COMPARISON OF RESISTANT, TOLERANT, AND SUSCEPTIBLE HOST RESPONSES TO CHERRY LEAF SPOT AND ASSESSMENT OF TRAIT INHERITANCE

By

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ABSTRACT

COMPARISON OF RESISTANT, TOLERANT, AND SUSCEPTIBLE HOST RESPONSES TO CHERRY LEAF SPOT AND ASSESSMENT OF TRAIT INHERITANCE

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Cherry Leaf Spot (CLS) is a devastating fungal disease of sour cherry where 'Montmorency', the major cultivar grown in the U.S., is highly susceptible. As many as 10 fungicide sprays can be required each growing season to combat this disease; therefore, developing CLS resistant cultivars is a top breeding priority. Past efforts identified cherry germplasm with resistance and tolerance to CLS; however, direct comparisons of the host disease responses had not been conducted. The goals of this study were to (*i*) compare CLS disease progression profiles of the susceptible 'Montmorency' and resistant and tolerant germplasm used in the MSU sour cherry breeding program, and (*ii*) gain an understanding of the inheritance of these traits by evaluating the host response of progeny individuals belonging to families derived from this germplasm. By observing host responses to CLS in a common environment with high disease pressure, significant differences were observed between the susceptible 'Montmorency' and the various tolerant and resistant selections in their response to CLS. These differences support previous reports that *P. canescens* derived resistance is consistent with a hypersensitive host response, and *P. avium*, *P. cerasus* 'North Star', and *P. maackii* derived tolerances are characterized by reduced infection and delayed defoliation compared to 'Montmorency'. Evaluation of the CLS host responses of progeny individuals derived from this germplasm supports a dominant two gene model for *P. canescens* derived resistance, and a recessive gene model for *P. avium* derived tolerance.

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CHAPTER 1
LITERATURE REVIEW

Introduction

Sour cherry (*Prunus cerasus*) is a major fruit crop in the U.S., with the vast majority of production concentrated in Michigan with the monoculture of one cultivar, ‘Montmorency’. In orchards of large acreage, this monoculture can intensify losses to devastating pathogens. The most important fungal pathogen of sour cherry in all humid production areas of the world is *Blumeriella jaapii* (Rhem) Arx (teleomorph *Phloeosporella padi* [Lib.] Arx), the causal agent of cherry leaf spot (CLS). CLS results in leaf chlorosis and defoliation, symptoms with devastating effects on both the short- and long-term production of an orchard (Keitt et al., 1937). To combat this pathogen, growers may use as many as 10 fungicide applications during the growing season. These fungicide applications are a major production expense to growers and in some seasons even an aggressive spray program does not provide full control of the pathogen. An emerging resistance of *B. jaapii* to these chemicals, as well as the possibility of their discontinuation, threatens the sustainability of the sour cherry industry (Proffer et al., 2006).

Cherry Leaf Spot: Causal Agent and Disease Symptoms

Blumeriella jaapii is a fungal pathogen that thrives in orchard environments. It overwinters in plant debris and begins a new infection cycle each spring with the production of sexual ascospores that come in contact with new leaves via rainwater splash (Holb, 2009). The fungus infects these young leaves through the stomata and becomes established in susceptible hosts. Upon establishment, small brown or purple spots are visible on the adaxial leaf surface and over time the lesions become evident on the abaxial leaf surface as well, where asexual conidia are produced in white masses from the center of the lesion. These conidia, which are also spread by rain splash, can then generate secondary infections allowing the pathogen to spread rapidly to all leaves in uninfected areas of the tree. Ultimately *B. jaapii* infection will cause early leaf chlorosis and defoliation, the consequences of which are observable in the same season and in subsequent years, impacting fruit quality, yield, and overall tree health. Short-term effects include low fruit yield and poor fruit quality, including insufficient fruit color, softness, and low soluble solid levels, as well the inability to detect photoperiod, which is necessary for the winter acclimation and spring dormancy break of twigs and buds (Holb, 2009; Howell & Stackhouse, 1973; Keitt et al., 1937). If winter bud damage occurs, fruit yields in several subsequent years can be significantly reduced. The loss of photosynthetic capability due to defoliation also halts carbohydrate production and increases the risk of winter tissue damage and ultimately tree death (Howell & Stackhouse, 1973; Keitt et al., 1937).

Fungicide treatments rarely eradicate the fungus completely but rather slow its progression long enough to delay defoliation, allowing the tree to accumulate the carbohydrates needed for fruit growth and winter survival. As a result of incomplete control, the fungus has an opportunity to evolve in response to the selection pressure presented by the fungicide modes of action, an issue becoming apparent in the case of some commonly used chemicals. The introduction of a cultivar with genetic resistance to CLS has the potential to prevent the pathogen’s devastating effects; however, this resistance can also act as an additional selection pressure, whereby the pathogen evolves to overcome the host genetic defenses.

The ability of a pathogen to overcome host genetic resistance lies in its reproductive and population characteristics. It has been observed that pathogens with the greatest capacity to overcome genetic resistance have a high evolutionary potential, mixed reproductive system, high genotypic flow, large effective population sizes, and high mutation rates, all of which increase the rate of evolution (McDonald & Linde, 2002). Based on what is known about these traits in *B.*

jaapii, the potential risk of the host's genetic resistance breaking down can be estimated. *Blumeriella jaapii* has a mixed reproductive system, meaning that its life cycle involves both sexual and asexual reproduction (Holb, 2009; Keitt et al., 1937). It has moderate genotypic flow, as it is spread by water rather than wind, and it has a moderate mutation rate, which is evidenced by the evolution of resistance to Demethylation Inhibitor (DMI) fungicides over a period of only a few years (Proffer et al., 2006). Although the pathogen has a moderate genotypic flow, there is still the problem of the monoculture environment in which it thrives. Crop rotations cannot be implemented in orchard systems, and climatic extremes cannot be relied upon for population control because the fungus is capable of surviving harsh winters. In some cases, sanitation through the combination of leaf debris removal and mulching can reduce existing levels of inoculum in an orchard (Holb, 2013); however, it is financially prohibitive for growers to implement and does not generate a large enough reduction in disease incidence to be worthwhile.

Considering these factors, the greatest threats to the durability of sour cherry resistance are the mixed reproductive system of *B. jaapii* and the monoculture growing environment of the crop, which each contribute to the pathogen's evolutionary potential. The risk of *B. jaapii* overcoming the genetic host resistance can be reduced if cultivars with complete, multigenic resistance are generated by identifying and pyramiding multiple CLS resistance and tolerance loci.

Cherry Taxonomy

Cherries are a member of the Rosaceae family in the *Prunus* genus, which includes other stone fruit crops such as peach, plum, almond, and apricot. Within this genus, cherry species comprise two subgenera, Cerasus Pers. and Padus (Moench) Koehne, and are divided into multiple sections (Rehder, 1974). The major fruit crop species belong to the Cerasus Pers. subgenus and Cerasus Koehne section which includes the diploid sweet cherry (2n=2x=16, *P. avium*), tetraploid sour cherry (2n=4x=32, *P. cerasus*), tetraploid ground cherry (2n=4x=32, *P. fruticosa*), and the wild diploid cherry species *P. canescens* (2n=2x=16). Sour cherry (*P. cerasus*) is a segmental allotetraploid resulting from the ancient hybridization of sweet cherry (*P. avium*) and ground cherry (*P. fruticosa*) (Beaver & Iezzoni, 1993; Olden & Nybom, 1968). The relationship between these three species and the wild species *P. canescens* is unknown; however, its breeding behavior suggests that it belongs in the Cerasus Koehne section (Schuster, 2005). Within the Padus subgenus, the major cherry species is *P. maackii* (2n=4x=32), which is important to rootstock breeding because it can be crossed with both sweet and sour cherry.

Host Plant Resistance and Tolerance

In an effort to combat the *B. jaapii* pathogen, unique germplasm sources of genetic resistance and tolerance to CLS were sought (Iezzoni, 2005). Among the acquired germplasm were selections derived from sweet cherry (*P. avium*), the wild species *P. canescens*, *P. maackii*, and the sour cherry (*P. cerasus*) cultivar ‘North Star’(Alderman et al., 1950), which were determined to be of interest for their tolerant response to the disease. Past studies determined that the selections that exhibit resistance to CLS were those derived from the wild diploid species *P. canescens* (Downey, 1999; Wharton et al., 2003) and those considered to be tolerant to the disease were several sweet cherry selections and the sour cherry cultivar ‘North Star’ (Sjulin et al., 1989), the *P. maackii* derived selection Almaz R1(1) (Schuster, 2004; Wharton et al., 2003), and the tetraploid sweet cherry selection ‘Csengodi’ (Apostol, 2000; Apostol & Iezzoni, 1992; Schuster, 2004).

The study conducted by Sjulin et al. (1989) showed that sweet cherry selections infected with *B. jaapii* exhibited fewer and smaller lesions than sour cherry selections, which further translated to fewer conidia produced per lesion. Visible lesions were evident on sweet cherry leaves between two to four days later than lesions appeared on sour cherry. In addition to delayed lesion development, sweet cherry selections also had a slower rate of defoliation than sour cherry selections. The sour cherry selection ‘North Star’ was identified as a slight exception to these observations, as although it exhibited an infection rate and lesion development characteristics similar to other sour cherry selections, it developed CLS lesions with fewer conidia and experienced less severe defoliation than the other sour cherry selections examined. From this study, the conclusion was made that selections with smaller lesions, longer latent periods, and reduced sporulation have improved tolerance to CLS (Sjulin et al., 1989). No additional studies have been conducted to further these conclusions regarding CLS tolerance, and results are yet to be documented with regard to the inheritance of these traits.

Detailed phenotypic evaluation of the hypersensitive defense response of the *P. canescens* derived cultivar GiSelA 6 (‘GI 148-1’) was conducted by Wharton et al. (2003). By monitoring lesion development on detached leaves of ‘Montmorency’ and ‘GI 148-1’, this study supported the hypothesis that, in susceptible hosts, *B. jaapii* infects the leaf through stomata and grows biotrophically for the first seven days following infection. This initial growth habit is believed to permit the fungus to become well established before host response reactions are triggered by the initiation of acervuli formation, thereby reducing the effects of host defense compounds. In resistant hosts, like ‘GI 148-1’, Wharton et al. (2003) suspect that following initial *B. jaapii* infection, further biotrophic proliferation is prevented by host defense compounds which lead to pigmentation and abscission of leaf cells surrounding the lesion. It is still possible for these lesions to produce acervuli, however, sporulation ultimately does not occur. This *P. canescens* derived hypersensitive resistance to CLS was further investigated through genotypic analysis of several more related individuals, leading to the identification of a major QTL associated with CLS resistance on linkage group 4 (Stegmeir et al., 2014). These authors developed genetic markers to allow the screening of individuals at this QTL, named *CLSR_G4*, for the *P. canescens* derived allele. By comparing marker and phenotype data it was determined that the presence of the *P. canescens* allele at this locus is required for an individual to express a CLS resistant phenotype. The limitation, however, is that the presence of the resistance allele at the *CLSR_G4* locus does not guarantee resistance. Approximately one-fifth to one-third of the individuals with the *P. canescens* derived allele at this locus were susceptible to CLS. This suggests that at least one additional QTL may be involved in complete resistance, a phenomenon which fits the anticipated segregation of a two gene model. Attempts were made by Stegmeir et al. (2014) to identify this second proposed gene using bulked segregant analysis, without success. They proposed that this failure was caused by the inability to detect minor gene effects characteristic of horizontal resistance in small populations.

Cherry Leaf Spot and the Sour Cherry Industry

Sour cherry growers commit significant time and funds to the control of CLS within their orchards where adequate control requires weekly fungicide treatments and complete spray coverage. If a single application is missed, or even delayed, the consequences can be substantial. The development of a sour cherry cultivar that is resistant to CLS is of great interest to growers as it would (*i*) eliminate the need for costly, repeated fungicide applications; (*ii*) prevent the death and consequent replacement of trees; (*iii*) result in healthy trees capable of reaching their full yield potential; (*iv*) eliminate the impact of variable disease severities on the consistency of crop yield and quality over adjacent years; and (*v*) reduce the environmental impact of sour cherry production. If a new resistant cultivar is to be easily integrated into existing production systems, it must also meet industry expectations for horticultural traits such as productivity and fruit quality suitable for processing.

Breeding a Durably Resistant Cultivar

The known sources of tolerance and resistance to CLS hold great promise for incorporating durable resistance into a sour cherry cultivar favored by the industry and consumers. To make progress toward this goal, efforts must be made to increase cherry breeding efficiency. In the short term, the development of informative phenotyping protocols for disease response permits effective and informative data collection regarding the expression of these traits in breeding selections. Detailed phenotypic evaluation provides the information needed to formulate hypotheses of trait inheritance and to identify the possible genes involved.

Because it takes several years for a tree to reach maturity, genetic markers are an important tool for tree breeders, allowing seedlings with undesirable traits to be culled from the breeding program before they are planted in the field. The time and funds saved by the exclusion of these individuals can then be directed toward the care of seedlings predicted to exhibit a resistant host response. Identification of the genetic controls of resistance and tolerance traits will permit the development of genetic markers for use in screening individuals and conducting marker assisted selection (MAS) within the breeding program, as has already been implemented with the *CLSR_G4* resistance locus (Basundari, 2015). The identification of the second resistance gene proposed to complement *CLSR_G4* (Stegmeir et al., 2014) would make possible the development of a completely predictive genetic test for resistance to be implemented in screening individuals and conducting MAS.

Knowledge regarding the genetic locations of genes involved in host resistance and tolerance, their mode of action (e.g. dominant or recessive), and the implementation of MAS are necessary to pyramid multiple disease resistance and/or tolerance loci into a durably resistant cultivar. The ability to identify individuals that have all genetic components of each trait will allow breeding programs to make selections that would not be possible through phenotyping alone. This increased ease of selection on the basis of CLS response will make the combination of CLS resistance and desirable horticultural traits more efficient and expedite the development of a commercial quality CLS resistant sour cherry cultivar.

Research Objectives

To build upon the existing knowledge of host response to CLS and improve the application of this knowledge within the MSU sour cherry breeding program, this study aimed to (*i*) directly compare CLS disease progression profiles of the susceptible ‘Montmorency’ and available resistant and tolerant germplasm, and (*ii*) gain an understanding of the inheritance of these traits by evaluating the host response of progeny individuals belonging to families derived from this germplasm.

CHAPTER 2

COMPARISON OF CHERRY LEAF SPOT PROGRESSION ON HOSTS WITH PUTATIVE RESISTANCE OR TOLERANCE TO THE DISEASE

Introduction

Cherry leaf spot (CLS), caused by the fungal pathogen *Blumeriella jaapii*, is a foliar disease that has long posed a challenge to the sour cherry industry. This problem is particularly widespread, as the sour cherry industry in the U.S. is almost entirely a monoculture of one cultivar, 'Montmorency', that is highly susceptible to CLS. With the aim of combating this disease, germplasm previously reported to be CLS resistant or tolerant was acquired and incorporated into the sour cherry breeding program at Michigan State University (MSU) in the 1980s (Iezzoni, 2005). This germplasm included individuals representing multiple species, including *Prunus avium* (sweet cherry), *P. canescens* (wild diploid cherry), *P. cerasus* (sour cherry), and *P. maackii*. *Prunus canescens*-derived selections have been shown to exhibit a hypersensitive resistance response when infected with *B. jaapii* (Downey, 1999; Wharton et al., 2003), while nine *P. avium* cultivars (Sjulin et al., 1989), the *P. avium* tetraploid cultivar 'Csengodi' (Apostol, 2000; Apostol & Iezzoni, 1992; Schuster, 2004), the *P. cerasus* cultivar 'North Star' (Sjulin et al., 1989), and the *P. maackii* selection Almaz R1(1) (Schuster, 2004) were shown to exhibit tolerance responses to CLS. These individuals, which have the potential to contribute alleles from different loci that can be pyramided to achieve CLS-resistant cultivars, were subsequently used as parents in the MSU sour cherry CLS resistance breeding program.

Accurate phenotypic data that provides a detailed understanding of the resistant and tolerant host responses is important foundational information for CLS resistance breeding programs. Accurate phenotypic data will aid in (*i*) the identification of parental germplasm and decisions of what crosses to make, (*ii*) the determination of which CLS tolerant or resistant elite selections to advance, and (*iii*) the identification of the loci responsible for the resistant and tolerant responses. Prior studies have reported data on CLS disease progression on one or more

CLS resistant or tolerant sources (Apostol & Iezzoni, 1992; Downey, 1999; Schuster, 2004; Sjulin et al., 1989; Stegmeir et al., 2014; Wharton et al., 2003); however, none of these studies evaluated these sources of resistance and tolerance simultaneously in a common environment, whereby differences in host response could be directly compared. Identification of these differences will permit the development of hypotheses of the inheritance and genetic complexity of each of these traits, as well as inform future studies of host-pathogen interactions at the cellular level.

The objective of this study was to obtain CLS disease progression profiles of the resistant and tolerant germplasm used in the MSU sour cherry breeding program, and compare them with that of the susceptible cultivar ‘Montmorency’. This information would be used to determine which parents will result in the development of the most durably resistant cultivars and to design a phenotyping protocol to aid in the identification of loci controlling CLS disease response.

Materials and Methods

Plant Materials

In 2015, CLS disease progression was evaluated on 14 clonal individuals that included the susceptible ‘Montmorency’, the *P. avium* cultivar ‘Krupnoplodnaya’, seven putatively tolerant individuals (‘North Star’, two ‘North Star’ derived individuals, ‘Csengodi’, one ‘Csengodi’ derived individual, and two *P. maackii* derived individuals), and five putatively resistant *P. canescens* derived individuals (Table 2.1). The trees, ranging from 6 to 17 years old, were growing at the MSU Clarksville Research Center (CRC) in Clarksville, Michigan. For each clone, the number of trees available for evaluation ranged from one to eight (Table 2.1). No CLS fungicides were applied on the trees in 2014 or 2015 (Appendix A).

Table 2.1: Clones selected for CLS evaluation and the number of trees of each included in the study. Evaluations were conducted at two orchard locations for 14 clones, totaling 43 trees.

Location	**Clones**	**Tolerance/ Resistance source**	**Type**	**CLS Trait** [1]	**Number of trees**
CRC [2]	‘Montmorency’	none	Sour	Susceptible	8
CRC	‘North Star’	unknown	Sour	Tolerant	1
CRC	27-27-44	‘North Star’ & *P. avium*	Sour	Tolerant	6
CRC	26eo-17-29	‘North Star’ & *P. avium*	Sour	Tolerant	2
CRC	‘Csengodi’	*P. avium*	Tetraploid sweet cherry	Tolerant	2
CRC	26eo-11-27	‘Csengodi’	Sour	Tolerant	2
CRC	Almaz R1(1)	*P. maackii*	Sour	Tolerant	1
CRC	Almaz R2(1) [3]	*P. maackii*	Sour	Tolerant	1
CRC	‘Krupnoplodnaya’	*P. avium*	Sweet	Tolerant	2
CRC	23-23-13	*P. canescens*	Sour	Resistant	2
CRC	24-32-37	*P. canescens*	Sour	Resistant	1
CRC	24-32-41	*P. canescens*	Sour	Resistant	1
CRC	24-32-43	*P. canescens*	Sour	Resistant	1
CRC	26e-11-10	*P. canescens*	Sour	Resistant	1
PPRC [4]	‘Montmorency’	none	Sour	Susceptible	6
PPRC	26eo-17-29	‘North Star’ & *P. avium*	Sour	Tolerant	6, 4 [5]

[1] Refer to Apostol (2000); Apostol & Iezzoni (1992); Downey (1999); Schuster (2004); Sjulin et al. (1989); Stegmeir et al. (2014); Wharton et al. (2003)

[2] CRC = Clarksville Research Center, Clarksville MI

[3] Almaz R2(1) is a half-sibling of Almaz R1(1). It is not a breeding parent, and therefore was not included in initial evaluations in 2015. This tree was later included because it proved more tolerant to CLS than Almaz R1(1); as a result, it was evaluated only 7, rather than 18 times, in 2015.

[4] PPRC = Plant Pathology Research Center, East Lansing MI

[5] At the PPRC, evaluation included six trees of 26eo-17-29 in 2015 and four trees in 2016.

To provide a replication of the tolerant response, CLS disease progression was evaluated in 2015 and 2016 on trees of two clones, ‘Montmorency’ and the ‘North Star’-derived tolerant clone 26eo-17-29. These trees were located at the MSU Plant Pathology Research Center (PPRC) in East Lansing, Michigan (Table 2.1). In 2015, a set of six trees were evaluated for each clone, while in 2016, a different set of trees consisting of six trees of ‘Montmorency’ and four trees of 26eo-17-29 were evaluated. No CLS fungicides were applied to these trees in their respective year of evaluation.

CLS Visual Phenotyping

To characterize the epidemiological traits of the fungus, the progression of CLS infection was monitored on 10 branches per tree, selected to represent different locations in the canopy (East/West; High/Middle/Low). In 2015, all trees were rated weekly (2015 season total of 18 ratings) with the exception of the six ‘Montmorency’ trees located at the PPRC which were rated three times per week to more precisely characterize the life cycle timing of *B. jaapii* (2015 season total of 41 ratings). In 2016, the 10 trees at the PPRC were evaluated two to three times per week (2016 season total of 33 ratings), while those at the CRC were evaluated approximately twice per month (2016 season total of 10 ratings, results in Appendix B). Data were collected solely from leaves on the current season’s growth. For each branch, this included a leaf count (with a leaf being included in the total if it had unfolded to an angle greater than 90°), as well as the number of those leaves with CLS symptoms, and the approximate number of lesions present on each infected leaf.

These data were used to calculate three measures of disease severity: incidence, lesion density, and defoliation. Incidence was calculated as the percent of leaves infected (where infection was defined as the presence of one or more lesions) out of the total leaf number for each branch. Lesion Density was calculated as the number of lesions per cm^2 of leaf area. Lesions were counted on all infected leaves of each of the 10 branches per tree. To obtain an estimate of leaf area to be used in the lesion density calculation, more than 100 leaves were randomly selected from unmonitored branches over the course of the season, and leaf area was determined using the Assess 2.0 Image Analysis Software for Plant Disease Quantification© (Lamari, 2008), permitting the value to be converted from average number of lesions per leaf to average number of lesions per cm^2 of leaf area. Defoliation was calculated as the percent of leaves lost out of the total leaf number. As the plant materials evaluated had different leaf numbers on each branch, the date when the average leaf number reached a maximum was considered to be the date with 0% defoliation for that tree.

CLS Digital Phenotyping

In addition to conducting visual ratings of selected branches, 10 leaves were collected from each of the trees every week from a canopy location near that of the branches being monitored. These leaves were digitally scanned and analyzed using the Assess 2.0 Image Analysis Software for Plant Disease Quantification©. Using this software, the 'percent area infected' of each individual leaf was obtained. Additionally, multiple photographs of each tree were taken weekly in order to capture an overall visual representation of the disease progression.

Environmental Data

At the conclusion of each season, weather data were obtained from the Michigan State University Enviro-weather Automated Weather Station Network (Michigan State University

Board of Trustees, 2011, mawn.geo.msu.edu) for the Clarksville and East Lansing sites, to aid in the interpretation of the CLS progression data.

Statistical Analyses

For each year, the data were grouped into observations made during one- or three-week intervals depending on rating frequency, thereby providing replicated observations for clones where only one tree was available for evaluation. Analyses comparing clones, locations and years were done using ANOVA with a Bonferroni adjustment in R (R Core Team, 2015). A Bonferroni adjustment was used as a multiple-comparison correction, providing a more conservative evaluation of significance and accounting for the comparison of all of the evaluated clones in the same analysis, as well as variability in the number of replicate trees.

Results

Progression of CLS on susceptible 'Montmorency' trees

In 2015, trees of the CLS susceptible 'Montmorency' located at the CRC were severely impacted by the high disease pressure that resulted from ample rainfall and no CLS fungicide applications (Figure 2.1A, Figure 2.2). Percent incidence increased dramatically in the spring, from about 9% on May 28 to 60% on June 11. Within a month (July 8), all 'Montmorency' leaves evaluated were infected with CLS to varying degrees. The lesion densities on these infected leaves were less than 1 lesion per cm^2 until mid-June when lesion densities exceeded 10 lesions per cm^2; the leaves that remained on the trees the longest ultimately reached a maximum of 20 lesions per cm^2 of leaf area. This increase in lesion density coincided with the start of defoliation, which began on June 17 and proceeded rapidly, reaching 60% on July 1, 90% on

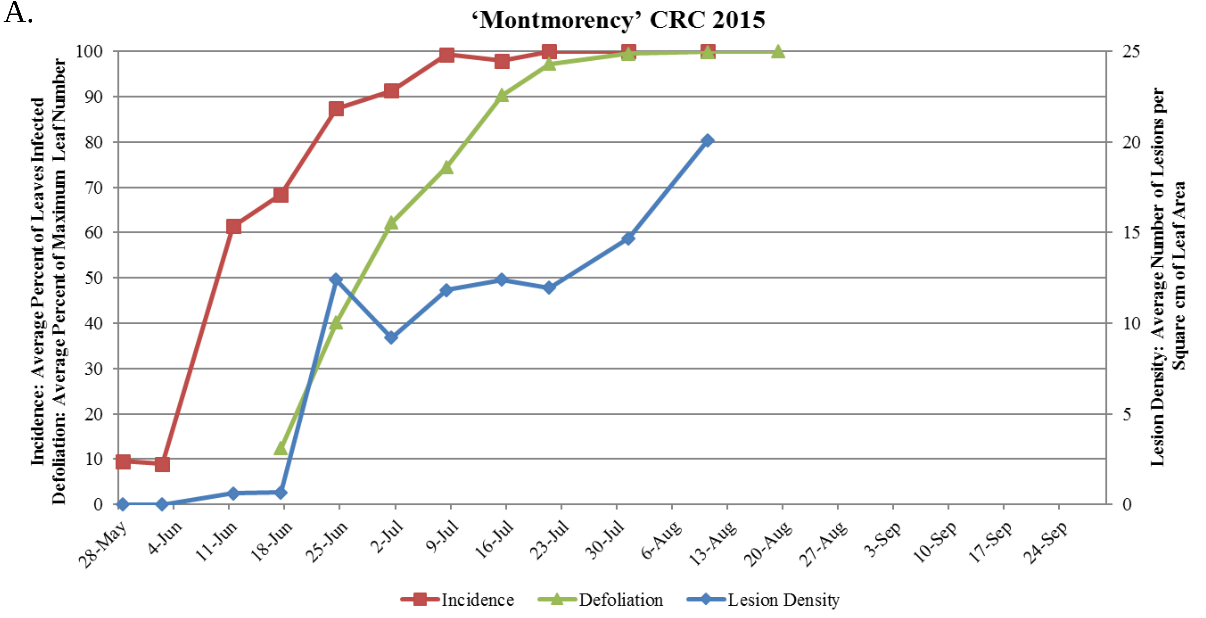
July 15, and 100% by August 19. After the ‘Montmorency’ trees were completely defoliated, they produced new leaves (refoliation) (Figure 2.3, see August 27 photograph). These leaves were not evaluated for CLS progression; however, it was observed that they were quickly infected with CLS and most defoliated within three weeks.

In 2015, the ‘Montmorency’ trees located at the PPRC also exhibited rapid CLS progression, although following a different time course than those at the CRC (Figure 2.1B). Incidence was 9% on June 11, 60% on June 26, and 100% on August 10. Lesion density was below or approaching 2 lesions per cm^2 for the majority of the season, then increased exponentially from the end of August to the end of September, with the leaves retained longest ultimately reaching a maximum average of 14.5 lesions per cm^2. Defoliation began on June 25, reached 60% on July 22, 90% on September 1, and 100% on September 22.

In 2016, dry weather delayed disease progression until later in the season than was observed in 2015 (Figure 2.2, Figure 2.4A). Percent incidence was 10% on June 30, 60% on July 30, and 100% on August 20. Lesion density remained below 1.5 lesions per cm^2 until mid-August, and the leaves retained the longest reached a maximum of 11.8 lesions per cm^2 on September 16. Defoliation began on June 28, reached 60% on August 26, and 100% on September 18.

Figure 2.1: Progression of CLS infection on ‘Montmorency’ in 2015 for the three disease severity parameters: incidence, defoliation, and lesion density; (A) ‘Montmorency’ trees located at the CRC, (B) ‘Montmorency’ trees located at the PPRC. Statistical analysis is presented in Table 2.5.

A.



B.

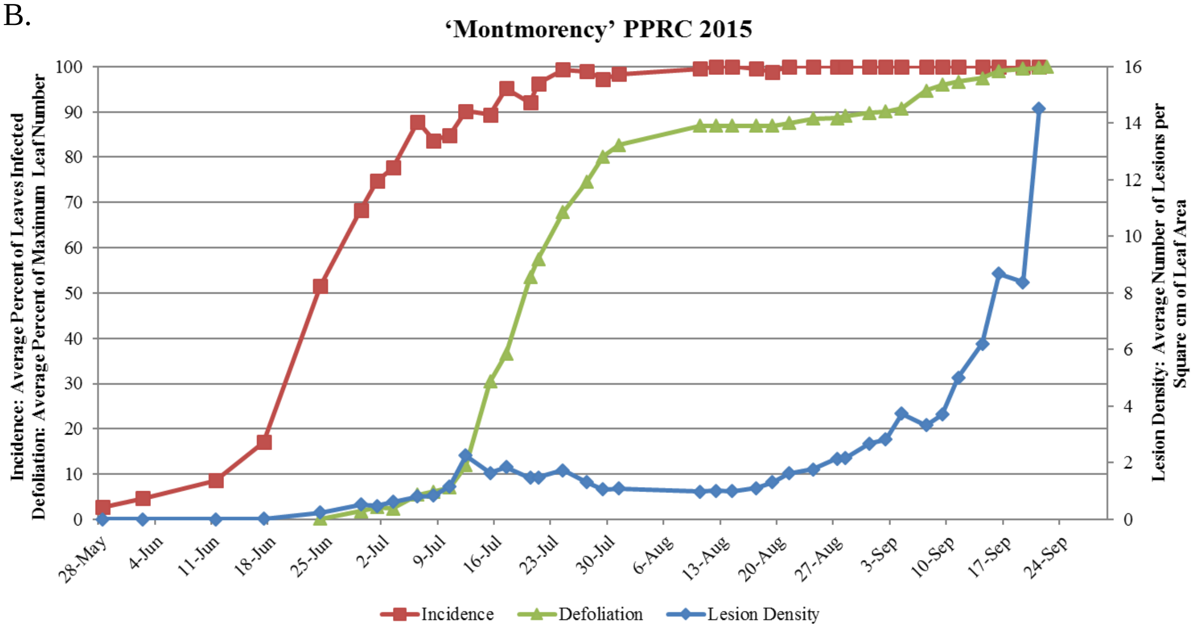
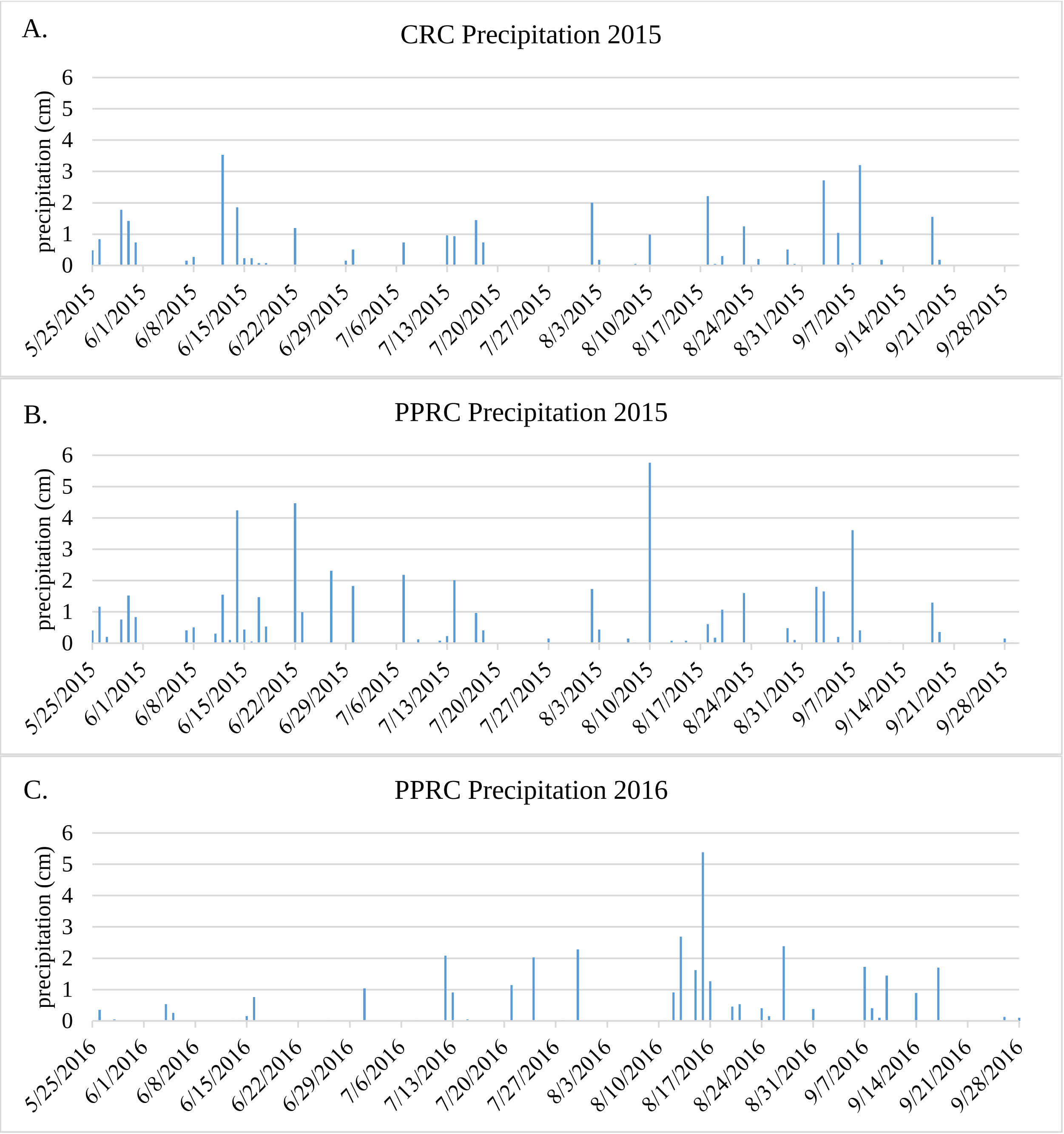


Figure 2.2: Precipitation (cm) experienced during the 2015 season at the CRC[1] (A) and the PPRC (B) and during the 2016 season at the PPRC (C). Data were obtained from the Michigan State University Enviro-weather Automated Weather Station Network (Michigan State University Board of Trustees, 2011).



[1] Due to equipment failures, the CRC precipitation data from 6/29/15 to 7/17/15 is that which was recorded at the weather station located in Belding, MI approximately 20 miles (32 km) North of the Clarksville Research Center.

Figure 2.3: Time series representation of disease severity in 2015 on a set of representative cherry clones growing at the CRC.

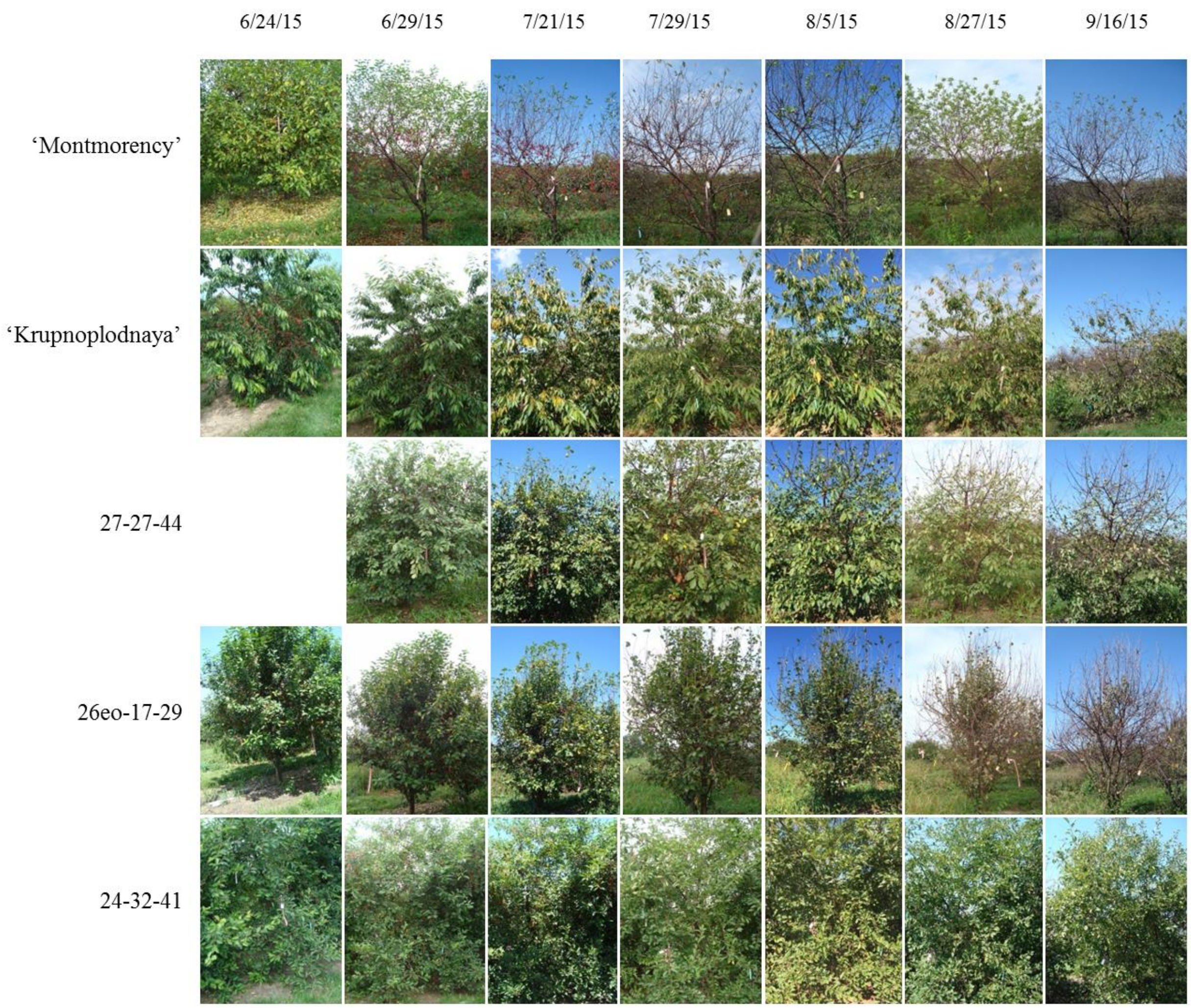
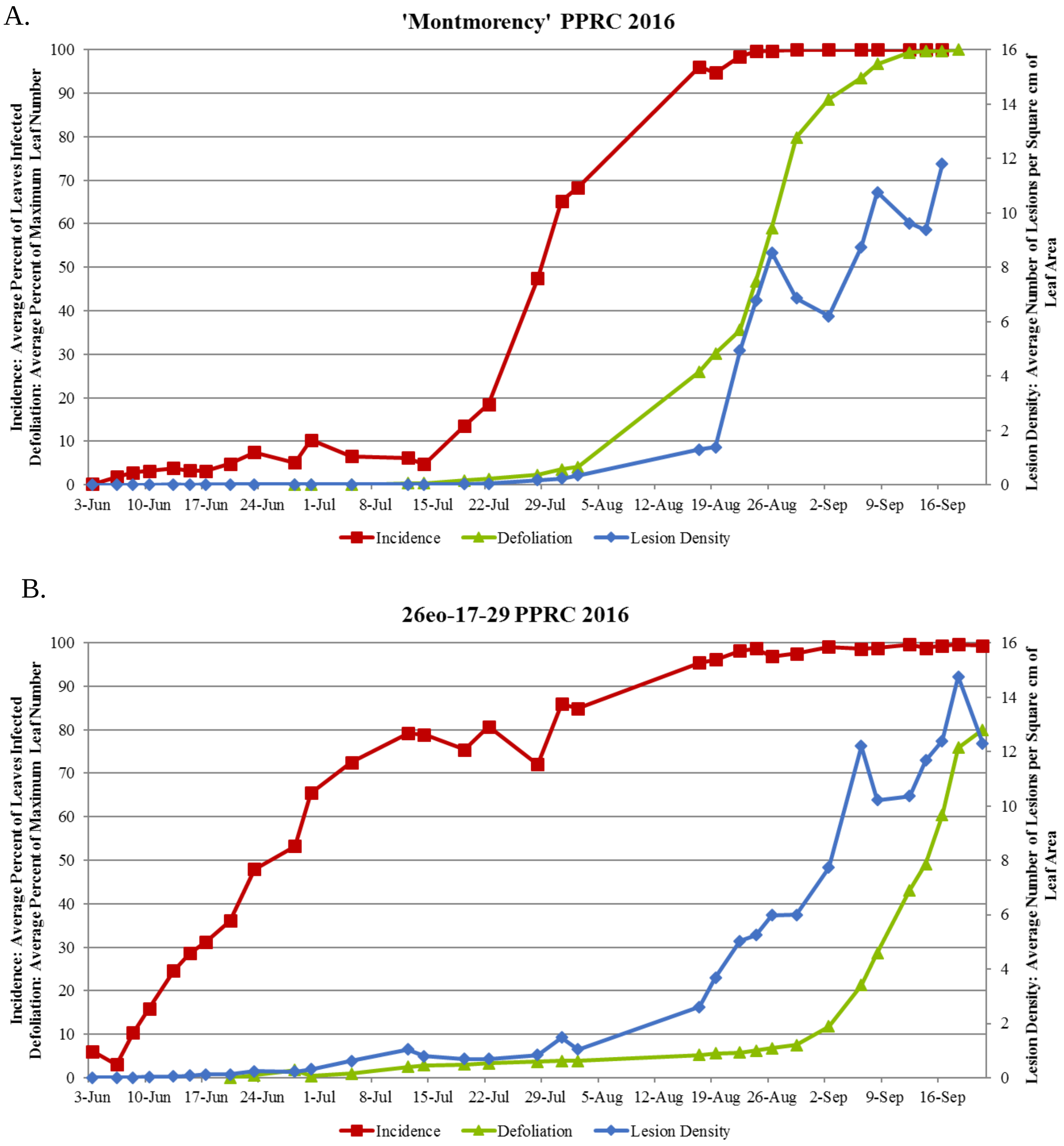


Figure 2.4: Progression of CLS infection in 2016 on clones of ‘Montmorency’ and 26eo-17-29 located at the PPRC for the three disease severity parameters: incidence, defoliation, and lesion density; (A) ‘Montmorency’ trees located at the PPRC, (B) 26eo-17-29 trees located at the PPRC. Statistical analysis is presented in Table 2.6.



Progression of CLS on P. maackii, 'North Star', and sweet cherry derived clones

The clones that were previously reported to be tolerant all experienced a disease progression similar to 'Montmorency', although in some cases disease progression was delayed by one to two weeks (Figure 2.5A, B, C). Significant differences in the extent of CLS incidence on these clones were observed during the weeks from June 17 to July 3; however, no differences were observed for the remainder of the season (Table 2.2). 'Montmorency' reached the highest mean lesion density with 20 lesions per cm^2 on August 10, which was not significantly different from that of 'North Star' or of Almaz R1(1), which reached maximum lesion densities of 19.1 lesions per cm^2 and 14.5 lesions per cm^2, respectively (Figure 2.5B, Table 2.3). The other clones exhibited lesion densities near or below 6 lesions per cm^2 for the majority of the season, with some clones acquiring additional lesions in September (Figure 2.5B). The sweet cherry cultivar 'Krupnoplodnaya' had significantly lower lesion densities than 'Montmorency' throughout the period of evaluation (Table 2.3). With the exception of 'North Star', all tolerant clones exhibited significantly slower defoliation than 'Montmorency' over the first ten weeks of the season (Table 2.4). Among the tolerant clones, 'North Star' exhibited the most rapid defoliation, while 'Krupnoplodnaya' exhibited the slowest defoliation (Figure 2.5C). By the end of September, three of the tolerant clones had not reached 100% defoliation; 'Csengodi' and 27-27-44 reached 91% and 93% defoliation, respectively, and 'Krupnoplodnaya' reached only 70% defoliation at that time.

Figure 2.5: Progression of CLS infection on tolerant clones in 2015, compared with the susceptible response of ‘Montmorency’: (A) incidence, (B) lesion density, and (C) defoliation. Statistical analyses of these three disease descriptors are presented in Tables 2.2, 2.3, and 2.4, respectively.

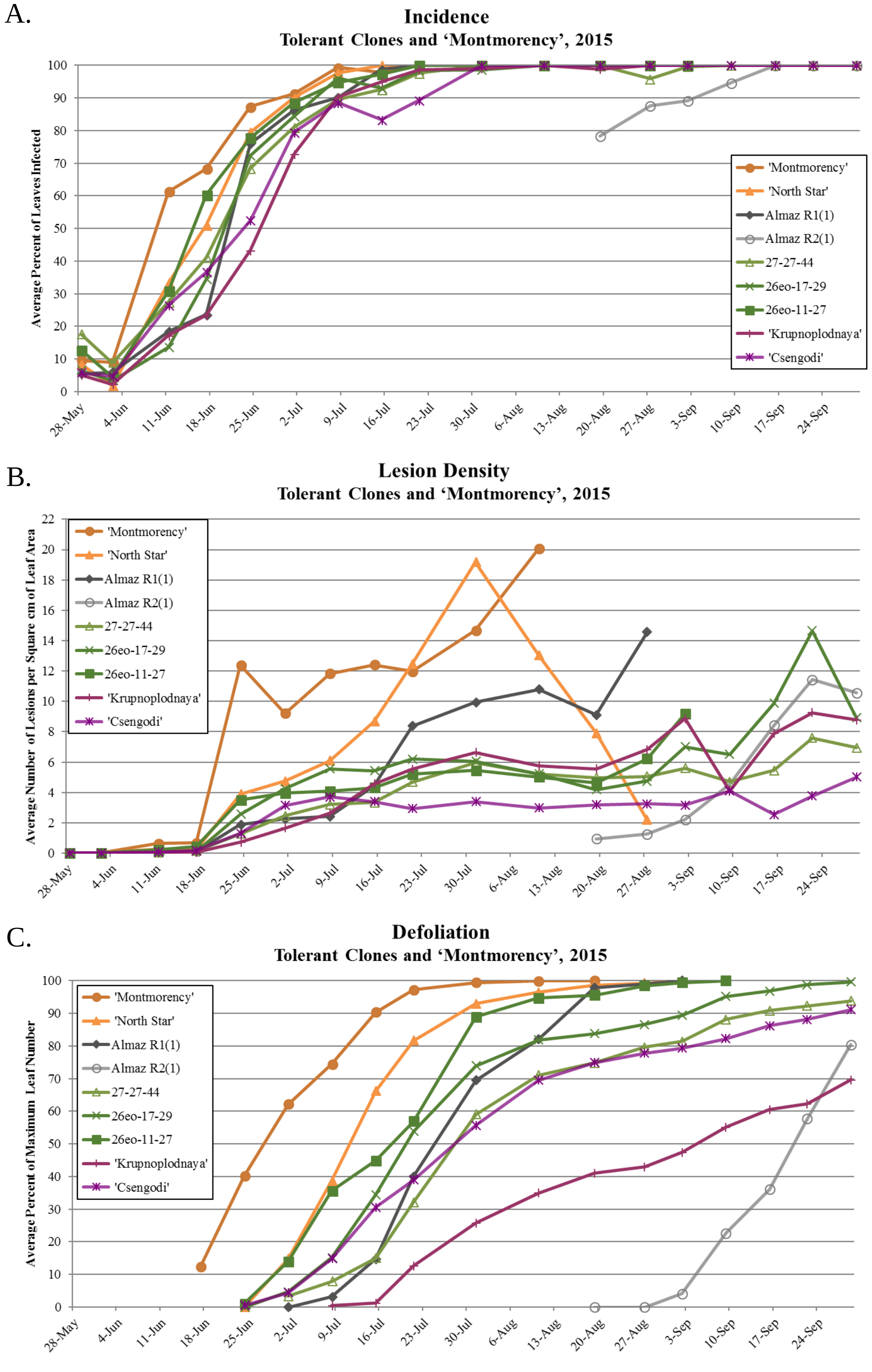


Table 2.2: Analysis of variance for percent incidence of CLS for all clones evaluated at the CRC in 2015 for their host response to the disease. Data averages for each clone were grouped into three week intervals prior to analysis.

	Weeks 1-3 5/28/15-6/11/15		**Weeks 4-6** 6/17/15-7/3/15		**Weeks 7-9** 7/6/15-7/24/15		**Weeks 10-12** 7/27/15-8/14/15		**Weeks 13-15** 8/17/15-9/4/15		**Weeks 16-18** 9/7/15-9/29/15	
‘Montmorency’	26.6	a[1]	82.4	a	99.1	a	100	a	-	-	-	-
26eo-17-29	7.9	ab	63.8	ab	95.9	a	99.4	a	100	a	100	a
‘North Star’	14.5	ab	73.6	ab	99.2	a	100	a	100	a	-	-
26eo-11-27	16.1	ab	75.6	ab	97.4	a	100	a	100	a	-	-
‘Csengodi’	12.3	ab	56.2	bc	87.1	a	100	a	100	a	100	a
27-27-44	18.0	ab	63.5	ab	93.3	a	100	a	98.5	a	100	a
‘Krupnoplodnaya’	8.2	ab	46.5	bcd	94.6	a	99.5	a	99.6	a	100	a
Almaz R1(1)	10.0	ab	62.0	abc	96.3	a	100	a	100	a	-	-
24-32-41	1.1	b	1.8	de	2.0	d	22.1	d	48.8	c	79.2	cd
24-32-43	2.2	b	2.5	de	8.0	d	44.4	c	64.7	b	91.0	ab
23-23-13	1.3	b	0.9	e	23.2	c	52.7	bc	70.9	b	84.7	bc
24-32-37	0.7	b	12.8	cde	50.8	b	92.3	a	95.7	a	100	a
26e-11-10	0.4	b	6.3	de	27.4	c	59.8	b	64.3	b	74.9	d

[1] Letters adjacent to values indicate statistical significance within columns at α=0.05.

Table 2.3: Analysis of variance for CLS lesion density (lesions/cm^2) for all clones evaluated at the CRC in 2015 for their host response to the disease. Data averages for each clone were grouped into three week intervals prior to analysis.

	Weeks 1-3 5/28/15-6/11/15		**Weeks 4-6** 6/17/15-7/3/15		**Weeks 7-9** 7/6/15-7/24/15		**Weeks 10-12** 7/27/15-8/14/15		**Weeks 13-15** 8/17/15-9/4/15		**Weeks 16-18** 9/7/15-9/29/15	
‘Montmorency’	0.2	a[1]	7.4	a	12.1	a	15.8	a	-	-	-	-
26eo-17-29	0.02	b	2.3	b	5.7	bc	5.7	bc	5.3	bc	11.4	a
‘North Star’	0.09	ab	2.9	ab	9.1	ab	16.1	a	5.1	bcd	-	-
26eo-11-27	0.08	ab	2.6	b	4.5	c	5.2	bc	6.2	bc	-	-
‘Csengodi’	0.03	ab	1.5	b	3.4	cd	3.2	cd	3.2	cde	3.9	bc
27-27-44	0.02	b	1.3	b	3.7	c	5.6	bc	5.2	bc	6.2	b
‘Krupnoplodnaya’	0.02	b	0.8	b	4.2	c	6.2	bc	7.1	b	7.5	ab
Almaz R1(1)	0.01	b	1.4	b	5.1	bc	10.4	ab	11.8	a	-	-
24-32-41	0.002	b	0.001	b	0.001	e	0.02	d	0.07	f	0.4	c
24-32-43	0.001	b	0.003	b	0.004	e	0.06	d	0.3	ef	2.3	bc
23-23-13	0.0008	b	0.0005	b	0.05	e	0.1	d	0.2	f	0.4	c
24-32-37	0.0005	b	0.02	b	0.3	de	0.5	d	0.8	def	4.1	bc
26e-11-10	0.0002	b	0.007	b	0.2	de	0.2	d	0.3	ef	0.9	c

[1] Letters adjacent to values indicate statistical significance within columns at α=0.05.

Table 2.4: Analysis of variance for percent defoliation caused by CLS for all clones evaluated at the CRC in 2015 for their host response to the disease. Data averages for each clone were grouped into three week intervals prior to analysis.

	Weeks 1-3 5/28/15-6/11/15		**Weeks 4-6** 6/17/15-7/3/15		**Weeks 7-9** 7/6/15-7/24/15		**Weeks 10-12** 7/27/15-8/14/15		**Weeks 13-15** 8/17/15-9/4/15		**Weeks 16-18** 9/7/15-9/29/15	
‘Montmorency’	-	-	30.4	a[1]	86.2	a	99.6	a	-	-	-	-
‘North Star’	-	-	7.4	ab	62.2	ab	94.7	ab	99.3	a	-	-
26eo-11-27	-	-	6.0	b	45.8	bc	91.8	ab	97.9	a	100	a
‘Csengodi’	-	-	2.0	b	28.1	bcde	62.6	c	77.4	b	86.9	a
27-27-44	-	-	6.9	b	18.3	cde	65.0	c	78.6	b	91.3	a
‘Krupnoplodnaya’	-	-	0.9	b	4.8	de	30.3	d	43.8	c	61.9	ab
26eo-17-29	-	-	2.3	b	34.5	bcd	77.9	bc	86.6	ab	97.6	a
Almaz R1(1)	-	-	0	b	19.3	bcde	75.8	bc	98.9	a	-	-
24-32-41	-	-	-	-	0	e	0.3	e	4.4	e	11.9	d
24-32-43	-	-	0	b	2.9	de	8.3	de	19.1	de	53.5	ab
23-23-13	-	-	-	-	1.4	e	5.4	e	14.3	e	21.9	cd
24-32-37	-	-	-	-	0	e	2.2	e	4.8	e	36.5	bcd
26e-11-10	-	-	-	-	3.0	de	22.9	de	36.1	cd	48.9	bc

[1] Letters adjacent to values indicate statistical significance within columns at $\alpha=0.05$.

In 2015, the trees of the tolerant clone 26eo-17-29 located at the PPRC exhibited a response comparable to the trees of the same clone located at the CRC, with the exception of rate of defoliation, which was significantly slower on the PPRC trees and never reached 100% (Figure 2.6, Table 2.5). In 2016, the trees of 26eo-17-29 located at the PPRC were infected significantly earlier in the season than the PPRC ‘Montmorency’ trees, as well as having higher lesion densities until mid-August (Figure 2.4B, Table 2.6). At this point in the season, the level of defoliation on the ‘Montmorency’ trees rapidly surpassed that of the tolerant 26eo-17-29 trees (Table 2.6). Between 2015 and 2016, significant differences in incidence and lesion density could be observed in both ‘Montmorency’ and 26eo-17-29 at various points in the season (Table 2.7). Alternatively, no significant differences in defoliation were observed between years for 26eo-17-29; however, differences were observed in the rate of defoliation of ‘Montmorency’.

Figure 2.6: Progression of defoliation due to CLS infection on the clones replicated by location, 'Montmorency' and '26eo-17-29', in 2015. Statistical analysis of CLS defoliation for these two clones at the two locations is presented in Table 2.5.

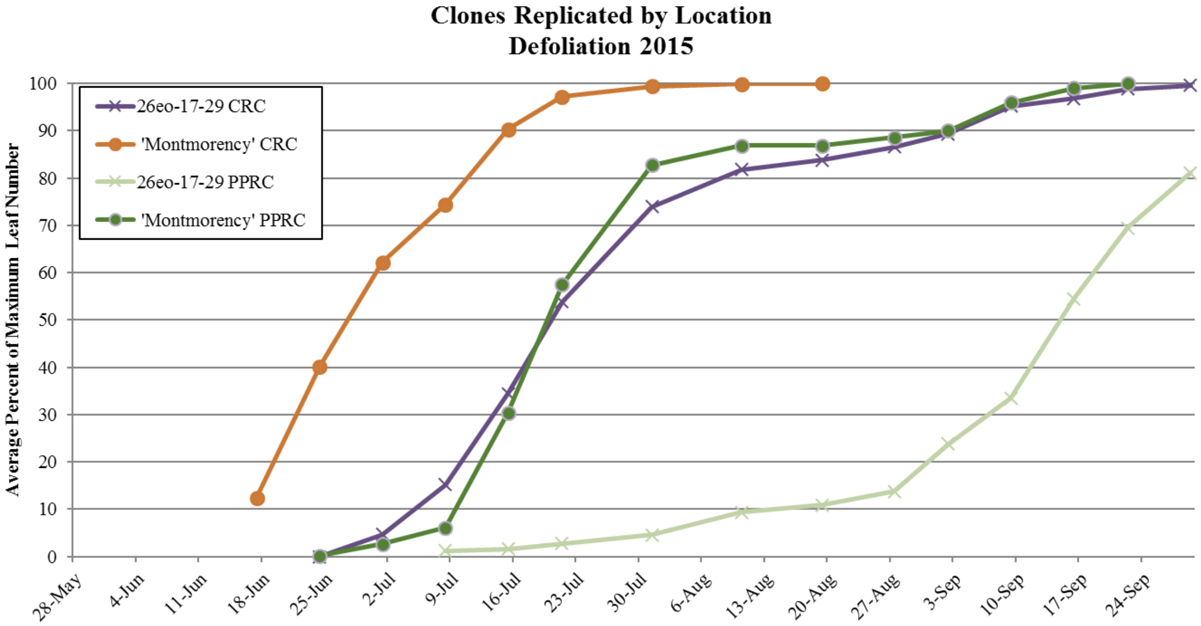


Table 2.5: Analysis of variance for 2015 data for three disease severity parameters, comparing the host responses of 'Montmorency' and 26eo-17-29 at two locations.

	Week 1 5/28		**Week 2** 6/2		**Week 3** 6/11		**Week 4** 6/17		**Week 5** 6/24		**Week 6** 6/29-7/3		**Week 7** 7/6-7/10		**Week 8** 7/12-7/17	
Incidence (%)																
'Montmorency' CRC	9.6	a[1]	9.0	a	61.4	a	68.4	a	87.4	a	91.3	a	99.3	a	98.0	a
26eo-17-29 CRC	6.5	ab	3.4	b	13.7	b	34.5	bc	72.4	b	84.3	ab	96.1	ab	93.0	ab
'Montmorency' PPRC	2.7	b	4.7	b	8.6	b	17.0	c	51.6	c	73.6	b	85.5	c	91.6	b
26eo-17-29 PPRC	2.1	b	3.2	b	15.1	b	40.1	b	62.7	b	86.3	a	90.4	b	93.2	ab
Lesion Density (lesions/cm^2)																
'Montmorency' CRC	0.007	a	0.007	a	0.6	a	0.7	a	12.4	a	9.2	a	11.8	a	12.4	a
26eo-17-29 CRC	0.004	a	0.01	a	0.03	b	0.1	b	2.6	b	4.3	b	5.5	b	5.4	b
'Montmorency' PPRC	0.001	a	0.004	a	0.01	b	0.02	b	0.2	b	0.5	c	0.9	c	1.9	c
26eo-17-29 PPRC	0.002	a	0.004	a	0.02	b	0.2	b	0.3	b	0.7	c	0.8	c	0.9	c
Defoliation (%)																
'Montmorency' CRC	-	-	-	-	-	-	-	-	33.9	a	57.5	a	71.7	a	89.8	a
26eo-17-29 CRC	-	-	-	-	-	-	-	-	0	b	4.7	b	15.2	b	34.4	b
'Montmorency' PPRC	-	-	-	-	-	-	-	-	0	b	2.4	b	6.2	b	26.3	b
26eo-17-29 PPRC	-	-	-	-	-	-	-	-	0	b	2.3	b	1.2	b	1.7	c

	Week 9 7/20-7/24		**Week 10** 7/27-7/31		**Week 11** 8/10-8/14		**Week 12** 8/17-8/21		**Week 13** 8/24-8/28		**Week 14** 8/31-9/4		**Week 15** 9/7-9/11		**Week 16** 9/14-9/19		**Week 17** 9/21-9/29	
Incidence (%)																		
'Montmorency' CRC	100	a	100	a	100	a	-	-	-	-	-	-	-	-	-	-	-	-
26eo-17-29 CRC	98.5	ab	98.7	a	100	a	100	a	100	a	100	a	100	a	100	a	100	a
'Montmorency' PPRC	96.0	ab	98.2	a	99.8	a	99.5	a	100	a	100	a	100	a	100	a	100	a
26eo-17-29 PPRC	93.6	b	96.4	a	98.6	ab	99.1	a	98.9	ab	99.8	a	99.6	ab	97.4	a	99.5	a
Lesion Density (lesions/cm^2)																		
'Montmorency' CRC	12.0	a	14.7	a	20.08	a	-	-	-	-	-	-	-	-	-	-	-	-
26eo-17-29 CRC	6.2	b	6.06	b	5.2	b	4.2	a	4.8	a	7.0	a	6.5	ab	9.9	a	15.7	a
'Montmorency' PPRC	1.6	b	1.2	b	1.0	d	1.3	c	2.0	b	3.1	b	4.0	b	7.6	a	14.5	ab
26eo-17-29 PPRC	1.1	b	2.0	b	3.6	c	3.08	b	5.3	a	5.6	a	7.1	a	8.4	a	11.7	b
Defoliation (%)																		
'Montmorency' CRC	97.1	a	99.5	a	99.8	a	-	-	-	-	-	-	-	-	-	-	-	-
26eo-17-29 CRC	53.8	b	73.9	b	81.8	b	83.8	a	86.6	a	89.4	a	95.2	a	96.8	a	99.2	a
'Montmorency' PPRC	59.6	b	79.2	b	86.9	b	87.1	a	88.7	a	90.2	a	95.8	a	98.6	a	99.8	a
26eo-17-29 PPRC	2.7	c	4.5	c	9.3	c	10.9	b	13.7	b	23.9	b	33.5	b	54.5	b	75.3	b

[1] Letters adjacent to values indicate statistical significance within columns for each of the three disease severity parameters at α=0.05.

Table 2.6: Analysis of variance for 2016 data for three disease severity parameters, comparing the host responses of ‘Montmorency’ and 26eo-17-29 at the PPRC.

	Week 1		**Week 2** 6/3		**Week 3** 6/6-6/10		**Week 4** 6/13-6/17		**Week 5** 6/20-6/23		**Week 6** 6/28-6/30		**Week 7** 7/5		**Week 8** 7/12-7/14	
Incidence (%)																
‘Montmorency’	-	-	0.2	b[1]	2.6	b	3.5	b	6.1	b	7.7	b	6.6	b	5.5	b
26eo-17-29	-	-	6.0	a	9.8	a	28.2	a	42.0	a	59.4	a	72.5	a	79.0	a
Lesion Density (lesions/cm^2)																
‘Montmorency’	-	-	0.00009	b	0.003	b	0.005	b	0.007	b	0.01	b	0.007	b	0.9	a
26eo-17-29	-	-	0.004	a	0.02	a	0.09	a	0.2	a	0.3	a	0.6	a	0.009	b
Defoliation (%)																
‘Montmorency’	-	-	-	-	-	-	-	-	0	a	0.1	b	0.3	b	1.6	b
26eo-17-29	-	-	-	-	-	-	-	-	1.7	a	3.0	a	3.5	a	6.4	a

	Week 9 7/19-7/22		**Week 10** 7/28		**Week 11** 7/31-8/2		**Week 12** 8/18-8/19		**Week 13** 8/22-8/26		**Week 14** 8/29-9/2		**Week 15** 9/6-9/8		**Week 16** 9/12-9/16		**Week 17** 9/18-9/21	
Incidence (%)																		
‘Montmorency’	16.0	b	47.5	b	66.8	b	95.4	a	99.3	a	100	a	100	a	100	a	-	-
26eo-17-29	78.1	a	72.0	a	85.4	a	98.4	a	97.9	b	98.3	a	98.7	b	99.2	a	99.5	-
Lesion Density (lesions/cm^2)																		
‘Montmorency’	0.7	a	0.8	a	0.3	b	0.3	b	6.9	a	6.6	a	9.8	a	10.4	a	-	-
26eo-17-29	0.03	b	0.2	b	1.3	a	1.3	a	5.4	a	6.9	a	11.2	a	11.5	a	13.5	-
Defoliation (%)																		
‘Montmorency’	1.5	b	2.5	a	4.0	a	27.8	a	46.9	a	84.1	a	95.1	a	99.6	a	100	a
26eo-17-29	5.6	a	6.2	a	6.3	a	9.3	b	9.0	b	12.5	b	27.4	b	51.3	b	77.1	b

[1] Letters adjacent to values indicate statistical significance within columns for each of the three disease severity parameters at $\alpha=0.05$.

Table 2.7: Analysis of variance for three disease severity parameters, comparing the host responses of 'Montmorency' and 26eo-17-29 located at the PPRC over the 2015 and 2016 seasons.

	Week 1		**Week 2**		**Week 3**		**Week 4**		**Week 5**		**Week 6**		**Week 7**		**Week 8**	
Incidence (%)																
'Montmorency' 2015	2.7	a[1]	4.7	a	8.6	a	17.0	c	51.6	ab	73.6	b	85.5	a	91.6	a
26eo-17-29 2015	2.1	a	3.2	ab	15.1	a	40.1	a	62.7	a	86.3	a	90.4	a	93.2	a
'Montmorency' 2016	-	-	0.2	b	2.6	b	3.5	d	6.1	c	7.7	d	6.6	c	5.5	c
26eo-17-29 2016	-	-	6.0	a	9.8	a	28.2	b	42.0	b	59.4	c	72.5	b	79.0	b
Lesion Density (lesions/cm^2)																
'Montmorency' 2015	0.001	a	0.004	a	0.01	ab	0.02	c	0.2	a	0.5	b	0.9	a	1.9	a
26eo-17-29 2015	0.002	a	0.004	a	0.02	a	0.2	a	0.3	a	0.7	a	0.8	a	0.9	b
'Montmorency' 2016	-	-	0.00009	a	0.003	b	0.005	c	0.007	b	0.01	d	0.007	b	0.009	c
26eo-17-29 2016	-	-	0.004	a	0.02	a	0.09	b	0.2	a	0.3	c	0.6	a	0.9	b
Defoliation (%)																
'Montmorency' 2015	-	-	-	-	-	-	-	-	0	a	2.4	a	6.2	a	26.3	a
26eo-17-29 2015	-	-	-	-	-	-	-	-	0	a	2.3	ab	1.2	b	1.7	b
'Montmorency' 2016	-	-	-	-	-	-	-	-	0	a	0.07	b	0.3	b	1.6	b
26eo-17-29 2016	-	-	-	-	-	-	-	-	1.7	a	3.0	a	3.5	ab	6.4	b

	Week 9		**Week 10**		**Week 11**		**Week 12**		**Week 13**		**Week 14**		**Week 15**		**Week 16**		**Week 17**	
Incidence (%)																		
'Montmorency' 2015	95.9	a	98.2	a	99.8	a	99.5	a	100	a	100	a	100	a	100	a	100	a
26eo-17-29 2015	93.6	a	96.4	a	98.6	a	99.1	a	98.9	ab	99.8	a	99.6	ab	97.4	a	99.5	a
'Montmorency' 2016	16.0	c	47.5	c	66.8	c	95.4	a	99.3	a	100	a	100	a	100	a	-	-
26eo-17-29 2016	78.1	b	72.0	b	85.4	b	98.4	a	97.9	b	98.3	a	98.7	b	99.2	a	99.5	a
Lesion Density (lesions/cm^2)																		
'Montmorency' 2015	1.6	a	1.2	b	1.0	b	1.3	b	2.0	b	3.1	b	4.0	c	7.6	b	14.5	a
26eo-17-29 2015	1.1	b	2.0	a	3.6	a	3.1	a	5.3	a	5.6	a	7.1	b	8.4	ab	11.7	a
'Montmorency' 2016	0.03	d	0.2	c	0.3	c	1.4	b	6.9	a	6.6	a	9.8	ab	10.4	ab	-	-
26eo-17-29 2016	0.7	c	0.8	b	1.3	b	3.2	a	5.4	a	6.9	a	11.2	a	11.5	a	13.5	a
Defoliation (%)																		
'Montmorency' 2015	59.6	a	79.2	a	86.9	a	87.1	a	88.7	a	90.2	a	95.8	a	98.6	a	99.8	a
26eo-17-29 2015	2.7	b	4.5	b	9.3	b	10.9	c	13.7	c	23.9	b	33.5	b	54.5	b	75.3	b
'Montmorency' 2016	1.5	b	2.5	b	4.0	b	27.8	b	46.9	b	84.1	a	95.1	a	99.6	a	100	a
26eo-17-29 2016	5.6	b	6.2	b	6.3	b	9.3	c	9.0	c	12.5	b	27.4	b	51.3	b	77.1	b

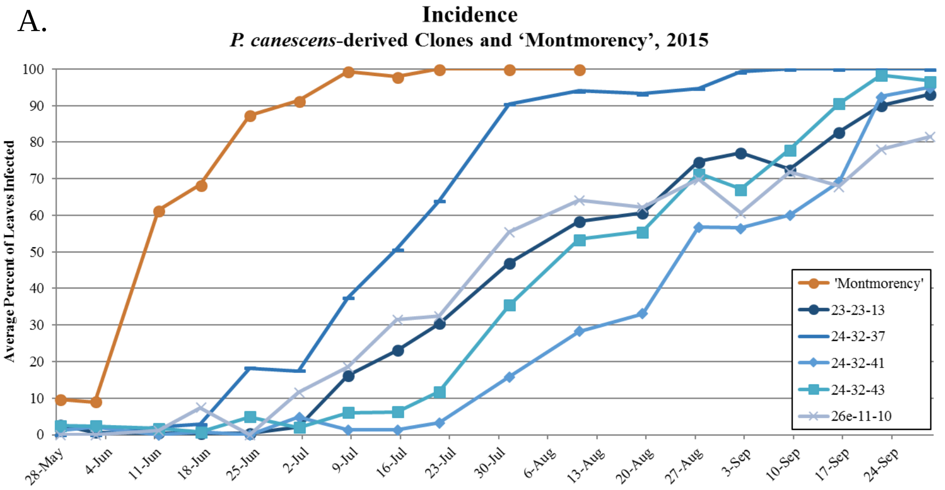
[1] Letters adjacent to values indicate statistical significance within columns for each of the three disease severity parameters at α=0.05.

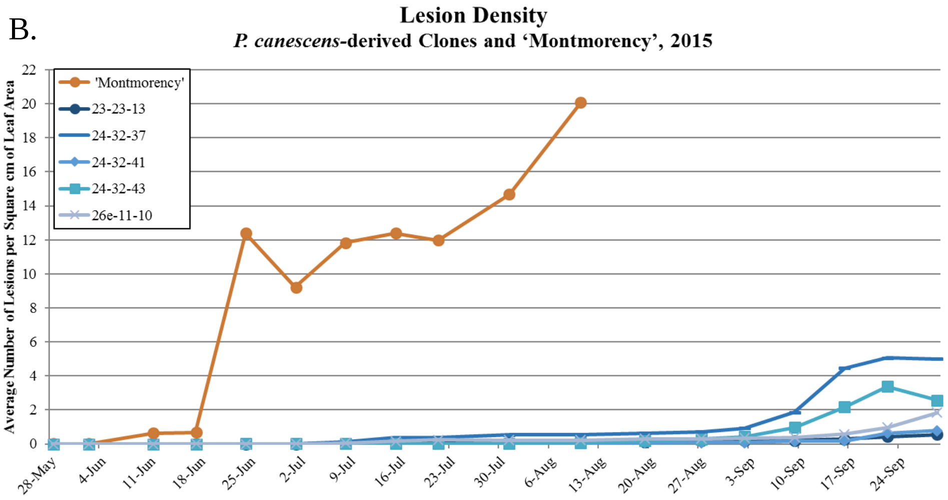
Progression of CLS on P. canescens derived clones

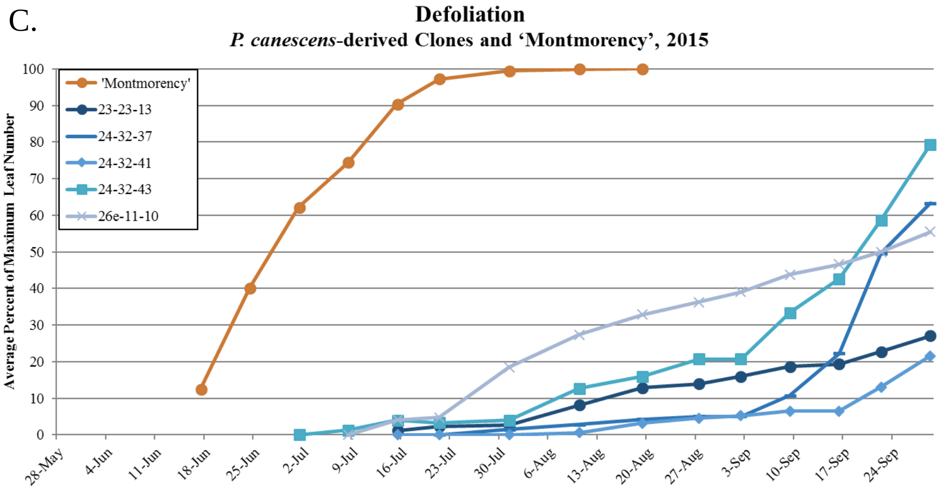
In 2015, all of the *P. canescens* derived clones previously reported to be resistant exhibited a high level of CLS resistance for the majority of the season. The clones monitored were 23-23-13 and four of its progeny (26e-11-10, 24-32-37, 24-32-41, and 24-32-43), therefore representing three and four generations of derivation from the ancestral resistance source, *P. canescens*. For all measures of CLS severity, 24-32-41 consistently exhibited the best resistance response, while all others had variable rankings; however, all resistant clones were significantly less susceptible than ‘Montmorency’ and nearly all of the tolerant clones for each of the disease severity parameters (Tables 2.2, 2.3, 2.4).

Infection of these previously reported CLS resistant clones occurred later in the season than that of ‘Montmorency’ (Figure 2.7A, Table 2.2). Of the resistant clones, 24-32-37 had the most rapid infection rate, reaching 90% incidence on July 31, while the clone with the slowest infection rate, 24-32-41, had only reached 16% on this date. 24-32-37 was also the only previously reported resistant clone to reach 100% infection at the end of September; however, this was not statistically different from that of 24-32-43 which ultimately reached an incidence of 98% (Table 2.2). The resistant clones maintained a significantly lower lesion density than all other clones for most of the season (Figure 2.7B, Table 2.3). All five resistant clones had fewer than one lesion per cm^2 until September 3 when 24-32-37 and 24-32-43 exhibited higher lesion densities. A similar time course was observed for defoliation, with all resistant clones exhibiting very little defoliation until later in the season (Figure 2.7C). 24-32-41 exhibited significantly less defoliation than 24-32-37 at the end of the season, reaching 22% on September 29 compared to the 80% defoliation reached by 24-32-37 on the same date (Table 2.4).

Figure 2.7: Progression of CLS infection on resistant *P. canescens* derived clones in 2015, compared with the susceptible response of 'Montmorency'; (A) incidence, (B) lesion density, (C) defoliation. Statistical analyses of these three disease descriptors are presented in Tables 2.2, 2.3 and 2.4, respectively.







Effects of Branch Location

The 2015 data associated with each of the six branch locations within the canopy (East/West, High/Middle/Low) were averaged among the ‘Montmorency’ trees located at the PPRC for each of the three disease severity parameters. While some statistical differences were identified for each of the parameters at certain time points, the results do not suggest differences in the progression of CLS incidence and lesion density within the tree canopy (Table 2.8).

Table 2.8: Analysis of variance for the 2015 PPRC ‘Montmorency’ data comparing the six branch locations across the three disease severity parameters.

	Weeks 1-3 5/28/15-6/11/15		**Weeks 4-6** 6/17/15- 7/3/15		**Weeks 7-9** 7/6/15- 7/24/15		**Weeks 10-12** 7/27/15- 8/14/15		**Weeks 13-15** 8/17/15- 9/4/15		**Weeks 16-18** 9/7/15- 9/29/15	
Incidence (%)												
West/High	8.0	a[1]	71.5	a	96.8	a	100	a	100	a	100	a
West/Middle	7.1	a	66.8	a	94.0	ab	100	a	100	a	100	a
West/Low	9.1	a	64.2	a	86.1	b	100	a	100	a	100	a
East/High	2.6	a	48.7	a	90.7	ab	98.9	ab	99.5	a	100	a
East/Middle	0.5	a	47.2	a	89.2	ab	98.0	ab	100	a	100	a
East/Low	4.0	a	51.1	a	87.8	b	95.9	b	99.0	a	100	a
Lesion Density (lesions/cm^2)												
West/High	0.02	a	0.7	a	1.8	a	1.6	a	3.2	a	6.0	a
West/Middle	0.02	a	0.5	ab	1.8	a	1.5	a	2.4	ab	10.5	a
West/Low	0.005	a	0.4	ab	1.1	a	1.1	b	2.4	ab	6.4	a
East/High	0.002	a	0.2	ab	1.4	a	0.8	b	1.4	b	4.2	a
East/Middle	0.0007	a	0.2	b	1.2	a	0.7	b	1.5	b	7.5	a
East/Low	0.002	a	0.2	ab	1.2	a	1.1	b	2.7	ab	5.7	a
Defoliation (%)												
West/High	-	-	5.7	a	45.7	a	91.4	a	96.6	a	98.6	a
West/Middle	-	-	2.4	a	37.5	a	92.2	a	95.9	a	99.1	a
West/Low	-	-	1.4	a	27.0	a	79.4	bc	86.5	c	97.1	ab
East/High	-	-	0	a	28.3	a	85.8	ab	90.9	b	98.1	a
East/Middle	-	-	0	a	22.5	a	71.1	c	76.3	e	90.6	ab
East/Low	-	-	0	a	19.2	a	73.0	c	80.0	d	95.4	ab

[1] Letters adjacent to values indicate statistical significance within columns for each of the three disease severity parameters at $\alpha=0.05$.

Assess 2.0 Image Analysis Software for Plant Disease Quantification©

The Assess 2.0 Software was used to evaluate the percent of leaf area infected for the leaves collected weekly in 2015. The software-derived data and the visual field counts were similar in terms of the final rankings of the clones and the general trajectories of the seasonal trends; however, higher variability was seen in the software-derived data than in the field-collected data for all clones and locations over the entirety of the season.

Discussion

The results from this study were consistent with previous observations of germplasm tolerance and resistance to CLS while further detailing the specific disease progression profiles and host response patterns in each of the studied cherry clones. As anticipated, ‘Montmorency’ was highly susceptible to CLS and, when unsprayed, lost nearly all of its leaves prior to fruit harvest. This rapid disease progression demonstrates the need for frequent fungicide applications in the commercial production of ‘Montmorency’ sour cherries, especially when weather conditions are favorable for disease occurrence. Infected trees receiving no fungicide treatment yield fewer and poorer quality fruits, are limited by the reduced amount of carbohydrates stored for winter survival, and experience altered acclimation to cold temperatures in the fall and breaking of dormancy in the spring (Howell & Stackhouse, 1973; Keitt et al., 1937). It is also important to note that those trees which were defoliated early in the season produced new leaves (refoliation). In sour cherry and other tree species, refoliation occurs at the expense of stored carbohydrates thereby negatively impacting tree physiology (Howell & Stackhouse, 1973; Wargo, 1972; Wargo, Parker, & Houston, 1972). When these new leaves are rapidly lost to disease, as observed in this study, this refoliation response is likely more harmful than beneficial to the tree. As testament to the detrimental effect of CLS on ‘Montmorency’ orchard longevity, the ‘Montmorency’ trees monitored in 2015 were observed to be of poor general health in the following 2016 season, having sparse leaves on old growth, a thin canopy, and substantially reduced fruit yield compared with the typical performance of healthy ‘Montmorency’ trees. This study clearly illustrates the limitations of the commercially-favored cultivar ‘Montmorency’ and the impossibility of producing quality sour cherries of this cultivar, in humid climates with frequent in-season rain events, without substantial use of fungicides for disease management.

The clones with putative tolerance to CLS were also observed as such in this study, exhibiting host responses that were less severe than ‘Montmorency’. The tolerant clones monitored in this study included the *P. maackii* derived Almaz R1(1) and Almaz R1(2), and the sweet cherry ‘Krupnoplodnaya’, tetraploid sweet cherry ‘Csengodi’, sour cherry ‘North Star’, and three sour cherry clones with recent sweet cherry ancestry. By quantitatively evaluating these tolerant clones, small differences were identified in the host responses to CLS that had not been previously recorded. In past studies (Sjulin et al., 1989), sweet cherry was documented as being tolerant to CLS, having smaller lesions, longer latent periods, and reduced sporulation compared with sour cherry. Therefore, it was expected that ‘Krupnoplodnaya’ and ‘Csengodi’ would exhibit high tolerance in this study, and that the sour cherry clones with recent sweet cherry ancestry would exhibit tolerance similar to that of sweet cherry. This hypothesis was confirmed with ‘Krupnoplodnaya’ being the most tolerant to CLS, followed by ‘Csengodi’ and the three sour cherry clones with sweet cherry ancestry. The sour cherry cultivar ‘North Star’ had the poorest response to CLS among the tolerant clones, which, under this hypothesis, was due to its lack of recent sweet cherry ancestry. The two *P. maackii* clones, Almaz R1(1) and Almaz R2(1), exhibited notably different responses to CLS, with the Almaz R1(1) host response closely resembling that of the other tolerant clones while the response of Almaz R2(1) was delayed by about 2 months. Because these clones are half-siblings, it is hypothesized that the genetics of the paternal parents are involved in these differences. Because Almaz R2(1) has not been evaluated in past studies, it’s response will need to be confirmed in additional seasons; however, *P. maackii* remains a potential genetic source for CLS tolerance. A sour cherry cultivar with the delayed disease severity that is characteristic of tolerance has the potential to reduce, but not eliminate, the use and expense of fungicide applications in sour cherry production. Ideally, this tolerance will be combined with *P. canescens* derived resistance with the goal of breeding a more durably resistant cultivar.

In the study conducted by Wharton et al. (2003), the host response of the *P. canescens* derived individual ‘GI 148-1’ to CLS infection was detailed as a hypersensitive response where biotrophic proliferation of the fungus following initial infection is prevented by host defense compounds which lead to pigmentation and abscission of the leaf cells surrounding the lesion. These findings were also supported by a more recent study (Stegmeir et al., 2014) which investigated the host response of 23-23-13, a progeny of ‘GI 148-1’. Because the *P. canescens* derived clones in this study included 23-23-13 and four of its progeny, the same level of resistance was expected. All five of the clones exhibited expected levels of resistance throughout most of the season; however, three clones deviated from the anticipated response at the season conclusion. These clones showed a sudden occurrence of secondary cycles of infection and rapid defoliation in early September, compared to the others which maintained their previous hypersensitive response and low infection levels.

Because these trees had been left unsprayed for several consecutive years preceding this study it was uncertain whether this loss of resistance was due to the evolution of the local *B.*

jaapii strain(s), leading to gain of virulence against the *P. canescens* clones, or whether previous evaluations of the resistant host response phenotype were incorrect. In order to determine the cause of this unexpected host response, a greenhouse study was conducted where grafted clones of the three trees of concern were inoculated with the common strain of *B. jaapii* or an isolate recovered from the infected trees at the CRC to evaluate their virulence. In this study, all of the resistant clones inoculated with the common strain of *B. jaapii* exhibited the expected hypersensitive host response; however, when inoculated with the CRC *B. jaapii* isolate, successful pathogen establishment was observed on two of the tested clones (T. J. Proffer, personal communication), suggesting that evolution of the CRC population of *B. jaapii* had occurred. These two clones were 24-32-37 and 24-32-43, those that exhibited the greatest deviation from the expected resistant response in the orchard evaluation. These results are consistent with the unexpected host response observed in the 2015 season and provide direction to future investigations.

Data collected in this study allowed the variable rates of CLS progression among clones to be illustrated with great detail. The adjustment of the lesion density data to account for variation in leaf size provided a consistent disease representation across the clones and permitted comparisons of the extent of infection. While Sjulin et al. (1989) used artificial inoculation and detached leaf assays to make similar comparisons of disease establishment, they did not include progeny individuals of ‘North Star’ or any individuals with *P. canescens* ancestry. The combined evaluation of lesion density and defoliation in this study revealed a possible connection between tolerance and the number of lesions a leaf can withstand before dropping, which additionally supports correlations noted by Sjulin et al. (1989) between the severity of defoliation in the field and lesion characteristics observed in detached leaf assays.

Monitoring selected branches for incidence of CLS provided important information not only regarding rate differences among clones but also of disease progression throughout the tree canopy. In commercial orchards, the first signs of infection are often seen at the top of the canopy, where fungicide coverage may be poor. This observation encouraged investigation into the advancement of CLS throughout unsprayed trees. By monitoring branches at specific locations within the canopy, this study successfully investigated this disease progression, finding that some differences between branch locations exist at certain time points in the season, however, these differences do not permit consistent rankings of branch locations among the three measurements of severity. This reinforces the need for good orchard management and complete spray coverage to limit the amount of inoculum present in an orchard as well as the number of leaves left unprotected.

The Assess 2.0 Image Analysis Software for Plant Disease Quantification© was used in this study to determine the average leaf area of each of the studied clones as well as to evaluate the average percent of the leaf area infected from the leaf samples collected. This software proved to be very accessible and effective in determining these values; however, when determining the percent of the leaf area infected, the software-derived data did not closely mirror the field collected data. While the trends and rankings were consistent between the two, the field-collected data had less variability than the software-derived data due to the greater number of leaves contributing to the average values, as compared to that of the software where a single leaf was representative of multiple leaves on the corresponding branch. Although these software results were not perfectly illustrative, the use of the software to calculate average leaf area was extremely valuable in the adjustment of the field collected lesion density data to account for variability in leaf size among the clones.

The comparison between ‘Montmorency’ and the tolerant clone 26eo-17-29 at two locations demonstrated that severity of CLS infection is likely dependent on the combined influences of weather, orchard management, and existing inoculum. In 2015, CLS infection of the trees at the PPRC was delayed in comparison to the corresponding trees located at the CRC. Although the weather was not substantially different between the two locations, minor variability can be influential if the conditions conducive to pathogen growth occur frequently. The factors believed to have had the largest effect, in this case, are existing inoculum and orchard management. The trees at the PPRC had been sprayed in all years prior to that of evaluation, whereas many of the trees at the CRC had been unsprayed for multiple years preceding the study. In terms of management, the orchard located at the PPRC is planted on a slight slope and at a wider within-row spacing that permits good air movement between trees. In contrast, the orchards at the CRC are not planted on a slope and have a denser tree spacing which limits air flow and lengthens the amount of time the orchard remains wet following a rain event.

In 2016, lack of precipitation at the start of the season caused CLS infection to be significantly delayed on the ‘Montmorency’ trees at the PPRC, compared with the 2015 season and other seasons with average weather conditions. Because *B. jaapii* spores are rain-dispersed and reliant on specific conditions for establishment within host leaves, the more severe effects of CLS were not observed on the unsprayed ‘Montmorency’ trees at the PPRC until more regular rainfall events occurred. However, even after delayed infection, CLS infection progressed rapidly on these trees, which were completely defoliated by mid-September. An unexpected host response was observed on the 26eo-17-29 trees at the PPRC in 2016, which had significantly higher incidence than ‘Montmorency’ at the beginning of the season. Although this response was inconsistent with the 2015 observations, the low lesion density on these leaves translates to relatively low disease severity. For example, on June 30, 2016 this equates to approximately 80 leaves with fewer than 2 lesions per cm^2 out of a total of 130 total rated leaves, compared to ‘Montmorency’ which had approximately 15 leaves with the same number of lesions out of 130 total rated leaves on this date. Because this higher incidence did not translate to higher defoliation at the end of the season, lesion density and defoliation should be preferentially considered when assigning the designation of tolerance.

At the CRC in 2016, the lack of precipitation and the application of fungicides reduced the establishment and spread of CLS throughout the orchard, resulting in a dataset inadequate for comparison to the 2015 season results. Therefore, these results have been excluded from this analysis, but are included in Appendix B.

The conditions experienced in 2015 were ideal for observing host responses to CLS under severely high disease pressure and wet conditions, which were likely more extreme than those that a typical commercial orchard would experience. These results therefore support a breeding approach to pyramid resistant and tolerant host responses in order to develop a more durably resistant cultivar for use in commercial production in the future.

CHAPTER 3

PROGENY EVALUATION FOR RESPONSE TO CHERRY LEAF SPOT AND SEGREGATION FOR HORTICULTURAL QUALITY TRAITS

Introduction

Pyramiding alleles that confer CLS resistance and tolerance to obtain a sour cherry cultivar with durable resistance requires a detailed understanding of the resistance or tolerance available from various donors. It also requires knowledge of the inheritance and ultimately the genetic control of these traits, such as whether the desirable alleles are dominant or recessive, and DNA diagnostic tools that allow for the identification of progeny that have all of these desired resistance and tolerance alleles. In Chapter 2, the first requirement, a detailed understanding of the levels of resistance or tolerance from different sources, was achieved. In this study, the goal was to address the second criterion: knowledge of the inheritance of the resistant and tolerant host responses. To achieve this goal, progeny individuals at different generations of derivation from the ancestral resistance or tolerance donors were evaluated for their response to CLS. This information was used to determine the inheritance of these traits from different parental donors, and the value of continued use of certain parents in future crosses. The evaluation of progeny in this manner permits the observation of trait segregation within families. By studying the distribution of this segregation and identifying trends spanning multiple generations, hypotheses can be developed regarding the genetic control of these traits.

Success in the development of a superior cultivar is defined not only by the achievement of durable resistance to CLS but by the concurrent maintenance of horticultural traits that meet industry standards. To assess the progress being made toward this goal, the progeny evaluated for their response to CLS were also evaluated for important horticultural traits. As a result, individuals to be maintained in the breeding program as parents, as well as any possible genetic linkages between disease resistance and horticultural characteristics, could be identified.

The objective of this study was to gain an understanding of the inheritance of CLS resistance and tolerance by evaluating the host response of progeny individuals belonging to several families over the course of a season. To evaluate the success of the breeding program in maintaining CLS resistance or tolerance in addition to horticultural quality, this study also aimed to evaluate fruit traits valuable to sour cherry production practices.

Materials and Methods

Plant Materials

Prior to this study, crosses were made between CLS susceptible individuals and tolerant or resistant individuals in an effort to combine these traits with horticultural quality, as well as between tolerant and resistant individuals in an effort to pyramid these desired CLS traits. These families, consisting of individuals of varying ages, were located at both the MSU Clarksville Research Center (CRC) in Clarksville, MI, and the MSU greenhouses.

In 2015, 267 progeny individuals located at the CRC were phenotyped for their response to CLS. This included 126 progeny derived from the *P. canescens* resistance donor, 129 progeny derived from sweet cherry, ‘North Star’, and *P. maackii* tolerance donors, and 12 progeny from a cross where one parent was resistant and one parent was tolerant, a family created in an effort to combine the two traits. Therefore, all families are descended from one or more of the known germplasm sources of resistance (*P. canescens*) or tolerance (*P. avium*, *P. cerasus*, and *P. maackii*) (Table 3.1).

In order to rate CLS host response, most of these progeny were not sprayed with fungicides in 2015, with the exception of particularly valuable or very young trees which could have been lost to the disease if no preventative action was taken. In 2016, all evaluated trees were sprayed with fungicides (details regarding each year's spray program are included in Appendix A). Although this treatment resulted in less disease pressure, valuable information was obtained regarding the host response under traditional disease management practices, as well as regarding fruit quality characteristics which could not have been evaluated on CLS stressed trees.

Table 3.1: List of the 28 families and 267 progeny individuals derived from four different germplasm sources of resistance or tolerance that were evaluated for their response to CLS.

Family	Trait(s) of Interest	Germplasm Source of Trait	Generation of Progeny from Source	Number of Progeny
26eo-11-27 [1] × 27e-09-47	Tolerance	*P. avium* (4x) 'Csengodi'	1	3
26eo-11-27 × 24-14-24	Tolerance	*P. avium* (4x) 'Csengodi'	1	1
26eo-11-27 × o.p. [2]	Tolerance	*P. avium* (4x) 'Csengodi'	1	1
'Újfehértói Fürtös' × **Almaz R1(1)**	Tolerance	*P. maackii* Almaz R1(1)	1	1
'Montmorency' × **Almaz R1(1)**	Tolerance	*P. maackii* Almaz R1(1)	1	3
Almaz R1(1) × o.p.	Tolerance	*P. maackii* Almaz R1(1)	1	1
25o-14-20 × **'North Star'**	Tolerance	*P. cerasus* 'North Star'	1	19
'North Star' × 27e-05-33	Tolerance	*P. cerasus* 'North Star'	1	4
'Újfehértói Fürtös' × **I-13-61**	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	2	9
26eo-17-29 × 27-03-08	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	21
I-63-05 × **26eo-17-29**	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	28
26eo-17-29 × o.p.	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	1
27-27-44 × 27e-15-38	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	10
27-27-44 × 27e-16-47	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	7
'Erdi Jubileum' × **27-27-44**	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	1
27-27-44 × 'Montmorency'	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	4
26eo-08-02 × **27-27-44**	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	5

Table 3.1 (cont'd)

Family	Trait(s) of Interest	Germplasm Source of Trait	Generation of Progeny from Source	Number of Progeny
27-08-30 × **27-27-44**	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	3
I-63-05 × **27-27-44**	Tolerance	*P. cerasus* 'North Star' and *P. avium* 'Kansas Sweet'	3	7
'Montmorency' × **23-23-13**	Resistance	*P. canescens*	3	28
27-27-10 × o.p.	Resistance	*P. canescens*	3	1
23-23-13 × **23-23-07**	Resistance	*P. canescens*	3	27
23-23-07 × **23-23-13**	Resistance	*P. canescens*	3	3
'Újfehértói Fürtös' × **23-23-13**	Resistance	*P. canescens*	3	38
24-32-37 × 27e-05-33	Resistance	*P. canescens*	4	12
M172 × **24-32-43**	Resistance	*P. canescens*	4	3
'Montmorency' × **24-32-41**	Resistance	*P. canescens*	4	14
Almaz R1(1) × **23-23-13**	Resistance & Tolerance	*P. canescens* and *P. maackii* Almaz R1(1)	3 1	12

[1] Parent(s) in bold contributed the trait(s) of interest.

[2] o.p. = open pollinated

Paternity Testing and Screening for the CLSR_G4 Resistance Allele

Numerous studies have identified various alleles at the self-incompatibility *S*-locus, which can be used to confirm seedling parentage in sour cherry (Table 3.2). Paternity tests were conducted for all progeny individuals using DNA tests for the *S*-locus alleles known to segregate in the parents of the families included in this study (Table 3.3). An individual was considered to be a true hybrid if it shared the expected *S*-alleles of the recorded paternal parent. Individuals with inconclusive results were considered to be potential hybrids; however, further testing is required to confidently confirm parentage. Progeny of *P. canescens* derived resistant clones were also genotyped for the previously identified *P. canescens* derived resistance allele at the

CLSR_G4 QTL using the SSR marker CLS028, if prior genotypic information was not available (Stegmeir et al., 2014).

Table 3.2: *S*-alleles and details of the *S-RNase*-based PCR DNA tests used for paternity testing the progeny of families evaluated for their CLS response.

Target Gene and Primer Name	Primer Sequence 5’→ 3’	Annealing Temp (°C)	Extension time (s)	Product Size (bp)	Reference
PruC2 Pce-R (Non-specific primer) [1]	CTATGGCCAAGTAATTATTCAAACC TCTTTGTTCCATTCGCYTTCCC	56	75	— [2]	Tao et al., 1999 Tsukamoto et al., 2008
S_1-RNase (630 bp) PaS1-F PaS1-R	GTAATTGCAACGGGTCAAAATATGAG ACAACTCAGTATTAGTTGCTGGATCA	56	75	817	Sonneveld et al., 2001
S_2-RNase PaS2-F PaS2-R	CCTGCTTACTTTGTCACGCA AAGTGCAATCGTTCATTTG	57	75	350	Sonneveld et al., 2001
S_4-RNase (826 bp) PaS4-F PaS4-R	CACTGGGTCGCTGTTTAACTTTAGG TTGCATTTGATTAAGTGAGGCTTCA	62	75	819	Sonneveld et al., 2001
S_6-RNase (339 bp) PaS6-F PaS6-R	ACTGGACCGCAATTTAAGCG AGTTGCGCTTTAATGGGTGCA	58	75	463	Sonneveld et al., 2001
S_9-RNase (~550 bp) PaS9-F PaS9-R	TTTGTTACGTTATGAGCAGCAG ATGAAACAATACATACCACTTTGCTA	62	75	781	Sonneveld et al., 2003
SFB_{13} PcSFB13-F PcSFB13-R	AGTTAATGACTGCAAGGCTGTAAGGG CCCCATTGTACGATAATTGTAATCC	58	75	439	Tsukamoto et al., 2006
S_{26}-RNase (543 bp) PcS26-F PcS26-R	CACCTGCATACTTCGCAAGA TGCTGCTTTAATGGGTGCTA	66	75	773	Hauck et. al., 2006
S_{33}-RNase (424 bp) PcS33-F PcS33-R	CACAGTTCGCAAGAAATGC ATGTTGGCATTTTGGTCGG	66	60	819	Tsukamoto et al., 2008
S_{35}-RNase PcS35-F PcS35-R	GACCCGATTTAGCAATAGTTTG GAGGCATCGTCAAGTTGTTAG	66	60	898	Tsukamoto et al., 2008
S_{36a} specific primer PcS36ab-F PcS36a-spR	GCTAGCCAACCACTTTTACG GAAACCCACATGATACAAACTG	66	60	898	Tsukamoto et al., 2010
S_{36b}/S_{36b2}/S_{36b3} specific primer PcS36ab-F PcS36b/b2/b3/R	GCTAGCCAACCACTTTTACG ATACATTGTAGGCCAGTCTGTG	66	60	S_{36b}: 760 S_{36b2}: 759 S_{36b3}: 760	Tsukamoto et al., 2010

[1] PruC2/Pce-R is a non-specific primer which amplifies several *S*-alleles in in both sour and sweet cherry. It is used here as a preliminary primer for paternity testing purposes.

[2] The fragment sizes of each of the amplification products from this primer pair can be found in the publications by Tao et al. (1999) and Tsukamoto et al. (2008).

Table 3.3: *S*-alleles known to be present in the parents of the families evaluated for their CLS response. Further information regarding *S*-allele discovery in a majority of these individuals can be found in the publication by Sebolt, Tsukamoto, and Iezzoni (Acta Hort, in press), with the *S*-alleles of the remaining individuals being identified as part of this study or obtained from A. Iezzoni (unpublished).

Parent	***S*-locus alleles identified**
Almaz R1(1)	2, 6, 36a
'Erdi Jubileum'	1, 6, 13', 36b
I-13-61	13m, 36a, 36b
I-63-05	6, 36a
M172	13', 35, 36a, 36b
'Montmorency'	6, 13m, 35, 36a
'North Star'	13', 36a, 36b, 35
'Újfehértói Fürtös'	1, 4, 35, 36b
23-23-07	9, 26, 36a
23-23-13	13', 26
24-32-37	4, 26, 36b
24-32-41	1, 26
24-32-43	4, 35
25-14-20	1', 6, 36a, 36b
26eo-08-02	-
26eo-11-27	6m2, 36a, 36b, 33
26eo-17-29	4, 13', 35, 36b
27-03-08	1', 13', 35, 36a
27-08-30	1, 33, 36b
27-14-24	-
27-27-10	4, 26, 36b
27-27-44	13', 36a, 35
27e-05-33	6, 13', 36x, 36x
27e-09-47	1, 13, 36a, 36b
27e-15-38	4, 13', 13', 36a
27e-16-47	13', 35, 36a, 36b

DNA used to conduct these genetic tests was extracted from young leaf tissue using the Silica Bead Method collection and extraction protocol developed by Edge-Garza et al. (2014). The PCR mixture used for both the *S*-locus and the *CLSR_G4* locus contained 1× PCR buffer (Invitrogen^{TM}, Thermo Fisher Scientific Inc., Waltham, MA, USA), 2.5 mM $MgCl_2$ (Invitrogen^{TM}), 0.2 mM of each deoxynucleotide triphosphate (Invitrogen^{TM}), 2.5 pmol of each primer (Integrated DNA Technologies Inc., Coralville, IA, USA), 100-120 ng of genomic DNA, and 0.3 U *Taq* polymerase (Invitrogen^{TM}) in a 15-µl reaction. PCR conditions for the *S*-locus were as follows: 94°C (5 min) followed by 35 cycles of 94°C (30 s), *X*°C (*Y* s), 72°C (1 min), followed by a final elongation step of 72°C (5 min), where *X* is the selected primer's published annealing temperature and *Y* is its respective extension time (Table 3.2). PCR fragments for the *S*-locus were then separated in a 2% agarose gel, stained with GelRed^{TM} Nucleic Acid Gel Stain (Biotium, Inc., Fremont, CA, USA), and visualized using UV illumination. For the CLS028 marker at the *CLSR_G4* locus, a touchdown PCR was used. Conditions were as follows: 94°C (5 min) followed by 9 cycles of 94°C (30 s), 60°C (45 s) (-1°C per cycle), 72°C (1 min) and then 24 cycles of 94°C (30 s), 55°C (45 s), 72°C (1 min), followed by a final elongation step of 72°C (5 min). PCR fragments for CLS028 were then separated in a 6% polyacrylamide gel and visualized with silver staining following the procedure outlined by Olmstead et al. (2008).

Phenotyping Protocol for Host Response to CLS

Progeny individuals were phenotyped every two weeks over the 2015 growing season for their response to CLS. This visual evaluation consisted of four parameters: percent infection, percent defoliation, incidence, and an overall qualitative disease score. Percent infection was an estimate of the percent of the tree that was infected based on visual assessment of the existing leaves. Percent defoliation was the approximate percent of leaves lost. Incidence was rated using a scale from 0 to 2 representing lesion characteristics (0 = no visible infection, 1 = infection present but no noticeable lesion sporulation, 2 = infection present with noticeable lesion sporulation). A qualitative categorization representing the overall CLS disease state of the tree at the time of evaluation was also assigned at each rating to provide a progressive assessment of the host disease response. This disease descriptor had the following five categories representing the range of possible host responses: S = Susceptible, MT = Mildly Tolerant, T = Tolerant, MR = Mildly Resistant, R = Resistant.

Assignment of a Comprehensive Disease Score

During the 2015 season, all trees were evaluated six times based on the four parameters described above. At the end of the season, these data were used to assign each tree a single comprehensive disease score on a scale from 1 to 5 (Figure 3.1). On this scale, 1 = Low level of infection without lesion sporulation (0-35% defoliation at the end of September), 2 = Low level of infection with lesion sporulation (<35% defoliation at the end of September), 3 = Moderate level of infection with lesion sporulation (40-65% defoliation at the end of September), 4 = Severe level of infection with slow progression and lesion sporulation (>65% defoliation at the end of September), 5 = Severe level of infection with fast progression and lesion sporulation (75% defoliation by mid-July or by mid-August if progression from 50 to 75% occurred in less than 2 weeks' time).

Once all progeny had been assigned a comprehensive disease score, all further analyses were conducted using these scores. The comprehensive disease scores were combined with pedigree information and visualized together using the Pedimap 1.2 © software (Voorrips et al., 2012); the resulting figures can be found in Appendix C.

Figure 3.1: Scale of the comprehensive disease scores. Individuals were assigned a score based on their response to CLS over the course of the season. For visualization of the data, each score was assigned a different color. As illustrated by the photographs from September 22, 2015, a disease score of 1 or 2 was assigned to individuals that showed high resistance or tolerance, respectively, to CLS through the end of the season. A disease score of 3 was assigned to individuals having a slight tolerance to CLS, and scores of 4 or 5 were assigned to individuals that showed high susceptibility to CLS.

1	2	3	4	5
Low infection without sporulation	**Low infection with sporulation**	**Moderate infection with sporulation**	**Severe infection, slow progression**	**Severe infection, fast progression**
0-35% final defoliation	< 35% final defoliation	40-60% final defoliation	> 65% final defoliation	75% defoliation by mid-July or by mid-August (if defoliation increased from 50% to 75% in < 2 weeks)

Evaluation of Horticultural Traits

In addition to the disease phenotyping protocol described above, several horticultural traits including bloom date, harvest date, days to fruit ripening, crop load, and various fruit quality characteristics were evaluated, following standard protocols (Stegmeir, Sebolt, & Iezzoni, 2014), for all progeny individuals that were of the appropriate maturity. For this study, the interpretation of this collected data was prioritized to focus on two major traits desired by sour cherry producers: high fruit firmness and freestone pit characteristics.

To evaluate these traits, a representative sample of fruit was collected from each tree on the day of harvest. Firmness was measured in g/mm using a BioWorks, Inc. FirmTech Fruit Firmness Tester, compressing each fruit from cheek to cheek. Level of free- or cling-stone was assigned using a scale from 1 to 5 (1 = pit completely free of flesh [freestone], 5 = very clingy flesh [clingstone]). These data were collected and averaged from 25 fruits for firmness and 5 fruits for freestone pit characteristics.

Results

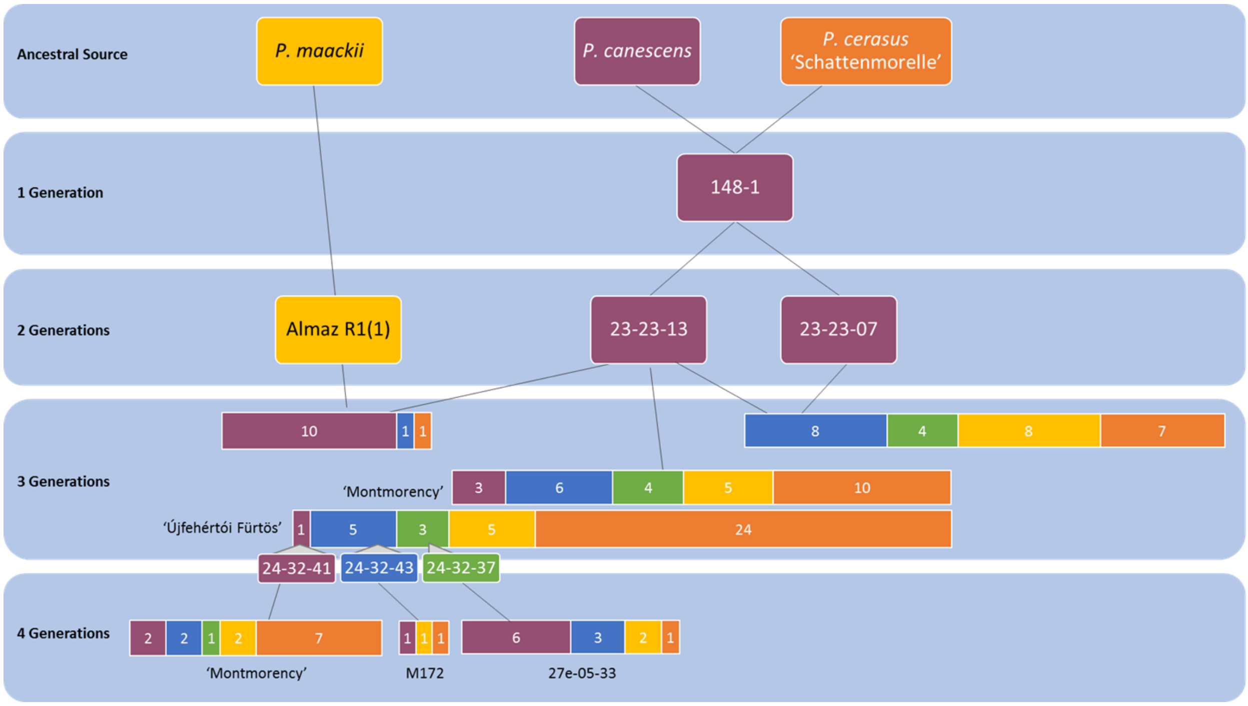
Paternity Testing and Screening for the CLSR_G4 Resistance Allele

By genotyping progeny individuals at the *S*-locus using the DNA tests listed in Table 3.2 many true hybrid progeny individuals could be identified within the families evaluated for CLS, in addition to those genotyped prior to this study. Progeny considered to be true hybrids have been determined to share at least one *S*-allele with their paternal parent. The *S*-alleles identified for each individual can be found in Appendix C; however, due to inconclusive test results, some individuals cannot be confidently considered true hybrids without additional genotyping. Individuals belonging to *P. canescens* derived families were also screened for the resistance allele at the *CLSR_G4* locus, if not genotyped prior to this study. The results of genotyping at the *CLSR_G4* locus for each tested individual can also be found in Appendix C, although additional genotyping is required to confirm the presence of the CLS resistance allele at this locus for some individuals. By confirming that the progeny evaluated in this study for CLS response are true hybrids or that they inherited the *CLSR_G4* resistance allele, conclusions can be confidently made regarding the inheritance of the resistance and tolerance traits within these families.

Evaluation of Host Response to CLS

Among the families evaluated for their response to CLS, disease resistance segregated in families derived from *P. canescens* and both *P. canescens* and *P. maackii* (Fig. 3.2). The progeny individuals from the *P. canescens* derived families exhibited the full range of host responses to CLS, with disease scores from 1 (highly resistant) to 5 (highly susceptible) (Figure 3.2, Appendix C). In some cases, individuals that had been reported as resistant (equivalent score of 1) prior to this study (Stegmeir et al. 2014) were observed to be slightly more susceptible to CLS (score of 2) due to the significant disease pressure experienced. Nearly all individuals receiving a disease score of 1 or 2 were confirmed to have the *P. canescens* derived resistance allele at the *CLSR_G4* locus (Appendix C), and when disease scores of 1 and 2 are considered to be resistant, the phenotypic segregation ratios of these families are consistent with those observed by Stegmeir et al. (2014) in two smaller families, reflecting the expected segregation ratios of a two gene model.

Figure 3.2: Families with *P. canescens* ancestry as well as *P. canescens* and Almaz R1(1) ancestry. Each family is represented by a horizontal bar which is divided according to the segregation of the comprehensive disease scores among progeny individuals (see Figure 3.1 for the color scale of comprehensive disease scores). Parents contributing resistance or tolerance are shown in the pedigree, while the susceptible parent of each family is listed to the left of the family's horizontal bar.



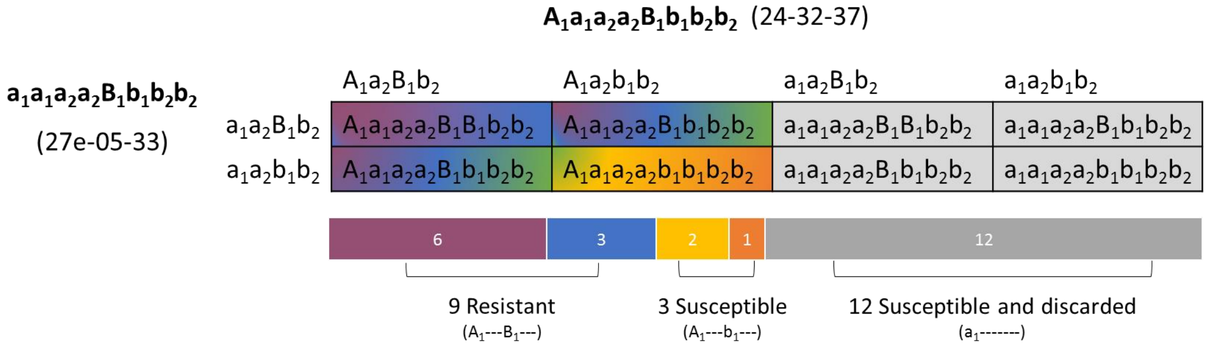
Based on the suspected parental genotypes of the five *P. canescens* derived segregating families examined here, a two gene model results in a segregation ratio of 3 resistant progeny to 5 susceptible progeny (Table 3.4); however, it is possible for this ratio to be obtained from two different combinations of parental genotypes. For example, individuals 23-23-13 and 23-23-07 are both known to have the resistance allele at the *CLSR_G4* locus (noted as *A* and determined using SSR marker CLS028). It is hypothesized that 23-23-13 also has the resistance allele at the proposed second locus (noted as *B*) and that 23-23-07 does not. Therefore, the expected 3:5 ratio of this cross is the result of the hypothesized combination *AB* × *Ab*. This differs from the other *P. canescens* derived families where the 3:5 ratio is due to hypothesized combinations of *aB* × *AB*, as illustrated in Figure 3.3 where resistant individuals have the *AB* genotype which translates to a disease score of 1 or 2 (purple and blue, respectively). The goodness of fit χ^2 values listed in Table 3.4 confirm that the observed segregation ratios are not significantly different from the expected ratios in all of the studied families, with the only exception being the 'Újfehértói Fürtös' × 23-23-13 family.

Table 3.4: Determination of χ^2 Goodness of Fit for families with *P. canescens* ancestry. Only progeny individuals confirmed to be true hybrids were included in the calculations (genotyping data in Appendix C). Ratios represent the number of resistant and number of susceptible progeny individuals, respectively, based on a two gene model for resistance (Figure 3.3).

Family	**No. of individuals**	**Expected ratio**	**Expected values**	**Observed values**	χ^2	**Probability (P value)**
24-32-37 × 27e-05-33	12	3 : 1	9 : 3	9 : 3	0	1.00
23-23-13 × 23-23-07	26	3 : 5	9.75 : 16.25	8 : 18	0.503	0.478
'Montmorency' × 23-23-13	19	3 : 5	7.1 : 11.9	7 : 12	0.004	0.953
'Montmorency' × 24-32-41	4	3 : 5	1.5 : 2.5	2 : 2	0.267	0.606
'Újfehértói Fürtös' × 23-23-13	27	3 : 5	10.1 : 16.9	4 : 23	5.928	0.015

α= 0.05, degrees of freedom=1, critical χ^2 value=3.841

Figure 3.3: The two gene CLS resistance model (as proposed by Stegmeir et al., 2014), illustrated using the segregation pattern of the family 24-32-37 × 27e-05-33. One-half of the progeny are predicted to have a dominant *P. canescens* derived resistance allele for the QTL *CLSR_G4* (represented here by 'A_1'). Absence of this A_1 allele predicts susceptibility, and those progeny are discarded during MAS (Basundari, 2015). The other half segregate in a ratio of 3 resistant to 1 susceptible individual based on the hypothesis of a second disease resistance locus (*B*) where both A_1 and B_1 are needed to confer resistance. See Figure 3.1 for the color scale of comprehensive disease scores.

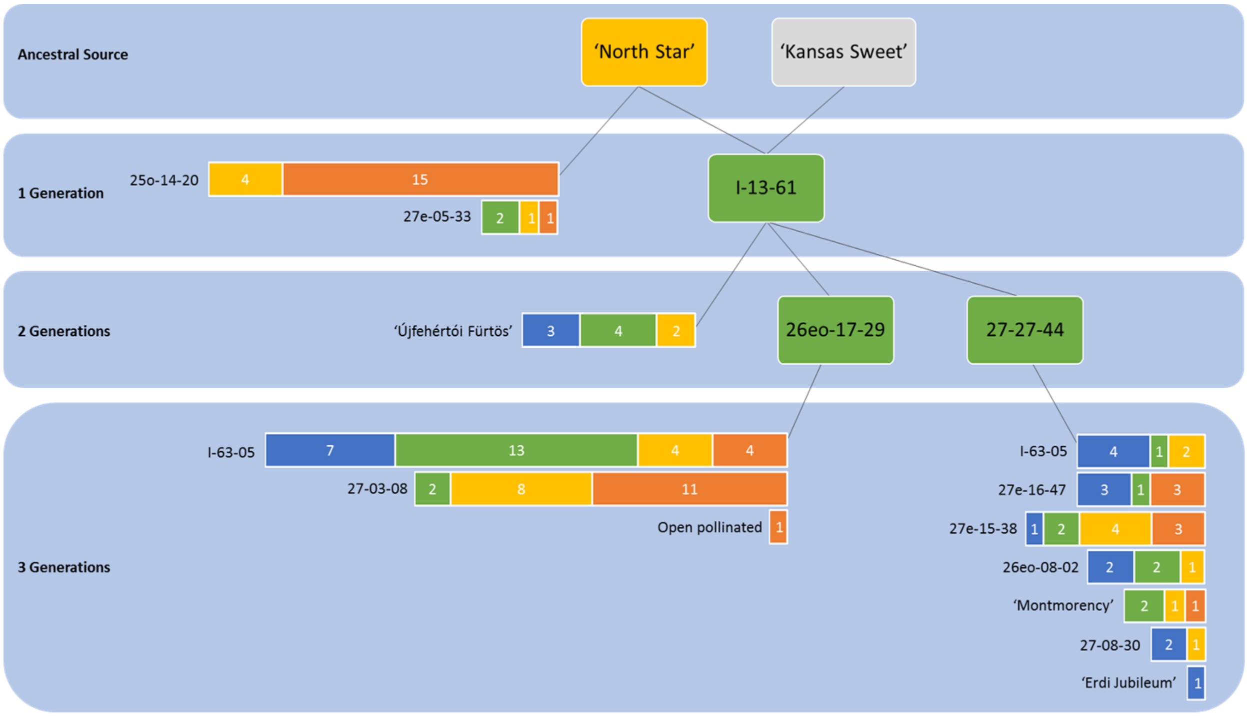


There was one family with both *P. canescens* and *P. maackii* ancestry, 23-23-13 × Almaz R1(1). This pyramided family, combining resistance and tolerance traits, consists of 12 individuals with 10 having a highly resistant disease score of 1, one having a score of 2, and one having a highly susceptible score of 5.

Among the families evaluated for their response to CLS, disease tolerance segregated in families derived from both *P. cerasus* and *P. avium* (Fig. 3.4). Individuals with both *P. cerasus* and *P. avium* ancestry belong to one of eleven different families and received comprehensive disease scores ranging from 2 to 5 (Figure 3.4, Appendix C). These families are all crosses between one susceptible parent and one parent that is derived from I-13-61, a hybrid between ‘North Star’ (*P. cerasus*) and ‘Kansas Sweet’ (*P. avium*). Of these eleven families, eight families included progeny that exhibited increased CLS tolerance (disease score of 2) compared with either of the parents (which had a disease score of 3 if I-13-61 derived, or a score of 4 or 5 if not a tolerance donor). The families derived from I-13-61 (‘North Star’ *P. cerasus* × ‘Kansas Sweet’ *P. avium)* had more tolerant progeny individuals than the two families where ‘North Star’ was the sole tolerance donor (‘North Star’ × 27e-05-33 and 25o-14-20 × ‘North Star’) which consisted primarily of individuals with highly susceptible disease scores of 4 or 5 (Figure 3.4).

In addition to the families with ‘North Star’ as the sole tolerance donor, families derived from the *P. avium* (4x) ‘Csengodi’ as well as from the *P. maackii* derived Almaz R1(1) did not segregate for tolerance, having only progeny individuals with high susceptibility to CLS, represented by comprehensive disease scores of 4 or 5 (Appendix C).

Figure 3.4: Families with *P. cerasus* ‘North Star’ ancestry as well as those with ‘North Star’ and *P. avium* ‘Kansas Sweet’ ancestry. Each family is represented by a horizontal bar that illustrates the segregation of comprehensive disease scores among the progeny individuals (see Figure 3.1 for the color scale of comprehensive disease scores). Parents contributing tolerance are shown in the pedigree, while the susceptible parent of each family is listed to the left of the family’s horizontal bar. The comprehensive disease score assigned to each parent is also represented; note that no disease score was assigned to ‘Kansas Sweet’, as it was not evaluated within this study.



Evaluation of Horticultural Traits

While data was obtained for several horticultural traits, in this study the interpretation of this collected data was prioritized to focus on two major traits desired by sour cherry producers: high fruit firmness and freestone pit characteristics. Among the families examined in this study, some demonstrated high within-family variability for these traits, while others showed more consistent trends. Overall, each of these traits were observed to be heritable over several generations in certain families; however, as was observed with CLS tolerance, certain parents seem to contribute more positively to the maintenance of these traits in the progeny than others.

For breeding purposes, a high level of fruit firmness is desired. This level must be greater than that of ‘Montmorency’, which has an average firmness of 121 g/mm. For example, ‘Újfehértói Fürtös’, has a higher fruit firmness than ‘Montmorency’ with an average value of 133 g/mm (Table 3.5A). Within the families in this study, the firmness of the progeny individuals ranged from a low of 99 g/mm to a high of 223 g/mm (Table 3.5B, Figure 3.5A). A majority of the progeny were of an acceptable firmness; however, the families with the greatest proportion of progeny firmer than 150 g/mm had 23-23-13 as a parent. The best families were Almaz R1(1) × 23-23-13, 23-23-13 × 23-23-07, and ‘Újfehértói Fürtös’ × 23-23-13. Oppositely, the families with ‘Montmorency’ as a parent tended to have progeny with softer fruits, although several still had an acceptable level of firmness. The families examined in this study for their CLS tolerance traits tended to have progeny individuals with a moderate, but acceptable, firmness level (Table 3.5B).

To improve ease of fruit pitting during processing, highly freestone individuals are desired. As described above, a score of 1 is assigned to the most freestone individuals, while a score of 5 is earned by highly clingstone individuals. For freestone pit characteristics, ‘Montmorency’ is the industry standard and has an average score of 1.9, therefore, this is the highest acceptable level of clingstone for breeding purposes. Regardless of ancestry, most of the earlier generations and parents of the families included in this study have a moderate freestone score between 2 and 3; however, the resulting progeny have highly variable freestone scores, falling across the entire range of 1 to 5 (Table 3.5, Figure 3.5B). The families with the highest proportion of progeny with acceptable freestone levels are *P. canescens* derived, particularly families with 23-23-13 as a parent. Among the 23-23-13 families, Almaz R1(1) × 23-23-13 and ‘Újfehértói Fürtös’ × 23-23-13 have some of the best freestone progeny individuals. From the available data, the families examined in this study for their tolerance traits were seen to have moderate levels of freestone, with some individuals exhibiting acceptable freestone traits. Many of these individuals belong to the I-63-05 × 27eo-17-29 family, although high clingstone individuals also arose from this cross (Table 3.5B).

Table 3.5: Fruit firmness and pit freestone characteristics for fruiting parents and progeny individuals from families evaluated for their response to CLS. (A) Mean firmness and freestone values for all parents, and (B) firmness and freestone mean, minimum, and maximum values of the progeny individuals belonging to each family.

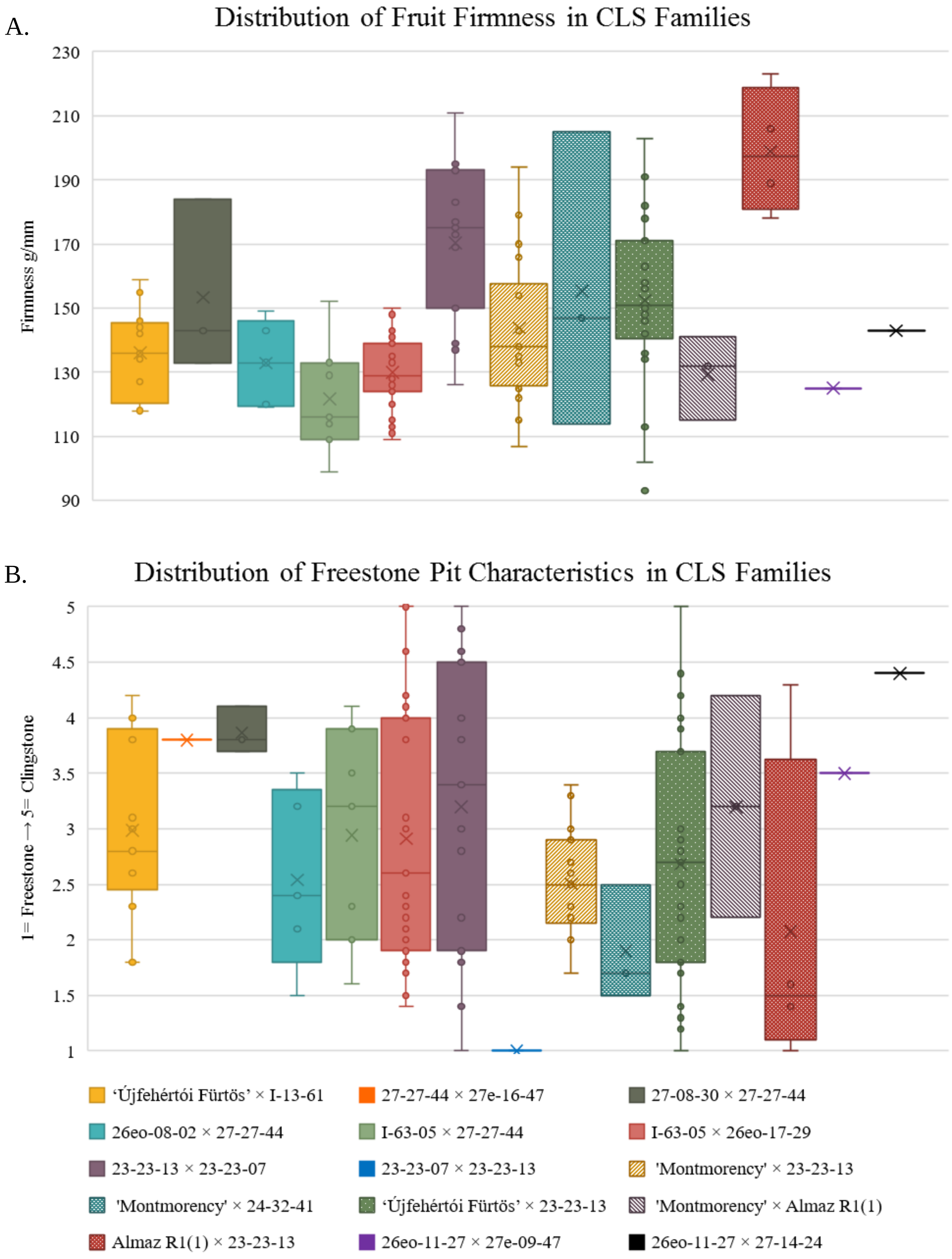
A.

Parent	**Mean Firmness** (g/mm)	**Mean Freestone** (scale from 1-5)
‘Újfehértói Fürtös’	133	2.7
I-13-61	-	2.2
27-27-44	144	3.7
27e-16-47	163	4.5
27-08-30	119	1.7
26e-08-02	-	-
I-63-05	132	2.0
26eo-17-29	141	2.3
23-23-13	144	2.1
23-23-07	171	1.9
‘Montmorency’	121	1.9
24-32-41	172	2.5
Almaz R1(1)	154	2.7
26eo-11-27	150	3.6
27e-09-47	124	1.6
27-14-24	-	-
‘Schattenmorelle’	170	2.2
P. canescens	119	2.5

B.

Family	**Firmness** (g/mm)				**Freestone** (scale from 1-5)			
	No. of progeny with data	**mean**	**min.**	**max.**	**No. of progeny with data**	**mean**	**min.**	**max.**
‘Újfehértói Fürtös’ × I-13-61	12	146	118	159	13	3.0	1.8	4.2
27-27-44 × 27e-16-47	-	-	-	-	1	3.8	-	-
27-08-30 × 27-27-44	3	153	133	184	3	3.9	3.7	4.1
26eo-08-02 × 27-27-44	5	133	119	149	5	2.5	1.5	3.5
I-63-05 × 27-27-44	7	122	99	152	7	2.9	1.6	4.1
I-63-05 × 26eo-17-29	29	130	109	150	29	2.9	1.4	5.0
23-23-13 × 23-23-07	15	170	126	211	15	3.2	1.0	5.0
23-23-07 × 23-23-13	-	-	-	-	1	1.0	-	-
‘Montmorency’ × 23-23-13	18	144	107	194	18	2.5	1.7	3.3
‘Montmorency’ × 24-32-41	3	155	114	205	3	1.9	1.5	2.5
‘Újfehértói Fürtös’ × 23-23-13	22	157	102	203	23	2.7	1.0	5.0
‘Montmorency’ × Almaz R1(1)	3	129	115	141	3	3.2	2.2	4.2
Almaz R1(1) × 23-23-13	4	199	178	223	4	2.1	1.0	4.3
26eo-11-27 × 27e-09-47	1	125	-	-	1	3.5	-	-
26eo-11-27 × 27-14-24	1	143	-	-	1	4.4	-	-

Figure 3.5: Box and whisker plots illustrating the distributions of fruit firmness (A) and freestone pit characteristics (B) in families evaluated for their CLS response.



Discussion

Host Response to CLS

By comparing the segregation patterns of the families belonging to each ancestral group, it was possible to gain insight to the inheritance of these traits. In the *P. canescens* derived families, these results support the hypothesis that the *P. canescens* resistance is controlled by two dominant loci, where the presence of resistance alleles at both loci is required to produce a resistant phenotype (Stegmeir et al., 2014). The goodness of fit χ^2 values listed in Table 3.4 for the ratios observed in this study support the two gene model hypothesis in all evaluated families with the exception of 'Újfehértói Fürtös' × 23-23-13, which segregated in a ratio that was significantly different from the proposed 3:5 ratio. Although further evidence is needed, this difference could potentially be accounted for through an alternative hypothesis that 'Újfehértói Fürtös' does not carry the resistance allele at the proposed second resistance locus (*B*). If this is the case, the expected ratio from the 'Újfehértói Fürtös' × 23-23-13 cross would be 1 resistant progeny to 3 susceptible progeny. Under this hypothesis, the 4:23 ratio observed in this study would not be significantly different from the expected 1:3 ratio, resulting in a χ^2 value of 1.494 and a probability of 0.22.

The hypothesized two gene model for CLS resistance is further supported by the segregation pattern of the 24-32-37 × 27e-05-33 family (Figure 3.3), which demonstrates the successful use of the SSR marker CLS028 in conducting marker assisted selection (MAS) at the *CLSR_G4* locus. In previous studies, this family was subjected to MAS where the 12 progeny individuals found to have the CLS028 allele at the *CLSR_G4* locus were maintained, while the 12 individuals without the allele were discarded (Basundari, 2015). By keeping only one half of the individuals after MAS, the expected ratio of resistant to susceptible individuals shifted from

3:5 to 3:1, which was observed exactly in the phenotypic segregation for CLS resistance (Table 3.3). While the use of MAS at the *CLSR_G4* locus was successful, the addition of a marker at the proposed second locus (currently noted as *B*) would permit more complete predictive capabilities. By screening large families under multifaceted rating parameters, we obtained a highly detailed and robust evaluation of host response to be used in support of the hypothesized two gene model for CLS resistance.

The crosses with the *P. maackii* derived Almaz R1(1) as a parent all resulted in progeny which had a mild CLS tolerance response similar to that of Almaz R1(1). This suggests that the *P. maackii* derived level of tolerance is a dominant trait. However, the pyramided family 23-23-13 × Almaz R1(1) which combines *P. canescens* derived resistance with *P. maackii* derived tolerance, exhibited a large ratio of highly resistant individuals. This indicates that by pyramiding these two traits the number of progeny with a high level of resistance to CLS can be increased; however, additional research will be needed to identify any underlying genetic mechanisms.

Progeny belonging to the *P. avium* (4x) ‘Csengodi’ derived families all exhibited high susceptibility to CLS (disease scores of 5). This suggests that sweet cherry tolerance is recessive or that it was not inherited in these progeny.

Through the comparison of families with *P. cerasus* ‘North Star’ ancestry and families with both *P. cerasus* ‘North Star’ and *P. avium* ‘Kansas Sweet’ ancestry, a model for the genetic control of tolerance was developed. The two families having ‘North Star’ as the tolerant parent exhibited no segregation within the progeny, with nearly all individuals being susceptible to CLS (Figure 3.4). Alternatively, the families which have both ‘North Star’ and ‘Kansas Sweet’ ancestry by means of I-13-61 were observed to segregate for tolerance, with some being highly tolerant, having a comprehensive disease score of 2. From this observation and the understanding that sweet cherry is reliably tolerant to CLS, it is hypothesized that the improved host response in these families is due to a contribution of tolerance alleles from *P. avium* via ‘Kansas Sweet’. The segregation patterns of tolerance in these families suggest that this *P. avium* derived tolerance is recessive and that the expression level of a tolerant phenotype may be directly related to the number of recessive tolerance alleles an individual has.

Among the families with both ‘North Star’ and ‘Kansas Sweet’ ancestry, differences in progeny phenotypes were also observed to be dependent on the susceptible parent included in the cross. When the tolerant parent (with tolerance derived from I-13-61) was crossed to certain susceptible parents, it resulted in many highly to moderately tolerant progeny (disease score of 2 or 3) as well as a few susceptible progeny (score of 4 and 5). However, when this same tolerant parent was crossed to a different set of susceptible parents, primarily susceptible progeny were observed. Upon investigation, it was discovered that in all of the families where highly tolerant progeny were observed, the susceptible parent is believed to be descendant from the *P. cerasus* landrace cultivar ‘Pandy’, accession number 38. This is particularly important because ‘Pandy’ is the grandparent of ‘North Star’ and exhibits many characteristics similar to sweet cherry.

Because *P. cerasus* is an allotetraploid of *P. avium* and *P. fruticosa* (Olden & Nybom, 1968), it is possible that certain alleles originating from *P. avium* are maintained within the *P. cerasus* genome. In this case, if the alleles inferring tolerance are recessive, it is conceivable that these recessive alleles are present in *P. cerasus* clones but are overcome by dominant susceptibility alleles and subsequently not expressed. Here we hypothesize that because ‘Pandy’ expresses several characteristics similar to sweet cherry, it inherited two *P. avium* tolerance alleles which were then conferred to its progeny, including ‘North Star’ and numerous more distant offspring. Therefore, when ‘North Star’ is crossed with ‘Kansas Sweet’, any ancient *P. avium* alleles in ‘North Star’ combine with the modern *P. avium* alleles in ‘Kansas Sweet’ and result in tolerant progeny, as the increased frequency of the tolerance alleles overcomes the effects of any dominant susceptibility alleles. This is taken a step further when these tolerant individuals are crossed with susceptible individuals who are also descendants of ‘Pandy’, where the possibility of additional recessive alleles increases the potential for high levels of tolerance, as was observed in these families.

Horticultural Traits

Although the horticultural trait data in this study was limited to those mature individuals capable of fruiting, several hypotheses can be drawn from the observations made in these resistant and tolerant families. To better confirm these hypotheses, further investigation using additional families consisting of more progeny will be needed.

By exploring the fruit firmness of several families and their progenitors, it was observed that *P. canescens*-derived families tend to have high fruit firmness. This trait appears to stem from ‘Schattenmorelle’ rather than *P. canescens*, however, as they have average values of 170 g/mm and 119 g/mm, respectively (Table 3.5A). In general, firmness is well maintained in the generations following this initial combination of ‘Schattenmorelle’ × *P. canescens*, resulting in firm breeding parents and families with a relatively large proportion of firm progeny individuals. Differences in the level of fruit firmness between families with common parents suggest that certain parents contribute more preferable firmness phenotypes to their progeny than others.

The freestone trait was also observed to be well maintained within *P. canescens* derived families. In general, these families consist of progenitors that are moderately freestone (score of

2-3) however the progeny segregate into the full range of freestone levels (1-5). Similar to the firmness data results, the available freestone data for the ‘North Star’ and *P. avium* derived families suggests that certain parents are less favorable than others for contributing to desirable freestone phenotypes in their progeny.

By combining CLS host response data with firmness and freestone data, specific individuals having desirable characteristics of two or all three of these traits could be identified. The Almaz R1(1) × 23-23-13 family has the best combination of CLS resistance (comprehensive disease scores of 1 and 2), fruit firmness (averages ranging from 178 g/mm to 223 g/mm), and pit freestone (most scores ranging from 1.0 to 1.6, on average). Although not aligning for all three traits, there are also families that are well aligned for two of the desired traits; 23-23-13 × 23-23-07 has progeny with desirable CLS response and high firmness, while ‘Montmorency’ × 23-23-13 has progeny with desirable CLS response and freestone pit characteristics. Individuals belonging to other families also fit the criteria for two or all three of the traits, even though their siblings do not express the same trait convergence. These individuals will be prioritized for investigation of their other horticultural traits and considered for their use as parents in the next generation of breeding crosses to be made toward further improvement.

Application of Results

The objectives of this study were to gain an understanding of the inheritance of CLS resistance and tolerance traits by evaluating progeny individuals of several families derived from different ancestral trait donors for their host response to the disease. In conjunction with host response to CLS, this study aimed to evaluate these families for fruit traits valuable to sour cherry production to determine the success of the breeding program in combining these disease and quality characteristics. Through the evaluation of host response to CLS, it was possible to determine the inheritance patterns of these traits when conferred through different ancestral donors and carried through several generations, information that can contribute to the future development of genetic markers for these resistance and tolerance traits, the design of crosses to maintain and combine these traits, and the accurate evaluation of the resulting progeny individuals. By adding select horticultural quality and production traits to the evaluation of these families, it is possible to further refine the selection of families and individuals for proliferation within the breeding program, which has the potential to shorten the time required to achieve a high quality, resistant cultivar favorable to modern sour cherry production practices. A cultivar of this caliber would reduce pesticide use, improve fruit processing efficiency, and ultimately reduce costs to growers and result in higher quality and more affordable sour cherry products for consumers.

APPENDICES

APPENDIX A

Orchard Pesticide Application Programs

Table A.1: Pesticide applications at the CRC in 2015. Applications were made in all of the orchards included in this study; however, fungicide treatments to control CLS were not applied to orchard rows where evaluated trees were located.

Application Date	Pesticide	Active Ingredient	Target	Rate/Acre	Applicator
4/13/15	Champ ® Dry Prill	Copper hydroxide	Bacterial Canker	6.85 lb.	D. Platte
5/7/15	Indar ® 2F	Fenbuconazole	Brown rot	3.6 fl. oz.	D. Platte
5/10/15	Assail ®	Acetamiprid	Plum Curculio (PC)	8 oz.	D. Platte
5/10/15	Bravo WeatherStik ®	Chlorothalonil	Cherry Leaf Spot (CLS)	4 pts	D. Platte
5/20/15	Actara ®	Thiamethoxam	PC	5 oz.	D. Platte
5/20/15	Bravo WeatherStik ®	Chlorothalonil	CLS, Brown rot	4 pts.	D. Platte
5/20/15	Bravo Ultrex ®	Chlorothalonil	CLS, Brown rot	3 lb.	D. Platte
5/28/15	Indar ® 2F	Fenbuconazole	CLS, Powdery Mildew	6 fl. oz.	D. Platte
5/28/15	Assail ®	Acetamiprid	PC	8 oz.	D. Platte
5/28/15	Belt ®	Flubendiamide	PC	4 oz.	D. Platte
6/9/15	Gem™	Trifloxystrobin	CLS, Powdery Mildew, Brown Rot	3.8 fl. oz.	D. Platte
6/9/15	Actara ®	Thiamethoxam	PC	5 oz.	D. Platte
6/9/15	Rimon ®	Novaluron	PC	30 oz.	D. Platte
6/9/15	Quintec™	Quinoxyfen	Powdery Mildew	7 fl. oz.	D. Platte
6/11/15 (Tier 25 only)	Gem™	Trifloxystrobin	CLS, Powdery Mildew	3.8 fl. oz.	D. Platte
6/11/15 (Tier 25 only)	Assail ®	Acetamiprid	PC, Leaf roller	8 oz.	D. Platte
6/26/15	Exirel™	Cyantraniliprole	Cherry Fruit Fly	10-20.5 fl. oz.	D. Platte
6/26/15	Luna® Sensation	Fluopyram, Trifloxystrobin	CLS, Brown Rot	5-5.6 fl. oz.	D. Platte
7/9/15	Luna® Sensation	Fluopyram, Trifloxystrobin	CLS, Brown Rot	5-5.6 fl. oz.	D. Platte
7/9/15	Sevin®	Carbaryl	Japanese Beetle	2 qt.	D. Platte
8/6/15 (Tier 25 and 26e only)	Sevin® XLR Plus	Carbaryl	Japanese Beetle	2 qt.	D. Platte
8/6/15 (Tier 25 and 26e only)	Sherpa ®	Imidacloprid	Japanese Beetle	8 fl. oz.	D. Platte
8/6/15 (Tier 25 and 26e only)	Bravo WeatherStik®	Chlorothalonil	CLS, Brown rot	3.12-4.12 pts.	D. Platte
8/14/15	Mustang® Maxx	Zeta-cypermethrin	Japanese Beetle, Spotted Wing Drosophila	4 fl. oz.	D. Platte
8/14/15	Bravo WeatherStik®	Chlorothalonil	CLS, Brown rot	3.12-4.12 pts.	D. Platte
8/25/15	Bravo Ultrex ®	Chlorothalonil	CLS, Brown rot	3.43 lb.	D. Platte
8/25/15	Mustang® Maxx	Zeta-cypermethrin	Japanese Beetle	4 fl. oz.	D. Platte

Table A.2: Pesticide applications at the CRC in 2016. Applications were made in all of the orchards included in this study, not excluding evaluated trees.

Application Date	Pesticide	Active Ingredient	Target	Rate/Acre	Applicator
4/27/16	Bravo Ultrex ®	Chlorothalonil	Cherry Leaf Spot (CLS), Brown rot	2.8 lb.	D. Platte
4/27/16	Indar ® 2F	Fenbuconazole	Blossom blight, Brown rot	6 oz.	D. Platte
5/9/16	Indar ® 2F	Fenbuconazole	Brown rot	6.12 oz.	D. Platte
5/9/16	Bravo Ultrex ®	Chlorothalonil	CLS, Brown rot	2.8 lb.	D. Platte
5/9/16	Assail ®	Acetamiprid	Plum Curculio (PC)	6.52 oz.	D. Platte
5/20/16	Initiate 720	Chlorothalonil	CLS	2 qt.	D. Platte
5/20/16	Assail ®	Acetamiprid	PC	6.5 oz.	D. Platte
5/26/16	Avaunt ®	Indoxacarb	Oriental Fruit Moth, PC	6 oz.	D. Platte
5/26/16	Luna® Sensation	Fluopyram, Trifloxystrobin	CLS, Powdery Mildew	5.7 oz.	D. Platte
5/26/16	Avaunt ®	Indoxacarb	Oriental Fruit Moth	6 oz.	D. Platte
6/3/16	Actara ®	Thiamethoxam	PC	5.5 oz.	D. Platte
6/3/16	Captan	Captan	CLS	2.5 lb.	D. Platte
6/10/16	Assail ®	Acetamiprid	PC, Cherry Fruit Fly	6 oz.	D. Platte
6/10/16	Luna® Sensation	Fluopyram, Trifloxystrobin	CLS, Powdery Mildew	5 oz.	D. Platte
7/1/16	Indar ® 2F	Fenbuconazole	Brown rot	6 oz.	D. Platte
7/1/16	Carbaryl	Carbaryl	Japanese Beetle, Cherry Fruit Fly	3 qt.	D. Platte
7/13/16	Carbaryl	Carbaryl	Japanese Beetle	2 qt.	D. Platte
7/13/16	Captan Gold ®	Captan	Brown Rot, CLS	2.5 lb.	D. Platte
8/4/16	Bravo WeatherStik®	Chlorothalonil	CLS, Brown rot	2 qt.	D. Platte
8/4/16	Sherpa ®	Imidacloprid	Japanese Beetle	1 fl. oz.	D. Platte
8/9/16	Envidor®	Spirodiclofen	European Red Mite	10.9 fl. oz.	D. Platte

Table A.3: Pesticide applications at the PPRC in 2015. Applications listed were made in the orchard block where the evaluated trees were located; however, fungicide treatments to control CLS were not applied to the row of evaluated trees.

Application Date	Pesticide	Active Ingredient	Target	Rate/Acre	Applicator
5/15/15	Bravo WeatherStik®	Chlorothalonil	Cherry Leaf Spot (CLS), Brown rot	4.125 pts.	C. Outwater
5/23/15	Pristine® 38WG	Pyraclostrobin Boscalid	Blossom blight, Brown rot, CLS, Powdery Mildew	14 oz.	C. Outwater
5/23/15	Indar ® 2F	Fenbuconazole	Blossom blight, Brown rot	8 fl. oz.	C. Outwater
5/23/15	Avaunt® 30 WG	Indoxacarb	Oriental Fruit Moth (OFM)	6 oz.	C. Outwater
5/29/15	Asana® XL 0.66 EC	Esfenvalerate, Benzeneacetate	Plum Curculio (PC), Cherry Fruit Fly, OFM, Leafrollers	13 fl. oz.	C. Outwater
5/29/15	Avaunt® 30 WG	Indoxacarb	OFM	6 oz.	C. Outwater
6/13/15	Syllit® FL	Dodine	CLS, Brown rot	27 fl. oz.	C. Outwater
6/13/15	Captan® 80WDG	Captan	Brown rot, CLS, Powdery Mildew	2.5 lb.	C. Outwater
6/13/15	Assail 30 SG	Acetamiprid	PC, Cherry Fruit Fly	8 oz.	C. Outwater
6/22/15	Merivon® 4.17SC	Fluxapyroxad Pyraclostrobin	Brown rot, CLS, Powdery Mildew	6.7 fl. oz.	C. Outwater
6/22/15	Provado ®	Imidacloprid	Japanese Beetle	8 fl. oz.	C. Outwater
7/03/15	Merivon® 4.17SC	Fluxapyroxad Pyraclostrobin	Brown rot, CLS, Powdery Mildew	6.7 fl. oz.	C. Outwater
7/23/15	Syllit® FL	Dodine	CLS, Brown rot	24 fl. oz.	C. Outwater
7/23/15	Captan® 80WDG	Captan	Brown rot, CLS, Powdery Mildew	2.5 lb.	C. Outwater
7/23/15	Asana® XL 0.66 EC	Esfenvalerate, Benzeneacetate	PC, Cherry Fruit Fly, OFM, Leafrollers	14.5 fl. oz.	C. Outwater
8/17/15	Bravo WeatherStik®	Chlorothalonil	CLS, Brown rot	4.125 pts.	C. Outwater

Table A.4: Pesticide applications at the PPRC in 2016. Applications listed were made in the orchard block where the evaluated trees were located; however, fungicide treatments to control CLS were not applied to the row of evaluated trees.

Application Date	Pesticide	Active Ingredient	Target	Rate/Acre	Applicator
5/26/16	Bravo WeatherStik®	Chlorothalonil	Cherry Leaf Spot (CLS), Brown rot	4 pts.	C. Outwater
5/26/16	Asana® XL 0.66 EC	Esfenvalerate, Benzeneacetate	Plum Curculio (PC), Cherry Fruit Fly, Oriental Fruit Moth (OFM), Leafrollers	10 fl. oz.	C. Outwater
5/26/16	Avaunt® 30 WG	Indoxacarb	OFM	6 oz.	C. Outwater
6/9/16	Syllit® FL	Dodine	CLS, Brown rot	24 fl. oz.	C. Outwater
6/9/16	Captan® 80WDG	Captan	Brown rot, CLS, Powdery Mildew	2.5 lb.	C. Outwater
6/9/16	Asana® XL 0.66 EC	Esfenvalerate, Benzeneacetate	PC, Cherry Fruit Fly, OFM, Leafrollers	8 fl. oz.	C. Outwater
6/9/16	Avaunt® 30 WG	Indoxacarb	OFM	5.5 oz.	C. Outwater
6/23/16	Syllit® FL	Dodine	CLS, Brown rot	24 fl. oz.	C. Outwater
6/23/16	Captan® 80WDG	Captan	Brown rot, CLS, Powdery Mildew	2.5 lb.	C. Outwater
6/23/16	Assail 30 WP	Acetamiprid	PC, Cherry Fruit Fly	4 oz.	C. Outwater
8/01/16	Bravo WeatherStik®	Chlorothalonil	CLS, Brown rot	4.125 pts.	C. Outwater
8/01/16	Asana® XL 0.66 EC	Esfenvalerate, Benzeneacetate	PC, Cherry Fruit Fly, OFM, Leafrollers	12 fl. oz.	C. Outwater
8/15/16	Bravo WeatherStik®	Chlorothalonil	CLS, Brown rot	4.125 pts.	C. Outwater

APPENDIX B

2016 Clarksville Research Center CLS Progression Results

Figure B.1: Progression of percent incidence of CLS for all clones located at the CRC and PPRC that were evaluated for their response to the disease in 2016. Statistical analyses can be found in Table B.1.

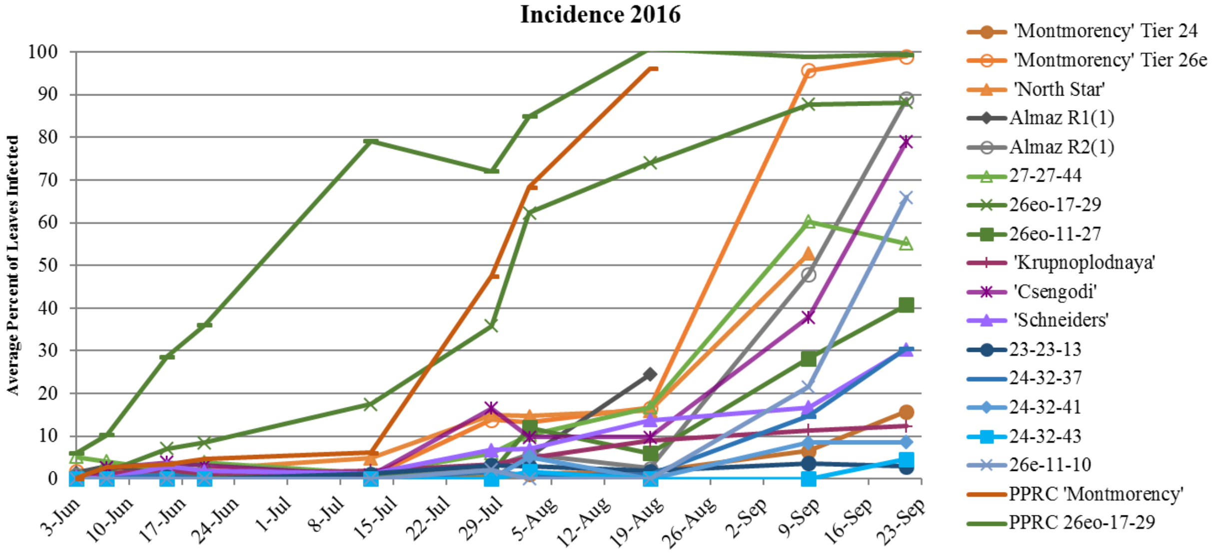


Table B.1: Analysis of variance for percent incidence of CLS for all clones located at the CRC and PPRC that were evaluated for their response to the disease in 2016.

	Weeks 1-3 6/3/16-6/17/16		Weeks 4-6 6/20/16-7/5/16		Weeks 7-9 7/12/16-7/28/16		Weeks 10-12 7/31/16-8/19/16		Weeks 13-15 8/22/16-9/8/16		Weeks 16-18 9/12/16-9/21/16	
‘Montmorency’	0.47	b[1]	0.53	b	0.17	c	1.51	c	6.58	h	15.76	de
‘Montmorency’ Tier 26e	1.52	b	1.17	b	7.25	bc	14.93	c	95.60	ab	98.96	a
‘Montmorency’ PPRC	2.63	b	6.84	b	18.08	b	81.08	ab	99.58	a	100	a
26eo-17-29	2.93	b	8.48	b	26.63	b	68.20	b	87.67	b	88.18	ab
26eo-17-29 PPRC	17.13	a	55.05	a	77.24	a	91.90	a	98.26	a	99.33	a
‘North Star’	1.65	b	2.43	b	9.93	bc	15.37	c	52.77	c	79.24	ab
26eo-11-27	0.38	b	0.62	b	1.05	c	9.025	c	28.20	ef	40.78	cd
‘Csengodi’	2.16	b	3.09	b	8.73	bc	9.83	c	37.92	de	79.09	ab
27-27-44	3.79	b	3.85	b	3.68	c	13.53	c	60.25	c	55.14	bc
‘Krupnoplodnaya’	1.31	b	1.33	b	2.63	c	6.91	c	11.42	gh	12.36	de
‘Schneiders’	1.15	b	1.84	b	4.02	bc	10.42	c	16.72	fg	30.41	cde
Almaz R1(1)	2.00	b	0	b	0	c	14.89	c	-	-	-	-
Almaz R2(1)	1.14	b	0	b	1.43	c	4.13	c	47.99	cd	88.98	ab
24-32-41	0	b	0	b	0	c	2.57	c	8.46	gh	8.64	de
24-32-43	0	b	0	b	0	c	0.86	c	0	h	4.69	de
23-23-13	0.15	b	0	b	2.20	c	2.38	c	3.66	h	2.92	e
24-32-37	0.33	b	0	b	0.83	c	0.92	c	14.69	fgh	30.56	cde
26e-11-10	0	b	0	b	1.11	c	0	c	21.52	efg	66.01	abc

[1] Letters adjacent to values indicate statistical significance within columns at α=0.05.

Figure B.2: Progression of CLS lesion density (lesions/cm^2) for all clones located at the CRC and PPRC that were evaluated for their response to the disease in 2016. Statistical analyses can be found in Table B.2.

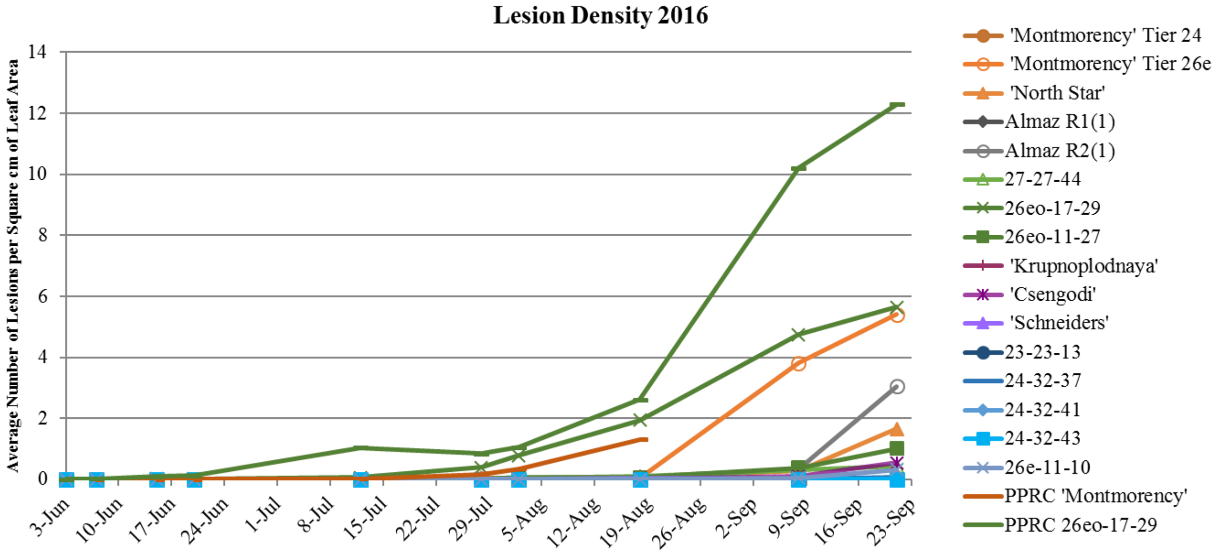


Table B.2: Analysis of variance for CLS lesion density (lesions/cm^2) for all clones located at the CRC and PPRC that were evaluated for their response to the disease in 2016.

	Weeks 1-3 6/3/16-6/17/16		**Weeks 4-6** 6/20/16-7/5/16		**Weeks 7-9** 7/12/16-7/28/16		**Weeks 10-12** 7/31/16-8/19/16		**Weeks 13-15** 8/22/16-9/8/16		**Weeks 16-18** 9/12/16-9/21/16	
‘Montmorency’	0.0004	b[1]	0.0003	b	0.00008	b	0.001	c	0.01	b	0.03	c
‘Montmorency’ Tier 26e	0.0008	b	0.001	b	0.004	b	0.02	c	3.81	ab	5.41	b
‘Montmorency’ PPRC	0.004	b	0.009	b	0.05	b	0.82	b	7.36	a	10.43	a
26eo-17-29	0.009	b	0.02	ab	0.2	b	1.37	ab	4.74	ab	5.65	b
26eo-17-29 PPRC	0.04	a	0.30	a	0.8	a	2.21	a	7.48	a	12.30	a
‘North Star’	0.001	b	0.004	b	0.02	b	0.06	c	0.28	b	1.66	bc
26eo-11-27	0.0005	b	0.0003	b	0.002	b	0.06	c	0.38	b	1.01	bc
‘Csengodi’	0.002	b	0.002	b	0.01	b	0.01	c	0.09	b	0.54	bc
27-27-44	0.004	b	0.004	b	0.01	b	0.07	c	0.31	b	0.42	c
‘Krupnoplodnaya’	0.0004	b	0.004	b	0.002	b	0.004	c	0.007	b	0.02	c
‘Schneiders’	0.001	b	0.002	b	0.009	b	0.02	c	0.02	b	0.05	c
Almaz R1(1)	0.001	b	0	b	0	b	0.04	c	-	-	-	-
Almaz R2(1)	0.0006	b	0	b	0.0008	b	0.002	c	0.35	b	3.05	bc
24-32-41	0	b	0	b	0	b	0.002	c	0.005	b	0.008	c
24-32-43	0	b	0	b	0	b	0.0008	c	0	b	0.004	c
23-23-13	0.00008	b	0	b	0.002	b	0.002	c	0.002	b	0.002	c
24-32-37	0.0005	b	0	b	0.001	b	0.001	c	0.01	b	0.06	c
26e-11-10	0	b	0	b	0.0005	b	0	c	0.04	b	0.31	c

[1] Letters adjacent to values indicate statistical significance within columns at α=0.05.

Figure B.3: Progression of percent defoliation caused by CLS for all clones located at the CRC and PPRC that were evaluated for their response to the disease in 2016. Statistical analyses can be found in Table B.3.

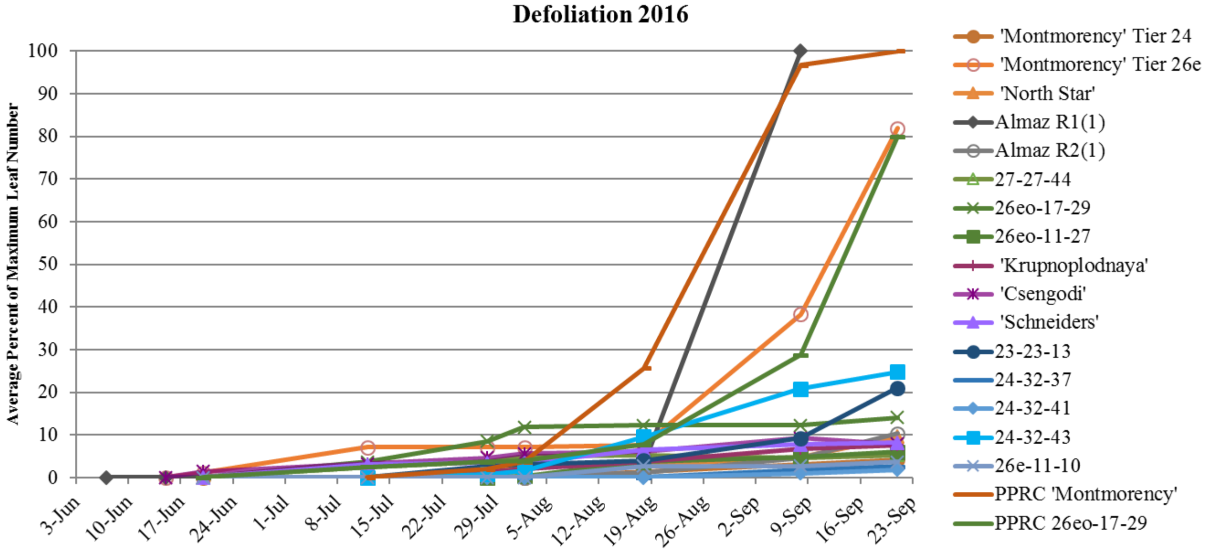
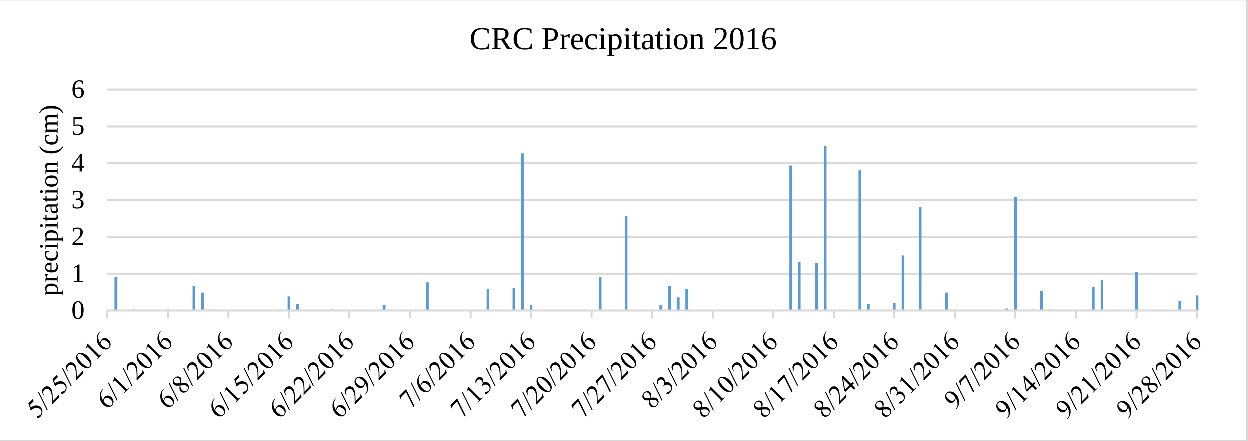


Table B.3: Analysis of variance for percent defoliation caused by CLS for all clones located at the CRC and PPRC that were evaluated for their response to the disease in 2016.

	Weeks 1-3 6/3/16-6/17/16		**Weeks 4-6** 6/20/16-7/5/16		**Weeks 7-9** 7/12/16-7/28/16		**Weeks 10-12** 7/31/16-8/19/16		**Weeks 13-15** 8/22/16-9/8/16		**Weeks 16-18** 9/12/16-9/21/16	
‘Montmorency’	0	a[1]	0.61	bc	3.38	ab	3.46	b	5.80	b	6.90	c
‘Montmorency’ Tier 26e	0	a	1.34	bc	7.29	a	7.64	ab	38.97	ab	82.14	a
‘Montmorency’ PPRC	-	-	0.11	c	1.73	ab	15.93	a	71.32	a	99.75	a
26eo-17-29	-	-	0	c	6.09	ab	12.00	ab	12.16	b	14.00	c
26eo-17-29 PPRC	1.52	a	2.64	ab	6.01	ab	7.84	ab	15.24	b	61.63	ab
‘North Star’	0	a	7.31	a	7.32	a	4.76	ab	8.11	b	12.29	c
26eo-11-27	-	-	-	-	0	ab	1.99	b	4.78	b	6.01	c
‘Csengodi’	0	a	1.42	bc	7.06	ab	5.84	ab	9.33	b	7.92	c
27-27-44	0.73	a	1.68	bc	4.46	ab	6.48	ab	5.94	b	6.68	c
‘Krupnoplodnaya’	-	-	0	c	0.63	ab	2.97	b	6.75	b	7.56	c
‘Schneiders’	0	a	1.29	bc	4.52	ab	6.27	ab	9.08	b	9.62	c
Almaz R1(1)	-	-	-	-	-	-	3.08	b	100	a	100	a
Almaz R2(1)	-	-	-	-	0	ab	0.57	b	4.59	b	10.34	c
24-32-41	-	-	-	-	0	ab	0	b	0.94	b	1.89	c
24-32-43	-	-	-	-	0.4	ab	5.6	ab	20.8	ab	24.8	bc
23-23-13	-	-	-	-	1.43	ab	3.76	ab	9.28	b	20.52	c
24-32-37	-	-	-	-	0	ab	0	b	1.77	b	2.65	c
26e-11-10	-	-	0	c	0.45	ab	1.36	b	2.73	b	3.64	c

[1] Letters adjacent to values indicate statistical significance within columns at α=0.05.

Figure B.4: Precipitation (cm) experienced during the 2016 season at the CRC [1]. Data were obtained from the Michigan State University Enviro-weather Automated Weather Station Network (Michigan State University Board of Trustees, 2011).



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[1] Due to equipment failures, the CRC precipitation data from 7/14/16 to 8/3/16 and from 8/21/16 to 9/30/16 is that which was recorded at the weather station located in Belding, MI approximately 20 miles (32 km) North of the Clarksville Research Center.

APPENDIX C

Family Pedigrees

Figure C.1: Pedigree of the 'North Star' × 27e-05-33 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

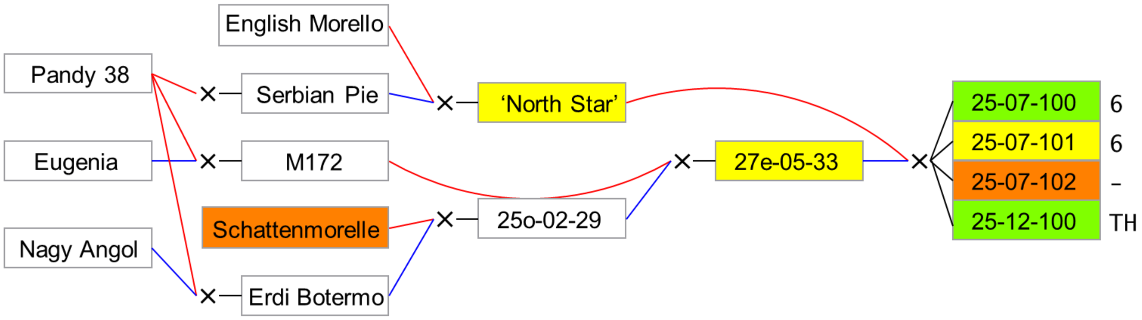


Figure C.2: Pedigree of the 25o-14-20 × 'North Star' family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

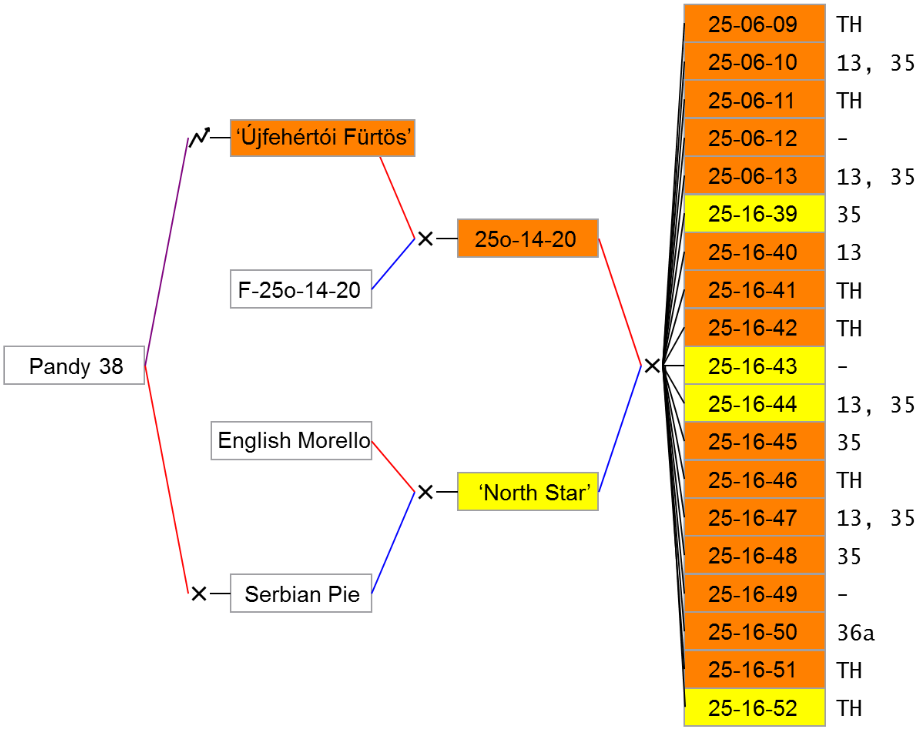


Figure C.3: Pedigree of the ‘Újfehértói Fürtös’ × I-13-61 family, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. No alleles at the *S*-locus have been identified for these progeny individuals (noted by the dash (-) to the right of each individual).

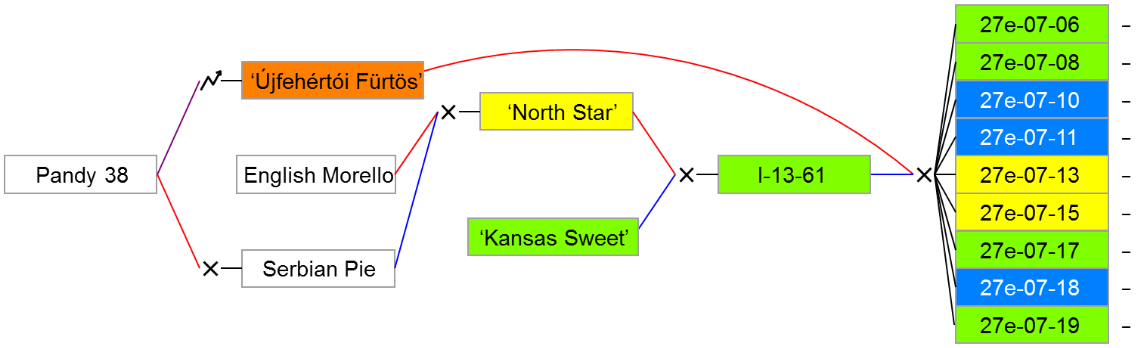


Figure C.4: Pedigree of half-sib families 27-27-44 × 27e-15-38 and 27-27-44 x 27e-16-47, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

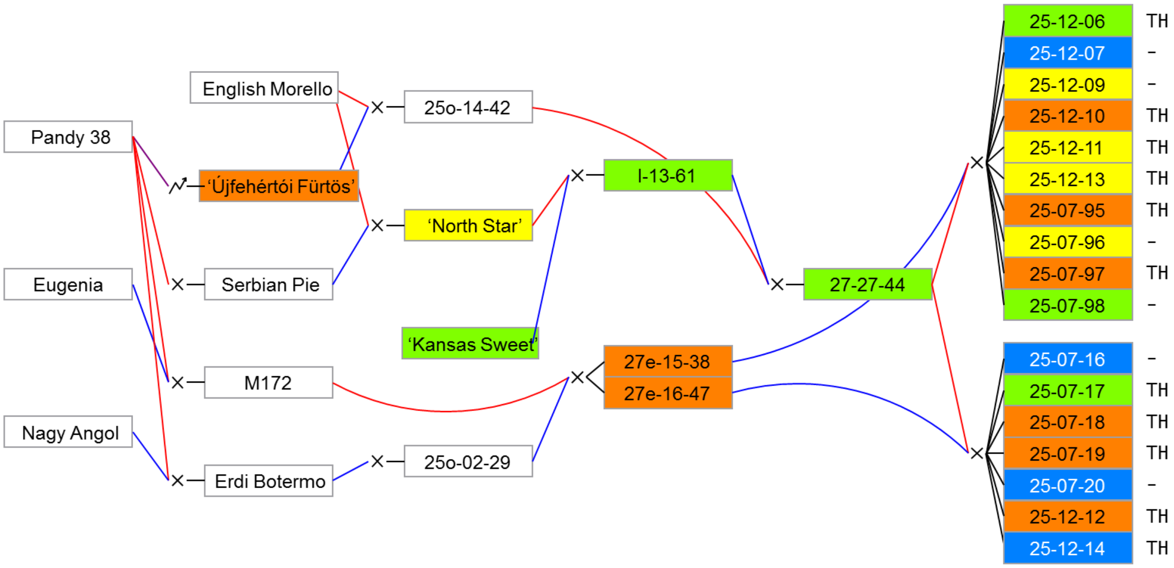


Figure C.5: Pedigree of the 27-27-44 × ‘Montmorency’ family, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

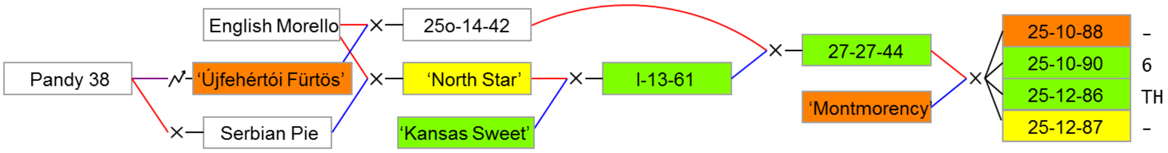


Figure C.6: Pedigree of the ‘Erdi Jubileum’ × 27-27-44 family, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. No alleles at the *S*-locus have been identified for the progeny individual (noted by the dash (-) to its right).

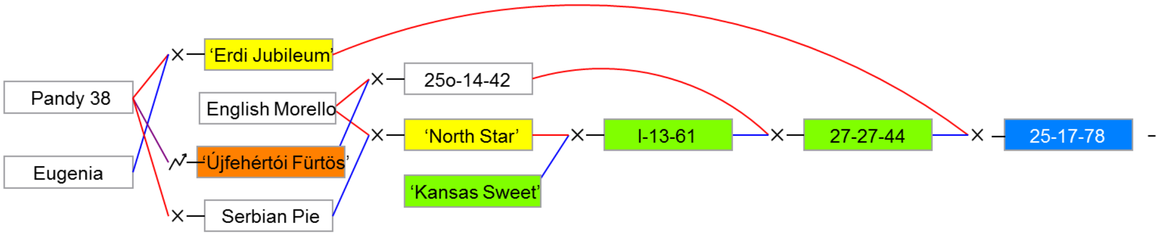


Figure C.7: Pedigree of the 27-08-30 × 27-27-44 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).

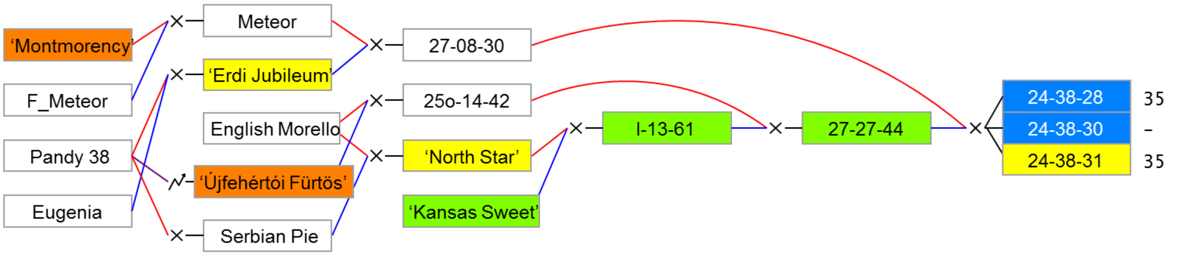


Figure C.8: Pedigree of the 26eo-08-02 × 27-27-44 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).

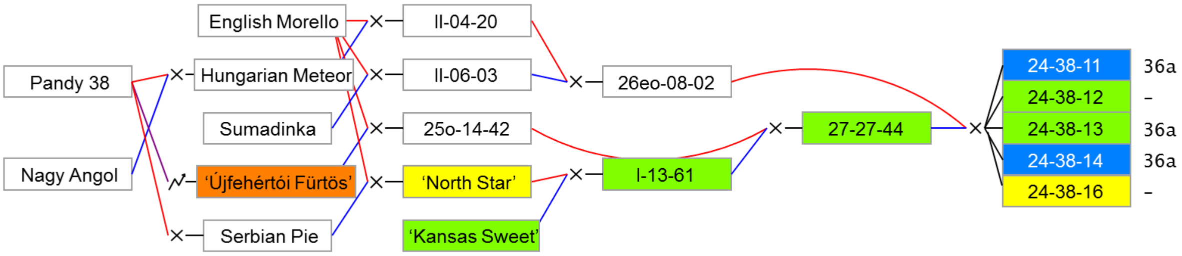


Figure C.9: Pedigree of the I-63-05 × 27-27-44 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).

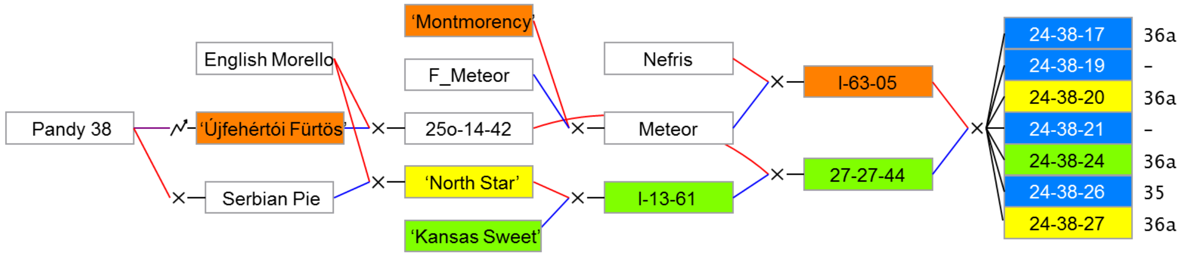


Figure C.10: Pedigree of the I-63-05 × 26eo-17-29 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).

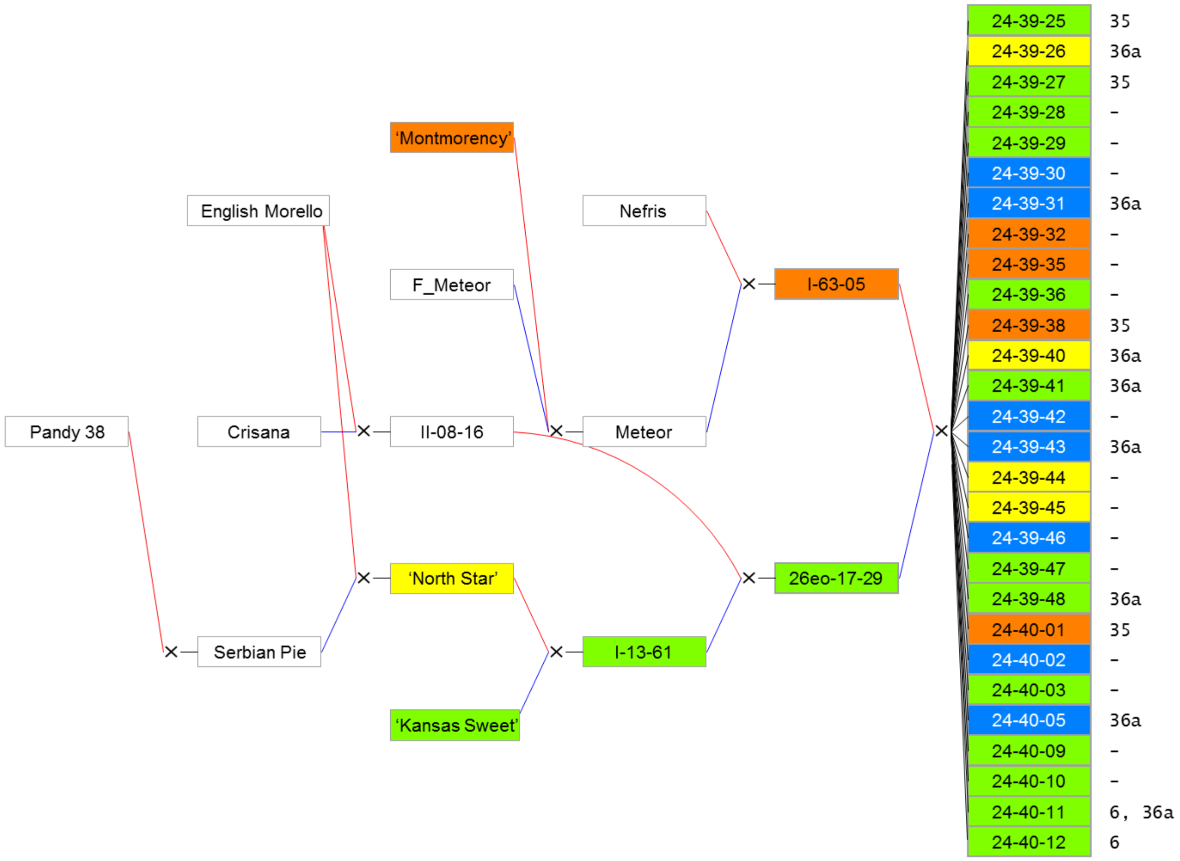


Figure C.11: Pedigree of the 26eo-17-29 × 27-03-08 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

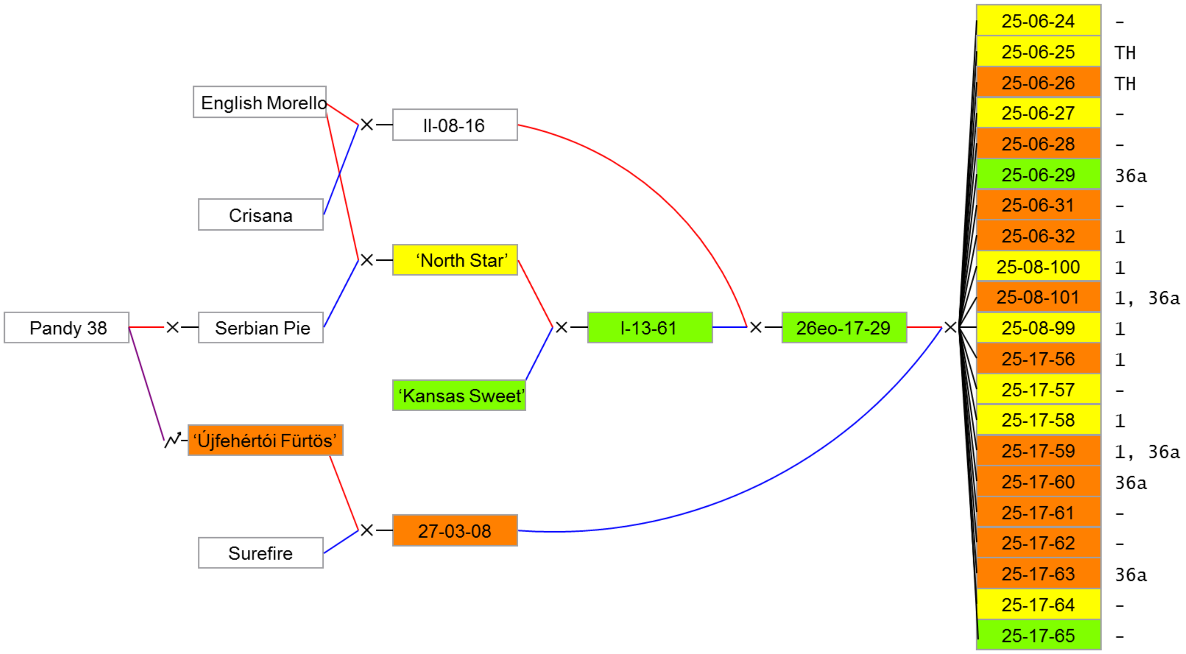


Figure C.12: Pedigree of the 26eo-17-29 × Open Pollinated family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. No alleles at the *S*-locus have been identified for the progeny individual (noted by the dash (-) to its right).

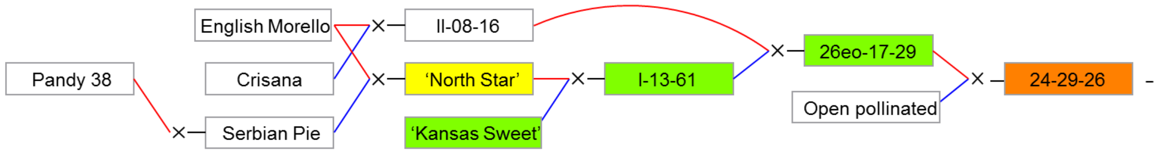


Figure C.13: Pedigree of the 24-32-37 × 27e-05-33 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted in the second column to the right of progeny individuals; those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

* CLS028 resistance allele at the *CLSR_G4* locus is present

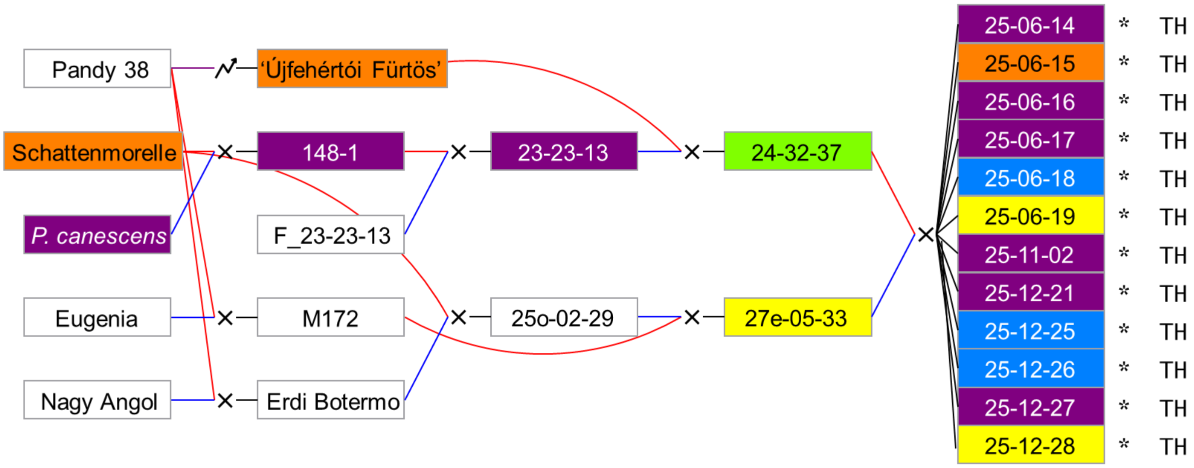


Figure C.14: Pedigree of 23-23-13 × 23-23-07 and the reciprocal cross 23-23-07 × 23-23-13, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted in the second column to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).

* CLS028 resistance allele at the *CLSR_G4* locus is present
† CLS028 resistance allele at the *CLSR_G4* locus is absent
- Results inconclusive for the CLS208 resistance allele at the *CLSR_G4* locus

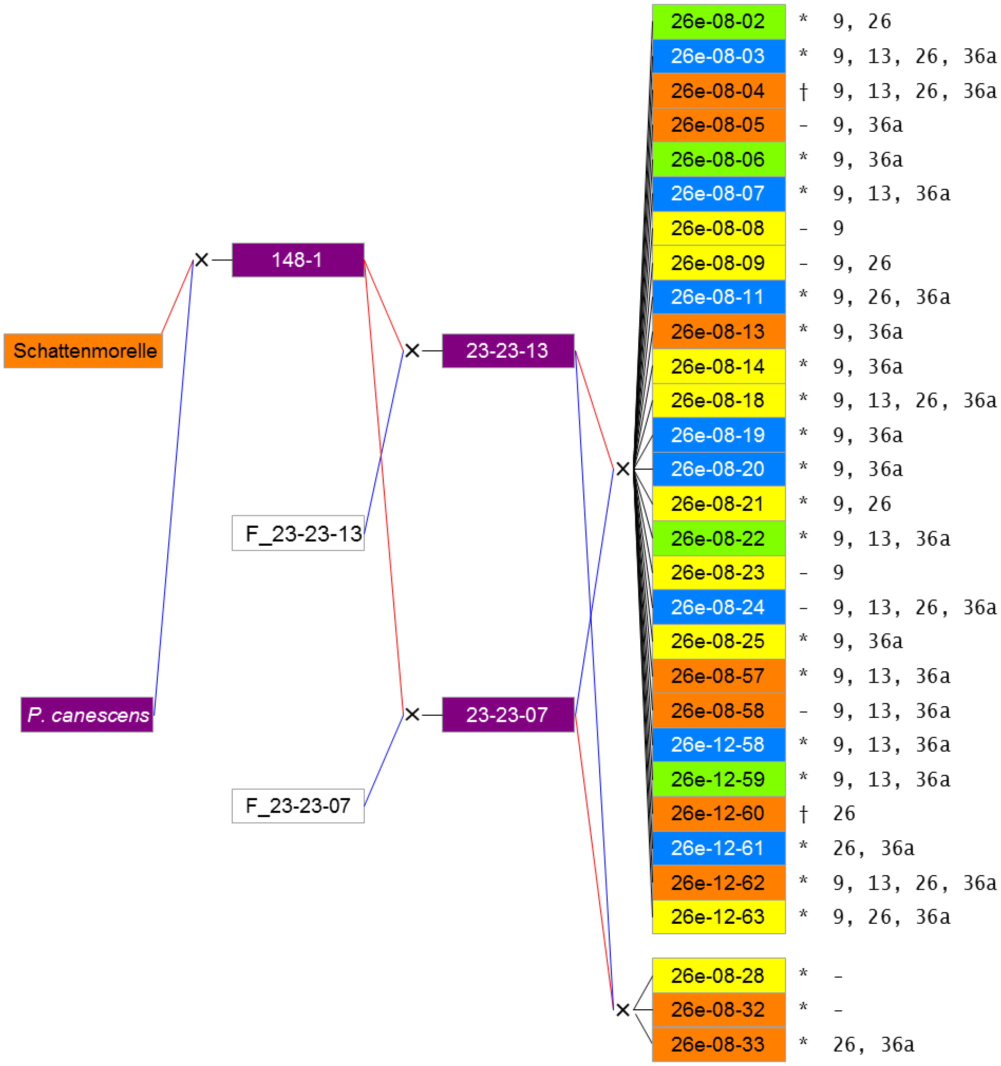


Figure C.15: Pedigree of the 27-27-10 × Open Pollinated family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. No alleles at the *S*-locus nor the CLS028 resistance allele at the *CLSR_G4* locus have been identified for the progeny individual (noted by the pair of dashes (-) to its right).

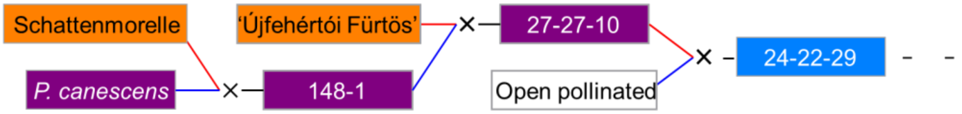


Figure C.16: Pedigree of the M172 × 24-32-43 family, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. No alleles at the *S*-locus nor the CLS028 resistance allele at the *CLSR_G4* locus have been identified for the progeny individuals (noted by the pair of dashes (-) to the right of each individual).

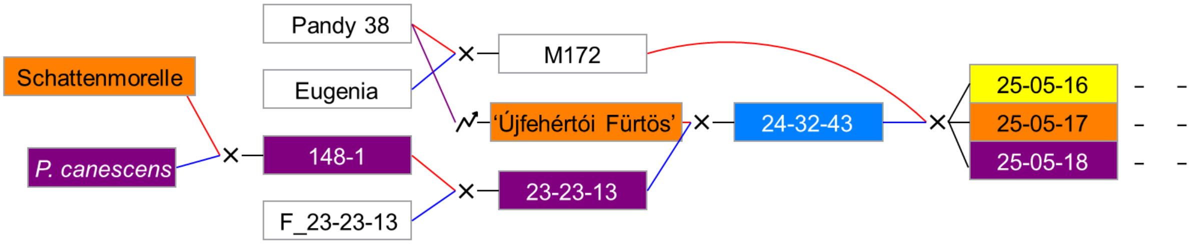


Figure C.17: Pedigree of the ‘Montmorency’ × 23-23-13 family, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted in the second column to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

* CLS028 resistance allele at the *CLSR_G4* locus is present

† CLS028 resistance allele at the *CLSR_G4* locus is absent

\- Results inconclusive for the CLS208 resistance allele at the *CLSR_G4* locus

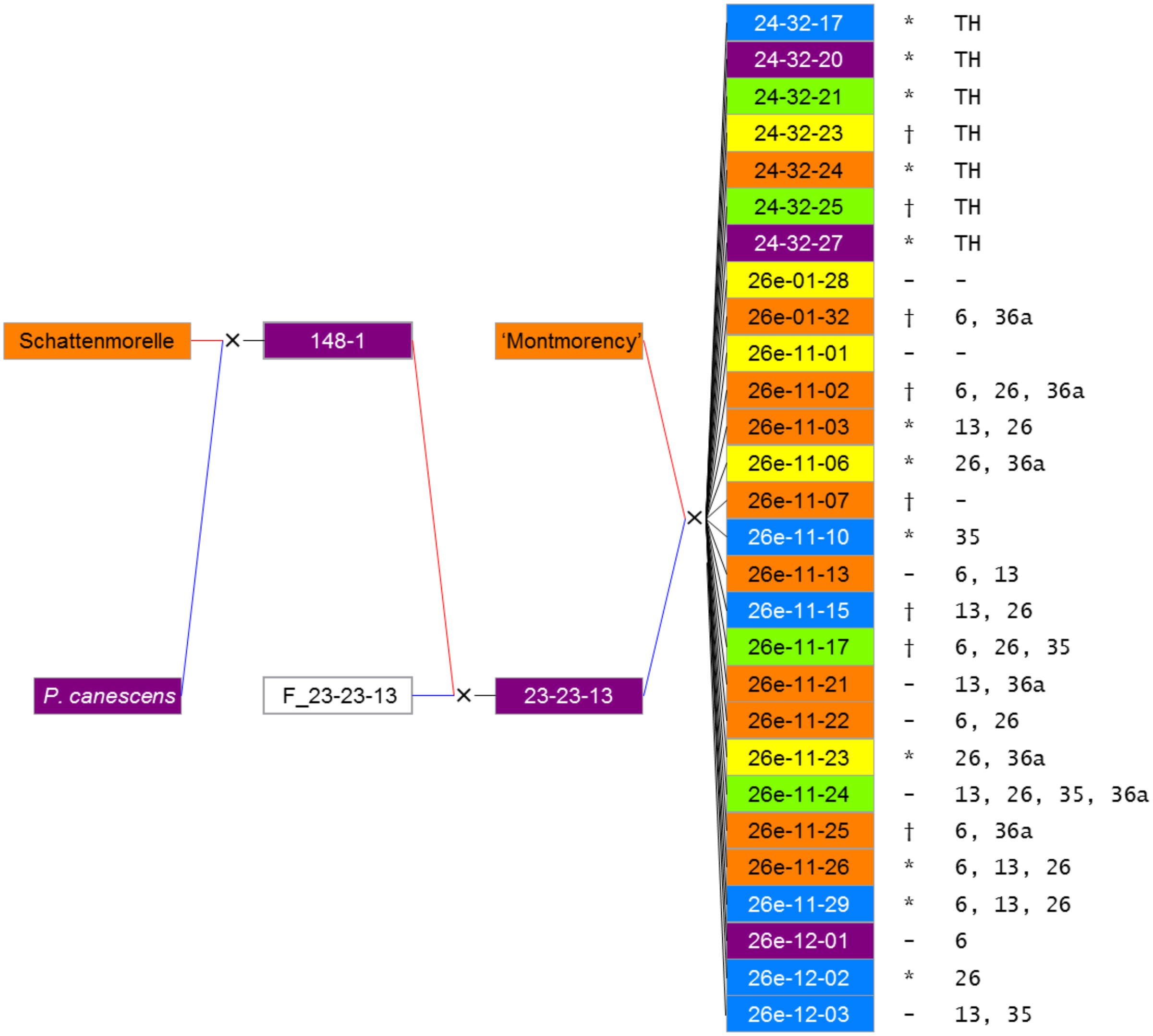


Figure C.18: Pedigree of the ‘Montmorency’ × 24-32-41 family, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted in the second column to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).

* CLS028 resistance allele at the *CLSR_G4* locus is present
† CLS028 resistance allele at the *CLSR_G4* locus is absent
\- Results inconclusive for the CLS208 resistance allele at the *CLSR_G4* locus

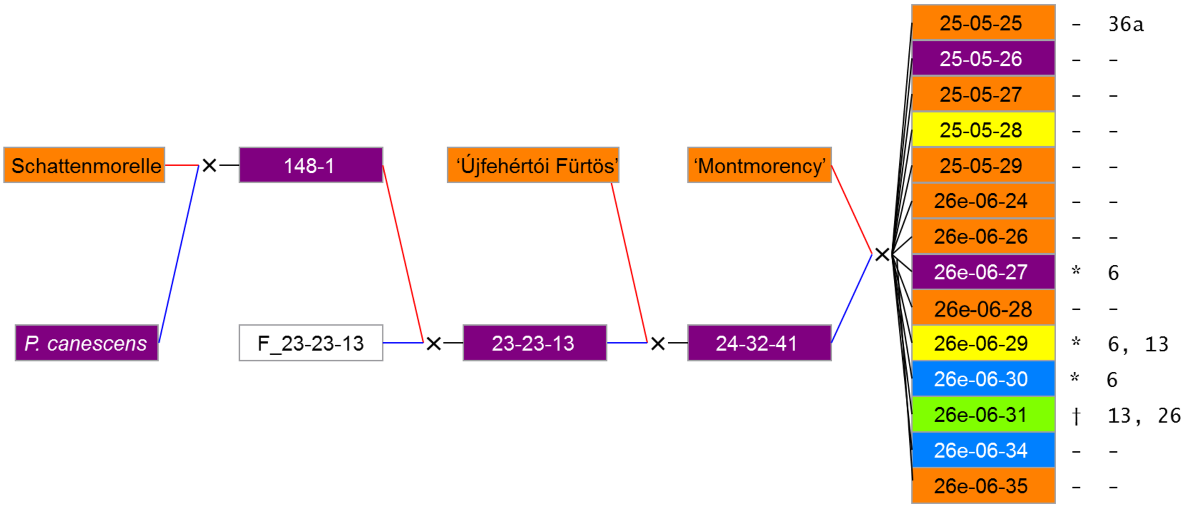


Figure C.19: Pedigree of the ‘Montmorency’ × 24-32-41 family, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted in the second column to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

* CLS028 resistance allele at the *CLSR_G4* locus is present
† CLS028 resistance allele at the *CLSR_G4* locus is absent
- Results inconclusive for the CLS208 resistance allele at the *CLSR_G4* locus

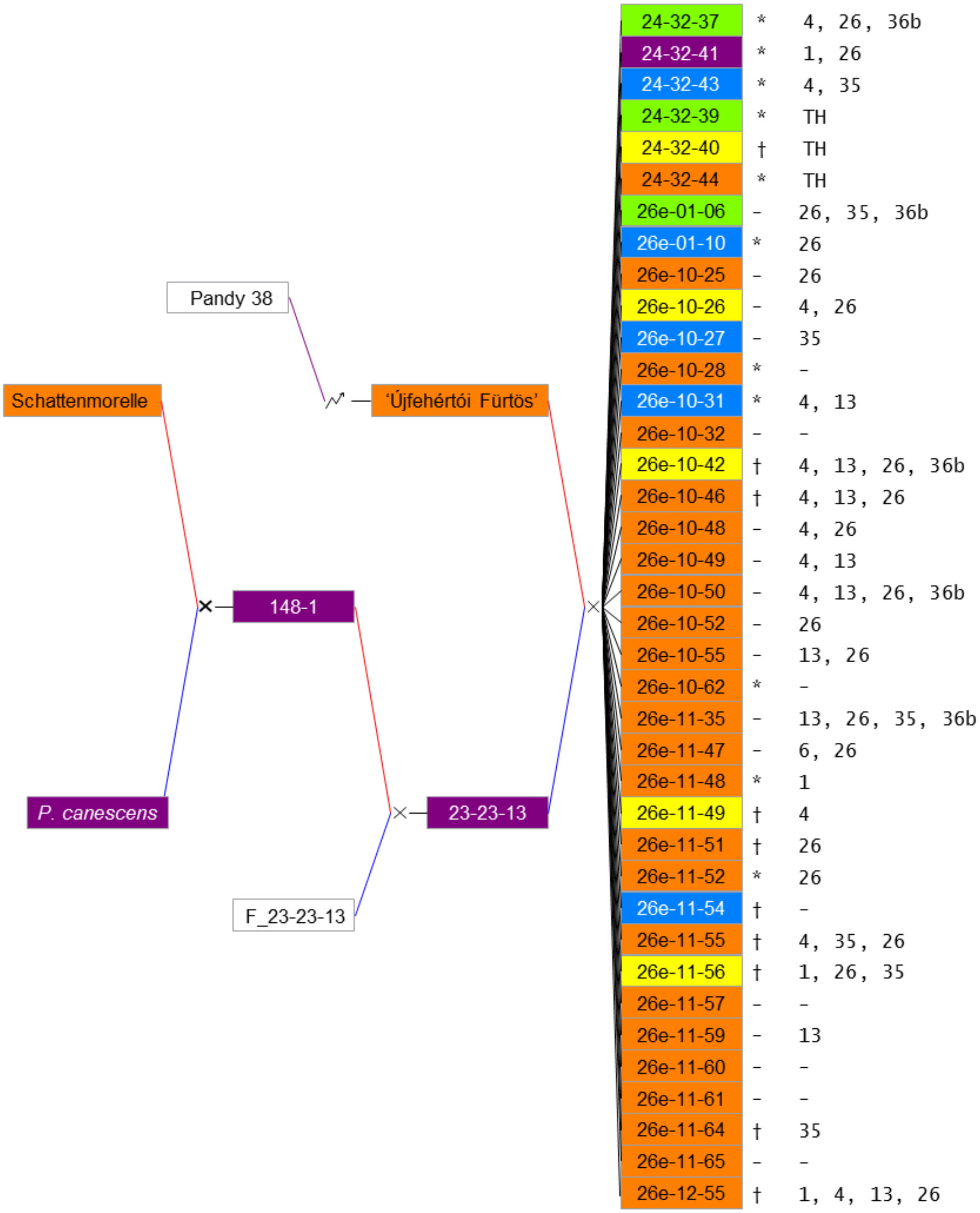


Figure C.20: Pedigrees of families with *P. maackii* derived Almaz R1(1) as a common parent, generated using Pedimap 1.2©. Colors indicate an individual's comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted in the second column to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-); those that were identified as likely true hybrids by T. Stegmeir prior to this study (per communication) are noted with a TH (True Hybrid), specific alleles are not known for these individuals.

* CLS028 resistance allele at the *CLSR_G4* locus is present
† CLS028 resistance allele at the *CLSR_G4* locus is absent
- Results inconclusive for the CLS208 resistance allele at the *CLSR_G4* locus

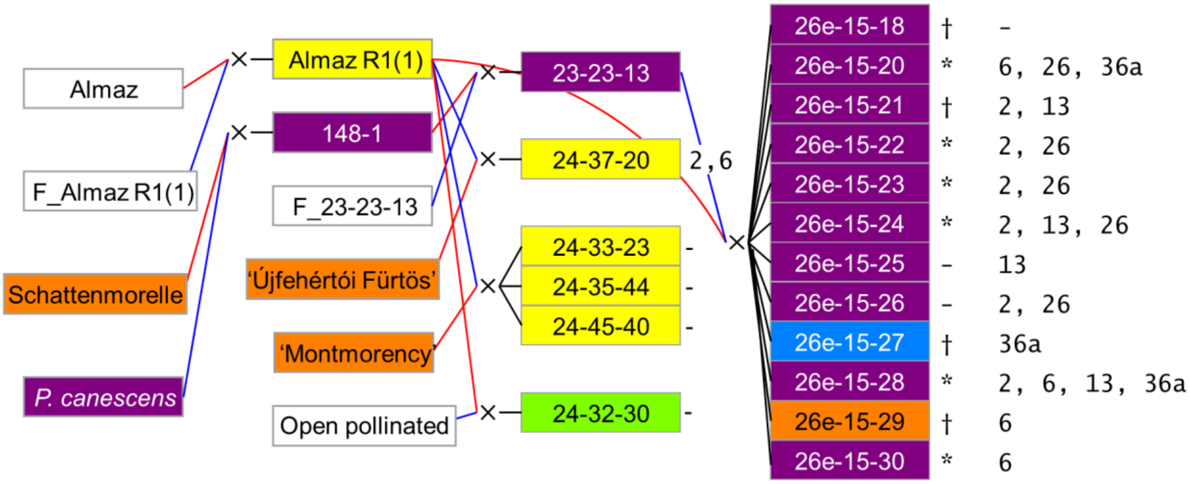
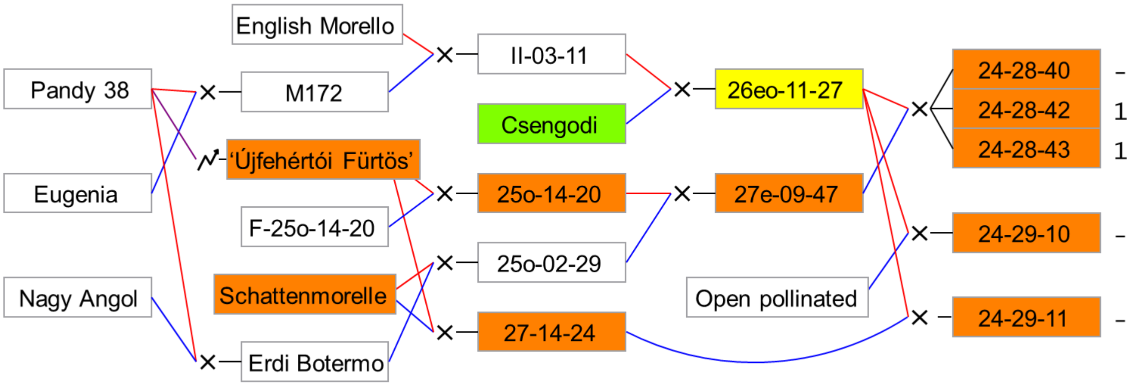


Figure C.21: Pedigrees of families with tetraploid *P. avium* cultivar ‘Csengodi’ derived 26eo-11-27 as a common parent, generated using Pedimap 1.2©. Colors indicate an individual’s comprehensive disease score, according to the scale outlined in Figure 3.1. Alleles identified at the *S*-locus are noted to the right of progeny individuals; individuals for which no *S*-locus alleles have been identified are noted with a dash (-).



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