RESISTANCE OF GARDEN ROSES TO CERCOSPORA LEAF SPOT

A Thesis

by

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ABSTRACT

Cercospora rosicola is a fungal pathogen that attacks roses and causes spotting on leaves, chlorosis and in severe cases, defoliation. This disease has become more prominent in roses after the widespread use of black spot resistant roses, most likely due to the reduction of fungicide applications on roses. The objective of this thesis is to evaluate garden roses and identify QTLs for resistance to cercospora leaf spot disease. Identification of susceptible and resistant roses to cercospora leaf spot is the first step for breeding cercospora resistant rose cultivars. Cercospora leaf spot was evaluated on a percentage-based rating scale of 0-9 (0=no disease and 9=foliage covered with lesions) during spring, summer and fall of 2016 through 2018 on 130 roses in Overton and College Station, TX. Average cercospora incidence in Overton increased from 2016 to 2017, but decreased in 2018, whereas in College Station, ratings increased three-fold from 2016 (0.6) to 2018 (2.2). Most cultivars showed tolerance to the disease, particularly *Rosa rugosa* hybrids. However, a number of cultivars including 'American Pillar', 'John Davis', 'Carefree Delight', 'Oso Happy Candy Oh', 'Oso Easy Cherry Pie' and 'Roxanne Veranda' were highly susceptible to cercospora. In College Station, moderately high repeatability and low coefficient of variance occurred in the later months of 2017 and 2018, while in Overton, all the evaluated months in 2016 except for April showed high repeatability and low coefficient of variance. This suggests that ratings during these months are consistent, and may be the more informative months for disease evaluation. Artificial inoculation in a greenhouse setting was also attempted to identify cercospora leaf spot resistant garden roses. Although the pathogen was successfully cultured, the low sporulation, high heat and low humidity during the inoculation

period slowed disease development. Thus, severe disease symptoms were not observed for this experiment.

In 2016, fifteen diploid inter-related rose populations were evaluated for cercospora leaf spot in June, September, October and November in College Station. The estimated broad sense and narrow sense heritability were 0.83 and 0.57, respectively. A pedigree-based analysis using Visual FlexQTL software was conducted on these populations. QTLs found on LG1, LG3 and LG7 appeared in different environments. In the overall analysis, QTLs found on LG1 (0-4cM) and LG3 (36-42cM) explained 8.5% and 7.7% of the total phenotypic variance, respectively. More studies are needed to improve the strength and consistency of QTL detection in this analysis.

DEDICATION

This thesis is dedicated to my father, Yang Woo Kang and my mother, Soo Young Kim for their love and unconditional support. I am also thankful to my sisters Swanie, Serena and Sharon Kang for their patience and encouragement during times of stress.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Economic importance of roses

Roses (*Rosa* spp.) belong to the Rosaceae family, an economically important family that includes apple, peach, strawberry, almonds, medicinal plants, ornamental plants and timber crops. Roses are one of the world's most popular flowering plants for their continuously blooming nature and versatility. They are used as ornamental plants for landscaping, gardening and potted plants, cut flowers for floral design, perfume and oil for aesthetics, and the rose hips are used as a source of vitamin C (Zlesak, 2006). Cut rose and the landscape rose are the largest markets. Their imported value to the USA is $US 519 million and $US 12 million, respectively (World Trade Map, 2015). According to the Floriculture Crop Summary (USDA and NASS, 2016), roses value around $US 203.5 million, and they contribute around 3.6% of horticultural plants produced in the USA (Green Industry Research Consortium, 2008).

Rose gardens and the recurrent blooming roses were cultivated in China for more than 2000 years (Guoliang, 2003). After the introduction of Asian roses to Europe, roses were exposed to the exchange of other traits that gave rise to new cultivars. Zlesak (2006) mentions that modern cultivars arose from the hybridization and interbreeding of 7 to 10 rose species such as *R. chinensis*, *R. damascena*, *R. multiflora*, *R. rugosa* and *R. foetida*, which are thought to have been chosen based on availability, physical features or higher seed set (de Vries and Dubois, 1996). Currently, there are more than 100 species of rose and over 44,000 commercial rose varieties (Cairns, 2000).

Roses have a base chromosome number x=7 and ploidy levels range from diploids to decaploids. Most rose cultivars are diploids, triploids or tetraploids (Jian et al., 2010; Zlesak,

2006). Roses are highly heterozygous and prone to moderate inbreeding depression. The inbreeding between modern rose classes may have led to reduced fertility (Zlesak, 2006). Self-incompatibility is common among diploid roses; however, self-fertilization is observed to be common in tetraploid roses suggesting a breakdown of this system at higher ploidy levels (Ueda and Akimoto, 2001).

Cercospora leaf spot of roses

Among the most common rose foliar diseases in the southeastern USA are black spot (*Diplocarpon rosae*), powdery mildew (*Sphaerotheca pannosa*) and cercospora leaf spot (*Rosisphaerella rosicola* (teleomorph: *Mycosphaerella rosicola*, syn: *Passalora rosicola*, *Cercospora rosicola* Pass. and *Pseudocercospora puderi*) (Mangandi and Peres, 2009; Videira et al., 2017). Compared to the effects of black spot and powdery mildew, cercospora has a lesser economic effect on roses. Recently this disease is becoming a larger threat to susceptible cultivars, most likely due to decreasing application of fungicides and the development of roses with higher black spot resistance. Unfortunately, little work has been done on either the pathogen or the disease.

Rosisphaerella rosicola was first described by Passerini in 1874 (Davis, 1938) and it was first collected in the USA in 1882 in Florida. Currently, it is distributed worldwide. The symptoms of cercospora leaf spot resemble those of black spot and thus it is often misdiagnosed. Cercospora leaf spot commonly appears on rose leaves, pedicels, stems and bracts (Mangandi and Peres, 2009). Lesions appear as dark circular spots with a dark red or purple halo and a tan center. Usually, the lesion size is 2-4 mm in diameter but it can reach up to 10 mm depending on the cultivar. Initially, the lesion will be a condensed dark red to purple color and gradually turn a dark brown to black with a tan necrotic spot. As the lesion grows, the tan area will widen and

dark spots, known as the stromata, appear scattered within the tan necrotic center. These dark stromata are the site of conidia growth and development. It mainly develops on the adaxial side of leaf but can also occur on the abaxial side. The conidia overwinter on the leaves and are dispersed by water splashing and wind (White and Klingeman, 2014). The infection typically starts from the bottom of the canopy and progresses upward to the newer growth.

Cercospora life cycle

Cercospora spp infect multiple plant species worldwide, and have an economic impact on the production of peanuts, coffee, sugar beets, corn, soybean and landscape plants such as hydrangea and roses (Pham et al., 2015; Rupe et al., 1982; Smith and Gaskill, 1970; Souza et al., 2012; Stalker, 1984; Vann, 2010). There are 659 species recognized in the genus, although approximately 3000 different names have been proposed (Crous and Braun, 2003). Chupp (1954) considered cercospora to be generally a host specific pathogen; however, this has been difficult to test (Crous, 2009). Recent studies indicate that the same pathogen species can be found in different hosts, such as *C. apii*, which is found on both celery and sugar beet, and some seem to operate at a strain level (Groenewald et al., 2006). This applies to *C. rodmanii* and its host *Eichhornia crassipes*, where some isolates of the pathogen also affected beet and sugar beet (Montenegro-Calderón et al., 2011).

C. rosicola conidiophore is straight, occasionally septate with a dark base and bound loosely in the fascicle. The conidia are linear or slightly curved; obclavate, long and narrow with 1 to 6 septations. They can be up to 45-120 µm long and 5 to 6 µm wide. *C. rosicola* is easily distinguishable by its slightly thickened conidial scars, long conidiophores, and lack of stromata (Nakashima, 2004).

Based on other cercospora diseases, factors such as leaf wetness, relative humidity, temperature and amount of inoculum play a crucial role for spore germination and disease development (Cooperman and Jenkins, 1986; Rupe et al., 1982). For cultures and sporulation, the species differ in their requirements for light, medium and temperature.

For *Cercospora* spp infection, high relative humidity and warm temperature are required. There is a wide temperature range (15-35°C) for *Cercospora* spp infection which varies with the species. The optimal range for infection for *C.zeae-maydis* on corn was 22-28°C (Beckman and Payne (1983), for *C. asparagi*, on asparagus was 25°C (Cooperman and Jenkins, 1986) and for *C. kikuchii* on soybean was from 22-31°C (Vathakos and Walters, 1979). Light and temperatures between 22-26°C increase production of cercosporin, a toxin that influences disease severity in soybean (*C. kikuchii*), sweet beet (*C. beticola*), coffee (*C. coffeicola*) and bananas (*C. musae*) (Daub and Ehrenshaft, 2000). *Cercospora* spp lack toxicity in dark, and the fungus does not produce cercosporin in temperatures greater than 30°C (Daub and Chung, 2007). It is critical to maintain high moisture for the first 96-168 hours for spore survival and germination and periods of dryness between the first hours or limited leaf wetness may inhibit germination or kill *C. zeae-maydis* spores (Rupe et al., 1982). In contrast, continuous free water reduces appressorial formation and penetration. The concentration of conidia is critical for disease development with most work on maize using $5x10^4$ conidia/mL (Beckman and Payne, 1983) and work with asparagus using $5x10^3$ conidia/mL or higher of *C. asparagi* causing defoliation and chlorosis (Cooperman and Jenkins, 1986).

Isolation and culturing the pathogen is difficult due to its slow growth, poor sporulation rate as well as the failure to obtain consistent lesions on the plant when artificially inoculated (Beckman and Payne, 1983; Cooperman and Jenkins, 1986). It is reported that cercospora

sporulates best in a media that is similar on which it sporulates in natural conditions (Vathakos and Walters, 1979). When compared with potato dextrose agar (PDA), V8, and other plant-based agar (carrot leaf decoction, asparagus decoction, soybean leaf decoction and green corn leaf decoction); generally, PDA had the least amount of sporulation while the other media had abundant sporulation. A photoperiod of 12 hours is optimum for sporulation however, depending on the *Cercospora* spp., the conidia can germinate in both complete darkness and light (Cooperman and Jenkins, 1986; El-Gholl et al., 1982; Souza et al., 2012).

With *C. asparagi*, virulence of the pathogen and ability to sporulate can be maintained for 10 to 20 transfers and will decline with further transfers (Cooperman and Jenkins, 1986). El-Gholl et al. (1982) and Vathakos and Walters (1979) were able to continue sporulation of *C. kikuchii* by selective subculturing and maintain sporulating cultures for 30 transfers and 2 years, respectively. However, the spore production declined after each transfer. Beckman and Payne (1983) were able to recover sporulating cultures of *C. zeae-maydis* by storing them on either culture slants or agar strips for 23 months or lyophilized strips for 8 months at 4°C.

Whole plant inoculation is a feasible method used for cercospora inoculation. *Cercospora* spp take from 10 days, in the case of soybean, to 30 days for cassava, to develop a lesion (Ayesu-Offei and Antwi-Boasiako, 1996; Vathakos and Walters, 1979). It is reported that *C. rosicola* takes 14 days for lesions to develop (Boelema, 1973).

Resistance to cercospora

Hagan and Akridge (2005) observed that shrub and groundcover roses seemed to have greater susceptibility to cercospora leaf spot as compared to hybrid tea and grandiflora roses, and noted variability between defoliation and spotting among cultivars. In South Africa, rootstock Basye #3 was highly susceptible to cercospora leaf spot (Boelema, 1973). It was first reported in

Brazil on *R. multiflora*, which showed severe leaf symptoms (Feres et al., 2017). In Tennessee, 88% of evaluated cultivars showed moderate cercospora leaf spot severity. ‘Carefree Delight’, ‘Fairy Queen’ and ‘Nearly Wild’ had high cercospora incidence while ‘All The Rage’, ‘Midas Touch’, ‘Baby Bloomer’, ‘Pascali’, ‘Belinda’s Dream’, ‘Pink Knock Out’, ‘Beloved’, ‘Pristine’, ‘Hansa’, ‘Sunbright’, ‘Honey Perfume’, ‘Tahitian Moon’, ‘Knock Out’ and ‘Wildberry Breeze’ had no cercospora lesions (Windham et al., 2017).

Breeding for resistance to cercospora

Conventional breeding for a cercospora resistant plant was described as difficult in several crops due to the amount of time needed for breeding, multigenic resistance and high environmental influence on disease development. Thus, locating QTL pertinent to resistance is crucial for selection of resistant plants (Duangsong et al., 2016; Holtschulte et al., 2010; Smith and Gaskill, 1970). To date, quantitative resistance to cercospora leaf spot has been described in cowpea (Duangsong et al., 2016), sugar beet (Taguchi et al., 2011), soybean (Pham et al., 2015), mungbean (Chankaew et al., 2011) and maize (Berger et al., 2014). Another way to confer resistance against pathogens are through the use of Resistance (R) genes that convey plant resistance by producing R proteins. R-genes conveying resistance to black spot (*Diplocarpon rosae*) have been identified (Whitaker et al., 2010) however, none have been identified for cercospora leaf spot. Currently, disease management includes monthly fungicide applications and cleaning of leaf debris.

Rose Map

Genetic maps and the genome sequence facilitate the identification of marker-trait associations leading to more efficient selection and breeding. Although the rose has a relatively

small genome size of ~560 Mb (Yokoya et al., 2000), mapping work and sequencing the rose genome is complicated by its high heterozygosity and ploidy levels (2x, 3x, and 4x).

Multiple rose maps have been created using the double pseudo test cross strategy which creates maps for each parent and then integrates the linkage groups (Debener and Linde, 2009). The first diploid rose map consisted of RAPD and AFLP markers and identified genes for double flowering and pink flower color (Debener and Mattiesch, 1999). This map was expanded by Yan et al. (2005). Another diploid map constructed in 2002, located QTLs for double corolla, thorns and recurrent blooming (Crespel et al., 2002). The same population was used by Hibrand-Saint Oyant et al. (2007) to augment the map using SSR markers. The next published map was in 2005 which also characterized recurrent blooming, flower size, leaf size and resistance to powdery mildew (Dugo et al., 2005). Spiller et al. (2011) constructed the first consensus map from diploid populations from Crespel et al. (2002); Linde et al. (2006); Shupert et al. (2007) and Yan et al. (2005). The diploid population from Shupert et al. (2007) and the tetraploid population from cross *Rosa hybrida* cv. 'Golden Gate' and *Rosa hybrida* cv. 'Fragrant Cloud' were used to create the first integrated map of diploid and tetraploid roses (Tsai, 2013). This was followed by a SNP based high density consensus map from integrating three populations, which improved marker density and order (Yan et al., 2018).

The first tetraploid map identified genomic regions controlling prickles (Rajapakse et al., 2001). The same population was used for development of SSR markers to anchor and combine the parental maps, which demonstrated high conservation of microsatellite regions in the rose genome (Zhang et al., 2006). Gar et al. (2011) created the second tetraploid map and established the synteny between *Rosa* and *Fragaria*. They located anther color, flower color and powdery mildew resistance on the map. Subsequent mapping work determined that the rose was a

segmental allopolyploid with both disomic and tetrasomic inheritance (Bourke et al., 2017; Koning-Boucoiran et al., 2012; Vukosavljev et al., 2016). This work was applied to a tetraploid map developed for identifying a novel black spot resistance locus (Zurn et al., 2018).

A draft of the rose genome was released by Nakamura et al. (2018) using *R. multiflora*. Genes involved in petal color, floral scent, floral development, flower opening and everblooming are described. Hibrand et al. (2018) and Raymond et al. (2018) sequenced the rose genome of a double haploid of *R. chinensis* 'Old Blush', therefore overcoming the obstacle of the high heterozygosity. Both assessed the genetic diversity of rose within the *Rosa* genus and identified potential loci controlling continuous flowering, double flowers, self-incompatibility, and prickle density. Raymond et al. (2018) constructed metabolic pathways for the regulation of scent and flower color, and proposed an interconnected regulation between the traits.

Methods for QTL analyses

There are multiple QTL mapping strategies including single marker analysis, simple interval mapping, composite interval mapping and Bayesian methods. Single marker analysis is the basic method of QTL detection; it does not require a linkage map and utilizes Student's t-test, analysis of variance or regression between the phenotype and a marker (McCough and Doerge, 1995). However, QTL detection is dependent on the distance between the marker and the QTL, thus the position of the QTL may not be accurate. Simple interval mapping and composite interval mapping require linkage maps and analyze the presence of QTL between linked markers (Lander and Botstein, 1989). While effects of additional QTLs bias the results from simple interval mapping, composite interval mapping increases the accuracy of QTL effects by integrating interval mapping with multiple regression analysis (Jansen, 1993).

A recent approach for QTL mapping is a pedigree-based analysis using the Bayesian method. This analysis incorporates multiple related populations with known pedigree to identify QTLs and its genetic components by tracing through alleles identical by descent. Using pedigree data improves statistical power, facilitates estimation of genetic parameters and detects presence of minor and major QTL (Bink et al., 2014). The Bayesian approach is implemented by the software FlexQTL (Bink et al., 2008) and has been applied on highly heterozygous, clonally propagated crops, such as apples, strawberry, peach, rose and sweet cherry (Cai et al., 2018; Fresnedo-Ramírez et al., 2015; Mangandi et al., 2017; Verma et al., 2019; Yan, 2017).

Conclusion

The objectives for this thesis are to evaluate cercospora leaf spot for garden roses in both field and artificially inoculated conditions, and to identify QTLs for resistance. There isn't a significant amount of information on the pathogen and the disease, thus understanding the optimal conditions for pathogen growth and the plant and pathogen interaction is an important step. Studies from corn, sugar beet, soybean and asparagus with cercospora leaf spot disease could help in determining these conditions. Furthermore, identifying cultivar susceptibility to cercospora leaf spot facilitates the development of resistant cultivars. Discovering QTLs for resistance to cercospora leaf spot and developing molecular markers can accelerate the selection process of identifying resistant genotypes.

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CHAPTER II

EVALUATION OF CERCOSPORA LEAF SPOT ON ROSES IN TEXAS

Introduction

Roses are one of the most popular landscape ornamental plants in the world. The first ornamental roses were cultivated in China during the Han dynasty (141-87 B.C.) (Guoliang, 2003). The introduction of Asian roses to Europe initiated the incorporation of the everblooming trait into the European rose. Since then, the hybridization and interbreeding of 7 to 10 rose species led to the rise of modern rose cultivars (Zlesak, 2006). Through genetic analyses, Vukosavljev et al. (2013) found Renaissance, Modern English, floribunda and Canadian Parkland cultivars to be similar and out of garden roses, hybrid tea roses are the most closely related to cut rose cultivars.

The most common and important foliar diseases of roses are black spot (*Diplocarpon rosae*), powdery mildew (*Sphaerotheca pannosa*) and cercospora leaf spot (*Rosisphaerella rosicola*) (Mangandi and Peres, 2009). Cercospora leaf spot of roses is caused by the fungus *R. rosicola* (syn: *Cercospora rosicola*, *Passalora rosicola*, teleomorph: *Mycosphaerella rosicola*) (Feres et al., 2017; Videira et al., 2017). It is a common disease worldwide, and until recently, it has been overlooked as it causes less severe damage as compared to black spot. The increased prevalence of this disease is most likely a consequence of the decreasing application of fungicides and the widespread plantings of black spot resistant roses.

Similarity between the symptoms of cercospora leaf spot and black spot may cause misdiagnoses for the disease (Figure 2.1). Cercospora leaf spot commonly appears as dark circular spots with a tan center on rose leaves. The spot has a defined border unlike black spot, which has a feathery edge. Usually, the lesion size is 2-4 mm in diameter but it can reach up to

10 mm. Initially, the lesion will be a condensed dark red to purple color and gradually turn dark brown to black with a tan necrotic spot. Occasionally, the edges of the lesion will have a dark red or purple halo with a dark center. As the lesion grows, the tan area will widen. On older lesions, dark spots, known as the stromata, appear scattered around the tan necrotic center, which is the site of conidia growth and development (Videira et al., 2017). Symptoms mainly develop on the adaxial side of the leaf but can also occur on the abaxial side. The conidia overwinter on the leaves and are dispersed by splashing water and wind (White and Klingeman, 2014). Typically, the infection starts from the bottom of the canopy and progresses upward to the newer growth (Mangandi and Peres, 2009).

A

B

Figure 2.1. Images of black spot (A) and cercospora leaf spot (B). Black spot is characterized by feathery edges, whereas cercospora leaf spot is characterized by a dark red/purple spot with defined edges.

Hagan et al. (2005) reported *Rosa wichurana,* 'Livin' Easy', 'Sweet Chariot', 'Hansa' and 'Carefree Wonder' as resistant to cercospora; 'Polar Ice', 'Fuchsia Meidiland' and 'Fire Meidiland' as tolerant, while 'Happy Trails', 'Petite Pink Scotch', 'The Fairy', 'Carefree Delight', and 'Therese Bugnet' were considered susceptible. Also, it was indicated that there was variability between defoliation and spotting among cultivars. Biweekly or monthly application of fungicides controlled the disease; however, it was noted that 'Therese Bugnet' was so susceptible to cercospora that the biweekly fungicide application failed to reduce cercospora severity. In Tennessee, 'Nearly Wild', 'Carefree Wonder' and 'Fairy Queen' were susceptible to cercospora leaf spot (Windham et al., 2017). In another study, Hagan and Akridge (2005) observed that cercospora severity was generally greater on shrub and ground cover roses compared with hybrid tea and grandiflora roses.

The objective of this study was to examine the incidence of cercospora leaf spot on garden roses in Texas. Data on disease severity over time and the conditions for cercospora leaf spot development in 2 different locations over 3 years of evaluations will be presented.

Material and Methods

130 rose cultivars were evaluated in both Overton and College Station, Texas. Most of the roses were planted in late summer 2015 in Overton and in fall 2015 in College Station. Roses that were received later were planted in early spring of 2016. The plants were evaluated during spring, summer and fall of 2016, 2017 and 2018 for several landscape traits, cercospora and black spot incidence and overall landscape quality. In College Station, the roses were evaluated in May-November in 2016, March-November in 2017 and April-November in 2018. In Overton, the roses were evaluated in April-October in 2016, June, July, September and November in 2017 and in April, June and October in 2018. The plants varied from shrub types, climbing, hybrid

rugosas, floribundas and modern hybrids (Table 2.1). The roses were planted in a randomized complete block design with 2 to 3 replications per location. In College Station, the plants were planted in double rows with weed barrier with a 4 ft spacing and 5 ft apart between rows in a double row. The double rows were around 8 ft apart. They were irrigated by overhead sprinklers to favor disease development. Throughout the study, no chemical amendments were applied. In Overton, the roses were planted in double or single rows based on their size. Large roses were planted in single rows with 6 ft spacing and the standard size roses were planted in double rows with 3 feet between plants within a row and 2 feet between the rows within a double row. The distance between the rows were 6 ft. Nitrogen fertilizer was applied weekly during the growing season. The roses were pruned between late February and early March each year with the rose canopy being reduced by at least one half.

Both locations lie within the USDA hardiness zone 8b. Although the weather is similar in both locations, Overton usually has a greater difference between its daily maximum and minimum temperature. The winters are 1-2°C cooler in Overton than in College Station, but their summers are similar, with the exception of 2017, where College Station had warmer temperatures by an average of 2°C. Overton had 77.3 cm of rain in 2016, compared to other years (102 cm and 128.8 cm in 2017 and 2018, respectively). College Station had higher rainfall than Overton in 2016 and 2017. High precipitation in summer 2017 was due to Hurricane Harvey (Fig. 2.2 and 2.3; Table 2.2).

Table 2.1. List of rose cultivars evaluated in College Station and Overton, Texas.

Cultivar	Classification	Cultivar	Classification
10043 N019	TAMU Selection	Miracle on the Hudson	Shrub
10043 N049	TAMU Selection	Moje Hammarberg	Hybrid Rugosa
Abbaye de Cluny	Hybrid Tea	Morden Centennial	Shrub
American Pillar	Hybrid Wichurana, Rambler	MORsoucrest	Hybrid Soulieana, Moss
Archbishop Desmond Tutu	Floribunda, Shrub	Munstead Wood	English rose, Shrub
Basye's Blueberry	Shrub	My Girl	Shrub
Basye's Purple	Hybrid Rugosa, Shrub	Nearly Wild	Floribunda
Belinda's Dream	Shrub	Old Blush	China / Bengale
Betty Prior	Hybrid Tea	ORA 05007	Floribunda
Beverly	Hybrid Tea	Oso Easy Cherry Pie	Floribunda, Shrub
Bonica	Floribunda, Shrub	Oso Easy Double Red	Floribunda
Brilliant Veranda	Floribunda, Patio	Oso Easy Fragrant Spreader	Shrub
Brite Eyes	Climber	Oso Easy Honey Bun	Shrub
Caldwell Pink	Polyantha	Oso Easy Italian Ice	Floribunda, Shrub
Carefree Beauty	Shrub	Oso Easy Lemon Zest	Shrub
Carefree Celebration	Shrub	Oso Happy Candy Oh	Shrub
Carefree Delight	Shrub	Oso Happy Petit Pink	Miniature
Carefree Sunshine	Shrub	Oso Happy Smoothie	Polyantha
Carmella Fairy Tale	Shrub	Papa Hemeray	China / Bengale
Champlain	Hybrid Kordesii, Shrub	Peachy Keen	Shrub
Charisma	Hybrid Tea	Phloxy Baby	Polyantha
Cherry Parfait	Floribunda, Grandiflora	Pink Enchantment	Hybrid Tea
Chuckles	Floribunda	Pink Home Run	Shrub
Darcey Bussell	English rose, Shrub	Plum Perfect	Floribunda
Dark Desire	Hybrid Tea	Polanaise	Shrub
Dee-Lish	Hybrid Tea	Poseidon	Floribunda
Dream Come True	Hybrid Tea	Purple Pavement	Hybrid Rugosa
Ducher	China / Bengale, Tea	Purple Rain	Shrub
Earth Angel	Floribunda	Raspberry Kiss	Floribunda, Hybrid Hulthemia persica
Elizabeth Taylor	Hybrid Tea	Raspberry Vigorosa	Floribunda
Elle	Hybrid Tea	Red Drift	Shrub
Europeana	Floribunda	Rise N Shine	Miniature
Fair Molly	Miniature, Polyantha	Rosarium Uetersen	Climber, Shrub
Falling In Love	Hybrid Tea	Roxanne Veranda	Shrub, Patio
Fame!	Grandiflora, Hybrid Tea	Ruby Vigorosa	Floribunda

Table 2.1. Continued

Cultivar	Classification	Cultivar	Classification
Fiji	Hybrid Tea	Sally Holmes	Shrub, Hybrid Musk
Flamingo Kolorscape	Floribunda, Shrub	Savannah	Hybrid Tea
Francis Meilland	Hybrid Tea	Sir Thomas Lipton	Hybrid Rugosa
Frau Dagmar Hastrup	Hybrid Rugosa, Shrub	Skylark	Shrub, English rose
GN15	Certified Roses Selection	Sky's the Limit	Climber
Golden Fairy Tale	Hybrid Tea	Solero Vigorosa	Floribunda, Shrub
Grande Amore	Hybrid Tea	Sophy's Rose	Shrub, English rose
Hansa	Hybrid Rugosa, Shrub	St. Patrick	Hybrid Tea
Home Run	Shrub	Star Delight	Hybrid Rugosa
Hot Cocoa	Floribunda	Stormy Weather	Climber, Shrub
Iceberg	Floribunda	Strawberry Hill	English rose, Shrub
Innocencia Vigorosa	Floribunda	Sunny Sky	Hybrid Tea
Intrigue	Floribunda	Sunrise Sunset	Shrub
J06-20-14-3	TAMU Selection	Sunset Celebration	Hybrid Tea
John Cabot	Hybrid Kordesii	Sweet Frances	Shrub
John Davis	Hybrid Kordesii	Sweet Vigorosa	Floribunda
Joseph's Coat	Climber, Floribunda	Tahitian Treasure	Grandiflora, Shrub
Julia Child	Floribunda	Tamango	Floribunda
Kashmir	Shrub	Teasing Georgia	English rose, Shrub
Knock Out	Shrub	Tequila	Floribunda
La Marne	Polyantha	Therese Bugnet	Hybrid Rugosa
Lafter	Hybrid Tea, Shrub	Tiffany	Hybrid Tea
Lemon Fizz	Floribunda, Shrub	Topolina Vigorosa	Miniature
Limoncello	Shrub	Toscana Vigorosa	Floribunda, Shrub
Linda Campbell	Hybrid Rugosa	Traviata	Hybrid Tea
Little Buckaroo	Miniature	Watercolors Home Run	Shrub
Livin' La Vida	Floribunda	Westerland	Climber, Shrub
M4-4	TAMU Selection	Windermere	English rose, Shrub
Mardi Gras	Floribunda	Winner's Circle	Climber
Mevrouw Nathalie Nypels	Floribunda, Polyantha	Winter Sunset	Shrub

The soil in the field located in College Station is a Westwood series, (NRCS and USDA, 2005) which are moderately alkaline and calcareous, with silty clay loam topsoil. The limitation

of this type of soil is the tendency to flood. In contrast, the field in Overton belongs to the Tenaha-Lilbert-Darco series, which are slightly acid with loamy, fine sand (NRCS and USDA, 2000). Challenges of this type of soil are droughtiness and low soil fertility.

Cercospora leaf spot and black spot incidence were rated on a percentage-based scale of 0 to 9. A score of 0 indicated no disease apparent in the plant, a score of 1 was given when 10% of the leaves of the plant canopy had lesions, a 2 indicated that 20% of canopy was covered with lesions, and a 9 indicated 90% of the rose canopy had disease symptoms and may also include some defoliation. Defoliation was also scored from 0 to 9 (0=plant covered with leaves, 1=10% defoliation apparent, 2-8=20%-80% of leaves fallen off canopy, 9=bare plant, with few leaves). Landscape ranking was determined using a 1 (desirable looking plant, full of leaves and flowers and little to no disease) to 5 (mostly defoliated or no flowers, diseased or dead plant) scale.

Statistical analyses were performed using JMP Pro 14.1.0 2018, SAS Institute Inc. Monthly ratings and comparison between rose accessions and months were done with an analysis of variance using a mixed model using REML (restricted maximum likelihood) analysis followed by student's t-test.

Variance components for the year analysis used the model $\gamma=\mu+\sigma^2_{Cultivar}+\sigma^2_{Month}+\sigma^2_{Block\ (Month)}+\sigma^2_{CultivarxMonth}+\sigma^2_{Error}$, where μ is the cercospora leaf spot incidence mean. The phenotypic variance (Vp) was the sum of the cultivar variance (Vcultivar), cultivar x month interaction variance (Vcultivarxmonth) and error variance (Ve) (Vp = Vcultivar + Vcultivarxmonth + Ve). Variance component for the month analysis was obtained from the model $\gamma=\mu+\sigma^2_{Cultivar}+\sigma^2_{Block}+\sigma^2_{Error}$ where μ is the cercospora leaf spot incidence mean. The Vp for the month was the sum of Vcultivar and Ve. Initially the analysis was run on data from all months and years at each site.

Upon inspection of the results, it was noticed that the level of cercospora varied dramatically so to assess the data quality, it was examined by month using the coefficient of variation and a repeatability estimate. Heritability/Repeatability was estimated by $H^2=V_{cultivar}/V_p$. Coefficient of variation (CV) was calculated by the dividing the root mean square error by the overall trait mean. Once the less informative data was eliminated, the best quality data was used for all subsequent analyses including Pearson's correlation analyses among traits (cercospora incidence, black spot incidence, defoliation and landscape rating), a REML variance analysis and finally a cluster analysis to separate the 130 cultivars into 3 classes of cercospora incidence. An estimated mean of cercospora leaf spot ratings from the mixed model was used for the heat maps. Heat maps were obtained from the R package 'gplots', function 'heatmap2' to assess cercospora leaf spot incidence over the months by year in each location. The 'heatmap2' function uses Euclidean distance to calculate the distance matrix. Patterns of high and low incidence of disease and dendrograms of the months and cultivars are shown in the heat map and were classified into three groups by the function 'cuttree'.

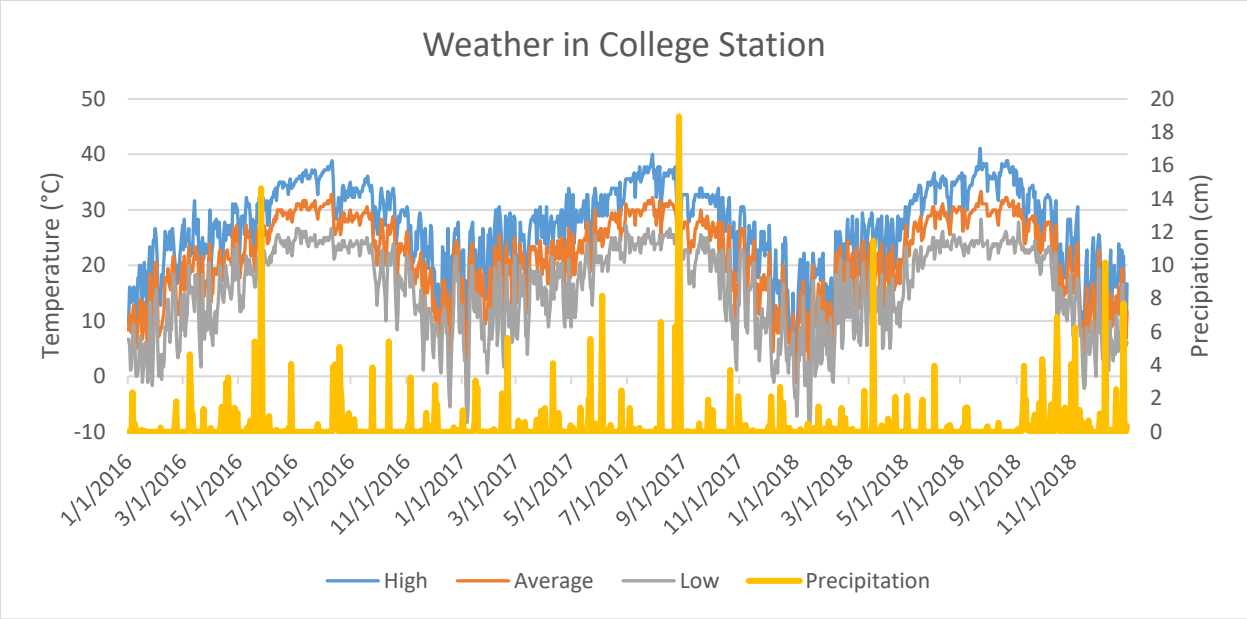

Figure 2.2. Daily maximum, minimum and average temperature and precipitation during 2016-2018 in College Station, Texas (Source: wunderground.com).

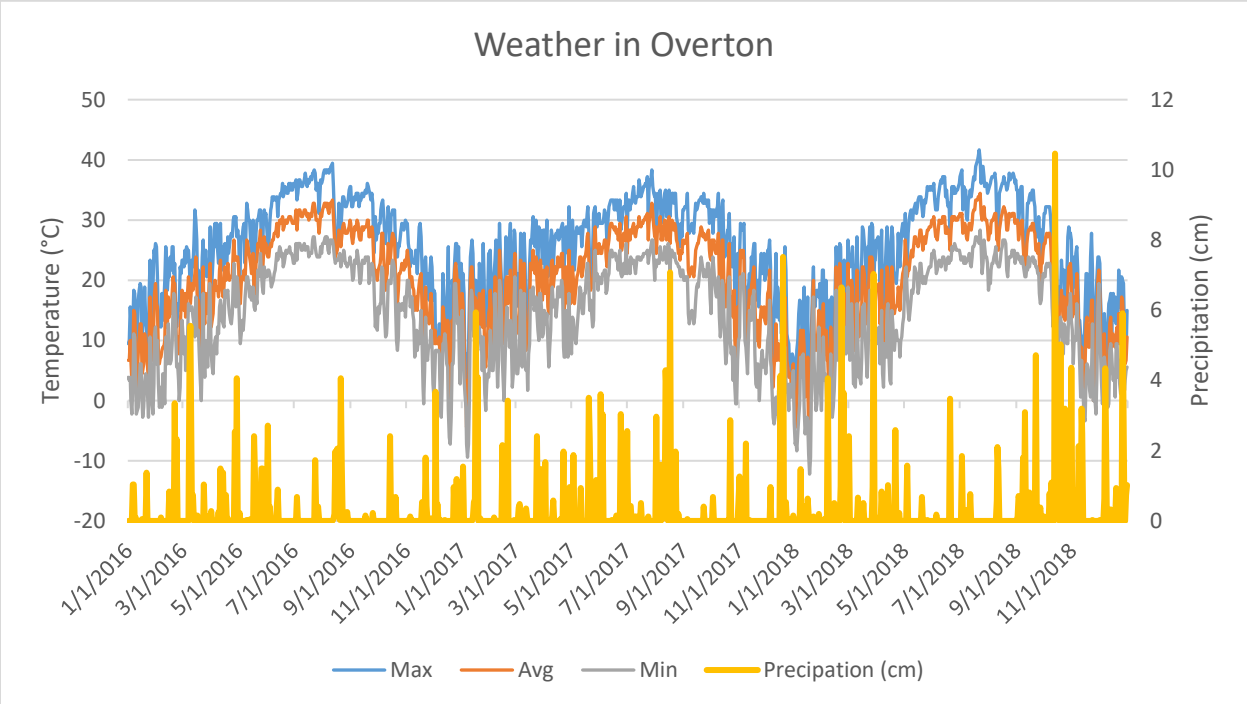

Figure 2.3. Daily maximum, minimum and average temperature and precipitation during 2016-2018 in Overton, Texas (Source: wunderground.com).

Table 2.2. Average maximum and minimum seasonal temperature, total precipitation and rain frequency (greater than 1mm of rain per day) in College Station (CS) and Overton (OT), Texas during 2016-2018.

	Temperature (° C)									
	Maximum		**Average**		**Minimum**		**Precipitation(cm) and days**			
	CS	OT	CS	OT	CS	OT	CS		OT	
Winter-2015-2016	18.4	16.7	12.9	11.1	7.0	5.3	13.7	8	17.4	9
Spring-2016	26.2	25.6	20.9	20.0	15.4	14.2	57.9	26	32.9	22
Summer-2016	34.4	34.5	29.3	29.5	24.0	24.2	29.5	18	21.1	18
Fall-2016	29.0	28.3	23.5	22.4	17.7	16.2	17.2	10	6.6	8
Winter-2016-2017	19.6	19.2	14.2	13.9	8.6	8.3	25.7	15	33.2	16
Spring-2017	27.3	25.0	21.5	19.1	15.8	12.8	23.4	21	22.9	21
Summer-2017	34.2	32.5	28.7	27.9	23.6	23.1	69.6	17	37.7	24
Fall-2017	28.0	29.3	22.2	22.7	16.7	15.8	11.6	9	4.6	6
Winter-2017-2018	16.4	14.5	11.4	9.0	6.1	3.3	30.7	23	23.3	25
Spring-2018	27.1	23.4	21.2	16.9	15.1	10.1	24.7	14	19.0	13
Summer-2018	35.6	35.5	29.8	29.8	23.9	23.9	9.7	12	11.1	9
Fall-2018	28.3	27.6	24.0	23.2	19.5	18.6	60.8	38	56.4	33

Months in spring are March, April and May; summer are June, July and August; fall are September, October and November and winter are January, February and December (Source: wunderground.com)

Results and Discussion

Levels of Cercospora Incidence

As expected, both the Cultivar and the CultivarxMonth effects were significant with only one exception (CultivarxMonth interaction effect for Overton in 2017) (Table 2.3). This reflects the differences in the cultivar's resistance to cercospora leaf spot as well as the necessity of having sufficient disease development to be able to distinguish among the range of resistance levels in this cultivar collection. In the yearly analysis, the month effect is significant at the 0.10 level in College Station for 2017 and 2018 and in Overton for one year (2016).

Although the climate is similar in Overton and College Station, Overton had twice the cercospora disease ratings of College Station (3.4 vs 1.4). This was due to low initial disease pressure in College Station as reflected in its very low mean cercospora incidence the first year (0.55) caused by a large number of rose entries not showing any cercospora symptoms. The planting location at College Station was previously a field crop production and grazing area without any ornamental plantings. The closest sources of cercospora inoculum were the Texas A&M campus (10 miles away) and the Antique Rose Emporium (17 miles away). In contrast, the field in Overton had a higher amount of inoculum due to its previous use in rose evaluations, and its proximity to residential areas and to Tyler, the rose capital of Texas and a major rose production zone.

It is reported that optimal disease conditions for cercospora species consist of warm temperatures (20-30°C), high relative humidity and high amount of inoculum (Cooperman and Jenkins, 1986; Rupe et al., 1982). Spread of the disease is initiated when the conidia are dispersed by splashing water (White and Klingeman, 2014). Temperature and precipitation during the evaluation period fluctuated greatly from year to year for both locations (Table 2.2).

There was a higher rainfall frequency and heavier rainfalls in College Station than Overton during 2016- 2018. Overton received 154 days of rain greater than 0.1 cm while College Station received 165 days of rain from March to November for the three years of evaluation. Also, Overton had 96 days of rain greater than 0.5 cm for the three years and College Station had 100 days of rain greater than 0.5 cm over the same time. Although College Station received slightly more rain than Overton, it would not explain the disease pressure difference between the locations. It was observed that Overton had a higher night-day temperature fluctuation than College Station.

For both locations, cercospora leaf spot had low incidence during March and April with higher levels appearing in May and later due to the inoculum levels building up in response to the warmer temperatures and increased rainfall in summer and fall (Figure 2.5). Thus, the disease was most prevalent during the later months of the year. Cercospora leaf spot was most evident in November in 2017 and 2018 (ratings of 2.7 and 2.8, respectively) in College Station and in September in 2016 and 2017 (ratings of 3.8 and 4.3, respectively) in Overton (Table 2.3). In addition, the larger inoculum presence encouraged disease development during the later years. This is especially true in College Station where the yearly average of cercospora leaf spot incidence increased from an average of 0.55 in 2016 to 2.2 in 2018 (Table 2.3).

Table 2.3. Significance of the components in a REML model and monthly ratings for cercospora leaf spot (0-9 rating scale) incidence in College Station and Overton, Texas for 2016-2018.

	College Station			Overton		
Year	**2016**	**2017**	**2018**	**2016**	**2017**	**2018**
Cultivar	***	***	***	***	***	***
Block(Month)	ns	ns	ns	ns	ns	ns
Month	ns	*	+	+	ns	ns
Cultivar x Month	***	***	***	***	ns	*
DF	129	129	129	129	129	129
Month						
March	-	0.18	-	-		
April	-	0.95	1.59	1.28	-	3.38
May	0.58	1.12	2.43	2.02	-	-
June	0.93	1.98	3.01	2.92	3.61	3.23
July	0.52	1.44	2.44	3.44	3.98	-
August	0.13	2.36	1.83	3.46	-	-
September	0.46	1.37	1.78	3.78	4.29	-
October	0.55	2.71	1.67	3.41	-	3.69
November	0.69	2.72	2.77	-	3.14	-
Year mean	0.56	1.65	2.2	3.04	3.74	3.43

NS = not significant, + = significant with a p <= 0.10, * = significant with a p<= 0.05, ** = significant with a p<= 0.01, and *** = significant with a p<= 0.001.
- indicates no evaluation in that month

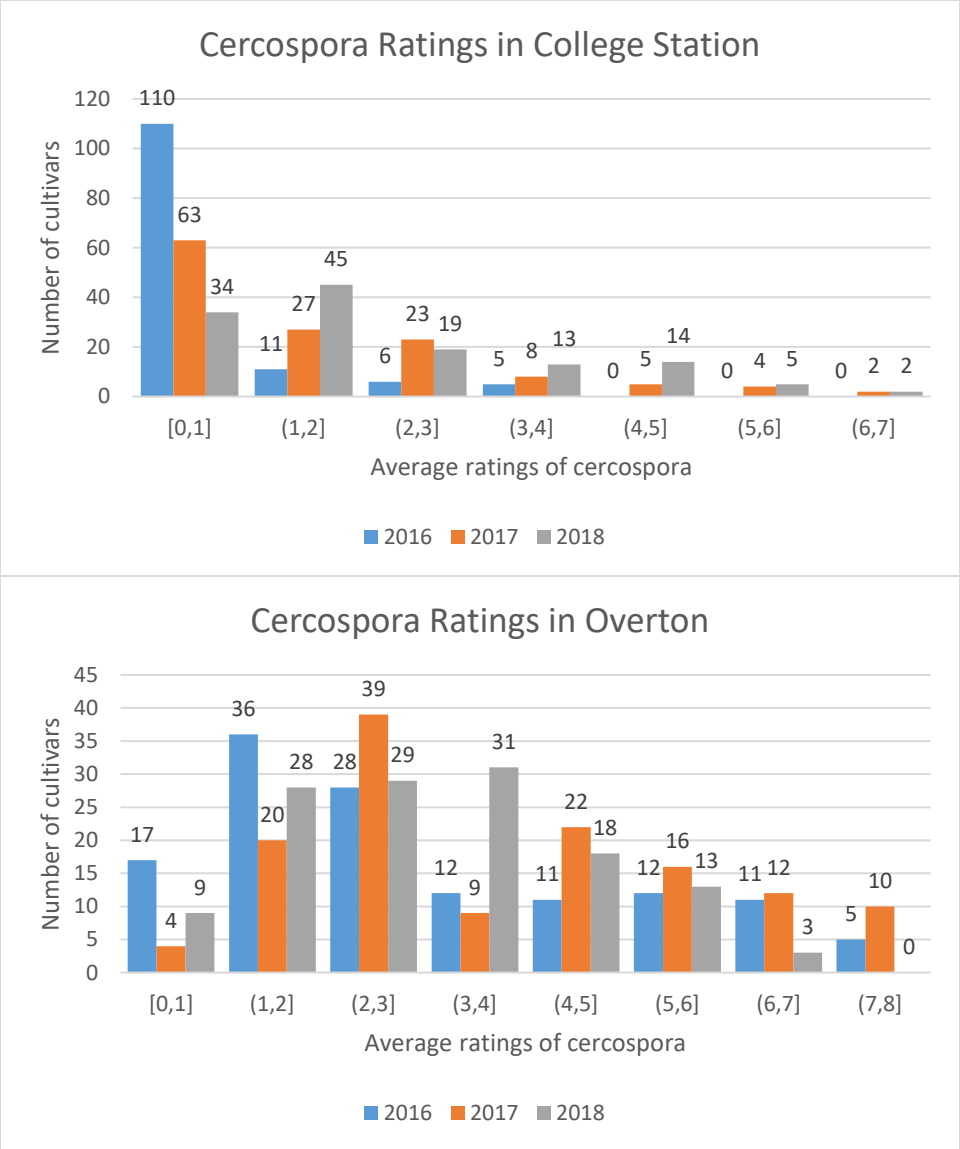

Figure 2.4. Average cercospora leaf spot incidence rating (0-9 scale) distribution in College Station and Overton, Texas (2016-2018).

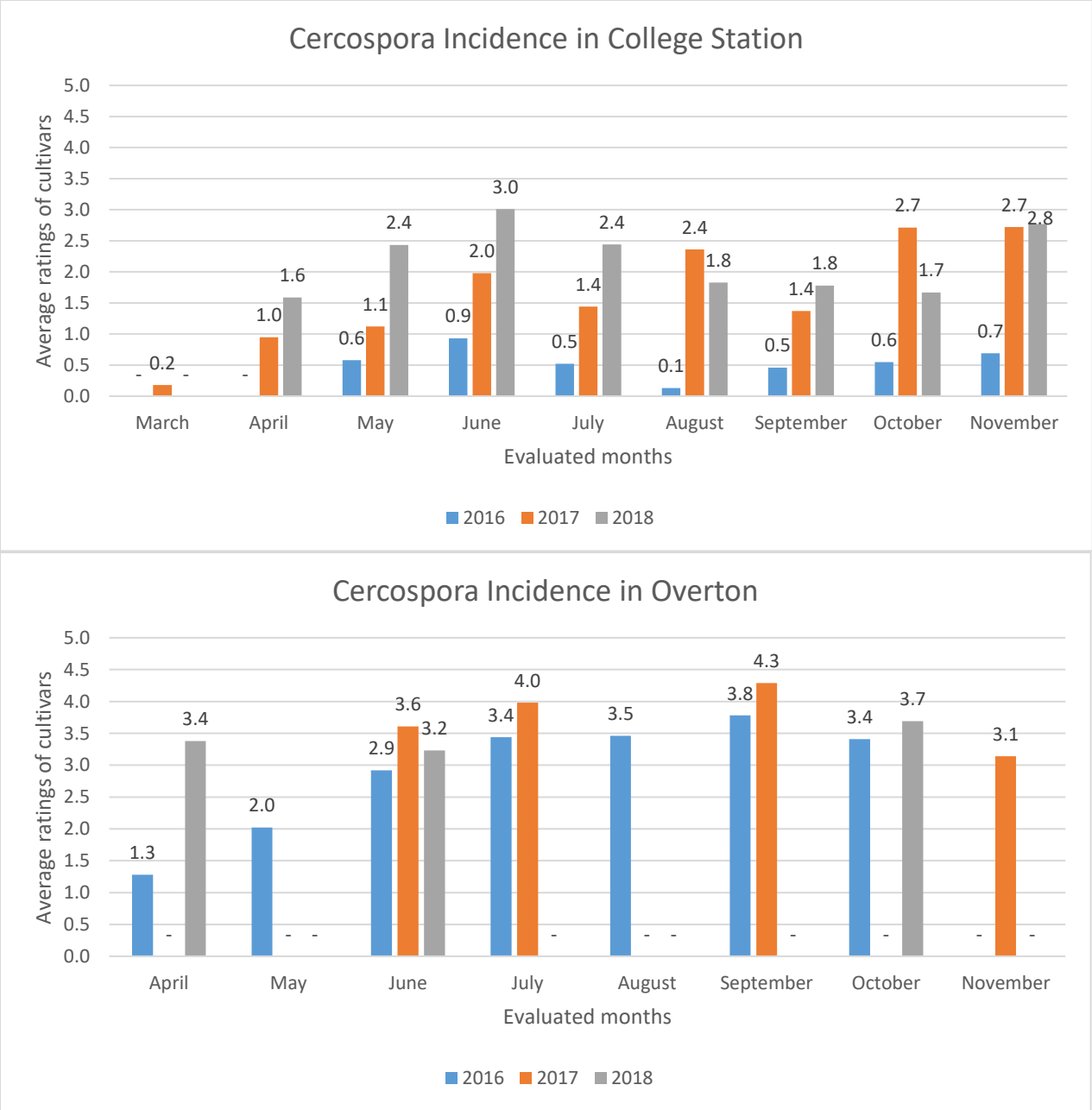

Figure 2.5. Cercospora leaf spot incidence for all cultivars in College Station and Overton, Texas during 2016, 2017 and 2018.
- indicates no observation during that month

Repeatability for Cercospora Leaf Spot

As previously mentioned, if the level of cercospora in the field is too low or its distribution in the field is not uniform, it will not be possible to distinguish among cultivars' inherent resistance to the pathogen. Thus, it is important to assess the quality of the data collected. For this we assessed the monthly data for cercospora leaf spot incidence for repeatability and its coefficient of variance. In College Station and Overton, the repeatability estimates varied between 0.06 to 0.73 and 0.02 to 0.79, respectively (Table 2.4 and 2.5) and the monthly coefficients of variation varied from 0.58 to 5.36 and 0.39 to 1.00, respectively. The coefficient of variation (CV) in the overall analysis were lower in Overton (0.47-0.67) than in College Station (0.73-1.5). The coefficient of variation decreased each year, which suggests less variation of cercospora leaf spot ratings and higher incidence among cultivars over time.

The most informative data would have a high repeatability (greater than 0.45) and a low coefficient of variation (less than 1.0). Thus, using this criterion, the 2016 data from College Station and the 2018 data from Overton should be eliminated from the dataset. In the case of College Station, as this was a new rose block in an area where roses had not been planted, the level of cercospora inoculum was too low to differentiate among cultivars. The 2018 data from Overton was plagued with missing data. In addition, other months that can be eliminated as less informative due to either a high CV or low repeatability would be March and April 2017 and August and September 2018 in College Station, and April 2017 in Overton.

Table 2.4. Variance components, repeatability (H^2) and coefficient of variation (CV) for monthly and overall cercospora leaf spot evaluations in College Station (CS), Texas 2016, 2017 and 2018.

CS2016										
Variance	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Overall
Cultivar	-	-	0.69	1.85	0.80	0.03	0.43	0.79	1.31	0.61
Block[x]	-	-	0.00	0.01	0.03	0.02	0.00	0.02	0.00	0.01
Month										0.05
Cultivar x Month										0.27
Error	-	-	0.61	0.91	0.74	0.46	0.54	0.59	0.98	0.70
V_p	-	-	1.30	2.76	1.55	0.49	0.97	1.39	2.30	1.58
H^2	-	-	0.53	0.67	0.52	0.06	0.45	0.57	0.57	0.39
CV	-	-	1.34	1.02	1.65	5.36	1.59	1.41	1.43	1.5
CS2017										
Cultivar	0.00	1.22	2.32	4.86	2.66	3.21	1.35	2.84	4.23	1.90
Block	0.01	0.00	0.08	0.22	0.13	0.22	0.21	0.43	0.07	0.15
Month										0.68
Cultivar x Month										0.62
Error	0.41	0.91	1.10	1.84	1.80	2.39	1.29	2.47	2.59	1.64
V_p	0.41	2.13	3.42	6.71	4.46	5.59	2.65	5.31	6.82	4.17
H^2	0.00	0.57	0.68	0.73	0.60	0.57	0.51	0.53	0.62	0.46
CV	3.55	1.00	0.93	0.69	0.93	0.66	0.83	0.58	0.59	0.78
CS2018										
Cultivar	-	0.95	3.36	4.15	2.89	1.06	1.37	3.24	3.92	1.95
Block	-	0.03	0.16	0.03	0.18	0.25	0.00	0.02	0.11	0.10
Month										0.25
Cultivar x Month										0.67
Error	-	1.09	2.54	3.36	3.44	2.80	2.30	1.52	3.23	2.55
V_p	-	2.05	5.90	7.51	6.33	3.86	3.66	4.76	7.15	5.17
H^2	-	0.47	0.57	0.55	0.46	0.27	0.37	0.68	0.55	0.38
CV	-	0.66	0.66	0.61	0.76	0.91	0.85	0.74	0.65	0.73

Error = cultivar x block interaction, Vp (phenotypic variance) = Vcultivar + Verror, H^2 = Vcultivar/Vp

- indicates no evaluation in that month

[x] indicates block(month) for overall analysis

Table 2.5. Variance components, and repeatability (H^2) and coefficient of variation (CV) for monthly cercospora leaf spot evaluations in Overton, Texas 2016, 2017 and 2018.

OT2016									
Variance	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Overall
Cultivar	2.64	5.60	6.63	5.76	4.61	3.86	2.35	-	3.77
Block [x]	-0.01	-0.01	0.05	-0.01	-0.02	-0.01	0.23	-	0.04
Month									0.92
Cultivar x Month									0.62
Error	1.62	1.46	1.89	3.25	3.39	3.10	2.57	-	2.59
V_p	4.26	7.06	8.52	9.01	8.00	6.96	4.93	-	6.99
H^2	0.62	0.79	0.78	0.64	0.58	0.55	0.48	-	0.54
CV	1.00	0.60	0.47	0.52	0.53	0.47	0.47	-	0.54
OT2017									
Cultivar	-	-	4.61	2.91	-	3.27	-	3.19	3.31
Block	-	-	-0.02	0.03	-	0.01	-	-0.02	0.00
Month									0.21
Cultivar x Month									0.04
Error	-	-	3.04	2.99	-	2.75	-	3.59	3.16
V_p	-	-	7.65	5.90	-	6.01	-	6.78	6.51
H^2	-	-	0.60	0.49	-	0.54	-	0.47	0.51
CV	-	-	0.48	0.43	-	0.39	-	0.60	0.47
OT2018									
Cultivar	4.80	-	0.62	-	-	-	0.16	-	1.10
Block	0.02	-	-0.03	-	-	-	-0.06	-	-0.03
Month									0.77
Cultivar x Month									5.29
Error	2.71	-	6.45	-	-	-	6.65	-	0.05
V_p	7.51	-	7.07	-	-	-	6.81	-	7.15
H^2	0.64	-	0.09	-	-	-	0.02	-	0.15
CV	0.49	-	0.79	-	-	-	0.7	-	0.67

Error = cultivar x block interaction, Vp (phenotypic variance) = Vcultivar + Verror, H^2 = Vcultivar/Vp

- indicates no evaluation in that month

[x] indicates block(month) for overall analysis

Thus, further analysis will focus on the College Station data from 2017 and 2018 minus data from March and April in 2017 and August and September in 2018 and on the Overton data

from 2016 and 2017 minus data from April in 2016. This data has higher repeatability and lower CV and thus represents the most informative data. It is noted that higher incidence of cercospora results in a lower CV, while a higher genetic variation and a smaller environmental variation could increase repeatability. Thus, evaluation of cercospora when disease incidence is highest will provide improved repeatability and CV.

Table 2.6. Revised variance components, and repeatability (H^2) and coefficient of variation (CV) for overall cercospora leaf spot evaluations in College Station (CS) and Overton (OT) for most informative months per year, by location and overall.

	CS			OT		
Variance	**2017**	**2018**	**Overall**	**2016**	**2017**	**Overall**
Cultivar	2.62***	2.38***	2.41***	3.98***	3.31***	3.49***
Block(month)	0.19*	0.09*	0.12*	0.04ns	0.00ns	0.03ns
Month	0.37ns	0.29ns	0.17ns	0.39ns	0.21ns	0.37ns
CultivarxMonth	0.46***	0.71***	0.36***	0.61***	0.04ns	0.29***
GxE	1.92	2.56	2.66	2.67	3.16	3.14
V_p	5.00	5.65	5.43	7.26	6.51	6.92
H^2	0.52	0.42	0.44	0.55	0.51	0.50
CV	0.71	0.69	0.77	0.51	0.47	0.52

Error = GxE interaction, Vp (phenotypic variance) = Vcultivar + Vcultivarxmonth + Verror, H^2 = Vcultivar/Vp
NS = not significant, * = significant with a p<= 0.05, ** = significant with a p<= 0.01, and *** = significant with a p<= 0.001.

Correlations

Correlations within traits among years and sites

Moderately high positive correlations were found for mean black spot (r = 0.63), cercospora leaf spot (r = 0.81), defoliation (r = 0.63) and landscape ratings (r = 0.62) between College Station and Overton (Table 2.7, Appendix A-1). Very strong correlations (r = 0.82) were

shown in College Station and Overton for all traits between years (Table 2.8 and 2.9, Appendix A-2 and A-3).

Correlations between cercospora leaf spot and black spot, defoliation and landscape rating

Black spot incidence shows a low to moderate negative correlation with cercospora incidence (r = -0.55 in College Station, r = -0.12 to -0.29 in Overton) among years and in the overall analysis, which suggests that different genes condition resistance to the diseases (Tables 2.7 - 2.9). Also, as black spot develops lesions after 7-10 days (Debener et al., 1998) as compared to 3-4 weeks (Feres et al., 2017) for cercospora leaf spot, it is possible that black spot can outcompete and inhibit cercospora leaf spot development on a plant. This may contribute to the negative correlations between these two traits.

Defoliation and cercospora leaf spot have a weak positive to moderate negative correlation (r= -0.42 to 0.24) whereas defoliation has a moderate to weak positive correlation (r= 0.17 to 0.49) with black spot. This indicates that defoliation is most likely induced by black spot rather than cercospora leaf spot. However, it is important to note that heat, wind and other environmental stressors cause defoliation.

In general, both diseases were weakly to moderately (r = -0.31 to 0.49) correlated with the landscape rating whereas the landscape rating and defoliation are strongly correlated.

Table 2.7. Pearson's correlation between College Station (CS) and Overton (OT) for mean values of black spot (BLS), cercospora leaf spot (CLS), defoliation (DF) and landscape (LR) ratings for garden roses in the field.

	CS-BLS	CS-CLS	CS-DF	CS-LR	OT-BLS	OT-CLS	OT-DF	OT-LR
CS-BLS		-0.55***	0.42***	0.34***	0.64***	-0.41***	0.11ns	0.08ns
CS-CLS			-0.28***	-0.2*	-0.28**	0.81***	0.11ns	0.28***
CS-DF				0.93***	0.35***	-0.32***	0.63***	0.51***
CS-LR					0.33***	-0.24**	0.63***	0.62***
OT-BLS						-0.28***	0.2*	0.28**
OT-CLS							0.08ns	0.38***
OT-DF								0.68***
OT-LR								

NS = not significant, * = significant with a p<= 0.05, ** = significant with a p<= 0.01, and *** = significant with a p<= 0.001

Table 2.8. Pearson's correlations in College Station (CS), Texas in 2017 and 2018 for mean black spot (BLS), cercospora leaf spot (CLS), defoliation (DF) and landscape (LR) ratings for garden roses in the field.

	BLS-17	BLS-18	CLS-17	CLS-18	DF-17	DF-18	LR-17	LR-18
BLS-17		0.82***	-0.55***	-0.53***	0.33***	0.44***	0.28**	0.4***
BLS-18			-0.53***	-0.55***	0.34***	0.49	0.26**	0.4***
CLS-17				0.91***	-0.12ns	-0.38***	-0.08ns	-0.29***
CLS-18					-0.15ns	-0.42***	-0.08ns	-0.31***
DF-17						0.78***	0.91***	0.72***
DF-18							0.79***	0.91***
LR-17								0.8***
LR-18								

NS = not significant, * = significant with a p<= 0.05, ** = significant with a p<= 0.01, and *** = significant with a p<= 0.001

Table 2.9. Pearson's correlations in Overton (OT), Texas 2016 and 2017 for black spot (BLS), cercospora leaf spot (CLS), defoliation (DF) and landscape (LR) ratings for garden roses in the field.

	BLS-16	**BLS-17**	**CLS-16**	**CLS-17**	**DF-16**	**DF-17**	**LR-16**	**LR-17**
BLS-16		0.51***	-0.12ns	-0.20*	0.22*	0.35***	0.29***	0.41***
BLS-17			-0.20*	-0.29***	-0.06ns	0.17ns	0.03ns	0.24**
CLS-16				0.83***	0.24**	0.04ns	0.49***	0.29***
CLS-17					0.06ns	-0.12ns	0.33***	0.17*
DF-16						0.65***	0.69***	0.44***
DF-17							0.46***	0.64***
LR-16								0.70***
LR-17								

NS = not significant, * = significant with a p<= 0.05, ** = significant with a p<= 0.01, and *** = significant with a p<= 0.001

Correlations between cercospora with black spot (r = -0.55 and -0.28 in College Station and Overton, respectively) and a correlation for defoliation in College Station (r = -0.28) would suggest that these two plant traits may influence the rating for cercospora incidence. Thus, these should be used as covariates in the analysis to assess differences among rose cultivars in their susceptibility (as measured by cercospora incidence) to cercospora leaf spot.

Cultivar Assessment

As expected, the Cultivar effect is highly significant and the Environment effect is highly significant in all cases except for Overton in 2017 which could be due to the smaller number of evaluations carried out that year. For the overall analysis both these effects and the Month effect were significant at both sites. The block effect was only significant in College Station, most probably due to the dust lifted from the road next to the field which limited the disease growth on the roses closer to the roadside.

As covariates, black spot ratings had a consistent and highly significant effect whereas defoliation ratings were significant in the overall analysis at College Station but not at Overton

(Table 2.10). This suggests that although both covariates explain some the variance of the cercospora leaf spot ratings, defoliation ratings do not account for as much as black spot ratings. This is consistent with the correlation analysis.

Table 2.10. Significance of the components in a REML model for the year ratings and overall analysis of cercospora leaf spot (0-9 rating scale) in College Station and Overton, Texas with black spot and defoliation as covariates.

	College Station			Overton		
Variance	**2017**	**2018**	**Overall**	**2016**	**2017**	**Overall**
Cultivar	***	***	***	***	***	***
Block (Month)	*	*	***	ns	ns	ns
Month	ns	ns	*	ns	ns	*
CultivarxMonth	***	***	***	***	ns	***
Black Spot	***	***	***	***	***	***
Defoliation	**	ns	**	ns	*	ns
Degrees of Freedom	129	129	129	129	129	129

NS = not significant, * = significant with a p<= 0.05, ** = significant with a p<= 0.01, and *** = significant with a p<= 0.001

The pattern of cercospora leaf spot in College Station 2017-2018 and in Overton in 2016-2017 are shown in Figures 2.6 and 2.7. In 2017 and 2018 in College Station, there is a more varied response to cercospora infection (Appendix A-4). August and November of 2017 and May, July, October and November of 2018 are clustered, which suggests that these have very similar disease development patterns. These months also have the highest disease ratings for that year. April, May and July 2017 are grouped together as the lowest months of cercospora incidence.

As mentioned, Overton has a higher cercospora leaf spot incidence than College Station, thus it may provide more insight into which cultivars are susceptible to the disease according to

disease pressure (Appendix A-5). In 2016, May, June and July are clustered together as the lowest months for cercospora infection, while July and September 2018 are clustered as the highest ratings for cercospora leaf spot. There is a distinctive divide between susceptible and more resistant plants.

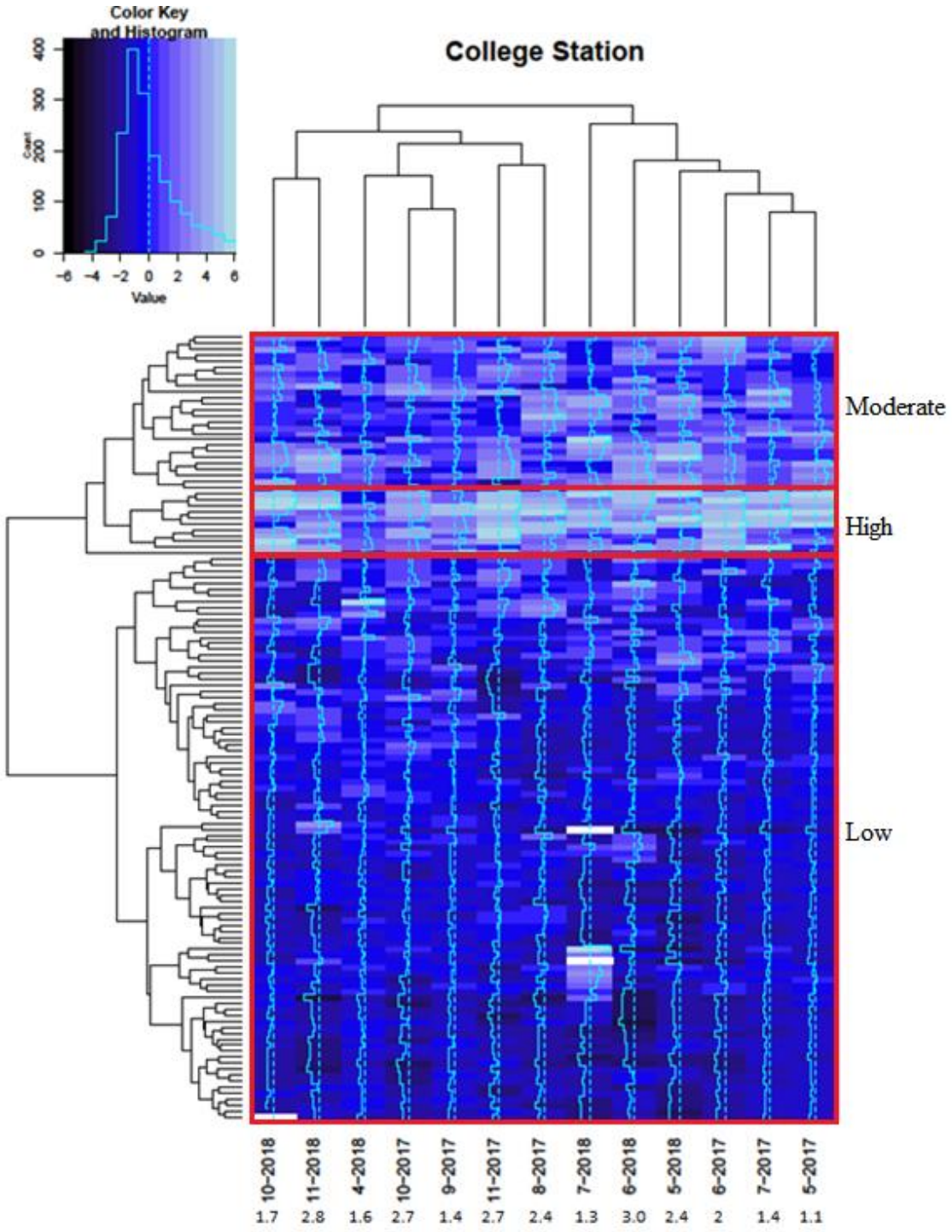

Figure 2.6. Heat map for cercospora leaf spot incidence clustered in groups (low, moderate and high) in College Station, Texas in May- November 2017 and 2018. The top shows a dendrogram for similar months and the right indicates which cultivars are clustered together. Month is shown on the bottom of the figure with their average rating. Dark blue indicates low rating and light blue indicates high rating. White signifies that no data was taken. Data found in Tables 2.12-2.14.

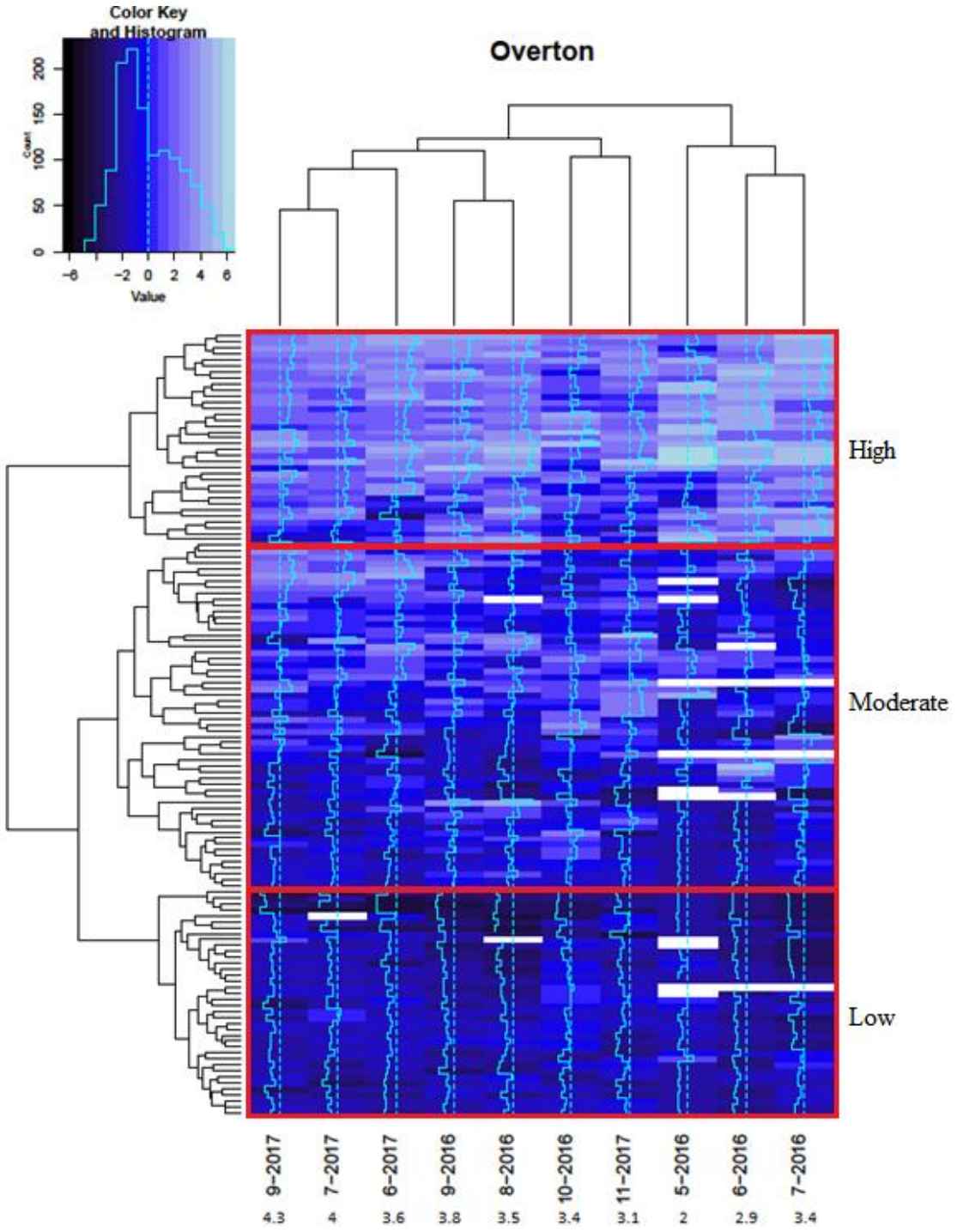

Figure 2.7. Heat map for cercospora leaf spot incidence clustered in groups (low, moderate and high) in Overton, Texas 2016-2017 from May to November. The top shows a dendrogram for similar months and the right indicates which cultivars are clustered together. Month is shown on the bottom of the figure with their average rating. Dark blue indicates low rating and light blue indicates high rating. White signifies that no data was taken. Data found in Tables 2.12-2.14.

On average, Overton had higher cercospora leaf spot ratings than in College Station. Consequently, when separating the clusters on the heat maps into 3 groups (high, moderate and low incidence), the cluster with low incidence in College Station was much larger than that seen in Overton (Table 2.11).

Table 2.11. Cercospora incidence (0-9 scale) and clusters at College Station versus Overton.

College Station		**Overton**		**Number of cultivars**	**Percent of cultivars**
Cluster	**Mean**	**Cluster**	**Mean**		
L	0.78e	L	1.47e	36	27.7
L	1.24d	M	3.04d	49	37.7
L	1.97c	H	5.43b	8	6.2
M	3.03b	L	1.74e	2	1.5
M	3.26b	M	4.32c	8	6.2
M	2.27b	H	5.66b	17	13.1
H	-	L	-	0	0
H	-	M	-	0	0
H	5.18a	H	6.62a	10	7.7

L – Low cluster, M – Moderate cluster, H – High cluster
Levels connected by same letter are not significantly different at $\alpha = 0.05$

Roses in the Low cluster in College Station

71.5% of rose accessions in College Station were clustered in the low cercospora incidence group whereas only 29.2 % of those in Overton were clustered in the low cercospora group (Table 2.11). Among those clustered in the low group in College Station, 28%, 38% and 6% were in the low, medium and high groups in the Overton ratings, respectfully. Interestingly, when these are put into subgroups according to the Overton clustering results, these were distinct subgroups with respect to their College Station cercospora ratings (Table 2.11). Thus, it might be possible to select out the most resistant roses with good accuracy, even in College Station.

‘Archbishop Desmond Tutu’, ‘Oso Easy Double Red’, ‘Winner's Circle’, ‘Lemon Fizz’, ‘Limoncello’, ‘Oso Easy Lemon Zest’ ‘Watercolors Home Run’ and ‘Flamingo Kolorscape’ were grouped as low in College Station but high in Overton. This may be due to the insufficient disease pressure for disease development for these cultivars in College Station. Within this cluster, the roses with the least incidence were all rugosa hybrids: ‘Moje Hammarberg’, ‘Purple Pavement’, ‘Frau Dagmar Hastrup’, ‘Hansa’ and ‘Sir Thomas Lipton’ (Table 2.12). This group also contained most of the English roses and about half of the hybrid tea cultivars along with a few shrubs, floribundas, miniatures, and ‘Old Blush’ the China rose.

Roses in the Moderate cluster at College Station

21% of the roses in College Station were clustered in the moderate cercospora incidence group while 44% of the roses in Overton were in this group. Within in this group in College Station, 2 (2%), 8 (6%) and 17 (13%) were in the low, moderate and high group in Overton, respectfully. Interestingly, ‘Basye’s Purple’ and ‘Earth Angel’ were in the moderate cluster in College Station, however in Overton, they were in the low cluster (Table 2.13). It is possible that these plants were stressed in College Station, which could have caused more cercospora leaf spot incidence.

Roses in the High cluster in College Station

All the roses with high cercospora incidence in College Station, had a high incidence in Overton although the reverse was not true. In College Station, only 10 (8%) roses were in the high cluster and in Overton 35 (27%) of the cultivars were in this group. Of the other 25 rated as having high cercospora incidence in Overton 2/3 were rated as moderate and 1/3 as low incidence in College Station. Overall, ‘John Davis’, ‘Oso Easy Cherry Pie’, ‘Oso Happy Candy Oh’ and ‘Roxanne Veranda’ had the highest rating during the 2 years (Table 2.14).

Conclusions about cercospora ratings in College Station versus Overton

Roses were separated into low, moderate and high clusters for cercospora leaf spot incidence in College Station and Overton. Due to the higher disease pressure in Overton, most of the roses in College Station showed higher cercospora incidence in Overton, thus belonging to a different, and generally higher disease incidence cluster. The only exception to this were 2 roses ('Basye's Purple' and 'Earth Angel') that were rated in the moderate cluster in College Station and the low cluster in Overton. Therefore, Overton could provide more informative data for cercospora leaf spot resistance than College Station. Nevertheless, the disease pressure in College Station is increasing, as seen over the evaluated years. From the evaluations in 2017 and 2018 in College Station, it is possible to identify cultivars that are resistant to cercospora leaf spot.

To obtain higher incidence for cercospora leaf spot, planting susceptible roses across the field could increase the level of inoculum. Maintenance of older, susceptible plants can also increase the disease pressure in the field as this can accumulate the genetic diversity of the pathogen with different races (Lühmann et al., 2009). In addition, not clearing debris and watering by sprinkling could increase disease levels as the pathogen spreads by splashing water. Increasing cercospora levels would help discern cultivar susceptibility to cercospora leaf spot and severity within cultivars.

Cercospora leaf spot rating by rose group

All the English roses in College Station were in the low cluster (Table 2.15), whereas in Overton, all the English roses were in the low cluster except for 'Munstead Wood' and 'Strawberry Hill', which were in the moderate cluster. In College Station, hybrid tea roses were

all the in the low cluster except for ‘Savannah’. However, in Overton, the hybrid tea roses were distributed among all three clusters.

Most of the *Rosa rugosa* hybrids were in the low cluster, with the exception of ‘Therese Bugnet’ and ‘Basye’s Purple’ in College Station and only ‘Therese Bugnet’ in Overton.

Overall, shrub, floribunda and climber type roses had the highest incidence of cercospora leaf spot in both College Station and Overton. As expected, few roses in College Station belong to the high cluster. In Overton, most of the roses in the high cluster are shrub, climber or floribunda types.

Roses with resistance to black spot disease

Roses in the low and moderate cercospora leaf spot incidence cluster showed a varied response to black spot incidence, with ratings ranging from 1-5 in College Station and 2-7 in Overton. Roses in the high cercospora leaf spot incidence cluster in College Station showed average black spot ratings less than 2. However, in Overton, the black spot incidence varied, with ratings ranging from 1-6.

Overall, ‘John Davis’ and ‘Oso Happy Candy Oh’ had high incidence of cercospora and black spot ratings of less than 2. ‘Poseidon’ and ‘Oso Easy Fragrant Spreader’ belonged to the high incidence cluster for cercospora leaf spot and had ratings greater than 5 for black spot in Overton. ‘Sir Thomas Lipton’, ‘Frau Dagmar Hastrup’, ‘Dark Desire’, ‘Moje Hammarberg’ and ‘Knock Out’ showed low ratings for both cercospora and black spot. Within the low cercospora leaf spot incidence cluster, ‘Linda Campbell’, ‘MORsoucrest’, ‘Carmella Fairy Tale’ and ‘Teasing Georgia’ had the highest incidence of black spot.

Roses in Tennessee were evaluated for cercospora leaf spot, black spot and defoliation by Windham et al. (2017). Similar results were found for cercospora leaf spot incidence, such as

‘Hansa’ and ‘Knock Out’ had low incidence of cercospora leaf spot, while ‘Nearly Wild’ had one of the higher incidences of cercospora leaf spot. In Alabama, ‘Carefree Delight’ and ‘Therese Bugnet’ had high cercospora leaf spot incidence (Hagan et al., 2005), which is comparable to what was observed in College Station and Overton. Also, the observation by Hagan and Akridge (2005) that shrub roses have greater cercospora leaf spot damage than hybrid tea roses is confirmed in this research.

Table 2.12. Accessions within the low cluster for cercospora leaf spot (CLS), black spot (BLS) and defoliation (DF) based on College Station heat map clustering with Overton cercospora leaf spot incidence.

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
Belinda's Dream	Shrub	L	1.3	2.4	2.7	L	1.6	3.1	2.3
Beverly	Hybrid Tea	L	1.4	3.0	4.0	L	1.9	3.3	2.0
Carefree Celebration	Shrub	L	1.4	2.7	4.3	L	1.5	4.0	4.2
Charisma	Hybrid Tea	L	0.9	3.9	5.2	L	2.1	5.4	2.9
Cherry Parfait	Floribunda, Grandiflora	L	0.5	4.1	3.7	L	1.7	5.4	2.7
Darcey Bussell	English rose, Shrub	L	0.5	3.5	4.9	L	2.0	4.6	4.0
Dee-Lish	Hybrid Tea	L	0.7	4.2	4.3	L	1.7	4.1	5.5
Dream Come True	Hybrid Tea	L	0.4	3.3	5.7	L	1.5	5.7	5.8
Elizabeth Taylor	Hybrid Tea	L	0.6	3.5	5.8	L	1.5	5.6	6.6
Fair Molly	Miniature, Polyantha	L	0.7	1.7	6.3	L	1.7	2.9	3.6
Fame!	Hybrid Tea, Grandiflora	L	0.6	2.1	5.2	L	2.7	3.0	4.7
Frau Dagmar Hastrup	Hybrid Rugosa, Shrub	L	0.3	0.7	3.9	L	0.4	2.4	3.4
GN15	Certified Roses Selection	L	0.6	4.5	5.3	L	1.7	5.6	5.7
Grande Amore	Hybrid Tea	L	1.1	3.3	5.2	L	2.0	4.3	2.4
Hansa	Hybrid Rugosa, Shrub	L	0.6	1.1	4.9	L	0.5	4.0	3.1
Kashmir	Shrub	L	1.2	2.2	2.3	L	1.1	3.6	1.3
Knock Out	Shrub	L	1.2	1.7	2.0	L	2.0	3.0	1.5
Linda Campbell	Hybrid Rugosa	L	0.6	4.9	5.1	L	1.7	7.2	3.7
Little Buckaroo	Miniature	L	0.8	2.8	5.1	L	1.5	4.0	5.0
Mardi Gras	Floribunda	L	0.9	2.4	6.5	L	1.3	4.9	5.9
Moje Hammarberg	Hybrid Rugosa	L	0.6	0.6	3.7	L	0.0	2.7	2.0
Morden Centennial	Shrub	L	2.4	3.4	6.1	L	1.3	5.9	5.2
Old Blush	China / Bengale	L	0.9	2.2	3.8	L	2.1	3.3	3.5
Purple Pavement	Hybrid Rugosa	L	0.6	1.2	3.2	L	0.3	3.7	2.3
Sir Thomas Lipton	Hybrid Rugosa	L	0.4	1.4	1.4	L	0.9	2.1	1.1
Skylark	English rose, Shrub	L	0.4	3.1	4.5	L	1.0	5.4	4.6

Table 2.12. Continued

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
Sophy's Rose	English rose, Shrub	L	0.6	3.2	5.0	L	1.6	4.9	4.3
St. Patrick	Hybrid Tea	L	0.6	1.4	4.5	L	1.7	3.2	4.0
Star Delight	Hybrid Rugosa	L	0.7	3.4	4.4	L	1.4	4.5	3.5
Stormy Weather	Climber, Shrub	L	0.8	3.3	5.0	L	1.4	4.6	3.7
Sunny Sky	Hybrid Tea	L	1.1	3.6	4.2	L	1.5	3.7	2.3
Sunset Celebration	Hybrid Tea	L	0.5	2.9	6.1	L	1.5	4.1	4.5
Sweet Frances	Shrub	L	0.6	4.2	4.4	L	2.2	3.6	1.8
Teasing Georgia	English rose,Shrub	L	0.5	5.2	4.7	L	1.4	6.2	3.2
Tiffany	Hybrid Tea	L	0.5	2.7	5.6	L	1.6	3.8	4.1
Windermere	English rose, Shrub	L	1.0	4.3	3.3	L	1.3	5.1	1.9
10043 N019	TAMU Selection	L	2.3	2.1	4.0	M	4.2	3.5	4.1
10043 N049	TAMU Selection	L	1.1	2.1	3.2	M	3.8	2.8	2.6
Abbaye de Cluny	Hybrid Tea	L	1.3	2.7	5.3	M	2.8	5.0	5.4
Basye's Blueberry	Shrub	L	1.1	3.2	4.0	M	3.0	4.4	2.5
Betty Prior	Hybrid Tea	L	1.2	3.7	4.7	M	3.2	5.1	2.6
Bonica	Floribunda, Shrub	L	1.9	2.2	5.2	M	2.4	3.9	3.6
Brite Eyes	Climber	L	1.6	1.3	2.8	M	3.8	4.3	3.0
Carmella Fairy Tale	Shrub	L	0.8	4.1	4.4	M	2.1	6.7	4.2
Champlain	Hybrid Kordesii, Shrub	L	2.0	3.0	5.2	M	3.4	4.3	4.3
Chuckles	Floribunda	L	0.9	2.8	5.2	M	2.9	4.7	4.8
Dark Desire	Hybrid Tea	L	2.4	1.7	3.8	M	4.0	2.6	3.2
Ducher	China / Bengale, Tea	L	2.2	2.1	1.8	M	2.3	3.8	2.8
Elle	Hybrid Tea	L	0.6	3.1	5.4	M	2.3	5.0	3.6
Europeana	Floribunda	L	0.8	3.5	5.5	M	2.6	5.4	5.5
Falling In Love	Hybrid Tea	L	0.4	2.4	6.2	M	1.9	4.3	6.1
Fiji	Hybrid Tea	L	1.2	2.6	4.3	M	5.3	3.7	1.7

Table 2.12. Continued

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
Francis Meilland	Hybrid Tea	L	0.5	3.2	4.2	M	2.4	4.8	4.1
Golden Fairy Tale	Hybrid Tea	L	1.5	2.8	4.5	M	3.0	4.5	3.2
Hot Cocoa	Floribunda	L	0.7	3.0	4.2	M	2.0	5.1	3.7
Iceberg	Floribunda	L	1.4	3.6	4.2	M	2.1	4.8	3.8
Intrigue	Floribunda	L	1.7	2.8	6.6	M	1.8	5.6	5.3
John Cabot	Hybrid Kordesii	L	0.5	2.4	4.0	M	3.8	4.3	4.7
Joseph's Coat	Climber, Floribunda	L	0.9	3.5	6.3	M	2.0	4.2	5.4
Julia Child	Floribunda	L	0.7	4.0	3.3	M	3.5	4.6	3.0
Lafter	Hybrid Tea, Shrub	L	1.2	2.4	3.6	M	3.1	3.8	2.7
M4-4	TAMU Selection	L	1.4	1.2	2.8	M	2.5	3.9	1.9
Mevrouw Nathalie Nypels	Floribunda, Polyantha	L	0.7	1.9	4.1	M	2.1	3.6	3.7
MORsoucrest	Hybrid Soulieana, Moss	L	1.5	4.0	5.1	M	3.8	7.2	5.3
Munstead Wood	English rose, Shrub	L	0.6	2.6	5.5	M	2.6	4.5	5.7
ORA 05007	Floribunda	L	0.7	2.6	5.8	M	2.0	4.6	4.8
Oso Happy Petit Pink	Miniature	L	2.5	1.5	2.2	M	4.5	4.1	2.3
Papa Hemeray	China / Bengale	L	0.8	2.2	5.6	M	2.3	3.8	4.7
Peachy Keen	Shrub	L	1.4	2.5	2.1	M	3.3	3.4	2.2
Pink Enchantment	Hybrid Tea	L	1.1	2.7	3.5	M	3.4	3.4	2.0
Pink Home Run	Shrub	L	2.5	1.5	2.4	M	3.7	3.1	3.5
Polanaise	Shrub	L	1.6	3.4	4.4	M	2.7	4.4	3.8
Raspberry Kiss	Floribunda, Hybrd Hulthemia persica	L	1.2	1.7	3.1	M	4.5	3.6	2.4
Red Drift	Shrub	L	2.1	2.3	3.2	M	4.6	3.5	3.8
Rise N Shine	Miniature	L	0.6	3.0	3.8	M	1.8	3.2	1.8
Sally Holmes	Shrub, Hybrid Musk	L	1.5	2.8	5.0	M	3.1	4.1	3.4
Sky's the Limit	Climber	L	0.9	4.0	5.7	M	1.8	4.7	5.9
Solero Vigorosa	Floribunda, Shrub	L	1.3	2.4	3.2	M	3.9	4.1	2.2

Table 2.12. Continued

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
Strawberry Hill	English rose, Shrub	L	0.6	3.5	5.0	M	2.3	5.1	4.4
Tahitian Treasure	Grandiflora, Shrub	L	1.3	1.9	3.2	M	4.3	3.4	2.5
Tamango	Floribunda	L	0.7	2.8	6.1	M	2.2	3.5	3.8
Tequila	Floribunda	L	1.1	4.1	3.2	M	3.8	5.0	4.2
Traviata	Hybrid Tea	L	0.7	4.2	5.1	M	3.5	4.4	2.9
Westerland	Climber, Shrub	L	1.4	2.9	5.2	M	2.7	5.4	4.8
Winter Sunset	Shrub	L	2.3	3.4	4.5	M	4.1	4.2	2.6
Archbishop Desmond Tutu	Floribunda, Shrub	L	1.6	2.9	4.7	H	6.7	4.1	5.4
Flamingo Kolorscape	Floribunda, Shrub	L	2.2	2.6	3.4	H	5.5	5.0	2.8
Lemon Fizz	Floribunda, Shrub	L	1.9	2.2	3.0	H	4.5	4.6	4.4
Limoncello	Shrub	L	1.8	2.8	2.8	H	6.4	3.0	2.7
Oso Easy Double Red	Floribunda	L	2.3	2.2	2.4	H	6.1	4.4	2.4
Oso Easy Lemon Zest	Shrub	L	2.4	3.0	4.2	H	4.1	4.6	4.0
Watercolors Home Run	Shrub	L	1.5	1.6	2.9	H	4.5	3.4	4.7
Winner's Circle	Climber	L	2.2	2.3	4.2	H	5.7	4.2	3.8

[x] Indicates overall mean for black spot (BLS), cercospora leaf spot (CLS) and defoliation (DF) from 2017 – 2018 in College Station and 2016 – 2017 in Overton, TX

Table 2.13. Accessions within the moderate cluster for cercospora leaf spot (CLS), black spot (BLS) and defoliation (DF) based on College Station heat map clustering with Overton cercospora leaf spot incidence.

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
Basye's Purple	Hybrid Rugosa, Shrub	M	2.7	2.5	4.7	L	1.5	6.1	2.4
Earth Angel	Floribunda	M	3.4	1.6	3.4	L	2.0	4.5	2.4
Caldwell Pink	Polyantha	M	2.8	2.4	3.3	M	4.5	4.6	4.5
Carefree Beauty	Shrub	M	2.9	2.2	4.1	M	4.1	4.2	5.0
Carefree Sunshine	Shrub	M	3.2	1.7	3.5	M	4.6	4.2	3.1
La Marne	Polyantha	M	2.7	2.8	2.3	M	4.0	4.5	3.3
My Girl	Shrub	M	3.3	2.1	2.7	M	4.6	3.8	2.3
Ruby Vigorosa	Floribunda	M	4.2	1.5	3.4	M	5.2	3.6	2.6
Sunrise Sunset	Shrub	M	3.7	2.1	3.0	M	3.8	3.8	2.8
Therese Bugnet	Hybrid Rugosa	M	3.4	0.9	3.3	M	3.9	6.1	2.8
Brilliant Veranda	Floribunda, Patio	M	3.9	3.0	4.2	H	6.9	3.9	3.6
Home Run	Shrub	M	2.8	2.0	2.4	H	4.9	3.3	4.6
Innocencia Vigorosa	Floribunda	M	4.1	1.2	2.0	H	6.3	3.7	1.6
J06-20-14-3	TAMU Selection	M	3.1	1.4	1.9	H	6.3	3.9	2.7
Livin' La Vida	Floribunda	M	3.8	1.3	4.4	H	5.3	3.2	4.8
Miracle on the Hudson	Shrub	M	2.6	2.0	2.4	H	4.3	3.3	2.4
Nearly Wild	Floribunda	M	2.8	3.3	2.9	H	4.4	5.7	2.8
Oso Easy Honey Bun	Shrub	M	3.5	1.7	3.0	H	4.7	5.0	3.8
Oso Easy Italian Ice	Floribunda, Shrub	M	3.1	1.6	3.0	H	6.1	3.5	5.2
Oso Happy Smoothie	Polyantha	M	4.2	1.9	4.4	H	6.3	2.4	5.5
Purple Rain	Shrub	M	4.1	1.0	4.0	H	5.8	2.4	4.2
Raspberry Vigorosa	Floribunda	M	3.5	1.8	3.7	H	4.9	4.2	4.7
Rosarium Uetersen	Climber, Shrub	M	3.1	2.8	4.3	H	6.5	3.1	3.7
Savannah	Hybrid Tea	M	3.2	2.3	3.3	H	5.9	4.0	4.6
Sweet Vigorosa	Floribunda	M	2.9	1.7	2.9	H	5.0	4.3	2.9
Topolina Vigorosa	Miniature	M	3.4	1.9	3.2	H	6.3	4.5	1.7

Table 2.13. Continued

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
Toscana Vigorosa	Floribunda, Shrub	M	3.3	1.8	2.9	H	6.7	3.1	2.1

[x] Indicates overall mean for black spot, cercospora leaf spot and defoliation from 2017 – 2018 in College Station and 2016 – 2017 in Overton, TX

BLS: Black spot, CLS: Cercospora leaf spot, DF: Defoliation

Table 2.14. Accessions within the high cluster for cercospora leaf spot (CLS), black spot (BLS) and defoliation (DF) based on College Station heat map clustering with Overton cercospora leaf spot incidence.

		College Station				Overton			
Cultivar	**Group**	**Cluster**	**CLS[x]**	**BLS**	**DF**	**Cluster**	**CLS**	**BLS**	**DF**
American Pillar	Climber, Hybrid Wichurana	H	4.6	1.3	4.0	H	7.7	2.5	2.5
Carefree Delight	Shrub	H	4.6	2.0	5.2	H	6.0	4.8	5.7
John Davis	Hybrid Kordesii	H	6.3	0.6	5.6	H	7.4	0.6	5.6
Oso Easy Cherry Pie	Floribunda, Shrub	H	5.9	1.1	4.0	H	7.5	3.5	6.1
Oso Easy Fragrant Spreader	Shrub	H	4.9	1.4	3.2	H	5.7	6.0	3.3
Oso Happy Candy Oh	Shrub	H	5.8	0.7	3.2	H	7.2	1.9	5.0
Phloxy Baby	Polyantha	H	4.6	1.5	2.7	H	6.2	2.6	4.1
Plum Perfect	Floribunda	H	5.2	0.9	4.1	H	6.8	3.5	5.1
Poseidon	Floribunda	H	4.1	1.9	5.0	H	4.7	5.0	5.0
Roxanne Veranda	Shrub, Patio	H	5.8	1.3	4.1	H	7.0	4.0	4.5

[x] Indicates overall mean for black spot, cercospora leaf spot and defoliation from 2017 – 2018 in College Station and 2016 – 2017 in Overton, TX

BLS: Black spot, CLS: Cercospora leaf spot, DF: Defoliation

Table 2.15. Comparative cercospora leaf spot ratings by rose type and cluster between College Station and Overton.

	College Station				Overton			
Rose Type	**Type Mean**	**Cluster**	**Cluster Mean**	**# of Cultivars**	**Type Mean**	**Cluster**	**Cluster Mean**	**# of Cultivars**
Climber	1.91ab	L	1.29b	6	3.94ab	L	1.44b	1
		M	3.05a	1		M	2.55b	4
		H	4.55a	1		H	6.62a	3
English rose	0.61c	L	0.61	7	1.73cd	L	1.45b	5
						M	2.43a	2
Floribunda	2.29a	L	1.21c	19	4.17a	L	1.65b	3
		M	3.50b	10		M	2.95b	14
		H	5.08a	3		H	5.82a	15
Hybrid Rugosa	1.10bc	L	0.53b	7	1.17d	L	0.84a	8
		M	3.06a	2		M	3.85b	1
Hybrid Tea	1.01bc	L	0.91b	22	2.62bc	L	1.79c	11
		M	3.16a	1		M	3.16b	11
						H	5.89a	1
Miniature	1.60abc	L	1.14a	4	3.15abc	L	1.58a	2
		M	3.44a	1		M	3.16a	2
						H	6.26a	1
Shrub	2.56a	L	1.60c	17	3.92a	L	1.60c	6
		M	3.26b	8		M	3.62b	12
		H	5.27a	4		H	5.50a	11

Levels connected by same letter in a column and by type are not significantly different at $\alpha = 0.05$

Conclusions

Most common foliar rose diseases found in Texas are black spot, powdery mildew and cercospora leaf spot. It has been observed that cercospora leaf spot has become more apparent with the introduction and increased plantings of black spot resistant roses, thus becoming a new concern for rose growers in the southeastern USA.

During the evaluations in 2016 -2018, Overton had higher cercospora incidence than College Station. In College Station, moderately high repeatability and low coefficient of variance occurred in the later months of 2017 and 2018, while in Overton, all the evaluated months in 2016 except for April showed high repeatability and low coefficient of variance. The covariate and the correlation analysis indicated that black spot incidence affected cercospora leaf spot ratings whereas defoliation had a lesser effect. Correlations for cercospora leaf spot between locations was 0.81, which shows that cercospora ratings are mostly consistent among locations. The heat map and the dendrograms showed the cercospora leaf spot development pattern where the months with the least disease incidence are the ones evaluated early on in the evaluation period (April, May and July 2017 in College Station and May, June and July 2016 in Overton). It also classified the cultivars by their rating. Most cultivars in College Station belonged to the low incidence group, however in Overton, most of the cultivars were in the moderate incidence group. Because of the higher disease pressure in Overton, there were more cultivars in the high incidence group than in College Station. Overall, around 28% of the roses evaluated showed a low incidence for cercospora leaf spot. *R. rugosa* hybrids had high tolerance to cercospora leaf spot, with the exception of 'Therese Bugnet' and 'Basye's Purple' which were grouped in the moderate cluster in Overton. The roses with the least disease incidence for both cercospora leaf spot and black spot are *R. rugosa* hybrids such as 'Frau Dagmar Hastrup', 'Moje Hammarberg'

and ‘Sir Thomas Lipton’. Most hybrid tea roses and the English roses grouped in the moderate cluster in Overton and low cluster in College Station.

Because of the consumer preference of less fungicide applications, this study can help consumers and landscapers identify cercospora resistant roses for hot and humid climates.

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CHAPTER III

EVALUATION OF CERCOSPORA LEAF SPOT IN GARDEN ROSES IN A CONTROLLED ENVIRONMENT

Introduction

Roses are one of the most important ornamental plants around the world for their versatile uses, including nutritional value, aroma, landscape and floral designs. They are valued at 203.5 million USD (USDA and NASS, 2016). One of the key traits in rose breeding is disease resistance as most consumers prefer low maintenance roses (Waliczek et al., 2018). Cercospora leaf spot is one of the primary foliar diseases on roses in the Southeastern area of the United States. It is a fungal disease caused by *Rosisphaerella rosicola* (syn=*Passalora rosicola*, *Cercospora rosicola*, teleomorph= *Mycosphaerella rosicola*) that produces lesions on leaves, pedicels, bracts and stems, eventually resulting in leaf chlorosis and defoliation (Mangandi and Peres, 2009). Cercospora leaf spot lesions are circular in shape and dark red/purple in color. Initially, they are around 2 mm in diameter. Although their size depends on the species or cultivar affected they can reach up to 10 mm in diameter (Davis, 1938). Mature lesions develop a tan necrotic center with a dark red edge. They are often accompanied by dark brown stromata, which produce clusters of conidiophores around the necrotic area. Lesions occur primarily on the adaxial side of the leaf. The conidia overwinter on leaves and the spores spread by water splashing (White and Klingeman, 2014).

Morphology of Rosisphaerella rosicola

Rosisphaerella rosicola conidiophore is straight, occasionally septate with a dark base and bound loosely in the fascicle. The conidia are linear or slightly curved; obclavate, long and narrow with 1 to 6 septations (Figure 3.1). They can be up to 20-98 µm long and 3 to 5 µm wide

(Videira et al., 2017). The pathogen is easily distinguishable by its slightly thickened conidial scars, long conidiophores, and subepidermal stromata (Feres et al., 2017; Nakashima, 2004).

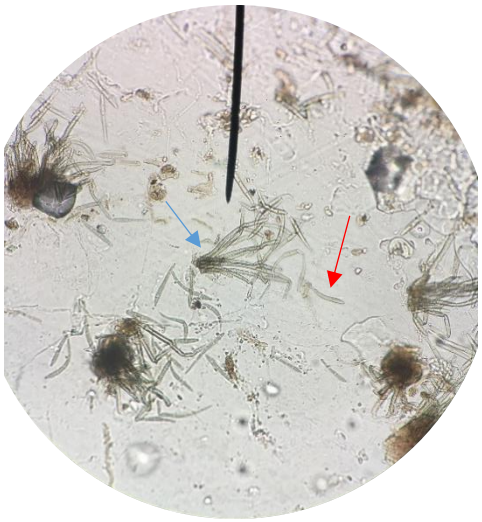


Figure 3.1. *Rosisphaerella rosicola* conidiophore and conidia at 400x magnification. The conidia are shown by the red arrow; it is characterized by its long needle like appearance. The blue arrow shows the conidiophore.

Cercospora life cycle

Members of the genus *Cercospora* infect multiple plant species worldwide, and have an economic impact on the production of peanuts (Stalker, 1984), coffee (Souza et al., 2012), sugar beets (Smith and Gaskill, 1970), corn (Rupe et al., 1982), soybean (Pham et al., 2015) and landscape plants such as hydrangea (Vann, 2010) and roses (Mangandi and Peres, 2009) . There are 659 species recognized in the genus, although approximately 3000 different names have been proposed (Crous and Braun, 2003). Chupp (1954) considered cercospora to be generally a host specific pathogen; however, in recent years, the same pathogen species have been found in

different hosts, as indicated by Groenewald et al. (2006) in celery and sugar beet and Montenegro-Calderón et al. (2011) in water hyacinth, beet and sugar beet.

Factors such as leaf wetness, relative humidity, temperature and amount of inoculum play a crucial role for spore germination and disease development (Cooperman and Jenkins, 1986; Rupe et al., 1982). At least 34 species, including *C. kikuchii*, *C. ricinella*, *C. beticola*, *C. nicotianae*, *C. asparagii* and *C. zeae-maydis*, produce cercosporin, a photoactivated toxin which can aggravate disease severity through peroxidation of membrane lipids (Daub and Ehrenshaft, 2000); (Jenns et al., 1989).

Cercospora species are able to infect in a wide temperature range with high relative humidity. In corn, it was reported that *C. zeae-maydis* spores germinated at temperatures between 17-30°C, but the optimum temperature for both spore germination and lesion development was between 22-30°C (Beckman and Payne, 1983). Optimal disease development seems to occur around 25°C, as reported for *C. asparagi* (25-30°C) (Cooperman and Jenkins, 1986), *C. zeae-maydis* (22-28°C) (Beckman and Payne, 1983) and *C. kikuchii* (22-31°C) (Vathakos and Walters, 1979). Leaf wetness is also important for disease development of some *Cercospora* species. In corn, continuous free water does not favor lesion development due to reduced appressorial formation and penetration by *C. zeae-maydis* (Beckman and Payne, 1983). It has been noted that high moisture for the first 24-48 hours is critical for spore survival and germination, as reported in *C. henningsii* where less than 50% of the spores germinated at 80% relative humidity after 48 hours, while 72% of the conidia germinated at 90% relative humidity (Ayesu-Offei and Antwi-Boasiako, 1996).

Additional factors known to influence germination of *Cercospora* conidia and subsequent disease development include light intensity and inoculum concentration and the interaction

between light intensity and temperature. Silva et al. (2016) reported that at a temperature of 17 °C and a light intensity of 320 µmol $m^{-2}s^{-1}$ optimum germination of *C. coffeicola* occurred, whereas Beckman and Payne (1983) demonstrated that constant fluorescent light at 0.36 µmol $m^{-2}s^{-1}$ (27 lx) inhibited spore germination of *C. zeae-maydis*. Light also activates cercosporin, thus increasing disease severity in crops (Daub and Herrero, 2006) such as coffee (Souza et al., 2012), banana (Churchill, 2011), and sugar beets (Calpouzo and Stallkne, 1967). The concentration of conidia is critical for disease development. Most research uses $5x10^4$ or higher concentration of conidia/mL (Beckman and Payne, 1983; Rupe et al., 1982); (Cooperman and Jenkins, 1986).

Means of infection is species dependent. For example, Souza et al. (2011) found that *C. coffeicola* infects the leaf through cracks or the stomata, while *C. henningsii* can either directly penetrate through the lower epidermis or form appressoria (Ayesu-Offei and Antwi-Boasiako, 1996; Babu et al., 2009). The conidia of *C. coffeicola* took around 4 hours to develop a germ tube, 36 hours after infection to penetrate the leaf and 35 days for lesion development and sporulation (Souza et al., 2011). The conidia emerged through or around the stomata. *C. henningsii* took around 9 hours to germinate, 1-3 days from inoculation to penetrate the leaf surface and the conidia were released 11 days after inoculation through ruptured epidermis at any part of the leaf surface (Babu et al., 2009).

Isolation and culturing the pathogen is difficult due to its slow growth, poor sporulation rate as well as the failure to obtain consistent lesions on the plant when artificially inoculated (Beckman and Payne, 1983; Cooperman and Jenkins, 1986). Vathakos and Walters (1979) observed that *C. kikuchii* sporulates best in senescent soybean leaf decoction agar under 8 hours of light, concluding that this pathogen yields abundant sporulation in conditions that are similar to natural conditions. When cultured, *Cercospora* had less sporulation on potato dextrose agar

(PDA) as compared to V8, and other plant-based agar (carrot leaf decoction, asparagus decoction, soybean leaf decoction and green corn leaf decoction). A photoperiod of 12 hours is optimum for sporulation however, depending on the *Cercospora* spp., the conidia can sporulate in both complete darkness and light.

Virulence of the pathogen and ability to sporulate can be maintained for 10 to 20 transfers and generally declines with further transfers (Cooperman and Jenkins, 1986). El-Gholl et al. (1982) and Vathakos and Walters (1979) were able to maintain sporulating cultures of *C. kikuchii* by selective subculturing for 30 transfers over 2 years. However, as the culture became older, the spore production decreased.

Whole plant inoculation has been successful with soybean, cassava, coffee and roses with the time from inoculation to lesion appearance ranging from 10 to 30 days (Ayesu-Offei and Antwi-Boasiako, 1996; Boelema, 1973; Souza et al., 2011; Vathakos and Walters, 1979).

Resistance for Cercospora Leaf Spot

Hagan et al. (2005) observed that shrub and groundcover roses had greater susceptibility to cercospora leaf spot compared to hybrid tea and grandiflora roses and noted variability between lesions and defoliation. In South Africa, rootstock Bayes #3 was highly susceptible to cercospora leaf spot (Boelema, 1973). It was first reported in Brazil on *R. multiflora*, which showed severe leaf symptoms (Feres et al., 2017). In Tennessee, most cultivars showed moderate cercospora leaf spot severity, and ‘Carefree Delight’, ‘Fairy Queen’ and ‘Nearly Wild’ had the highest scores for severity. ‘All The Rage’, ‘Midas Touch’, ‘Baby Bloomer’, ‘Pascali’, ‘Pink Knock Out’, ‘Beloved’, ‘Pristine’, ‘Hansa’, ‘Sunbright’, ‘Honey Perfume’, ‘Tahitian Moon’, ‘Knock Out’ and ‘Wildberry Breeze’ had no cercospora lesions (Windham et al., 2017).

The objective for this research was to evaluate resistance of selected rose plants to cercospora leaf spot under controlled environment conditions. Artificial inoculation was used in order to prevent black spot disease development that can occur during natural field evaluations as detailed in Chapter 2.

Material and Methods

Plant Material

Three diploid populations from parents 'J06-20-14-3' (J14-3), 'Little Chief' (LC), 'Vineyard Song' (VS), 'Red Fairy' (RF) and 'Old Blush' (OB) and a collection of cultivars (Tables 3.1 and 3.2) were screened for their resistance to cercospora leaf spot in a greenhouse at HortTREC (Horticulture Teaching, Research and Extension Center) in College Station, TX. 30 to 50 young plants from each population were propagated in the spring and fall of 2017 by rooting 3- to 4-node cuttings under mist. When the roots were established (around 4 weeks), they were planted into 4x4 inch pots with Sungrow Horticulture media with a slow release fertilizer (Osmocote® 20-20-20). Seedlings were grown for 3 months before inoculation. Three plants were kept for each inoculation. The cultivars were provided by Greenheart Farms as liners. The liners were transplanted into 4x4 inch pots and 5 plants from each cultivar were used for inoculation. Two weeks after transplanting, the cultivars were inoculated.

Table 3.1. Number of plants from the three diploid populations and cultivars used for *Rosisphaerella rosicola* inoculations.

Crosses	**Number of plants**	
	Summer 2018	**Winter 2018**
J14-3 x VS	53	58
J14-3 x LC	34	31
OB x RF	44	46
Cultivars		
Belinda's Dream		5
Burgundy Iceberg		5
Carefree Delight		5
Carefree Wonder		5
Cherry Parfait		5
Don Juan		5
Fiji		5
Firecracker Kolorscape		5
Hot Cocoa		5
Josephs Coat		5
Knock Out		5
Nearly Wild		5
Pink Enchantment		5
Sally Holmes		5
Sunny Knock Out		5
Westerland		5

Table 3.2. List of progenies used for inoculation of *Rosisphaerella rosicola* in Summer 2018 (S2018) and Winter 2018 (W2018). Plants used are marked with an X.

Progeny	**Crosses**	**S2018**	**W2018**	**Progeny**	**Crosses**	**S2018**	**W0218**	**Progeny**	**Crosses**	**S2018**	**W2018**
12046 N026	J14-3xLC	X	X	10073-N003	J14-3xVS	X		12062 N003	OBxRF	X	X
12046 N031	J14-3xLC	X	X	10073-N007	J14-3xVS	X	X	12062 N004	OBxRF	X	X
12046 N033	J14-3xLC	X	X	10073-N010	J14-3xVS	X	X	12062 N005	OBxRF		X
12046 N037	J14-3xLC	X		10073-N015	J14-3xVS		X	12062 N007	OBxRF		X
12046 N044	J14-3xLC	X		10073-N023	J14-3xVS	X	X	12062 N008	OBxRF	X	
12046 N047	J14-3xLC	X	X	10073-N029	J14-3xVS	X	X	12062 N009	OBxRF	X	X
12046 N049	J14-3xLC	X	X	10073-N030	J14-3xVS	X	X	12062 N010	OBxRF	X	X
12046 N054	J14-3xLC	X	X	10073-N031	J14-3xVS	X	X	12062 N011	OBxRF	X	X
12046 N055	J14-3xLC		X	10073-N032	J14-3xVS		X	12062 N014	OBxRF	X	X
12046 N057	J14-3xLC	X	X	10073-N035	J14-3xVS	X	X	12062 N015	OBxRF	X	X
12046 N063	J14-3xLC		X	10073-N037	J14-3xVS	X	X	12062 N016	OBxRF	X	X
12046 N068	J14-3xLC	X		10073-N043	J14-3xVS	X	X	12062 N017	OBxRF		X
12046 N071	J14-3xLC	X	X	10073-N046	J14-3xVS	X	X	12062 N018	OBxRF	X	X
12046 N072	J14-3xLC	X	X	10073-N048	J14-3xVS	X	X	12062 N021	OBxRF	X	X
12046 N073	J14-3xLC	X	X	10073-N051	J14-3xVS	X	X	12062 N022	OBxRF	X	X
12046 N078	J14-3xLC		X	10073-N056	J14-3xVS	X	X	12062 N024	OBxRF	X	X
12046 N079	J14-3xLC	X	X	10073-N057	J14-3xVS	X		12062 N027	OBxRF	X	X
12046 N080	J14-3xLC	X	X	10073-N060	J14-3xVS	X	X	12062 N034	OBxRF	X	X
12046 N083	J14-3xLC	X		10073-N062	J14-3xVS	X	X	12062 N035	OBxRF	X	X
12046 N086	J14-3xLC	X	X	10073-N066	J14-3xVS	X	X	12062 N036	OBxRF	X	
12046 N088	J14-3xLC	X	X	10073-N067	J14-3xVS	X	X	12062 N053	OBxRF	X	X
12046 N090	J14-3xLC	X	X	10073-N068	J14-3xVS	X	X	12062 N057	OBxRF	X	X
12046 N095	J14-3xLC		X	10073-N069	J14-3xVS	X	X	12062 N067	OBxRF	X	X
12046 N096	J14-3xLC	X		10073-N073	J14-3xVS	X		12062 N072	OBxRF	X	X
12046 N098	J14-3xLC	X	X	10073-N074	J14-3xVS	X	X	12062 N073	OBxRF	X	X
12046 N099	J14-3xLC	X		10073-N078	J14-3xVS	X	X	12062 N076	OBxRF	X	X
12046 N103	J14-3xLC		X	10073-N079	J14-3xVS	X	X	12062 N077	OBxRF	X	X

Table 3.2. Continued

Progeny	Crosses	S2018	W2018	Progeny	Crosses	S2018	W0218	Progeny	Crosses	S2018	W2018
12046 N104	J14-3xLC	X	X	10073-N080	J14-3xVS	X	X	12062 N084	OBxRF	X	X
12046 N107	J14-3xLC		X	10073-N087	J14-3xVS		X	12062 N085	OBxRF	X	X
12046 N116	J14-3xLC	X	X	10073-N088	J14-3xVS	X	X	12062 N089	OBxRF	X	X
12046 N117	J14-3xLC	X	X	10073-N089	J14-3xVS	X	X	12062 N090	OBxRF	X	X
12046 N121	J14-3xLC	X		10073-N091	J14-3xVS	X	X	12062 N099	OBxRF	X	
12046 N122	J14-3xLC	X		10073-N092	J14-3xVS	X	X	12062 N101	OBxRF	X	X
12046 N123	J14-3xLC	X	X	10073-N096	J14-3xVS	X	X	12062 N103	OBxRF	X	X
12046 N124	J14-3xLC	X	X	10073-N097	J14-3xVS	X	X	12062 N105	OBxRF	X	X
12046 N125	J14-3xLC	X	X	10073-N099	J14-3xVS	X	X	12062 N108	OBxRF		X
12046 N130	J14-3xLC	X	X	10073-N101	J14-3xVS	X	X	12062 N111	OBxRF	X	X
12046 N152	J14-3xLC	X		10073-N103	J14-3xVS	X	X	12062 N126	OBxRF	X	X
12046 N155	J14-3xLC	X	X	10073-N104	J14-3xVS	X	X	12062 N127	OBxRF	X	
12046 N157	J14-3xLC	X	X	10073-N105	J14-3xVS		X	12062 N128	OBxRF		X
				10073-N106	J14-3xVS	X	X	12062 N129	OBxRF	X	X
				10073-N108	J14-3xVS	X	X	12062 N131	OBxRF	X	X
				10073-N110	J14-3xVS	X	X	12062 N132	OBxRF	X	X
				10073-N111	J14-3xVS	X	X	12062 N138	OBxRF	X	X
				10073-N114	J14-3xVS	X	X	12062 N139	OBxRF	X	
				10073-N116	J14-3xVS	X	X	12062 N148	OBxRF	X	X
				10073-N118	J14-3xVS	X	X	12062 N151	OBxRF	X	X
				10073-N123	J14-3xVS	X	X	12062 N152	OBxRF		X
				10073-N124	J14-3xVS	X	X	12062 N155	OBxRF	X	X
				10073-N125	J14-3xVS	X	X	12062 N156	OBxRF	X	X
				10073-N127	J14-3xVS	X					
				10073-N130	J14-3xVS	X	X				
				10073-N132	J14-3xVS	X	X				
				10073-N135	J14-3xVS	X	X				
				10073-N137	J14-3xVS	X	X				

Table 3.2. Continued

Progeny	Crosses	S2018	W2018	Progeny	Crosses	S2018	W0218	Progeny	Crosses	S2018	W2018
				10073-N139	J14-3xVS	X	X				
				10073-N140	J14-3xVS	X	X				
				10073-N141	J14-3xVS		X				

Isolation and verification of the pathogen

Lesions with *Rosisphaerella rosicola* spores were collected from infected leaves from the Horticulture Farm along Hwy 2818 and from HORTTREC on Hwy 50. They were excised with a sterilized blade and pressed into water agar. The detached conidia were transferred to V8 media using a sterilized needle and incubated at 25°C in the dark for one month.

The pathogen was verified morphologically and with molecular data. The molecular confirmation was done by extracting the fungal DNA based on the protocol from the Zymo Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA). The primer sets ITS (Internal Transcribed Spacer) 1 and 4 and LSU (Large Subunit) were used to amplify the 18S ribosomal RNA (rRNA) region and the LSU 28S of the nuclear rRNA region (Table 3.3). After amplification of these regions via PCR, the PCR products were screened on a 2% agarose gel. The PCR products were sequenced by Sanger sequencing (Eton Bioscience Inc, San Diego, CA) and the nucleotides were assembled using Geneious software version 11.0.5 (Biomatters Inc., Newark, NJ). The nucleotide sequences were subjected to a BLAST search against the NCBI database.

Table 3.3. Primer sequences used to amplify fungal rRNA.

Primer	Primer Sequence (5'-3')	Reference
ITS1	TCC GTA GGT GAA CCT GCG G	White et al. (1990)
ITS1F	CTT GGT CAT TTA GAG GAA GTA A	Gardes and Bruns (1993)
ITS4	TCC TCC GCT TAT TGA TAT GC	White et al. (1990)
LR0R	ACC CGC TGA ACT TAA GC	Cubeta et al. (1991)
LR5	ATC CTG AGG GAA ACT TC	Vilgalys and Hester (1990)

Whole Plant Inoculation

For the Summer 2018 experiment, single spores were isolated and cultured in V8 media under 25°C in darkness for 2 -3 weeks. Mycelial discs (0.5 cm in diameter) were collected and placed on top of 5 young leaves for each plant. (Feres et al., 2017) (Figure 3.2). Once inoculated, the plants were put into a plastic tent (6ft wide, 23.5ft long and 3ft tall) with water trays to increase humidity for the first 72 hours after inoculation. These plants were inoculated in late July and were evaluated for disease incidence weekly from August to October 2018.

Figure 3.2. Mycelial discs (shown by red arrows) of cercospora placed on top of two leaves.

For the Winter 2018 experiment, fungal colonies were grown at 25°C in darkness for 2 weeks. Then the mycelium was inoculated onto a V8 media petri dish (Marcuzzo et al., 2015) to

induce sporulation (light intensity of 12.14 µmol s^{-1} and 25°C). After 1 week, the spores were collected by pouring 10 mL of water with 0.01% Tween 20 onto each plate and gently scraping the spores off with a scalpel. A concentration of $2x10^4$ conidia/mL (Cooperman and Jenkins, 1986) was atomized onto the plants in late November, 2018. The plants were put into a plastic tent with a misting fan and water trays for 2 weeks after inoculation to maintain a high relative humidity. The plants were evaluated for disease incidence from December to March.

Environmental conditions inside the plastic tent were monitored with a HOBO Temperature/ Relative Humidity logger.

All plants for both experiments were evaluated for disease incidence by counting the number of lesions on each plant.

Statistical Analyses

Descriptive statistics and ANOVA were calculated with JMP Pro 14.10.0 (SAS Institute Inc., Cary, NC, 2019). Tukey's honest significance test was used to compare the means. Pearson's correlations were calculated to assess associations between greenhouse and field evaluations (Chapter IV).

Results

Pathogen

Rosisphaerella rosicola conidia harvested from infected leaves were 32-50 µm long and 3-6 µm wide, had 0-4 septations, were pale brown in color and cylindrical and obclavate in shape (Figure 3.3) as expected (Davis, 1938; Feres et al., 2017; Videira et al., 2017). The conidia were mostly found unattached to the conidiophore when lesions were pressed onto the agar. The colonies were characterized by forming dense dark brown clusters, and slow growth (Figure 3.4).

The ITS and LSU regions were amplified by PCR following fungal DNA extraction. A BLAST search of the sequenced purified PCR products indicated at least 98% nucleotide sequence identity with *Passalora rosicola* and *Rosisphaerella rosicola* (Sequence ID: MF370214.1, MF951388.1 and MF951387.1) with the ITS regions and 100% identity with the LSU region of *Passalora rosicola* (Sequence ID: MF370215.1) (data not shown). Thus, the isolated colonies were confirmed to be *Rosisphaerella rosicola*.

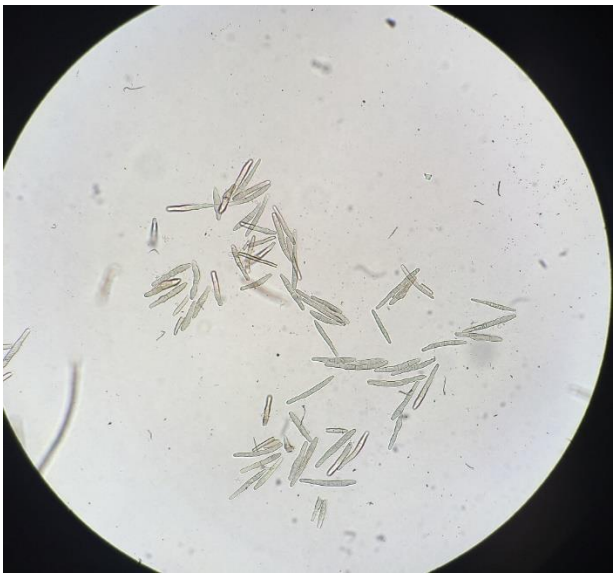


Figure 3.3. *Rosisphaerella rosicola* conidia at 400x magnification

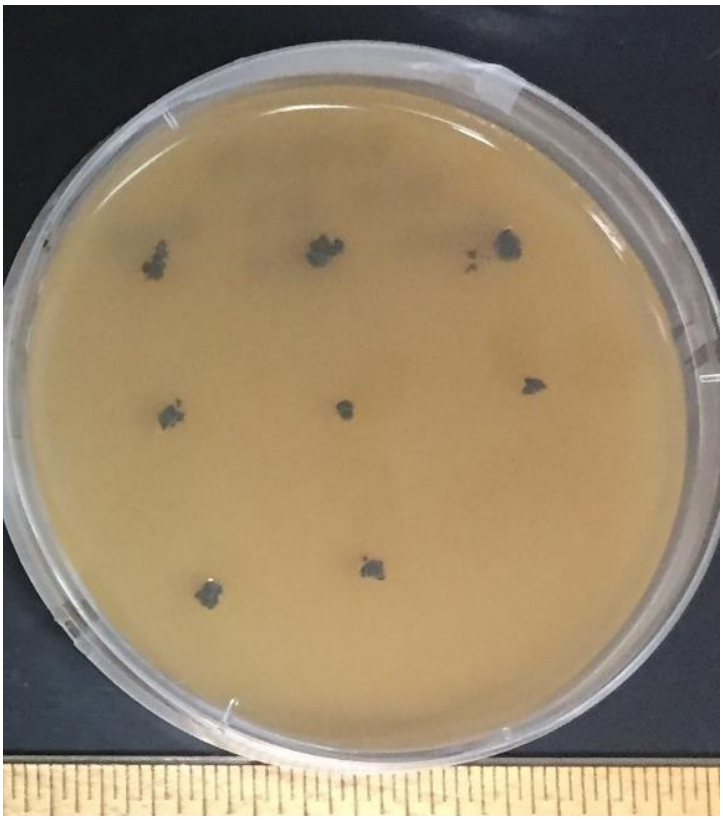


Figure 3.4. A 2-week-old culture of *Rosisphaerella rosicola*. It is distinguished by its slow growth, dark color and compact size.

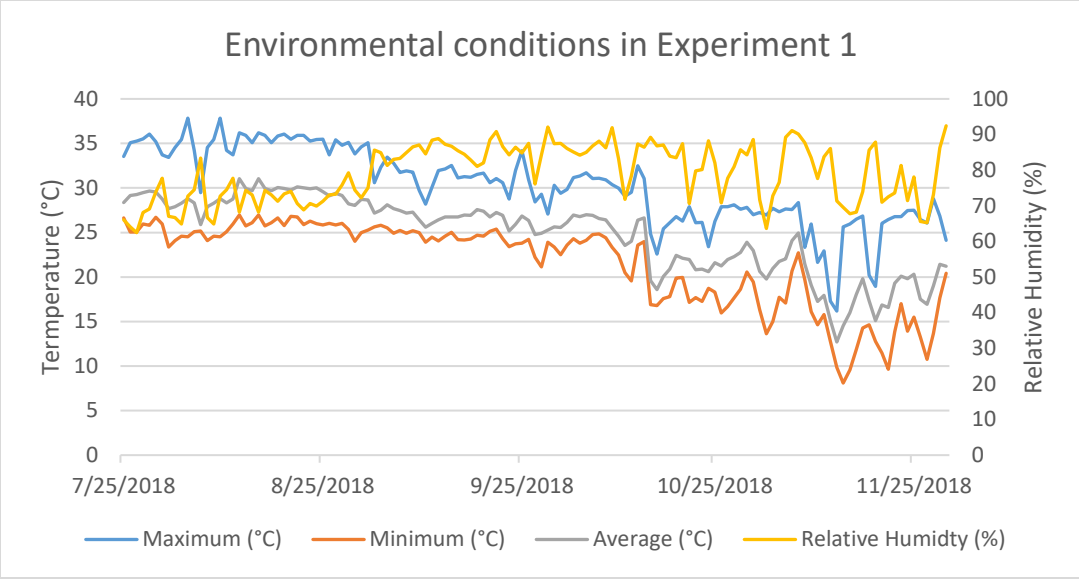
Environmental Conditions

Favorable disease development is dependent on the amount of inoculum, warm temperatures (optimal between 22-28ºC) and high relative humidity (Beckman and Payne, 1983; Cooperman and Jenkins, 1986; Vathakos and Walters, 1979). Lesion development is favored by long periods (4-7 days) of high relative humidity (90 ± 5%) and/or free water after inoculation (Cooperman and Jenkins, 1986) (Marcuzzo et al., 2016) (Beckman and Payne, 1983).

The greenhouse temperature varied substantially during the two experimental inoculation and evaluation periods (Figure 3.5). During the Summer 2018 trial, the temperatures ranged from 16ºC to 37ºC with a relative humidity between 60%-70% during the first weeks after the

inoculation. For the Winter 2018 experiment, the relative humidity was higher (70-80%) and the temperature was cooler (10ºC to 27ºC) during the first week after inoculation.

Overall observations indicated that lesion development occurred more in the cooler temperatures of 18-20ºC than in the high heat (28-31°C). The mycelial discs desiccated quickly under the high temperatures of July and the low relative humidity (Table 3.4), which lead to the low number of lesions produced in Summer 2018. However, it is unknown whether spraying the inoculum compared to placing mycelial discs on the leaves had an effect on disease development.



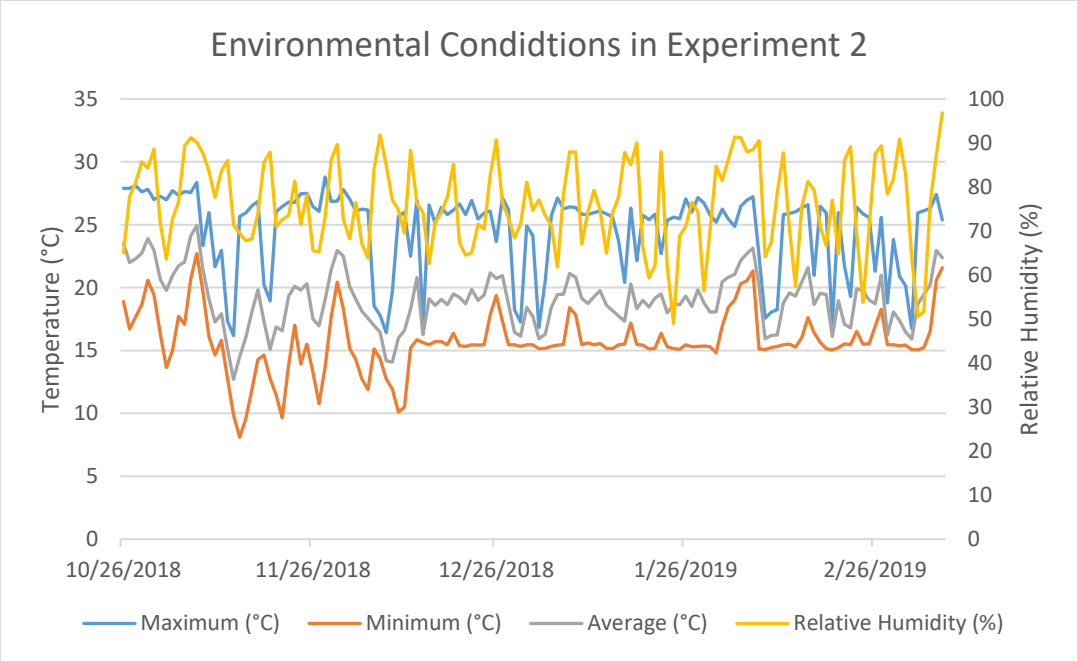


Figure 3.5. Maximum, minimum and average temperature and relative humidity recorded inside the greenhouse at HortTREC from July 2018 to March 2019.

Table 3.4. Maximum and minimum temperature and monthly average of relative humidity recorded in the greenhouse at HortTREC from inoculation to evaluations.

	Temperature (°C)		**Relative Humidity (%)**
	Maximum	Minimum	
July	34.9	25.9	68.8
August	35.1	25.5	72.4
September	31.1	24.4	85.1
October	28.3	20.4	83.7
November	25.3	14.7	77.6
December	24.1	15.0	75.0
January	25.0	15.6	73.4
February	23.8	16.6	78.0
March	23.6	16.7	75.8

Whole Plant Inoculation

All inoculated plants had lesions similar (Figure 3.6), although slightly smaller than the lesions observed in the field. Tiny dark spots started appearing around the third week after inoculation. Most lesions emerged after five weeks of inoculation (Tables 3.5 and 3.6).

The populations showed no to low counts of lesions (Figures 3.7 and 3.8). During the Winter 2018 experiment, most of the roses from population J14-3xVS had higher lesion counts compared to the other populations, however, in the Summer 2018 experiment, this population had the lowest number of lesions. In the field, J14-3xVS had the highest ratings for cercospora leaf spot and OBxRF and J14-3xLC had low incidence (Chapter IV). Cultivars that had the highest number of lesions were ‘Sunny Knock Out’ followed by ‘Fiji’ (Figure 3.9). Most cultivars showed very little disease development. Pathogenicity was demonstrated by both inoculations; however, the pathogen was not able to infect the plant well, most likely due to high temperatures (Summer 2018 experiment), low relative humidity during the first weeks after inoculation (65-75% in the Summer 2018 experiment and 70-80% in the Winter 2018 experiment) and insufficient inoculum.

Correlations between the greenhouse experiments and the field evaluations were very low (Figure 3.10), due to the poor disease development in the greenhouse experiments.

A 

B 

Figure 3.6. Cercospora lesions on ‘Sunny Knock Out’ rose 6 weeks after inoculation by spores. (A) Lesions observed on leaf. (B) Lesions observed on bract.

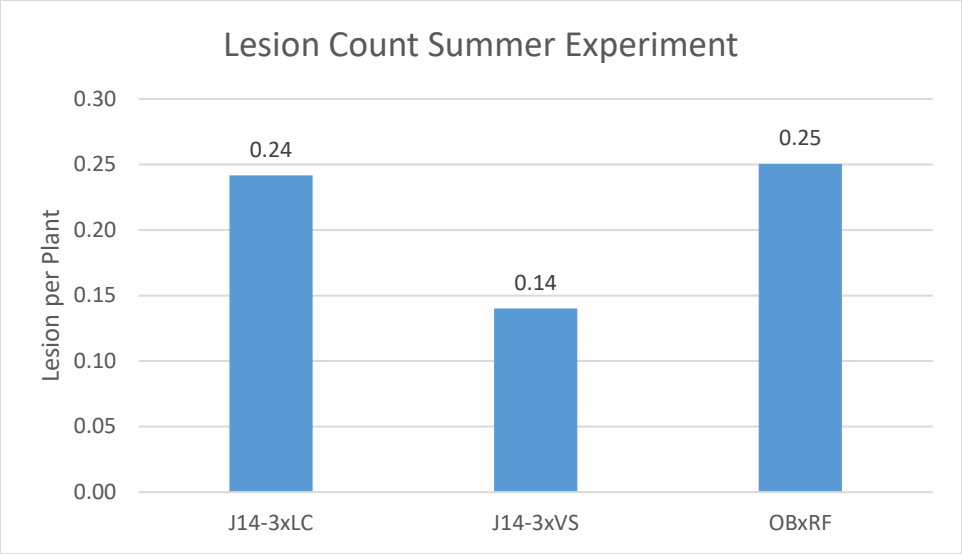


Figure 3.7. Average cercospora lesion counts/plant on the diploid rose populations after inoculation with mycelial discs evaluated weekly from August to October 2018 in the greenhouse in College Station, TX (Summer 2018 experiment).

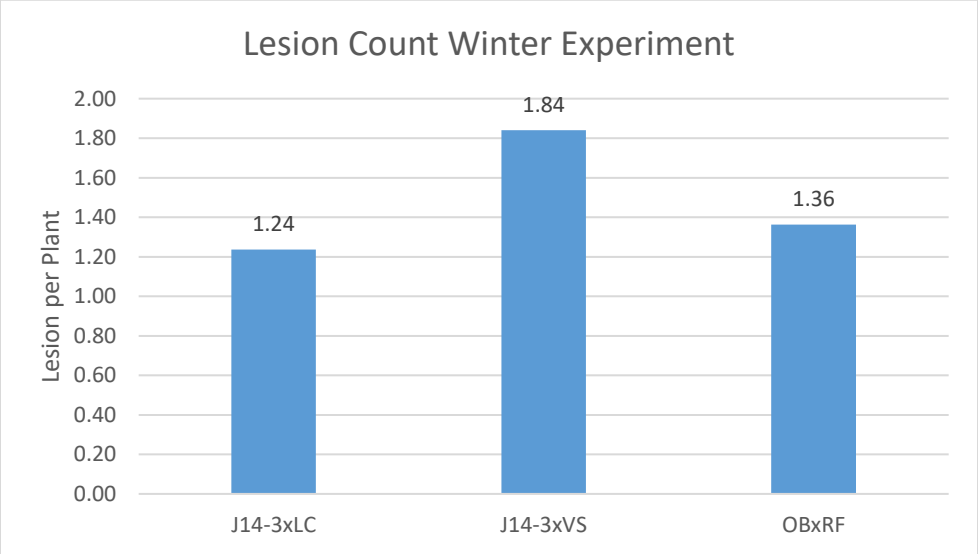


Figure 3.8. Average cercospora lesion counts/plant on the diploid rose populations after conidia inoculation evaluated weekly from December 2018 to March 2019 in the greenhouse in College Station, TX (Winter 2018 experiment).

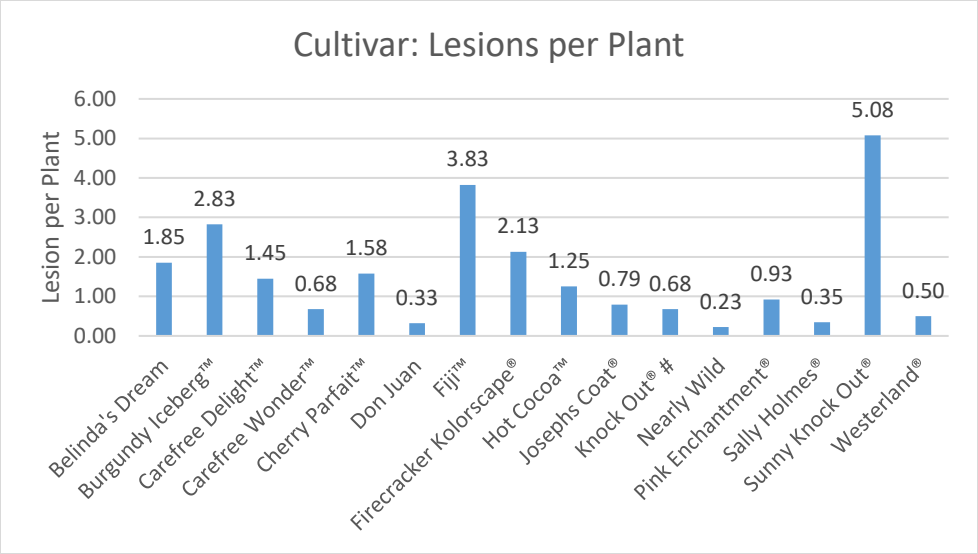


Figure 3.9. Average cercospora lesion counts/plant evaluated weekly from December 2018 to March 2019 on the rose cultivars after conidia inoculation in the greenhouse in College Station, TX (Winter 2018 experiment).

Table 3.5. Average number of lesions/plant and standard error during each evaluation in Summer 2018 experiment for the three diploid rose populations.

Evaluation date	Lesion Count	Standard Error
23-Aug-18	0.00e	0.09
30-Aug-18	0.00e	0.09
7-Sep-18	0.03de	0.09
13-Sep-18	0.03de	0.07
21-Sep-18	0.07cde	0.06
27-Sep-18	0.17bcd	0.06
4-Oct-18	0.27b	0.06
11-Oct-18	0.23bc	0.06
2-Nov-18	0.64a	0.06

Levels connected by same letter are not significantly different at $\alpha = 0.05$

Table 3.6. Average number of lesions/plant and standard error during each evaluation in Winter 2018 experiment for the three diploid rose populations and rose cultivars.

Evaluation date	Lesion Count			
	Population	Standard Error	Cultivar	Standard Error
20-Dec-18	0.52cd	0.19	0.45c	0.20
9-Jan-19	0.75d	0.09	0.45c	0.20
16-Jan-19	1.19c	0.09	1.30b	0.20
23-Jan-19	1.62b	0.09	2.19a	0.20
30-Jan-19	1.74b	0.09	2.01ab	0.20
6-Feb-19	2.15a	0.09	2.21a	0.20
13-Feb-19	1.75b	0.09	2.03ab	0.20
20-Feb-19	1.74b	0.09	1.60ab	0.20

Levels connected by same letter are not significantly different at $\alpha = 0.05$

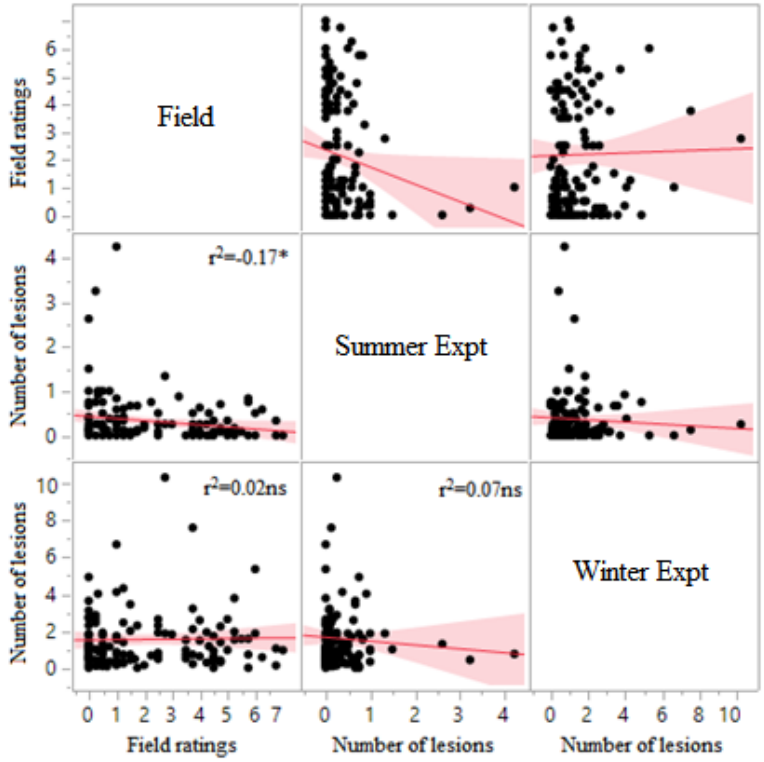


Figure 3.10. Pearson's correlations between the overall mean of lesions produced per plant from the two greenhouse experiments and the overall mean ratings from field evaluations (0-9 rating scale based on percentage of foliage covered with lesions) for diploid populations phenotyped in College Station 2016 in June, September, October and November. NS and * indicate not significant and significant at 0.05=p, respectively.

Discussion

Although *Rosisphaerella rosicola* was successfully isolated and cultured, sporulation and inoculation techniques need further research to discover the optimum conditions for spore production and germination for rose.

Culture medium influences the growth and sporulation of fungus. For sporulation, most describe V8 as an ideal medium for cercospora species sporulation, and with a 12-hour dark and light photoperiod, produced abundant spores for *C. beticola, C. coffeicola, C. zeae-maydis* and *C. asparagii* (Beckman and Payne, 1983; Conway, 1976; Cooperman and Jenkins, 1986; Souza et al., 2012). However, this method produced low sporulation rates for *R. rosicola*. Further work needs to be done to enhance sporulation by altering techniques (constant agitation, spreading mycelium over the plate and seeding) and environmental conditions (light intensity, temperature and media pH).

The amount of inoculum applied to the plants was limited to $2x10^4$ conidia/mL due to poor sporulation in culture. Although Cooperman and Jenkins (1986) observed moderate to severe cercospora ratings using this concentration, other studies used higher concentrations ($4.5x10^4$ to 10^5 conidia/mL) of inoculate to obtain good infection (Beckman and Payne, 1983; Rodriguez and Barreto, 2017; Souza et al., 2012). More work needs to be conducted to identify the optimum inoculum concentration required for infection of *R. rosicola* on garden roses.

Temperature and humidity play a major role in the success of Cercospora to germinate and successfully grow (Cooperman and Jenkins, 1986; Meredith, 1970; Rupe et al., 1982) . Multiple *Cercospora* species have optimal germination and infection at high relative humidity (greater than 90%) and warm temperatures (25-30°C) with temperatures greater than 34°C inhibiting germination (Beckman and Payne, 1983; Cooperman and Jenkins, 1986). Although

for most cercospora leaf spot species optimal disease development occurs between 25-30°C, this may not be accurate for those that affect roses. For example, *C. coffeicola* has a lower optimal temperature (18-22°C) for germination (Souza et al. (2011). Thus, it is likely that the low level of disease development in these experiments was due to not only low levels of inoculum but also suboptimal temperature and humidity for spore germination and disease development. Although a detached leaf assay would help control these environmental factors, this method would not be viable for this disease because it takes ~1 month for lesions to develop (Feres et al., 2017). Due to the poor disease development, the greenhouse assessments did not reflect field performance as indicated by the low correlations among greenhouse and field observations of disease incidence.

There were many challenges with this research such as slow disease development both in culture and on the plant, poor sporulation, and unfavorable environmental conditions for disease development. Although this study demonstrates pathogenicity of *Rosisphaerella rosicola,* severe disease symptoms were not expressed following inoculation of plants with the fungus. More research needs to be conducted on *Rosisphaerella rosicola* growth, infection process and optimum environment for disease development and spread on rose for efficient greenhouse screening.

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CHAPTER IV

QTL DISCOVERY FOR CERCOSPORA LEAF SPOT USING PEDIGREE BASED ANALYSIS FOR AN INTERRELATED POPULATION OF DIPLOID ROSES

Introduction

In the southeastern USA, the most common rose foliar diseases are black spot (*Diplocarpon rosae*), powdery mildew (*Sphaerotheca pannosa*) and cercospora leaf spot (*Rosisphaerella rosicola* (teleomorph: *Mycosphaerella rosicola*, syn: *Passalora rosicola* and *Cercospora rosicola* Pass) (Mangandi and Peres, 2009; Videira et al., 2017). The symptoms of cercospora leaf spot resemble those of black spot and thus it is often misdiagnosed. Cercospora leaf spot commonly appears on rose leaves, pedicels, stems and bracts (Mangandi and Peres, 2009). Lesions appear as dark circular spots with a dark red or purple halo and a tan necrotic center. As the lesion grows, the tan area will widen and dark spots, known as the stromata, appear scattered within the tan necrotic area. These dark stromata are the site of conidia growth and development. Cercospora leaf spot mainly develops on the adaxial side of the leaf but can also occur on the abaxial side. The conidia overwinter on the leaves and are dispersed by splashing water and wind (White and Klingeman, 2014). The infection typically starts from the bottom of the canopy and progresses upward to the newer growth.

Compared to the effects of black spot and powdery mildew, cercospora has a lesser economic effect on roses. Although recently, this disease is becoming a larger threat to susceptible cultivars, most likely due to decreasing application of fungicides resulting from the development of roses with higher black spot resistance. Unfortunately, little work has been done on either the pathogen or the disease.

Roses are highly heterozygous and prone to moderate inbreeding depression. The inbreeding between modern rose classes may have led to reduced fertility (Zlesak, 2006). Other challenges for breeders include germination difficulties, the need for large progeny size and the long breeding cycle. Knowledge of trait inheritance and identifying genetic markers for marker assisted breeding are valuable tools that can be used to identify desirable parents, make early selections of progeny, conduct gene pyramiding and select essential traits including those with low heritability (Collard et al., 2005). Improved mapping techniques and a large number of markers allow for the identification for more tightly linked markers, which may provide for wider adoption of marker-assisted selection.

Thus far genes and QTLs have been described in roses for black spot (Whitaker et al., 2010; Zurn et al., 2018) and powdery mildew resistance (Dugo et al., 2005; Gar et al., 2011; Linde et al., 2006), flower color and size (Debener and Mattiesch, 1999; Gar et al., 2011), recurrent blooming, prickles (Crespel et al., 2002) and leaf size (Dugo et al., 2005) but nothing is known about the inheritance of resistance to cercospora in rose. QTLs for resistance have been discovered in corn, soybean, sugar beet, mungbean and cowpea, and cercospora resistance has been described as complex due to the multiple genes involved and different races (Berger et al., 2014; Chankaew et al., 2011; Duangsong et al., 2016; Han et al., 2018; Taguchi et al., 2011).

A recent approach for QTL mapping is a pedigree-based analysis using the Bayesian method (Bink et al., 2002). This analysis incorporates multiple related populations with known pedigrees to identify QTLs and their genetic components by tracing alleles identical by descent. Using pedigree data improves statistical power and facilitates the estimation of genetic parameters and the detection of minor and major QTLs (Bink et al., 2014). The Bayesian approach is implemented by the software FlexQTL (Bink et al., 2008) and has been applied with

highly heterozygous, clonally propagated crops, such as apples, strawberry, peach, rose and sweet cherry (Cai et al., 2018; Fresnedo-Ramírez et al., 2015; Mangandi et al., 2017; Verma et al., 2019).

The objectives of this study are to estimate the heritability for cercospora leaf spot resistance in interrelated diploid rose populations and identify QTL(s) for cercospora leaf spot resistance.

Material and Methods

Plant Material and Field Assessment

Fifteen interrelated diploid rose populations (Table 4.1 and Figure 4.1) were evaluated in the field for cercospora leaf spot and black spot incidence during June, September, October and November 2016. These populations were located at the Horticulture Farm at Texas A&M University, College Station, TX. The plants were grown with weed barrier. No fungicides or pesticides were sprayed. Pruning was performed to remove dead wood and to synchronize the growth of the seedlings early during the year (February or March). One plant per seedling from each of the 15 populations was planted in the field.

The disease incidence was rated with a 0 to 9 scale, where 0 indicates no disease symptoms on the rose canopy, 1 = few lesions on the rose canopy, 2-8 = lesions across the rose canopy based on percentage of leaves with lesions and 9 = greater than or equal to 90% of the foliage infected with some defoliation and chlorosis. J06-30-3-3 (J3-3), J06-30-3-6 (J3-6), M4-4, Old Blush' (OB) and 'Sweet Chariot' are slightly susceptible (average field ratings less than 2) to cercospora leaf spot. 'Sweet Chariot' (SC) is reported to be resistant by Hagan et al. (2005). J06-20-14-3 (J14-3) is susceptible to the disease (average field rating greater than 3). The

susceptibility of ‘Red Fairy’ (RF), ‘Little Chief’ (LC), ‘Vineyard Song’ (VS) and J06-28-4-6 (J4-6) are unknown.

Table 4.1. Fifteen interrelated diploid rose populations used for genetic analysis for cercospora resistance.

Cross	Female Parent	Male Parent	Number of Progeny
J14-3xLC	J06-20-14-3	Little Chief	71
J14-3xRF	J06-20-14-3	Red Fairy	121
J14-3xSC	J06-20-14-3	Sweet Chariot	58
J14-3xVS	J06-20-14-3	Vineyard Song	83
J3-3xRF	J06-30-3-3	Red Fairy	19
J4-6xRF	J06-28-4-6	Red Fairy	61
M4-4xSC	M4-4	Sweet Chariot	10
M4-4xVS	M4-4	Vineyard Song	5
OBxJ3-6	Old Blush	J06-30-3-6	82
OBxM4-4	Old Blush	M4-4	10
OBxRF	Old Blush	Red Fairy	67
SCxJ14-3	Sweet Chariot	J06-20-14-3	24
SCxJ4-6	Sweet Chariot	J06-28-4-6	10
SCxM4-4	Sweet Chariot	M4-4	52
VSxJ14-3	Vineyard Song	J06-20-14-3	6

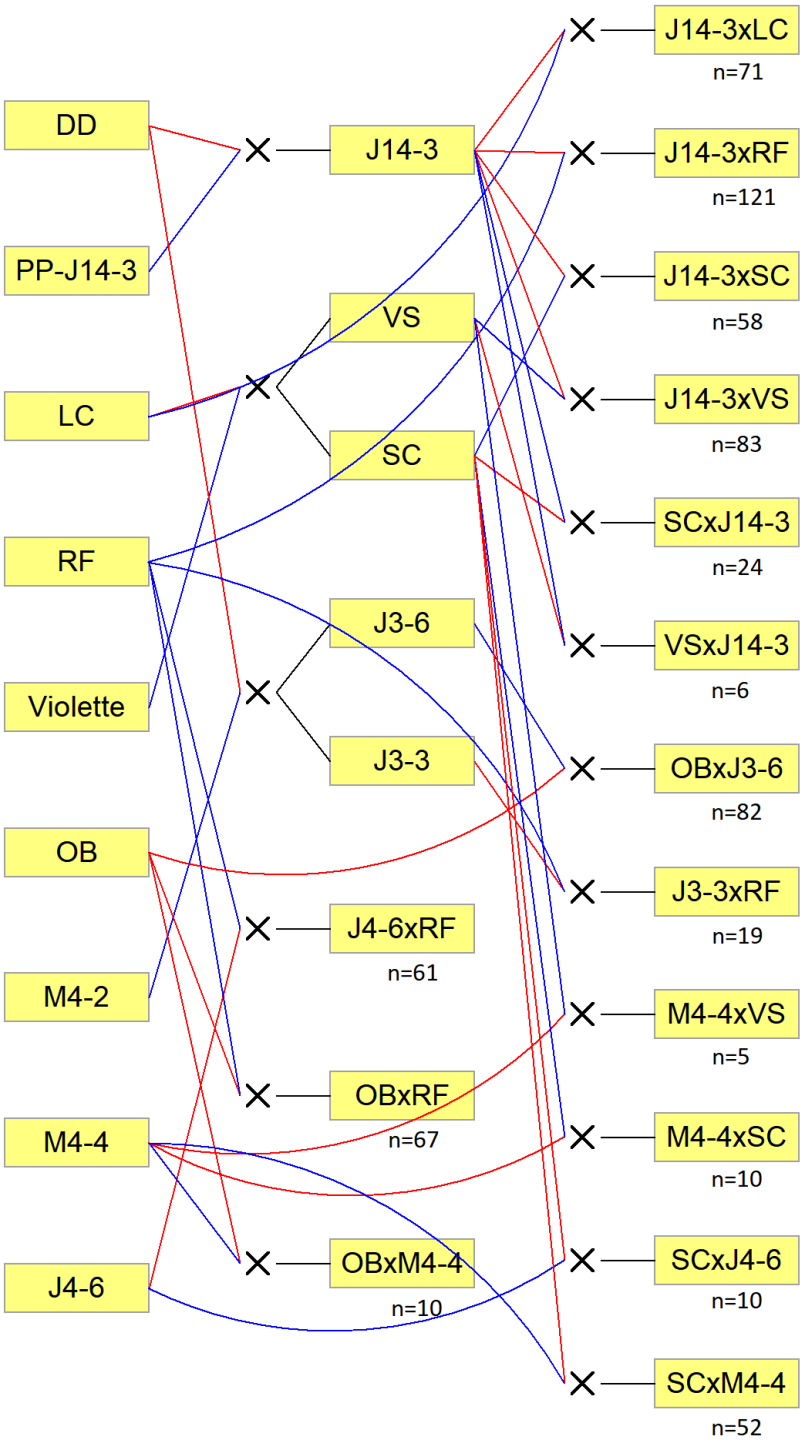


Figure 4.1. Pedigree of the 15 diploid rose populations and their progeny number. Red and blue lines link progeny to female and male parents, respectively.

The soil at the Texas A&M Horticulture Farm belongs to the Robco series, which has a slightly acid, loamy, sandy topsoil and strongly acid clay loam subsoil (Web Soil Survey, 2017). Challenges with this type of soil is the need for lime, low fertility and high possibility of drought. The climate in College Station is warm and humid (USA hardiness 8b), with winters infrequently going under 0°C. The monthly mean maximum temperature ranged from 24-28°C in spring, 33-36°C in summer, 24-33°C in fall and 16-24°C in winter while the monthly mean minimum temperature was 13-18°C, 23-25°C, 13-23°C, and 5-9°C during spring, summer, fall and winter, respectively. Total precipitation was 801 mm and although it was distributed throughout the growing season, it peaked in late May, followed by August (Table 4.2, Figure 4.2).

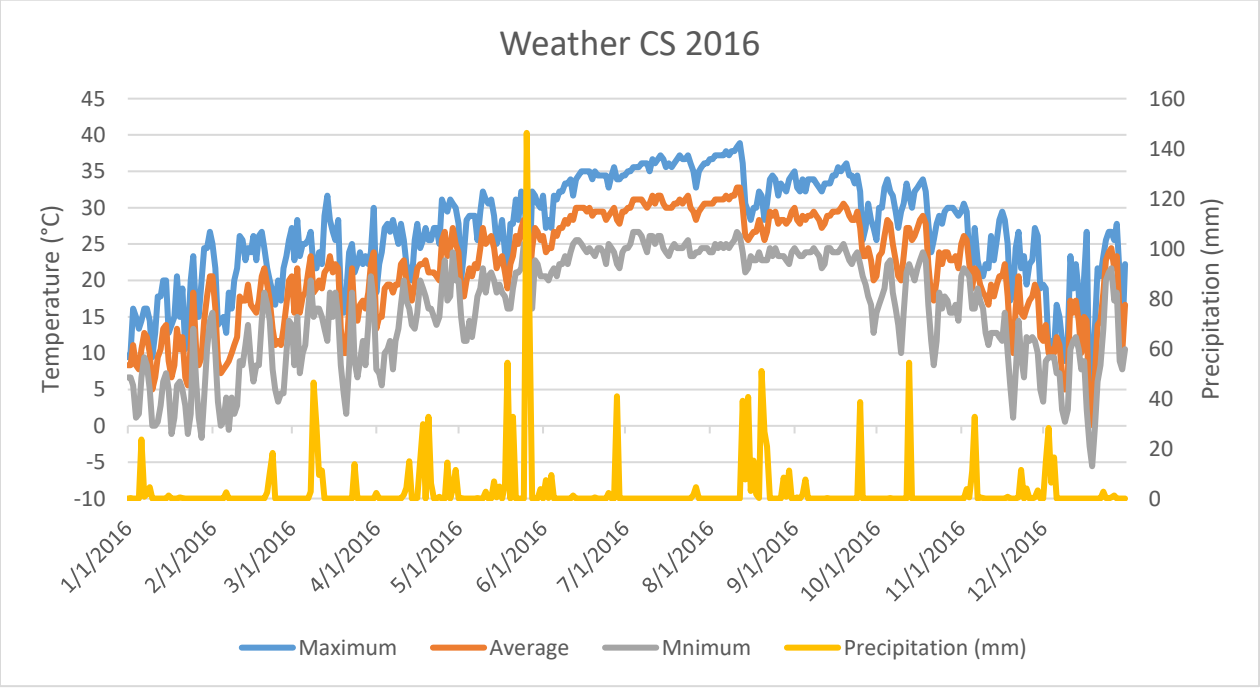


Figure 4.2. Climate in College Station, TX during 2016 (Source: wunderground.com).

Table 4.2. Temperature and total precipitation during 2016 in College Station, TX. (Source: wunderground.com).

	Temperature (°C)			
Month	**High**	**Average**	**Low**	**Precipitation (cm)**
January	16.5	10.67	4.5	3.3
February	21.1	14.6	7.9	3.4
March	24.3	18.6	12.7	11.3
April	26.2	20.8	15.1	13.8
May	28.1	23.4	18.5	32.8
June	33.1	28.3	23.2	6.2
July	35.9	30.5	24.9	0.6
August	34.1	29.2	23.8	22.7
September	32.8	27.9	22.6	4.9
October	30.1	23.9	17.5	5.5
November	24.1	18.7	12.9	6.9
December	17.9	13.4	8.7	7.1

Statistical Analysis

Statistical analyses for the phenotypic data were performed in JMP software Pro 14.0.0 (SAS Institute Inc). Normality analysis of the monthly ratings and overall means data (original and transformed by square root or log 10) was assessed by the Shapiro-Wilks test. Monthly ratings and comparisons between populations or accessions were done using an analysis of variance followed by a Student t test for mean separation. Pearson's correlations were assessed to explore any associations between cercospora and black spot incidence as well as between evaluated months. The variance components for the monthly evaluations were calculated using restricted maximum likelihood (REML) estimation with the model $\gamma=\mu+ \sigma^2_{Population}+\sigma^2_{Error}$, where μ is the cercospora leaf spot incidence mean for the month and $\sigma^2_{Population}$ is the variance among populations and σ^2_{Error} is the residual error from segregation, dominance and environmental conditions (Walsh, 2007). Phenotypic variance (Vp) was estimated by the sum of the variance among populations (Vpop) and the residual variance (Ve). Broad sense heritability (H^2) was

calculated by 2(Vpop) divided by phenotypic variance (Vp). Variance components from the overall evaluation were estimated using restricted maximum likelihood (REML) estimation with the model $\gamma=\mu+ \sigma^2_{FP}+\sigma^2_{MP}+\sigma^2_{Progeny[FP, MP]} + \sigma^2_{Month}+ \sigma^2_{FPxMonth}+ \sigma^2_{MPxMonth}+\sigma^2_{ProgenyxMonth}+\sigma^2_{Error}$, where μ is the cercospora leaf spot incidence mean, σ^2_{FP} and σ^2_{MP} are the variances for the female (FP) and male (MP) parent, respectively, $\sigma^2_{Progeny[FP, MP]}$ is the variance for progenies of a given cross, σ^2_{Month} is the variance due to the month of assessment, $\sigma^2_{FPxMonth}$, $\sigma^2_{MPxMonth}$, and $\sigma^2_{ProgenyxMonth}$ are variances due to the interaction of female and male parents and progenies with the month of assessment, and σ^2_{Error} is the error variance.

The additive variance (Va) was calculated as the sum of the variance of the parents, the non-additive variance (Vd) was the variance of progeny and the sum of the variances for the interaction between the genotype (FP, MP, and progeny) and the month of assessment was treated as the genetic-environmental variance (Vg x e) since the variance due to the interaction of progeny x month was confounded with residual error. Phenotypic variance (Vp) was calculated by Vp = Va + Vd + Vg x e/E, where E indicates the number of months used in the analysis. Narrow sense heritability (h^2) was calculated by dividing Va by the phenotypic variance (Vp), and broad sense heritability (H^2) was calculated as Vg (Va + Vd) divided by Vp (Hallauer et al., 2010).

QTL Detection

Progenies were genotyped by genotyping-by-sequencing (GBS) using the methylation sensitive restriction enzyme *Ngo*MIV (Yan et al., 2018). The rose consensus map developed by Yan et al. (2018) was constructed using SNP data from 5 families (J14-3 x LC, J14-3 x VS, OB x RF, J4-6 x RF and OB x J3-6) which resulted in 4538 SNP markers across the 7 rose linkage groups. At the time of map construction, the *Fragaria vesca* genome sequence was used as a

'proxy' reference genome to identify SNP within the rose progeny since the rose genome sequence was unavailable (Yan et al., 2018). The SNP markers were named according to their position on the *Fragaria vesca* genome sequence. Markers were removed if they had more than 10% missing data, double recombination, singletons or inheritance conflicts, which were obtained from FlexQTL. After data curation, 791 SNP markers spread over 7 linkage groups (Table 4.3) were used for the FlexQTL analysis.

Phenotypic and genotypic data were analyzed by FlexQTL software to identify QTLs (Bink et al., 2008; Bink and van Eeuwijk, 2009; Roach et al., 2016). The analysis was run at least 4 times on each evaluation date and the overall data, with different chain length, prior and maximum number of QTL to obtain an effective chain size of at least 100 for the phenotypic mean, variance of the error, number of QTLs and the variance for the number of QTLs to ensure valid statistical inferences and conclusions (Sorensen and Gianola, 2002). Markov chain length varied from 200,000 to 475,000 iterations, from which 1,000 simulations were sampled for statistical inference. A mixed model that incorporates both the additive and dominant model was applied. An additive only model was also run, however, due to low effective chain size, this model was not used. Evidence of QTL presence was determined by 2ln Bayes Factor (2lnBF) values greater than 5 for at least one environment or a 2ln(BF) greater than 2 for at least 2 environments and consistent co-localization of ±4cM (Rawandoozi, 2019).

From the FlexQTL software, additive variance (Va) was calculated by subtracting the residual variance (Ve) from the phenotypic variance (Vp) and the narrow sense heritability (h^2) was calculated by:

$$h^2 = {}^{Va}/_{Vp}$$

Phenotypic variance explained (PVE) by each QTL was estimated with FlexQTL™ software outputs and calculated as described (Rawandoozi, 2019):

$$\text{PVE} = {}^{\text{Va}}/_{\text{Vp}} \times 100$$

where Va is additive variance (AVT1) of QTL.

Table 4.3. Number of SNP markers and length of each linkage group on the diploid rose map used for FlexQTL analysis (Yan et al., 2018).

Linkage Group	Markers	Length
1	78	49
2	204	77
3	54	52
4	110	55
5	114	76
6	132	63
7	99	58

Haplotype Analysis

The SNPs within the QTL interval were selected to study haplotypes. The haplotypes in the progeny were identified manually and were assigned a QTL genotype (QQ for high ratings, Qq for segregation or qq for low ratings). Analysis of variance and student's t-test was conducted in JMP software Pro 14.0.0 (SAS Institute Inc) to determine differences between the QTL genotype effects on cercospora leaf spot disease incidence. The haplotypes and the QTL genotype prediction were generated by the FlexQTL software using the "mhaplotypes.cvs" and "Gtp_Genome.cvs" files.

Results

Cercospora Field Assessment

Neither the raw or transformed data exhibited a normal distribution (Table 4.4), consequently all statistical analyses were based on the original data. Monthly distributions of cercospora evaluations are skewed to the right (Figure 4.3). This indicates that most of the progeny had low to moderate cercospora leaf spot incidence. In the overall mean evaluation, 40% of the plants had a rating less than 1. Disease severity varied between month and populations. The month with the highest disease severity was November and the lowest severity was September (Table 4.5). The families with the highest disease incidence rating were VSxJ14-3 (4.71) and its reciprocal cross J14-3xVS (4.38) while OBxM4-4, SCxM4-4, OBxRF, OBxJ3-6 and J14-3xLC had average cercospora ratings less than 1 (Table 4.6).

For cercospora leaf spot disease development, a warm climate (20-30°C), high relative humidity and leaf wetness for spore germination, and amount of inoculum are important factors (Cooperman and Jenkins, 1986; Rupe et al., 1982). Also, cercospora leaf spot interaction with black spot may play a role as black spot develops faster than cercospora (7-10 days compared to 3-4 weeks) thus limiting cercospora growth (Debener et al., 1998; Feres et al., 2017).

There is a moderately high correlation between cercospora ratings between months (r=0.44-0.67) and the overall mean (r=0.72-0.85), however there is a consistently low negative correlation (r=-0.39-0.02) between blackspot and cercospora (Table 4.7 and Figure 4.4). The correlations between cercospora ratings and black spot ratings, although significantly different from zero, are low suggesting that different genes condition resistance to the diseases.

Table 4.4. Normality (Shapiro-Wilk) analysis of the raw data and data transformation (square root and log 10) for percent of foliage infected with cercospora leaf spot of the diploid rose populations in June, September, October and November and the overall mean of the four evaluated months in College Station in 2016.

	June			September			October			November			Average		
Population	**Raw**	**$x^{0.5}$**	**log10**	**Raw**	**$x^{0.5}$**	**log10**	**Raw**	**$x^{0.5}$**	**log10**	**Raw**	**$x^{0.5}$**	**log10**	**Raw**	**$x^{0.5}$**	**log10**
J14-3xLC	***	***	***	***	***	**	***	***	**	***	***	***	***	**	*
J14-3xRF	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***
J14-3xSC	**	***	*	***	***	***	***	***	**	***	***	***	*	NS	**
J14-3xVS	***	***	***	***	***	***	**	***	***	**	***	***	*	***	***
J3-3xRF	**	***	**	**	**	**	*	*	*	*	***	**	NS	NS	*
J4-6xRF	***	***	NS	***	***	***	***	***	***	***	***	***	**	**	***
M4-4xSC	***	***	*	***	**	**	***	**	**	NS	**	NS	***	NS	NS
M4-4xVS	NS	NS	NS	***	***	.	*	NS	**	**	**	***	NS	NS	NS
OBxJ3-6	NS	***	***	***	***	***	***	***	***	***	***	***	***	***	***
OBxM4-4	***	***	.	***	***	.	***	***	.	***	***	***	***	***	NS
OBxRF	***	***	NS	***	***	***	***	***	***	***	***	***	***	***	**
SCxJ14-3	**	***	NS	***	***	NS	***	***	***	*	***	**	NS	NS	NS
SCxJ4-6	*	*	**	**	**	*	*	**	NS	**	***	**	NS	NS	NS
SCxM4-4	***	***	**	***	***	***	***	***	NS	***	***	***	***	***	**
VSxJ14-3	**	***	**	NS	NS	NS	*	**	**	NS	**	NS	NS	*	**

NS, *, **, *** not significant, significant at p<0.05, 0.01, and 0.001, respectively

- indicates one or no observation present

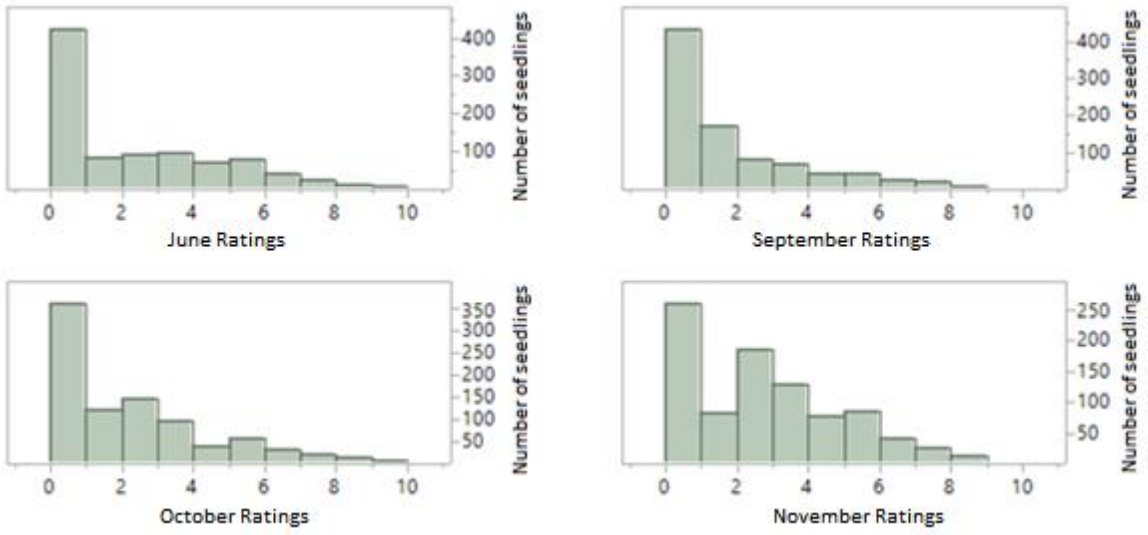


Figure 4.3. Monthly (June, September, October and November 2016) distribution of cercospora leaf spot incidence based on percentage of infected foliage (0 to 9 scale) in field plots in College Station, TX on 15 interrelated diploid rose populations.

Table 4.5. Mean comparison of cercospora leaf spot ratings (0 to 9 scale) and standard error based on percentage of infected leaves on plant in June, September, October and November 2016 for 15 diploid rose populations in College Station, TX.

Month	Mean	Standard error
June	1.87b	0.09
September	1.32c	0.09
October	1.74b	0.09
November	2.30a	0.10

Levels connected by same letter are not significantly different at $\alpha = 0.05$

Table 4.6. Mean comparison of cercospora leaf spot rating (0 to 9 scale) for each diploid rose population among June, September, October and November 2016 and their average in College Station, Texas.

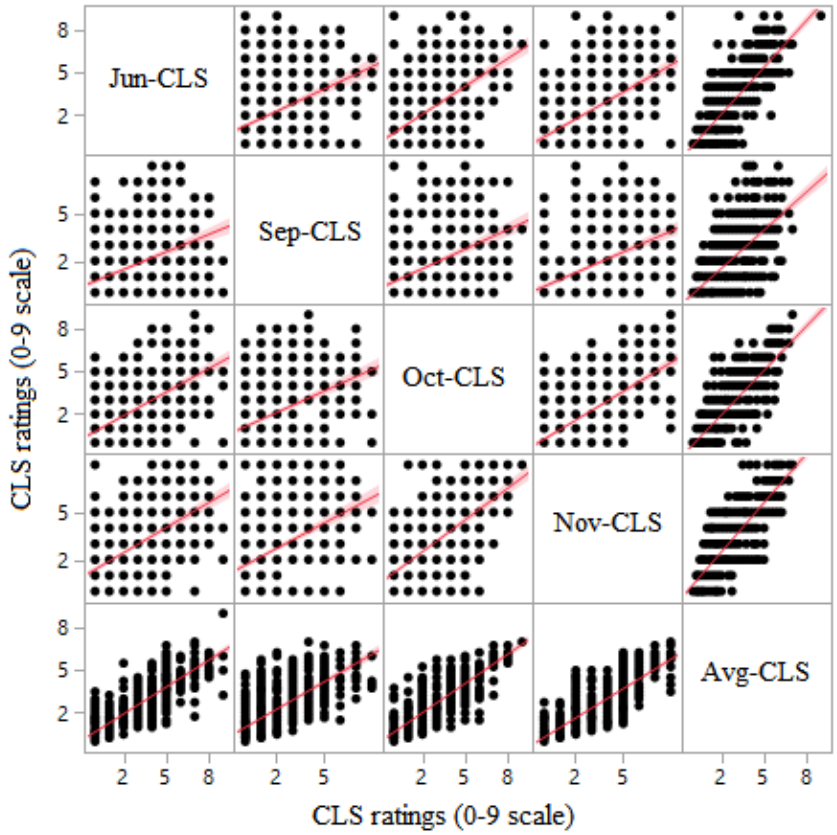
Population	June	September	October	November	Average
J14-3xLC	1.53ce	0.86cde	0.42f	0.96def	0.97efg
J14-3xRF	2.62bd	2.09b	2.69c	2.82c	2.55b
J14-3xSC	3.40b	0.89cde	2.92c	2.84c	2.58bd
J14-3xVS	4.67a	3.60a	4.06ab	5.19a	4.38a
J3-3xRF	1.89bcde	2.67ab	2.72bcd	3.33bc	2.64bcd
J4-6xRF	0.54ef	1.05cde	1.39def	2.82c	1.45e
M4-4xSC	2.11bcdef	1.63abcde	2.88bcde	3.88abc	2.61bcd
M4-4xVS	2.60abcdef	0.40bcde	1.00cdef	1.00cdef	1.25cdefgh
OBxJ3-6	0.48ef	0.60de	0.45f	1.62de	0.78fgh
OBxM4-4	0.10ef	0.11de	0.13ef	0.38def	0.17gh
OBxRF	0.08f	0.56de	0.51f	0.50f	0.43gh
SCxJ14-3	2.88bcd	1.67bcd	1.96cde	2.08cd	2.15bcd
SCxJ4-6	1.30cdef	0.89bcde	1.22cdef	2.78bcd	1.54cef
SCxM4-4	0.40ef	0.12e	0.36f	0.69ef	0.39h
VSxJ14-3	4.50ab	3.17abc	6.00a	5.17ab	4.71a

Levels connected by same letter within a column are not significantly different at $\alpha = 0.05$

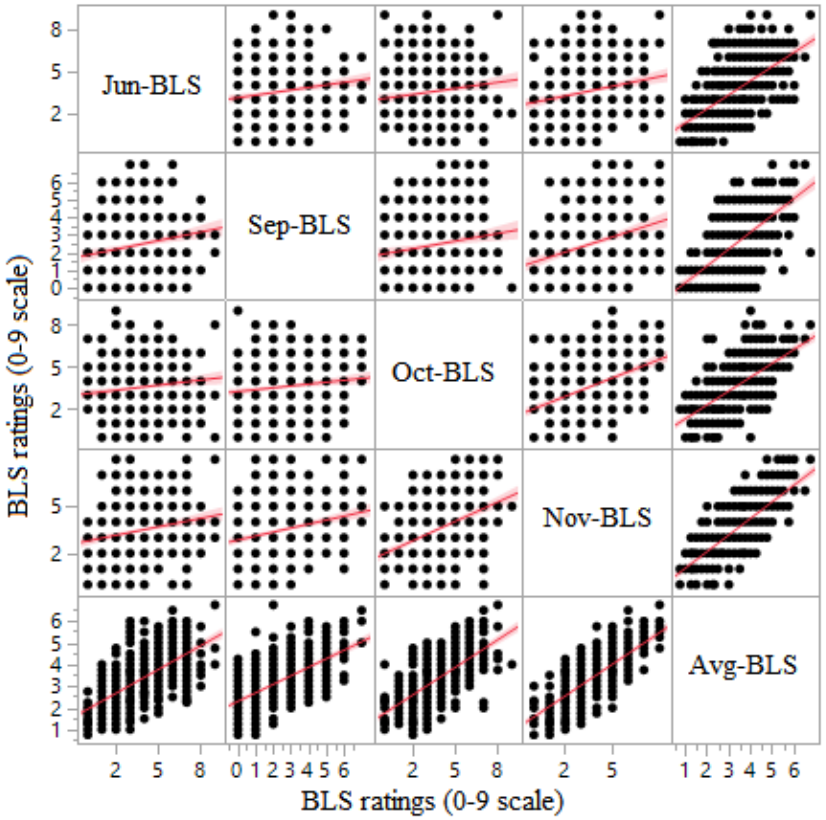
Table 4.7. Correlations between cercospora leaf spot (CLS) and black spot (BLS) incidence, and between evaluated months (June, September, October and November) and their mean for 15 diploid rose populations (n = 679) in College Station, Texas in 2016.

	Jun-CLS	Sep-CLS	Oct-CLS	Nov-CLS	Avg-CLS	June-BLS	Sep-BLS	Oct-BLS	Nov-BLS	Avg-BLS
Jun-CLS		0.44***	0.60***	0.58***	0.83***	-0.39***	-0.18***	-0.06ns	-0.23***	-0.34***
Sep-CLS			0.45***	0.48***	0.72***	-0.22***	0.02ns	-0.05ns	-0.20***	-0.18***
Oct-CLS				0.67***	0.85***	-0.23***	-0.16***	-0.10*	-0.15***	-0.25***
Nov-CLS					0.84***	-0.32***	-0.22***	-0.05ns	-0.24***	-0.32***
Avg-CLS						-0.36***	-0.17***	-0.09*	-0.25***	-0.34***
June-BLS							0.17***	0.13**	0.20***	0.61***
Sep-BLS								0.14**	0.27***	0.61***
Oct-BLS									0.42***	0.65***
Nov-BLS										0.72***

NS, *, **, *** not significant, significant at p<0.05, 0.01, and 0.001, respectively.



A



B

Figure 4.4. Scatterplot for correlations between the evaluations in June, September, October and November for cercospora leaf spot (CLS) (A) and black spot (BLS) (B). Both were rated according to the percentage of infected leaves on the rose canopy (0 to 9 scale).

Genetic Variance and Heritability Estimations for Cercospora Leaf Spot

The broad sense heritability varied from 0.52 - 0.9 for the monthly evaluations (Table 4.8) and broad and narrow sense heritability estimates from the variance component analysis for the diploid rose populations over the 4 months were 0.83 and 0.57, respectively (Table 4.9). The moderately high heritabilities indicate the importance for both additive and non-additive genetic effects. There was higher additive variance than non-additive variance for the overall mean ratings. In the monthly evaluations, the error variance was larger than the population variance. As expected with disease incidences that are dependent on the environmental conditions for expression, a moderately high GxE interaction was seen (2.14). The high broad heritability indicates that resistance for cercospora leaf spot is heritable.

Table 4.8. Genetic variance components and broad sense heritability for cercospora evaluations of the 15 diploid populations for June, September, October and November in College Station, Texas in 2016.

	June	**Sept**	**Oct**	**Nov**
Vpop	1.84	0.89	2.06	1.91
Ve	2.87	2.52	2.54	2.48
Vp	4.71	3.41	4.61	4.39
H^2	0.78	0.52	0.90	0.87

Vp (phenotypic variance) = Vpop + Ve, $H^2 = 2(Vpop)/Vp$

Table 4.9. Genetic variance components and, broad sense and narrow sense heritability for cercospora evaluations of the 15 diploid populations from the overall evaluation in College Station, Texas in 2016.

Percentage of total variance	
FP	17.13
MP	18.77
Progeny	16.81
Month	4.36
FPxMonth	4.03
MPxMonth	2.77
ProgenyxMonth	36.12
Genetic Variances	
Va	1.79
Vd	0.84
Vg	2.63
Vgxe	2.14
Vp	3.17
Heritability	
h^2	0.57
H^2	0.83

FP = female parent, MP = male parent, Va = parental variances, Vd = progeny variance, Vg = variance due to parents and progeny, Vgxe = variance due to the interaction of genotype and environment, sum of the FPxMonth, MPxMonth, ProgenyxMonth effects and residual, Vp (phenotypic variance) = Va+Vd+(Vgxe/E), h^2 = Va/Vp, H^2 = (Va+Vd)/Vp, E = number of months.

QTL Analysis

Three QTLs were found on LG 1, 3 and 7, where the QTL on LG 1 was seen in June and the overall combined analysis, the QTL on LG 7 was found only in the September analysis, and the QTL on LG3 was observed in October, November and the overall combined analysis (Figure 4.5). The narrow sense heritability ranged from 0.44 - 0.62 (Table 4.10), which is similar to the REML model estimates.

The proportion of phenotypic variation explained ranged between 7.1-8.5% for LG1, 6.3-12.4% for LG 3 and 21.3% for LG 7 (Table 4.11). The highest posterior QTL intensity was

found in the September evaluation and the overall analysis. A partial negative dominance effect was shown across the 4 months of evaluation and the overall analysis. The QTL on LG 1 was located between 0-4cM, flanked by the markers chr7_5726635 and chr7_9417814. The QTL on LG 3 peaked at 34 and 36 cM and ranged between 32-38 cM (markers chr6_8977697 and chr6_10299130). On LG 7, the interval was between 52-56 cM with the nearest markers being chr5_26042126 and chr5_28914204 (Table 4.12). The QTLs on LG 1, 3 and 7 were located on the distal end of each LG (Figures 4.6-4.8). It is important to note that the markers are based on the *Fragaria vesca* genome sequence, thus their physical location on the rose genome is unknown.

Table 4.10. QTLs mapped for cercospora resistance in 15 interrelated diploid rose families grown in College Station, TX in 2016.

								2ln(BF)		
Trait	**MCMC**	**Mean**	**Vp**	**Ve**	**Va**	**h^2**	**LG**	**1/0**	**2/1**	**3/2**
June	250,000	1.97	5.21	2.321	2.89	0.55	1	5.5	3.7	3.2
September	200,000	1.43	3.66	1.442	2.22	0.61	7	8.2	3.1	-0.4
October	475,000	1.84	4.38	2.021	2.36	0.54	1	4.2	-0.3	NA
							3	6.7	2.0	0.1
							4	3.7	1.9	-0.9
November	200,000	2.39	4.39	2.456	1.93	0.44	3	12.7	3.6	1.4
Overall	200,000	1.86	2.96	1.12	1.84	0.62	1	5.6	1.2	-0.5
							3	8.2	3.5	1.2

Markov chain Monte Carlo (MCMC) run length, phenotypic mean, phenotypic variance (Vp), residual variance (Ve), additive variance (Va), narrow-sense heritability (h^2), the linkage groups (LG) that QTLs were mapped on, and the QTL evidence [2ln(BF)] which is hardly any (0-2); positive (2-5); strong (5-10); and decisive (>10). 1/0, 2/1 and 3/2 indicates the QTL evidence for one QTL compared to none, 2 QTLs compared to 1 QTL, and 3 QTLs compared to 2 QTLs, respectively.

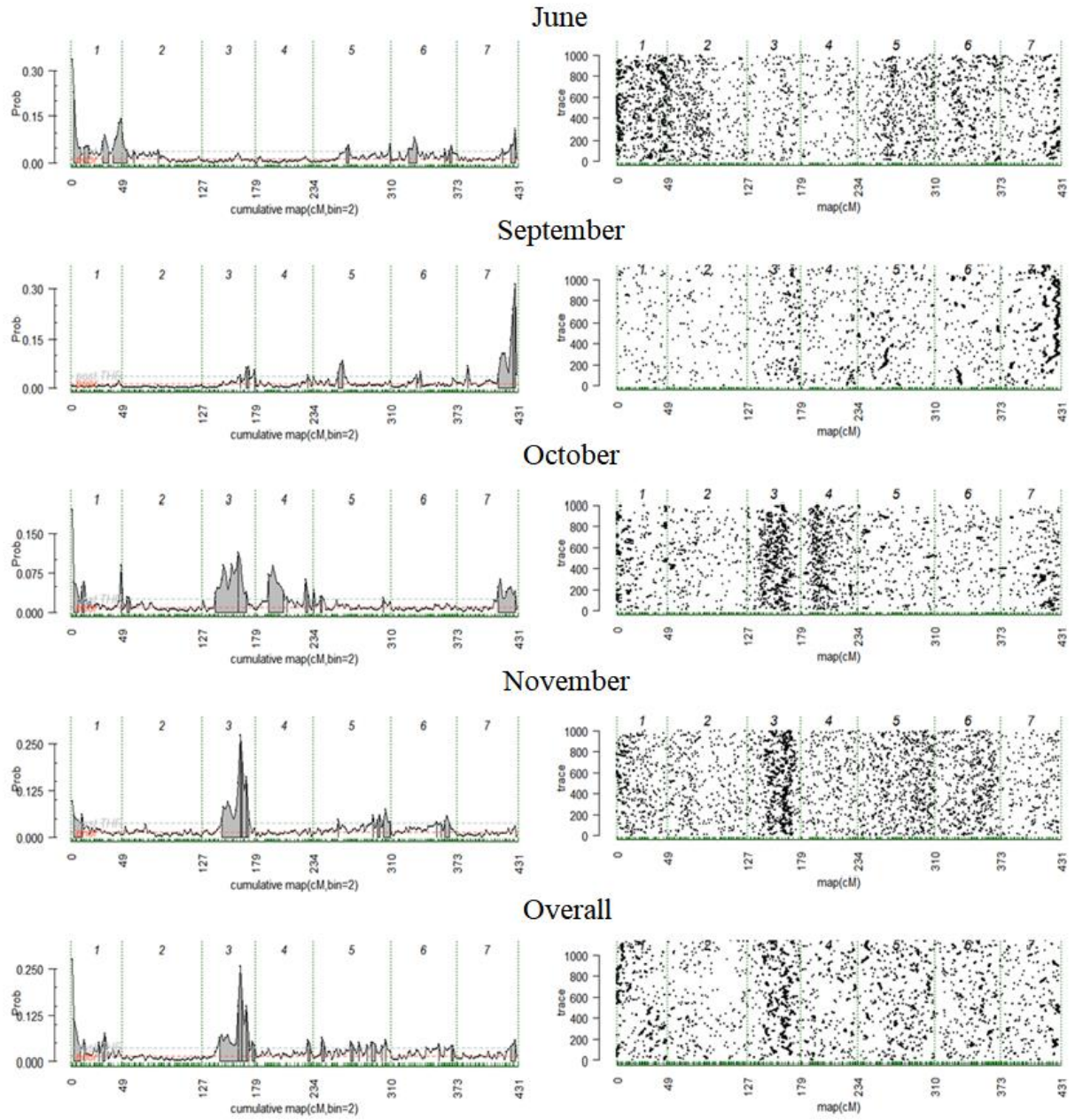


Figure 4.5. Posterior positions (left) and Trace sample QTL positions (right) based on an additive/dominance model performed using Visual FlexQTL software (Bink et al., 2008) for cercospora incidence for June, September, October and November, and the overall combined mean for 15 interrelated diploid rose populations grown in the field in College Station, TX. 2016.

Table 4.11. QTLs, linkage group, interval, QTL peak, intensity, additive effect, dominance effect and phenotypic variance explained (PVE) for cercospora leaf spot (CLS) evaluated in four months (June, September, October and November), and the overall combined mean for 15 interrelated diploid rose families grown in College Station, TX in 2016.

QTL	LG	Interval (cM)	QTL Peak (cM)	Intensity[x]	Additive Effect	Dominance Effect	PVE
CLS_June	1	[0, 4]	0	0.65	1.03	-0.64	7.12
CLS_Sep	7	[52, 56]	56	0.69	1.59	-1.31	21.27
CLS_Oct	3	[32, 38]	34	0.36	0.89	-0.58	6.34
CLS_Nov	3	[34, 38]	36	0.64	1.15	-0.39	12.41
CLS_Overall	1	[0, 4]	0	0.48	0.89	-0.69	8.45
	3	[36, 42]	36	0.69	0.74	-0.23	7.67

[x] Intensity refers to the probability of the QTL in the interval

Table 4.12. QTLs, linkage group, QTL interval, SNP marker and genetic position (cM) of flanking markers, and nearest marker to the QTL peak for cercospora leaf spot evaluated during four months (June, September, October and November), and the overall combined mean for 15 interrelated diploid rose families grown in College Station, TX.

			Flanking markers		Nearest marker	
QTL	**LG**	**Interval (cM)**	**Name**	**Genetic Position (cM)**	**Name**	**Genetic Position (cM)**
CLS_June	1	[0, 4]	chr7_5726635	0	chr7_7531798	0.63
			chr7_9417814	4.11		
CLS_Sep	7	[52, 56]	chr5_26042126	51.79	chr5_28914204	55.83
			chr5_28914204	55.83		
CLS_Oct	3	[32, 38]	chr6_8977697	31.26	chr6_10886313	34.03
			chr6_10299130	37.06		
CLS_Nov	3	[34, 38]	chr6_10886313	34.03	chr6_10110076	37.06
			chr6_10299130	37.06		
CLS_Overall	1	[0, 4]	chr7_5726635	0	chr7_7531798	0.63
			chr7_9417814	4.11		
	3	[36, 42]	chr6_10110076	37.06	chr6_10110076	37.06
			chr6_16820517	41.3		

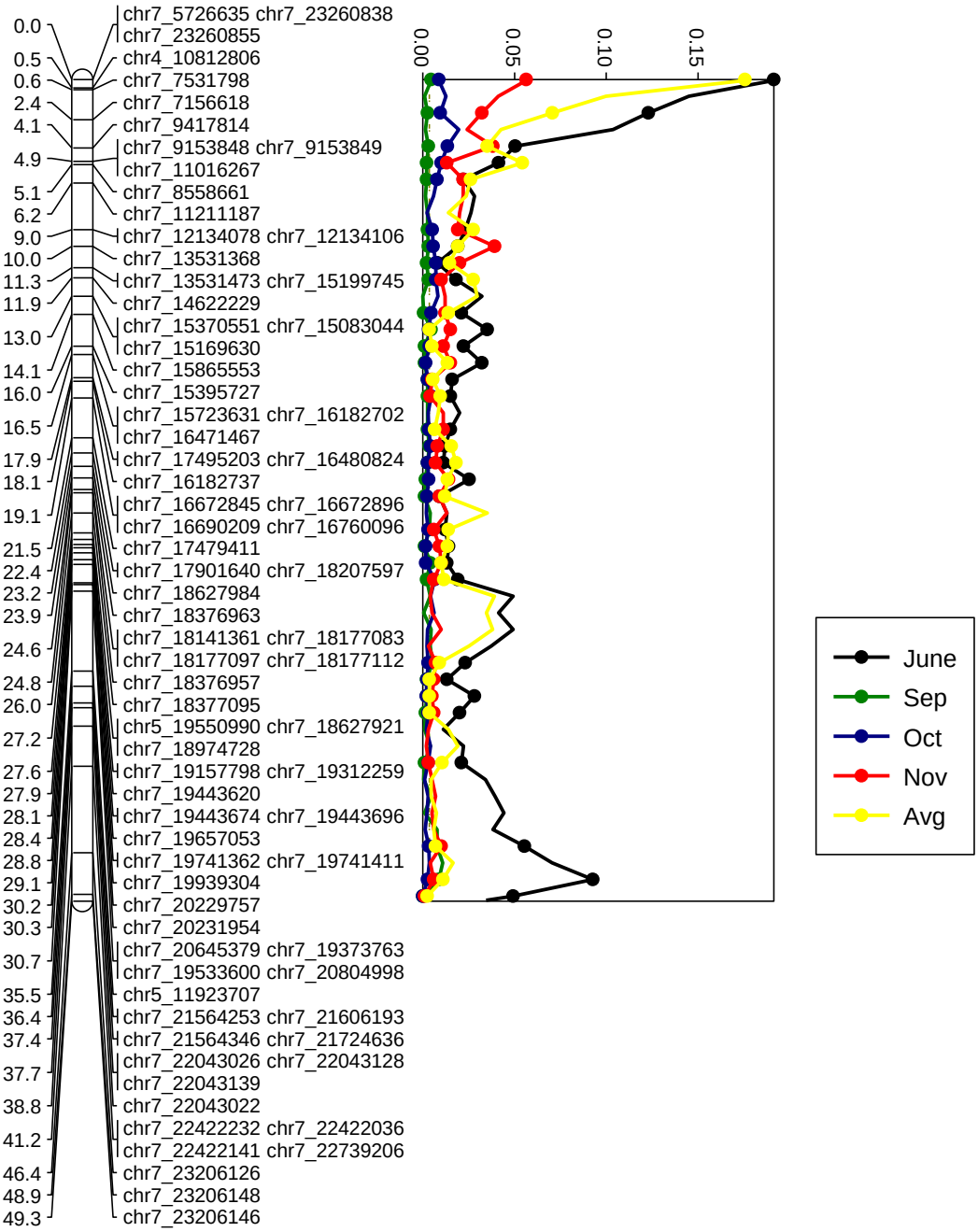


Figure 4.6. The position of putative QTLs for cercospora leaf spot on LG1 from the four evaluation months and the overall combined mean created by MapChart software (Voorrips, 2002) for 15 interrelated diploid rose families grown in College Station, TX in 2016.

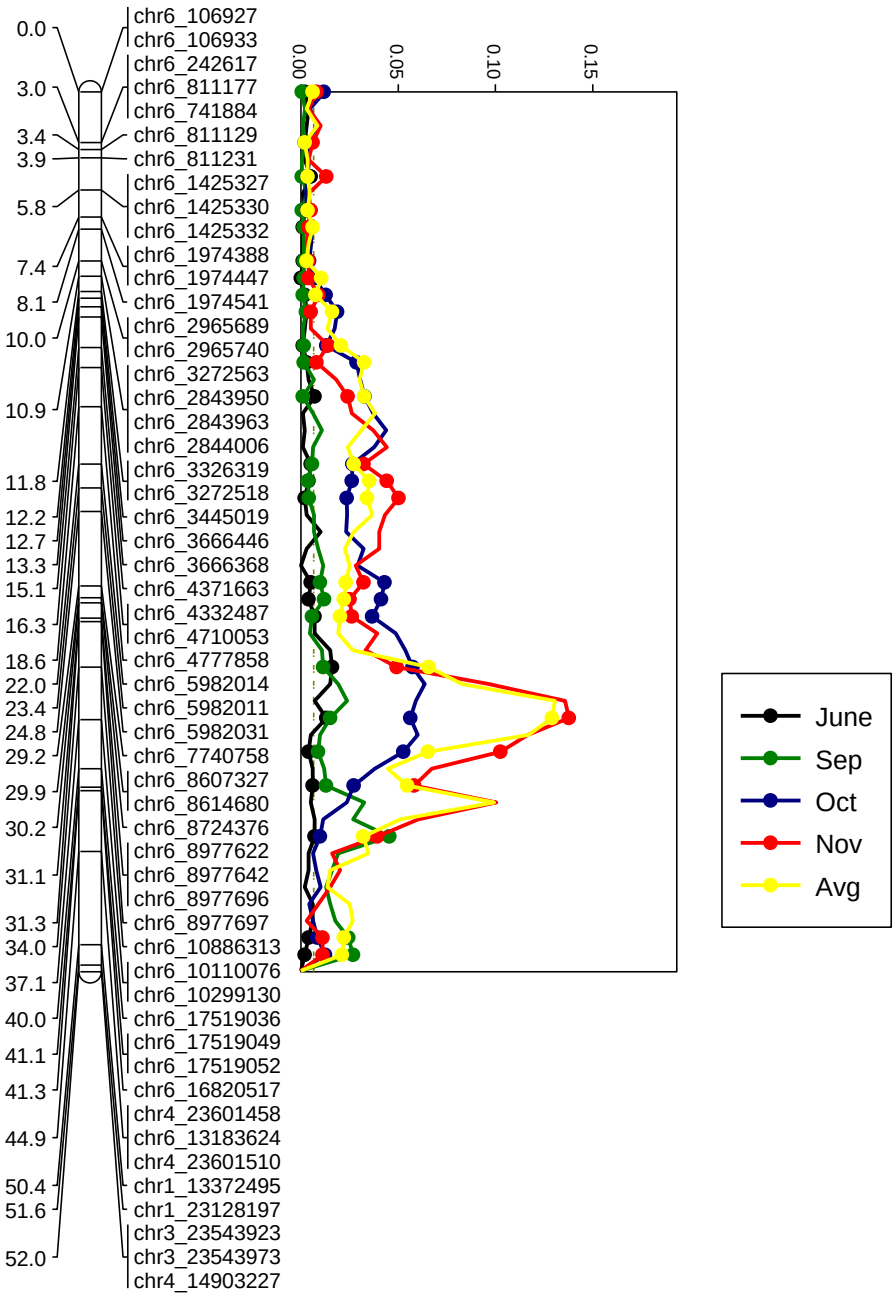


Figure 4.7. The position of putative QTLs for cercospora leaf spot on LG3 from the four evaluation months and the overall combined mean created by MapChart software (Voorrips, 2002) for 15 interrelated diploid rose families grown in College Station, TX in 2016.

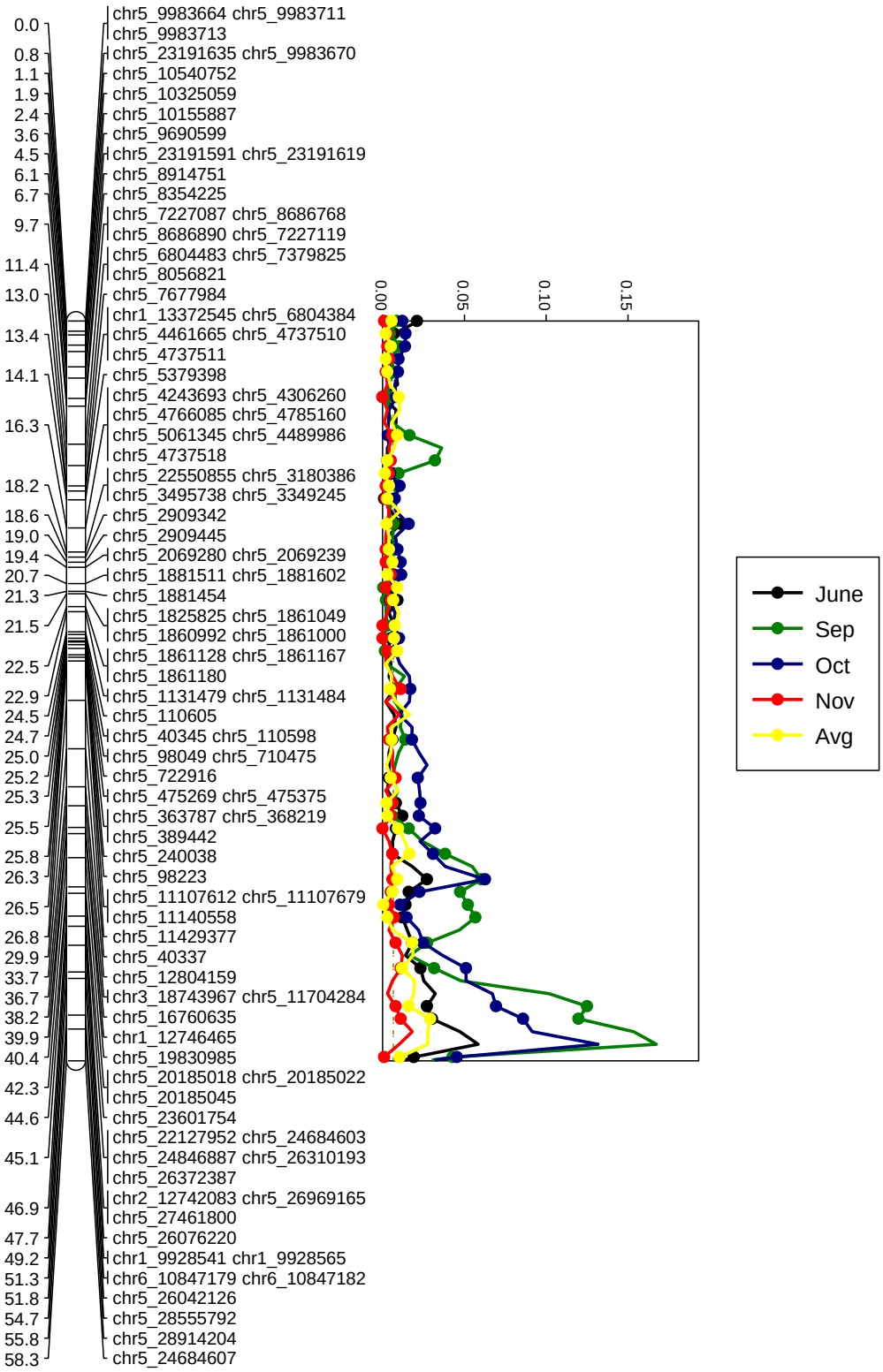


Figure 4.8. The position of putative QTLs for cercospora leaf spot on LG7 from the four evaluation months and the overall combined mean created by MapChart software (Voorrips, 2002) for 15 interrelated diploid rose families grown in College Station, TX in 2016.

Haplotype Analysis

In the overall QTL analysis, QTLs were found on LG1 and LG3 between 0-4cM and 36-42cM, respectively. QTL genotypes (QQ, Qq or qq) were assigned to each individual based on the overall combined analysis ratings with q alleles leading to a decrease in disease rating and Q alleles resulting in an increase in disease rating. Table 14.3 shows the probable genotypes within the LG1 and LG3 QTL regions for the parents of the 15 interrelated populations. Parents J14-3 and Violette had homozygous QQ genotypes in the LG1 QTL region while no parents had the homozygous QQ genotypes in the QTL region on LG3. J3-6 and J4-6 had homozygous qq at the signal peak on LG1 and "Little Chief", M4-4, "Old Blush" and "Red Fairy" were homozygous qq at both LG QTLs. 'Sweet Chariot' and 'Vineyard Song' were heterozygous Qq at the QTL peak on LG1, and 'Vineyard Song' was also Qq at the QTL peak on LG3. Nevertheless, the QTL genotype predictions for many parents were not available, which could be due to small population size for several populations and/or no phenotypic data for the parents. As shown in Figures 4.9 and 4.10, there was segregation for the disease, as progenies in group qq had significantly lower disease ratings than those in group QQ. However, there were many progenies that were not classified in the QTL genotypes, especially in LG3. This could be due to the low density of markers.

8 and 13 putative haplotypes were discovered on LG1 (Table 4.14) and LG3 (Table 4.15) respectively. On LG1, 4 of the haplotypes were parental and were found on J14-3, J3-6, J4-6, M4-4, OB, RF and VS. On LG3, 6 haplotypes were parental and were discovered among J14-3, J4-6, OB and RF. However, it is difficult to confirm these haplotypes and their origin of resistance due to missing marker data and unknown phenotypic data of the progenitors and several progenies (Figures 4.11 and 4.12).

Table 4.13. Probable QTL genotype (QQ, Qq, qq) for the parents of the 15 diploid rose populations at the signal peak on LG1 (1cM) and LG 3 (37cM) from the overall cercospora leaf spot evaluations. This data was found in the "Gtp_Genome.cvs" file from FlexQTL software.

Parents	LG1	LG3
DD	NA	NA
J14-3[x]	QQ	NA
J3-3	NA	NA
J3-6	qq	NA
J4-6	qq	NA
LC	qq	qq
M4-2	NA	NA
M4-4	qq	qq
OB	qq	qq
PP-J14-3	NA	NA
RF	qq	qq
SC	Qq	NA
Violette	QQ	NA
VS	Qq	Qq

NA indicates no sufficient data to categorize into QTL genotype

[x] J14-3: J06-20-14-3, J3-3: J06-30-3-3, J3-6: J06-30-3-6, J4-6: J06-28-4-6, LC: 'Little Chief', OB: 'Old Blush', PP-J14-3: Pollen Parent of J14-3, RF: 'Red Fairy', SC: 'Sweet Chariot', VS: 'Vineyard Song'

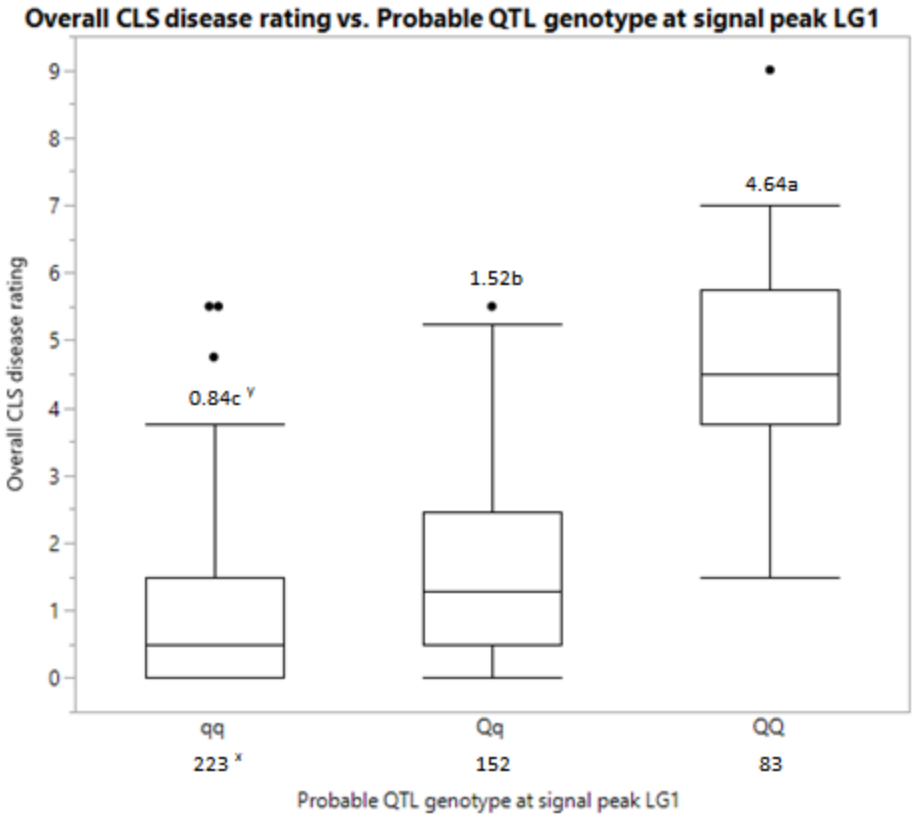


Figure 4.9. Box and whisker plot of 2016 field overall cercospora leaf spot rating for the three probable QTL genotypes at signal peak 1cM on LG1 among all diploid rose mapping materials. Dots outside the maximum and minimum lines are outliers.

[x] Number of progenies in each QTL genotype class

[y] Student's t-test mean comparison. Levels not connected by the same letter are significantly different (α=0.05)

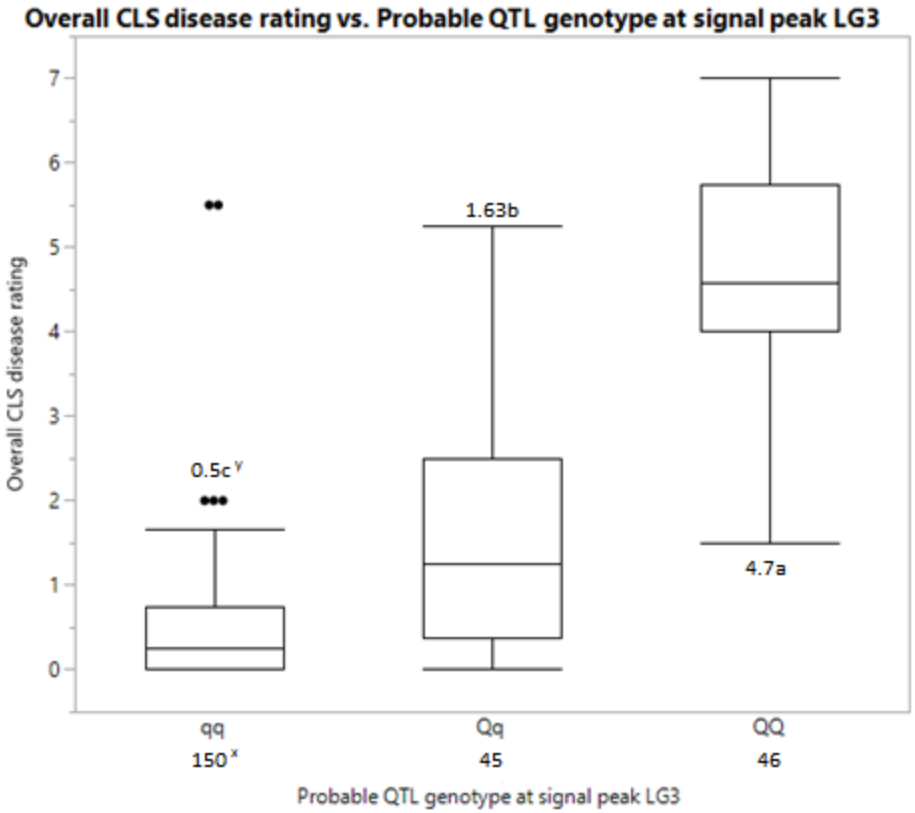


Figure 4.10. Box and whisker plot of 2016 field overall cercospora leaf spot rating for the three probable QTL genotypes at signal peak 37cM on LG3 among all diploid rose mapping materials. Dots outside the maximum and minimum lines are outliers.

[x] Number of progenies in each QTL genotype class

[y] Student's t-test mean comparison. Levels not connected by the same letter are significantly different (α=0.05)

Table 4.14. Haplotypes identified on LG1 within the QTL interval (0-4.11cM) from the overall QTL analysis. The QTL peak is located at 1cM.

Position	**0**	**0**	**0**	**0.5**	**0.63**	**2.36**	**4.11**
SNP	chr7_5726635	chr7_23260838	chr7_23260855	chr4_10812806	chr7_7531798	chr7_7156618	chr7_9417814
H1	G	C	A	C	C	T	A
H2	G	C	T	C	C	T	A
H3	G	G	A	C	C	T	G
H4	T	C	A	T	C	A	A
H5	G	C	A	C	C	A	A
H6	T	C	A	C	C	T	A
H7	T	C	A	T	C	T	A
H8	G	C	T	C	C	T	G

Table 4.15. Haplotypes identified on LG3 within the QTL interval (31-41.3cM) from the overall QTL analysis. The QTL peak is located at 36cM.

Position	**31.26**	**34.03**	**37.06**	**37.06**	**39.99**	**41.09**	**41.09**	**41.3**
SNP	chr6_8977697	chr6_10886313	chr6_10110076	chr6_10299130	chr6_17519036	chr6_17519049	chr6_17519052	chr6_16820517
H1	C	G	A	G	C	G	A	G
H2	G	G	G	A	C	T	C	A
H3	C	A	A	G	C	G	A	G
H4	G	A	G	A	A	G	A	A
H5	G	A	G	A	C	G	A	A
H6	G	G	G	A	C	G	A	A
H7	G	A	G	A	C	T	C	A
H8	C	A	A	G	C	G	A	A
H9	C	A	A	G	A	G	A	A
H10	C	A	G	A	A	G	A	A
H11	G	G	G	A	A	G	A	A
H12	G	G	G	A	A	T	C	A
H13	G	G	G	A	C	T	C	G

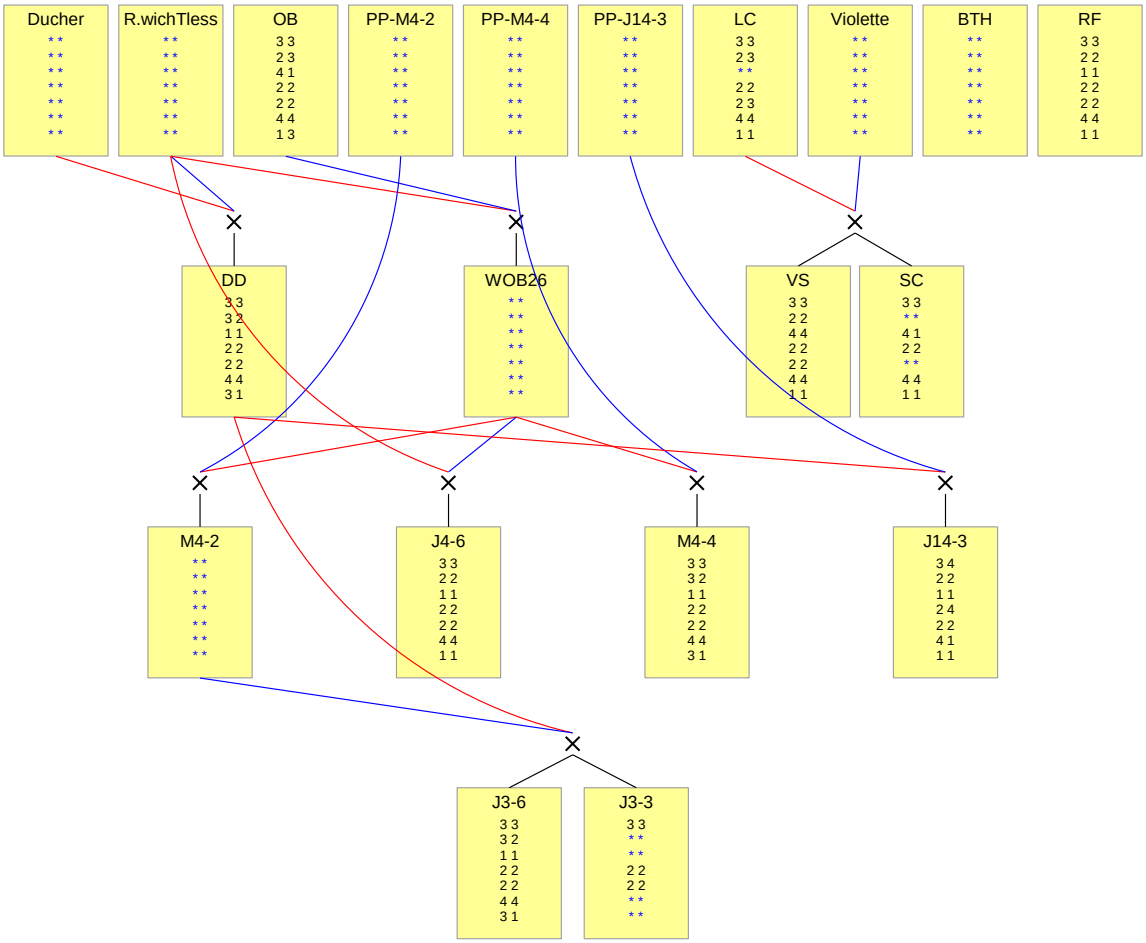


Figure 4.11. Pedigree of the parents of the diploid rose populations generated by Pedimap 1.2 (Voorrips et al., 2012) with the genotypic data of the 7 SNP markers for the QTL interval in LG1. 1=A, 2=C, 3=G, 4=T and *=missing data.

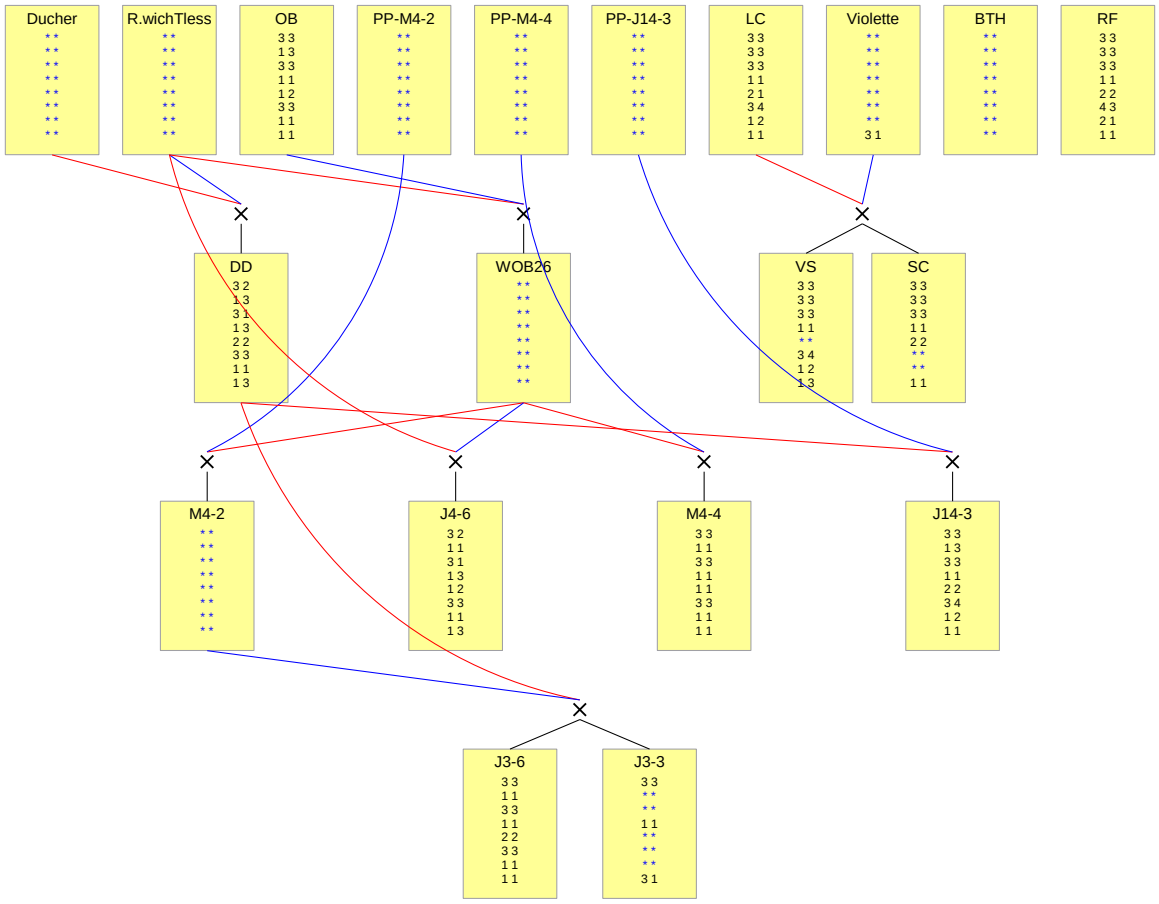


Figure 4.12. Pedigree of the parents of the diploid rose populations generated by Pedimap 1.2 (Voorrips et al., 2012) with the genotypic data of the 8 SNP markers for the QTL interval in LG3. 1=A, 2=C, 3=G, 4=T and *=missing data.

Discussion

In this study, it was observed that most plants had low or moderate level of cercospora incidence in the field. The populations with highest disease incidence were VSxJ14-3 and J14-3xVS and most disease appeared in November, followed by June. Cercospora leaf spot is dependent on environmental conditions and the presence of inoculum. Feres et al. (2017) observed that it takes around a month for disease to show lesions after inoculation. May had high precipitation and warm temperatures, which could have encouraged disease development. October had warm temperatures but low precipitation, however, the accumulation of inoculum from September and October could have favored disease development in November.

Resistance to cercospora was moderately heritable (h^2 = 0.57 and H^2=0.83). Three QTLs for cercospora leaf spot resistance were detected: one each on LG 1, 3 and 7. The phenotypic variance explained for these QTLs varied from 6-21%. This suggests that selection for a resistant rose is possible. Interestingly, the QTL on LG7 had the highest phenotypic variance explained (21%), but it only occurred in September, which had the lowest overall rating for cercospora leaf spot. Also, the partial negative dominance effect observed on all the QTL analyses suggests that there is a partially dominant resistance gene(s) for cercospora leaf spot.

The QTL for cercospora leaf spot on LG 3 is mapped to the same interval as the QTL for black spot (interval 36-42 for cercospora leaf spot and 34-44 for black spot) (Yan, 2017). This suggests that this region may provide generalized resistance to fungal diseases, as reported in cowpea, where the resistance for the pathogens *Cercospora canescens* and *Pseudocercospora cruenta* were mapped at the same position. (Duangsong et al., 2016).

The QTLs located on LG1 and LG7 may be specific to cercospora leaf spot resistance, as the QTL on LG1 was observed on the June and the overall analysis, and the one on LG7

appeared only on the September analysis. More studies are needed to determine what environmental factors such as precipitation, temperature, light, nutrition, and biotic stressors enhance disease pressure and/or trigger the expression of these QTLs. In June and the overall analysis, the correlation between cercospora leaf spot and black spot is low (-0.39 and -0.34, respectively) and in September, there is no correlation between the two diseases. Also, as the QTL on LG1 is located on the end of the linkage group, this QTL may be misplaced as there are not sufficient marked to accurately place it.

Further evaluations are needed to improve the strength and consistency of QTL detection in this analysis. One replicate per seedling and field design may have affected the analysis and using a randomized complete block design with replicated progenies might allow for more accurate phenotypic data. Also, acquiring the missing phenotypic data for the parents, would help with understanding the segregation for cercospora leaf spot in the populations and improve the QTL analysis. In addition, a linkage map based on the rose genome would increase the number of markers and create a more robust map. This would enhance the detection of haplotypes for better understanding the inheritance of resistance to cercospora leaf spot and discover genes associated with disease resistance (Zurn et al., 2018)

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CHAPTER V

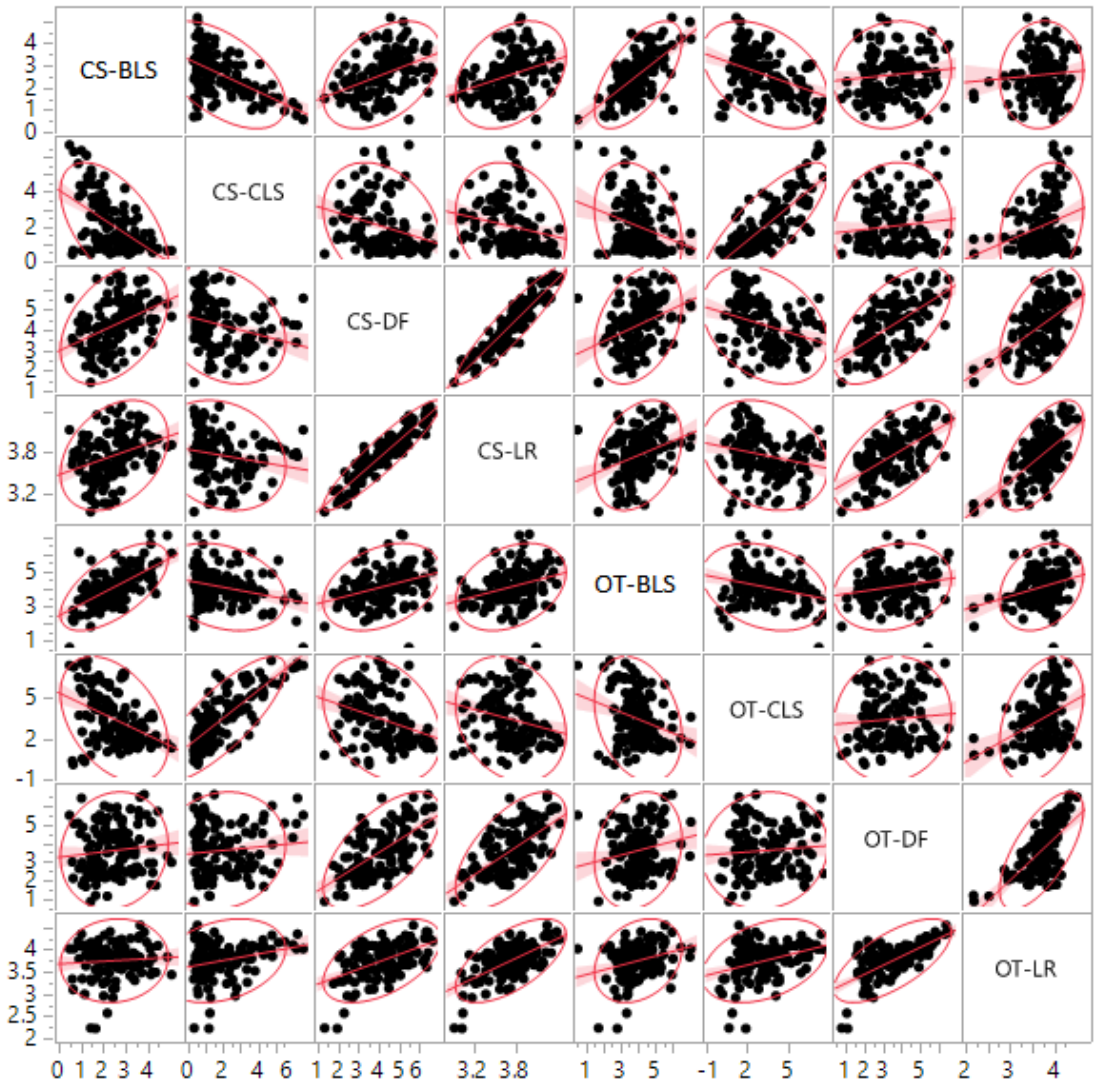
SUMMARY

In this research, cercospora leaf spot for roses was evaluated in both a field and greenhouse environment, and heritability and QTLs were studied in interrelated diploid populations. A collection of 130 cultivars were evaluated for this disease as well as black spot, defoliation and landscape rating in College Station and Overton, TX during 2016, 2017 and 2018. Most of the cultivars showed lower ratings for cercospora leaf spot in College Station compared to Overton, although the ratings in College Station increased over time. High correlations (0.8) were found for cercospora leaf spot between location, which shows that cercospora ratings are consistent among locations. Negative correlations were found for black spot, defoliation and landscape ratings with cercospora in College Station while defoliation and landscape ratings had positive correlations with cercospora leaf spot in Overton. The cultivar groups that had higher incidence of cercospora leaf spot were shrub, floribunda and climber type roses while the roses with lower incidence were the *Rosa rugosa* hybrids. In the greenhouse evaluations, 16 cultivars as well as 3 diploid populations were inoculated with the pathogen *R. rosicola*. These plants did not get infected successfully in the greenhouse possibly due to low conidia concentrations, high temperatures, and low humidity especially immediately after inoculation of the plant.

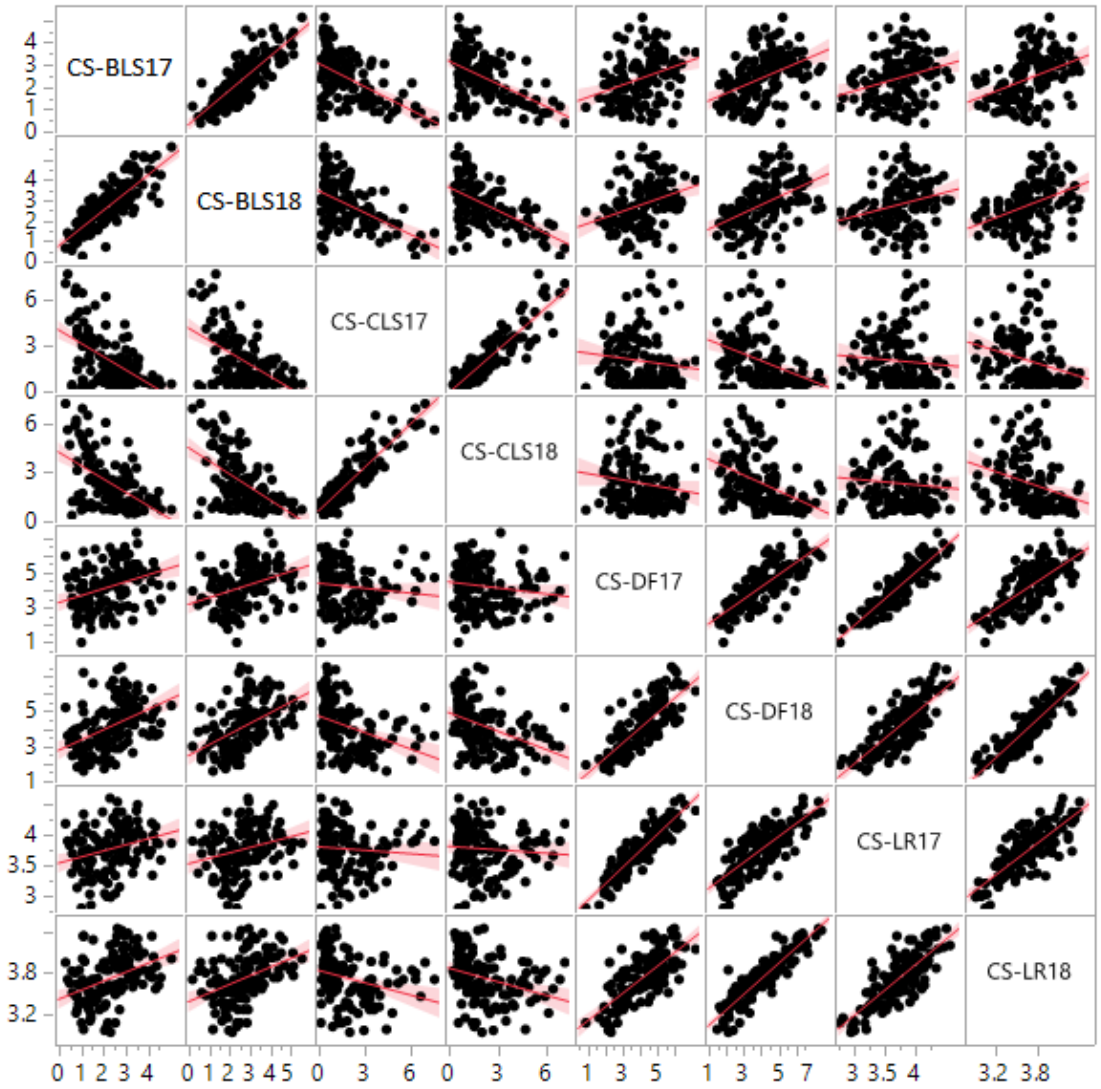
15 interrelated diploid populations generated in a partial diallel design were evaluated for cercospora leaf spot and were assessed for heritability and QTL discovery. Overall, broad sense heritability was 0.83 and narrow sense heritability was 0.57. The large GxE interaction suggests the importance of environment for this disease. A preliminary study was conducted for QTL analysis using a pedigree-based Bayesian approach. 3 QTLs were found, each one on linkage

groups 1, 3 and 7. The QTL on LG1 appeared on the June evaluation and the overall analysis. The QTL on LG3 appeared on the October and November evaluations and the overall analysis while the QTL on LG7 was found only on the September evaluation. The QTLs on LG1 and LG3 explained around 6-10% of the phenotypic variation while the QTL on LG7 explained 20% of the phenotypic variation. Although haplotypes were discovered on LG1 and LG3, their progenitor's origin could not be confirmed. Further research is needed to improve strength and consistency of the QTLs detected in this study and these results should be validated with other studies.

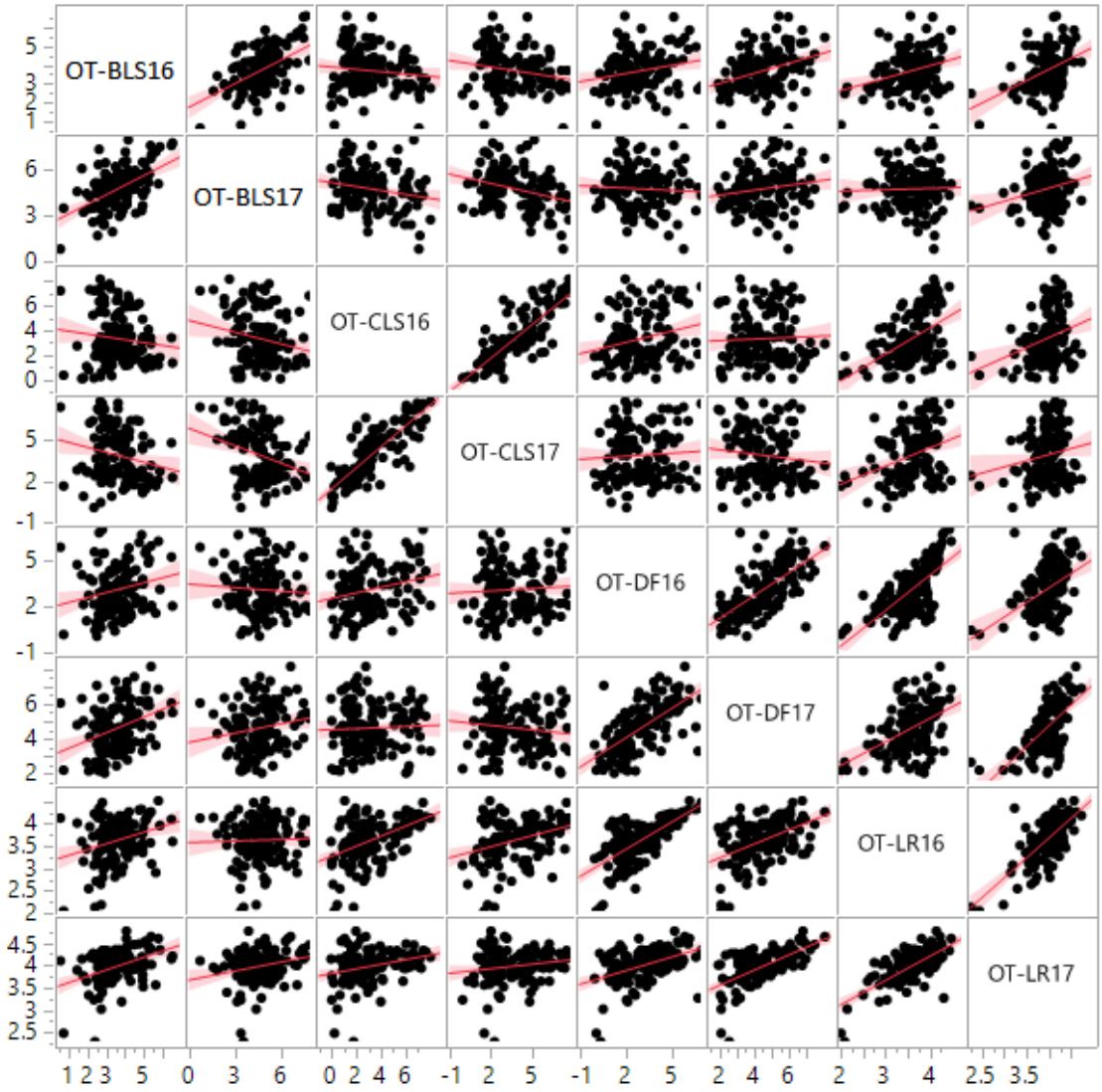
APPENDIX A



Appendix A-1. Overall correlations for ratings of mean black spot (BLS), cercospora leaf spot (CLS), defoliation (DF) and landscape rating (LR) for garden roses in the field in College Station (CS) 2017-2018 and Overton (OT), Texas 2016-2017.



Appendix A-2. Overall correlations for ratings of mean black spot (BLS), cercospora leaf spot (CLS), defoliation (DF) and landscape rating (LR) for garden roses in the field in College Station (CS), Texas in 2017 and 2018



Appendix A-3. Overall correlations for ratings of mean black spot (BLS), cercospora leaf spot (CLS), defoliation (DF) and landscape rating (LR) for garden roses in the field in Overton (OT), Texas in 2016 and 2017

Appendix A-4. College Station ratings for BLS (black spot), CLS (cercospora leaf spot), DF (defoliation) and LR (landscape rating) in 2016, 2017 and 2018 as well as the overall combined ratings for 130 rose cultivars.

	2017				2018				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
10043 N019	1.89	2.15	3.17	3.67	2.67	2.10	4.81	3.88	2.28	2.13	3.99	3.78
10043 N049	0.59	1.85	2.30	3.23	1.50	2.38	4.08	3.55	1.05	2.12	3.19	3.39
Abbaye de Cluny	0.94	3.00	5.11	4.19	1.63	2.44	5.56	4.00	1.29	2.72	5.34	4.10
American Pillar	4.26	0.89	3.81	3.74	4.83	1.71	4.21	4.02	4.55	1.30	4.01	3.88
Archbishop Desmond Tutu	1.28	3.22	4.61	3.97	1.88	2.56	4.81	3.97	1.58	2.89	4.71	3.97
Basye's Blueberry	0.56	3.80	3.93	3.78	1.71	2.67	4.04	3.77	1.14	3.24	3.99	3.78
Basye's Purple	2.52	1.81	5.46	3.97	2.88	3.19	4.00	3.63	2.70	2.50	4.73	3.80
Belinda's Dream	1.19	2.22	2.26	3.11	1.33	2.63	3.06	3.17	1.26	2.43	2.66	3.14
Betty Prior	1.17	3.44	4.17	3.58	1.13	4.00	5.31	3.88	1.15	3.72	4.74	3.73
Beverly	0.85	3.11	3.59	3.69	1.90	2.94	4.38	3.64	1.38	3.03	3.99	3.67
Bonica	1.37	1.85	5.07	4.06	2.33	2.62	5.33	4.00	1.85	2.24	5.20	4.03
Brilliant Veranda	3.59	3.26	3.35	3.56	4.17	2.77	5.12	4.14	3.88	3.02	4.24	3.85
Brite Eyes	1.61	0.81	2.63	3.37	1.50	1.69	3.04	3.46	1.56	1.25	2.84	3.42
Caldwell Pink	2.41	2.19	2.93	3.30	3.08	2.62	3.62	3.65	2.75	2.41	3.28	3.48
Carefree Beauty	2.74	1.81	3.81	3.54	2.98	2.48	4.44	3.65	2.86	2.15	4.13	3.60
Carefree Celebration	0.93	2.78	4.33	3.69	1.96	2.52	4.29	3.73	1.45	2.65	4.31	3.71
Carefree Delight	4.56	1.57	5.37	3.89	4.58	2.50	5.04	3.85	4.57	2.04	5.21	3.87
Carefree Sunshine	3.39	1.17	3.17	3.53	3.06	2.13	3.75	3.50	3.23	1.65	3.46	3.52
Carmella Fairy Tale	0.56	3.89	4.39	3.87	1.13	4.21	4.33	3.88	0.85	4.05	4.36	3.88
Champlain	1.37	3.07	5.41	4.05	2.66	2.91	4.97	3.91	2.02	2.99	5.19	3.98
Charisma	0.74	3.63	4.96	3.92	1.02	4.08	5.40	4.19	0.88	3.86	5.18	4.06
Cherry Parfait	0.30	4.33	3.11	3.48	0.63	3.81	4.38	3.67	0.47	4.07	3.75	3.58
Chuckles	0.75	2.72	5.28	4.04	1.10	2.85	5.05	4.12	0.93	2.79	5.17	4.08
Darcey Bussell	0.37	3.37	3.96	3.72	0.67	3.60	5.85	3.98	0.52	3.49	4.91	3.85

Appendix A-4. Continued

	2017					2018				Average			
Name	**CLS**	**BLS**		**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Dark Desire	2.52	1.44	3.93	3.78	2.21	1.92	3.75	3.54	2.37	1.68	3.84		3.66
Dee-Lish	0.41	3.89	3.85	3.67	1.04	4.54	4.75	3.83	0.73	4.22	4.30		3.75
Dream Come True	0.41	3.48	5.00	4.28	0.38	3.13	6.31	4.41	0.40	3.31	5.66		4.35
Ducher	1.39	2.33	1.83	3.22	3.00	1.81	1.69	3.25	2.20	2.07	1.76		3.24
Earth Angel	2.48	1.11	3.48	3.56	4.25	2.12	3.29	3.48	3.37	1.62	3.39		3.52
Elizabeth Taylor	0.67	3.56	5.44	4.31	0.44	3.38	6.06	4.28	0.56	3.47	5.75		4.30
Elle	0.56	3.00	4.89	4.06	0.63	3.25	5.88	4.25	0.60	3.13	5.39		4.16
Europeana	0.50	4.17	4.67	4.14	1.06	2.88	6.38	4.34	0.78	3.53	5.53		4.24
Fair Molly	0.50	1.56	5.50	4.25	0.88	1.93	7.19	4.41	0.69	1.75	6.35		4.33
Falling In Love	0.15	2.19	5.93	4.55	0.58	2.54	6.54	4.27	0.37	2.37	6.24		4.41
Fame!	0.28	2.58	4.44	4.25	0.88	1.69	5.94	4.22	0.58	2.14	5.19		4.24
Fiji	0.78	2.70	3.78	3.91	1.63	2.42	4.83	3.81	1.21	2.56	4.31		3.86
Flamingo Kolorscape	2.07	2.48	3.04	3.39	2.29	2.79	3.83	3.54	2.18	2.64	3.44		3.47
Francis Meilland	0.33	2.85	3.52	3.61	0.58	3.54	4.92	3.83	0.46	3.20	4.22		3.72
Frau Dagmar Hastrup	0.30	0.48	3.13	3.31	0.38	0.81	4.60	4.04	0.34	0.65	3.87		3.68
GN15	0.52	4.11	4.74	3.81	0.77	4.81	5.75	4.06	0.65	4.46	5.25		3.94
Golden Fairy Tale	1.41	2.37	4.11	3.81	1.58	3.25	4.88	4.06	1.50	2.81	4.50		3.94
Grande Amore	0.81	2.83	4.96	3.75	1.42	3.71	5.44	3.94	1.12	3.27	5.20		3.85
Hansa	0.37	0.93	3.89	3.57	0.75	1.25	5.81	4.47	0.56	1.09	4.85		4.02
Home Run	2.89	1.33	1.93	3.02	2.60	2.58	2.92	3.65	2.75	1.96	2.43		3.34
Hot Cocoa	0.67	3.11	3.19	3.60	0.73	2.79	5.25	4.10	0.70	2.95	4.22		3.85
Iceberg	1.26	3.22	4.52	3.81	1.46	3.87	3.87	3.74	1.36	3.55	4.20		3.78
Innocencia Vigorosa	3.96	0.96	1.93	3.07	4.31	1.33	2.15	3.09	4.14	1.15	2.04		3.08
Intrigue	1.07	3.15	6.09	4.50	2.31	2.35	7.13	4.46	1.69	2.75	6.61		4.48
J06-20-14-3	3.07	0.81	1.63	3.04	3.13	2.05	2.25	3.35	3.10	1.43	1.94		3.20

Appendix A-4. Continued

	2017				2018				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
John Cabot	0.37	2.52	4.22	3.52	0.54	2.33	3.81	3.67	0.46	2.43	4.02	3.60
John Davis	5.81	0.44	5.74	4.15	6.75	0.67	5.42	4.17	6.28	0.56	5.58	4.16
Joseph's Coat	0.94	3.50	5.92	4.29	0.94	3.56	6.75	4.44	0.94	3.53	6.34	4.37
Julia Child	1.00	3.61	2.67	3.50	0.44	4.44	3.94	3.72	0.72	4.03	3.31	3.61
Kashmir	0.78	2.07	2.19	2.93	1.71	2.38	2.38	3.13	1.25	2.23	2.29	3.03
Knock Out	1.11	1.37	1.46	2.69	1.21	2.08	2.46	3.30	1.16	1.73	1.96	3.00
La Marne	2.17	3.33	2.00	3.22	3.13	2.31	2.50	3.66	2.65	2.82	2.25	3.44
Lafter	1.00	1.94	3.67	3.54	1.31	2.79	3.58	3.57	1.16	2.37	3.63	3.56
Lemon Fizz	1.74	2.00	2.70	3.27	1.96	2.35	3.33	3.48	1.85	2.18	3.02	3.38
Limoncello	1.04	2.67	2.35	2.97	2.63	2.83	3.17	3.37	1.84	2.75	2.76	3.17
Linda Campbell	0.26	4.67	4.63	3.80	0.87	5.13	5.50	3.90	0.57	4.90	5.07	3.85
Little Buckaroo	0.41	2.76	5.19	3.97	1.17	2.75	4.96	3.92	0.79	2.76	5.08	3.95
Livin' La Vida	2.93	1.33	4.26	3.94	4.69	1.19	4.50	4.00	3.81	1.26	4.38	3.97
M4-4	1.22	0.89	2.06	3.44	1.50	1.44	3.63	3.75	1.36	1.17	2.85	3.60
Mardi Gras	0.44	2.89	5.56	4.28	1.38	2.00	7.50	4.44	0.91	2.45	6.53	4.36
Mevrouw Nathalie Nypels	0.50	2.39	4.06	3.97	0.86	1.38	4.19	3.88	0.68	1.89	4.13	3.93
Miracle on the Hudson	2.41	1.81	1.78	2.81	2.79	2.21	3.00	3.12	2.60	2.01	2.39	2.97
Moje Hammarberg	0.17	0.56	3.78	3.64	1.00	0.63	3.69	3.75	0.59	0.60	3.74	3.70
Morden Centennial	1.52	3.59	6.09	4.07	3.25	3.15	6.12	4.15	2.39	3.37	6.11	4.11
MORsoucrest	1.67	3.85	5.19	3.98	1.35	4.19	5.00	4.02	1.51	4.02	5.10	4.00
Munstead Wood	0.44	2.81	5.15	4.09	0.79	2.37	5.75	4.08	0.62	2.59	5.45	4.09
My Girl	3.07	1.52	2.48	3.07	3.50	2.62	2.94	3.38	3.29	2.07	2.71	3.23
Nearly Wild	2.93	3.26	2.70	3.50	2.63	3.42	3.04	3.67	2.78	3.34	2.87	3.59
Old Blush	0.67	2.44	3.81	3.35	1.09	1.97	3.78	3.47	0.88	2.21	3.80	3.41
ORA 05007	0.56	2.67	5.78	3.94	0.88	2.50	5.75	4.13	0.72	2.59	5.77	4.04

Appendix A-4. Continued

	2017				2018				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Oso Easy Cherry Pie	6.44	0.52	4.00	3.63	5.33	1.65	3.92	3.69	5.89	1.09	3.96	3.66
Oso Easy Double Red	1.94	2.11	1.94	2.83	2.63	2.38	2.75	3.13	2.29	2.25	2.35	2.98
Oso Easy Fragrant Spreader	5.06	1.67	2.78	3.56	4.75	1.13	3.63	4.06	4.91	1.40	3.21	3.81
Oso Easy Honey Bun	2.89	1.83	2.83	3.47	4.19	1.56	3.19	3.50	3.54	1.70	3.01	3.49
Oso Easy Italian Ice	2.63	1.19	2.67	3.37	3.65	2.10	3.33	3.60	3.14	1.65	3.00	3.49
Oso Easy Lemon Zest	2.37	3.07	3.59	3.72	2.42	3.00	4.71	4.00	2.40	3.04	4.15	3.86
Oso Happy Candy Oh	5.44	1.00	3.37	3.67	6.23	0.38	3.08	3.77	5.84	0.69	3.23	3.72
Oso Happy Petit Pink	1.63	1.30	1.81	2.88	3.29	1.71	2.54	3.21	2.46	1.51	2.18	3.05
Oso Happy Smoothie	4.11	1.89	3.70	3.69	4.33	1.92	5.17	4.08	4.22	1.91	4.44	3.89
Papa Hemeray	0.37	1.81	4.89	3.94	1.13	2.60	6.33	4.21	0.75	2.21	5.61	4.08
Peachy Keen	1.26	2.11	1.78	2.96	1.58	2.83	2.33	3.19	1.42	2.47	2.06	3.08
Phloxy Baby	4.19	0.81	2.11	3.37	5.08	2.21	3.37	3.29	4.64	1.51	2.74	3.33
Pink Enchantment	0.85	2.44	3.37	3.50	1.25	3.02	3.67	3.58	1.05	2.73	3.52	3.54
Pink Home Run	2.44	1.48	2.19	3.00	2.50	1.54	2.67	3.27	2.47	1.51	2.43	3.14
Plum Perfect	5.44	0.81	4.22	3.72	5.02	1.06	3.98	3.78	5.23	0.94	4.10	3.75
Polanaise	1.44	3.15	4.30	3.89	1.73	3.67	4.58	3.92	1.59	3.41	4.44	3.91
Poseidon	4.33	1.37	4.85	3.83	3.88	2.33	5.13	3.85	4.11	1.85	4.99	3.84
Purple Pavement	0.17	1.17	2.89	3.22	1.06	1.25	3.56	3.63	0.62	1.21	3.23	3.43
Purple Rain	3.80	0.48	4.15	3.72	4.35	1.50	3.79	3.73	4.08	0.99	3.97	3.73
Raspberry Kiss	0.81	1.78	2.67	3.15	1.54	1.58	3.60	3.47	1.18	1.68	3.14	3.31
Raspberry Vigorosa	2.81	1.33	3.48	3.44	4.25	2.21	3.88	3.71	3.53	1.77	3.68	3.58
Red Drift	2.00	1.70	2.63	3.41	2.25	2.92	3.75	3.50	2.13	2.31	3.19	3.46
Rise N Shine	0.52	2.52	2.83	3.43	0.71	3.38	4.75	3.94	0.62	2.95	3.79	3.69
Rosarium Uetersen	1.96	2.74	4.56	3.91	4.15	2.85	4.00	3.84	3.06	2.80	4.28	3.88
Roxanne Veranda	6.06	0.89	4.04	3.80	5.46	1.79	4.08	3.65	5.76	1.34	4.06	3.73

Appendix A-4. Continued

	2017				2018				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Ruby Vigorosa	3.19	1.22	3.04	3.46	5.17	1.79	3.79	3.73	4.18	1.51	3.42	3.60
Sally Holmes	1.59	2.37	5.02	3.80	1.37	3.21	4.96	3.60	1.48	2.79	4.99	3.70
Savannah	2.94	2.44	3.06	3.47	3.38	2.13	3.63	3.66	3.16	2.29	3.35	3.57
Sir Thomas Lipton	0.15	0.85	1.04	2.78	0.60	2.00	1.83	3.04	0.38	1.43	1.44	2.91
Skylark	0.19	3.33	4.67	3.87	0.60	2.79	4.42	3.73	0.40	3.06	4.55	3.80
Sky's the Limit	0.44	3.56	5.19	3.94	1.29	4.35	6.12	4.17	0.87	3.96	5.66	4.06
Solero Vigorosa	0.74	2.44	2.63	3.46	1.81	2.44	3.75	3.66	1.28	2.44	3.19	3.56
Sophy's Rose	0.48	3.48	4.70	3.94	0.75	2.81	5.33	3.94	0.62	3.15	5.02	3.94
St. Patrick	0.56	1.37	4.04	4.19	0.58	1.44	5.00	3.96	0.57	1.41	4.52	4.08
Star Delight	0.39	3.89	4.33	3.58	1.00	2.94	4.50	3.69	0.70	3.42	4.42	3.64
Stormy Weather	0.85	3.37	4.78	3.76	0.67	3.25	5.12	3.85	0.76	3.31	4.95	3.81
Strawberry Hill	0.52	3.15	4.70	3.78	0.67	3.92	5.21	3.85	0.60	3.54	4.96	3.82
Sunny Sky	0.52	3.41	4.30	3.74	1.58	3.79	4.08	3.86	1.05	3.60	4.19	3.80
Sunrise Sunset	2.93	1.56	2.63	3.39	4.54	2.54	3.42	3.63	3.74	2.05	3.03	3.51
Sunset Celebration	0.37	2.52	5.19	4.17	0.67	3.25	6.92	4.17	0.52	2.89	6.06	4.17
Sweet Frances	0.33	3.85	4.04	3.66	0.92	4.63	4.71	3.79	0.63	4.24	4.38	3.73
Sweet Vigorosa	2.41	1.00	2.52	3.31	3.41	2.34	3.22	3.66	2.91	1.67	2.87	3.49
Tahitian Treasure	0.67	1.89	3.17	3.61	1.88	2.00	3.19	3.69	1.28	1.95	3.18	3.65
Tamango	0.44	2.96	5.59	3.99	1.02	2.71	6.50	4.00	0.73	2.84	6.05	4.00
Teasing Georgia	0.33	5.11	4.19	3.86	0.75	5.19	5.17	4.00	0.54	5.15	4.68	3.93
Tequila	0.85	3.93	2.37	3.25	1.38	4.29	4.00	3.75	1.12	4.11	3.19	3.50
Therese Bugnet	3.15	0.70	3.11	3.39	3.71	1.08	3.40	3.64	3.43	0.89	3.26	3.52
Tiffany	0.26	2.67	4.78	4.10	0.71	2.67	6.33	4.34	0.49	2.67	5.56	4.22
Topolina Vigorosa	2.44	1.11	2.57	3.44	4.44	2.71	3.79	3.79	3.44	1.91	3.18	3.62
Toscana Vigorosa	2.96	1.11	2.26	3.19	3.54	2.50	3.58	3.64	3.25	1.81	2.92	3.42

Appendix A-4. Continued

	2017				2018				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Traviata	0.48	4.07	4.83	3.83	0.88	4.29	5.29	4.08	0.68	4.18	5.06	3.96
Watercolors Home Run	1.48	1.00	2.78	3.25	1.46	2.27	3.06	3.52	1.47	1.64	2.92	3.39
Westerland	0.93	3.37	5.04	3.87	1.96	2.50	5.29	3.90	1.45	2.94	5.17	3.89
Windermere	0.37	4.37	3.02	3.69	1.54	4.19	3.67	3.73	0.96	4.28	3.35	3.71
Winner's Circle	2.00	1.70	3.89	3.60	2.29	2.79	4.42	3.79	2.15	2.25	4.16	3.70
Winter Sunset	2.04	2.54	3.96	3.70	2.58	4.29	4.94	4.03	2.31	3.42	4.45	3.87

Appendix A-5. Overton ratings for BS (black spot), CLS (cercospora leaf spot), DF (defoliation) and LR (landscape rating) in 2016, 2017 and 2018 as well as the overall combined ratings for 130 rose cultivars.

	2016				2017				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
10043 N019	3.43	4.00	4.14	3.93	5.00	3.00	4.00	4.13	4.22	3.50	4.07	4.03
10043 N049	2.57	3.14	2.14	3.57	5.00	2.50	3.00	3.38	3.79	2.82	2.57	3.48
Abbaye de Cluny	2.74	4.38	4.62	3.98	2.92	5.58	6.21	4.15	2.83	4.98	5.42	4.07
American Pillar	7.67	2.33	1.81	3.90	7.79	2.67	3.25	4.17	7.73	2.50	2.53	4.04
Archbishop Desmond Tutu	6.14	4.00	4.07	4.05	7.25	4.25	6.71	4.21	6.70	4.13	5.39	4.13
Basye's Blueberry	2.92	2.54	1.21	3.14	3.00	6.25	3.88	3.50	2.96	4.40	2.55	3.32
Basye's Purple	1.40	5.07	1.29	2.83	1.58	7.08	3.58	3.54	1.49	6.08	2.44	3.19
Belinda's Dream	0.62	2.74	2.43	2.74	2.63	3.38	2.13	3.31	1.63	3.06	2.28	3.03
Betty Prior	2.61	4.19	1.78	3.22	3.83	5.92	3.42	3.75	3.22	5.06	2.60	3.49
Beverly	1.82	2.88	1.83	3.56	2.00	3.75	2.25	3.69	1.91	3.32	2.04	3.63
Bonica	1.93	3.39	3.00	3.46	2.90	4.44	4.17	3.74	2.42	3.92	3.59	3.60
Brilliant Veranda	6.02	4.21	2.52	3.98	7.67	3.58	4.58	4.15	6.85	3.90	3.55	4.07

Appendix A-5. Continued

	2016				2017				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Brite Eyes	3.43	3.75	1.14	3.45	4.06	4.81	4.81	3.92	3.75	4.28	2.98	3.69
Caldwell Pink	3.83	3.64	3.52	3.74	5.13	5.50	5.38	3.94	4.48	4.57	4.45	3.84
Carefree Beauty	3.07	4.38	4.38	3.76	5.13	4.00	5.54	4.00	4.10	4.19	4.96	3.88
Carefree Celebration	0.81	3.62	3.69	3.37	2.08	4.42	4.71	3.86	1.45	4.02	4.20	3.62
Carefree Delight	5.61	4.46	5.31	4.11	6.35	5.08	6.11	4.46	5.98	4.77	5.71	4.29
Carefree Sunshine	3.40	3.05	1.76	3.31	5.75	5.25	4.38	4.00	4.58	4.15	3.07	3.66
Carmella Fairy Tale	1.48	6.05	3.05	3.81	2.67	7.42	5.33	4.33	2.08	6.74	4.19	4.07
Champlain	3.33	2.97	3.47	3.69	3.50	5.67	5.17	3.83	3.42	4.32	4.32	3.76
Charisma	1.52	5.01	1.90	3.55	2.58	5.87	3.79	4.05	2.05	5.44	2.85	3.80
Cherry Parfait	1.24	5.48	2.05	3.43	2.21	5.33	3.25	3.65	1.73	5.41	2.65	3.54
Chuckles	1.86	4.67	3.79	3.67	4.00	4.67	5.75	4.17	2.93	4.67	4.77	3.92
Darcey Bussell	1.71	4.62	2.95	3.36	2.21	4.67	5.00	3.90	1.96	4.65	3.98	3.63
Dark Desire	3.00	3.38	2.13	3.75	5.00	1.88	4.25	3.88	4.00	2.63	3.19	3.82
Dee-Lish	1.67	4.55	3.74	3.52	1.75	3.67	7.21	4.40	1.71	4.11	5.48	3.96
Dream Come True	0.71	5.10	4.93	4.05	2.29	6.33	6.71	4.52	1.50	5.72	5.82	4.29
Ducher	1.79	4.14	1.92	2.96	2.88	3.50	3.69	3.75	2.34	3.82	2.81	3.36
Earth Angel	1.13	4.25	1.13	3.50	2.83	4.83	3.75	3.88	1.98	4.54	2.44	3.69
Elizabeth Taylor	0.95	6.12	6.31	4.50	2.00	5.00	6.79	4.58	1.48	5.56	6.55	4.54
Elle	2.00	4.62	2.33	3.60	2.50	5.33	4.83	3.94	2.25	4.98	3.58	3.77
Europeana	3.43	5.67	4.81	4.17	1.67	5.08	6.13	4.42	2.55	5.38	5.47	4.30
Fair Molly	0.57	2.86	2.79	3.79	2.75	3.00	4.50	4.13	1.66	2.93	3.65	3.96
Falling In Love	1.74	4.67	5.33	4.14	2.08	3.92	6.83	4.75	1.91	4.30	6.08	4.45
Fame!	2.67	1.50	4.67	4.00	2.75	4.50	4.75	3.88	2.71	3.00	4.71	3.94
Fiji	5.00	3.50	0.33	3.25	5.50	3.88	3.00	4.25	5.25	3.69	1.67	3.75
Flamingo Kolorscape	4.57	4.17	2.00	3.48	6.42	5.92	3.54	3.98	5.50	5.05	2.77	3.73

Appendix A-5. Continued

	2016				2017				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Francis Meilland	2.00	5.24	3.40	3.52	2.75	4.42	4.83	3.92	2.38	4.83	4.12	3.72
Frau Dagmar Hastrup	0.05	1.57	1.77	3.05	0.83	3.15	4.99	3.74	0.44	2.36	3.38	3.40
GN15	1.26	5.24	5.43	4.10	2.13	5.88	5.88	4.00	1.70	5.56	5.66	4.05
Golden Fairy Tale	2.89	4.21	1.79	3.71	3.00	4.88	4.63	4.00	2.95	4.55	3.21	3.86
Grande Amore	1.78	3.36	0.72	3.47	2.25	5.17	4.17	4.04	2.02	4.27	2.45	3.76
Hansa	0.19	2.86	1.90	3.05	0.83	5.08	4.21	3.58	0.51	3.97	3.06	3.32
Home Run	4.40	3.36	2.88	3.40	5.38	3.25	6.38	4.31	4.89	3.31	4.63	3.86
Hot Cocoa	1.86	4.62	2.00	3.33	2.21	5.50	5.42	4.00	2.04	5.06	3.71	3.67
Iceberg	1.87	4.36	2.76	3.68	2.41	5.17	4.77	4.09	2.14	4.77	3.77	3.89
Innocencia Vigorosa	5.46	2.61	1.10	3.62	7.21	4.79	2.00	3.99	6.34	3.70	1.55	3.81
Intrigue	1.42	5.54	4.78	4.19	2.21	5.63	5.85	4.33	1.82	5.59	5.32	4.26
J06-20-14-3	6.64	2.90	2.05	3.74	5.92	4.83	3.33	3.92	6.28	3.87	2.69	3.83
John Cabot	4.00	3.48	3.81	3.71	3.67	5.17	5.58	4.11	3.84	4.33	4.70	3.91
John Davis	7.33	0.52	5.17	3.95	7.38	0.75	6.00	4.08	7.36	0.64	5.59	4.02
Joseph's Coat	2.52	5.12	6.10	4.26	1.50	3.25	4.63	4.17	2.01	4.19	5.37	4.22
Julia Child	2.76	5.55	2.29	3.62	4.29	3.58	3.71	3.77	3.53	4.57	3.00	3.70
Kashmir	0.43	2.81	0.48	2.29	1.83	4.42	2.17	3.00	1.13	3.62	1.33	2.65
Knock Out	1.57	2.38	0.33	2.17	2.33	3.58	2.63	2.27	1.95	2.98	1.48	2.22
La Marne	2.83	4.67	2.83	3.75	5.25	4.25	3.75	3.50	4.04	4.46	3.29	3.63
Lafter	2.52	2.57	0.98	2.74	3.58	5.08	4.50	3.71	3.05	3.83	2.74	3.23
Lemon Fizz	5.57	4.00	4.21	3.86	3.50	5.25	4.50	4.00	4.54	4.63	4.36	3.93
Limoncello	5.26	2.67	1.86	3.21	7.50	3.33	3.46	3.92	6.38	3.00	2.66	3.57
Linda Campbell	1.10	6.76	1.83	3.64	2.21	7.67	5.46	4.19	1.66	7.22	3.65	3.92
Little Buckaroo	0.00	2.00	3.64	3.57	3.00	6.00	6.25	4.13	1.50	4.00	4.95	3.85
Livin' La Vida	5.21	3.50	3.93	4.00	5.38	2.88	5.75	4.00	5.30	3.19	4.84	4.00

Appendix A-5. Continued

	2016				2017				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
M4-4	2.79	3.57	1.00	3.89	2.25	4.25	2.75	4.25	2.52	3.91	1.88	4.07
Mardi Gras	1.14	5.26	4.74	3.93	1.38	4.58	6.96	4.48	1.26	4.92	5.85	4.21
Mevrouw Nathalie Nypels	2.04	3.36	3.93	3.64	2.25	3.75	3.50	3.63	2.15	3.56	3.72	3.64
Miracle on the Hudson	3.62	3.45	1.86	2.76	4.92	3.17	3.00	3.17	4.27	3.31	2.43	2.97
Moje Hammarberg	0.05	2.05	1.79	2.90	0.00	3.42	2.25	3.21	0.03	2.74	2.02	3.06
Morden Centennial	0.90	4.76	3.69	3.40	1.71	7.08	6.79	3.98	1.31	5.92	5.24	3.69
MORsoucrest	2.86	6.86	4.57	3.93	4.75	7.50	6.00	4.13	3.81	7.18	5.29	4.03
Munstead Wood	2.52	3.60	4.07	3.60	2.71	5.33	7.25	4.33	2.62	4.47	5.66	3.97
My Girl	2.57	2.60	1.39	2.67	6.58	4.92	3.17	4.04	4.58	3.76	2.28	3.36
Nearly Wild	4.35	4.93	1.88	3.70	4.50	6.42	3.71	4.08	4.43	5.68	2.80	3.89
Old Blush	1.55	2.81	2.48	2.95	2.63	3.69	4.50	3.69	2.09	3.25	3.49	3.32
ORA 05007	1.52	4.07	3.36	3.64	2.50	5.08	6.25	4.33	2.01	4.58	4.81	3.99
Oso Easy Cherry Pie	7.43	2.71	5.96	4.25	7.63	4.25	6.25	4.31	7.53	3.48	6.11	4.28
Oso Easy Double Red	5.75	4.00	1.43	3.32	6.38	4.88	3.38	3.63	6.07	4.44	2.41	3.48
Oso Easy Fragrant Spreader	6.07	4.18	1.43	3.89	5.25	7.88	5.13	4.44	5.66	6.03	3.28	4.17
Oso Easy Honey Bun	5.11	2.71	2.57	3.61	4.25	7.25	5.00	4.25	4.68	4.98	3.79	3.93
Oso Easy Italian Ice	7.00	2.07	4.71	4.04	5.13	5.00	5.63	4.13	6.07	3.54	5.17	4.09
Oso Easy Lemon Zest	4.89	2.86	2.93	3.79	3.25	6.38	5.13	4.31	4.07	4.62	4.03	4.05
Oso Happy Candy Oh	7.21	2.21	4.57	3.96	7.25	1.63	5.38	3.94	7.23	1.92	4.98	3.95
Oso Happy Petit Pink	4.14	3.43	1.50	3.00	4.88	4.75	3.00	3.69	4.51	4.09	2.25	3.35
Oso Happy Smoothie	6.17	2.52	4.79	3.74	6.33	2.33	6.29	3.96	6.25	2.43	5.54	3.85
Papa Hemeray	1.86	2.86	6.14	4.29	2.75	4.75	3.25	3.25	2.31	3.81	4.70	3.77
Peachy Keen	2.19	2.43	1.76	3.07	4.42	4.42	2.67	3.58	3.31	3.43	2.22	3.33
Phloxy Baby	5.83	2.76	3.31	3.76	6.58	2.33	4.92	3.96	6.21	2.55	4.12	3.86
Pink Enchantment	1.67	3.00	1.58	3.71	5.13	3.88	2.50	3.63	3.40	3.44	2.04	3.67

Appendix A-5. Continued

	2016				2017				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Pink Home Run	2.67	3.26	2.40	2.88	4.67	2.83	4.50	4.00	3.67	3.05	3.45	3.44
Plum Perfect	6.39	2.79	4.75	4.11	7.13	4.13	5.50	4.25	6.76	3.46	5.13	4.18
Polanaise	2.67	3.33	4.33	4.00	2.63	5.38	3.25	3.81	2.65	4.36	3.79	3.91
Poseidon	6.42	4.25	5.75	4.25	3.00	5.75	4.25	4.13	4.71	5.00	5.00	4.19
Purple Pavement	0.14	2.36	0.93	2.54	0.38	5.00	3.63	3.63	0.26	3.68	2.28	3.09
Purple Rain	5.17	2.33	3.58	3.70	6.38	2.50	4.75	4.25	5.78	2.42	4.17	3.98
Raspberry Kiss	4.33	2.76	1.29	3.14	4.75	4.50	3.46	3.65	4.54	3.63	2.38	3.40
Raspberry Vigorosa	5.14	3.98	3.81	3.93	4.63	4.38	5.50	4.25	4.89	4.18	4.66	4.09
Red Drift	4.25	3.50	3.33	3.67	5.00	3.50	4.25	4.00	4.63	3.50	3.79	3.84
Rise N Shine	1.00	2.00	1.00	3.42	2.63	4.38	2.63	3.63	1.82	3.19	1.82	3.53
Rosarium Uetersen	6.74	2.00	3.21	3.79	6.21	4.17	4.08	4.17	6.48	3.09	3.65	3.98
Roxanne Veranda	7.57	2.81	3.74	3.98	6.50	5.25	5.25	4.29	7.04	4.03	4.50	4.14
Ruby Vigorosa	4.25	3.00	1.42	3.79	6.08	4.25	3.75	4.31	5.17	3.63	2.59	4.05
Sally Holmes	2.33	3.33	2.45	3.60	3.83	4.92	4.25	3.83	3.08	4.13	3.35	3.72
Savannah	5.36	4.81	4.00	3.79	6.42	3.08	5.17	3.92	5.89	3.95	4.59	3.86
Sir Thomas Lipton	0.24	0.86	0.10	2.07	1.58	3.42	2.17	2.46	0.91	2.14	1.14	2.27
Skylark	1.00	5.12	3.19	3.36	1.08	5.67	6.08	4.17	1.04	5.40	4.64	3.77
Sky's the Limit	1.86	4.74	4.31	4.02	1.67	4.67	7.50	4.44	1.77	4.71	5.91	4.23
Solero Vigorosa	3.10	3.83	1.10	3.94	4.75	4.38	3.38	4.25	3.93	4.11	2.24	4.10
Sophy's Rose	1.24	4.86	2.40	3.48	1.87	4.83	6.25	4.02	1.56	4.85	4.33	3.75
St. Patrick	1.42	3.00	2.81	3.57	1.89	3.47	5.19	4.30	1.66	3.24	4.00	3.94
Star Delight	0.86	3.57	2.64	3.11	2.00	5.38	4.25	3.56	1.43	4.48	3.45	3.34
Stormy Weather	0.88	3.60	3.21	3.24	2.00	5.63	4.25	3.67	1.44	4.62	3.73	3.46
Strawberry Hill	1.62	4.86	3.14	3.69	2.88	5.33	5.71	4.10	2.25	5.10	4.43	3.90
Sunny Sky	1.30	3.31	1.37	3.43	1.79	4.13	3.29	3.77	1.55	3.72	2.33	3.60

Appendix A-5. Continued

	2016				2017				Average			
Name	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**	**CLS**	**BLS**	**DF**	**LR**
Sunrise Sunset	1.81	2.90	2.52	3.26	5.75	4.63	3.13	4.00	3.78	3.77	2.83	3.63
Sunset Celebration	1.43	4.52	4.00	3.64	1.50	3.67	4.92	3.88	1.47	4.10	4.46	3.76
Sweet Frances	1.80	2.55	0.00	3.20	2.50	4.63	3.63	3.69	2.15	3.59	1.82	3.45
Sweet Vigorosa	4.08	4.08	2.11	3.94	5.83	4.50	3.75	4.19	4.96	4.29	2.93	4.07
Tahitian Treasure	2.90	3.33	1.57	3.38	5.75	3.50	3.33	3.63	4.33	3.42	2.45	3.51
Tamango	2.10	2.54	0.60	3.31	2.38	4.38	7.00	4.13	2.24	3.46	3.80	3.72
Teasing Georgia	0.86	5.38	1.71	3.07	1.92	6.92	4.71	4.00	1.39	6.15	3.21	3.54
Tequila	3.14	3.89	2.71	3.29	4.50	6.13	5.63	3.88	3.82	5.01	4.17	3.59
Therese Bugnet	3.00	4.76	1.57	3.33	4.71	7.50	4.00	4.35	3.86	6.13	2.79	3.84
Tiffany	1.00	3.79	2.62	3.52	2.25	3.83	5.67	3.96	1.63	3.81	4.15	3.74
Topolina Vigorosa	6.36	3.58	0.94	3.80	6.17	5.50	2.42	4.21	6.27	4.54	1.68	4.01
Toscana Vigorosa	6.28	2.67	2.14	3.78	7.13	3.50	2.13	4.00	6.71	3.09	2.14	3.89
Traviata	2.75	3.86	1.56	3.82	4.25	4.83	4.25	3.94	3.50	4.35	2.91	3.88
Watercolors Home Run	4.45	3.14	3.67	3.67	4.58	3.67	5.75	4.13	4.52	3.41	4.71	3.90
Westerland	2.10	4.38	3.05	3.86	3.25	6.50	6.63	4.58	2.68	5.44	4.84	4.22
Windermere	1.29	3.81	0.76	3.02	1.29	6.42	3.13	4.10	1.29	5.12	1.95	3.56
Winner's Circle	5.67	3.81	2.67	3.64	5.63	4.67	4.96	3.94	5.65	4.24	3.82	3.79
Winter Sunset	2.15	3.20	1.90	3.75	6.00	5.25	3.25	4.00	4.08	4.23	2.58	3.88