been used with success in many plant breeding programs and examples in perennial fruit crops include apple (K. Evans, pers. comm. 2013), cherry (A. Iezzoni, pers. comm.), hazelnut (Sathuvalli et al., 2007) and to a small extent strawberry (Whitaker, 2011).

While MAB has not been widely used in cultivated strawberry (*F.* ×*ananassa*), a few breeding programs are using markers. Breeders in collaboration with the Institut National de la Recherche Agronomique (INRA) in France have used molecular markers linked to *Rca2*, a single gene for resistance to anthracnose (*Colletotrichum acutatum* J.H. Simmonds) for parent selection (Whitaker, 2011). Driscoll Strawberry Associates, Inc. (Watsonville, CA) used *Rca_240* and other *Rca2* markers developed in-house to screen 20% of their seedlings (Whitaker, 2011). Other molecular markers have been discovered for red stele resistance (*Phytophthora fragariae* Hickman) (van de Weg, pers. comm.) and remontancy (Hancock, pers. comm. and Denoyes pers. comm.) and are being validated to assess their applicability across diverse germplasm.

The value of marker-assisted breeding is achieved due to the increase in efficiency by allowing breeders to identify a trait in a genotype that might takes years of evaluation to visualize or screen. Breeders can therefore run a smaller breeding program that should have the same chances of success (fewer seedlings) for the traits of interest or make more rapid advances by planting the same number of seedlings but knowing that all the seedlings contain the traits of interest.

## RosBREED

"RosBREED: Enabling marker-assisted breeding in Rosaceae" is a multistate, multi-institutional, multi-national project dedicated to the improvement of U.S. rosaceous crops by targeted applications of genomics knowledge and tools to enhance efficiency of breeding programs and was funded through the USDA-NIFA Specialty Crops Research Initiative (Iezzoni et al., 2010). Within the Rosaceae, *Malus* sp. (apple), *Prunus* sp. (peach and cherry), and *Fragaria* sp. (strawberry) are being studied and the project encompasses 12 breeding programs.

The overall belief of RosBREED is that an "Integration of modern genomics tools with traditional breeding approaches will transform crop improvement in the Rosaceae, significantly improving profitability of U.S. rosaceous crop industries." Marker assisted breeding will yield long-term economic, social and environmental benefits as well as developing superior fruit cultivars more efficiently and contributing to increased consumption and enjoyment of these fruit, nut and floral products (Iezzoni, et al., 2010).

The scenario where a breeder can enter the desired phenotype into a "Breeders Toolbox" database and be able to identify all seedlings or cultivars that have that trait as well as all the associated information like genotypes, markers available to identify this trait and companies that can provide such a genotyping service is already available for strawberry through the Genome Database for Rosaceae website at http://www.rosaceae.org/breeders_toolbox. This toolbox also incorporates a Breeding Information Management (BIM) System that is being trialed at this time and allows the breeder to use the phenotypic and genotypic for all the strawberry individuals included in the database in addition to socio-economic information about the traits obtained by the socio-economic team and the technology portfolio to make more rapid and informed crossing decisions with the "Cross-assist" function, which suggests the best parents for desired traits and the number of seedlings that would be needed to achieve the trait of interest (Jung et al., 2008).

RosBREED is using Pedigree-based Analysis (PBA) to identify and validate quantitative trail loci (QTL) and the favorable allele(s) responsible for a phenotype of interest in a group of pedigree-linked genotypes (Iezzoni et al., 2010; van de Weg et al., 2004). Pedimap™ is a program used to visualize and track the relatedness of the pedigree linked germplasm set and works in conjunction with FlexQTL™ which find associations between phenotypic traits and regions in the genome that are responsible for that trait (Bink et al., 2002; 2008). PBA uses identity by descent (IBD) to relate alleles across pedigree-linked individuals to determine their impact on phenotype. The attractiveness of the PBA approach is that it utilizes multiple breeding populations that are linked by

pedigree and typically used by breeders and thus segregate for multiple traits as opposed to making a single large mapping population that segregates only for traits present in the two parents (Whitaker, 2011).

In order to pursue this MAB approach, phenotypic and molecular data are needed and markers for traits of interest need to be discovered and developed. RosBREED's strawberry group identified 947 *Fragaria* genotypes to include in the Crop Reference Set (CRS) and Breeding Pedigree Set (BPS). The CRS includes genotypes of selected native species that have desirable traits, cultivars, founders and breeding families from the USDA-ARS (OR) and Michigan State University that are linked by pedigree and representative of all US breeding programs. The BPS consists of five mapping populations that are segregating for traits of interest to strawberry production in the geographical region represented by the programs that developed them. These genotypes were provided from multiple institutions including: the USDA-ARS Horticulture Crops Research Unit (HCRU; Corvallis, OR), Michigan State University, the USDA-ARS National Clonal Germplasm Repository (NCGR; Corvallis, OR), the University of Florida, the University of New Hampshire, the Instituto de Investigación y Formación Agraria y Pesquera (IFAPA; Málaga, Spain), East Malling Research (EMR) in the United Kingdom, INRA (Maison Jeannette, France) and the Center for Genetic Resources (CGN; Wageningen, The Netherlands. Driscoll Strawberry Associates (Watsonville, CA) provided critical support, as they were able to bring the European material into the US using their long established quarantine and propagation facilities.

Characterizing this diverse germplasm will help strawberry breeders gain a better understanding of the diversity available for breeding. The database that is assembled for the phenotypic traits associated with these genotypes, when combined with genome-wide genotypic data will be critical for identification of marker-locus trait associations. High-throughput genome scan methods were developed for octoploid strawberry and are being evaluated in this germplasm and include a 90K Axiom® (Affymetrix, Santa Clara, CA) chip, IStraw90 (Nahla Bassil, per. comm., 2013) and genotyping-by-sequencing (Elshire et al., 2011). Once marker-locus trait associations are identified for traits of economic importance, they will be converted to molecular markers that can be easily and cheaply adopted by breeders in parent or seedling selection. These molecular tools will allow breeders a more efficient method to breed for traits that are high in demand for growers, food processors, retailers and consumers or that are problematic to phenotype using traditional breeding approaches.

Genetic analysis of data for octoploid strawberry is complicated by its ploidy level. However, its allopolyploid nature means its chromosomes pair in a disomic fashion making it possible to "diploidize" it to simplify analysis. The process of microsatellite allele dose and configuration establishment (MADCE) approach was created to allow genotype data from allopolyploids that act like a diploid to be analyzed. Once diploidized, strawberry data can be analyzed with tools such as FlexQTL (van Dijk et al., 2012).

# CONCLUSIONS

Strawberries have been an important fruit crop worldwide for well over a century and have a long history of breeding, selection, and cultivation. The goals of this thesis were to characterize 947 strawberry individuals for traits that will eventually be used to identify QTL through pedigree-based analysis. These traits could then be integrated into a marker assisted breeding program. One of these traits, day-neutrality, will in addition to eventual QTL studies, be analyzed for GCA, SCA and heritability in four different environments.

This thesis presents a standardized phenotyping protocol for strawberry as part of RosBREED, a marker-trait association for a proposed SSR marker linked to the *Rpf1* gene for *Phytophthora fragariae* resistance and genotype by environment interaction as well as heritability and general and specific combining ability for 36 families crossed to a set of day-neutral parents by cultivars adapted to the Pacific Northwest region and those adapted to the Midwest.

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# Large-Scale Standardized Phenotyping of Strawberry in RosBREED

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## Abstract

In an effort to implement marker-assisted breeding in Rosaceae, many traits need to be characterized in diverse germplasm. The USDA-NIFA Specialty Crop Research Initiative-funded RosBREED project includes breeding programs of four Rosaceae crops (apple, peach, cherry and strawberry). Phenotyping each crop for specific horticultural and commercial traits is an important process needed to translate genomic knowledge through marker-assisted breeding into enhanced breeding efficiency. This data will directly aid in the identification of quantitative trait loci or marker-trait associations that will be used to assist breeding programs in the future. Large-scale, standardized phenotyping protocols have been set up for each crop. The standardized phenotyping protocol for strawberries was agreed upon by the breeding teams in Oregon, Michigan, New Hampshire, California and Florida and includes four trait categories: phenology and other flower-related traits, plant characteristics, fruit characteristics, and fruit chemistry traits. We describe how each of the traits in the categories was evaluated. A summary of mean values for 37 traits of the genotypes planted at the RosBREED locations in 2011 and 2012 is provided. The phenotypic data for widely used founder germplasm is available through the "Breeders Toolbox" at the Genome Database for Rosaceae (http://www.rosaceae.org/breeders_toolbox).

INTRODUCTION

Plant breeding programs strive to identify the ideal genotypes for the environments where the crop will be grown and that meet the ideal characteristics of the crop for the industry in that region. The plants' phenotype is the result of the interaction between its genotype and environment. A breeding program will be successful if it can reliably identify genotypes with the optimum traits needed for an individual to be released as a cultivar. Most breeding programs utilize traditional approaches to identify desirable phenotypes. Marker-assisted breeding (MAB) can facilitate and expedite the release of new cultivars. This technology is becoming easier to integrate into traditional breeding programs due to decreased costs, increased efficiency, and greater molecular marker availability (Bliss, 2010).

The U.S. Department of Agriculture-National Institute of Food and Agriculture (USDA–NIFA) Specialty Crop Research Initiative-funded RosBREED project aims to 'bridge the chasm' between genomics and traditional rosaceous crop breeding programs (Iezzoni et al., 2010). Large-scale phenotyping protocols have been published for apple (Evans et al., 2011) and peach (Frett et al., 2012) and presentations of the standardized phenotyping can be viewed for all crops (apple, peach, cherry and strawberry) at www.rosbreed.org.

While MAB has not been widely used in strawberry (*Fragaria ×ananassa* Duch. ex Rozier), a few breeding programs are using markers. Breeders in collaboration with Institut National de la Recherche Agronomique (INRA) in France have used molecular markers linked to *Rca2*, a single gene for resistance to *Colletotrichum acutatum* J.H.

Simmonds, the causal agent of anthracnose on fruit and plant, for parent selection (Whitaker, 2011). Driscoll Strawberry Associates Inc. (Watsonville, CA) use *Rca_240* and other *Rca2* markers developed in-house to screen 20% of their seedlings (Whitaker, 2011). Other molecular markers have been discovered for red stele resistance (*Phytophthora fragariae* Hickman) (van de Weg, pers. comm.) and remontancy (Hancock, pers. comm. and Denoyes pers. comm.) and are being validated to assess their applicability across diverse germplasm.

Valuable genotypic resources are becoming available in strawberry and include the *F. vesca* 815 × *F. bucharica* 601 (FV × FB) reference map for diploid strawberry (Sargent et al., 2008), a reference genome sequence for *F. vesca* L. (woodland strawberry) (Shulaev et al., 2011), and a large number of identified and mapped microsatellites for the octoploid strawberry (Isobe et al., 2013). Resources in the diploid strawberry can be used to better understand the subgenome structure and composition of the octoploid cultivated strawberry genome.

One of the issues complicating MAB in the cultivated strawberry is its ploidy level (2*n*=8x=56) which complicates linkage map construction. A method called Microsatellite Allele Dose and Configuration Establishment (MADCE) has been used to map the homeologs in high ploidy individuals (van Dijk et al., 2012). This method relies on quantitative analysis to identify allele dosage and assign alleles to each of the four subgenomes in strawberry (van Dijk et al., 2012).

Despite the complexity of creating octoploid linkage maps, recent advances include linkage map construction for five *F. ×ananassa* populations, all of which are

included in RosBREED as part of the Breeding Pedigree Set (BPS) and Crop Reference Set (CRS). Linkage maps have been reported for populations derived from the following crosses: 'Capitola' × CF1116 (Lercerteau-Köhler et al., 2003; Rousseau-Gueutin et al., 2008), 'Tribute' × 'Honeoye' (Weebadde et al., 2008), 'Redgauntlet' × 'Hapil' (Sargent et al., 2009) and '232' × '1392' (Zorrilla-Fontanesi, et al., 2011). A linkage map is currently being constructed using the 'Holiday' × 'Korona' progeny (Thijs van Dijk, unpublished). Synteny was observed between the 'Redgauntlet' × 'Hapil' octoploid map and the FV × FB diploid reference map (Sargent et al., 2009; Sargent et al., 2012). Quantitative Trait Loci QTL were found for day-neutrality (Gaston et al., 2013; Weebadde et al., 2008) and yield, as well as important fruit quality characters such as soluble solids, ascorbic acid, titratable acidity, color, and firmness (Lerceteau-Köhler et al., 2012; Zorrilla-Fontanesi, et al., 2011). Furthermore, the gene underlying one QTL controlling the variation in a flavor compound has been identified (Zorrilla-Fontanesi et al., 2012). These resources are essential for enabling MAB and its use in developing new strawberry cultivars with increased quality.

RosBREED has used Pedigree-Based Analysis (PBA) to identify the favorable alleles present in a group of pedigree-linked genotypes (Iezzoni et al., 2010; van de Weg et al., 2004). Using PBA allows for the identification of QTL by analyzing populations with the same founding parents. Markers near QTL linked to traits of interest can be further used in MAB, after their validation, e.g. presence of the marker in genetic resources showing the trait of interest.

In strawberry, we used two SSR markers to confirm accession identity and further eliminate outcrosses and misidentified accessions. However, these two markers alone provided only a small amount of genomic representation. Therefore, an Axiom® 90,000 SNP chip was developed through the efforts of RosBREED and the international strawberry community and will be evaluated for genome scanning.

The lack of reliable phenotypic data can hinder the use of statistical methods for identifying the associations between phenotypic and genotypic data for breeders and geneticists. This shortage may lead to lack of understanding of the genetic basis of biological processes (Bassil and Volk, 2010). RosBREED aims to close this gap between genomics research and traditional breeding programs (Iezzoni et al., 2010). The following paper describes the standardized phenotyping protocol developed for strawberry and reports descriptive statistics for 34 traits based on evaluation of the genotypes planted at the RosBREED locations.

## MATERIALS AND METHODS

### Strawberry germplasm

RosBREED's strawberry group identified 890 *Fragaria* individuals to include in two reference germplasm sets, the Crop Reference Set (CRS, freely distributed data through the Genome Database for Rosaceae www.rosaceae.org) and the Breeding Pedigree Set (BPS, data kept private by breeding programs). Both sets of germplasm were provided from multiple institutions including: the USDA-ARS Horticulture Crops Research Unit (HCRU), Michigan State University (MSU), the USDA-ARS National

Clonal Germplasm Repository (NCGR), the University of Florida (UF), the University of New Hampshire (UNH), the Instituto Andaluz de Investigación y Formación Agraria y Pesquera (IFAPA) in Spain, East Malling Research (EMR) in the United Kingdom, the Institut National de la Recherche Agronomique (INRA) in France, and the Center for Genetic Resources (PB-WUR) in The Netherlands. Driscoll Strawberry Associates (Watsonville, CA) provided critical support, as they imported the European material into the U.S. using their long-established quarantine and propagation facilities.

The BPS is made up of four European mapping populations. The INRA population comprised of 56 genotypes from 'Capitola' × CF1116 ('Pajaro' × ['Earliglow' × 'Chandler']) (B. Denoyes). The EMR population is comprised of 51 genotypes from 'Red Gauntlet' × 'Hapil' (D.J. Sargent). The PB-WUR population is comprised of 29 genotypes from 'Holiday' × 'Korona' (W.E. van de Weg). The IFAPA population is comprised of 69 genotypes from '232' (Sel. 4-43 × 'Vilanova') & '1392' ('Gaviota' × 'Camarosa') (I. Amaya).

The CRS is comprised of the MSU population, 65 genotypes from 'Tribute' × 'Honeoye', plus 10 randomly selected genotypes from 17 populations from MSU and 19 populations from the USDA-ARS, HCRU breeding programs. The rest of the CRS included germplasm that represented important founding genotypes (165 cultivars) and 52 accessions of wild origin including individuals from the USDA-ARS-NCGR "supercore" collection (Hancock et al., 2002).

Two plants of each genotype in the form of rooted runners were planted in Michigan, Oregon, New Hampshire, California, and Florida, in August 2010 (Table 1.1).

In June 2011, the INRA, EMR, and PB-WUR populations were distributed and planted at each location as multi-crowned plants. The IFAPA population was distributed and planted in September 2011 as bare-root runners.

Phenology and flowering-related traits

All phenotypic traits and methods for scoring along with the location and year each were scored as shown in Table 1.1. Collection of flowering data began when at least one flower was open, and was recorded weekly until the end of July or the first week of August in 2011 and 2012 for the two plants of each genotype. Presence of newly opened flowers on each date was noted. The time period of the evaluation was chosen to identify short-day plants that flowered only in the spring and those that continued to flower until the days began to shorten in late summer. Day-neutrality was quantified by summing the number of weeks flowering after week 11 from the first bloom at all locations. Growing degree days for first bloom and for harvest date was calculated as the mean of the maximum and minimum daily temperature minus the base 50 °F (10 °C). Growing degree day accumulation began 1 January, and accumulated throughout the year. Data for growing degree days were generated from weather stations in each region: Oregon (Hyslop Farm, Corvallis, OR) (Coop, 2012), Enviro-weather at Michigan (MSU Southwest MI Research & Extension Center, Benton Harbor, MI) (Andresen, 2012), and in New Hampshire (Woodman Farm, Durham, NH). Days from 1 January for bloom and harvest dates were calculated by summing the number of days from 1 January to the calendar date on which the first bloom and harvest was observed. The presence of anthers

was noted when flowers of each accession were open to determine the flower's sex. In addition, the average flower number per truss from three trusses was used to determine truss size. The location of the truss in relation to the canopy (above or below) and the relative peduncle length were also noted. Fruit was assessed once to twice weekly for when fruit color (red, yellow, white) was fully developed and 30-50% of fruit on the plant were ripe. An average of five fruit was attempted for harvested from each genotype. Fruit was harvested only once, per genotype harvesting the first available fruit. The fruit from each plant were harvested and stored in resealing freezer bags, and kept on ice until stored in a -20 °C freezer for further evaluation.

Plant characteristics

Plant characteristics were evaluated in the field and scored on a scale from 1-9 based on an average of the two plants (Table 1.1). Traits evaluated included: estimated crop load, plant vigor, number of runners, and incidence of diseases. Crop load was estimated based on a scale from having no fruit to being over-cropped. Vigor was determined on the basis of the plant being dead to vigorously growing. The number of runners per plot was estimated from having no runners to having hundreds of runners. Disease was scored in the field at each location from having no disease symptoms to having extreme symptoms for particular diseases in each area. These included: anthracnose (*Colletotrichum* spp.), powdery mildew (*Podosphaera aphanis* [Wallr.] U. Braun & S. Takamatsu), leaf scorch (*Diplocarpon earliana* [Ell. & Ev.] Wolf), leaf spot (*Mycosphaerella fragariae* [Tul.] Landau) with a subset of genotypes screened for

response to red stele root rot (*Phytophthora fragariae* Hickman) at Agriculture and Agri-Food Canada, Atlantic Food and Horticulture Research Centre' Kentville, Nova Scotia.

Fruit characteristics

Fruit characteristics were evaluated in the field and scores or measurements were based on an average from assessing two to three berries per plot (Table 1.1). Techniques for evaluation were based on methods breeders would typically use in the field and that can be quickly and simply performed. External fruit traits evaluated included: shape, appearance, fruit deformation due to unfilled achenes, achene color, achene position, percent of filled achenes, external color, gloss, skin strength, cap size, position of the calyx and ease of cap removal. Fruit shape was scored as long conic, globose, globose conic, cordiform, or oblate. Appearance was scored ranging from very malformed to symmetrical and attractive. Fruit malformation denotes deformed fruit resulting from unset achenes and was scored for presence/absence. Achene color was scored from dark to light brown or green. Achene position was scored as protruding, even, or sunken in relation to the surface of the fruit. The percentage of filled achenes was estimated in 10% increments. External color was scored from white to dark red, and gloss was scored from dull to very shiny. Skin strength was based on how easily fruit was abraded when the evaluator's thumb was firmly dragged over surface of the fruit. Cap size was scored as greater than, less than or equal to the width of the fruit. The position of the calyx was scored as raised, even or sunken relative to the shoulder of the fruit.

The internal fruit traits evaluated included: fruit firmness, internal color, depth of internal color, and flavor. Fruit firmness was scored by compressing fully ripe fruit between thumb and forefinger and scored from hard to mushy. Ease of "capping" was scored by removing the calyx ("cap") of the fruit. The berries were then sliced down the meridian and internal color was scored ranging from white to deep red. The estimation of depth of internal color was based on the percent of the flesh that was colored. Flavor scores were largely based on the perception of sweetness but also the presence/absence of off-flavors and aromas was considered.

Fruit chemistry

Frozen fruit samples were removed the evening before to fully thaw at room temperature. The number of fruit per bag was noted and the fruit were weighed for use in calculating average berry weight and percent drip loss. At this point fruit was put in a plastic clamshell and stored back in the plastic bag to assist in evaluating drip loss. Drip loss, pH, soluble solids and titratable acidity were measured on the thawed fruit and samples were saved to measure total anthocyanins and total phenolics at a later date.

For all samples drip loss was measured first. Clamshells were used to remove the thawed fruit from the bag, separating it from the juice without squeezing the fruit, eliminating the release of excess juice or changing the fruits integrity. The juice was then weighed. Total drip loss was calculated as a percentage of fruit weight by the formula $W_1$-$W_2/W_1$ where $W_1$ was the average berry weight before drip loss occurred and $W_2$ was the average berry weight after drip loss.

After measuring drip loss, the fruit in the clamshell was emptied back into the bag with the juice and homogenized by rolling a weighted glass bottle over the bag, grinding the fruit to pulp and crushing the achenes. The pH was measured on all fruit pulp samples by placing the pH probe from the DL 115 auto titrator (Mettler Toledo, Oakland, CA) in the bag of the strawberry homogenate. Percent soluble solids was measured from the pulp by placing a few drops on a refractometer (ATAGO "Pocket" Digital Refractometer PAL-S, Tokyo, Japan). Titratable acidity was measured using the DL 115 auto titrator. Titrations were performed using 5 g of fruit pulp and 45 mL of $CO_2$-free water. Samples were then titrated with 0.1 N NaOH to pH 8.1 (Plotto et al., 2008). After titrations, 1.5 mL of the homogenate was saved in three 2 mL tubes and stored at -80 °C for later measurement of total anthocyanins and phenolics.

Total anthocyanins and total phenolics were measured simultaneously. Thirty minutes prior to testing, one vial of each of the stored samples was thawed, vortexed and centrifuged. Total anthocyanins were measured using the pH differential method as described by Lee et al. (2005) and the determination of total phenolics was performed using the Folin-Ciocalteu method as described by Waterhouse (2002). Both methods were modified for use in 96-well, flat-bottomed microplates (Greiner Bio-One, NC).

## STATISTICAL ANALYSIS

In order to illustrate the ranges in trait expression, trait means were calculated from the combined locations of Oregon, Michigan, New Hampshire, and California data for the CRS in 2011 and 2012 and BPS in 2012 using the PROC Means in SAS 9.3 (SAS

Institute, Cary, NC). In addition to trait means from the combined locations, the total number of individuals used to calculate each mean and the range of means are reported (Table 1.2). A full analysis of variance that examines the variability in this germplasm was conducted but will be presented in a separate paper.

## RESULTS AND DISCUSSION

The strawberry germplasm used was extremely diverse and included four European mapping populations, one mapping population from MSU and breeding populations from both MSU and USDA-ARS Corvallis breeding programs. Germplasm also included cultivars, founders or the individuals that can be traces back to the beginning of the pedigrees of modern cultivars (Sjulin and Dale, 1987) as well as intermediate ancestors and wild species. The germplasm was evaluated at multi-location field sites. The diversity in germplasm and environment led to a wide range of minimum and maximum trait values. Mean trait values for locations are presented in Table 1.1 to provide and example of how diverse this germplasm is.

A summary of the means of all traits from the RosBREED OR, MI, CA and NH locations for the CRS in 2011 and 2012 and the BPS for 2012 is reported in Table 1.2. Mean values for traits significantly differed among genotypes ($P \leq 0.0001$) in both the CRS and BPS in 2011 and 2012. This is seen by the trait values encompassing in many cases the entire measurement range for each trait. The mean phenology and flowering related traits were fairly similar between 2011 and 2012. The number of days from 1 January to the first bloom was on average 10 days earlier in 2012 for the CRS. The BPS

set bloomed in 2012 four days earlier than the 2011 CRS bloom and approximately four days after the 2012 CRS bloom. This shift in dates is similar to the days from 1 January for harvest date, which was on average 10 days earlier in 2012 across locations for the CRS. The BPS harvest was approximately seven days earlier than the 2011 CRS harvest and approximately five days later than the 2012 CRS harvest. The means for plant characteristics are in general similar for each year and between each reference set. However, a slightly greater runner number was detected for 2011 for the CRS and though not exactly comparable the BPS runner number for its first year is higher than the 2012 CRS runner number. The mean for external fruit characteristics did not differ between years. The mean for internal fruit characteristics differed slightly such that removal of caps was easier and the internal color was slightly darker in 2011, however, the percent of internal color was greater in 2012. The means for fruit chemistry and lab measured traits differed. Fruit weight, was on average 4.7 g greater in 2011 for the CRS. The BPS had a high average weight but no data for the second year. The percent drip loss, was on average 15.9% greater in 2011. Total phenolics and total anthocyanins differed between 2011 and 2012 for the CRS. Total anthocyanins were on average 24.7 $mg \bullet L^{-1}$ Pg-3-gluc Equivalents greater in 2011, and total phenolics were 32.8 $mg \bullet L^{-1}$ Gallic Acid Equivalents greater in 2012. The total anthocyanins and phenolics in the BPS were less than both years of the CRS.

A lack of reliable and available phenotypic data can hinder the use of statistical methods for identifying the associations between phenotypic and genotypic data for breeders and geneticists. Having standardized protocols in place that can be followed and

used among different locations allows great power in data replication. When evaluators at separate locations follow the same phenotyping protocol the limiting factor for combined QTL analysis comes from differences due to genotype by environment interactions.

A complete presentation on these strawberry phenotyping protocols can be viewed at http://www.rosbreed.org/resources/fruit-evaluation. RosBREED strawberry genomic and phenotypic data for all described traits from 2011 and 2012 is being integrated into the Genome Database for Rosaceae "Breeder's Toolbox" at (http://www.rosaceae.org/breederstoolbox; 2013) (Jung et al., 2008). This database is meant to store and integrate private (BPS) and public (CRS) phenotypic and genotypic data for apple, peach, cherry and strawberry so breeders can more accurately and efficiently assess parent and progeny performance and selection. Phenotypic data collected using this protocol will be useful to identify marker-trait associations and QTL using FlexQTL$^{TM}$ (Bink et al., 2002; 2008) or other software.

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Table 1.1. Ratings and measurements for 37 strawberry phenotypic traits evaluated on 890 genotypes of strawberry (*Fragaria* sp.). Traits were evaluated in 2011 and 2012, as indicated, at locations in California (CA), Michigan (MI), New Hampshire (NH), Oregon (OR) and Nova Scotia (NS) in 2011 and 2012 as part of the USDA-funded RosBREED project.

| Trait | Evaluation criteria | 2011 | 2012 |
|---|---|---|---|
| Phenology and flowering related traits | | | |
| Peduncle length | Scored 1-5; 1= divides close to crown, 2= divides at 25% from crown, 3= divides 50% from crown, 4= divides 75% from crown, 5= divides right before flower/fruit | MI, OR, NH | MI, OR, NH |
| Flowering location | Determined at bloom; Scored 1-2; 1= above, 2= below canopy | MI, OR, NH | MI, OR, NH |
| Presence of anthers | Determined at bloom; Scored 1-2; 1= yes, 2= no | MI, NH | MI, OR, NH |
| Period of flowering | Yes/no, evaluated weekly | MI, OR, NH | CA, MI, OR, NH |
| Growing degree days for first bloom and harvest date | Mean of daily maximum and minimum temperature, minus the base 50 °F (10 °C). Beginning 1 January and accumulated throughout the year | MI, OR, NH | MI, OR, NH |
| Days from 1 January for first bloom and for harvest date | Calculated by summing the number of days from 1 January to the calendar date the first bloom was observed or the harvest date | MI, OR, NH | CA, MI, OR, NH |
| Truss size | Number of flowers per truss | MI, OR | MI, OR, NH |
| Harvest date | Date fruit were harvested based on when 30-50% of the fruit were ripe | MI, OR, NH | MI, OR, NH |
| Day neutrality | Quantitative weeks of flowering after week 11 from the first week of bloom at each location | CA, MI, OR, NH | CA, MI, OR, NH |
| Plant characteristics | | | |
| Crop estimate | Amount of fruit on plant; Scored 1-9; 1= no fruit; 9= over-cropped | CA, MI, OR | MI, OR, NH |
| Vigor | Plant health/growth; Scored 1-9; 1= dead; 9= extremely vigorous | MI, OR | MI, OR, NH |
| Number of runners | Visual estimation of runners; Scored 1-9; 1= none; 9= hundreds | MI, OR, NH | MI, OR, NH |
| Disease | Dependent on the disease naturally present at each location, diseases scored separately when multiple diseases were present. Anthracnose (*Colletotrichum acutatum*), powdery mildew (*Podosphaera aphanis*), leaf scorch (*Diplocarpon earliana*), leaf spot (*Mycosphaerella fragariae*); Scored 1-9; 1= severe disease; 9= no symptoms. | MI, OR, NH, FL | MI, OR, NH, FL |

| Table 1.1 continued | | | |
|---|---|---|---|
| Trait | Evaluation criteria | 2011 | 2012 |
| Red stele root rot | A subset was screened for response to red stele root rot (*Phytophthora fragariae*) in greenhouse bench tests; Scored 0-5; 0= death, 5= no symptoms | NS | NS |
| External fruit characteristics | | | |
| Shape | Scored 1-9; 1= long conic, 3= globose, 5= globose conic, 7= cordiform, 9= oblate | CA, MI, OR, NH | CA, MI, OR, NH |
| Appearance | Scored 1-9; 1= very malformed; 9= symmetrical and attractive | CA, MI, OR, NH | CA, MI, OR, NH |
| Malformation | Deformation due to unfilled achenes in 1/3 or more ripe fruit; Scored 1-2; 1= yes, 2= no | MI, OR, NH | MI, OR, NH |
| Achene color | Scored 1-9; 1= dark; 9= very light brown or green | MI, OR, NH | MI, OR, NH |
| Achene position | In relation to fruit surface; Scored 1-3; 1= sunken, 2= even, 3= protruding | MI, OR, NH | MI, OR, NH |
| Percent filled achenes | Percent of all achenes that were filled (10% increments) | CA, MI, OR, NH | MI, OR, NH |
| External color | Scored 1-9; 1= white; 9= dark red | CA, MI, OR, NH | CA, MI, OR, NH |
| Gloss | Scored 1-9; 1=dull; 9=very glossy | CA, MI, OR, NH | MI, OR, NH |
| Skin strength | How easily fruit was abraded when thumb firmly dragged over flesh; Scored 1-9; 1=soft; 9= tough | MI, OR, NH | MI, OR, NH |
| Cap size | Calyx size in relation to fruit width; Scored 1-3; 1=calyx smaller, 2=calyx equal, and 3=calyx larger | MI, OR, NH | MI, OR, NH |
| Calyx position | Scored 1-5; 1=raised (necked), 3= flat (even with shoulders), 5= sunken | CA, MI, OR, NH | CA, MI, OR, NH |
| Internal fruit characteristics | | | |
| Firmness | Firmness of flesh when a fully ripe fruit was compressed between thumb and forefinger Scored 1-9; 1= very soft; 9= very firm | MI, OR, NH | MI, OR, NH |
| Ease of capping | Ease with which cap was removed when pulled by fingers; Scored 1-9; 1= does not remove; 9= very easily removed | MI, OR, NH | MI, OR, NH |
| Internal color | Fruit sliced down the meridian; Scored 1-9; 1= white; 9= "black" | MI, OR, NH | MI, OR, NH |
| Depth of internal color | Percentage of flesh with solid color (10% increments) | MI, OR, NH | MI, OR, NH |
| Flavor | Perception of sweetness and presence of off-flavors; Scored 1-9; 1= not sweet, bad off-flavors; 9= very sweet, no off-flavors | OR, NH | MI, OR, NH |

| Table 1.1 continued | | | |
|---|---|---|---|
| Fruit chemistry and weight | | | |
| Fruit weight | Average weight (g) of five primary fruit harvested when 30-50% of the fruit on each plant were ripe | MI, OR, NH | CA, MI, OR, NH |
| Trait | Evaluation criteria | 2011 | 2012 |
| Drip loss | Percent weight lost when frozen berries were thawed | OR | MI, OR, NH |
| pH | pH of fruit pulp | MI, OR, NH | MI, OR, NH |
| Percent soluble solids | Percent soluble solids of fruit puree as determined using refractometer | MI, OR, NH | MI, OR, NH |
| Titratable acidity | $g \bullet L^{-1}$citric acid of fruit puree; determined using auto-titrator with pH 8.1 end-point | MI, OR, NH | CA, MI, OR, NH |
| Total anthocyanins | $mg \bullet L^{-1}$ Pg-3-gluc equivalents; determined using pH differential method (Lee et al., 2005) | OR | OR |
| Total phenolics | $mg \bullet L^{-1}$ Gallic Acid Equivalents; determined using Folin-Ciocalteu method (Waterhouse, 2012) | OR | OR |

Table 1.2. Mean, minimum and maximum values for 34 traits of the combined crop reference (CRS) and breeding pedigree sets (BRS) from Oregon, Michigan, California and New Hampshire RosBREED locations in 2011 and 2012. Mean performance of genotypes over locations varied significantly ($P \leq 0.0001$) for all traits in both years.

| | CRS harvest 2011 | | | CRS harvest 2012 | | | BPS harvest 2012 | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | n[z] | Mean | Range | n | Mean | Range | n | Mean | Range |
| Phenology | | | | | | | | | |
| Peduncle length | 1336 | 3.6 | 1-5 | 2090 | 2.9 | 1-5 | 534 | 2.9 | 1-5 |
| Total flowering weeks | 1399 | 5.0 | 1-16 | 2151 | 7.9 | 1-17 | 538 | 8.0 | 1-17 |
| Flowering cycles | 1399 | 1.1 | 0-3 | 2152 | 1.2 | 0-3 | 538 | 1.1 | 1-2 |
| Days from 1 January for first bloom | 1399 | 128.5 | 104-216 | 2151 | 119.3 | 87.0-359.1 | 537 | 123.5 | 95-164 |
| Growing degree days for first bloom | 1399 | 194.8 | 0-1247 | 1508 | 223.9 | 0-790 | 353 | 252.2 | 90.9-570.3 |
| Truss size | 543 | 5.2 | 2-14 | 1466 | 4.6 | 1-17 | 350 | 7.0 | 1-33 |
| Growing degree days for harvest date | 937 | 554.3 | 325.0-1097.0 | 1067 | 639.9 | 256.0-1667.7 | 247 | 670.5 | 400.0-1851.2 |
| Days from 1 January for harvest date | 937 | 168.8 | 154-199 | 1067 | 157.3 | 138-208 | 247 | 161.9 | 149-200 |
| Plant characteristics | | | | | | | | | |
| Crop estimate | 1440 | 4.0 | 1-9 | 1474 | 3.5 | 1-9 | 292 | 4.9 | 1-9 |
| Vigor | 1244 | 4.6 | 1-9 | 2167 | 4.0 | 1-9 | 417 | 5.9 | 1-9 |
| Number of runners | 983 | 4.1 | 1-9 | 2142 | 2.9 | 1-9 | 536 | 3.4 | 1-9 |
| External fruit characteristics | | | | | | | | | |
| Shape | 1430 | 5.7 | 1-9 | 1669 | 5.8 | 1-9 | 377 | 5.6 | 1-9 |
| Appearance | 1430 | 4.6 | 1-9 | 1670 | 4.6 | 1-9 | 376 | 5.4 | 1-9 |
| Achene color | 906 | 4.8 | 1-9 | 1023 | 4.4 | 1-9 | 120 | 4.8 | 2-7 |
| Achene position | 907 | 2.0 | 1-3 | 1204 | 2.2 | 1-3 | 247 | 2.2 | 1-3 |

Table 1.2 continued

| | CRS harvest 2011 | | | CRS harvest 2012 | | | BPS harvest 2012 | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | n[z] | Mean | Range | n | Mean | Range | n | Mean | Range |
| Percent filled achenes | 1437 | 89.2 | 10-100 | 1200 | 85.0 | 10-100 | 246 | 85.2 | 40-100 |
| External color | 1467 | 6.6 | 1-9 | 1404 | 6.3 | 1-9 | 350 | 6.2 | 2-9 |
| Gloss | 1479 | 5.9 | 1-9 | 1146 | 5.5 | 1-9 | 245 | 6.2 | 2-9 |
| Skin strength | 935 | 4.5 | 1-9 | 881 | 5.4 | 1-9 | 220 | 4.8 | 1-9 |
| Cap size | 885 | 1.3 | 1-3 | 1202 | 1.5 | 1-3 | 246 | 1.4 | 1-3 |
| Calyx position | 1429 | 3.4 | 1-5 | 1725 | 3.3 | 1-5 | 375 | 3.3 | 1-5 |
| Internal fruit characteristics | | | | | | | | | |
| Firmness | 900 | 5.2 | 1-9 | 881 | 5.5 | 1-9 | 220 | 5.0 | 2-8 |
| Ease of capping | 938 | 6.0 | 1-9 | 878 | 4.7 | 1-9 | 221 | 4.7 | 1-9 |
| Internal color | 934 | 5.5 | 1-9 | 880 | 4.7 | 1-9 | 221 | 4.0 | 1-8 |
| Depth of internal color % | 933 | 72.3 | 10-100 | 881 | 82.7 | 10-100 | 221 | 89.5 | 20-100 |
| Flavor | 688 | 4.2 | 1-9 | 852 | 4.5 | 1-9 | 214 | 3.3 | 1-8 |
| Fruit characteristics measured in the lab including weight and chemistry | | | | | | | | | |
| Fruit weight g | 926 | 11.4 | 0.3-37.6 | 1582 | 6.7 | 0.2-29.3 | 375 | 13.3 | 1.9-34.9 |
| Drip loss % | 549 | 35.8 | 0.0-61.7 | 1016 | 19.9 | 0.3-60.8 | 239 | 32.2 | 5.8-65.8 |
| pH | 909 | 3.5 | 2.8-4.1 | 1555 | 3.5 | 2.9-4.2 | 423 | 3.5 | 3.0-4.3 |
| Percent soluble solids | 1480 | 10.2 | 3.1-19.5 | 1578 | 9.3 | 1-16.6 | 424 | 8.6 | 4.6-15.6 |
| Titratable acidity (gŸL$^{-1}$ citric acid) | 910 | 1.0 | 0.2-2.2 | 1557 | 0.9 | 0.3-2.2 | 425 | 0.8 | 0.4-2.2 |
| Total anthocyanins (mgŸL$^{-1}$ Pg-3-gluc Equivalents) | 531 | 328.8 | 5.3-1109.3 | 380 | 304.1 | 19.5-1631.5 | 125 | 272.0 | 61.7-542.2 |
| Total phenolics (mgŸL$^{-1}$ Gallic Acid Equivalents) | 537 | 347.4 | 2.0-638.8 | 379 | 380.2 | 1.0-742.6 | 125 | 331.6 | 123.2-589.8 |

Table 1.2 continued

| | CRS harvest 2011 | | | CRS harvest 2012 | | | BPS harvest 2012 | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | n[z] | Mean | Range | n | Mean | Range | n | Mean | Range |
| Ratio soluble solids/titratable acidity | 821 | 10.8 | 3.0-35.1 | 1557 | 10.6 | 3.1-37.3 | 424 | 11.5 | 5.1-26.6 |

[z]n is the total number of genotypes among the OR, MI, CA and NH locations evaluated for each trait.

# Association of *Rpf1* marker with Red Stele (*Phytophthora fragariae*) Resistance in Strawberry (*Fragaria* spp.)

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Abstract

Red stele (*Phytophthora fragariae* Hickman var. *fragariae)* is a devastating root rot disease in strawberry (*Fragaria* L.). Several sources of genetic resistance are exploited in strawberry breeding and five race-specific resistance (R)-genes are known. Recently, a tightly linked SSR marker was found for the *Rpf1* gene at Wageningen-UR, The Netherlands. In this study, 130 individuals with known and unknown response to the red stele pathogen were selected to validate this SSR marker association with the *Rpf1* gene and included cultivars and selections from the USDA-ARS-HCRU (Corvallis, OR) breeding program of the domestic strawberry *F.* ×*ananassa*, as well as some supercore accessions of the octoploid progenitor species *F. chiloensis* and *F. virginiana*. These individuals were screened in bench tests for response to two races of the pathogen Canadian race 4 (Cdn-4) isolate ONT-3, and Canadian race 5 (Cdn-5) isolates BC-23 and NOV-77. Given that Cdn-4 has avirulence (Avr) factors 1, 4 and 5 while Cdn-5 lacks Avr 1 but has Avr 4 and 5, the *Rpf1* gene will confer resistance to Cdn-4 and will be ineffective against Cdn-5. Of the inoculated individuals, eight genotypes of wild origin and five MSU selections and 'Allstar' were resistant to Cdn-5, possibly indicating presence of other resistance factors. These resistant genotypes may be valuable for widening the genetic base of resistance in commercial cultivar development. To avoid epistatic effects, these individuals were excluded from the validation along with 45 other

individuals that had intermediate disease scores. None of the wild species had the *Rpf1* SSR marker. Two cultivars were not true-to-type and were excluded from the analysis. For the remaining 56 individuals that showed severe disease scores for Cdn-5, 18 were resistant to Cdn-4 and susceptible to Cdn-5 while 38 were susceptible to both races. The *Rpf1* SSR marker was present in 17 of 18 individuals that were resistant to Cdn-4 and susceptible to Cdn-5 and absent in 32 of 38 accessions that were susceptible to both races of this disease for a marker-trait association of 87.5% (49/56). Based on this large MTA, *Rpf1* SSR marker can be used confidently to predict R1 resistance in strawberry.

The soil oomycete *Phytophthora fragariae* Hickman var. *fragariae* is the causal agent of red stele disease in strawberry. Lanarkshire disease was the name it was commonly given when it was first studied in the Lanarkshire district in Scotland in 1920 (Nickerson, 1998). The first reported infection of *P. fragariae* in the United States was in Illinois (U.S.) in 1935, and by the early 1980s, the disease had caused serious losses to strawberry growers on almost every continent (Nickerson, 1998). Isolates have been race-typed in the U.S. (Converse, 1970), Canada (Nickerson and Murray, 1993) and Europe (Kennedy and Duncan, 1993; van de Weg, 1997a).

The symptoms of *P. fragariae* infection vary depending on the severity of the infection. Symptoms can be observed above ground as stunted growth and in some cases the youngest leaves appear bluish-green while the older leaves begin to turn red, yellow or orange (Nickerson, 1998). Below ground, the infection begins at the root tip and moves towards the crown, and when the root is sliced lengthwise, the stele is red above the rot (Nickerson, 1998). When the infection is not as severe, plants show retarded

growth without a color change. However, both cases lead to eventual plant death (Nickerson, 1998).

*Phytophthora fragariae* can persist in a dormant state in the soil for over 10 years due to the thick cell walls in its oospores (Newton et al., 2010). Cool weather, soil temperatures from 7 to 15 °C, and water-saturated soil conditions bring the dormant oospores to the active zoospore stage (Wilcox, 1991). The zoospores can move through the soil from field to field infecting strawberry root tips and laterals (Wilcox, 1991). This active state usually occurs in the fall and spring due to the preferred conditions of this pathogen. The organism can also be moved from field to field by farming equipment and on infected nursery stock. *Phytophthora fragariae* is a quarantine disease in the US and Europe. Europe has a zero tolerance policy on nursery stock. An outbreak of the pathogen has the greatest negative impact on nurseries as they are no longer allowed to grow on infected land (van de Weg, pers. comm.). Current management practices to reduce disease include good drainage, planting disease-free nursery stock, and planting resistant cultivars (Nickerson, 1998). Fungicides have been used to prevent *P. fragariae* infections for many years. Fosetyl-Al (Aliette®) and metalaxyl (Ridomil®) have been used since the 1980's for control. However, *P. fragariae* has been known to develop resistance to metalaxyl (Seemüller and Sun, 1989). A combination of cultural and chemical controls allows growers to grow relatively susceptible cultivars in red stele infested areas (Hokanson and Finn, 2000).

The first program to breed for red stele resistance in strawberry began in Scotland in 1933 and selection No. 52 of unknown parentage now known as 'Frith' was the source

of resistance (Scott et al., 1984). Breeding for resistance in the U.S. began in 1938 when George Waldo initiated a bench screening program as a joint program between USDA-ARS Beltsville, MD and the University of Maryland (Darrow, 1966). In 1943, this program expanded to include the USDA-ARS Corvallis, OR where Waldo had moved (Scott et al., 1984).

The USDA-ARS breeding program in Beltsville, MD had a major emphasis on breeding for *P. fragariae* with resistant cultivars being released from the 1950's to the 1990's under breeders George M. Darrow, Don H. Scott, Arlen D. Draper, Gene J. Galletta, and continued releases under Stan Hokanson. Initially breeding was focused on developing cultivars resistant to only Eastern races of *P. fragariae* (Maas et al., 1989). However, with cultivar movement resistance to multiple races was important. Maas et al. (1989) investigated available cultivars with resistance to Eastern races and screened with races occurring on the West coast and found many had multiple sources of resistance. Well-known resistant cultivars from their breeding efforts include: Stelemaster (1954), Surecrop (1956), Sunrise (1964), Redchief (1968), Guardian (1969), Atlas (1970), Darrow (1974) Earliglow (1975), Scott (1979), Allstar (1981), Tribute (1981), Tristar (1981), Lateglow (1987), Delmarvel (1994), Mohawk (1994), Northeaster (1994), Latestar (1995), Primetime (1995), and Winona (1996) (Galletta et al., 1997; Haymes, et al., 2000; Maas, 2004; van de Weg, 1997a). Bench screening was the major means of selection for resistant seedlings. As many as 30,000 seedlings from up to of 100 crosses could be evaluated each year (Galletta et al., 1994). In vitro selection was investigated in 1993 and proved to be an efficient means for detecting resistance. However, this method

proved challenging to screen large numbers of seedlings but could be used to check new cultivar releases or selections to be used as parents (Maas et al., 1993).

Breeding for resistance to *P. fragariae* was also a major objective of the USDA-ARS Corvallis, OR where seedlings were screened for resistance to a mixture of *P. fragariae races* in sand-filled benches in the 1960's and 1970's. Waldo (1960) reported in the proceedings of the Western Washington Horticultural Association on selections that were promising against *P. fragariae.* The selections included OSC 2414 (a parent of 'Benton' and 'Linn'), 'Molalla', then known as OSC 2416 (a parent of 'Olympus'), and OSC 2433. In addition to their resistance, these three selections were also noted for high processing quality. The two selections, 'Vale', then known as OSC 2331 (the other parent of 'Benton'), and OSC 2234, were noted as some of the most resistant individuals tested. Four other cultivars from this program had notable *P. fragariae* resistance: 'Siletz' (1955), 'Hood' (1965), 'Benton' (1975), and 'Linn' (1976) (Galletta et al., 1997; Haymes, et al., 2000, Maas, 2004; van de Weg, 1997a). These cultivars showed resistance to one, or even a few races of *P. fragariae*. However, they were not necessarily completely resistant to the disease, as multiple races of the pathogen exist as well as complications from incomplete resistance genes.

The existence of multiple races along with regional adaption of cultivars complicates breeding efforts (Scott et al., 1984). In addition to multiple races, resistance to some races is known to be quantitative or incomplete (Maas and Galletta, 1989, van de Weg, 1997b). Resistance occurs in native *F. chiloensis* (L.) Duch. and *F. virginiana* Duch. and integrating resistance from these native species is possible (Scott et al., 1984).

However, multiple generations of selection are required to recover marketable pomological characteristics (Scott et al., 1984). In a study testing the resistance of 88 unique *F. chiloensis* individuals from locations in California, Oregon, Washington, and British Columbia, an accession collected from Yaquina, OR showed the most resistance to three *P. fragariae* races (Daubeny and Pepin, 1965). Daubeny (1964) reported that the Yaquina A clone yielded the largest amount of red stele resistant progeny compared to 25 other parents.

Breeding with Yaquina A began in the 1950's shortly after Cdn-5 was discovered. Yaquina A proved to be a good parent yielding two selections, MDUS 3022, and MDUS 3023 in 1958. These selections were backcrossed to commercially promising selections and three selections were made in the second generation, (MDUS 3316, MDUS 3603, and MDUS 3748) in 1962. Further backcrossing produced third generation selections MDUS 4116 and MDUS 4457 in 1968 and a fourth-generation selection MDUS 4579 in 1973 (Scott et al., 1984). It was noted that fruit quality was unacceptable for most of these selections. While MDUS 4579 may be the most promising resistant selection, Yaquina A is not in the background of any modern selections or cultivars to date (K. Lewers, pers. comm.).

A gene-for-gene model for host-pathogen interaction was first observed by Flor (1956) for flax and flax rust. The gene-for-gene hypothesis presumes that for every resistance factor, R, in the host, there is a corresponding avirulence factor (Avr) in the pathogen (Flor, 1956). The gene-for-gene model for red stele was proposed by van de Weg (1997a) to explain observed interactions between strawberry cultivars and races of

*P. fragariae*. This was confirmed by using a combination of sets of US, Canadian and UK strawberry genotypes, referred to as differentials that varied in susceptibility to the various races and were also used to identify races (van de Weg et al., 1993; van de Weg, 1997a). In order to be a complete differential set, each genotype was required to have a single, unique resistance factor and as a group, comprise all known resistance factors (van de Weg, 1997a). The US differential set consists of 'Blakemore' (R0), 'MD-683' (R1), 'Aberdeen' (R2.3), 'Stelemaster' (R1.2) and 'Yaquina A' (R5) lacking a single factor differential for R2 and R3 (Converse 1970, van de Weg, 1997a). The Canadian set consisted of the same genotypes as in the US, however 'Sparkle' (R2) was used in place of 'Aberdeen', but still lacking the differential for R3. Therefore, both the U.S. and Canadian sets were incomplete which may have led to early misidentification of races (van de Weg, 1997a). Various UK differentials existed, and of those, one was complete and consisted of *F. vesca* (R0), MD-683 (R1), 'Climax' (R2), '52AC18' (R3), 'Del Norte' (R4), 'Yaquina B' (R5), 'Siletz' (R1.2), 'Perle de Prague' (R1.3), and 'Aberdeen' (R2.3), (Kennedy and Duncan, 1993; van de Weg, 1997a). van de Weg (1997a) postulated the existence of five interacting resistance (R1-R5) and avirulence (Avr1-Avr5) factors that can explain the interaction between the strawberry host genotypes and pathogen isolates evaluated so far.

Two of the five known resistance factors R1 and R2, were characterized by van de Weg (1997b, 1997c). R1 is encoded by the *Rpf1* gene segregating 1:1 in a cross between MD-683 (R1), and 'Senga Sengana' (susceptible, R0), which supports the single resistance gene hypothesis (van de Weg, 1997b). The *Rpf1* allele is dominant as selfing

MD-683 gave rise to resistant and susceptible offspring (Scott et al., 1950). The *Rpf1* gene confers incomplete resistance with response ranging from 0-2 for the resistant MD-683 parent upon infection, on a 0-6 rating scale from resistant to susceptible (van de Weg et al., 1993; van de Weg, 1997b). Incomplete resistance meant restricted sporulation or slight to moderate mycelial development (van de Weg, 1997b). With complete resistance, however, no sporulation is observed. Therefore, this incomplete resistance needs to be quantified. Resistance and susceptibility were classified by comparing the response of the inoculated individual to that of the controls used in the study. The susceptible control ('Blakemore') and resistant (MD-683) exhibited a range of disease responses, relative either to the resistant or susceptible end of the scale. Resistance to R2 was studied using 12 segregating populations from pairwise crosses of four resistant and three susceptible parents (van de Weg, 1997c). American race A7 (Avr2.4.5) was used to test the resistance response in these populations (van de Weg, 1997a, 1997c). In his study, infected root tips were analyzed for presence of zoospores of *P. fragariae* and thus deemed resistant or susceptible. Plants in this study were not scored quantitatively as *Rpf2* possesses complete resistance (van de Weg et al., 1993; van de Weg, 1997c, 1997b). *Rpf2* like *Rpf1* was confirmed as a single dominant gene that is responsible for R2 resistance based on 1:1 segregation in the tested populations (van de Weg 1997c).

Molecular markers that detect the *Rpf1* gene for resistance can be used for screening instead of the more challenging greenhouse inoculations. Bulked segregant analysis was used to identify eight random amplified polymorphic DNA (RAPD) markers linked to the *Rpf1* gene from a cross of MD-683 (R1) × 'Senga Sengana' (susceptible)

(Haymes et al., 1997). In 2000, a sequence characterized amplified region (SCAR) marker was cloned from one of the developed RAPD markers, OPO-16C, that was linked in repulsion to the *Rpf1* allele but linked in coupling to the SCAR. This SCAR marker was more specific, easier to score and was more reproducible compared to the RAPDs (Haymes et al., 2000). The three RAPD markers, OPO-16C, OPC-8A and OPC-8D, as well as the SCAR marker SCAR-R1A were tested in 34 European and North American cultivars with known *Rpf1* resistance and in 53 susceptible cultivars (Haymes et al., 2000). The SCAR-R1 marker was present in 23 of the 34 *Rpf1* red stele resistant genotypes and was absent in all but 'Cambridge Vigour' of the susceptible cultivars. The authors recommended use of two markers flanking the *Rpf1* locus, OPO-8A and SCAR-R1, to identify resistant cultivars and to minimize false positives. In some cultivars like Stelemaster and its descendants, OPO-16C was linked to *Rpf1* instead of SCAR-R1A. In this case, OPO-8A and OPO-16C should be used as the flanking markers. OPO-8A is 1.7cM from the *Rpf1* gene while SCAR-R1 is 3 cM from the other side of the *Rpf1* gene. Crossover between *Rpf1* and either one of these markers was reported as the likely cause of the loss of the gene or marker in some cultivars (Haymes et al., 2000). Recently, a simple sequence repeat (SSR) marker was developed and found to detect presence of R1 with a 99% marker trait association (MTA) in the tested strawberry samples (van de Weg, pers. comm.). Compared to RAPD and SCAR markers, SSRs are more reproducible and allow differentiation of homozygous from heterozygous alleles. In the octoploid strawberry, a method entitled Microsatellite Allele Dose and Configuration Establishment (MADCE) was recently developed that allows identification of the full

allelic configuration at a locus including null alleles, homozygous loci and alleles that are present on multiple homeologues (van Dijk et al., 2012). This method enhances the efficiency and information content obtained from an SSR primer pair and enables use of software and algorithms developed for diploid crops. Identifying markers linked to each of the five resistance factors would allow pyramiding resistance in new cultivars. Seedlings could then be screened with these markers for the presence of multiple resistance factors, saving the time and the labor needed for inoculation trials with each known race. Pyramiding resistance genes has been done traditionally in bench tests for resistance factors 1, 2 and 3 by crosses of MD-683 (R1) and 'Aberdeen' (R2.3) and screening seedling progeny for resistance to isolates A3 (Avr1.4.5), A8 (Avr3.4.5) and A7 (Avr2.4.5) (van de Weg, 1997a).

The U.S. Department of Agriculture-National Institute of Food and Agriculture (USDA–NIFA) Specialty Crop Research Initiative-funded RosBREED project is using an SSR marker associated with *Rpf1* as one of two SSR primer pairs to confirm trueness-to-type of 947 strawberry individuals selected to represent the breadth of diversity in breeding germplasm (Mathey et al., 2013). The objective of this study is to investigate the association of this SSR marker with presence of the *Rpf1* gene in 130 diverse strawberry accessions and the potential of these markers for marker-assisted breeding (MAB) programs.

## Material and Methods

### Plant material

A total of 153 individuals representing *Fragaria* diversity were chosen to investigate the usefulness of the *Rpf1* marker and to identify potential new sources of resistance to *P. fragariae*. Unrooted runners of this germplasm set were obtained from the USDA-ARS National Clonal Germplasm Repository (NCGR), and rooted runners obtained from the USDA-ARS Horticultural Crops Research Laboratory (HCRL), and Michigan State University (MSU) and sent to Agriculture and Agri-Food Canada, Kentville, Nova Scotia, CA (AAFC). In Nova Scotia, attempts were made to root eight runners from each of the 153 individuals (four for each race). When an individual failed to runner, large multi-crowned plants were divided to produce replicates if possible. Propagation was unsuccessful in 23 genotypes and they were eliminated from the study leaving 130 individuals to test (Appendix Table 4.1). In addition to these 130 individuals, two controls, 'Honeoye', which is known to be susceptible to Cdn-4 and Cdn-5 and 'Mira', with resistance to Cdn-4 and susceptibility to Cdn-5, were included (Jamieson et al., 2001; Nickerson and Jamieson 1995). The remaining individuals included three cultivars with known resistance and two with known susceptibility to R1, as proposed by van de Weg (1997a). The three cultivars and their proposed resistance alleles are: 'Allstar' (proposed R1), 'Earliglow' (R1.2/1.2.3), and 'Perle de Prague' (R1.3). 'Totem' is believed to have *Rpf2* due to its resistance response to Cdn-6 (A-7) (Nickerson and Jamieson, 1995). This conclusion was drawn from van de Weg (1997c) validation of *Rpf2* using only A-7 that possesses Avr2.4.5. 'Totem' is not expected to have *Rpf1*

resistance based on the absence of *Rpf1* SCAR marker (Haymes et al., 2000). A 'Honeoye' clone from the RosBREED set was included in addition to the control 'Honeoye' and is was reported as susceptible to *P. fragariae* (Dathe, 1999; Nickerson and Jamieson, 1995). Nine other cultivars with unknown resistance were included along with 21 seedlings from the Michigan State University (MSU) breeding program (E. Lansing, MI), 10 advanced selections mainly from the Oregon USDA-ARS breeding program, 51 seedlings from the USDA-ARS (ORUS) breeding program (Corvallis, OR), and 34 accessions from the USDA-ARS National Clonal Germplasm Repository (NCGR) "supercore" collection. The included individuals from the "supercore" collection are 15 native *F. chiloensis* and 19 *F. virginiana* individuals that were identified by Hancock et al. (2002) to represent the range of diversity within these octoploid species that are the progenitors of *F. x ananassa* (Appendix Table 4.1). Also included in the "supercore" category for this study is JH101-1, a *F. chiloensis* hybrid that was not part of the original supercore.

Isolates

Two races of *P. fragariae* were chosen: Cdn-4 isolate ONT-3 and Cdn-5 represented by a mixture of two isolates BC-23 and NOV-77. Isolate ONT-3 originated from a single-zoospore isolate in 1990 from a root of 'Kent' found in a commercial strawberry field in Fort Erie, ON that was showing symptoms of red stele (Nickerson and Jamieson, 1995). Isolate BC-23 was acquired in 1992 from a single-zoospore isolate from a root of 'Cavendish' growing in a commercial strawberry field in Aldergrove, BC

(Nickerson and Jamieson, 1995). Isolate NOV-77 originated from a hyphal-tip isolate from a root also from 'Cavendish', originally isolated in 1994 from a commercial field in Nine Mile River, Hants County, NS (Nickerson and Jamieson, 1995). Isolates of Cdn-4 and Cdn-5 were race typed using the Canadian differential set (Nickerson and Jamieson, 1995). Based on the responses of this set, we used the gene-for-gene model to assign Cdn-4 avirulence 1, 4 and 5 (Avr1.4.5) and Cdn-5 avirulence factors 4 and 5 (Avr4.5). Individuals with resistance to Cdn-4 and susceptibility to Cdn-5 should have the *Rpf1* gene responsible for R1.

The isolates were grown on kidney bean agar as described in Nickerson and Murray (1993). The inoculum mycelial slurry was prepared using 0.47 L of chilled distilled water. The preparation of each inoculum involved blending the distilled water on low for 10 s with seven plates of ONT-3 cultures for Cdn-4, and four plates of BC-23 plus four plates of NOV-77 for Cdn-5. The slurry of each inoculum was poured into 1 L glass beakers, brought up to volume with additional cold distilled water, and homogenized with a large glass-stirring rod. This process was repeated, creating two separate batches of inoculum per isolate on each inoculation date.

Inoculation and screening

AAFC has been screening for root rot resistance for two decades using a procedure similar to that reported by Scott et al. (1976). For this study, two sand-filled benches were designated for the disease screening. Roots of each plant were dipped in the inoculum and planted into either the bench for race Cdn-4 or race Cdn-5. Each bench

consisted of two blocks. Each block was divided into six sub-blocks that were planted at the same time, contained two plants of each 'Honeoye' and 'Mira' as controls, and were inoculated with a separately prepared batch of inoculum. Each sub-block included a set of 30 genotypes (two plants of each genotype, in staggered rows of 15). A total of 180 plants, including 12 control plants were spaced 10.2 cm apart within the row, and 7.6 cm between the rows in each sub-block. Plants in rows 1-4 (block 1, sub-block 1) were planted at the same time as those in rows 25-28 (block 2, sub-block 1), but with a different batch of inoculum.

Soilless potting media was washed from the rooted runners. The plants were inoculated by dipping their roots into the inoculum slurry and then planted into the sand bench. Planting and inoculation took place on five separate dates. Difference in times of runner development was the reason between the first and the second planting. The first planting date was 14 February 2012 and consisted of two sub-blocks (1 and 2) for each race. Sub-blocks 3, 4, 5, and 6 were then planted on 19, 20, 21, and 22 March, respectively. The benches were flooded beginning 11 April and contining every-other day until 11 May. On the day of flooding a rubber stopper is placed in a hole in the underside of the bench. The benches begin being filled at 9:00 am until water was seen at the top of the sand. The stopper was pulled at 11:00 am to drain. Plants were dug for analysis, rinsed off and rated for disease severity on 23-25 May. Plants were scored as follows: 0 (dead), 1 (0-24% healthy roots), 2 (25-49%), 3 (50-74%), 4 (75-99%), and 5 (no disease) (Fig. 1). The scoring is similar to that of Nickerson and Jamieson (1995) yet inversed [in their study 0 = no symptoms or a few necrotic spots; 1 = necrotic tips plus rotting and red

stele involving less than 3% of the length of the adventitious roots; 2 = 4-25% rotting and red stele; 3 = 26-50%; 4 = 51-75%; and 5 = 76-100%].

DNA extraction and molecular screening

Young leaf tissue was collected from plants growing at the USDA-ARS, NCGR, the USDA-ARS, HCRU, and MSU. Genomic DNA was extracted in Fall 2010 using E-Z 96 Plant DNA Kits (Omega Bio-Tek Inc., Norcross, GA) as previously described by Gilmore et al. (2011). DNA was quantified using the absorbance optical density, (OD) at 260 nm wavelength (Teare et al., 1997) using Victor Multilabel Plate Reader (Perkin Elmer, Waltham, MA). DNA was diluted to create working stock concentration of 3 ng/µL. DNA was amplified with the Type-It Multiplex Microsatellite PCR kit (Qiagen, Valencia, CA) in a total volume of 15 µL. The PCR reaction contained: 8.3 µL of 2x Type-it Multiplex PCR Master Mix (Qiagen, Valencia, CA), 1.7 µL of a 10x multiplex primer mix containing 2 µM and 1 µM respectively of each fluorescently labeled forward primer (*Rpf1* SSR-Hex, and ARSFL007-Fam) and standard reverse primer (pig-tailed for the *Rpf1* SSR to prevent split peaks [Brownstein et al., 1996]), 1.7 µL Q-Solution and 3.3 µL of 3 ng/µL template DNA. The SSR primer sequences for *Rpf1* were provided by van de Weg. ARSFL007 has been linked to male sterility in strawberry and was included to identify outcrossers, or non-true-to-type individuals in the 947 individuals being evaluated in the RosBREED project (Goldberg et al., 2010). The forward ARSFL007 primer sequence is GCGCGCATAAGGCAACAAAG and reverse primer GCGAATGGCAATGACATCTTCTCT (Ashley et al., 2003).

Thermocycler amplification was performed in a PTC-225 Thermal Cycler (MJ Research, Inc., Waltham, MA) using a Touch Down 52 program, which consisted of an initial denaturing of (1) 95 °C for 5 min; (2) 95 °C for 30 s; (3) 62 °C for 1.5 min minus 1 °C per cycle; (4) 72 °C for 30 s; (5) then back to (2) for nine cycles; followed by (6) 95 °C for 30 s; (7) 52 °C for 1.5 min; (8) 72 °C for 30 s; (9) then back to (6) for 28 cycles; (10) 60 °C for 30 min; (11) 4 °C until removed from thermocycler.

Success of the PCR was confirmed by 3% agarose gel electrophoresis. The PCR products were diluted 1:124 and 1.05 µL was submitted to Oregon State University's Center for Genome Research and Biocomputing core laboratory where they were separated with an ABI 3730 (Life Technologies Corp.) capillary electrophoresis machine. GeneScan™ -500 ROX® internal size standard (Life Technologies Corp.) was included in each sample during capillary electrophoresis to enable automated data analysis and precise DNA fragment size comparisons between electrophoresis runs. GeneMapper ID-X v. 4.1 software (Life Technologies Corp., Carlsbad, CA) was used for allele visualization and scoring.

Statistical analysis

Analysis of variance was conducted using R 2.10.1 version 2009, to test for block and sub-block effects of each control (R Development Core Team. 2009). Standard deviations were performed in Microsoft® Excel® 2008 for Mac version 12.2.4 for the replicated results of each individual for each race of *P. fragariae.*

Results

'Honeoye' and 'Mira' were used as controls in each sub-block of the disease screen. Mean disease scores for 'Honeoye', expected to be susceptible to Cdn-4 and Cdn-5, were 1.54 and 3.17, respectively (Fig. 2.4, Table 2.5). Mean disease scores of 'Mira', expected to be resistant to Cdn-4 and susceptible to Cdn-5 were 4.62 and 1.17, respectively (Table 2.5). There were no differences among the blocks for 'Honeoye' and for 'Mira' separately. A sub-block effect was found for 'Honeoye' for Cdn-4 ($P \leq 0.018$) and Cdn-5 ($P \leq 0.028$), while no significant sub-block effect was found for 'Mira'. Therefore blocking was ignored for all samples and disease scores were averaged across replicates.

Based on averages of the controls, plants scoring 0-2 were deemed susceptible and those scoring 4-5 were deemed resistant. A score of three in at least half of the replicates inoculated was considered intermediate and we considered the response of these plants inconclusive as to resistance or susceptibility. This scale is similar to Nickerson and Jamison (1995) except in reverse order. No universal standard scale exists. However, pathologists generally want to see high disease, therefore, they use the high end of the scale to indicate severe infection, whereas, horticulturists want low disease and healthy plants and use the high end of the scale to indicate low or no infection.

The number of replicates varied among the tested genotypes. Of the 130 individuals screened with Cdn-4, 71 had one replicate, eight had two replicates and 51 had three replicates. For those screened with Cdn-5, 75 had one replicate, seven had two replicates and 48 had three replicates (Table 2.1). Because of varied responses of

susceptibility and resistance between replicates of individuals screened, standard deviations were calculated on the scores for the 130 replicated genotypes for response to Cdn-4 and Cdn-5. A standard deviation greater than 0.8 indicated at least one point difference in replicated scores. Only those individuals that had both susceptible and resistant ratings were eliminated. A few individuals with standard deviations less than 0.8 but intermediate values of 3 in at least half of the replicates and were eliminated. On this basis, 45 individuals were eliminated from the study (Appendix Table 4.1, Table 2.1) due to the difficulty of distinguishing resistance from susceptibility. These included three cultivars with unknown response, 11 MSU seedlings, two ORUS selections, 15 ORUS seedlings, and 14 supercore accessions (Table 2.1, Appendix Table 4.1).

Of the remaining 85 accessions (after excluding the 45 individuals with variable disease scores among replicates) 14 individuals with resistance to Cdn-5 were eliminated from validation (Table 2.2). These individuals included 12 accessions that were found resistant to both races and referred to as RR (Table 2.2, Appendix 4.1) and two that were susceptible to Cdn-4 and resistant to Cdn-5, indicated by SR (Table 2.2, Appendix 4.1). Individuals with resistance to Cdn-5 included 'Allstar', five MSU seedlings, and eight supercore accessions (6 *F. chiloensis* and 2 *F. virginiana*). All of these accessions except for MSU 9-9-5 and MSU 9-16-25 were resistant to both races. Seventy-one accessions remained and included: three cultivars with known resistance factors, seven cultivars with unknown resistance, five MSU seedlings, seven selections, 36 ORUS seedlings, and 13 supercore accessions (Table 2.3).

Association of the *Rpf1* marker was indicated by presence of the marker in individuals that expressed resistance to Cdn-4 (scores 4-5) and susceptibility to Cdn-5 (scores 0-2) and its absence in individuals that were susceptible to both races.

Of the three cultivars with known disease response factors, 'Perle de Prague' and 'Totem' were susceptible to both races and lacked the *Rpf1* marker and 'Earliglow' was susceptible to both races but unexpectedly had the *Rpf1* marker. Of the nine cultivars with unknown disease response, 'Puget Reliance', 'Sweet Bliss' and 'Valley Red' were resistant to Cdn-4 and susceptible to Cdn-5 and had the *Rpf1* marker; 'Charm', 'Strawberry Festival', 'Sweet Charlie', 'Tillamook' and 'Tufts' were susceptible to both races and lacked the *Rpf1* marker*;* and 'Melody' was susceptible to both races and unexpectedly had the *Rpf1* marker (Table 2.3).

Of the five MSU seedlings, marker presence and absence matched the rating in the disease screen (Table 2.3). MSU 9-5-1 was resistant to Cdn-4, susceptible to Cdn-5 and had the *Rpf1* marker*,* while MSU 9-1-4, MSU 9-5-4, MSU 9-9-10, and MSU 9-12-8 were susceptible to both races and lacked the *Rpf1* marker.

In the ORUS genotypes, association between the *Rpf1* marker, presence or absence and resistance or susceptibility to *Rpf1* disease response, respectively, was found in four of the five selections. Two selections, NW 90054-37, and ORUS 2742-1 were resistant to Cdn-4 and susceptible to Cdn-5 and had the *Rpf1* marker. Two selections, ORUS 1267-236, and ORUS 2490-1 were susceptible to both races and lacked the *Rpf1* marker. One selection, ORUS 1239 R-21 was susceptible to both races and unexpectedly had the *Rpf1* marker (Table 2.3).

Of the 36 ORUS seedlings, 11 of 12 seedlings that were resistant to Cdn-4 and susceptible to Cdn-5 had the *Rpf1* marker while 20 of 24 individuals that were susceptible to both races lacked the *Rpf1* marker (Table 2.3). Marker association with *Rpf1* disease resistance and susceptibility was found to segregate in progeny of the same cross. For example, the SSR marker was present in ORUS 3306-6 that was resistant to Cdn-4 and susceptible to Cdn-5; the marker was absent in its full-sib ORUS 3306-14 that was susceptible to Cdn-4 and Cdn-5. The *Rpf1* marker was absent in each of the 13 supercore accessions tested irrespective of resistance or susceptibility to Cdn-4 (Table 2.3). Ten supercore accessions were susceptible to Cdn-4 while three *F. virginiana* supercore accessions (FRA 1408, FRA 1699 and FRA 1701) were resistant to Cdn-4 and susceptible to Cdn-5 (Table 2.3).

## Discussion

'Honeoye' was selected as a control based on its reported susceptibility to isolates representing Cdn-4 and Cdn-5 races of *P. fragariae* (Dathe 1999; Nickerson and Jamieson, 1995). 'Mira' was chosen as a control because of its resistance to isolates representing Cdn-4 and susceptibility to isolates representing Cdn-5 (Jamieson et al., 2001). 'Mira' should have R1 or possibly R1.3 based on the races to which it is resistant (Jamieson et al., 2001) and using the proposed avirulence in van de Weg (1997a) as represented in Table 2.6. In 'Honeoye', a variable response among replicates to the two races of *P. fragariae* was observed leading to a mean intermediate disease score (Table 2.5) while in 'Mira' the disease response to Cdn-4 and Cdn-5 agreed with previous

reports (Jamieson et al., 2001). This variable response in ‘Honeoye’ was confirmed in a second trial using five replicates planted randomly in five blocks (unpublished). To eliminate the possibility of multiple genotypes of ‘Honeoye’ used for the inoculations, we fingerprinted the two stock plants of ‘Honeoye’ with the two SSR primer pairs used for confirming trueness-to-type in the RosBREED strawberry set in addition to five other SSR primer pairs (unpublished) and found them to have an identical genetic fingerprint to that of ‘Honeoye’ maintained at the USDA-ARS NCGR.

There is a possibility that the isolates used in this study were improperly race-typed as the differential used in Nickerson and Jamieson (1995) lacked a differential for R3 meaning the ONT-3 isolate of Cdn-4 from this study has potential to be A1 (Avr1.3.4.5) as interpreted using van de Weg (1997a) (Table 2.6). Also, either Cdn-5 isolate (BC-23 or NOV-77) could be A8 (Avr3.4.5) based on its response to the Nickerson and Jamieson (1995) differential set, and interpreted using the proposed avirulence factors of van de Weg (1997a) (Table 2.6). This new classification would coincide with the control ‘Mira’ having R1.3 and control ‘Honeoye’ having R3. In order to confirm this incorrect race typing, the isolates need to be tested on a cultivar with R3 such as Kent, Micmac, or 52AC18. If the R3 differential is S to the isolates our current classification is correct. However, an R response would indicate a different avirulence. It is important to state that if any of these isolates were race-typed incorrectly they still have the potential to validate R1. An R response to Cdn-4 (Avr1.3.4.5 or Avr1.4.5) and an S response to Cdn-5 (Avr3.4.5 or Avr4.5) will still validate that resistance is R1.

The intermediate response to Cdn-4 and Cdn-5 observed in the 'Honeoye' control leads us to believe that 'Honeoye' has incomplete resistance to a race of red stele. Due to the potential of Cdn-4 having Avr1.3.4.5 and Cdn-5 having Avr3.4.5, combined with the response of 'Mira' from this and previous studies showing that its resistance could be R1.3 it is proposed that 'Honeoye' has incomplete resistance to R3.

An alternative explanation for the variable disease response obtained for 'Honeoye' is that its vigorous growth and corresponding root regrowth masked its susceptibility during the 10-14 weeks following inoculation and prior to assessing the pathogen response. In previous studies pathogenicity was assessed three weeks after inoculation instead of the 10-14 weeks used here, possibly capturing susceptibility before plants had time to regrow (Nickerson and Jamieson, 1995; Nickerson and Murray, 1993). Gooding (1971) reported that in the relatively resistant, 'Crusader', 'Talisman' and 'Templar', resistance was associated with a high capacity for root regeneration, which could support our observations in 'Honeoye'. Varied response could be caused by declining isolate aggressiveness of Cdn-4 isolate ONT-3 and Cdn-5 isolates BC-23 and NOV-77, as they were first isolated in the 1990's. This is not likely, however, as we have cases of severe infection. In extreme cases, certain plant pathogens have been known to lose their ability to infect within a few months when maintained on agar medium, therefore, re-isolating from the host needs to be done annually (Hildebrand, pers. comm.). *Phytophthora fragariae* is relatively stable but the loss of pathogenicity in culture is still a possibility and occasional re-isolation would help prevent this decline in aggressiveness (Jamieson pers. comm.). Loss of isolate aggressiveness was also thought to have occurred

when weaker infection occurred at the USDA in Beltsville, MD after repeated subculturing and cold storage for over 30 years (Galletta et al., 1994).

Like the 'Honeoye' control, variable response among replicates in the 130 individuals was observed for Cdn-4 and Cdn-5. Such variable response could have resulted from incomplete resistance in *Rpf1,* as well as different capacity of these accessions for regrowth and corresponding root regeneration.

According to van de Weg et al. (1996), four replicates are sufficient for reliable classifications and to avoid misclassifications due to incomplete resistance. However, a larger number of replicates were recommended for assessing moderate levels of resistance (van de Weg, 1997b). In this experiment, we were able to propagate enough plants for 2-4 replicates. To avoid misclassification of disease response, standard deviations greater than 0.8 among the replicated individuals for each race as well as intermediate scores (Table 2.1) as described in the materials and methods were used to exclude individuals that could have been misclassified from the analysis. Cdn-4 and Cdn-5 were chosen based on their avirulence factors and are equivalent to American races A3 and A5 respectively (Nickerson and Jamieson, 1995). From this assignment we used the avirulence factors proposed by van de Weg (1997a) to assign avirulence to each race (Avr1.4.5) and (Avr4.5), to Cdn-4 and Cdn-5 respectively. Resistance to Avr1.4.5 and susceptibility to Avr4.5 indicates that the resistant response is caused by *Rpf1*. However, resistance to both races Cdn-4 and Cdn-5, as observed in 12 of our accessions, indicates the presence of resistance factor 4 or 5 or both in the host. These isolates could also have had an additional avirulence factor that was recognized by a matching resistance factor in

the host and these have not yet been identified. Susceptibility to Cdn-4 (Avr1.4.5) and resistance to Cdn-5 (Avr4.5) indicates that Cdn-5 could have an additional avirulence factor not present in Cdn-4, an unlikely scenario that was found in only two MSU seedlings in our study (Table 2.1 and Table 2.2). The 14 individuals that expressed high resistance to Cdn-5 and were eliminated from the validation for resistance to *Rpf1* could be tested with a complete set of isolate and host differentials to determine resistance. 'Allstar' was the only cultivar resistant to both races which does not agree with its previous classification as resistant to Cdn-4 but susceptible to Cdn-5 (van de Weg, 1997a). This discrepancy is the result of genotype differences in 'Allstar' used in this study from that previously reported (Fig. 2.5, Fig. 2.6., Table 2.4) (van de Weg, 1997a). SSR-based fingerprints of 'Allstar' obtained from the NCGR were different from those observed for the 'Allstar' provided by van de Weg (NL).

We sought pedigree information to explain observations of resistance or susceptibility in our test individuals based on presence of cultivars with known red stele resistance sources in their pedigree. Ten of the 14 individuals with Cdn-5 resistance were wild *F. chiloensis* or *F. virginiana* or progeny from crosses with these species. The parents of the two resistant MSU 9-5 seedlings are 'Earliglow' and FRA 1702. 'Earliglow' came from the USDA Beltsville, MD efforts for breeding resistance to *P. fragariae* with proposed R1.2/1.2.3 (van de Weg, 1997a). FRA 1702 (PI 612500 *F. virginiana* subsp. *glauca)* was collected in Alberta, Canada, and known to be resistant to black root rot, scorch and leaf spot (Hancock et al., 2001). FRA 1702 has not been tested for resistance to *P. fragariae*. The presence of undiscovered sources of red stele

resistance in *F. chiloensis* or *F. virginiana* needs further investigation. Pedigree information from the remaining seedlings that expressed Cdn-5 resistance could not explain the source of the observed resistance response. 'Tribute' is a parent of two other MSU seedlings, MSU 9-15-5 and MSU 9-16-25 with resistance to Cdn-5, . 'Tribute' has a few resistant individuals in its pedigree including 'Surecrop' (R1.2.3), 'Sunrise' (R1), and 'Stelemaster' (R1.2) (van de Weg, 1997a). When screened with markers, 'Tribute' had RAPD marker OPO-8A for R1 resistance but it was not tested in this experiment (Haymes, et al., 2000). While only one of its progeny, MSU 9-15-5 had the *Rpf1* SSR marker, the presence of *Rpf1* resistance in 'Tribute' needs testing with controlled inoculations in this cultivar and several seedlings from segregating populations. The last MSU seedling with resistance to Cdn-5 was MSU 9-9-5 ('Fort Laramie' × 'Honeoye'). Pedigree information from 'Fort Laramie' and 'Honeoye' could not obviously explain the source of the observed resistant response.

Of the cultivars with known R1 response, 'Totem' had the expected susceptible response to the *P. fragariae* races, as well as, the *Rpf1* marker (Table 2.3). Neither, 'Perle de Prague' or 'Earliglow', with known *Rpf1* resistance (van de Weg, 1997a) responded as we expected (R Cdn-4 and S to Cdn-5). 'Perle de Prague' was susceptible to both races and did not have the *Rpf1* SSR marker and 'Earliglow' was susceptible to both races but had the marker. We believe the cause of this discrepancy is existence of multiple clones for both cultivars. van de Weg et al. (1997) reported multiple clones for 'Perle de Prague' as the cause of discrepancies in response to the red stele pathogen in several studies (Converse and Scott 1962; Hickman 1962; Hickman and English, 1951; Milholland et al.,

1989). ‘Perle de Prague’ from the NCGR did not have any of the RAPD and SCAR markers (Haymes et al., 2000) and was susceptible to *Rpf1* (Milholland et al., 1989). ‘Perle de Prague’ obtained from D.M. Kennedy James Hutton Institute (formerly the Scottish Crop Research Institute), Invergowrie, Scotland) expressed resistance to A-3 (Avr1.4.5) and susceptibility to NS-4 (Avr4.5) indicating resistance to *Rpf1* (van de Weg, 1997b). Different clones of ‘Earliglow’ are suspected in this study as plants were obtained from two sources (NCGR and the USDA-ARS Corvallis breeding program).

Of the nine cultivars with unknown red stele resistance, Puget Reliance, Sweet Bliss and Valley Red were resistant to Cdn-4 and Cdn-5 and had the *Rpf1* marker and will be valuable for providing sources of *Rpf1*. ‘Valley Red’, an offspring of ‘Puget Reliance’, has many founders with known R1, R2 and/or R3 in its pedigree including; ‘Linn’ (R1.3/2.3/1.2.3) (van de Weg, 1997a), ‘Totem’ (R2) (Nickerson and Jamison, 1995), ‘Siletz’ (R1.2) (van de Weg, 1997a) and ‘Sparkle’ (R2) (van de Weg 1997a). Testing ‘Puget Reliance’ with a complete set of differential isolates to identify its exact R factors would be valuable. While ‘Melody’ was susceptible to both *P. fragariae* races, it had the *Rpf1* marker. There is nothing in the pedigree of ‘Melody’ that suggests possible resistance and one of its parents ‘Senga Sengana’ is known to be susceptible. The ‘Melody’ clones in this study were only sent from the USDA-ARS Corvallis breeding program and not from multiple locations; therefore, this discrepancy could be a labeling error or recombination event between the marker and the gene. An additional challenge that could have led to a susceptible score in resistant plants is the presence of *Pythium* or

*Rhizoctonia* on the roots of some genotypes as seen in Fig. 2.3 with the 'Honeoye' and could be the cause of results in Table 2.1.

Segregation of *Rpf1* resistance in one of the five MSU seedlings used in the validation, MSU 9-5-1 is possible given its maternal parent 'Earliglow', is known to have R1 resistance (van de Weg, 1997a).

*Rpf1* marker-disease association was observed in four of five ORUS selections. The marker was present in two selections that were R to Cdn-4 and S to Cdn-5. NW 90054-37 have 'Puget Reliance' in their pedigree, and ORUS 2742-1 is the offspring of 'Sweet Bliss' × 'Valley Red' and tested positive for the *Rpf1* marker and for R1 resistance in the disease screen. Absence of the marker was detected in ORUS 1267-236, ORUS 2490-1 that were susceptible to both races. The last selection, ORUS 1239 R-21, had two replicates for each race and scored S to Cdn-4 and Cdn-5, yet had *Rpf1*. This discrepancy is most likely due to the low number of replicates, a possible labeling error, a recombination of the marker and the gene, or possible infection from other pathogens (Fig. 3) (Jamieson, pers. comm.).

R1 resistance was found in 11 ORUS seedlings as indicated by presence of the *Rpf1* marker and R to Cdn-4 and S to Cdn-5. Seven are offspring of 'Puget Reliance': ORUS 3315-4, ORUS 3315-10, ORUS 3315-11, ORUS 3317-2, ORUS 3317-3, ORUS 3318-1 and ORUS 3323-4. One, ORUS 3324-9 has 'Puget Reliance' as a grandparent. A lack of association was detected in five genotypes: ORUS 3324-1, ORUS 3325-2 and ORUS 3326-13 only had one replicate, insufficient for unequivocal detection of incomplete resistance (van de Weg, 1997b). ORUS 3321-8 had three replicates for each

race, was R to Cdn-4 and S to Cdn-5 but lacked the *Rpf1* marker; and ORUS 3317-6, replicated four times for each race, responded as susceptible yet had the *Rpf1* marker. Recombination between *Rpf1* and the gene or labeling errors could explain why despite adequate replication in these two seedlings, there were no marker trait associations. It is important to state the genotyping was done from samples growing at the USDA-ARS-NCGR or in the field and not necessarily on the same clone that was inoculated with the disease providing room for such labeling error to occur.

The *Rpf1* marker was absent in all of the 34 *F. chiloensis* and *F. virginiana* accessions tested in this study as well as the remaining 18 species representatives included in the RosBREED strawberry germplasm set under evaluation (Mathey et al., 2013). None of the alleles amplified by the *Rpf1* SSR marker were associated with resistance or susceptibility to *Rpf1* based on the disease screen response in these "supercore" individuals. Sequence variations in these species at the primer site(s) of the *Rpf1* SSR could have prevented amplification of this marker and was possible given the large number of null alleles obtained in representative accessions of these species genotyped with this marker. Still, a disease response characteristic of *Rpf1* or a similar resistance factor was observed in each of four replicates per race in *F. virginiana* accessions FRA 1408, FRA 1699 and FRA 1701 (Table 2.3). This suggests the presence of *Rpf1* or a closely related resistance factor in *F. virginiana* that cannot be detected with this *Rpf1* marker. Therefore, this marker cannot be used in *F. chiloensis* and *F. virginiana* to identify *Rpf1* resistance. The *Rpf1* SSR marker association with resistance or susceptibility to *Rpf1* appears effective only in *F. ×ananassa*.

The 56 *F.* ×*ananassa* individuals examined had an MTA of 87.5% (49/56) for the *Rpf1* SSR marker indicating its usefulness in predicting *Rpf1* resistance in highly diverse *F.* ×*ananassa* germplasm. The germplasm used in the current study was extremely diverse and the individuals were not closely related and the resistance factors they were expressing were unknown. Using this germplasm was a robust test of the SSR marker and allowed MTA validation with *Rpf1* as well as identification of previously undiscovered sources of *Rpf1* resistance that will require additional characterization.

Bench screening strawberry seedlings has proven very useful for selecting genotypes with resistance to multiple races of *P. fragariae*. While bench screening was extensively utilized in the 1900s throughout North America, currently it is only used by the AAFC-Kentville program due to loss of isolate pathogenicity and because it is time consuming, seedling limiting, and requires repeated isolation and typing of isolates and multiple handling and transplanting of seedlings. Scoring genotypes for partial or incomplete disease resistance like that expressed by *Rpf1* is challenging and ideally requires a large number of replicates. Using marker-assisted selection for the complex of multiple genes associated with resistance would solve the above issues.

The *Rpf1* SSR marker with a high MTA within such diverse germplasm has strong potential to be integrated into a marker assisted breeding program for selection of parents or offspring with resistance factor 1 and for pyramiding resistance from different sources. Screening germplasm with this marker coupled with disease screening allowed the identification of other possible sources of *P. fragariae* resistance in some of the MSU

seedlings and in the supercore collection. Future integration of SSR markers for the other known sources of resistance would make this an even more valuable tool.

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Table 2.1. Disease response for the four replicates to Cdn-4 and Cdn-5 races in the 45 individuals removed from validation because replicated results yielded both susceptible (0-2) and resistant (4-5) for at least one race, an intermediate score of 3 in at least half of the replicates or a standard deviation > 0.8 among replicates (Std). The presence (+) or absence (-) of the SSR marker for *Rpf1* resistance (*Rpf1*) is also indicated.

| Genotype | Cdn-4[z] replicate | | | | STD[y] | Cdn-5[z] replicate | | | | STD[y] | *Rpf1*[x] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | |
| Cultivars | | | | | | | | | | | |
| Albion | 4 | 0 | 2 | NA | 2.00[w] | 1 | 1 | 1 | NA | 0 | - |
| Honeoye | 2 | 1 | NA | NA | 0.71[u] | 2 | 3 | NA | NA | 0.71[u] | - |
| Jewel | 2 | 2 | NA | NA | 0 | 4 | 2 | NA | NA | 1.41[w] | + |
| MSU seedlings | | | | | | | | | | | |
| MSU 9-1-7 | 2 | 4 | NA | NA | 1.41[w] | 2 | 2 | NA | NA | 0 | - |
| MSU 9-1-8 | 4 | 1 | NA | NA | 2.12[w] | 3 | 4 | NA | NA | 0.71[v] | - |
| MSU 9-5-8 | 3 | 3 | 1 | 1 | 1.15[u] | 5 | 5 | 5 | 4 | 0.50 | - |
| MSU 9-8-1 | 2 | 1 | NA | NA | 0.71 | 4 | 3 | NA | NA | 0.71[v] | + |
| MSU 9-8-4 | 3 | 1 | NA | NA | 1.41[u] | 2 | 1 | NA | NA | 0.71 | + |
| MSU 9-8-5 | 3 | 2 | NA | NA | 0.71[u] | 4 | 5 | NA | NA | 0.71 | - |
| MSU 9-8-6 | 5 | 5 | NA | NA | 0 | 3 | 3 | NA | NA | 0.00[u] | + |
| MSU 9-12-6 | 4 | 2 | NA | NA | 1.41[w] | 3 | 3 | NA | NA | 0 | - |
| MSU 9-12-9 | 4 | 2 | NA | NA | 1.41[w] | 3 | 5 | NA | NA | 1.41[w] | - |
| MSU 9-16-17 | 3 | 1 | NA | NA | 1.41[u] | 2 | 3 | NA | NA | 0.71[u] | - |
| MSU 9-16-21 | 3 | 2 | NA | NA | 0.71 | 3 | 4 | NA | NA | 0.71[v] | - |
| ORUS advanced selections | | | | | | | | | | | |
| ORUS 2427-4 | 3 | 2 | NA | NA | 0.71[u] | 2 | 2 | NA | NA | 0 | - |
| ORUS 2781-1 | 3 | 2 | NA | NA | 0.71[u] | 1 | 1 | NA | NA | 0 | + |
| ORUS seedlings | | | | | | | | | | | |
| ORUS 3304-5 | 4 | 4 | 5 | 5 | 0.58 | 3 | 3 | 3 | 3 | 0.00[u] | + |
| ORUS 3304-10 | 3 | 2 | NA | NA | 0.71[u] | 2 | 2 | NA | NA | 0 | - |
| ORUS 3306-2 | 4 | 3 | NA | NA | 0.71[v] | 3 | 2 | NA | NA | 0.71 | + |
| ORUS 3306-4 | 1 | 3 | NA | NA | 1.41[u] | 3 | 2 | NA | NA | 0.71[u] | + |
| ORUS 3316-3 | 2 | 3 | NA | NA | 0.70[u] | 1 | 1 | NA | NA | 0 | + |
| ORUS 3316-10 | 2 | 5 | NA | NA | 2.12[w] | 1 | 2 | NA | NA | 0.71 | + |
| ORUS 3317-1 | 4 | 3 | 2 | 3 | 0.82[w] | 1 | 2 | 1 | 1 | 0.50 | + |
| ORUS 3317-10 | 1 | 2 | 1 | 1 | 0.50 | 2 | 3 | 1 | 3 | 0.96[u] | - |
| ORUS 3320-4 | 3 | 2 | NA | NA | 0.71[u] | 2 | 2 | NA | NA | 0 | - |
| ORUS 3320-8 | 1 | 1 | NA | NA | 0 | 1 | 3 | NA | NA | 1.41[u] | - |
| ORUS 3321-4 | 3 | 2 | NA | NA | 0.71 | 3 | 4 | NA | NA | 0.71[v] | - |
| ORUS 3323-1 | 2 | 5 | NA | NA | 2.12[w] | 2 | 2 | NA | NA | 0 | + |
| ORUS 3323-7 | 1 | 3 | NA | NA | 1.41[u] | 1 | 1 | NA | NA | 0 | + |

| Genotype | Cdn-4[z] replicate | | | | STD[y] | Cdn-5[z] replicate | | | | STD[y] | *Rpf1*[x] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | |
| ORUS 3326-4 | 3 | 1 | NA | NA | 1.41[u] | 1 | 1 | NA | NA | 0 | + |
| ORUS 3326-15 | 2 | 4 | NA | NA | 1.41[w] | 1 | 2 | NA | NA | 0.71 | + |
| "Supercore" genotypes | | | | | | | | | | | |
| FRA 24 | 4 | 4 | 3 | 4 | 0.50 | 2 | 2 | 2 | 2 | 0 | - |
| FRA 42 | 5 | 5 | 2 | 3 | 1.50[w] | 4 | 5 | 4 | 5 | 0.58 | - |
| FRA 48 | 5 | 5 | 2 | 3 | 1.50[w] | 4 | 5 | 4 | 4 | 0.50 | - |
| FRA 357 | 3 | 3 | 4 | 4 | 0.58[v] | 5 | 5 | 5 | 5 | 0 | - |
| FRA 982 | 4 | 0 | 1 | 2 | 1.71[w] | 3 | 2 | 1 | 1 | 0.96 | - |
| FRA 1092 | 4 | 0 | 1 | 1 | 1.73[w] | 1 | 1 | 1 | 1 | 0 | - |
| FRA 1414 | 5 | 5 | 2 | 3 | 1.50[w] | 1 | 2 | 1 | 2 | 0.58 | - |
| FRA 1455 | 4 | 4 | 2 | 3 | 0.96[w] | 1 | 1 | 1 | 1 | 0 | - |
| FRA 1557 | 4 | 4 | 2 | 2 | 1.15[w] | 1 | 1 | 1 | 1 | 0 | - |
| FRA 1580 | 4 | 4 | 3 | 3 | 0.58[v] | 4 | 4 | 4 | 3 | 0.5 | - |
| FRA 1694 | 5 | 5 | 3 | 2 | 1.50[w] | 1 | 2 | 1 | 1 | 0.5 | - |
| FRA 1695 | 4 | 4 | 3 | 3 | 0.58[v] | 1 | 1 | 1 | 1 | 0 | - |
| FRA 1700 | 3 | 4 | 1 | 1 | 1.50[w] | 1 | 1 | 2 | NA | 0.58 | - |
| FRA 1703 | 4 | 1 | NA | NA | 2.12[w] | 1 | 1 | NA | NA | 0 | - |

Table 2.2. Mean resistance scores and disease response to Cdn-4 and Cdn-5 for 14 *Fragaria* genotypes that expressed resistance to Cdn-5. Plants were scored 0 (highly susceptible or dead) to 5 (healthy no symptoms). An average disease score ≥4 was considered resistant (R) and < 2.5 susceptible (S). Presence (+) or absence (-) of the SSR marker for *Rpf1* resistance is also indicated. These individuals were eliminated from the validation of *Rpf1* due to possible epistatic effects.

| | | | Cdn-4 | | Cdn-5 | | |
|---|---|---|---|---|---|---|---|
| Genotype | Female parent | Male parent | Mean | Response | Mean | Response | *Rpf1*[x] |
| Cultivar | | | | | | | |
| Allstar | US 4419 | MDUS 3184 | 5.00 | R | 4.00 | R | - |
| MSU seedlings | | | | | | | |
| MSU 9-5-2 | Earliglow | FRA 1702 | 5.00 | R | 4.50 | R | + |
| MSU 9-5-5 | Earliglow | FRA 1702 | 4.50 | R | 5.00 | R | - |
| MSU 9-15-5 | Tribute | Earliglow | 4.50 | R | 4.00 | R | + |
| MSU 9-9-5 | Fort Laramie | Honeoye | 2.00 | S | 4.50 | R | - |
| MSU 9-16-25 | Tribute | Honeoye | 1.50 | S | 4.50 | R | - |
| Supercore | | | | | | | |
| JH 101-1 | *F. chiloensis* | OP | 5.00 | R | 5.00 | R | - |
| FRA 34 | *F. chiloensis* | OP | 4.75 | R | 5.00 | R | - |
| FRA 372 | *F. chiloensis* | OP | 4.50 | R | 4.50 | R | - |
| FRA 1690 | *F. chiloensis pacifica* | OP | 4.50 | R | 5.00 | R | - |
| FRA 1691 | *F. chiloensis* | OP | 5.00 | R | 5.00 | R | - |
| FRA 1692 | *F. chiloensis* | OP | 4.25 | R | 4.50 | R | - |
| FRA 1697 | *F. virginiana* | OP | 5.00 | R | 4.25 | R | - |
| FRA 1698 | *F. virginiana* | OP | 4.50 | R | 5.00 | R | - |

Table 2.3. Mean disease scores and responses for 58 *Fragaria ×ananassa* individuals screened with *Phytophthora fragariae* races Cdn-4 and Cdn-5 and used to validate association of the *Rpf1* SSR marker with resistance to *Rpf1*. Plants were scored 0 (highly susceptible or dead) to 5 (healthy no symptoms). An average disease score ≥4 was considered resistant (R) and < 2.5 susceptible (S). Presence (+) or absence (-) of the *Rpf1* SSR marker are indicated as well as association between the marker presence and resistance to *Rpf1* (Y for yes) and between marker absence and susceptibility (N for no). A (-) indicates known cause for the lack of association.

| | Cdn-4[z] | | Cdn-5[z] | | | |
|---|---|---|---|---|---|---|
| Genotype | Mean | Response | Mean | Response | *Rpf1*[y] | Association |
| Cultivar | | | | | | |
| Charm | 1.00 | S | 1.00 | S | - | Y |
| Puget Reliance | 5.00 | R | 1.00 | S | + | Y |
| Strawberry Festival | 1.00 | S | 1.00 | S | - | Y |
| Sweet Bliss | 5.00 | R | 2.00 | S | + | Y |
| Sweet Charlie | 1.00 | S | 1.00 | S | - | Y |
| Tillamook | 1.00 | S | 1.50 | S | - | Y |
| Totem | 1.50 | S | 1.00 | S | - | Y |
| Tufts | 2.33 | S | 1.25 | S | - | Y |
| Valley Red | 4.25 | R | 1.00 | S | + | Y |
| Earliglow | 0.75 | S | 1.00 | S | + | - |
| Perle de Prague | 1.50 | S | 1.50 | S | - | - |
| Melody | 1.50 | S | 1.25 | S | + | N |
| MSU seedlings | | | | | | |
| MSU 9-1-4 | 1.00 | S | 2.00 | S | - | Y |
| MSU 9-5-1 | 5.00 | R | 1.50 | S | + | Y |
| MSU 9-5-4 | 2.00 | S | 1.50 | S | - | Y |
| MSU 9-9-10 | 1.50 | S | 2.00 | S | - | Y |
| MSU 9-12-8 | 1.00 | S | 1.00 | S | - | Y |
| ORUS selections | | | | | | |
| ORUS 2742-1 | 5.00 | R | 1.00 | S | + | Y |
| NW 90054-37 | 4.50 | R | 1.50 | S | + | Y |
| ORUS 1267-236 | 1.50 | S | 1.00 | S | - | Y |
| ORUS 2490-1 | 1.00 | S | 1.50 | S | - | Y |
| ORUS 1239 R-21 | 1.00 | S | 1.50 | S | + | N |
| ORUS seedlings | | | | | | |
| ORUS 3306-6 | 4.75 | R | 1.75 | S | + | Y |
| ORUS 3315-4 | 4.50 | R | 1.50 | S | + | Y |
| ORUS 3315-10 | 4.00 | R | 1.00 | S | + | Y |
| ORUS 3315-11 | 4.75 | R | 1.00 | S | + | Y |

| Genotype | Cdn-4[z] Mean | Cdn-4[z] Response | Cdn-5[z] Mean | Cdn-5[z] Response | *Rpf1*[y] | Association |
|---|---|---|---|---|---|---|
| ORUS 3316-2 | 4.50 | R | 1.00 | S | + | Y |
| ORUS 3316-5 | 4.50 | R | 1.00 | S | + | Y |
| ORUS 3317-2 | 5.00 | R | 1.25 | S | + | Y |
| ORUS 3317-3 | 5.00 | R | 1.75 | S | + | Y |
| ORUS 3318-1 | 5.00 | R | 1.50 | S | + | Y |
| ORUS 3323-4 | 4.50 | R | 1.00 | S | + | Y |
| ORUS 3324-9 | 4.50 | R | 1.00 | S | + | Y |
| ORUS 3304-1 | 1.50 | S | 1.50 | S | - | Y |
| ORUS 3304-5 | 1.50 | S | 2.00 | S | - | Y |
| ORUS 3304-8 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3305-13 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3306-14 | 1.50 | S | 2.00 | S | - | Y |
| ORUS 3315-1 | 1.67 | S | 2.33 | S | - | Y |
| ORUS 3316-1 | 1.67 | S | 1.00 | S | - | Y |
| ORUS 3318-4 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3318-9 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3320-1 | 1.50 | S | 1.00 | S | - | Y |
| ORUS 3320-7 | 1.00 | S | 1.50 | S | - | Y |
| ORUS 3321-6 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3323-14 | 1.00 | S | 1.75 | S | - | Y |
| ORUS 3323-3 | 1.67 | S | 1.00 | S | - | Y |
| ORUS 3324-14 | 1.50 | S | 1.00 | S | - | Y |
| ORUS 3324-3 | 1.00 | S | 1.50 | S | - | Y |
| ORUS 3325-13 | 2.00 | S | 1.00 | S | - | Y |
| ORUS 3325-4 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3326-2 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3326-3 | 1.00 | S | 1.00 | S | - | Y |
| ORUS 3321-8 | 4.67 | R | 2.00 | S | - | N |
| ORUS 3317-6 | 1.50 | S | 1.25 | S | + | N |
| ORUS 3324-1 | 1.00 | S | 1.00 | S | + | N |
| ORUS 3325-2 | 1.50 | S | 1.00 | S | + | N |
| ORUS 3326-13 | 1.50 | S | 1.00 | S | + | N |

Table 2.4. Fingerprints of two plants labeled as 'Allstar'. One from Center for Genome Research the Netherlands (PB-WUR) and one from National Clonal Germplasm Repository USA (NCGR) screened with *Rpf1* SSR and ARSFL007. The PB-WUR clone has allele 140 for *Rpf1* resistance, and the NCGR clone does not.

| | *Rpf1* SSR | | | | | |
|---|---|---|---|---|---|---|
| | A-1 | A-2 | B-1 | B-2 | C-1 | C-2 |
| 'Allstar' PB-WUR | 149 | 213 | 161 | 161 | 140 | 170 |
| 'Allstar' NCGR | $$ | $$ | 161 | $ | 170 | $ |

| | ARSFL007 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A-1 | A-2 | B-1 | B-2 | C-1 | C-2 | D-1 | D-2 |
| 'Allstar' PB-WUR | 212 | 216 | 216 | 216 | 221 | 221 | 246 | 254 |
| 'Allstar' NCGR | 216 | 216 | 216 | 216 | 221 | 221 | 246 | 254 |

Table 2.5. Two replicates of clones of control individuals of 'Honeoye' and 'Mira' planted in each block and sub-block and their response to Cdn-4 and Cdn-5 on a 0-5 susceptible to resistant scale.

| | | Honeoye | | Mira | |
|---|---|---|---|---|---|
| **Block** | **Sub-block** | **Cdn-4** | **Cdn-5** | **Cdn-4** | **Cdn-5** |
| Expected response: | | Susceptible | Susceptible | Resistant | Susceptible |
| 1 | 1 | 4 | 4 | 5 | 1 |
| 1 | 1 | 4 | 5 | 5 | 2 |
| 1 | 2 | 0 | 3 | 4 | 1 |
| 1 | 2 | 1 | 2 | 3 | 1 |
| 1 | 3 | 3 | 4 | 4 | 1 |
| 1 | 3 | 3 | 4 | 3 | 1 |
| 1 | 4 | 1 | 4 | 5 | 1 |
| 1 | 4 | 1 | 4 | 5 | 2 |
| 1 | 5 | 1 | 1 | 5 | 1 |
| 1 | 5 | 2 | 1 | 5 | 1 |
| 1 | 6 | 1 | 1 | - | 1 |
| 1 | 6 | 1 | 3 | - | 1 |
| **Mean block 1** | | 1.83 | 3.00 | 4.40 | 1.17 |
| 2 | 1 | 3 | 5 | 5 | 1 |
| 2 | 1 | 4 | 5 | 5 | 2 |
| 2 | 2 | 0 | 2 | 5 | 1 |
| 2 | 2 | 0 | 3 | 5 | 1 |
| 2 | 3 | 1 | 4 | 5 | 1 |
| 2 | 3 | 1 | 5 | 4 | 1 |
| 2 | 4 | 1 | 1 | 5 | 1 |
| 2 | 4 | 1 | 2 | 5 | 1 |
| 2 | 5 | 1 | 2 | 5 | 1 |
| 2 | 5 | 2 | 3 | 4 | 1 |
| 2 | 6 | 0 | 4 | 5 | 1 |
| 2 | 6 | 1 | 4 | 5 | 1 |
| **Mean block 2** | | 1.25 | 3.33 | 4.83 | 1.08 |
| **Overall mean** | | 1.54 | 3.17 | 4.62 | 1.13 |

[z]ANOVA sub-block effects were observed for 'Honeoye' for each race $p \leq$ 0.018 and 0.028 respectively

[y]No ANOVA differences were detected by block 1 or 2 in either race or control

Table 2.6. Avirulence factors for American *Phytophthora fragariae* races based on responses to Canadian differentials Nickerson and Jamieson, 1995) with resistance factors proposed by van de Weg (1997a).

| | | | | Cdn-4 | Cdn-5 | | |
|---|---|---|---|---|---|---|---|
| Race (1-7) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | . | 1 | 1 | 1 | . | . | . |
| | . | . | 2 | . | . | 2 | 2 |
| | ? | ? | ? | ? | ? | ? | ? |
| | . | . | . | 4 | 4 | 4 | 4 |
| Differentials (Resistance Factors) | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Blakemore (R0) | S | S | S | S | S | S | S |
| MD-683 (R1) | S | R | R | R | S | S | S |
| Sparkle (R2) | S | S | R | S | S | R | R |
| Missing (R3) | ? | ? | ? | ? | ? | ? | ? |
| Del Norte (R4) | S | S | S | R | R | R | R |
| Yaquina A (R5) | R | R | R | R | R | R | R |


Fig. 2.1. Scale used to score strawberry plants for *Phytophthora fragariae* disease response: highly susceptible = 0-2, intermediate = 3, resistant = 4-5.


Fig. 2.2. Varied disease responses of ‘Honeoye’ (susceptible control) to *Phytophthora fragariae* Cdn-4 bench screen.

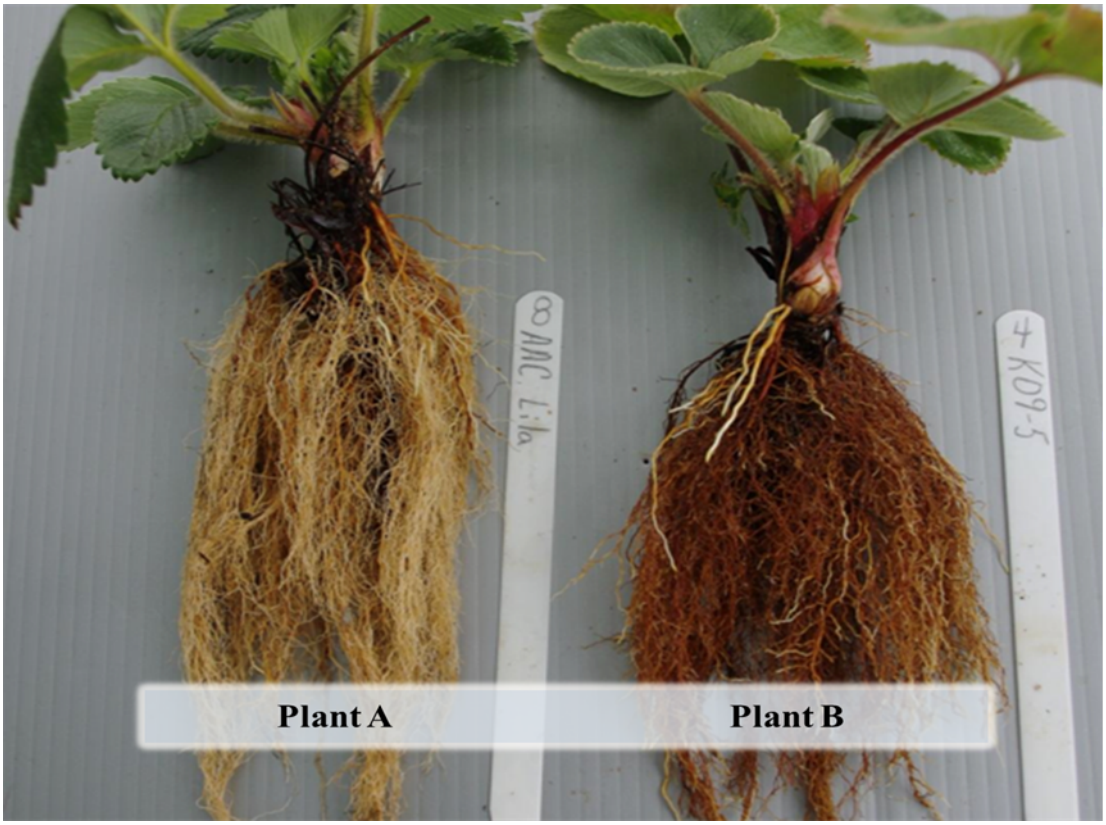


Fig. 2.3. Two 'healthy' appearing plants, plant A 'healthy' white roots, plant B with bronze roots suggesting *Pythium* sp. infection with no sign of *Phytophthora fragariae*. Occurrence of symptoms of disease organisms other than *P. fragariae.*

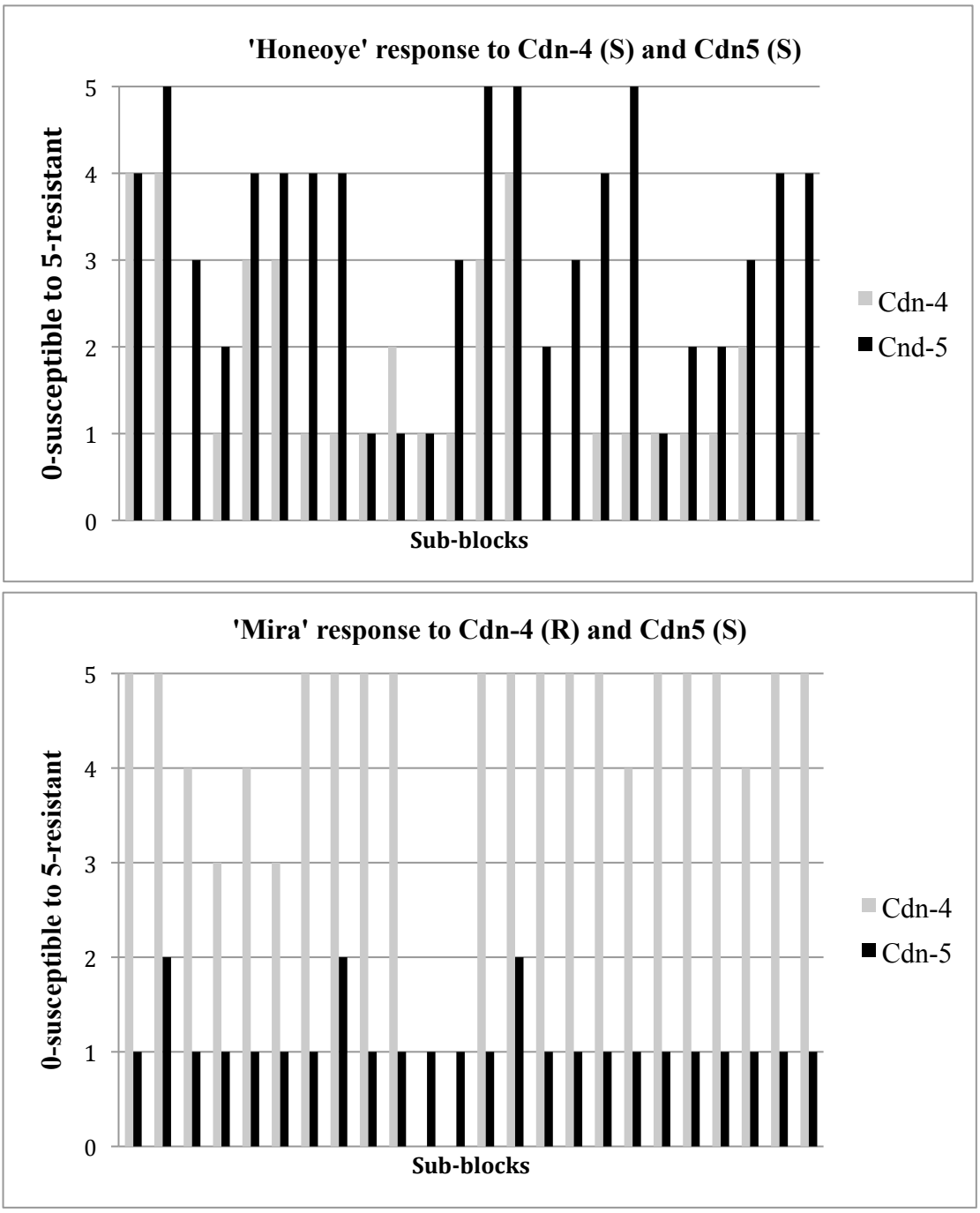


Fig. 2.4. Disease response to Cdn-4 and Cdn-5 on a 0-5 susceptible to resistant scale of the control individuals 'Honeoye' (S to both races) and 'Mira' (R to Cdn-4, S to Cdn-5) planted in each block and sub-block. Mean for the scores in each block are listed and block effects were calculated using ANOVA for ‘Honeoye’ and ‘Mira’ for each race. Sub-block effects were found in ‘Honeoye’ for Cdn-4 ($P = 0.018$) and Cdn-5 ($P = 0.028$).

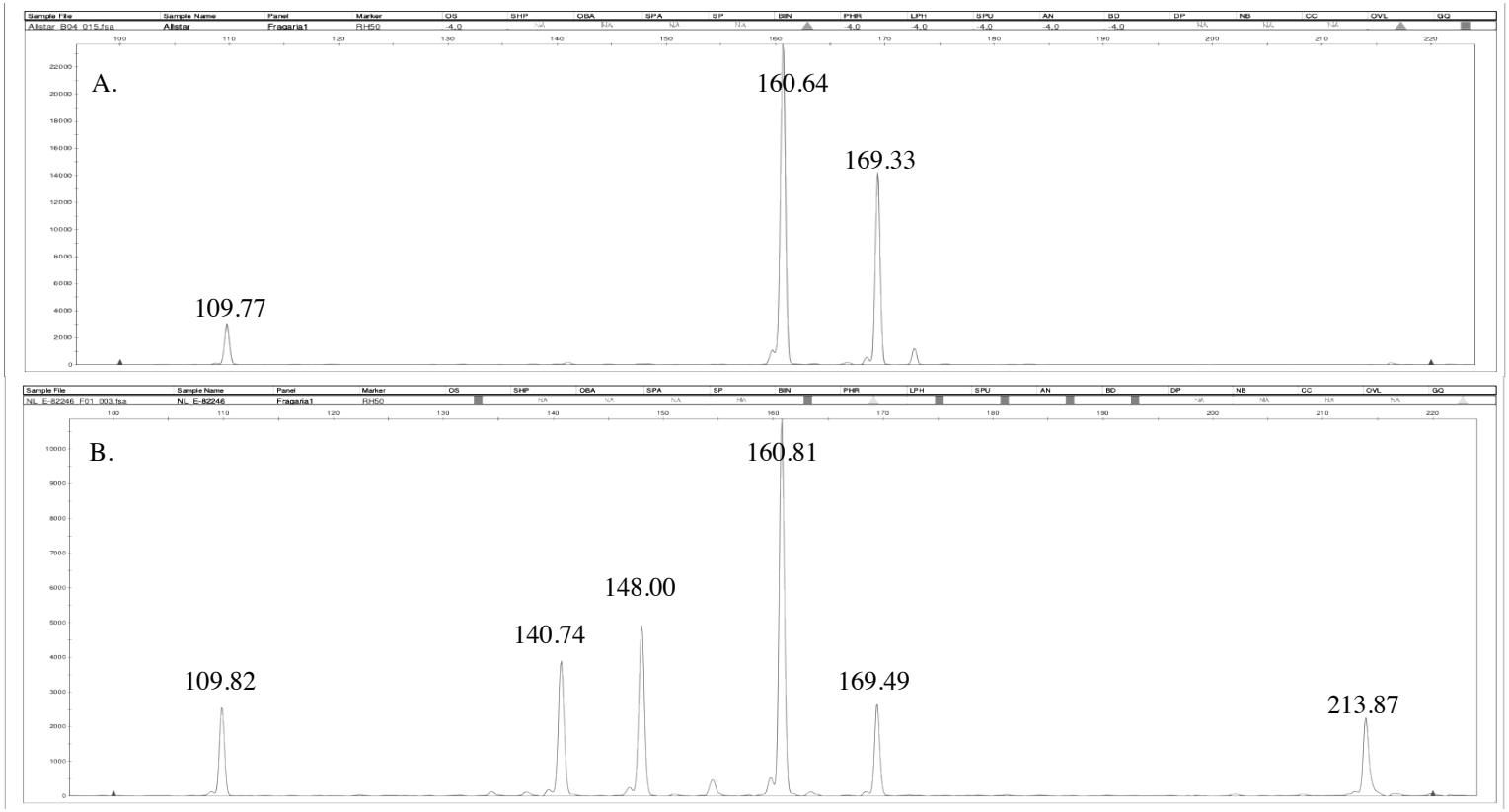


Fig. 2.5. Electropherograms of two clones of 'Allstar' screened with *Rpf1* SSR marker. Clone A was provided from the NCGR (USA) and clone B from PB-WUR (Netherlands). Note PB-WUR clone has *Rpf1* at allele 140, and the NCGR clone lacks that allele.

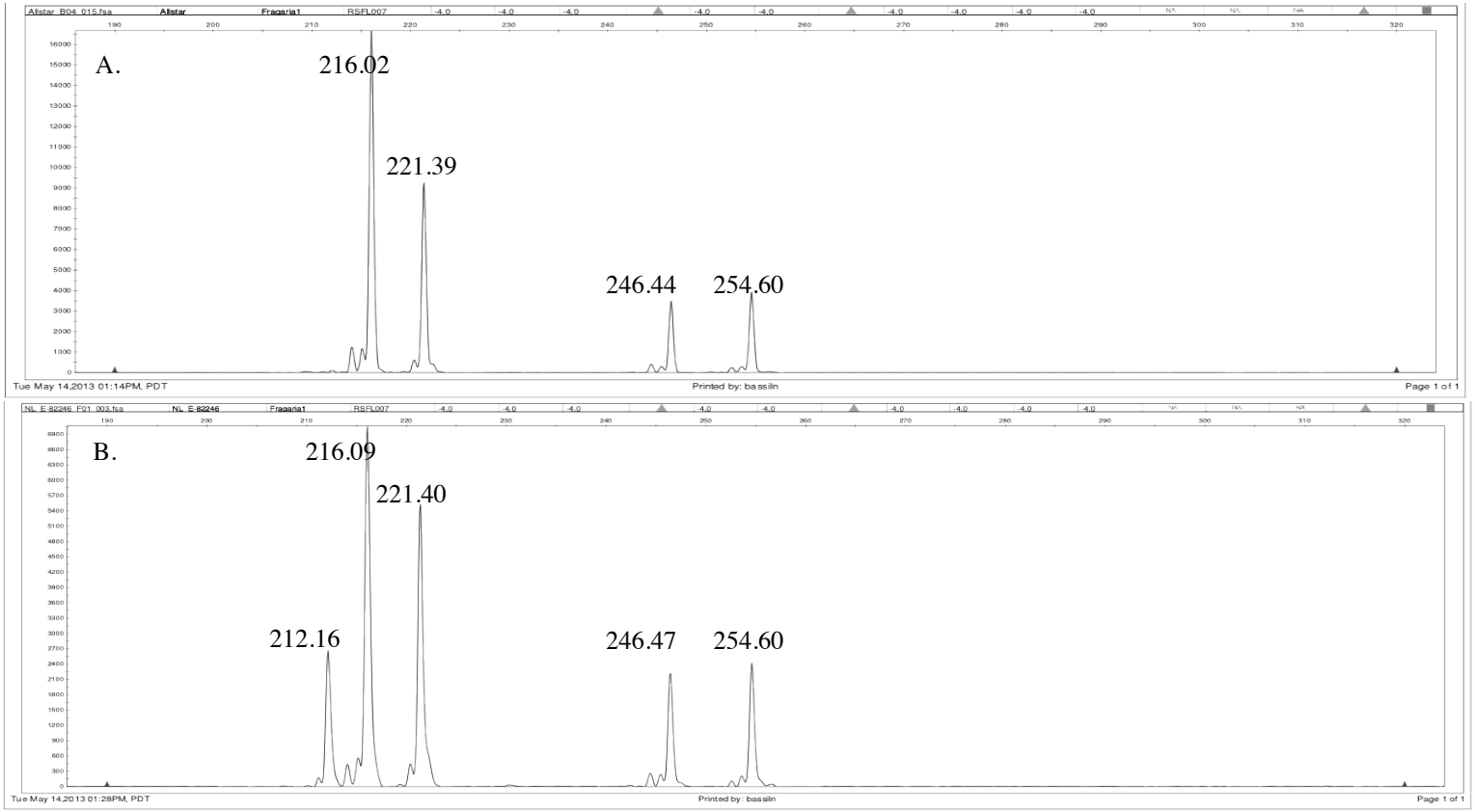


Fig. 2.6. Electropherograms of two clones of 'Allstar' screened ARSFL007. Clone A from NCGR (USA) and clone B from PB-WUR (Netherlands). Genotypes differ at allele 212 where NCGR clone lacks it and the PB-WUR clone has it.

# Genotype by Environment Interactions and Combining Ability for Strawberry Populations in RosBREED

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*Additional index words*. *Fragaria* sp., fruit quality, remontancy, heritability

*Abstract*

Strawberry is one of the five fruit crops included in the USDA-NIFA Specialty Crop Research Initiative-funded, multi-institutional and trans-disciplinary project, "RosBREED: Enabling Marker-Assisted Breeding in Rosaceae". A diverse germplasm set of 890 individuals was propagated, distributed and phenotyped in California, Florida, Michigan, New Hampshire and Oregon. Individual native species and cultivars were included along with 10 seedlings from each of 36 crosses representing eastern and western North American and European short day and remontant cultivars. In addition, four populations that had been created to allow genetic mapping were planted in 2011 and evaluated in 2012. Plants were phenotyped in 2011 and 2012 for phenology, other flower related traits, plant characteristics, fruit characteristics and fruit chemistry traits. An analysis of variance identified genotype, location and year variability and genotype × location interactions as significant. There was significant variability among genotypes, locations and evaluation year for all of the characteristics. However, minimal genotype × location and genotype × year interactions were detected. Genetic variance components and the general combining ability effects varied among traits. 'Sarian' was identified as the best contributing parent for day-neutrality. Narrow-sense heritability estimate ranged from 0-0.78 and was highest for day-neutrality and titratable acidity. Having a better understanding of these attributes will provide breeders guidance on the most effective

breeding strategies for incorporating superior traits from this germplasm into their programs.

Breeding for the ideal strawberry (*Fragaria ×ananassa* Duch. ex Rozier) cultivar is a challenge. Breeders must prioritize all of the potential traits, decide where to place their effort, and choose the germplasm that will help them meet their goals. Having a better understanding of the genetic variability, and parental effects for traits aids breeders in directing this effort and in choosing the best parents for crosses.

The U.S. Department of Agriculture-National Institute of Food and Agriculture (USDA–NIFA) Specialty Crop Research Initiative-funded project RosBREED, aims to 'bridge the chasm' between genomics and traditional rosaceous crop breeding programs (Iezzoni et al., 2010). Through the efforts of this project, large scale standardized phenotyping protocols were established by the breeding teams for strawberry at Oregon (Corvallis), Michigan (Benton Harbor), California (Watsonville), New Hampshire (Durham) and Florida (Wimauma) to capture phenology and flowering related traits, plant characteristics, fruit characteristics and fruit chemistry (Mathey et al., 2013).

The RosBREED's strawberry group identified 890 *Fragaria* genotypes to evaluate. They were meant to encompass and represent the breadth of relevant diversity in breeding germplasm and common ancestors (Mathey et al., 2013). This large germplasm set is divided into two groups: the crop reference set (CRS) and the breeding pedigree set (BPS). The CRS data is downloadable and readily available to anyone from the "Breeders Toolbox" at the Genome Database for Rosaceae (GDR)

(http://www.rosaceae.org/breeders_toolbox). The BPS includes four European mapping populations for which data will be reported but not freely downloadable. The germplasm comprising the CRS and BPS were provided from multiple institutions including: the USDA-ARS Horticulture Crops Research Unit (HCRU), Michigan State University (MSU), the USDA-ARS National Clonal Germplasm Repository (NCGR), the University of Florida, the University of New Hampshire, the Investigación y Formación Agraria y Pesquera (IFAPA) in Spain, East Malling Research (EMR) in the United Kingdom, the Institut National de la Recherche Agronomique (INRA) in France and the Center for Genetic Resources (CGN) in The Netherlands. Driscoll Strawberry Associates Inc. (Watsonville, CA) provided critical support, as they imported the European material into the U.S. using their long-established quarantine and propagation facilities.

The germplasm sets (BPS and CRS) were planted in five distinct growing regions in the U.S. (CA, OR, MI, NH and FL) to assess environmental influences on the performance of the germplasm. The genotype, the environment and their interaction determine the phenotype of a plant. Having an understanding of the genotype × environment interaction is important for traditional and marker-assisted breeding. In traditional breeding, screening germplasm in multiple sites allows the breeder to know how the environments influence the traits of interest.

In molecular breeding or when phenotyping plants for quantitative trait loci (QTL) discovery, datasets generated from multiple sites and replicates are more powerful to detect and characterize the QTL because of the increased number of data points (Schmitz et al., 2013). However, if data from multiple environments is going to be

compiled for a QTL analysis it is important to first determine genotype × environment interactions for each trait. If the genotype × environment interaction for a trait is significant, it may be best to run separate QTL analyses by location. Multiple QTL may exist for a particular trait and be influenced by specific environments. QTL effects can be controlled by more than one gene pathway and by interactions among genes, traits and environments (Mathews et al., 2008).

General and specific combining ability

Evaluating general and specific combining ability (GCA and SCA) variance components and heritability in populations allows the breeder to identify traits they have the most control of in order to achieve the greatest gains for a particular trait. The GCA effect is the average contribution a parent makes to the offspring in a series of crosses and the GCA variance component is a measure of the additive genetic variance for the trait. The GCA effect reflects how useful a parent would be for passing a trait on to the next generation based on the value and significance (Masny et al., 2005). Narrow-sense heritability refers to the amount of additive genetic variance expressed as a proportion of the total phenotypic variance. Narrow-sense heritability determines the amount of progress that can be made from selecting and recombining the best individuals.

The SCA effect shows the value of the interaction a pair of parents has for passing along a specific trait. The SCA variance component is a measure of non-additive effect of genes for a trait. In a situation where the GCA variance component for a trait is greater than the SCA variance component, additive genetic variance is more important than non-

additive genetic variance, therefore, strong gains can be made by crossing among the best parents (Watkins and Spangelo, 1967).

The combining ability components can be considered as fixed or random in the linear model and the associated variance components can be estimated by (1) ordinary least squares computations (ANOVA) (2) maximum likelihood estimation and (3) restricted maximum likelihood (REML). REML is the most versatile method of estimation (Little et al., 2006). Whether parents involved in combining ability estimates are considered as fixed or random depends upon which inferences are to be made (Backer, 1978). In fixed effect models, inferences about the parents involved in the crosses are made, while in random effect models inferences are made about the populations the parents represent (Baker, 1978).

Multiple studies have investigated general and specific combining ability effects and heritability for many strawberry traits such as disease resistance, number of flowers, fruit yield, berry size, fruit firmness, total soluble solids, ascorbic acid, appearance, harvest date and internal and external color (Bestfleisch et al., 2012; Hasing et al., 2011; Lal and Seth, 1981, Lal and Seth, 1982; Masny et al., 2005; Murti et al., 2012; Shaw, 1989; Simpson and Sharp, 1988; Spangelo et al., 1971; Watkins and Spangelo, 1967). Simpson and Sharp (1988) identified short day and everbearing strawberry genotypes with significant GCA effects in determining blooming and yield-related traits, whereas SCA was more important for runner production. The results suggested that it was possible to combine early fruiting with runner production in an everbearing genotype (Simpson and Sharp, 1988). Firmness, total soluble solids and color were found to have

high narrow-sense heritabilities by Murti et al. (2012). Hasing et al. (2011) investigated internal and external fruit color and found narrow-sense heritability of juice color to be high, internal color to have intermediate heritability and external color to have low heritability.

Identification of important traits

Over 30 different traits were measured as part of RosBREED (Mathey et al., 2013). These traits are divided into four categories: phenology and other flowering traits, plant characteristics, internal and external fruit characteristics, and fruit chemistry traits (Table 1.1). Each of these traits is important to the grower, nursery producer, processor or fresh market packer as well as the end consumer. The objective of this study is to estimate the genetic variance components, narrow-sense heritability and combining ability effects for these traits in 35 populations representing crosses between Midwestern and Pacific Northwestern adapted short-day and remontant individuals from a number of diverse genetic backgrounds.

Prior to this study the traits to be evaluated were selected and debated and while every trait is important, several traits were considered to have major importance. The group of flowering traits was chosen because flowering is key to fruit production and repeat flowering/remontancy was especially important to evaluate in these diverse populations and environments. Remontancy, the characteristic of reblooming that has often been described as day-neutrality or everbearing, is affected by day length and temperature (Durner et al., 1984; Hancock and Warner, 2010). While runnering and vigor are not that important in annual strawberry production, they are crucial to success in parts

of the country where the crop is overwintered in a perennial matted row system. In order for consumers to want to buy strawberries, they need to find them desirable. Firm, beautiful fruit with abrasive resistant skins that can ship across the country while maintaining attractive appearance are critical to the wholesale fruit packers. Local fresh strawberry producers want outstanding flavored fruit that are easy for pickers to pick. While the processed industry does not need fruit that ships well fresh, they do need fruit that cap well, have intense flavor and color, low pH and a high soluble solids levels in order to be suited as a processed product. These industry needs drove the decision-making on which traits to focus on.

Evaluating a wide variety of sources of remontancy in breeding populations is important for understanding the best germplasm and approaches used to develop widely adapted remontant cultivars. In coastal California and to a certain extent the Pacific Northwest, where mild temperatures predominate even in the summer, continuous flower initiation is strong. However, in the Midwest, high temperatures reduce the amount of flower initiation in the same strawberry genotypes (Weebadde et al., 2008). In order to have reliable remontancy in environments that have hot summers, it is important to identify genotypes that are remontant and heat tolerant.

Runnering is an important characteristic for propagation of strawberry. Low runner numbers have been associated with remontant genotypes. If plants do not runner, they cannot be clonally propagated and are not suited for the perennial matted row production system. The remontant flowering and runnering traits were recently found to

be controlled by the same QTL, FaPFRU, in cultivated octoploid strawberry (Gaston et al., 2013).

The success of a strawberry breeding program, whether using traditional or molecular approaches, depends on identifying genetic variation for a trait and understanding how the trait is impacted by environmental parameters. This research investigated genotypic and environmental differences and their interactions across MI, OR, NH and CA for 36 populations developed by the USDA-ARS (Corvallis, OR) and MSU breeding programs. Also presented is initial genotype and location effects for four European mapping sets for the 2012 growing season in MI, OR and CA. Lastly, GCA, SCA and heritability are reported for 35 of these crosses using a line × tester design. These crosses will be used to identify remontant genotypes that are widely adapted and to better understand the breeding behavior of these traits along with other plant and fruit characteristics.

## Materials and Methods

### Plant materials

Thirty-six crosses were made for this study (Table 3.1) and were included as part of the RosBREED CRS. The crosses between Midwest adapted genotypes (‘Earliglow’, ‘Honeoye’, MSU 49, and MSU 56) were treated as lines and the remontant set of parents (‘Fort Laramie’, ‘Sarian’, ‘Seascape’, ‘Tribute’, and FRA 1702 [PI 612500]) as testers were done in Michigan along with a tester × tester (‘Seascape’ × ‘Fort Laramie’) and the crosses between Pacific Northwest adapted genotypes (ORUS 2427-1, ‘Puget Reliance’, ‘Tillamook’, and ‘Totem’) as lines by the tester remontant set were done in Oregon. All

36 crosses were used for the evaluation of genotype × environment analysis, however, the tester × tester cross was removed for the GCA and SCA analysis. At each location, the seedlings were germinated and grown until all began to runner. Ten random genotypes were chosen from each of the crosses for propagation, and rooted runners of each genotype were distributed to each of the field trialing sites in 2010.

The four European mapping populations make up the BPS and included: the INRA population, 56 seedlings from 'Capitola' × CF1116 ('Pajaro' × ['Earliglow' × 'Chandler']) (Lercerteau-Köhler et al., 2003); the EMR population, 51 seedlings from 'Redgauntlet' × 'Hapil' (Sargent et al., 2009); the PB-WUR population, 29 seedlings from 'Holiday' × 'Korona' (Thijs van Dijk, unpublished); and the IFAPA population, 69 seedlings from '232' (Sel. 4-43 × 'Vilanova') & '1392' ('Gaviota' × 'Camarosa') (Zorrilla-Fontanesi et al., 2011). The INRA, EMR, and PB-WUR populations were distributed to each location in spring 2011 as vegetative plants and the IFAPA population was distributed in fall 2011.

Field locations

The fields were located in Oregon, Michigan, New Hampshire, California, and Florida. Due to limited field space, only 11 of the 36 populations were planted in New Hampshire. A computer failure claimed some California data, and due to poor adaptation of the plant material to the production system, only three disease responses were evaluated in Florida in 2011 before the field was removed due to rapid plant decline.

Two plants of each genotype were planted at the Oregon State University Vegetable Farm in Corvallis, OR (44°34’21” N, 123°14’44” W), Southwest Michigan Research & Extension Center in Benton Harbor, MI (42°05’17” N, 86°21’28” W), University of New Hampshire Woodman Farm in Durham, NH (43°08’45” N, 70°56’59” W) and The Company Ranch in Watsonville, CA (36°55’39” N, 121°40’45” W). Only one genotype from the pair was evaluated representing a single replicate at each location. Plants in OR and MI were spaced 0.91 meters apart in row and 0.91 meters between rows. Plants in NH were spaced 1.5 meters in row and 1.5 meters between rows. Plants in CA were planted in double rows system with 0.31 m between plants and 1.2 m between the centers of each row. At each location, the standard commercial practices for the region were used as far as fertilization, irrigation, and weed control. While these locations are in the northern U.S., Oregon and California are much milder in the summer and winter than Michigan and New Hampshire. The average high and low for summer (July) and winter (December) temperatures for each location are as follows: Oregon (28 °C, 11 °C; 8 °C, 1 °C), California (22 °C, 12 °C; 16 °C, 4 °C), Michigan (28 °C, 17 °C; 3 °C, -4 °C), and New Hampshire (28 °C, 22 °C; 3 °C, -7 °C). Precipitation also varied among the locations with Oregon (1,110 mm) and New Hampshire (1,087 mm) having the highest annual moisture, California (592 mm) the lowest and Michigan (942 mm) was intermediate.

Traits

Traits are presented in four different categories: phenology and other flowering traits, plant characteristics, internal and external fruit characteristics and fruit chemistry traits. A list of these traits and how they were measured is outlined in great detail in Mathey et al. (2013) and can be referenced in Table 1.1.

Data analysis

Analyses of variance (ANOVA) were performed for all genotypes, environments, and years and the genotype × environment interactions using R 2.10.1 version 2009 (R Development Core Team, 2009). Genotypic differences were detected using a model where genotypes were fixed effects and environments were random effects. Differences in environment and the interaction of genotypes × environments were detected as fixed effects in a linear model. Differences in years and the interaction of genotype × years were also detected in a fixed effects model as well as the interaction of year × environment.

Calculations for GCA and SCA variance components were performed as outlined by Xiang and Li (2003). Specific SAS code is outlined in Dr. Fikret Isik class notes, NC State University, Raleigh, NC (F. Isik, pers. comm.). However, instead of a diallel design, a line × tester model was used where lines (Midwest and Pacific Northwest adapted individuals n = 8) were crossed with remontant testers (n = 5) to create 35 cross combinations one cross made twice and five crosses were missing (Table 3.1).

General and specific combining ability can be accounted for using fixed or random effects. Typically a fixed effects model involves parents that are non-random

members of a mating population such as specifically selected cultivars or parents for which the GCA and SCA effects are estimated. Ideally, a random model involves parents that are randomly sampled from a random mating population and reports the GCA and SCA variance. We selected the random effect model in estimating combining ability because we are interested in the population they represent and our crosses were unbalanced as well as include a large number of missing data. Therefore, mixed effect estimates were more appropriate (Littell, 2002).

Calculations for GCA and SCA variance components were performed using the line × tester design. A line × tester analysis is similar to diallel analysis, however, it accounts for the unbalanced crossing scheme. Data from the OR and MI environments were used for the analyses, for each year (2011 and 2012).

The matrix for mixed model is: $Y = X\beta + Z\gamma + \varepsilon$

Where:

Y is a vector of observed data, β is unknown fixed-effect parameters with design matrix X. $\gamma$ is random effect parameters with random effect design matrix Z including GCA and SCA, and ε is a random error term. Here $\gamma$ and ε are Gaussian random variables, uncorrelated with expectation of 0, and variance G and R. In our case, we considered year as a fixed effect, while general and specific combining abilities were considered random effects. The Banded Toeplitz covariance structure was used.

Thus the model can be presented as: $y_{ijkl} = B_i + p_{jk} + c_{jk} + e_{ijkl}$

or

$y_{ijkl} = B_i + l_j + t_k + c_{jk} + e_{ijkl}$

$y_{ijkl}$ is the overall mean where l is the trait observation, i is the year, and jk is the cross.

$B_i$ is effect of the $i^{th}$ year (fixed effect)

$p_{ij}$ is general combining ability of m parents (line + tester)

$l_i$ is general combining ability of j lines (random effect)

$t_k$ is general combining ability of k testers (random effect)

$c_{jk}$ is specific combining ability of line j and tester k, and

$e_{ijkl}$ is the random within plot error term.

The REML procedure implemented in SAS 9.3 MIXED procedure (SAS Institute, Cary, NC) was used for the analysis. Thus, the GCA and SCA effects are the best linear unbiased predictions (BLUP). The procedure allows for comparisons among genotypes from different breeding populations. The BLUP methodology allows for the estimation of genetic variances from the data that are routinely generated in breeding programs, therefore eliminating the need for mating designs (Bernardo, 2010).

The model calculates GCA ($\sigma^2_G$) and SCA variance ($\sigma^2_S$) components. From these, additive ($\sigma^2_A$), dominance ($\sigma^2_D$), and phenotypic variances ($\sigma^2_{P)}$ and narrow-sense heritability ($h^2$) were estimated as:

$$\sigma^2_A = 4\sigma^2_G$$

$$\sigma^2_D = 4\sigma^2_S$$

$$\sigma^2_P = 2\sigma^2_G + \sigma^2_S + \sigma^2_E$$

$$h^2 = \sigma^2_A/\sigma^2_P$$

(Hasing et al., 2011).

Results and Discussion

Genotype, location and year differences were evaluated, as well as the genotype × location, genotype × year and environment × year interactions (Table 3.2). Genotypic differences were detectable for all traits except peduncle length, cap size, calyx position and flavor (Table 3.2). Either there really is not much variability among the genotypes for these four traits or a more stringent evaluation method is needed to evaluate them.

The variability due to location was significant for all traits (Table 3.2). California on average had the longest continual weeks in flower; and percent soluble solids were greater in California and Michigan than in Oregon and New Hampshire. Oregon had the highest fruit weight and percent drip loss. The long continual flowering in CA was expected due to the cooler average summer temperatures that do not inhibit day-neutral types from blooming.

Significant differences between 2011 and 2012 were detected for all traits except fruit shape, fruit appearance, calyx position, titratable acidity and the ratio of soluble solids to titratable acidity (Table 3.2). Evaluation of these traits in a single year may be adequate in future studies. Internal color was on average darker in 2011 for all locations. However, the percent depth of internal color was greater in all locations in 2012. Fruit weight was greater in 2012 for MI, but was greater in 2011 in NH and OR. Runner number greatly increased in MI in the 2012 season but declined rapidly in NH and OR as plant vigor declined.

Genotype × location interactions were observed for five of the 34 traits: flowering cycles, growing degree days for harvest (GDDH), day-neutrality, cap size, and calyx

position. Michigan had the highest number of GDDH followed by OR, while NH had the least number of GDDH. Growing degree days for harvest in MI were almost 100 degree days more in the 2012 season, while in NH GDDH were about 40 degree days less in 2012 and for OR GDDH were only one degree day different between the years. Day-neutrality was least expressed in NH and most in CA. This finding is consistent with Weebadde (2008). Although they did not use the exact same environments, there were Eastern and Western differences; California and Oregon had the highest percentage of DN progeny and Maryland had the least. Having strong genotype × location interactions for flowering cycles, GDDH, cap size, and calyx position shows the complexity of these traits and that the genotypes are responding differentially in each environment. Cap size and calyx position had a significant genotype × location interaction this could mean these traits are complex and respond differentially in each environment. However because of the lack of genotype differences and the significant location differences, there is a possibility that variation in estimation of these traits among the different locations were due to inconsistency among scorers.

Significant genotype × year effects were only found for fruit weight. Fruit weight averaged over two years was around 6.5 g for MI and NH but in OR was double this at 12.4 g. Fruit weight increased from 2011 to 2012 in MI, however it decreased in OR and NH, possibly due decline in plant vigor. The mean fruit weight was very small in MI the first year (4.9 g) and increased by 47% in the second year while the fruit size in NH and OR decreased 54% and 44%, respectively. This is likely due to the fact that the plants took longer to reach full size in Michigan due to a shorter growing season than in

Oregon. Furthermore, in Oregon there is a tendency for non-adapted 2nd year plants to decline in size due to viruses and other diseases. New Hampshire has similar environmental conditions to MI but may have succumbed to more disease or virus pressure in 2012.

Significant location × year interactions were detected for all traits evaluated in both years except for day-neutrality, calyx position, and flavor. The number of runners had a significant location × year interaction as runner number greatly increased in MI in 2012, while they greatly decreased in NH and OR coincident with a decline in plant vigor.

Genotypic and environmental differences were evaluated for the first production season (2012) for the four European mapping populations (Table 3.3). Crop estimate, external color, and soluble solid/titratable acidity ratio and pH were the only traits that had significant genotypic variability. All traits were significantly different across environments, except flowering cycles, truss size, crop estimate, skin strength and internal color. This was slightly unexpected as these are four distinct populations that segregate for disease resistance and fruit quality traits including sugars, acids, anthocyanins and l-ascorbic acid, productivity and fruit firmness (Lercerteau-Köhler et al., 2003; Zorrilla-Fontanesi et al., 2011) and thus, we expected more detectable variability. Since these plants were established late, major differences might be detectable in year two.

For the combining ability analyses, residual vs. predicted values plots for each trait were normally distributed. Therefore, no data transformation was needed. General

combining ability variance components were significant and greater than SCA variance components for peduncle length, total flowering weeks, flowering cycles, truss size, growing degree days for harvest data, day-neutrality, achene position, ease of capping, fruit weight, percent soluble solids, titratable acidity and soluble solids/titratable acidity (Table 3.4). This suggests additive effects are more important than non-additive effects allowing the breeder to cross parents with the best trait values over several generations (Watkins and Spangelo, 1967).

Narrow-sense heritability estimates were moderate to high (0.33-0.78) for total flowering weeks, flowering cycle, truss size, day-neutrality, number of runners, fruit weight, pH, and titratable acidity (Table 3.4). These higher values are important to the breeder as narrow-sense heritability determines the amount of progress that can be made from selecting and recombining the best individuals in a population (Bernardo, 2010). Therefore, these values indicate a high likelihood of progress for these traits.

Parents with positive GCA effects for day-neutrality included ‘Earliglow’ and ‘Sarian’ while the remontant parent FRA 1702 had negative effects. This contribution of day-neutrality from ‘Earliglow’ is surprising as it was not included as a remontant parent, however acquiring day-neutrality from two non-day-neutral parents is not unheard of as J. Hancock (pers. comm.) has found this in previous work. The GCA effect for vigor was positive for FRA 1702 but negative for ‘Sarian’. For number of runners, ‘Puget Reliance’ and FRA 1702 had positive GCA effects while progeny of ‘Sarian’ produced far fewer runners. Since flowering and runnering in strawberry genotypes usually are strongly negatively correlated, it was not surprising that ‘Sarian’s progenies were strongly day-

neutral and produced few runners, ‘Seascape’, selected in part for its shipability, not surprisingly contributed positively to skin strength while the wild selection FRA 1702, had progeny with tender skin. Fruit from the progeny of ORUS 2427-1 and ‘Seascape’ tended to be firmer than in the other progenies while those from FRA 1702 were softer. FRA 1702 had positive influence for ease of capping while the progeny of ‘Sarian’ and ‘Seascape’ were hard to cap. ORUS 2427-1, ‘Fort Laramie’ and ‘Seascape’ all had positive GCA effects for heavier fruit while progeny from ‘Earliglow’ and FRA 1702 were lighter. ORUS 2427-1 and ‘Seascape’ are modern genotypes and their progenies were expected to be heavier, while those of ‘Earliglow’ and ‘Fort Laramie’ released in the 1970s, were expected to have progenies with smaller fruit. ‘Fort Laramie was surprising as its progenies tended to be heavier than might have been expected. While not significant, ‘Earliglow’, ‘Tillamook’ and ORUS 2427-1 had progenies with low drip losses while those from ‘Puget Reliance’ and ‘Honeoye’ were significant and large. While the low drip loss from two of the Northwest parents was expected, those from ‘Earliglow’ were a surprise and suggest that it might be a good parent to incorporate into the Northwest breeding programs for this characteristic. While ‘Puget Reliance’ is a Northwest cultivar, it is known to have higher than desirable drip loss and so the poor performance of its offspring for this trait were not surprising. MSU 49 and ‘Sarian’ had positive GCA effects for pH, and FRA 1702 and ‘Tribute’ contributed to lower pH levels in their progeny. Breeding programs interested in cultivars for processing where a pH at or below 3.5 is desirable are more likely to benefit from using FRA 1702 and ‘Tribute’ as parents than ‘Sarian’ or MSU 49. FRA 1702 contributed to high percent soluble solids

and MSU 49 and ‘Fort Laramie’ contributed to lower soluble solids. ‘Puget Reliance’ and FRA 1702 contributed to progenies with higher titratable acidity and MSU 49, MSU 56, and ‘Sarian’ contributed to lower titratable acidity. MSU 49 and ‘Sarian’ tended to contribute to higher soluble solid/titratable acidity in their progenies and FRA 1702 contributed negatively. When breeding for markets where fruit perceived as being sweeter, rather than tart, is desirable, then MSU 49 and ‘Sarian’ would be better choices as parents but they would not be good parents for breeders developing cultivars for the processed fruit market.

In general the Midwest adapted parents had the most positive GCA effects for phenology and flowering related traits mainly with significant GCA effects attributed to ‘Earliglow’ for total flowering weeks, flowering cycles, truss size, and day-neutrality while having negative significant GCA effects for days from 1 January for first bloom, harvest, and growing degree days for harvest meaning early bloom, and harvest. Pacific Northwest adapted parents tended to contribute to later ripening and smaller truss size. For plant characteristics, GCA effects for crop estimate and vigor were not significant, however they were all positive for Midwest adapted parents and were negative for PNW parents with the exception of ‘Puget Reliance’. The GCA effects for number of runners in all of the Midwest adapted parents were negative while all were positive for PNW parents. External fruit characteristics did not differ much between Midwest and PNW adapted parents except for ‘Puget Reliance’ which contributed greater to sunken achenes, and Midwest adapted parents all had positive GCA effects for external color. Parental contribution for internal fruit characteristics were not greatly distinct between Midwest

adapted parents and PNW. For firmness, three of the Midwest parents contributed negatively and MSU 49 contributed with positive GCA effects. Two of the PNW parents contributed negatively for firmness, 'Tillamook' and ORUS 2427-1 had significant GCA effects contributing to offspring with firmer fruit. For fruit weight the Eastern adapted parent 'Earliglow' had significant negative GCA effects and the PNW parent ORUS 2427-1 had positive GCA effects.

## Conclusions

The flowering and day-neutrality traits (total weeks flowering, flowering cycles and day-neutrality) had high heritability estimates. 'Sarian' was identified as the best parent for contributing strong flowering habit as it had the highest positive GCA effects for each of those three traits. This could be because 'Sarian' is grown from an F1 hybrid seed population (Bentvelsen, 2006) and is probably homozygous dominant for day-neutrality.

Similar to what was found in Simpson and Sharp (1988), day-neutrality and runner production were heritable and parents with significant GCA effects were identified suggesting possible integration of each of these traits with recurrent selection. Overall, we found very little genotype × environment and genotype × year interactions for a majority of the traits in this study. This suggests that future studies to identify QTL could be done with this distinct set of germplasm combined across locations and years for a more powerful analysis. With this diverse population, it may only be necessary to evaluate in one location in one year to get a good assessment of breeding behavior. The

results presented provide a better understanding of the heritability of these traits. Valuable parents for most traits could be identified and those for day-neutrality, high runner number, and fruit chemistry attributes among the Midwest, PNW and the remontant parents will be especially valuable and used future crosses.

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Tables:

Table 3.1. The 35 strawberry (*Fragaria* x*ananass*a) families evaluated were produced using line by tester cross design where Midwest adapted and Pacific Northwest adapted parent (Lines) were crossed with remontant parents (Tester).

| Tester | Lines | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Midwest | | | | Pacific Northwest | | | |
| | Earliglow | Honeoye | MSU 49 | MSU 56 | ORUS 2427-1 | Puget Reliance | Tillamook | Totem |
| FRA 1702 | X | X | X | X | X | X | X | X |
| Fort Laramie | X | X | X | X | X | X | X | X |
| Sarian | | | | | X | X | X | X |
| Seascape | X | X | X | X | 2X | X | X | |
| Tribute | X | X | X | X | X | X | X | |

Table 3.2. Analysis of variance of genotype (ID), location (L), genotype × environment (ID×L), genotype × year (ID×Y) and location × year (L×Y) interaction based on trait means for 36 strawberry (*Fragaria ×ananassa*) families consisting of 10 individuals each (17 from MSU and 19 from USDA-ARS-HCRU) grown in Michigan (MI), New Hampshire (NH), Oregon (OR) and California (CA) for 2011 and 2012.

| | **Significance** | | | | | | **Means - location and years** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trait** | **ID** | **L** | **ID × L** | **Y** | **ID × Y** | **L × Y** | **MI 2011** | **MI 2012** | **NH 2011** | **NH 2012** | **OR 2011** | **OR 2012** | **CA 2011** | **CA 2012** |
| **Phenology and flowering related traits** | | | | | | | | | | | | | | |
| Peduncle length | ns | *** | ns | *** | ns | *** | 4.2 | 2.4 | 3.8 | 2.6 | 2.8 | 3.0 | NA | 3.4 |
| Total flowering weeks | *** | *** | ns | *** | ns | *** | 3.6 | 8.2 | 6.1 | 4.5 | 6.5 | 7.0 | NA | 11.0 |
| Flowering cycles | *** | *** | *** | *** | ns | * | 1.2 | 1.3 | 1.2 | 1.2 | 1.1 | 1.2 | NA | 1.3 |
| Days from 1 January for first bloom | *** | *** | ns | *** | ns | *** | 134.9 | 97.8 | 128.1 | 128.7 | 120.5 | 115.6 | NA | 137.1 |
| Growing degree days for first bloom | *** | *** | ns | *** | ns | *** | 219.0 | 270.5 | 96.8 | 188.6 | 184.2 | 185.0 | NA | NA |
| Truss size | *** | *** | ns | *** | ns | NA | 5.1 | 4.4 | NA | 4.6 | NA | 4.4 | NA | NA |
| Days from 1 January for harvest | *** | *** | ns | *** | ns | *** | 163.2 | 154.5 | 161.0 | 146.8 | 172.3 | 162.2 | NA | NA |
| Growing degree days for harvest date | *** | *** | *** | *** | ns | *** | 554.5 | 750.4 | 411.7 | 355.9 | 579.2 | 580.1 | NA | NA |
| Day-neutrality | *** | *** | *** | *** | ns | ns | 1.02 | 1.08 | 0.30 | 0.37 | 0.64 | 0.71 | NA | 2.28 |
| **Plant characteristics** | | | | | | | | | | | | | | |
| Crop estimate | ** | *** | ns | ** | ns | *** | 5.4 | 5.0 | 2.5 | 3.7 | 4.5 | 2.8 | 3.4 | NA |
| Vigor | *** | *** | ns | *** | ns | *** | 4.2 | 4.1 | NA | 4.0 | 4.7 | 3.9 | NA | 4.5 |
| Number of runners | *** | *** | ns | *** | ns | *** | 1.8 | 4.4 | 6.2 | 1.8 | 3.4 | 1.7 | NA | 2.7 |
| **External fruit characteristics** | | | | | | | | | | | | | | |
| Shape | *** | *** | ns | ns | ns | *** | 4.8 | 5.5 | 5.6 | 6.4 | 5.8 | 6.0 | 6.5 | 6.1 |
| Appearance | *** | *** | ns | ns | ns | *** | 3.8 | 5.2 | 5.2 | 5.7 | 5.2 | 5.2 | 4.2 | 3.2 |
| Achene color | *** | *** | ns | ** | ns | *** | 1.8 | 3.9 | 6.4 | 6.5 | 6.0 | 4.7 | NA | NA |
| Achene position | *** | *** | ns | *** | ns | *** | 2.5 | 2.3 | 2.6 | 2.4 | 1.7 | 2.0 | NA | NA |
| Percent filled achenes | *** | *** | ns | *** | ns | *** | 76.6 | 91.0 | 73.0 | 71.1 | 90.2 | 84.5 | 97.6 | NA |

| Table 3.2 continued | Significance | | | | | | Means - location and years | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trait** | **ID** | **L** | **ID × L** | **Y** | **ID × Y** | **L × Y** | **MI 2011** | **MI 2012** | **NH 2011** | **NH 2012** | **OR 2011** | **OR 2012** | **CA 2011** | **CA 2012** |
| External color | *** | *** | ns | *** | ns | *** | 7.4 | 6.5 | 6.8 | 7.2 | 7.0 | 6.4 | 5.9 | 6.1 |
| Gloss | *** | *** | ns | *** | ns | *** | 7.1 | 6.2 | 5.9 | 6.3 | 6.2 | 4.2 | 5.5 | NA |
| Skin strength | *** | *** | ns | *** | ns | *** | 6.5 | 5.2 | 5.6 | 7.0 | 3.5 | 4.7 | NA | NA |
| Cap size | ns | *** | *** | *** | ns | *** | 1.9 | 1.9 | 1.2 | 1.7 | 1.0 | 1.0 | NA | NA |
| Calyx position | ns | *** | *** | ns | ns | ns | 2.9 | 3.2 | 2.9 | 2.6 | 3.5 | 3.6 | 3.7 | 3.7 |
| **Internal fruit characteristics** | | | | | | | | | | | | | | |
| Firmness | *** | *** | ns | ns | ns | *** | 7.2 | 5.2 | 6.7 | 6.9 | 4.5 | 5.3 | NA | NA |
| Ease of capping | *** | *** | ns | *** | ns | *** | 6.5 | 4.1 | 4.4 | 4.8 | 6.2 | 6.1 | NA | NA |
| Internal color | *** | *** | ns | *** | ns | *** | 7.8 | 4.8 | 6.3 | 5.6 | 4.7 | 4.4 | NA | NA |
| Depth of internal color % | *** | *** | ns | *** | ns | *** | 54.8 | 81.2 | 61.4 | 79.6 | 85.0 | 92.5 | NA | NA |
| Flavor | ns | *** | ns | *** | ns | ns | NA | 4.6 | 6.0 | 6.3 | 3.6 | 3.4 | NA | NA |
| **Fruit characteristics measured in the lab including weight and chemistry** | | | | | | | | | | | | | | |
| Fruit weight (g) | *** | *** | ns | *** | *** | *** | 4.9 | 7.2 | 9.4 | 4.3 | 15.2 | 8.5 | NA | 6.5 |
| Drip loss % | *** | *** | ns | *** | ns | NA | NA | 16.8 | NA | 9.5 | 35.7 | 26.9 | NA | NA |
| pH | *** | *** | ns | *** | ns | *** | 3.5 | 3.5 | 3.4 | 3.5 | 3.5 | 3.3 | NA | NA |
| Percent soluble solids | *** | *** | ns | *** | ns | * | 11.5 | 10.5 | 9.3 | 9.0 | 8.6 | 7.5 | 11.2 | 10.0 |
| Titratable acidity ($g \bullet L^{-1}$ citric acid) | *** | *** | ns | ns | ns | *** | 0.9 | 1.1 | 1.1 | 1.0 | 0.9 | 0.8 | NA | 0.9 |
| Total anthocyanins ($mg \bullet L^{-1}$ Pg-3-gluc Equivalents) | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 341.1 | 308.7 | NA | NA |
| Total phenolics ($mg \bullet L^{-1}$ Gallic Acid Equivalents) | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | 345.7 | 390.7 | NA | NA |
| Soluble solid/titratable acidity | *** | *** | ns | ns | ns | *** | 13.0 | 10.1 | 8.9 | 9.3 | 10.6 | 10.4 | NA | 12.1 |

ns, *, **,***, Non significant and significant at $P \leq 0.05$, 0.01 or 0.001 respectively

Table 3.3. Analysis of variance for genotype (ID), environment (L) based on trait means of four European strawberry (*Fragaria ×ananassa*) mapping populations (INRA population, 56 progeny from 'Capitola' × CF1116 ('Pajaro' × ['Earliglow' × 'Chandler']); the EMR population, 51 progeny from 'Redgauntlet' × 'Hapil'; the CGN population, 29 progeny from 'Holiday' × 'Korona'; and the IFAPA population, 69 progeny from '232' (Sel. 4-43 × 'Vilanova') × '1392' ('Gaviota' × 'Camarosa')) grown in Michigan (MI), Oregon (OR) and California (CA) for 2012.

| | **Significance** | | **Locations** | | |
|---|---|---|---|---|---|
| **Trait** | **ID** | **L** | **MI 2012** | **OR 2012** | **CA 2012** |
| **Phenology and flowering related traits** | | | | | |
| Peduncle length | ns[z] | *** | 3.0 | 2.2 | 3.6 |
| Total flowering weeks | ns | *** | 4.6 | 5.7 | 13.2 |
| Flowering cycles | ns | ns | 1.2 | 1.1 | 1.1 |
| Days from January first for first bloom | ns | *** | 112.0 | 121.0 | 132.0 |
| Growing degree days for first bloom | ns | *** | 315.2 | 227.9 | NA |
| Truss size | ns | ns | 6.9 | 7.1 | NA |
| Days from January first for harvest | ns | *** | 159.9 | 166.0 | NA |
| Growing degree days for harvest date | ns | *** | 825.6 | 619.1 | NA |
| Day-neutrality | ns | *** | 0.4 | 0.3 | 3.3 |
| **Plant characteristics** | | | | | |
| Crop estimate | *** | ns | 4.8 | 5.2 | NA |
| Vigor | ns | *** | NA | 6.6 | 5.3 |
| Number of runners | ns | *** | 4.5 | 2.7 | 2.4 |
| **External fruit characteristics** | | | | | |
| Shape | ns | ** | 5.5 | 6.0 | 5.3 |
| Appearance | ns | *** | 5.4 | 5.9 | 4.7 |
| Achene color | NA | NA | 4.2 | NA | NA |
| Achene position | ns | *** | 2.4 | 2.0 | NA |
| Percent filled achenes | ns | *** | 95.5 | 80.5 | NA |
| External color | *** | * | 6.3 | 6.2 | 5.9 |
| Gloss | ns | *** | 7.2 | 5.4 | NA |
| Skin strength | ns | ns | 4.8 | 4.6 | NA |
| Cap size | ns | *** | 1.9 | 1.0 | NA |
| Calyx position | ns | *** | 4.2 | 2.5 | 3.8 |

Table 3.3 continued

| | Significance | | Locations | | |
|---|---|---|---|---|---|
| **Trait** | **ID** | **L** | **MI 2012** | **OR 2012** | **CA 2012** |
| **Internal fruit characteristics** | | | | | |
| Firmness | *** | * | 4.7 | 5.1 | NA |
| Ease of capping | ns | *** | 3.0 | 6.1 | NA |
| Internal color | *** | ns | 3.9 | 4.2 | NA |
| Depth of internal color (%) | ns | *** | 88.0 | 95.4 | NA |
| Flavor | ns | *** | 3.4 | 2.4 | NA |
| **Fruit chemistry** | | | | | |
| Fruit weight (g) | ns | *** | 8.6 | 17.3 | 13.0 |
| Drip loss (%) | ns | *** | 19.5 | 43.5 | NA |
| pH | ** | *** | 3.4 | 3.3 | NA |
| Percent soluble solids | ns | *** | 9.4 | 7.2 | 9.4 |
| Titratable acidity ($g \bullet L^{-1}$ citric acid) | ns | *** | 1.0 | 0.7 | 0.7 |
| Total anthocyanins ($mg \bullet L^{-1}$ Pg-3-gluc Equivalents) | NA | NA | NA | 269.0 | NA |
| Total phenolics ($mg \bullet L^{-1}$ Gallic Acid Equivalents) | NA | NA | NA | 330.2 | NA |
| Soluble solid/titratable acidity | *** | *** | 9.1 | 10.2 | 13.8 |

[z]ns, *, **,*** Non significant and significant at $P \leq 0.05$, 0.01 or 0.001 respectively

Table 3.4. The GCA, SCA, additive, dominance and phenotypic variance and narrow-sense heritability of 33 flowering, vegetative, fruit characteristics and chemistry traits for 2011 and 2012 of 35 strawberry (*Fragaria ×ananassa*) populations grown in Benton Harbor, MI and Corvallis, OR.

| | GCA ($\sigma^2_G$) | SCA ($\sigma^2_S$) | Error | $\sigma^2_A$ | $\sigma^2_D$ | $\sigma^2_P$ | $h^2$ |
|---|---|---|---|---|---|---|---|
| Phenology and flowering related traits | | | | | | | |
| Peduncle length | 0.04*[z] | 0.01 | 1.66 | 0.16 | 0.03 | 1.74 | 0.09 |
| Total flowering weeks | 0.80* | 0.50** | 5.02 | 3.20 | 2.00 | 7.12 | 0.45 |
| Flowering cycles | 0.02* | 0.00* | 0.12 | 0.08 | 0.02 | 0.17 | 0.47 |
| Days from Jan. 1 to first bloom | 5.15* | 6.54** | 90.95 | 20.60 | 26.16 | 107.79 | 0.19 |
| GDD first bloom | 193.72 | 384.78** | 4532.78 | 774.88 | 1539.12 | 5305.00 | 0.15 |
| Truss size | 0.39* | 0.13* | 2.71 | 1.56 | 0.52 | 3.62 | 0.43 |
| Days from Jan. 1 to harvest | 1.58* | 0.46 | 23.12 | 6.32 | 1.83 | 26.74 | 0.24 |
| GDD for harvest date | 257.76* | 175.64 | 5297.05 | 1031.04 | 702.56 | 5988.21 | 0.17 |
| Day-neutrality | 0.47* | 0.06* | 1.42 | 1.88 | 0.24 | 2.42 | 0.78 |
| Plant characteristics | | | | | | | |
| Crop estimate | 0.04 | 0.12* | 2.58 | 0.14 | 0.48 | 2.77 | 0.05 |
| Vigor | 0.08 | 0.28** | 1.62 | 0.31 | 1.12 | 2.05 | 0.15 |
| Number of runners | 0.57* | 0.63** | 2.86 | 2.28 | 2.52 | 4.63 | 0.49 |
| External fruit characteristics | | | | | | | |
| Shape | 0.07 | 0.06 | 2.98 | 0.29 | 0.22 | 3.18 | 0.09 |
| Appearance | 0.00 | 0.18* | 3.21 | 0.01 | 0.72 | 3.40 | 0.00 |
| Achene color | 0.04 | 0.16* | 2.50 | 0.14 | 0.64 | 2.73 | 0.05 |
| Achene position | 0.02* | 0.02* | 0.39 | 0.09 | 0.08 | 0.45 | 0.20 |
| Percent filled achenes | 1.98 | 4.52 | 186.62 | 7.93 | 18.08 | 195.10 | 0.04 |
| External color | 0.06 | 0.13** | 0.83 | 0.26 | 0.52 | 1.09 | 0.24 |

| Table 3.4 continued | GCA ($\sigma^2_G$) | SCA ($\sigma^2_S$) | Error | $\sigma^2_A$ | $\sigma^2_D$ | $\sigma^2_P$ | $h^2$ |
|---|---|---|---|---|---|---|---|
| Gloss | 0.13* | 0.13* | 2.07 | 0.52 | 0.52 | 2.46 | 0.21 |
| Skin strength | 0.10* | 0.16* | 2.71 | 0.40 | 0.64 | 3.07 | 0.13 |
| Cap size | 0.00 | 0.01** | 0.29 | 0.00 | 0.04 | 0.30 | 0.00 |
| Calyx position | 0.01 | 0.00 | 1.37 | 0.03 | 0.00 | 1.38 | 0.02 |
| Internal fruit characteristics | | | | | | | |
| Firmness | 0.11* | 0.13* | 1.96 | 0.44 | 0.52 | 2.31 | 0.19 |
| Ease of capping | 0.28* | 0.22* | 3.38 | 1.12 | 0.88 | 4.16 | 0.27 |
| Internal color | 0.13 | 0.35* | 2.40 | 0.51 | 1.40 | 3.01 | 0.17 |
| Depth of internal color % | 17.71 | 29.32* | 430.09 | 70.86 | 117.28 | 494.84 | 0.14 |
| Flavor | 0.02 | 0.00 | 3.38 | 0.08 | 0.00 | 3.42 | 0.02 |
| Fruit characteristics measured in the lab including weight and chemistry | | | | | | | |
| Fruit weight g | 3.11* | 1.35* | 16.85 | 12.44 | 5.40 | 24.42 | 0.51 |
| Drip loss % | 4.92 | 9.53* | 97.04 | 19.68 | 38.12 | 116.41 | 0.17 |
| pH | 0.00* | 0.00* | 0.02 | 0.01 | 0.01 | 0.02 | 0.33 |
| Percent soluble solids | 0.22* | 0.11* | 3.00 | 0.88 | 0.44 | 3.55 | 0.25 |
| Titratable acidity g•$L^{-1}$ citric acid | 0.01* | 0.00** | 0.04 | 0.04 | 0.02 | 0.06 | 0.64 |
| Soluble solid/titratable acidity | 0.75* | 0.39* | 8.52 | 3.00 | 1.56 | 10.41 | 0.29 |

[z]*, ** Significant at $P \leq 0.05$ or 0.01, respectively

Table 3.5. General combining ability effects (GCA) for 13 strawberry (*Fragaria ×ananassa*) parents used in line x tester mating scheme grown in Benton Harbor, MI and in Corvallis, OR in 2011 and 2012.

| Trait | Midwest parents | | | | Pacific Northwest parents | | | | Remontant parents | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Earliglow | Honeoye | MSU 49 | MSU 56 | ORUS 2427-1 | Puget Reliance | Tillamook | Totem | FRA 1702 | Fort Laramie | Sarian | Seascape | Tribute |
| Phenology and flowering related traits | | | | | | | | | | | | | |
| Peduncle length | 0.37**[z] | -0.07 | -0.34 | -0.01 | 0.01 | -0.01 | 0.03 | 0.02 | 0.22* | -0.04 | -0.15 | -0.14 | 0.11 |
| Total flowering weeks | 0.80* | 0.16 | -0.15 | 0.76 | -0.60 | -0.27 | -0.43 | -0.28 | -0.91* | -0.9* | 1.96*** | -0.14 | -0.01 |
| Flowering cycles | 0.13* | 0.03 | -0.02 | 0.10 | -0.05 | -0.08 | -0.09 | -0.02 | -0.23 | -0.08 | 0.32*** | -0.01 | 0.00 |
| Days from 1 Jan. to first bloom | -2.55* | -0.89 | 0.90 | -1.73 | 2.18 | -0.18 | 2.26* | 0.01 | -3.88** | 1.29 | 1.57 | 1.09 | -0.07 |
| GDD first bloom | -10.31 | -3.82 | 4.88 | -10.79 | 13.37 | -4.39 | 12.69 | -1.63 | -22.11* | 5.27 | 15.18 | 2.79 | -1.12 |
| Truss size | 1.12*** | 0.27 | -0.22 | 0.05 | -0.71* | -0.16 | -0.21 | -0.14 | 1.13*** | -0.25 | -0.58* | -0.39 | 0.09 |
| Days from 1 Jan. to harvest | -1.91** | -0.68 | -0.63 | -0.94 | 2.04** | 1.15* | 0.38 | 0.58 | -0.77 | -0.26 | 1.40* | 0.40 | -0.75 |
| GDD for harvest date | -16.47* | -6.49 | -6.51 | -11.91 | 25.21** | 10.34 | 1.70 | 4.12 | -9.51 | -5.74 | 25.82* | 0.21 | -10.79 |
| Day-neutrality | 0.49* | 0.06 | 0.01 | 0.39 | -0.26 | -0.38 | -0.21 | -0.11 | -1.01** | -0.52 | 1.71*** | -0.17 | -0.01 |
| Plant characteristics | | | | | | | | | | | | | |
| Crop estimate | 0.05 | 0.07 | 0.01 | 0.09 | -0.08 | 0.01 | -0.06 | -0.09 | -0.10 | 0.32* | -0.14 | 0.03 | -0.11 |
| Vigor | 0.10 | 0.03 | 0.03 | 0.13 | -0.13 | 0.13 | -0.11 | -0.17 | 0.33* | 0.21 | -0.43* | 0.03 | -0.13 |
| Number of runners | -0.27 | -0.22 | -0.42 | -0.36 | 0.23 | 0.71* | 0.02 | 0.30 | 1.24** | 0.51 | -1.42** | -0.20 | -0.13 |
| External fruit characteristics | | | | | | | | | | | | | |
| Shape | -0.36* | -0.09 | -0.18 | 0.22 | 0.08 | 0.19 | -0.16 | 0.31 | -0.33* | 0.08 | 0.18 | 0.07 | 0.00 |

| Table 3.5 continued | Midwest parents | | | | Pacific Northwest parents | | | | Remontant parents | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Earliglow | Honeoye | MSU 49 | MSU 56 | ORUS 2427-1 | Puget Reliance | Tillamook | Totem | FRA 1702 | Fort Laramie | Sarian | Seascape | Tribute |
| Appearance | 0.00 | 0.01 | 0.01 | 0.00 | -0.01 | 0.00 | -0.01 | 0.00 | -0.01 | 0.00 | -0.01 | 0.01 | 0.00 |
| Achene color | 0.10 | 0.12 | 0.04 | 0.10 | 0.05 | -0.22 | -0.08 | -0.11 | -0.03 | 0.01 | -0.17 | 0.13 | 0.07 |
| Achene position | 0.13 | 0.07 | -0.02 | -0.12 | -0.05 | -0.17* | -0.01 | 0.15 | -0.19* | -0.14 | 0.08 | 0.04 | 0.21* |
| Percent filled achenes | -0.66 | 0.60 | 0.41 | -0.11 | -0.22 | -0.38 | -0.82 | 1.18 | 0.83 | 0.51 | -1.85 | 1.41 | -0.89 |
| External color | 0.14 | 0.17 | 0.09 | 0.12 | -0.20 | -0.14 | -0.08 | -0.11 | -0.47** | 0.19 | 0.18 | 0.14 | -0.04 |
| Gloss | -0.01 | 0.06 | 0.28 | 0.09 | -0.18 | 0.13 | -0.22 | -0.15 | -0.78*** | -0.08 | 0.23 | 0.35* | 0.28 |
| Skin strength | 0.09 | -0.01 | 0.17 | -0.07 | 0.20 | -0.28 | -0.08 | -0.02 | -0.40* | -0.14 | -0.06 | 0.61** | -0.01 |
| Cap size | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Calyx position | -0.02 | -0.11 | 0.00 | 0.02 | -0.02 | 0.04 | 0.07 | 0.02 | 0.11* | -0.02 | -0.04 | -0.05 | -0.01 |
| Internal fruit characteristics | | | | | | | | | | | | | |
| Firmness | -0.03 | -0.13 | 0.22 | -0.25 | 0.35* | -0.30 | 0.23 | -0.09 | -0.49* | -0.09 | 0.07 | 0.43* | 0.08 |
| Ease of capping | 0.30 | 0.11 | -0.48 | 0.21 | -0.48 | 0.08 | 0.03 | 0.22 | 0.93** | 0.24 | -0.62* | -0.66* | 0.11 |
| Internal color | 0.16 | 0.06 | 0.05 | -0.13 | -0.17 | -0.05 | -0.05 | 0.13 | -0.71** | 0.35 | -0.03 | 0.29 | 0.10 |
| Depth of internal color % | 3.31 | 1.48 | -0.83 | -0.23 | -5.53* | 1.03 | -0.12 | 0.89 | -7.68** | 1.88 | 0.14 | 3.25 | 2.41 |
| Flavor | 0.13 | -0.11 | -0.04 | 0.00 | 0.06 | 0.07 | -0.12 | 0.01 | 0.09 | 0.08 | -0.08 | -0.06 | -0.03 |
| Fruit characteristics measured in the lab including weight and chemistry | | | | | | | | | | | | | |
| Fruit weight (g) | -1.84* | 0.10 | 0.78 | 0.61 | 1.62* | -0.01 | 0.08 | -1.34 | -3.42*** | 1.69* | -1.24 | 2.10* | 0.87 |
| Drip loss % | -2.42 | 2.57* | 0.11 | 1.59 | -1.67 | 2.44* | -1.87 | -0.76 | -0.97 | 2.03 | -1.04 | -0.28 | 0.26 |
| pH | 0.01 | 0.00 | 0.06* | 0.01 | -0.03 | -0.03 | 0.01 | -0.02 | -0.06* | 0.03 | 0.08** | 0.00 | -0.05* |

| Table 3.5 continued | Midwest parents | | | | Pacific Northwest parents | | | | Remontant parents | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Earliglow | Honeoye | MSU 49 | MSU 56 | ORUS 2427-1 | Puget Reliance | Tillamook | Totem | FRA 1702 | Fort Laramie | Sarian | Seascape | Tribute |
| Percent soluble solids | 0.48* | -0.25 | -0.54* | -0.13 | -0.04 | 0.17 | -0.18 | 0.49* | 0.80** | -0.43* | 0.12 | -0.43* | -0.07 |
| TA g•L-1citric acid | -0.01 | -0.03 | -0.14** | -0.10* | 0.05 | 0.09* | 0.04 | 0.09 | 0.20*** | -0.08 | -0.13* | -0.04 | 0.05 |
| SS/TA | 0.55 | -0.19 | 0.96* | 0.75 | -0.49 | -0.62 | -0.51 | -0.46 | -1.17* | 0.54 | 1.35* | -0.05 | -0.68 |

[z]*, **,*** Significant at $P \leq 0.05$, 0.01 or 0.001 respectively

# CONCLUSIONS; Phenotyping Diverse Strawberry (*Fragaria* spp.) Germplasm, for Aid in Marker-Assisted Breeding, and Marker-Trait Association for SSR Marker *Rpf1* for Red Stele (*Phytophthora fragariae*) Resistance

Strawberry has been an important fruit crop worldwide for well over a century and have a long history of breeding, selection, and cultivation. The first goal of this thesis was to characterize 947 diverse strawberry individuals and generate phenotypic data that will eventually be used to identify QTL through pedigree based analysis across multiple years and environments. The second goal was to validate an SSR marker linked to the *Phytophthora fragariae Rpf1* resistance.

The establishment of a standardized phenotyping protocol along with successful data collection from the RosBREED replicated fields (OR, MI, CA, NH and FL) was integrated into the Genome database for Rosaceae. This data is now available for public and private breeders to utilize and provides standard methods each can use to generate additional data from their program.

A marker-trait association with the *Rpf1* SSR marker and disease screening for detection of R1 was 87.5%. This is a high MTA value and proves that this SSR marker can successfully be used in *F.* ×*ananassa* for parent or seedling selection for this resistance gene. Along with this high MTA, the detection of 14 individuals with other potential valuable sources of *Phytophthora fragariae* resistance, were identified.

Minimal genotype × environment and genotype × year interactions were detected for the 36 families studied. This suggests that data from multi-year and the multiple locations can be combined for a robust QTL analysis. High narrow-sense heritability was also detected for total flowering weeks, flowering cycle, truss size, day-neutrality,

number of runners, fruit weight, pH, and titratable acidity. Valuable parents for most traits were identified and those for day-neutrality, high runner number, and fruit chemistry attributes among the Midwest, PNW and the remontant parents will be used in future cross.

# Appendix

Appendix Table 4.1. List of the 153 *Fragaria* individuals that were selected to screen by bench inoculation with Cdn-4 and Cdn-5 *Phytophthora fragariae* races, scores for each of the four plant replicates, standard deviation of the scores (Std), response to the inoculation (R for resistance or S for susceptibility) and presence (+) or absence (-) of the SSR marker for *Rpf1* resistance (*Rpf1*). For each replicate, NA indicates plant replicate not available. Plants were scored between 0 (highly susceptible or dead) and 5 (healthy no symptoms) and a disease score ≥ 4 indicated resistance. Plants that could not be propagated and thus could not be inoculated are highlighted in grey. Under Remarks, we indicate plants that were excluded from the association testing of marker to R1 resistance due to an intermediate score of 3 (I), lack of replication (NR), variability in disease response among replicates (standard deviation >0.8, SD), and presence of additional resistance factors indicated by resistance to both races (RR), susceptibility to Cdn-4 but resistance to Cdn-5 (SR) and null alleles in non *F.* ×*ananassa* (NW). The 56 individuals with "MA" (marker association) or "MN" (marker not associated) were used in validating the association of the *Rpf1* SSR marker with R1. Genotypes are listed in alphabetical order by section and by remarks.

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| **Cultivar** | | | | | | | | | | | | | | | | |
| Charm | BC 91-14-31 | WA 94023-1 | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| Perle de Prague[v] | unknown | unknown | 2 | 2 | 1 | 1 | 0.58 | 1 | 2 | 2 | 1 | 0.58 | S | S | - | MA |
| Puget Reliance | WSU 1945 | BC 77-2-72 | 5 | 5 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | R | S | + | MA |
| Strawberry Festival | Rosa Linda | Oso Grande | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| Sweet Bliss | BC753 | ORUS 1735-1 | 5 | 5 | 5 | NA | 0 | 2 | 2 | 2 | NA | 0 | R | S | + | MA |
| Sweet Charlie | FL 80-456 | Pajaro | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| Tillamook | Cuesta | Puget Reliance | 1 | 1 | NA | NA | 0 | 1 | 2 | NA | NA | 0.71 | S | S | - | MA |
| Totem[u] | Puget Beauty | Northwest | 2 | 1 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| Tufts | Tioga | Cal 46.5-1 | 3 | 2 | NA | 2 | 0.58 | 1 | 2 | 1 | 1 | 0.50 | S | S | - | MA |
| Valley Red | Anaheim | Puget Reliance | 4 | 4 | 5 | 4 | 0.50 | 1 | 1 | 1 | 1 | 0 | R | S | + | MA |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| Earliglow[t] | MDUS 2359 | MDUS 2713 | 0 | 1 | 1 | 1 | 0.50 | 1 | 1 | 1 | 1 | 0 | S | S | + | - |
| Melody | SCRI66 M1 | Senga Sengana | 3 | 1 | 1 | 1 | 1.00 | 1 | 2 | 1 | 1 | 0.50 | S | S | + | - |
| Allstar[s] | US 4419 | MDUS 3184 | 5 | 5 | NA | NA | 0 | 4 | 4 | NA | NA | 0 | R | R | - | RR |
| Albion | Diamant e | Cal 94.16-1 | 4 | 0 | 2 | NA | 2.00 | 1 | 1 | 1 | NA | 0 | | | - | SD |
| Jewel | NY 1221 | Holiday | 2 | 2 | NA | NA | 0 | 4 | 2 | NA | NA | 1.41 | | | + | SD |
| Honeoye | Vibrant | Holiday | 2 | 1 | NA | NA | 0.71 | 2 | 3 | NA | NA | 0.71 | | | - | I |
| Firecracker[r] | ORUS 850-48 | Totem | 5 | 5 | NA | NA | 0 | 5 | NA | NA | NA | NA | | | + | NR |
| MD-683[s] | Fairfax | Scot BK-46 | 1 | NA | NA | NA | NA | 2 | 1 | NA | NA | 0.71 | | | + | NR |
| Sweet Sunrise | Puget Reliance | B754 | 2 | 3 | NA | NA | 0.71 | 5 | NA | NA | NA | NA | | | + | NR |
| **MSU seedlings** | | | | | | | | | | | | | | | | |
| MSU 9-1-4 | MSU 49 | FRA 1702 | 1 | 1 | NA | NA | 0 | 2 | 2 | NA | NA | 0 | S | S | - | MA |
| MSU 9-5-1 | Earliglo w | FRA 1702 | 5 | 5 | 5 | 5 | 0 | 1 | 2 | NA | NA | 0.71 | R | S | + | MA |
| MSU 9-5-4 | Earliglo w | FRA 1702 | 2 | 2 | NA | NA | 0 | 2 | 1 | NA | NA | 0.71 | S | S | - | MA |
| MSU 9-9-10 | Fort Laramie | Honeoye | 2 | 1 | NA | NA | 0.71 | 2 | 2 | NA | NA | 0 | S | S | - | MA |
| MSU 9-12-8 | Honeoye | FRA 1702 | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| MSU 9-5-2 | Earliglo w | FRA 1702 | 5 | 5 | 5 | 5 | 0 | 4 | 4 | 5 | 5 | 0.58 | R | R | + | RR |
| MSU 9-5-5 | Earliglo w | FRA 1702 | 5 | 5 | 4 | 4 | 0.58 | 5 | 5 | NA | NA | 0 | R | R | - | RR |
| MSU 9-15-5 | Tribute | Earliglo w | 5 | 4 | NA | NA | 0.71 | 4 | 4 | NA | NA | 0 | R | R | + | RR |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| MSU 9-9-5 | Fort Laramie | Honeoye | 2 | 2 | NA | NA | 0 | 5 | 4 | NA | NA | 0.71 | S | R | - | SR |
| MSU 9-16-25 | Tribute | Honeoye | 1 | 2 | NA | NA | 0.71 | 5 | 4 | NA | NA | 0.71 | S | R | - | SR |
| MSU 9-1-7 | MSU 49 | FRA 1702 | 2 | 4 | NA | NA | 1.41 | 2 | 2 | NA | NA | 0 | | | - | SD |
| MSU 9-1-8 | MSU 49 | FRA 1702 | 4 | 1 | NA | NA | 2.12 | 3 | 4 | NA | NA | 0.71 | | | - | SD |
| MSU 9-8-1 | Fort Laramie | Earliglow | 2 | 1 | NA | NA | 0.71 | 4 | 3 | NA | NA | 0.71 | | | + | SD |
| MSU 9-12-6 | Honeoye | FRA 1702 | 4 | 2 | NA | NA | 1.41 | 3 | 3 | NA | NA | 0 | | | - | SD |
| MSU 9-12-9 | Honeoye | FRA 1702 | 4 | 2 | NA | NA | 1.41 | 3 | 5 | NA | NA | 1.41 | | | - | SD |
| MSU 9-16-21 | Tribute | Honeoye | 3 | 2 | NA | NA | 0.71 | 3 | 4 | NA | NA | 0.71 | | | - | SD |
| MSU 9-5-8 | Earliglow | FRA 1702 | 3 | 3 | 1 | 1 | 1.15 | 5 | 5 | 5 | 4 | 0.5 | | | - | I |
| MSU 9-8-4 | Fort Laramie | Earliglow | 3 | 1 | NA | NA | 1.41 | 2 | 1 | NA | NA | 0.71 | | | + | I |
| MSU 9-8-5 | Fort Laramie | Earliglow | 3 | 2 | NA | NA | 0.71 | 4 | 5 | NA | NA | 0.71 | | | - | I |
| MSU 9-8-6 | Fort Laramie | Earliglow | 5 | 5 | NA | NA | 0 | 3 | 3 | NA | NA | 0 | | | + | I |
| MSU 9-16-17 | Tribute | Honeoye | 3 | 1 | NA | NA | 1.41 | 2 | 3 | NA | NA | 0.71 | | | - | I |
| MSU 9-1-1 | MSU 49 | FRA 1702 | 2 | NA | NA | NA | NA | 1 | NA | NA | NA | NA | | | + | NR |
| MSU 9-8-8 | Fort Laramie | Earliglow | 4 | 1 | NA | NA | 2.12 | 5 | NA | NA | NA | NA | | | - | NR |
| MSU 9-12-5 | Honeoye | FRA 1702 | 4 | 3 | NA | NA | 0.71 | 5 | 4 | NA | NA | 0.71 | | | - | NR |
| MSU 9-15-3 | Tribute | Earliglow | 2 | 1 | NA | NA | 0.71 | 2 | NA | NA | NA | NA | | | - | NR |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| MSU 9-16-22 | Tribute | Honeoye | 5 | 5 | NA | NA | 0 | 3 | NA | NA | NA | NA | | | - | NR |
| **ORUS selections** | | | | | | | | | | | | | | | | |
| NW 90054-37 | WA 87010-7 | ORUS 984-49 | 4 | 5 | NA | NA | 0.71 | 2 | 1 | NA | NA | 0.71 | R | S | + | MA |
| ORUS 1239 R-21 | Sumas | ORUS 973-1 | 1 | 1 | NA | NA | 0 | 2 | 1 | NA | NA | 0.71 | S | S | + | MN |
| ORUS 1267-236 | Redcrest | ORUS 869-13 | 1 | 2 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 2490-1 | ORUS 1722-2 | Pinnacle | 1 | 1 | NA | NA | 0 | 2 | 1 | NA | NA | 0.71 | S | S | - | MA |
| ORUS 2742-1 | Sweet Bliss | Valley Red | 5 | 5 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | R | S | + | MA |
| ORUS 2427-4 | Pinnacle | ORUS 1723-3 | 3 | 2 | NA | NA | 0.71 | 2 | 2 | NA | NA | 0 | | | - | I |
| ORUS 2781-1 | Puget Summer | ORUS 2016-1 | 3 | 2 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | | | + | I |
| ORUS 2427-1 | Pinnacle | ORUS 1723-3 | 2 | NA | NA | NA | NA | 1 | NA | NA | NA | NA | | | - | NR |
| **ORUS seedlings** | | | | | | | | | | | | | | | | |
| ORUS 3306-6 | Totem | FRA 1702 | 4 | 5 | 5 | 5 | 0.5 | 2 | 2 | 1 | 2 | 0.50 | R | S | + | MA |
| ORUS 3315-10 | Fort Laramie | Puget Reliance | 4 | 4 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | R | S | + | MA |
| ORUS 3315-11 | Fort Laramie | Puget Reliance | 5 | 4 | 5 | 5 | 0.50 | 1 | 1 | 1 | 1 | 0 | R | S | + | MA |
| ORUS 3315-4 | Fort Laramie | Puget Reliance | 4 | 5 | NA | NA | 0.71 | 1 | 2 | NA | NA | 0.71 | R | S | + | MA |
| ORUS 3316-2 | Fort Laramie | Totem | 4 | 5 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | R | S | + | MA |
| ORUS 3316-5 | Fort Laramie | Totem | 4 | 5 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | R | S | + | MA |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| ORUS 3317-2 | Puget Reliance | FRA 1702 | 5 | 5 | 5 | 5 | 0 | 1 | 1 | 1 | 2 | 0.50 | R | S | + | MA |
| ORUS 3317-3 | Puget Reliance | FRA 1702 | 5 | 5 | 5 | NA | 0 | 1 | 2 | 2 | 2 | 0.50 | R | S | + | MA |
| ORUS 3318-1 | Puget Reliance | Sarian | 5 | 5 | NA | NA | 0 | 2 | 1 | NA | NA | 0.71 | R | S | + | MA |
| ORUS 3323-4 | Seascape | Puget Reliance | 4 | 5 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | R | S | + | MA |
| ORUS 3324-9 | Tillamook | Fort Laramie | 4 | 5 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | R | S | + | MA |
| ORUS 3304-1 | Tillamook | FRA 1702 | 1 | 2 | NA | NA | 0.71 | 1 | 2 | NA | NA | 0.71 | S | S | - | MA |
| ORUS 3304-5 | Tillamook | FRA 1702 | 2 | 1 | NA | NA | 0.71 | 2 | 2 | NA | NA | 0 | S | S | - | MA |
| ORUS 3304-8 | Tillamook | FRA 1702 | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3305-13 | Tillamook | Seascape | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3306-14 | Totem | FRA 1702 | 2 | 1 | NA | NA | 0.71 | 2 | 2 | NA | NA | 0 | S | S | - | MA |
| ORUS 3315-1 | Fort Laramie | Puget Reliance | 1 | 2 | 2 | NA | 0.58 | 2 | 3 | 2 | NA | 0.58 | S | S | - | MA |
| ORUS 3316-1 | Fort Laramie | Totem | 2 | 1 | 2 | NA | 0.58 | 1 | 1 | 1 | NA | 0 | S | S | - | MA |
| ORUS 3318-4 | Puget Reliance | Sarian | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3318-9 | Puget Reliance | Sarian | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3320-1 | Sarian | Totem | 1 | 2 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3320-7 | Sarian | Totem | 1 | 1 | NA | NA | 0 | 1 | 2 | NA | NA | 0.71 | S | S | - | MA |
| ORUS 3321-6 | Sarian | Tillamook | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| ORUS 3323-14 | Seascape | Puget Reliance | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 2 | 2 | 0.50 | S | S | - | MA |
| ORUS 3323-3 | Seascape | Puget Reliance | 2 | 1 | 2 | NA | 0.58 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3324-14 | Tillamook | Fort Laramie | 1 | 2 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3324-3 | Tillamook | Fort Laramie | 1 | 1 | NA | NA | 0 | 1 | 2 | NA | NA | 0.71 | S | S | - | MA |
| ORUS 3325-13 | Tillamook | Tribute | 2 | 2 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3325-4 | Tillamook | Tribute | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3326-2 | Tribute | Puget Reliance | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | - | MA |
| ORUS 3326-3 | Tribute | Puget Reliance | 1 | 1 | 1 | NA | 0 | 1 | 1 | 1 | NA | 0 | S | S | - | MA |
| ORUS 3317-6 | Puget Reliance | FRA 1702 | 1 | 2 | 1 | 2 | 0.58 | 1 | 1 | 1 | 2 | 0.50 | S | S | + | MN |
| ORUS 3324-1 | Tillamook | Fort Laramie | 1 | 1 | NA | NA | 0 | 1 | 1 | NA | NA | 0 | S | S | + | MN |
| ORUS 3325-2 | Tillamook | Tribute | 1 | 2 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | S | S | + | MN |
| ORUS 3326-13 | Tribute | Puget Reliance | 2 | 1 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | S | S | + | MN |
| ORUS 3321-8 | Sarian | Tillamook | 5 | 5 | 4 | NA | 0.58 | 2 | 1 | 3 | NA | 1.0 | R | S | - | MN |
| ORUS 3306-2 | Totem | FRA 1702 | 4 | 3 | NA | NA | 0.71 | 3 | 2 | NA | NA | 0.71 | | | + | SD |
| ORUS 3316-10 | Fort Laramie | Totem | 2 | 5 | NA | NA | 2.12 | 1 | 2 | NA | NA | 0.71 | | | + | SD |
| ORUS 3317-1 | Puget Reliance | FRA 1702 | 4 | 3 | 2 | 3 | 0.82 | 1 | 2 | 1 | 1 | 0.5 | | | + | SD |
| ORUS 3321-4 | Sarian | Tillamook | 3 | 2 | NA | NA | 0.71 | 3 | 4 | NA | NA | 0.71 | | | - | SD |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| ORUS 3323-1 | Seascape | Puget Reliance | 2 | 5 | NA | NA | 2.12 | 2 | 2 | NA | NA | 0 | | | + | SD |
| ORUS 3326-15 | Tribute | Puget Reliance | 2 | 4 | NA | NA | 1.41 | 1 | 2 | NA | NA | 0.71 | | | + | SD |
| ORUS 3304-2 | Tillamook | FRA 1702 | 4 | 4 | 5 | 5 | 0.58 | 3 | 3 | 3 | 3 | 0 | R | S | + | I |
| ORUS 3304-10 | Tillamook | FRA 1702 | 3 | 2 | NA | NA | 0.71 | 2 | 2 | NA | NA | 0 | | | - | I |
| ORUS 3306-4 | Totem | FRA 1702 | 1 | 3 | NA | NA | 1.41 | 3 | 2 | NA | NA | 0.71 | | | + | I |
| ORUS 3316-3 | Fort Laramie | Totem | 2 | 3 | NA | NA | 0.71 | 1 | 1 | NA | NA | 0 | | | + | I |
| ORUS 3317-10 | Puget Reliance | FRA 1702 | 1 | 2 | 1 | 1 | 0.50 | 2 | 3 | 1 | 3 | 0.96 | | | - | I |
| ORUS 3320-4 | Sarian | Totem | 3 | 2 | NA | NA | 0.71 | 2 | 2 | NA | NA | 0 | | | - | I |
| ORUS 3320-8 | Sarian | Totem | 1 | 1 | NA | NA | 0 | 1 | 3 | NA | NA | 1.41 | | | - | I |
| ORUS 3323-7 | Seascape | Puget Reliance | 1 | 3 | NA | NA | 1.41 | 1 | 1 | NA | NA | 0 | | | + | I |
| ORUS 3326-4 | Tribute | Puget Reliance | 3 | 1 | NA | NA | 1.41 | 1 | 1 | NA | NA | 0 | | | + | I |
| ORUS 3305-1 | Tillamook | Seascape | 1 | 1 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3305-3 | Tillamook | Seascape | 2 | NA | NA | NA | NA | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3305-10 | Tillamook | Seascape | 1 | 1 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3305-11 | Tillamook | Seascape | 1 | 1 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3306-5 | Totem | FRA 1702 | 3 | NA | NA | NA | NA | 1 | 1 | NA | NA | NA | | | + | NR |
| ORUS 3315-12 | Fort Laramie | Puget Reliance | 5 | NA | NA | NA | NA | NA | NA | NA | NA | NA | | | + | NR |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| ORUS 3320-2 | Sarian | Totem | 2 | NA | NA | NA | NA | NA | NA | NA | NA | NA | | | - | NR |
| ORUS 3320-3 | Sarian | Totem | 2 | 1 | NA | NA | 0.71 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3321-2 | Sarian | Tillamook | 1 | 1 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3321-9 | Sarian | Tillamook | 2 | 2 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3324-13 | Tillamook | Fort Laramie | 2 | 1 | NA | NA | 0.71 | 1 | NA | NA | NA | NA | | | + | NR |
| ORUS 3325-9 | Tillamook | Tribute | 2 | 1 | NA | NA | 0.71 | 1 | NA | NA | NA | NA | | | - | NR |
| ORUS 3325-15 | Tillamook | Tribute | 1 | 1 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |
| **Supercore** | | | | | | | | | | | | | | | | |
| FRA 58 | *F. virginiana* | OP | 0 | 0 | 0 | 0 | 0 | 1 | 0 | NA | NA | 0.71 | S | S | - | NW |
| FRA 110 | *F. virginiana* | OP | 0 | 0 | 0 | 1 | 0.50 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 688 | *F. chiloensis* | OP | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 796 | *F. chiloensis* | OP | 0 | 1 | 1 | 1 | 0.50 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 1088 | *F. chiloensis* | OP | 2 | 2 | 1 | 1 | 0.58 | 1 | 2 | 1 | 2 | 0.58 | S | S | - | NW |
| FRA 1100 | *F. chiloensis* | OP | 2 | 2 | 1 | 1 | 0.58 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 1108 | *F. chiloensis* | OP | 0 | 1 | 1 | 1 | 0.50 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 1435 | *F. virginiana* | OP | 2 | 2 | 2 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 1620 | *F. virginiana* | OP | 2 | 2 | 1 | 2 | 0.50 | 1 | 2 | 1 | 1 | 0.50 | S | S | - | NW |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| FRA 1696 | *F. virginiana* | OP | 2 | 3 | 1 | 1 | 0.96 | 1 | 1 | 1 | 1 | 0 | S | S | - | NW |
| FRA 1408 | *F. virginiana* (MS) | OP | 5 | 5 | 4 | 5 | 0.50 | 2 | 1 | 2 | 1 | 0.58 | R | S | - | NW |
| FRA 1699 | *F. virginiana* | OP | 4 | 5 | 5 | 4 | 0.58 | 1 | 2 | 1 | 1 | 0.50 | R | S | - | NW |
| FRA 1701 | *F. virginiana* | OP | 5 | 4 | 5 | 5 | 0.50 | 2 | 3 | 1 | 2 | 0.82 | R | S | - | NW |
| JH 101-1 | *F. chiloensis* | unknown | 5 | 5 | 5 | 5 | 0 | 5 | 5 | 5 | 5 | 0 | R | R | - | RR |
| FRA 34 | *F. chiloensis* | OP | 5 | 5 | 4 | 5 | 0.50 | 5 | 5 | 5 | 5 | 0 | R | R | - | RR |
| FRA 372 | *F. chiloensis* | OP | 5 | 5 | 4 | 4 | 0.58 | 5 | 5 | 4 | 4 | 0.58 | R | R | - | RR |
| FRA 1690 | *F. chiloensis pacifica* | OP | 5 | 5 | 4 | 4 | 0.58 | 5 | 5 | 5 | 5 | 0 | R | R | - | RR |
| FRA 1691 | *F. chiloensis* | OP | 5 | 5 | 5 | 5 | 0 | 5 | 5 | 5 | 5 | 0 | R | R | - | RR |
| FRA 1692 | *F. chiloensis* | OP | 5 | 4 | 4 | 4 | 0.50 | 4 | 4 | 5 | 5 | 0.58 | R | R | - | RR |
| FRA 1697 | *F. virginiana* | OP | 5 | 5 | 5 | 5 | 0 | 5 | 5 | 4 | 3 | 0.96 | R | R | - | RR |
| FRA 1698 | *F. virginiana* | OP | 5 | 5 | 4 | 4 | 0.58 | 5 | 5 | 5 | 5 | 0 | R | R | - | RR |
| FRA 24 | *F. chiloensis* | OP | 4 | 4 | 3 | 4 | 0.5 | 2 | 2 | 2 | 2 | 0 | | | - | SD |
| FRA 42 | *F. chiloensis* | OP | 5 | 5 | 2 | 3 | 1.5 | 4 | 5 | 4 | 5 | 0.58 | | | - | SD |
| FRA 48 | *F. chiloensis* | OP | 5 | 5 | 2 | 3 | 1.5 | 4 | 5 | 4 | 4 | 0.5 | | | - | SD |
| FRA 357 | *F. chiloensis* | OP | 3 | 3 | 4 | 4 | 0.58 | 5 | 5 | 5 | 5 | 0 | | | - | SD |

| Genotype | Female parent | Male parent | Cdn-4[z] replicate | | | | Std.[y] | Cdn-5 replicate | | | | Std. | Disease response | | *Rpf1* | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 | | Cdn-4 | Cdn-5 | | |
| FRA 982 | *F. virginiana* | OP | 4 | 0 | 1 | 2 | 1.71 | 3 | 2 | 1 | 1 | 0.96 | | | - | SD |
| FRA 1092 | *F. chiloensis* | OP | 4 | 0 | 1 | 1 | 1.73 | 1 | 1 | 1 | 1 | 0 | | | - | SD |
| FRA 1414 | *F. virginiana* (MS) | OP | 5 | 5 | 2 | 3 | 1.5 | 1 | 2 | 1 | 2 | 0.58 | | | - | SD |
| FRA 1455 | *F. virginiana* | OP | 4 | 4 | 2 | 3 | 0.96 | 1 | 1 | 1 | 1 | 0 | | | - | SD |
| FRA 1557 | *F. virginiana* (AL) | OP | 4 | 4 | 2 | 2 | 1.15 | 1 | 1 | 1 | 1 | 0 | | | - | SD |
| FRA 1580 | *F. virginiana* (SC) | OP | 4 | 4 | 3 | 3 | 0.58 | 4 | 4 | 4 | 3 | 0.5 | | | - | SD |
| FRA 1694 | *F. virginiana* | OP | 5 | 5 | 3 | 2 | 1.5 | 1 | 2 | 1 | 1 | 0.5 | | | - | SD |
| FRA 1695 | *F. virginiana* | OP | 4 | 4 | 3 | 3 | 0.58 | 1 | 1 | 1 | 1 | 0 | | | - | SD |
| FRA 1700 | *F. virginiana* | OP | 3 | 4 | 1 | 1 | 1.5 | 1 | 1 | 2 | NA | 0.58 | | | - | SD |
| FRA 1703 | *F. virginiana* | OP | 4 | 1 | NA | NA | 2.12 | 1 | 1 | NA | NA | 0 | | | - | SD |
| FRA 1104 | *F. chiloensis* | OP | 1 | 1 | NA | NA | 0 | 1 | NA | NA | NA | NA | | | - | NR |

[z]Proposed resistance factor 1.3 (van de Weg, 1997a).
[y]Proposed to have resistance factor 2 due to resistance to Cdn-3,4,6 (Nickerson and Jamieson, 1995) and absence of the SCAR-R1 *Rpf1* marker (Haymes et al., 2000).
[x]Proposed resistance factor of 1.2/1.2.3 (van de Weg, 1997a).
[w]Proposed resistance factor of 1 (van de Weg, 1997a).
[v]Grayed lines represent individuals that failed to produce runners.
[U] OP=open pollinated