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Fungal Disease Management Strategies of Fresh Market Raspberries and Processing Tomatoes

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by

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ABSTRACT OF THE THESIS

Fungal Disease Management Strategies of Fresh Market Raspberries and Processing Tomatoes

by

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In the first chapter we evaluate leaf pruning for management of cane Botrytis on fresh-market raspberries. In the study we determine the influence of removal of lower leaves from primocanes on: (i) incidence and severity of cane Botrytis caused by *B. cinerea*; and (ii) the air temperature and relative humidity within the raspberry canopy. The studies were conducted under two tunnel-row configurations: two studies under tunnels with three rows and one under tunnels with two rows. The leaf removal treatments consisted of a non-treated control and three methods twine, manual, and blade removal. We found that removal of leaves in the lower canopy of raspberry primocanes may affect cane Botrytis incidence or severity, but effect may be positive or negative depending on context.

The second chapter evaluates southern blight, a disease of processing tomato caused by the soilborne fungus *Athelia rolfsii*. The objectives of this study were to: (i) evaluate susceptibility of commercial processing tomato cultivars to southern blight; and

(ii) evaluate grafting and increased height of the graft union with the resistant rootstock Maxifort for southern blight management in processing tomato. Objective (i) greenhouse experiments evaluated 20 commercial processing tomato cultivars and six processing tomato breeding lines in pots inoculated with 10 *A. rolfsii* sclerotia per 100 cm^3 soil. Cultivars exhibited a range of susceptibility, and several commercial cultivars performed similarly to the breeding lines. For objective (ii) we evaluated two cultivars (Heinz 5608 or Heinz 8504), three graft treatments (standard height graft to Maxifort, tall height graft to Maxifort, and non-grafted) in both greenhouse and field studies. Southern blight incidence was drastically reduced by grafting treatments regardless of height. Based on our studies, the approach of grafting for management of southern blight may not be the best application. The use of resistant cultivars is a better and accessible approach for California processing tomato growers.

Table of Contents

Abstract xiii

General Introduction 1

References 3

Chapter 1 *Botrytis cinerea* Management on Fresh Market Red Raspberry (*Rubus spp*.) Primocanes

Page

Abstract 4

Introduction 5

Materials and Methods 10

Results 15

Discussion 19

References 27

Tables 30

Figures 35

Chapter 2 Evaluation of Cultivar Resistance and Grafting for Management of *Athelia rolfsii* in Processing Tomatoes

Abstract 47

Introduction 48

Materials and Methods 54

Results 64

Discussion 66

References 71

Tables 74

Figures 79

General Conclusions 88

List of Tables

Chapter 1 Page

Table 1.1 Fixed effects analysis of cane Botrytis incidence as influenced by leaf removal in the lower canopy and rating date at two locations near Camarillo, CA 30

Table 1.2 Incidence of cane Botrytis of red raspberry as influenced by leaf removal in the lower canopy at two locations near Camarillo, CA ... 31

Table 1.3 Fixed effects analysis of cane Botrytis severity as influenced by leaf removal in the lower canopy and rating date at two locations near Camarillo, CA 32

Table 1.4 Severity of cane Botrytis of raspberry as influenced by leaf removal in the lower canopy at two ranches near Camarillo, CA 33

Table 1.5 Number of raspberry receptacles as influenced by leaf removal in the lower canopy at ranch 1 and ranch 2 34

Chapter 2

Table 2.1 List of cultivars and treatments evaluated in the 2018 and 2019 greenhouse experiments 74

Table 2.2 Fixed effects analysis of cultivar and inoculum on severity of southern blight of processing tomato in the greenhouse 75

Table 2.3 Fixed effects analysis of severity of southern blight of processing tomato influenced by cultivar and grafting in the grafted greenhouse experiment of 2018 and 2019........ 76

Table 2.4 Fixed effects analysis of severity of southern blight of processing tomato in the grafted field experiment in 2018 and 2019 77

Table 2.5 Influence of cultivar and grafting treatment on southern blight incidence in field trials 78

List of Figures

Chapter 1 Page

Figure 1.1 Primocane node with cane Botrytis lesion showing concentric rings inside dark brown watermark border (left). Cane Botrytis lesion growth from broken petiole and gray *B. cinerea* sporulation on base of petiole (middle). Black sclerotia on 'bleached' cane Botrytis lesions growing on primocane.. 35

Figure 1.2 Twine leaf removal treatment on the lower canopy of the primocanes. The red circles show the tissue injury around the nodes caused by using the twine for leaf removal.. 36

Figure 1.3 Manual leaf removal treatment on the lower canopy of the Primocanes 37

Figure 1.4 Blade leaf removal treatment on the lower canopy of the primocanes showing attached petioles (top) and control treatment with no leaf removal (bottom) 38

Figure 1.5 Twine removed treatment in the lower raspberry canopy. Canes facing toward the furrow are removed by the side scraping using the black trellising twine. The leaves on the inside of the cane facing the drip tape are manually removed due to not being accessible to the twine 39

Figure 1.6 Incidence of cane Botrytis of raspberry as influenced by leaf removal in the lower canopy at ranch 1 and ranch 2. Lines represent raw data from individual plots with leaf treatments blade (solid red), control (dotted green), manual (dotted blue), and twine (dotted purple) 40

Figure 1.7 Severity of cane Botrytis of raspberry as influenced by leaf removal in the lower canopy at ranch 1(A-2-row and B-3-row) and ranch 2 (C-3-row). Lines represent total lesion length from individual lesions per cane with leaf treatments blade (solid red), control (dotted green), manual (dotted blue), and twine (dotted purple)................................ 41

Figure 1.8 Number of receptacles per cane across all leaf removal treatments for ranch 1 (2-row and 3-row) and ranch 2 (3-row). Twenty canes were counted for ranch 1 and ten canes were counted for ranch 2. Open circles represent total number of receptacles from a single cane 42

Figure 1.9 Time per day that temperature was different between the three treatments and the control. The 'change from control' was calculated for each 10 min time point by subtracting the value in a control plot from the value in each of the three treatment plots within the same block. The mean and 95% confidence interval of the change from control among blocks was calculated for each treatment-control pair at each time point. A treatment was determined to be different from the control if the confidence interval of the mean change from control did not include 0. Time per day was graphed separately based on direction of difference (higher or lower) and day (0600 to 1800 hours) or night (1800 to 0600 hours) 43

Figure 1.10 Time per day that relative humidity was different between the three treatments and the control. The 'change from control' was calculated for each 10 min time point by subtracting the value in a control plot from the value in each of the three treatment plots within the same block. The mean and 95% confidence interval of the change from control among blocks was calculated for each treatment-control pair at each time point. A treatment was determined to be different from the control if the confidence interval of the mean change from control did not include 0. Time per day was graphed separately based on direction of difference (higher or lower) and day (0600 to 1800 hours) or night (1800 to 0600 hours) 44

Figure 1.11 Weather data before and during the experiments. Data for each ranch period was converted to days after treatment and overlaid. For each ranch, each line represents one of two weather stations in Camarillo, CA located 7.5 miles or less from each study site 45

Chapter 2

Figure 2.1 Examples of the nongrafted transplant (left), standard grafted transplant (middle), and tall grafted plant (right). The red arrow indicates the height of the standard graft union at approximately 2.54 cm and at approximately 7.62 cm for the tall grafted union.. 79

Figure 2.2 Examples of the 0 to 7 rating scale used to assess severity of southern blight of processing tomato in the greenhouse. From left to right ratings 0, 1, 2, 3, 4, 5, 6, and 7... 80

Figure 2.3 Examples of colonized tomato stems by *A. rolfsii* in the greenhouse (top images) and symptoms of southern blight observed on processing tomato in the field. White mycelia and white sclerotia growing around a tomato stem in the greenhouse (top left). Mature tan to reddish brown sclerotia around a dried tomato stem (top right). Image of a wilting tomato plant due to southern blight (bottom left) and image of a collapsed and dead tomato plant due to southern blight (bottom right). Image on bottom right photo credit to Alexander I. Putman 81

Figure 2.4 Percent symptomatic plants on the last rating date in the 2018 and 2019 cultivar evaluation studies in the greenhouse. **Top panels,** cultivars evaluated in soil inoculated with 10 sclerotia per 100 cm^3 soil. **Bottom panel**, cultivars evaluated in non-inoculated soil. Within each year or cultivar, means followed by the same letter are not significantly different. 82

Figure 2.5 Relative treatment effects and 95% confidence intervals of disease severity under four inoculum levels of 0, 5, 10, and 20 sclerotia per 100 cm^3 soil from the 2017 greenhouse study. **Solid red line**, HZ 5608 grafted to Maxifort rootstock; **dotted green line**, non-grafted HZ 5608; **dashed blue line**, HZ 8504 grafted to Maxifort; **dashed purple line**, non-grafted HZ 8504 83

Figure 2.6 Disease severity of processing tomato cultivars HZ 5608 and HZ 8504 under two inoculum levels (0 and 10 sclerotia per 100 cm^3 soil) from the 2018 and 2019 greenhouse graft studies. Data was collected using a 0 to 7 rating scale; 0 = healthy and no disease, and 7 = completely wilted and dry, dead. Each line represents a replicate pot 84

Figure 2.7 Incidence of southern blight of processing tomato cultivars HZ 5608 and HZ 8504 alone (dotted blue line) or grafted to Maxifort rootstock (solid red) in the 2017 field study. Each line represents the mean incidence of four 15.4 m segment within each of seven replicate plots 85

Figure 2.8 Yield per plot of processing tomato either non-grafted (**none**) or grafted to Maxifort rootstock (**Maxi**) collected in the field in October 2017. Each data point represents one of seven replicate plots across two cultivars (HZ 5608 and HZ 8504), and numbers and horizontal bars indicate average yield of each grafting treatment across replicate plots and cultivars. 86

Figure 2.9 Incidence of southern blight of processing tomato cultivars HZ 5608 and HZ 8504 as influenced by grafting treatments: non-grafted (**red solid lines**), grafted to Maxifort rootstock at a standard height (**dotted green lines**), or grafted to Maxifort rootstock at a tall height (**dashed blue lines**) in the 2018 and 2019 field studies. Each line represents a replicate plot. Incidence was assessed for each plant by visually determining the presence or absence of southern blight symptoms and tracking the same plant across all rating dates.. 87

GENERAL INTRODUCTION

Raspberries (*Rubus spp.*) are an important crop for California, where it is among the top 20 commodities with an average annual value of $448 million from 2015 to 2017 (California Agricultural Statistics Review 2017-2018). Maintaining a high yielding, disease-free crop has been difficult due to low availability of farm workers to harvest and maintain the fresh market raspberry canopies. As a method to adjust to their limited field workers, some growers in California began experimenting with new cultural practices, such as pruning leaves near the base of the canes. In Ventura County, the common pruning practice is to remove mature and senescent leaves using the twine that is part of the trellis. The influence of these experimental cultural practices on incidence and severity of cane Botrytis is not known. Managing *B. cinerea* cane and fruit infections during this early period can be important for reducing severe epidemics during the rest of the crop. The first chapter focuses on our studies that evaluate the influence of pruning leaves on incidence and severity of cane Botrytis.

In the previous decades, soilborne diseases were commonly managed with the use of chemical fumigation, but the widening restrictions on the use of fumigants in the San Joaquin Valley of California has posed a challenge for growers. The California processing tomato industry averaged $1.1 billion in value from 2013 to 2017, and accounted for 93% of the production in the United States in 2017 (California Agricultural Statistics Review 2017-2018). Southern blight is a soilborne disease of processing tomato that has long been an economic concern in the San Joaquin Valley, and recently caused a widespread epidemic in both the San Joaquin and Sacramento Valleys (Swett and Nunez

2017). The threat of southern blight has caused reductions in acres planted with processing tomato in the southern San Joaquin Valley (J. Nunez, personal communication). The objectives of the second chapter were to: (i) evaluate susceptibility of commercial processing tomato cultivars to southern blight; and (ii) evaluate grafting and increased height of the graft union with the resistant rootstock Maxifort for southern blight management in processing tomato.

REFERENCES

California Agricultural Statistics Review, 2017-2018. California Department of Food and Agriculture. 11-104.

Swett, C. L., and Nunez, J. 2017. Southern Blight Cliff Notes-2017. Sacramento Valley Agronomy Notes. (1):1-6.

Chapter 1.

Botrytis cinerea Management on Fresh Market Red Raspberry *(Rubus* spp.) Primocanes

ABSTRACT

The fungal pathogen *Botrytis cinerea* causes cane Botrytis, a disease of raspberry canes, but the influence of pruning leaves on incidence and severity of cane Botrytis is not known. In this study we: (i) determine the effect of leaf removal in raspberry primocanes on incidence and severity of cane Botrytis caused by *B. cinerea*; and (ii) determine the influence of removal of lower leaves from primocanes on air temperature and relative humidity of the raspberry canopy. The studies were conducted under two tunnel-row configurations: two studies under tunnels with three rows (3-row) and one under tunnels with two rows (2-row). The treatments consisted of a non-treated control and three methods (twine, manual, and blade) of leaf removal. Under 2-row tunnels the percentage of canes infected by *B. cinerea* was significantly lower in plots treated with twine removal relative to control plots. In 3-row tunnels, disease severity as measured by length of lesions was significantly lower in twine removal plots compared to control plots at one location, but was significantly higher at a second location. Leaf removal did not have a significant effect on cane Botrytis incidence for 3-row density. Environmental monitoring revealed that the effect of leaf removal on relative humidity within the canopy was not consistently influenced by leaf removal treatments and did not correlate with disease severity. We found that removal of leaves in the lower canopy of raspberry

primocanes may affect cane Botrytis incidence or severity, but the direction of the effect may vary with context.

INTRODUCTION

Raspberries (*Rubus spp*.) are an important crop for California, where it is among the top 20 commodities with an average annual value of $448 million from 2015 to 2017 (California Agricultural Statistics Review 2017-2018). This represented 82% to 88% of the domestic raspberry production. The four California counties where raspberry is produced are Ventura, Santa Cruz, Santa Barbara, and Monterey. Specifically in Ventura and Santa Cruz counties, raspberries are among the top commodities (California Agricultural Statistics Review 2017-2018). On the West Coast of the United States, raspberry is typically produced in two stages from a single planting that is grown for a maximum of two years. In the primocane stage or first year cycle, harvest generally begins four months after planting of bare root transplants and continues for approximately three to five months. After harvest, the primocane growth is pruned near the last fruiting lateral or is mown at the soil line. The growth that follows this pruning begins the floricane stage or second cycle, which has a harvest period that generally begins three to four months after pruning and can last approximately four months. In Ventura County, a crop can be planted during four periods throughout the year: early spring, late spring, mid-summer, or late summer.

The ascomycete fungal pathogen *Botrytis cinerea* Pers.:Fr causes multiple diseases of raspberry. The first is grey mold or Botrytis fruit rot, a widespread and damaging disease of fruits and flowers of many hosts including raspberry (Harrison and Williamson 1986; Williamson et al. 1987). The second is cane Botrytis, a disease of the raspberry cane that was first reported in England in 1931 (Williamson 2017). Cane Botrytis is reportedly more severe on red raspberry (*Rubus idaeus* L.) canes compared to other *Rubus spp*. (Williamson 2017). Cane botrytis is known to more commonly affect the floricane stage in the U.S. Pacific Northwest (Pscheidt and Ocamb 2018), but in coastal California and in eastern Canada *B. cinerea* causes disease primarily to the primocane stage of raspberry (Julien Mercier, *personal communication*; Carisse et al. 2018). The primocane stage is critical for healthy establishment of the raspberry crop. Managing *B. cinerea* cane and fruit infections during this early period can be important for reducing severe epidemics during the rest of the crop. For example, a study evaluating *B. cinerea* conidia dispersal in raspberries showed locally-produced inoculum was important for controlling fruit infections (Jarvis 1962a).

New raspberry plantings are often established next to older plantings where mummified fruits, damaged canes, and dead plant debris potentially colonized by *B. cinerea* sclerotia are prevalent (Xu et al. 2012). These infected debris from older plantings are an important source for primary spore inoculum (Jarvis 1962b). Resting mycelia and sclerotia can also be found on weeds (Xu et al. 2012). When weather conditions are favorable in spring, sclerotia germinate and develop conidia that serve as inoculum for new infections (Jennings and Carmichael 1975; Jensen 2018). Sporulation on infected fruit or receptacles can also contribute to airborne inoculum able to infect canes (Pscheidt and Ocamb 2018). In geographic regions where raspberry is produced in a single cycle during the summer, symptoms of cane Botrytis first appear in mid to late summer as pale brown lesions around the nodes with concentric ‘watermark’ or banding patterns (Jennings and Williamson 1982; Pscheidt and Ocamb 2018; Williamson 2017) (Figure 1.1). Lesions can expand to three to four internodes, and have been reported to expand to 15.3 cm 14 days after inoculating a wound with mycelium (Harrison and Williamson 1986). The portion of the lesion with the watermark often turns gray or white as the cane matures and is often referred to as ‘bleached’ (Williamson 2017, Hockey 1952). Sclerotia, the vegetative resting structures of *B. cinerea*, form in the lesion beneath the cane epidermis and appear as black blister-like structures during the winter (Williamson 2017, Koike et al. 2009). The subepidermal sclerotia have been described in canes inoculated by conidia to be 1 to 2 mm by 2 to 15 mm in size (Hockey 1952). *Botrytis cinerea*-inoculated petioles from young canes were reported to impair nodal lengths, suppress axillary bud growth, and suppress lateral shoot development following spring (Williamson and Jennings 1986).

Two routes of infection by *B. cinerea* have been suggested in the disease cycle of cane Botrytis. In the first route, *B. cinerea* directly infects non-wounded cane tissue (Xu et al 2009, O’Neill et al 2009). In the second route, *B. cinerea* can begin by infecting cane wounds or mature leaves (Pscheidt and Ocamb 2018). In the case of mature leaves, infections spread from the leaf blade through the petiole to the cane (Pscheidt and Ocamb 2018), where the pathogen colonizes the primary cortex of the cane surrounding the node, just below the epidermis (Williamson 2017). This route has been partially (Pscheidt and

Ocamb 2018) or wholly (Williamson 2017) described from extensive field observations in the Pacific Northwest and the United Kingdom, respectively.

Raspberry is a vigorous plant that often develop dense canopies. The leaves near the base of the canes yellow, and as they senesce as they age they become more susceptible to infection by *B. cinerea* (Hockey 1952). Cane Botrytis infections are also known to be most severe inside a dense canopy, specifically near the lower area of the canes (Pscheidt and Ocamb 2018). Maintaining a high yielding, disease-free crop has been difficult due to low availability of farm workers to harvest and maintain the canopies. As a method to adjust to their limited field workers, some growers in California began experimenting with new cultural practices, such as pruning leaves near the base of the canes. In Ventura County, the common pruning practice is to remove mature and senescent leaves using the twine that is part of the trellis. However, this typically creates wounds along the base of the cane, and any wound on a cane can serve as an infection court for *B. cinerea* (O'Neill et al 2009). The influence of these experimental cultural practices on incidence and severity of cane Botrytis is not known. In a modification of this practice, some growers are using pruning methods such as high-powered blowers that are designed to reduce wounding. This method removes leaf blades but petioles are retained on the cane. It is unclear if pruning methods designed to reduce wounding offer improved management of cane Botrytis.

Temperature and relative humidity are important factors in development of diseases caused by *B. cinerea*, and practices that alter the raspberry canopy environment could influence cane Botrytis. To reduce infections, Washington State University

Integrated Pest Management strategies for cane Botrytis and fruit rot include the use of pruning and trellising to improve air circulation (WSU Whatcom County Extension). In strawberry, narrow plant spacing exhibited higher incidence of Botrytis fruit rot compared to wider plant spacing (Legard et al. 2000). In the United Kingdom, the incidence of cane Botrytis has been shown to be higher under dense canopy production (20 canes/m) that promotes high ambient humidity conditions compared to the lower density (10 canes/m) (O'Neill et al. 2009). The most severe cane botrytis infections in Scotland have been reported in high density nursery plantings (Pscheidt and Ocamb 2018). The growing conditions in these reports promote high relative humidity, and therefore a higher risk for pathogen sporulation (O'Neill et al. 2009). Similarly, fresh market raspberry production in Ventura County is commonly grown on 3 rows of densely-planted raspberries under one plastic hoop tunnel. Some growers in Ventura County are interested in experimenting by using fewer rows of plants per tunnel to maintain healthy crops. Although the role of planting density on disease appears well established, it is unknown if the pruning methods described above influence environmental conditions in the raspberry canopy in plantings with different row spacings.

Cultural management strategies to minimize cane infections by *B. cinerea* have yet to be characterized for fresh market raspberry production in California. The objectives of this study are to: (i) determine the effect of leaf removal in raspberry primocanes on incidence and severity of cane Botrytis caused by *B. cinerea*; and (ii) determine the influence of removal of lower leaves from primocanes on air temperature and relative humidity of the raspberry canopy.

MATERIALS AND METHODS

Study Locations. Field studies were conducted within commercial plantings on ranches in Camarillo, Ventura County, California. At ranch 1, the study was conducted on the primocane of proprietary red raspberry cultivar 1 that was planted as roots in August of 2017. At this location, the study was conducted under two tunnel-row configurations: tunnels with three rows (3-row), which are 7.3 m (24 ft) wide with 2.13 m (7 ft) row spacing, and tunnels with two rows (2-row), which are 5.48 m (18 ft) wide with 2.7 m (9 ft) row spacing. At ranch 2, the study was conducted on the primocane of proprietary red raspberry cultivar 2 that was planted as roots in May of 2018. At this location, the study was conducted only on the 3-row configuration as described for ranch 1. Hoop tunnels on both ranches were formed with clear polyethylene plastic.

Treatments. The treatments consisted of a non-treated control and three methods of leaf removal. The ‘twine’ treatment was performed by removing whole leaves protruding toward the furrow by manually scraping the lowest run of synthetic twine, which is used to trellis the canopy, up and down along the canes (Figure 1.2). The ‘manual’ treatment was executed by pulling the whole leaf in an upward direction from the base of the petiole (Figure 1.3). This treatment was designed to mimic the twine leaf removal with less severe wounding. The ‘blade’ treatment was executed by manually pinching the blades off in the direction of the apex, leaving only the petioles attached to the cane (Figure 1.4). The blade removal treatment was designed to mimic the results from using a power leaf blower for leaf removal. No leaves were removed in the non-treated control. The twine treatment removed leaves facing toward the furrow but did not remove leaves facing in other directions To isolate the hypothesized influence of wounding in twine removal from the presence or absence of leaf blades in blade removal, the leftover leaves were removed manually in the same manner as the 'manual' treatment (Figure 1.5). Treatments were applied from the soil line up to the height of the lowest twine, which was 30.5 cm (1 ft) at ranch 1 and 48 cm (19 inches) at ranch 2. Treatments were performed on January 4th and 5th in 2018 at ranch 1 and September 24th and 25th in 2018 at ranch 2. At both locations experimental units measured 1 tunnel × 14.6 m (48 ft) row length, and treatments were assigned to experimental units in a randomized complete block design with 5 replications, with each tunnel forming a block.

Young shoots from the raspberry roots commonly called 'suckers' were removed from treated plots on the same day leaf removal treatments were applied for ranch 1, whereas young shoots from the control treatments were removed in April 2018. Young shoots were not removed from cultivar 2 at ranch 2 because this practice is not commonly performed on this cultivar.

Data Collection. To evaluate objective (i), the incidence and severity of cane botrytis were assessed three times beginning approximately six (ranch 1) and five (ranch 2) months after planting and one month after treatments were performed. Specific assessment dates for ranch 1 were February 13, March 13, and April 11 2018 and for ranch 2 were October 17, November 7, and November 28 2018. Data were collected on

December 18 at ranch 2 for both incidence and severity but data was discarded due to difficulty in differentiating lesions from the brown cane tissue that progresses as the cane begins to harden due to aging. To avoid edge effects, the observational units were confined to the center of each experimental unit. Parallel to the rows, the observational unit consisted of the center 4.9 m of row length of each 14.6 m long experimental unit. Perpendicular to the rows, the observational unit consisted of the center row in the 3-row tunnels and the interior half (facing the center furrow) of each row in the 2-row tunnels. Incidence was assessed by counting the number of canes exhibiting symptoms of cane Botrytis on each date. Percent incidence was calculated by dividing the number of symptomatic canes by the total number of canes in each observational unit. The total number of canes in the observational units was determined on the first rating date only. Severity was assessed by arbitrarily tagging 10 symptomatic canes and measuring the length of each lesion with a tape measure. This process was difficult for some lesions at ranch 2 because the canes of that cultivar quickly turn brown and harden progressively upward from the soil line. Therefore, for some lesions at ranch 2, the top half of the lesion length was measured to avoid potential confusion with the hardening tissue. These half lengths were then doubled for analysis. Disease assessments for ranch 1 were made on the whole cane while in ranch 2 only lesions up to 48 cm above the soil were recorded.

Objective (ii) was evaluated with Hobo MX2301 data loggers (Onset Computer Corporation, Bourne, MA, USA) that were mounted to the trellis stake in the center row of the 3-row tunnels and on the west row of the 2-row tunnels facing south (down the row). At each ranch the loggers were mounted at the height of the lowest twine, or the maximum height to which pruning treatments were applied. Loggers took air temperature and relative humidity readings on 30 s intervals, and every 10 min recorded averages of these readings. To serve as an analog for yield, the number of receptacles on 20 arbitrarily chosen canes in each observational unit was counted on May 29 and 30 for ranch 1 and 10 arbitrarily chosen canes on December 19 and 20 for ranch 2.

Statistical Analysis. The influence of leaf removal treatment on disease incidence, disease severity, and receptacles was analyzed with generalized linear mixed models using PROC GLIMMIX in SAS v9.4. For disease data, leaf removal, rating date, and leaf removal × rating date were analyzed as fixed effects. Disease data could not be modeled with repeated measures despite attempts with various combinations of program options. Random effects for incidence data were block and block × leaf removal, the latter to account for clustering within observational units when using the binomial distribution (Madden and Kriss, 2016). For severity data, block was included as a random effect for the ranch 1 3-row and ranch 2 experiments, but was omitted from the ranch 1 2-row experiment due to poor fit of the model when included. Severity data were pooled by summing the total length of all lesions for each cane within each plot and rating date. Canes at ranch 1 with lesions measured above the leaf removal area were omitted from the severity analysis. The three experiments were analyzed separately. Incidence and severity data was evaluated using the binomial and log normal distributions, respectively, and the logit and identity link functions, respectively, in the *model* statement. Receptacle data was analyzed using the negative binomial distribution and log link function. When evidence for a leaf removal × rating date interaction was observed, the *slice* statement was used to analyze the effect of leaf removal within each rating date. When evidence for an influence of leaf removal was observed, means were separated using the least significant difference test with Tukey-Kramer adjustment for multiple comparisons with the *lsmeans* statement.

For objective (ii), air temperature and relative humidity data was analyzed based on the differences between each of the three leaf treatments and the control. The 'change from control' was calculated for each 10 min time point by subtracting the value in a control plot from the value in each of the three treatment plots within the same block. The mean and 95% confidence interval of the change from control among blocks, defined as the region between the mean plus and minus the standard error of the mean times 1.96, was calculated for each treatment-control pair at each time point. A treatment was determined to be different from the control if the confidence interval of the mean change from control did not include 0. The total time per day that the change from control was different was determined for both positive or negative values of change from control. Because analysis of the mean and confidence interval results indicated distinct patterns of change from control associated with time of day, the total time per day value was also calculated separately for day (0600-1800 hours) and night (1800-0600 hours) for both positive and negative values.

At ranch 1, three of the control plots (one in 2-row and 2 in 3-row) were compromised early in the season due to accidental removal of leaves by the farm crew. Disease, receptacle, and environmental data from these plots was discarded as missing for analysis. In addition, some environmental data was missing or modified due to mistaken settings on the loggers. First, the four data loggers placed in the plots in one block at ranch 2 stopped recording in October. Second, one of the loggers in 3-row at ranch 1 and one at ranch 2, both in manual removal plots, only recorded instantaneous readings at each time point and not 10 minute averages, therefore no data was available from these plots. Third, one logger in 3-row at ranch 1 (control plot) and at ranch 2 (blade removal) recorded values approximately 4 and 1 minutes, respectively offset from the rest of the loggers. These timestamps were adjusted to the nearest 10 minutes to align with the rest of the loggers.

RESULTS

Incidence was generally low on the first two rating dates in all three experiments (Table 1.1). At the last rating date, disease incidence was higher in ranch 1 (Feb to Apr) compared to ranch 2 (Oct to Nov), although the ranches had differences in timing intervals between treatment application from final rating date of 96 and 64 days for ranch 1 and 2 respectively. (Figure 1.6). On the final rating date, incidence in individual plots ranged from 41.9 to 91.5% in ranch 1 and from 8.6 to 77.8% in ranch 2. Analysis of fixed effects showed evidence for a significant leaf removal × date interaction for ranch 1 2-row and ranch 2, but no evidence for an effect of leaf removal or leaf removal × date for ranch 1 3-row (Table 1.1). Examination of the interactions showed that for ranch 1 2-row, evidence for a significant effect of leaf removal was observed on the final rating date only (Table 1.2). On this date (April 11), the percentage of canes infected by *Botrytis* *cinerea* was significantly lower (by 13 percentage points) in plots treated with twine removal relative to control plots (Table 1.2). At ranch 2, some evidence for an effect of leaf removal was observed on the first two rating dates (Table 1.1), but mean separation showed that incidence was similar among all treatments on these dates at $P \leq 0.05$. However, examination of individual pairwise comparisons showed some evidence (P = 0.0554 and 0.0859 on Oct. 17 and Nov. 7, respectively) that incidence was higher in manual treated plots compared to the control on Oct 17 (data not shown). Although leaf removal did not have a significant main or interaction effect on cane Botrytis incidence for 3-row tunnels at ranch 1 (Table 1.1), there was a numeric trend of lower incidence in blade removal and control plots on the first two rating dates compared to manual and twine plots (Table 1.2).

Overall, disease severity levels were similar across ranches during the same time period after treatment. But, for the last rating date at ranch 1 when disease increased, more time had elapsed compared with the last rating date at ranch 2 (Figure 1.7). In 2-row tunnels, the shortest lesion length on the final rating date was 0.64 cm at ranch 1 and at ranch 2, while the 3-row tunnels at ranch 1 was 0.95 cm. The longest lesion lengths were 112.39 cm at ranch 1 and 68.58 cm at ranch 2. Evidence of a significant effect of leaf removal treatments on disease severity was observed in all three experiments (Table 1.3). In 2 row tunnels at ranch 1, total length of cane Botrytis lesions per cane in manual removal plots were significantly lower than the blade removal treatment (Table 1.4). At ranch 1 in 3-row tunnels, lesion length was significantly lower in the blade, manual, and twine removal (by 57.9, 70.6, 70.2 percent, respectively) compared to the control treatment. In contrast, at ranch 2, lesion length in twine and manual removal plots were significantly higher than the control plots, whereas lesion length in blade removal plots did not differ from other treatments or the control. There was a non-significant pattern of lower severity in manual removal plots at ranch 1 when compared to twine and control in the 2-row tunnels and to blade in the 3-row tunnels (Table 1.4).

There were no statistical or numerical differences in the number of receptacles between treatments in any of the three experiments (Table 1.5; Figure 1.8).

The air temperature change from control in individual blocks when averaged by hour generally fell within the range of -2°C to 4°C, although the overall minimum and maximum values over all three experiments were -6.06°C and 7.35°C, respectively (data not shown). In each of the three experiments, the direction of the change from control did not always agree among blocks, and this disagreement was typically due to one block only. Analysis of the change from control using 95% confidence intervals showed that air temperature in plots treated with leaf removal significantly differed from the air temperature in control plots for appreciable lengths of time per day (Figure 1.9). However, the direction was opposite at different times of day. Throughout each of the three experiments, air temperatures in leaf removal treated plots were generally higher than in control plots during daytime hours but lower relative to control plots during nighttime hours. The day-to-day pattern of time with significantly different change from control was similar among treatments, but was generally highest in manual removal plots and lowest in twine removal plots. In 3-row tunnels at ranch 2, for example, temperature in manual plots had the most hours with a significant difference from the control during the night compared to the other treatments.

The relative humidity change from control in individual blocks as hourly averages generally ranged from -10 to 10 percentage points, but the absolute range over all three experiments was -22.1 to 50.3 percentage points (data not shown). Similar to air temperature, the direction of the change from control typically disagreed for one of the five blocks. Analysis of change from control showed that relative humidity was different between treatment and control plots for noticeable lengths of time per day, but the patterns were dissimilar from air temperature and were not consistent among experiments (Figure 1.10). In 2-row tunnels, all treatments followed a similar pattern of higher relative humidity in treated plots compared to the control during nighttime hours but lower during daytime hours, but in the final month of the experiment relative humidity in all treatments was higher than the control during both day and night. In 3-row at ranch 1, relative humidity was generally higher in treated plots compared with the control through the first three months of the experiment, regardless of time of day. At ranch 2, relative humidity in blade and manual treated plots was higher than the control at night, but during daytime hours was both higher and lower throughout the experiment. In general, day-to-day patterns among treatments were similar. However, a clear trend was observed in which relative humidity in twine removal plots was higher than in control plots for very little time compared with blade or manual treatments, and only in 3-row tunnel at ranch 1 and ranch 2. In contrast, for 3-row tunnels at both ranches, the time that relative humidity was lower in treated plots compared to the control was similar among all three treatments.

Shifts were observed in change from control patterns in the 2-row and 3-row experiments at ranch 1 beginning in mid-April. A shift was most pronounced for the 3-row tunnels where, in treated plots relative to the control, relative humidity shifted from higher to lower, whereas temperature shifted to exclusively higher, regardless of time of day. In 2-row tunnels, a similar shift was observed for relative humidity only during daytime hours, but for temperature no shift was observed. The onset of the shifts observed in April to May in relative humidity and temperature coincided with the removal of suckers by the farm crew that occurred sometime between April 5 and April 11.

DISCUSSION

We found that removal of leaves in the lower canopy of raspberry primocanes may affect cane Botrytis incidence or severity, but the direction of the effect may vary with context. Leaf removal may reduce severity of cane Botrytis in cultivar 1 and may increase severity in cultivar 2 in 3-row production systems. Additionally, leaf removal may reduce incidence in cultivar 1 in 2-row production systems. In our experiments we observed the opposite effect by twine removal between the two ranches for high density canopies. There are numerous aspects that differed among the experiments that might explain the conflict. The main differences are the plant density, the cultivar grown, and the different planting cycles between the two ranches.

Variation in plant density among the experiments may have affected the response of cane Botrytis to leaf removal. Within ranch 1, leaf removal treatments provided some reductions in cane Botrytis incidence in the less compact 2-row tunnels but did not influence severity. In contrast, in the more compact 3-row tunnels, leaf removal treatments influenced severity but not incidence. In a study evaluating use of a contact herbicide to control cane vigor in Scotland, cane Botrytis incidence was 65% in control plots compared to 10% incidence in herbicide-treated plots (Williamson 1979). Although we did not evaluate chemical removal treatments, our results in a lower density 2-row tunnel system showed lower incidence of cane Botrytis in leaf removal treatments opposed to no leaf removal. Therefore, it is possible that practices that modify the canopy would have a greater impact on the environment within a dense canopy compared with a less dense canopy. This suggestion is supported by our finding that the influence of leaf removal treatments on relative humidity was modest in the 2-row experiment relative to the 3-row experiments.

Similar to the tunnel configurations, canopy density varied between ranch 1 and ranch 2 in experiments with the same tunnel configuration. The cultivar at ranch 1 produces a denser canopy than the cultivar at ranch 2 due to a higher number of both canes and suckers. Unlike the 2-row tunnels at ranch 1, however, at ranch 2 disease severity was higher in plots where leaves were removed. It is unlikely that canopy density alone accounted for the opposite response between the two ranches.

In addition to agronomic practices that vary between cultivars, the two cultivars we used in our experiments may explain the conflicting direction of influence of leaf removal in two ways. First, the cultivars may differ in their resistance to colonization by *B. cinerea* and expression of cane Botrytis symptoms, but this information is not publicly available. Second, the cultivars may respond differently to wounding, which in turn may lead to a difference in susceptibility to *B. cinerea* infection. Thornless cultivars have previously been reported to be less vulnerable to mechanical damage that in turn results in lower incidence of Botrytis-caused disease (Jennings 1962; Knight and Keep 1958; Anon 1946). However, Knight and Keep (1958) and Anonymous (1946) evaluated the disease of fruits whereas only Jennings (1962) evaluated cane Botrytis, and it is unclear what kind of mechanical damage the plants were subjected to. In our study, we caused mechanical damage to canes and evaluated disease of the canes. As measured from severity in twine removal treatments, the thorny cultivar (ranch 1) appeared less susceptible compared with the thornless cultivar (ranch 2) that experienced increased severity. It is possible that susceptibility to mechanical damage is not correlated with thorn production in these cultivars. Genetic and physiological characteristics of red raspberry cultivars should be considered when applying leaf removal treatments using twine.

The time of year in which the experiments were conducted, specifically when the treatments were applied, may also have contributed to the conflicting results between ranches. The treatments at ranch 1 were applied during the winter, which in Ventura county is marked by cool ambient temperatures (daily maximum of 18.3°C), lower average relative humidity, and increased chance of rain and clouds. In contrast, ambient conditions at the end of summer when treatments were applied at ranch 2 are marked by warm temperatures (daily maximum temperature of 23.9°C), consistently moderate relative humidity (~75%), no rain, little cloud cover, and calm winds. Actual conditions during 2018 generally reflected these long-term averages. Ambient temperature was generally lower during the ranch 1 experiments compared to the ranch 2 experimental period after treatment application (Figure 1.11). Relative humidity was much lower for ranch 1 compared to ranch 2 between about 30 and 10 days before treatment application, but was not different between the ranches on the day of treatment application or after. A total of 2.6 cm rain over two days fell in the area within one week of treatment application at ranch 1. There are two possible ways that ambient weather conditions differentially influenced cane Botrytis development between the ranches. One way is that the favorability to disease development of ambient weather conditions may combine with other factors, such as wounding, to influence colonization and symptom expression. However, conditions were slightly more favorable for ranch 1 after treatments were applied due to lower temperatures and rain, therefore wounding from leaf removal treatments would have been expected to increase disease severity. Another way is that the higher relative humidity before treatment application at ranch 2 could have led to higher levels of inoculum present in the environment, which in turn could lead to higher disease. Thirdly, conditions for ranch 2 may have been more favorable for infection on the days of treatments application and shortly afterward. While relative humidity, daily maximum temperature, precipitation, and wind were similar between the two ranches, daily minimum temperature was higher by about 10°C at ranch 2. If it is assumed that the optimal temperature of 20°C (Bulger et al. 1987) for infection of strawberry flowers also applies to raspberry canes, then conditions at ranch 2 were more favorable than ranch 1 in the days after treatment application, which could have led to higher incidence of cane Botrytis.

We hypothesized the removal of lower leaves would shift environmental conditions within the canopy to be less favorable for cane Botrytis development. Unexpectedly, in our study leaf removal treatments appeared to increase relative humidity within the raspberry canopy, especially within dense plantings. However, this effect was not consistent across experiments. In Northwest Washington, minimum air temperature, night air temperature, cumulative rain, leaf wetness, and duration of leaf wetness was correlated with *B. cinerea* colonization of processing raspberry fruit (Kozhar and Peever 2018). In strawberry, incidence of flower infections by *B. cinerea* has been reported to be correlated at relative humidity >80% and >90% (Bulger et al. 1987; Wilcox and Seem 1994; Xu et al. 2000). Given that leaf wetness typically forms at night and that leaf wetness is important in development of many diseases caused by *B. cinerea*, we would expect disease to be more severe in 3-row treatments that had higher relative humidity at night. In our study, however, relative humidity was higher at night for only two of the treatments at ranch 2 but disease was more severe compared to the control for all three treatments. Therefore, relative humidity may not be a significant factor for cane Botrytis severity.

Leaf removal treatments also influenced temperature, but the effect was more consistent than relative humidity across experiments. Temperature was higher in treated canopies than the control during the day but were lower than the control at night. This suggests that the leaves in the raspberry canopy serve to moderate temperatures, or reduce variability, with respect to daily fluctuations in ambient temperature. This assertion is supported by the generally greater time per day of significant change from control in manual treatments, given that the manual treatment removed the most material from the lower canopy. Because the effect was generally consistent across experiments and treatments yet few significant differences in disease incidence were observed, the influence of canopy on temperature is may not an important factor for cane Botrytis incidence.

We found few significant differences in cane Botrytis incidence among treatments despite finding a strong influence of leaf removal on disease severity. However, a non-significant trend of higher incidence in the manual and twine treatments versus blade removal and the control was observed in all three experiments on the first rating date. Because the manual and twine treatments removed petioles from the cane whereas petioles remained attached to the cane in the control and blade treatments, the wounding caused by petiole removal may increases disease incidence early in the season. By the last rating date, however, this trend had dissipated or reversed at ranch 1, but remained a trend at ranch 2, especially between the blade and twine treatments. Taken together, this suggests leaf removal treatments may have an impact on disease incidence right after leaf removal or early in the season but that the impact may diminish as the season progresses. Furthermore, the length of time the trend was observed supports our suggestion above that the cultivars used in our experiment differ in susceptibility or response to wounding.

Leaf removal treatments did not affect receptacle counts despite large differences in severity. While this suggests that primocane yield is not influenced by cane Botrytis, it is unknown if receptacle counts are representative of marketable yield. Even if actual marketable fruit could have been collected, collecting yield data from small plots of raspberry is known to be logistically challenging and may not be a reliable measure of yield or representative of the potential raspberry yield. Though there were many infected canes that developed sclerotia near the end of each experiment, the impact of the sclerotia on the floricane is unknown. Additional studies are needed to understand the effects of sclerotia on bud break and bud elongation in fresh market raspberry. Because we did not perform a true replication of any of our experimental conditions, future research is needed to confirm the influence of leaf removal on incidence and severity of cane Botrytis in different production conditions.

Currently, in California fresh market raspberry production there are no management practices commonly used to manage cane Botrytis. The twine treatment is the current experimental method of leaf removal practiced by growers in Ventura County due to its labor efficiency. Because it is an aggressive practice, we hypothesized that a practice that causes less wounding would improve cane Botrytis management. The method we examined was the manual removal treatment, which was designed as an equivalent canopy treatment but with less wounding. Across all three experiments there were no significant differences in cane Botrytis severity between the twine and manual treatments. The use of high powered blowers to remove leaves in the lower raspberry canopy would be beneficial to evaluate for novel management practices of cane Botrytis. Our study indicates that wounding by twine leaf removal is not important for disease severity. Our experiments have shown leaf removal methods can be applied for certain cultivars and row spacings.

REFERENCES

Anonymous. 1946. Small Fruits. Rep. Wash. agr. Exp. Sta., being Bull. 482, 54.

Bulger, M. A., Ellis, M. A., and Madden, L. V. 1987. Influence of temperature and wetness duration on infection of strawberry flowers by Botrytis cinerea and disease incidence of fruit originating from infected flowers. Phytopathology. 77(8): 1225-1230.

Carisse, O., McNealis, V., and Kriss, A. 2018. Association between weather variables, airborne inoculum concentration, and raspberry fruit rot caused by Botrytis cinerea. Phytopathology. 108(1): 70-82.

Harrison, J. G. and Williamson, B. 1986. Botrytis spp. On red raspberry: survival in fruits and infection of canes. Transactions of the British Mycological Society. 86(1): 171-173.

Hedrick U. P., Howe, G. H., Taylor, O. M., Berger, A., Slate, G. L. and Einset, O. 1925. The Small Fruits of New York. Report of the New York State Agricultural Experiment Station. Lyon Comp Printers, Albany, 614.

Hockey, J. F. 1952. Grey mould wilt of raspberry. Scientific Agriculture. 32 (1116):150-152.

Jarvis, W. R. 1962a. The dispersal of spores of Botrytis cinerea Fr. in a raspberry plantation. Transactions British Mycological Society. 45(4): 549-559.

Jarvis, W. R. 1962b. The infection of strawberry and raspberry fruits by Botrytis cinerea Fr. Annals of Applied Biology. 50: 569-575.

Jennings, D. L. 1962. Some evidence on the influence of the morphology of raspberry canes upon their liability to be attacked by certain fungi. Horticultural Research. 1: 100-111.

Jennings, D. L. and Williamson, B. 1982. Resistance to Botrytis cinerea in canes of Rubus idaeus and some related species. Annals of Applied Biology. 100: 375-381.

Jennings, D. L. and Charmichael, E. 1975. Resistance to grey mould (Botrytis cinerea Fr.) in red raspberry fruits. Horticultural Research. 14:109-115.

Jensen, S. 2018. Botrytis Blight. Plant Disease Diagnostic Clinic Fact Sheets. Cornell University, Ithaca, NY.

Knight, R. L., and Keep, E. 1958. Developments in soft fruit breeding at East Malling. Rep. E. Malling Res. Sta. for 1957. 62.

Koike, S. T., Bolda, M. P., Gubler, W. D., and Bettiga, L. J. 2009. Botrytis Fruit Rot. UC IPM Pest Management Guidelines: Caneberries. UC ANR Publication 3437. http://ipm.ucanr.edu/PMG/r71100211.html

Kozhar, O. and Peever, T. L. 2018. How does Botrytis cinerea Infect Red Raspberry? Phytopathology. 108:1287-1298.

Legard, D. E., Xiao, C. L., Mertely, J. C., and Chandler, C. K. 2000. Effects of Plant Spacing and Cultivar on Incidence of Botrytis Fruit Rot in Annual Strawberry. Plant Disease. 84: 531-538.

Madden, L.V. and A.B. Kriss. 2016. Materials from: Workshop on generalized linear mixed models (GLMM), 30 July 2016, Tampa, FL. Available: https://www.oardc.ohio-state.edu/aps-statsworkshop/downloads/downloads.htm. Accessed: 13 July 2019.

O'Neill, T., Berrie, A. M., Wedgwood, E., Allen, J., Xu, X-M. 2009. Effect of canopy manipulation on cane and fruit botrytis in protected raspberry. Communications in Agricultural and Applied Biological Sciences. 74(3): 633-643.

Pscheidt, J.W., and Ocamb, C.M. (Senior Eds.). Raspberry (*Rubus spp.)* -Fruit rot and cane botrytis. 2018 Pacific Northwest Plant Disease Management Handbook. © Oregon State University.
https://pnwhandbooks.org/plantdisease/host-disease/raspberry-rubus-spp-fruit-rot-cane-botrytis

Washington State University Whatcom County Extension. Integrated Pest Management for Raspberries. Botrytis Cane & Fruit Rot (*Botrytis cinerea*). 2019. http://whatcom.wsu.edu/ipm/manual/rasp/botrytis.html

Wilcox, W. F. and R. C. Seem. 1994. Relationship between strawberry gray mold incidence, environmental variables, and fungicide applications during different periods of the fruiting season. Phytopathology. 84:264-270.

Williamson, B. 2017. Cane Botrytis. Compendium of raspberry and blackberry diseases and pests. 17-19. Second Edition. American Phytopathological Society, St. Paul.

Williamson, B. and Jennings, D. L. 1986. Common resistance in red raspberry to Botrytis cinerea and Didymella applanata, two pathogens occupying the same ecological niche. Annals of Applied Biology. 109(3):581-593.

Williamson, B., Lawson, H. M., Woodford, J. A. T., Hargreaves, A. J., Wiseman, J. S., and Gordon, S. C. 1979. Vigour control, an integrated approach to cane, pest and disease management in red raspberry (*Rubus idaeus*). Annals of Applied Biology. 92:359-368.

Williamson, B., McNicol, R. J., and Dolan, A. 1987. The effect of inoculating flowers and developing fruits with Botrytis cinerea on postharvest gray mold of red raspberry. Annals of Applied Biology. 111(2): 285-294.

Xu, X., Harris, D. C., and Berrie, A. M. 2000. Modeling infection of strawberry flowers by Botrytis cinerea using field data. Phytopathology 90:1367-1374.

Xu, X., Wedgwood, E., Berrie, A. M., and O'Neill, T. M. 2009. Infection of raspberry leaves by *Botrytis cinerea* in relation to leaf and cane age. Communications in Agricultural and Applied Biological Sciences. 74(3): 761-770.

Xu, X., Wedgwood, E., Berrie, A. M., Allex, J., and O'Neill, T. M. 2012. Management of raspberry and strawberry grey mould in open field and under protection. A review. Agronomy for Sustainable Development 32:531-543.

Table 1.1. Fixed effects analysis of cane Botrytis incidence as influenced by leaf removal in the lower canopy and rating date at two locations near Camarillo, CA.

Type III Tests of fixed effects						
Ranch	*Tunnel*	*Effect*[a]	*Num DF*	*Den DF*	*F Value*	*Pr > F*
1	2-row	Leaf Removal	3.0	14.22	0.58	0.6364
		Date	2.0	45.00	565.96	<.0001
		Leaf removal x Date	6.0	45.00	2.35	0.0461
		Feb. 13	3.0	45.00	1.07	0.3730
		Mar. 13	3.0	45.00	0.51	0.6743
		Apr. 11	3.0	45.00	3.02	0.0394
1	3-row	Leaf Removal	3.0	10.11	0.37	0.7755
		Date	2.0	42.00	625.04	<.0001
		Leaf removal x Date	6.0	42.00	1.34	0.2597
2	3-row	Leaf Removal	3.0	15.98	3.04	0.0595
		Date	2.0	48.00	76.27	<.0001
		Leaf removal x Date	6.0	48.00	2.89	0.0174
		Oct. 17	3.0	48.00	3.15	0.0334
		Nov. 7	3.0	48.00	2.41	0.0788
		Nov. 28	3.0	48.00	1.12	0.3504

[a] The main effect was evaluated by fitting a generalized linear mixed model with a binomial distribution. Slicing evaluations were done on leaf removal within each rating date.

Table 1.2. Incidence of cane Botrytis of red raspberry as influenced by leaf removal in the lower canopy at two locations near Camarillo, CA.

			Cane Botrytis Incidence (%)[a]		
Ranch	*Tunnel*	Treatment	Feb. 13	Mar. 13	Apr. 11
1	2-row	Control	4.6	15.2	76.7 a
		Blade	4.8	12.2	63.9 ab[b]
		Manual	6.3	11.6	66.9 ab
		Twine	8.7	14.0	63.7 b
1	3-row	Control	7.8	14.0	71.8
		Blade	9.9	16.1	73.4
		Manual	12.8	19.1	68.1
		Twine	13.8	23.1	71.1
			Oct. 17	Nov. 7	Nov. 28
2	3-row	Control	1.6	5.0	20.8
		Blade	1.6	4.8	14.4
		Manual	5.5	10.8	18.0
		Twine	4.4	7.6	28.2

[a] Percent incidence was calculated within the observational unit in the center of each plot by dividing the number of canes with symptoms of cane Botrytis on each rating date by the total number of canes determined at the first rating date.

[b] Within each experiment (ranch and tunnel type) and rating date, means followed by the same letter are not significantly different according to a least significant difference ($P \leq 0.05$) test with Tukey's adjustment for multiple comparisons. Experiments and rating dates lack letters if no effect of leaf removal was detected by slicing.

Table 1.3. Fixed effects analysis of cane Botrytis severity as influenced by leaf removal in the lower canopy and rating date at two locations near Camarillo, CA.

		Type III Tests of fixed effects				
Ranch	*Tunnel*	*Effect*[a]	*Num DF*	*Den DF*	*F Value*	*Pr > F*
1	2-row	Leaf Removal	3.0	395.0	2.49	0.0293
		Date	2.0	395.0	38.17	<.0001
		Leaf removal x Date	6.0	395.0	0.14	0.9757
1	3-row	Leaf Removal	3.0	435.2	11.75	<.0001
		Date	2.0	453.4	36.21	<.0001
		Leaf removal x Date	6.0	453.4	0.68	0.6790
2	3-row	Leaf Removal	3.0	244.6	4.62	0.0045
		Date	2.0	245.4	17.66	<.0001
		Leaf removal x Date	6.0	244.3	1.51	0.1628

[a] The main effect was evaluated by fitting a generalized linear mixed model with a log normal distribution.

Table 1.4. Severity of cane Botrytis of raspberry as influenced by leaf removal in the lower canopy at two ranches near Camarillo, CA.

Total Lesion Length (cm)[a]			
	Ranch 1	*Ranch 1*	*Ranch 2*
Leaf Removal	*2-row*	*3-row*	*3-row*
Control	7.91 ab	23.64 a	3.94 b
Blade	8.18 a[b]	9.93 b	6.86 ab
Manual	6.10 b	6.96 b	9.87 a
Twine	7.71 ab	7.04 b	11.10 a

[a] Severity was determined by measuring the length (cm) of each lesion on 10 arbitrarily-selected canes in the observational unit in the center of each plot, and summing the length of all lesions for each cane. The average lesion length over all dates is reported.

[b] Within each tunnel type and leaf removal treatment, means followed by the same letter are not significantly different according to a least significant difference ($P \leq 0.05$) test with Tukey's adjustment for multiple comparisons.

Table 1.5. Number of raspberry receptacles as influenced by leaf removal in the lower canopy at ranch 1 and ranch 2.

		Receptacles per Cane			
Ranch	*Tunnel*[c]	*Num DF*	*Den DF*	*F Value*	*Pr > F*
1[a]	2-row	3.0	372.0	0.52	0.6662
1[a]	3-row	3.0	352.0	0.23	0.8732
2[b]	3-row	3.0	192.0	1.52	0.2100

[a] The number of receptacles on 20 arbitrarily-selected canes per observational unit in the center of each plot was counted on May 29 and 30.

[b] The number of receptacles on 10 arbitrarily-selected canes per observational unit in the center of each plot was counted on December 19 and 20.

[c] The main effect was evaluated by fitting a generalized linear mixed model with binomial distribution and subjecting the model to test with Tukey's adjustment for multiple comparisons.

Figure 1.1. Primocane node with cane Botrytis lesion showing concentric rings inside dark brown watermark border (left). Cane Botrytis lesion growth from broken petiole and gray *B. cinerea* sporulation on base of petiole (middle). Black sclerotia on 'bleached' cane Botrytis lesions growing on primocane.

Figure 1.2. Twine leaf removal treatment on the lower canopy of the primocanes. The red circles show the tissue injury around the nodes caused by using the twine for leaf removal.


Figure 1.3. Manual leaf removal treatment on the lower canopy of the primocanes.

Figure 1.4. Blade leaf removal treatment on the lower canopy of the primocanes showing attached petioles (top) and control treatment with no leaf removal (bottom).

Figure 1.5. Twine removed treatment in the lower raspberry canopy. Canes facing toward the furrow are removed by the side scraping using the black trellising twine. The leaves on the inside of the cane facing the drip tape are manually removed due to not being accessible to the twine.

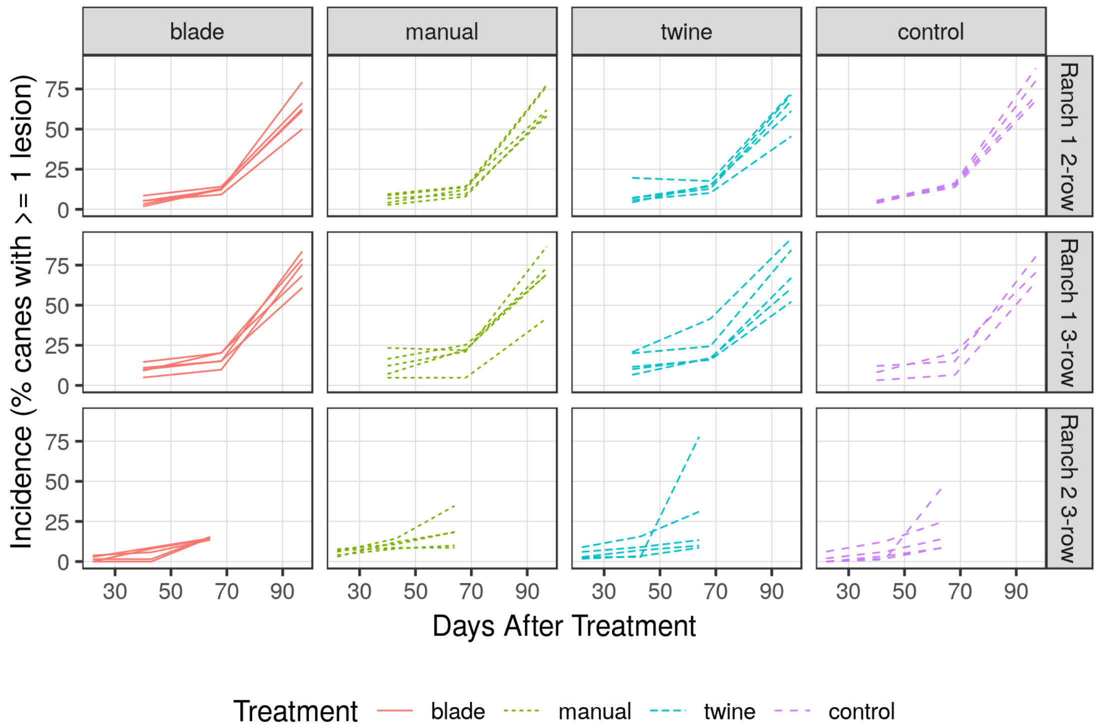


Figure 1.6. Incidence of cane Botrytis of raspberry as influenced by leaf removal in the lower canopy at ranch 1 and ranch 2. Lines represent raw data from individual plots with leaf treatments blade (solid red), control (dotted green), manual (dotted blue), and twine (dotted purple).

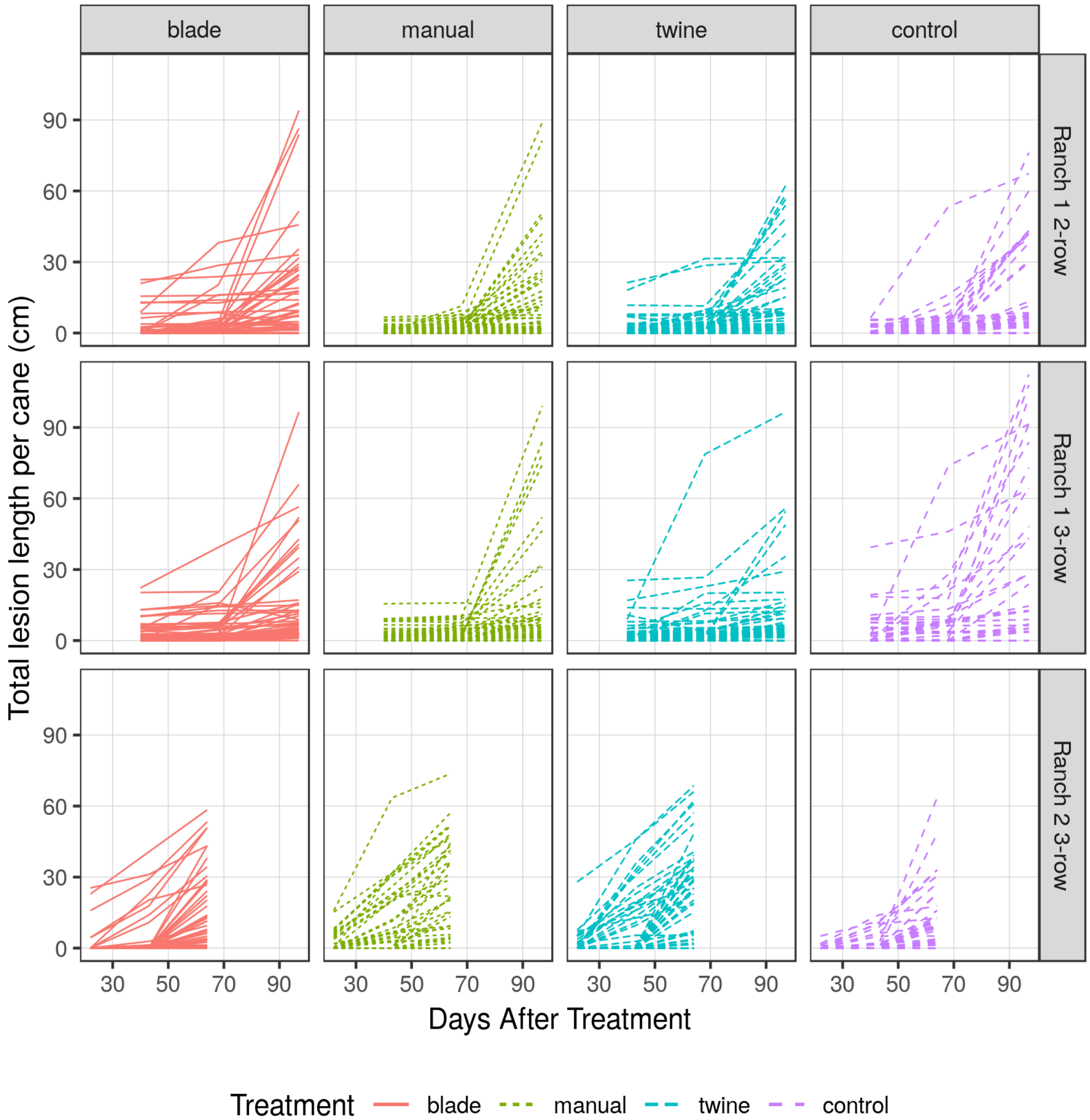


Figure 1.7. Severity of cane Botrytis of raspberry as influenced by leaf removal in the lower canopy at ranch 1(A-2-row and B-3-row) and ranch 2 (C-3-row). Lines represent total lesion length from individual lesions per cane with leaf treatments blade (solid red), control (dotted green), manual (dotted blue), and twine (dotted purple).

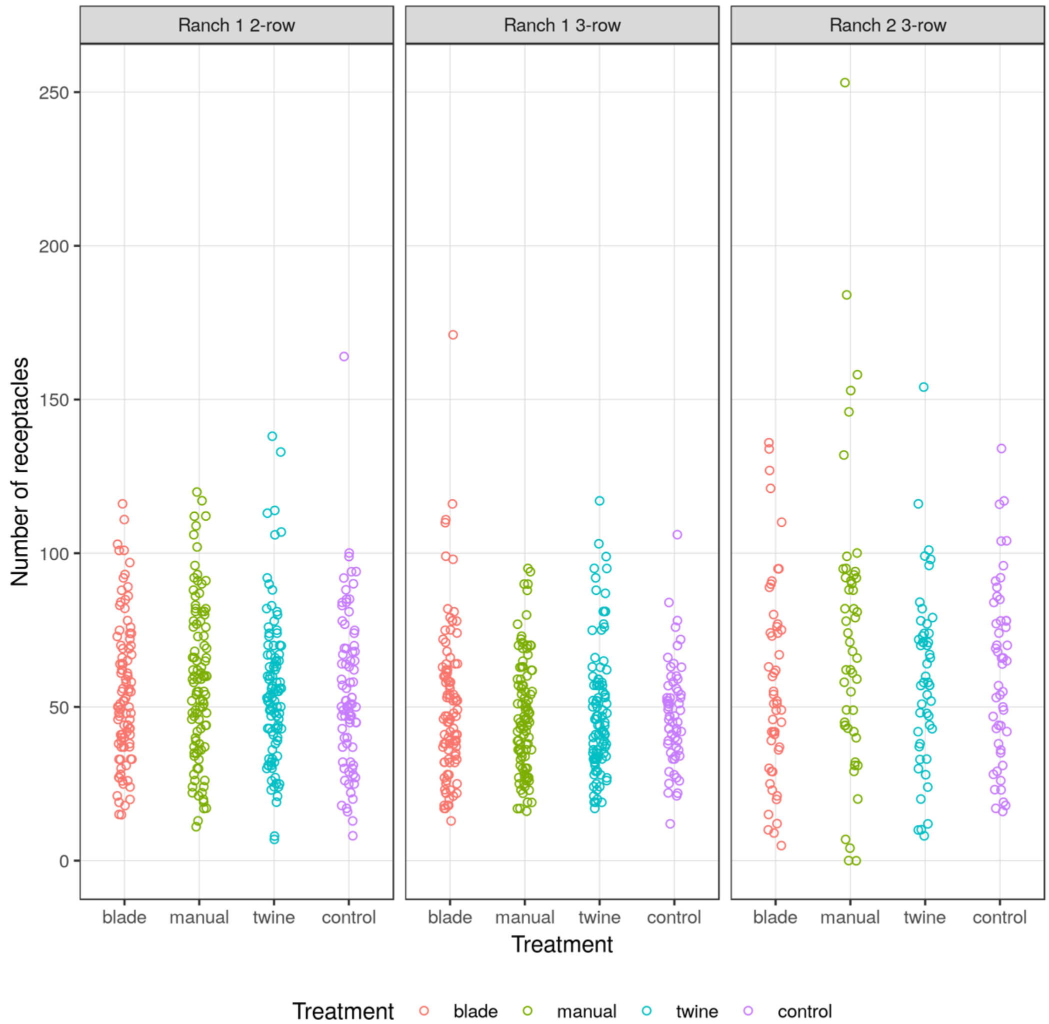


Figure 1.8. Number of receptacles per cane across all leaf removal treatments for ranch 1 (2-row and 3-row) and ranch 2 (3-row). Twenty canes were counted for ranch 1 and ten canes were counted for ranch 2. Open circles represent total number of receptacles from a single cane.

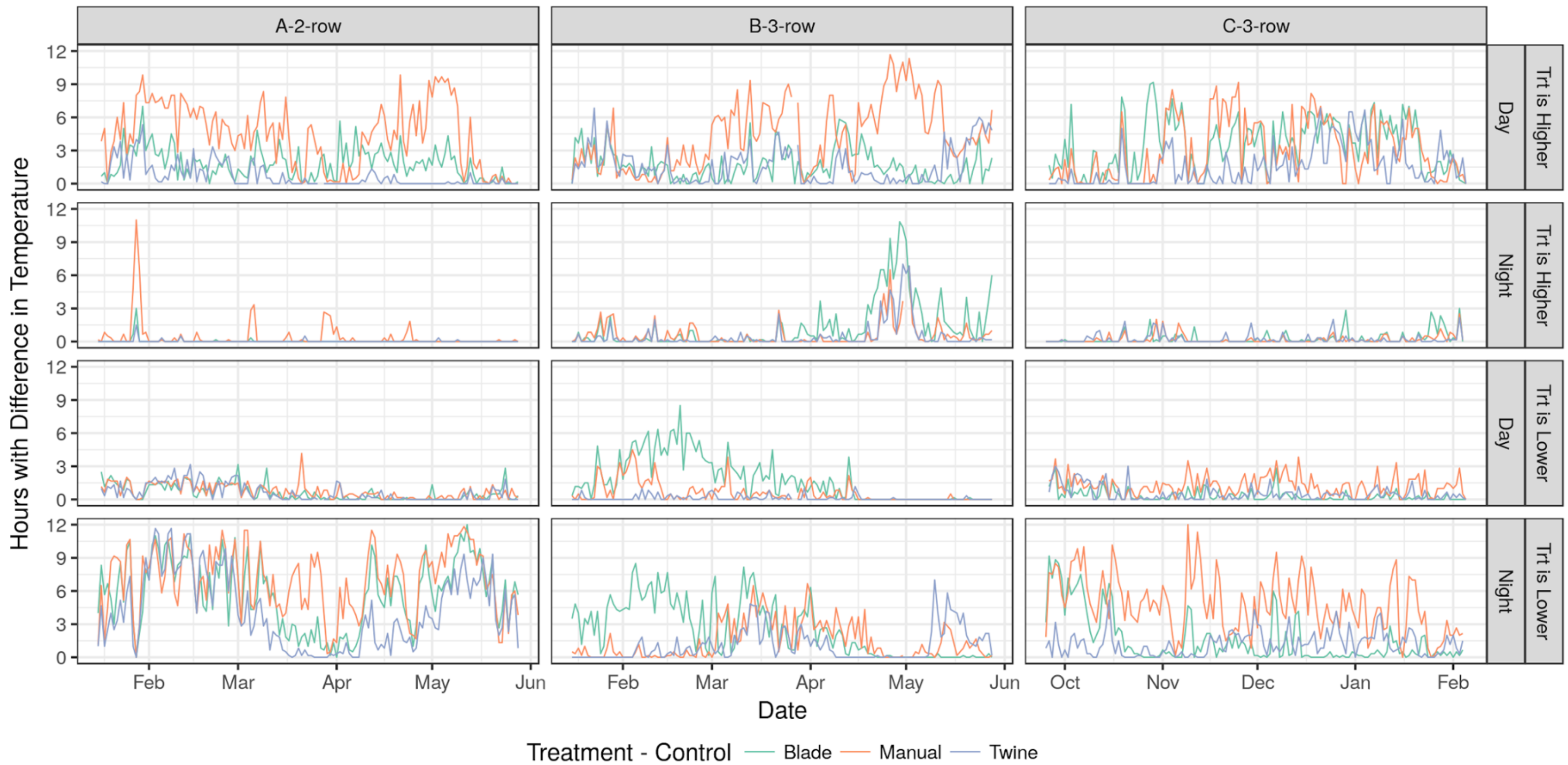


Figure 1.9. Time per day that temperature was different between the three treatments and the control. The 'change from control' was calculated for each 10 min time point by subtracting the value in a control plot from the value in each of the three treatment plots within the same block. The mean and 95% confidence interval of the change from control among blocks was calculated for each treatment-control pair at each time point. A treatment was determined to be different from the control if the confidence interval of the mean change from control did not include 0. Time per day was graphed separately based on direction of difference (higher or lower) and day (0600 to 1800 hours) or night (1800 to 0600 hours).

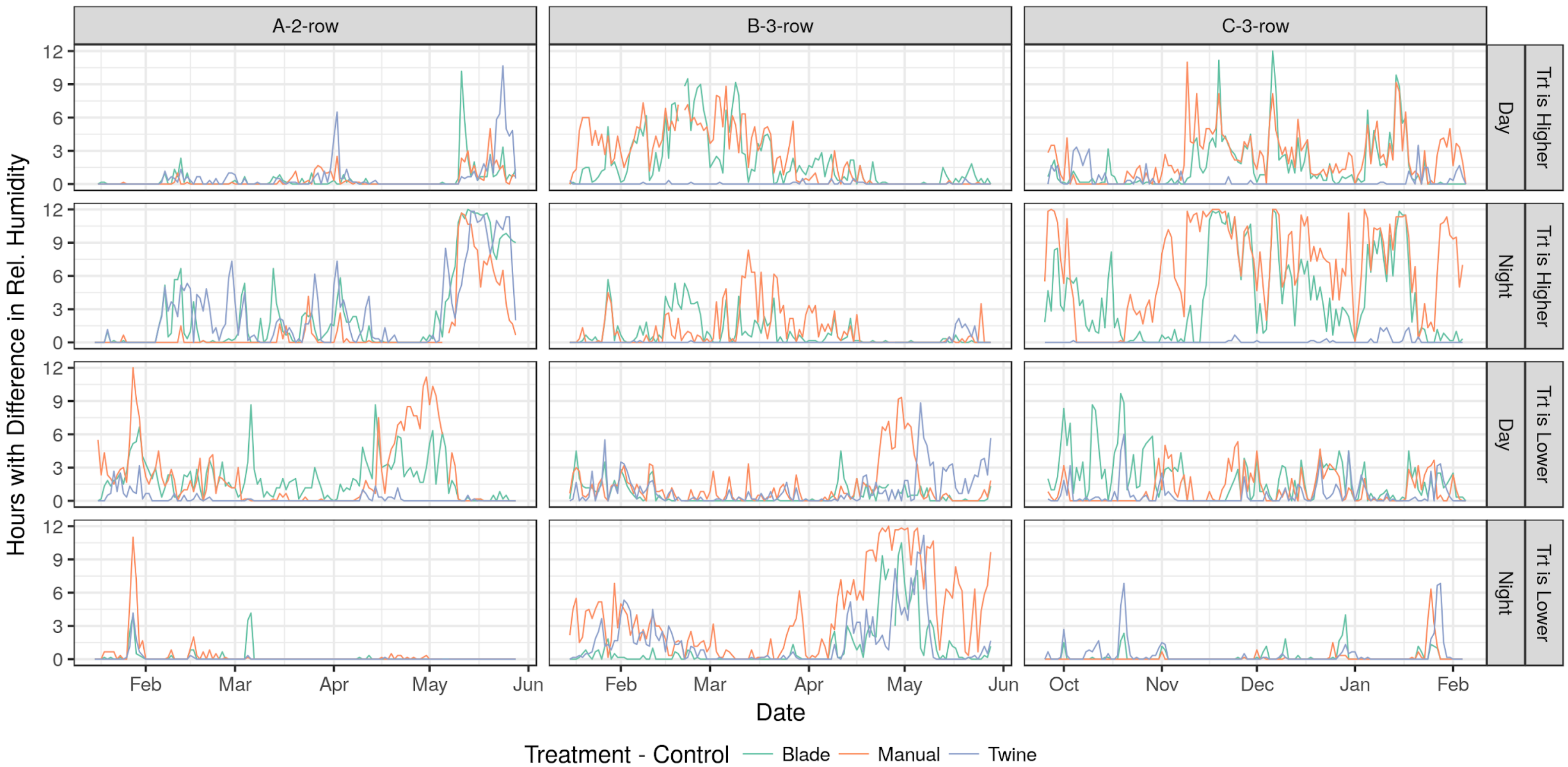
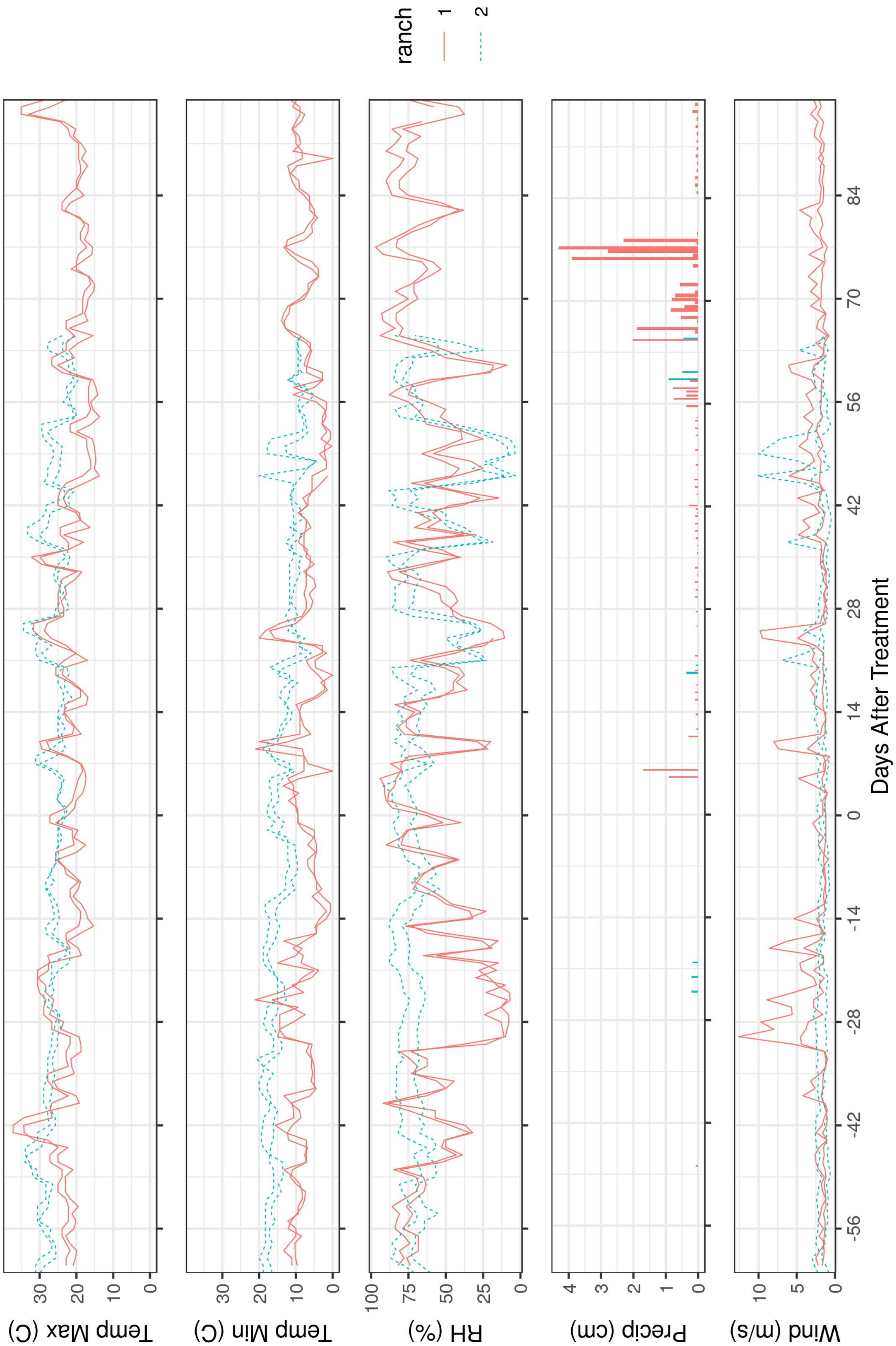


Figure 1.10. Time per day that relative humidity was different between the three treatments and the control. The 'change from control' was calculated for each 10 min time point by subtracting the value in a control plot from the value in each of the three treatment plots within the same block. The mean and 95% confidence interval of the change from control among blocks was calculated for each treatment-control pair at each time point. A treatment was determined to be different from the control if the confidence interval of the mean change from control did not include 0. Time per day was graphed separately based on direction of difference (higher or lower) and day (0600 to 1800 hours) or night (1800 to 0600 hours).



Temp Max (C)
Temp Min (C)
RH (%)
Precip (cm)
Wind (m/s)
Days After Treatment
ranch
1
2

Figure 1.11. Weather data before and during the experiments. Data for each ranch period was converted to days after treatment and overlaid. For each ranch, each line represents one of two weather stations in Camarillo, CA located 7.5 miles or less from each study site.

Chapter 2.

Evaluation of Cultivar Resistance and Grafting for Management of Southern Blight in Processing Tomatoes

ABSTRACT

Southern blight is a disease of processing tomato caused by the soilborne fungus *Athelia rolfsii*, but options for managing this disease in California are limited. The objectives of this study were to: (i) evaluate susceptibility of commercial processing tomato cultivars to southern blight; and (ii) evaluate grafting and increased height of the graft union with the resistant rootstock Maxifort for southern blight management in processing tomato. For objective (i) the susceptibility of 20 commercial processing tomato cultivars (two years) and six processing tomato breeding lines from Texas A&M (one year only) to *A. rolfsii* was evaluated in greenhouse experiments in pots inoculated with 10 *A. rolfsii* sclerotia per 100 cm^3 soil. For objective (ii) we evaluated two cultivars (Heinz 5608 or Heinz 8504), three graft treatments (grafted to Maxifort rootstock with standard scion height, grafted to Maxifort rootstock at a tall height, and non-grafted) in a field studies with natural inoculum or in inoculated greenhouse experiments. Greenhouse experiments found a range in susceptibility of tested cultivars. One cultivar in 2018 (H 4707) and three in 2019 (HMX 1892, 5737 M, and 5876 M) did not develop any symptoms. Although several commercial cultivars performed similarly to the resistant breeding lines, there were few differences among cultivars in both years, and relative differences among some cultivars varied between years. Disease severity was low in both greenhouse grafted experiments in 2018 and 2019 and no consistent trends were

observed. In the field experiments, mean incidence in the non-grafted plots was approximately 7.5 and 11.5 times higher in 2018 and 2019, respectively, regardless of the height of the graft union. Based on our studies, the approach of grafting for management of southern blight may not be the best application. The use of resistant cultivars is a better and accessible approach for California processing tomato growers.

INTRODUCTION

California agriculture is in a time of opportunity to adopt sustainable practices for the management of challenging issues such as soilborne disease. In the previous decades, soilborne diseases were commonly managed with the use of chemical fumigation, but the widening restrictions on the use of fumigants in the San Joaquin Valley of California has posed a challenge for growers. One of the affected crops in California is processing tomato (*Solanum lycopersicum* L.), which averaged 1.1 billion dollars in value from 2013 to 2017, and accounted for 93% of the production in the United States in 2017 (California Agricultural Statistics Review 2017-2018). Despite the total value of processing tomato statewide, growers face challenges due to the low market return on a per area basis. Southern blight is a disease of processing tomato that has long been an economic concern in the San Joaquin Valley, and recently caused a widespread epidemic in both the San Joaquin and Sacramento Valleys (Swett and Nunez 2017). The threat of southern blight has caused reductions in acres planted with processing tomato in the southern San Joaquin Valley (J. Nunez, personal communication).

Southern blight is caused by the soilborne fungus *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. (anamorph *Sclerotium rolfsii*) that has a host range of over 500 different plant species (Punja and Rahe 1992). The fungus produces a white, typically fan-shaped mycelial mat and distinctive tan to reddish brown sclerotia with walls are composed of chitin and laminarin (Roberts et. al 2014, Punja and Rahe 1992). The sclerotia survive and germinate at soil depths of 0 to 8 cm and are commonly dispersed by the movement of infested soil or plant material (Punja 1985, Roberts et al. 2014). Initial infections in the field most commonly occur on plant tissues that are in contact with the soil surface where sclerotia are stimulated to germinate by drying and remoistening (Roberts et al. 2014, Abeygunawardena et al. 1957). Following germination from sclerotia, mycelia of *A. rolfsii* colonizes aboveground plant tissue and releases cell wall degrading enzymes. The enzymes disintegrate host tissues and when colonizing stem tissue form a lesion around the stem near the soil line that advances rapidly to the point of girdling the stem (Punja 1985, McCarter 1991, Roberts et al 2014). The pathogen is most damaging when it infects stems or crowns, in which it causes wilting, cankers, rot, or whole-plant necrosis on various crops (Ristaino et al. 1994). In processing tomato, the most common symptom of southern blight is rapid wilt of vegetation above the ground. The temperature range for mycelial growth is from 8 to 40ºC, and the optimal temperatures for sclerotia formation is from 27 to 30ºC (Punja 1985). Overall, temperatures 25 to 35ºC are most conducive to disease development (Roberts et al 2014). Sclerotia form on mycelial mats that are 5 to 6 days old (Roberts et al. 2014). When mature, sclerotia can persist in the soil for many years (Punja 1985; Xu et al. 2008). Additionally, *A. rolfsii* can persist in the soil saprophytically as mycelium on plant debris (Punja 1985, Roberts et al. 2014, Jenkins and Averre 1986). If management practices are not adopted, within a season or two a single infected plant can produce thousands of sclerotia, potentially resulting in serious yield loss due to southern Blight (Swett and Nunez 2017).

There are several methods to reduce losses caused by *A. rolfsii,* but their uses are limited in processing tomato production in California. These include rotation with non-host crops, minimizing soil moisture on the soil surface, and deep plowing to bury the sclerotia. Rotating with crops that are non-hosts including corn, barley, wheat, and small grains has been shown to reduce sclerotia density in subsequent years (Punja 1985, Roberts et al. 2014). However, these rotation crops are not economically viable as regular rotation partners in the San Joaquin Valley of California, and few alternatives are available due to the wide host range of the pathogen. Although reducing soil moisture has been shown to reduce southern blight (Smith 1972; Swett and Nunez 2017), the use of subsurface drip irrigation has already become the standard practice in California. The drip lines are buried at a depth of 25.4 cm to 30.48 cm (10 to 12 inches) for weed management, water efficiency, and to reduce *A. rolfsii* inoculum accumulating in the furrow (Sutton et al. 2006, Davis et al. 2013). *Athelia rolfsii* sclerotia cannot survive long periods under anaerobic conditions, thus deep plowing of infected plant tissue and sclerotia to at least 20 cm depth has shown to reduce inoculum (Roberts et al. 2014, Punja et al. 1986, Gurkin and Jenkins 1985). However, this approach is not feasible in California production systems because the drip lines remain buried for two to three consecutive crop seasons and could be subject to damage from deep plowing. Taken together, agronomic methods have little potential to further improve the management of southern blight.

Fumigation with metam sodium or metam potassium was traditionally relied upon to effectively manage *A. rolfsii* in processing tomato in the San Joaquin Valley. For effective control the product must be applied through sprinklers, however, sprinkler application is restricted. The alternative method to sprinkler application is shanking the product into the soil, but this approach does not allow for effective dispersion of metam sodium into the soil, therefore it is not effective (Swett and Nunez 2017). There are effective fungicides such as flutolanil, penthiopyrad, and tebuconazole available to manage southern blight in vegetable crops (Roberts et al. 2014, Swett and Nunez 2017). However, processing tomato cultivars are determinant, not trellised, and the canopy is often full late in the season when the pathogen is most active. These characteristics prevent chemical application to the vulnerable stem tissue at or above the soil line and is the main reason why chemical management of southern blight has proven ineffective in processing tomato (Swett and Nunez 2017). The processing tomato industry in the San Joaquin Valley of California would benefit from having new efficient and sustainable approaches to manage southern blight.

Host plant resistance is the most sustainable option in managing soilborne disease (Gullino et al. 2003), but like other crops it is believed there is little resistance to southern blight within commercial processing tomato cultivars. However, some resistance is available in the tomato germplasm. The Texas A&M breeding program released several breeding lines that have shown superior resistance to southern blight under field conditions (Leeper et al. 1992). The mechanism of resistance is associated with the development of secondary tissue on the basal mainstem called the phellem barrier

(Leeper et al. 1992). The six Texas A&M selections 5635M, 5707M, 5719M, 5737M, 5876M, and 5913M were screened for two years in fields infested with *A. rolfsii* and showed resistance commensurate to a resistant wild accession PI 126432 (Leeper et al. 1992). Additionally, the six selections showed field resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W. C. Snyder & H.N. Hansen race 1 including good average plant yields for 5719M and 5876M (Leeper et al. 1992). The relative susceptibility of commonly grown commercial processing tomato cultivars to southern blight is unknown but would be beneficial for disease management.

Grafting is another option for management of soilborne diseases. Disease control by grafting has already shown to be a beneficial alternative to the soil fumigant methyl bromide in Asia and much of Europe (King et al. 2008). Grafting is a fusion of two plant segments, the shoot of the plant with desired fruit quality called the ‘scion’ and the root system with desired root traits as the ‘rootstock,’ that functions as a single plant (Goldschmidt 2014, Mudge et al. 2009). Grafting is commonly used for perennial crops and has since the early 20th century become a technique for vegetable production in *Cucurbitae* and *Solanaceae* species (Goldschmidt 2014; Mudge et al. 2009). Grafting to a resistant rootstock has previously been shown to reduce diseases causes by soilborne pathogens and has potential to be a sustainable alternative to fumigants for the control of many soilborne diseases (Ioannou 2001, Cohen et al. 2002, Louws et al. 2010, King et al. 2008; Rivard and Louws 2008). In tomatoes, grafting has been used to augment growth under low potassium environments (Schwarz et al 2013), improve tomato resistance to root-knot-nematodes (Rivard et al. 2010), and increase tolerance to drought (Cantero-

Navarro et al. 2016). The main mechanism of disease control by grafted plants is speculated to be by avoidance by having the resistant rootstock come into contact with the pathogen instead of the susceptible scion tissues (King et al. 2008). Maxifort is an interspecific hybrid of tomato and a wild *Solanum* species (*S. lycopersicum* × *S. habrochaites*) developed as a rootstock for greenhouse tomato (Higashide et al. 2014). In a study in the southeastern United States, heirloom tomato grafted to the rootstock-specific Maxifort exhibited 0 to 5% southern blight incidence whereas incidence in non-grafted plants was 27 to 79% (Rivard et al. 2010). To our knowledge grafting processing tomatoes to a southern blight-resistant rootstock has not been explored in processing tomatoes in the San Joaquin Valley.

Although rootstocks like Maxifort are highly resistant, some plants often develop southern blight symptoms (Rivard et al. 2010). In our preliminary work, we observed that the graft union was planted below the soil line, possibly rendering the susceptible scion vulnerable to infection by *A. rolfsii* in the field. We hypothesized that raising the height of the graft union would reduce southern blight incidence. To our knowledge, raising the height of the graft union in other crops has yet to be evaluated in processing tomato.

The use of resistant rootstock for an annual crop has been studied in fresh-market and heirloom tomatoes for improvement on yield but has yet to be explored for disease resistance for processing tomato in California. The objectives of this study were to: (i) evaluate susceptibility of commercial processing tomato cultivars to southern blight; and (ii) evaluate grafting and increased height of the graft union with the resistant rootstock Maxifort for southern blight management in processing tomato.

MATERIALS AND METHODS

Inoculation. *Athelia rolfsii* sclerotia were produced in culture media using the oat seed method (Punja and Rahe, 1992). The three *Athelia rolfsii* isolates used were each obtained from a different processing tomato field in Kern County, California in 2017. Briefly, for each trial the isolates were grown from infested filter paper maintained at -80ºC, hyphal tipped from mycelium actively growing on potato dextrose agar, and incubated at 25ºC under continuous light for approximately six days. Two plugs from the edge of the purified colonies were inoculated into Erlenmeyer flasks containing oat seeds and 1% water agar that had been autoclaved twice for 60 min on a 24 hr interval. Flasks were then incubated at room temperature for approximately 33 days. The sclerotia grown on oats were moved into sterile 5.7 L plastic containers placed in a biosafety cabinet to dry for approximately 14 days, and sclerotia were separated from oats by pressing the dried oat-sclerotia mixture with a gloved hand over a 2.0 mm and 850 μm sieves. Sclerotia were stored at room temperature in a plastic Ziploc bag until experiment set up. Viability of the inoculum was evaluated by germinating surface disinfested sclerotia on water agar.

Cultivar greenhouse experiment. The susceptibility of 19 commercial processing tomato cultivars to *A. rolfsii* was evaluated in a greenhouse study in 2018 (Table 2.1). In 2019, 19 commercial cultivars and six processing tomato breeding lines from Texas A&M were evaluated. The commercial cultivars were chosen based on highest total yield in California counties affected by southern blight. Treatments consisted of the 19 of these commercial cultivars grown in inoculated soil and a selection of 6 of the 19 commercial cultivars grown in non-inoculated soil as negative controls. The 2018 trial included two hybrid tomato cultivars grown in inoculated soil as positive (resistant) controls, but were not included in 2019 due to poor germination. The rate of inoculum was 10 sclerotia per 100 cm^3 soil based on recommendations by Punja and Rahe (1992). The plants were started from seed using an organic seed starter soil mix (EB Stone Organics, San Jose, California) in a tray with 200 22 mL, 2.22 cm x 2.22 cm cells. Two seeds were planted per cell. The trays were placed on a clear plastic-lined chamber in the greenhouse on a warming mat set at 24ºC and misted three times per day for 15 seconds. Emergence began five days post seeding. Eleven days post seeding the trays were moved to an open misting bench where the plants could receive more sunlight, thinned to one plant per cell using sterile metal scissors, and sprinkled with one tablespoon of granular Osmocote Flower and Vegetable fertilizer 14-14-14 (The Scotts Company, Marysville, Ohio) per 90 cells on the trays. The Osmocote rate used for germination was recommended by colleagues with tomato germination experience. Three weeks post seeding the plants were transplanted into trays with 36 166 mL, 5.72 cm x 5.08 cm cells to allow for advanced root development. In these larger trays, the soil substrate used was UC Soil Mix III, composed of 50:50 plaster sand:peat moss that was pasteurized at 100ºC for two hours. Inoculation and transplanting occurred five weeks post seeding. The day before inoculation, sclerotia were surface disinfested using 0.5% sodium hypochlorite solution for 1 minute, subsequently rinsed twice in sterile deionized water, and dried with sterile paper towels. The number of sclerotia needed to total 1 g was determined by manually counting, the amount to be added was weighed and incorporated to the top 10.2 cm (4 inch) of soil in 15.2 cm (6 inch) diameter pots to reach the target sclerotia count per 100 cm^3 soil. Plants were then transplanted into 15.2 cm (6 in), 2.7 L pots with UC Soil Mix III at one plant per pot.

Plants were grown in a greenhouse with the temperature set at 33ºC. Temperature data loggers were installed, but in 2018 they malfunctioned. In 2018, the plants were arranged in a randomized complete block design with seven replications across four benches and with six replications across three benches in 2019 oriented east-west, one or two blocks per bench. Blocking was designed to capture potential confounding factors of light, temperature, and watering differences across the different benches. All benches had their own irrigation sub-line connected to a main line. A drip system was installed 15 days post transplanting with one JAIN Twist Weight emitter (Jain Irrigation, Inc., Jalgaon, India) per pot and was set to water daily for 2 minutes early in the morning. Each plant was fertilized once per week for 3 weeks with 15 mL of a solution containing Jack's Classic Professional Water Soluble Plant Food 20-20-20 (JR Peters Inc., Allentown, PA) at the recommended rate of 1 tablespoon per gallon of water. The volume of fertilizer was chosen based on observation of adding a volume of liquid that would not leach through the openings of the pots. Four days after the drip system was installed approximately one tablespoon of granular Osmocote Flower and Vegetable fertilizer 14-14-14 was added around the drip emitter of each pot. The same growing methods from 2018 were used for 2019 with adjustments in using only granular Osmocote Flower and Vegetable fertilizer 14-14-14. The plants were maintained in the greenhouse for 126 days in 2018 and the plants from 2019 were maintained in the greenhouse for 107 days.

In 2018, because little disease development was observed in blocks 5 and 6, additional inoculum was added to the inoculated pots in these blocks 77 days after initial inoculum was added. Inoculum for each individual pot was calculated by multiplying the volume of the top four inches of the pots (1853 cm^3) by the rate of inoculum (10 sclerotia per 100 cm^3 soil), then divided by the average number of sclerotia (1484 sclerotia) from one gram of sclerotia. The sclerotia from all three isolates were evenly mixed. The mixed sclerotia were then weighed to 0.12 g for each individual pot, inoculum per pot were placed in a ziplock bag, then one bag of inoculum was carefully poured around the previously inoculated tomato stem.

Plant material for grafting experiments. The processing tomato cultivars used as scions or non-grafted controls in grafting experiments were Heinz 5608 and Heinz 8504, which are commonly grown in the San Joaquin Valley of California. The hybrid cultivar Maxifort (De Ruiter Seeds, Bergschenhoek, The Netherlands) served as the rootstock in grafted treatments. For all greenhouse and field grafting experiments, transplants and grafting were produced by Growers Transplanting Inc. in Salinas, CA using the tube grafting technique (Rivard and Louws, 2006) with the modification of using a clip that applies minimal pressure on the graft union. Grafting for the high-union grafted treatment consisted of plants with a union approximately 2.54 cm (1 inch) above the standard graft. These high-union grafted plants were produced by stretching the rootstock 2.54 cm to 5.08 cm before the grafting process, applying extra fertilizer to the rootstock, and cutting the rootstock approximately 6.35 cm to 7.62 cm from the plug (Juan Pablo Caballero, *personal communication from Growers Transplanting Inc*.).

Grafting greenhouse experiments. In 2017 a preliminary study was conducted that evaluated two cultivars (Heinz 5608 or Heinz 8504), two graft treatments (grafted to Maxifort rootstock or non-grafted control), and four inoculum levels (0, 5, 10, or 20 *A. rolfsii* sclerotia per 100 cm^3 of soil) in a full factorial treatment arrangement. On June 5, 2017 a single plant was transplanted into each 2733 mL pot with UC Soil Mix III that was inoculated as described above for the cultivar trial and grown in a greenhouse at 32ºC. One-plant pots were arranged in a randomized complete block design with 8 replications across two benches oriented east-west, four blocks per bench. The pots were watered via a drip system beginning 24 days post planting. The plants were fertilized every 2 to 3 weeks with 100 mL of Jack's Classic Professional Water Soluble Plant Food 20-20-20.

The treatment structure was modified based for 2018 and 2019 to include a grafted treatment with a high-union, referred to as 'tall'. These studies evaluated two cultivars (Heinz 5608 or Heinz 8504), three graft treatments (grafted to Maxifort rootstock with standard scion height, grafted to Maxifort rootstock at a tall height, and non-grafted), and two inoculum levels (0 and 10 *A. rolfsii* sclerotia per 100 cm^3 of soil) in a full factorial arrangement (Figure 2.1). Plants were transplanted into one-plant pots on July 18 in 2018 and April 29 in 2019. Plants were arranged in a randomized complete block design with 6 and 8 replications in 2018 and 2019, respectively, on benches oriented east-west with two or three blocks per bench. The 2018 experiment was conducted in a greenhouse set to 21ºC for 120 days, which was increased to 26ºC for 34 days due to low disease pressure. The 2019 experiment was conducted in a greenhouse set to 35ºC for 83 days. Hobo MX2301 data loggers (Onset Computer Corporation, Bourne, MA, USA) monitored temperature in the greenhouse and reported the average temperature maximum 38ºC and minimum 15ºC in 2018. In 2018, four days post planting drip irrigation was used to water daily with fertilized water for 21 days before switching to industrial water. After adjusting the drip system, the plants were fertilized once every week then adjusted to fertilizing twice a week with 100 mL solution of Jack's Classic Professional Water Soluble Plant food. In 2019, approximately one tablespoon of granular Osmocote Flower and Vegetable fertilizer 14-14-14 was added around the drip emitter of each pot. In 2019 additional inoculum was added to the inoculated pots 64 days after transplant to encourage disease development. The same procedure executed for the 2018 cultivar experiment was used to calculate and re-inoculate the pots.

Field grafting experiment. Field experiments were performed in a commercially-owned field south of Bakersfield, Kern County, CA. The field has historically been under consistent tomato production and typically experiences southern blight. The soil was a sandy clay loam with a pH of 6.37 and 2.19% organic matter. In 2017, the preliminary field experiment evaluated two cultivars (Heinz 5608 or Heinz 8504) under two graft treatments (grafted to Maxifort rootstock or non-grafted control) that were mechanically transplanted on May 15, 2017. Plots were 165 m long with 30.4 cm plant spacing and were arranged in a randomized complete block with 7 replications. Plants were irrigated with a buried drip system at a depth of 26 cm.

The field experiment in 2018 and 2019 evaluated the same treatment structure as the 2018 and 2019 greenhouse experiments. The field experiments consisted of treatments arranged in a randomized complete block design with 6 replications with plots that measured 34 m long in 2018 and 30.5 m long in 2019. In both the 2018 and 2019 field trials, the plants were mechanically transplanted at a spacing of 60.9 cm in single-row beds. Transplants were established with a towed water tank (2018) or sprinklers (2019), then irrigation was switched to drip. Although not located within an active production field, the experiments were maintained by the commercial grower using standard practices for processing tomato in the southern San Joaquin Valley.

Data Collection. In both the cultivar and grafting greenhouse experiments, Southern blight severity was rated using the following 0 to 7 ordinal rating scale: 0 = no disease symptoms; 1 = chlorosis of the older leaflets; 2 = wilting of the older chlorotic leaflets; 3 = wilting of the older leaves with a wilted (drooping) apex; 4 = necrotic older leaflets with a wilted apex, and apex leaflets showing chlorosis; 5 = all leaflets are dry; 6 = all leaflets are wilted and dry with a chlorotic stem; and 7 = a dead plant that is completely wilted and dry (Figure 2.2). Data was collected weekly after southern blight symptoms began to develop for the greenhouse cultivar experiments in 2018 and 2019. For the grafting greenhouse experiments, disease severity was rated every 2 weeks after southern blight symptoms began to develop for the 2017 and weekly for the greenhouse graft study in 2018 and 2019.

For the field trials of objective (ii), in 2017 data was collected weekly beginning six weeks after planting. Strike counts, defined as plants observable as infected or not infected, were collected from four 15.2 m sections per plot in the 2017 field trial. In the 2018 and 2019 trials, data collection began two and five weeks after transplanting after transplanting, respectively, and approximately every one to two weeks thereafter. In these trials, the status of each plant was individually recorded on each rating date. Plants that were wilting, collapsed, and lime-colored were rated as exhibiting southern blight symptoms (Figure 2.3). Other diseases were also observed in these trials. Plants with crisp leaves that roll or curl upwards with or without appearing stunted were rated as symptomatic of curly top, and plants with crinkled leaves having interveinal yellowing and typically with stunted growth were rated as symptomatic of unknown virus(es). Plants completely brown and dry were rated as dead. When a dead plant was observed, it was marked with a flag to ensure it would be counted on subsequent rating dates.

Yield data was collected from 165 m long plots from the 2017 field trial on September 18, 2017. Yield data was not collected in 2018 due to quick collapse of plants ending in poor fruit quality for harvest. Yield data was not collected in 2019.

Data Analysis. For the 2018 and 2019 cultivar and grafting trials in the greenhouse, the influence of experimental factors on southern blight severity was analyzed with generalized linear mixed models with PROC GLIMMIX in SAS 9.4 using the multinomial distribution and the cumulative logit link function. The cultivar trial was analyzed as a nested model, with inoculum as a main effect and inoculum × cultivar as an interaction effect, because only a small set of the cultivars were evaluated in non-inoculated control plots. The grafting trial was analyzed as a factorial. For both trials, rating date was included separately as an additional main effect and not included as an interaction to reduce complexity of attempting to model ordinal data. Block was included as a random effect for both trials. When interactions were significant, the effect of cultivar within inoculum and the effect of graft within cultivar were examined with the *slice* statement for the cultivar and grafting trials, respectively. Levels of significant main effects or interactions were separated by obtaining odds ratios for all pairwise comparisons with the *model* statement. Due to the large number of treatments in the cultivar study, odds ratios were summarized with the *lsmeans* statement in PROC PLM with the Tukey-Kramer adjustment for multiple comparisons. In the cultivar trials, the effect of cultivar among inoculated plants was analyzed using a dataset with non-inoculated cultivars removed because odds ratios cannot be determined for interaction terms. Initial analysis of the greenhouse grafting trials did not detect statistical evidence for an effect of inoculum despite a total lack of symptoms in control pots, therefore all non-inoculated pots were removed for analysis. In addition, initial analysis of the 2019 cultivar trial did not find statistical evidence for separation of cultivars despite clear variation in the raw data. Therefore, three cultivars which possessed all 0 ratings on all dates were excluded from analysis.

For the 2018 and 2019 field trials, individual plant status data was first subjected to quality control. In some cases, the same plant was rated with more than one disease over the course of each trial. This was generally due to lack of clarity of the symptoms when they are first observed or a secondary disease affecting plants following the first. Quality control consisted of assigned the true or primary pathogen retroactively to all symptomatic ratings. Then, for dead plants, the cause of death was determined from ratings on previous dates when the plant was symptomatic but alive. Following quality control, ratings were summarized at the plot level. The total number of plants in each plot with a given rating was determined, and southern blight incidence was determined by adding the number of plants exhibiting southern blight symptoms and the number of plants dead due to southern blight. The influence of cultivar, graft, rating date, and all interactions on southern blight incidence in the 2018 and 2019 field trials was analyzed with a generalized linear mixed model in PROC GLIMMIX with the binomial distribution and the logit link function. Block was included as a random effect. The effect of graft within significant cultivar × graft interactions was examined with the *slice* statement. Means of significant main or sliced effects were separated using the least significant difference test with Tukey-Kramer adjustment for multiple comparisons with the *lsmeans* statement.

For the 2017 field trial, yield and strike count data were analyzed with PROC GLIMMIX procedure in SAS v9.4 using the log normal and binomial distributions, respectively.

The 2017 greenhouse experiments were analyzed as relative treatment effects (also known as relative marginal effects) with repeated measures using the *nparLD* package v2.1 (Kimihiro et al., 2012) in R v3.3.2 (R Core Team, 2016). An analysis of variance (ANOVA)-type statistic was used to determine the effect of treatment, and means will be separated using 95% confidence intervals calculated from the *nparLD* package.

RESULTS

Cultivar greenhouse experiment. Disease severity was moderate in 2018 but more severe in the 2019 trial. By the end of the experiments, some inoculated plants from almost all cultivars had died from southern blight, but many plants did not develop any symptoms (Figure 2.4). The raw data showed that cultivars differed primarily in the number that did not develop any symptoms and that most died after exhibiting disease symptoms. One cultivar in 2018 (H 4707) and three in 2019 (HMX 1892, 5737 M, and 5876 M) did not develop any symptoms. No symptoms were observed in any non-inoculated plants. In both trial years, analysis of fixed effects showed that the interaction of cultivar and inoculum had a significant effect on disease severity, and slicing these interactions showed inoculum of 10 sclerotia per 100 cm^3 of soil had an effect on disease severity (Table 2.2).

There were few differences among cultivars in the multiple comparison analyses in both years, and relative differences among cultivars varied between years (Figure 2.4). In 2018, HZ 4707 had the lowest risk of developing disease, but was not different from SUN 6366, HZ 1428, and N 6428. In 2019, risk of both HZ 4707 and HZ 1428 was relatively low but was similar to several commercial cultivars and Texas A&M breeding lines. In contrast, N 6428 had the highest risk in 2019 but was not significantly different from six other cultivars. Cultivar N 6416 had the highest risk in 2018 and relatively high risk in 2019, but was not different from 9 or 12 other cultivars, respectively. Although Maxifort and Multifort were included as positive controls in 2018, their risk of developing southern blight was similar to all but 1 and 4 of the remaining cultivars, respectively. Of the Texas A&M breeding lines that were not excluded from analysis in 2019, 5635M and 5913M exhibited the least risk, but were not significantly different from the two remaining breeding lines (5719M and 5707M) and 8 commercial cultivars.

Grafting greenhouse experiments. The preliminary 2017 study showed under moderate inoculum pressure, disease severity was significantly higher in non-grafted HZ 5608 compared to HZ 5608 grafted to Maxifort, but was similar for H 8504 grafted and non-grafted (Figure 2.5).

Disease severity was low in both 2018 and 2019 experiments. The Type III analysis of fixed effects detected a significant effect of grafting on disease severity in 2018 ($P < 0.0001$) and 2019 ($P = 0.0059$) (Table 2.3). However, odds ratio estimates and confidence intervals of the pairwise comparisons control-standard and standard-tall were not sensical (e.g., either missing or >999.999), and the control-tall comparison suggested that control had significantly greater odds to develop disease in 2018 but significantly lower odds in 2019. HZ 5608 had numerically higher incidence of southern blight compared to HZ 8504 for both replicate trials in inoculated pots across all grafted treatments (Figure 2.6), however a statistical effect of cultivar was not detected in either experiment.

Field grafting experiment. Disease incidence was significantly lower ($P <$ 0.0001 to 0.0122) on four of five rating dates in grafted plots compared to non-grafted in 2017 (Figure 2.7). On the final rating date, southern blight incidence was 52% and 58% lower in grafted compared to non-grafted plots. A significant effect of grafting ($P =$

0.0382) was observed on yield, in which yield was 30.0% higher in grafted plots compared to non-grafted (Figure 2.8).

In 2018 and 2019, disease severity was moderate to high (Figure 2.9). The Type III analysis of fixed effects on the 2018 field trial showed a significant interaction of cultivar and grafting ($P = 0.0143$) on disease incidence, whereas in 2019 only the main effect of grafting was significant ($P < 0.0001$) (Table 2.4). For both cultivars in 2018 and in 2019, disease incidence was significantly lower in grafted plots regardless of height when compared to the non-grafted control. Mean incidence in non-grafted plots was approximately 7.5 and 11.5 times higher in 2018 and 2019, respectively, when averaged over cultivar and height of the graft union. Additionally, for HZ 8504 in 2018, incidence in tall grafted plots was significantly lower than incidence in standard plots (Table 2.5). This numeric trend was also observed for HZ 5608 in 2018 and in 2019, but the difference was not significant.

DISCUSSION

This study presents options for the management of southern blight of processing tomato in California. We found that grafting to resistant rootstocks dramatically reduced southern blight in processing tomato. Our finding agrees with previous literature on the benefit of grafting for management of southern blight and other diseases. In addition, our results suggest that raising the height of the graft union may reduce southern blight incidence. Finally, we observed variation in susceptibility to southern blight among commercial cultivars currently planted in California.

While our findings in processing tomato agree with previous research in fresh market tomato, the utility of grafting to processing tomato production may be lower due to the relative costs and returns between the two systems. Although we did not perform a comprehensive economic analysis of production using grafted transplants, the current cost of F1 hybrid seed and the grafting operation exceeds returns under reasonable price and yield scenarios. Grafting can increase overall plant vigor and yield in the absence of disease or other stress, but studies in California focusing on grafting for increasing yield have found both increases and occasional decreases in yield when grafted to fresh market tomatoes (Grieneisen et al. 2018). Therefore, absent developments that reduce the costs of these inputs, grafting is currently not economically feasible for processing tomato production in California.

In addition to economic challenges with grafting, we encountered cultural issues with grafting in our experiments. Notably, we observed that the grafted and the tall grafted plants can outgrow the scion. The plants in the greenhouse and the field developed shoot growth from the rootstock, particularly in the tall grafted plants, likely due to the higher number of nodes on its rootstock stem compared to the standard grafted plants. This overgrowth was especially prevalent in 2019, in which a month after planting in the field, outgrowths of Maxifort were observed to emerge from the soil in between the grafted transplants. The different procedure used by the nursery to produce the transplants in 2019 compared to 2018 may explain the greater incidence of overgrowing the scion. Thus, while the grafted plants reduce southern blight incidence, overgrown plants develop an undesirable taller vegetative canopy and produce unwanted fruit of the rootstock.

Although statistical and numeric trends suggest that increasing the height of the graft union may reduce southern blight incidence, the magnitude of this difference is small and not likely to make a practical difference in commercial production. The tall grafted plants did not develop as much southern blight as the non-grafted plants, but there are additional cultural issues with using the resistant rootstock in a processing tomato field such the risk of transplants breaking at their union while being transplanted or quickly after transplanting. Therefore, increasing the height of the graft union is likely not an economical approach for processing tomato production.

We found a range of variation in susceptibility to southern blight among commercial cultivars. One Heinz cultivar was promising in 2018, although it developed disease in 2019. In 2019 one Harris Moran cultivar showed promise in its resistance to southern blight, but it was not evaluated in 2018 due to poor germination. We found the breeding lines reported by Leeper et al. 1992 to also perform well in one replicate year in greenhouse conditions, therefore these breeding lines may be beneficial for southern blight resistance breeding for California processing tomatoes. However, three of the breeding lines performed similarly to two Heinz and one Nunhmens cultivars that are already planted in the southern San Joaquin Valley. The cultivars that performed well in our experiments may have lower susceptibility to southern blight compared to other cultivars and thus may be options for growers to plant in fields with a history of southern blight.

While results from the cultivar experiments were promising, cultivars could not be separated easily when evaluating their risk of developing disease. The disease risk ranking of some cultivars changed drastically between the two experimental replications: a few cultivars with low risk in 2018 developed more disease in 2019 and vice versa, but several cultivars were in the highest risk tier in both years. Although cultivars were difficult to separate, the study in 2019 developed more disease than the study in 2018. This may have been due to excessively high irrigation pressure in two blocks of the 2018 study that is suspected to have washed the inoculum out of pots, resulting in minimal disease development. Further evaluation of these cultivars in the greenhouse and the field is needed to confirm these findings.

Disease incidence was low overall in the greenhouse grafting studies, especially in HZ 8504 compared to HZ 5608. This may be due to our experimental setup in which plants were trellised because of space constraints on the greenhouse bench. When plants were trellised, the stem did not branch near the soil line, and vegetative tissues did not touch inoculated soil, while in the field the vegetative canopy is at risk of coming into contact with sclerotia in the soil. The utility of greenhouse experiments for evaluating the effect of grafting on southern blight may be limited.

These studies showed promising results for the management of southern blight in California. Based on our studies, the approach of grafting for management of southern blight may not be the best application. The use of resistant cultivars is a better and accessible approach for California processing tomato growers. We recommend the development of field studies to evaluate the promising cultivars in the greenhouse under natural conditions.

REFERENCES

Abeygunawardena, D. V. W. and Wood, R. K. S. 1957. Factors affecting the germination of sclerotia and mycelial growth of Sclerotium rolfsii sacc. Transactions of the British Mycological Society. 40(2): 221-231.

California Agricultural Statistics Review, 2017-2018. California Department of Food and Agriculture. 11-104.

Cantero-Navarro, E., Romero-Aranda, R., Fernandez-Munoz, R., Martinez-Andujar, C., Perez-Alfocea, F., Albacete, A. 2016. Improving agronomic water use efficiency in tomato by rootstock-mediated hormonal regulation of leaf biomass. Plant Science. 251:90-100.

Cohen, R., Horev, C., Burger, Y., Shriber, S., Hershenhorn, J., Katan, J., and Edelstein, M. 2002. Horticultural and Pathological Aspects of Fusarium Wilt Management Using Grafted Melons. HortScience. 37(7):1069-1073.

Davis, R. M., Miyao, G., Subbarao, K. V., Stapleton, J. J., Aegerter, B. J. 2013. UC IPM Pest Management Guidelines: Tomato. Southern Blight. UC ANR Publication 3470. http://ipm.ucanr.edu/PMG/r783100711.html

Goldschmidt, E. E. 2014. Plant Grafting: New Mechanisms, Evolutionary Implications. Frontiers in Plant Science 5(727): 1-9.

Grieneisen, M., Aegerter, B., Stoddard, S. Zhang, M. 2018. Growers' Guide to Grafted Tomatoes. Second Edition. UC ANR Publication 1-12

Gullino, M. L., Camponogara, A., Gasparrini, G., Rizzo, V., Clini, A., Garibaldi, A. 2003. Replacing methyl bromide for soil disinfestation: the Italian Experience and Implications for other countries. Plant Disease 87(9): 1012-1021.

Gurkin, R. S. and Jenkins, S. F. 1985. Influence of Cultural Practices, Fungicides, and Inoculum Placement on Southern Blight and Rhizoctonia Crown Rot of Carrot. Plant Disease 69(6): 477-481.

Higashide, T., Nakano, A., Yasuba, K. 2014. Yield and Dry Matter Production of a Japanese Tomato 'Momotaro York' Are Improved by Grafting onto a Dutch Rootstock 'Maxifort'. Journal of the Japanese Society for Horticultural Science 83(3): 235-243.

Ioannou, N. 2001. Integrating soil solarization with grafting on resistant rootstocks for management of soil-borne pathogens of eggplant. Journal of Horticultural Science and Biotechnology 76(4): 396-401.

Jenkins, S. F., and Averre, C. W. 1986. Problems and Progress in Integrated Control of Southern Blight of Vegetables. Plant Disease 70(7): 614-619.

Kimihiro Noguchi, Yulia R. Gel, Edgar Brunner, Frank Konietschke. 2012. nparLD: An R Software Package for the Nonparametric Analysis of Longitudinal Data in Factorial Experiments. Journal of Statistical Software 50:1-23. http://www.jstatsoft.org/v50/i12/.

King, S. R., Davis, A. R., Liu, W., Levi, A. 2008. Grafting for Disease Resistance. HortScience 43(6): 1673-1676.

Leeper, P. W., Phatak, S. C., Bell, D. K., George, B. F., Cox, E. L., Oerther, G. E., and Scully, B. T. 1992. Southern Blight-resistant Tomato Breeding Lines: 5635M, 5707M, 5719M, 5737M, 5876M, and 5913M. HortScience 27(5): 475-478.

Louws, F. J., Rivard, C. L., and Jubota, C. 2010. Grafting Fruiting Vegetables to Manage Soilborne Pathogens, Foliar Pathogens, Arthropods and Weeds. Scientia Horticulturae 127: 127-146

McCarter, S. 1991. Southern Blight. Compendium of Tomato Diseases: 22-23. American Phytopathological Society, St. Paul, MN.

Mudge, K., Janick, J., Scofield, S., and Goldschmidt, E. E. 2009. A History of Grafting. Horticultural Reviews. 35: 437-493.

Punja, Z. K. 1985. The biology, ecology, and control of sclerotium rolfsii. Phytopathology 23:97-127.

Punja, Z. K., Carter, J. D., Campbell, G. M., and Rossell, E. L. 1986. Effects of Calcium and Nitrogen Fertilizers, Fungicides, and Tillage practices on Incidence of Sclerotium rolfsii on Processing Carrots. Plant Disease 70(9): 819-824.

Punja, Z. K. and Grogan, R. G. 1981. Eruptive germination of Sclerotia of Sclerotium rolfsii. Phytopathology 71(10): 1092-1099.

Punja, Z. K. and Rahe, J. E. 1992. Sclerotium. In: Methods for research on soilborne phytopathogenic fungi, ed. L. L. Singleton, J. D. Mihail, and C. M. Rush, 166-171. St. Paul MN: American Phytopathological Society Press.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ristaino, J. B., Lewis, J. A., Lumsden, R. D. 1994. Influence of Isolates of Gliocladium virens and Delivery Systems on Biological Control of Southern Blight on Carrot and Tomato in the Field. Plant Disease. 78:153-156.

Rivard, C.L. and F.J. Louws. 2006. Grafting for disease resistance in heirloom tomatoes. North Carolina Coop. Ext. Serv. Bul. Ag-675.

Rivard, C. L., and Louws, F. J. 2008. Grafting to Manage Soilborne Diseases in Heirloom Tomato Production. HortScience 43(7): 2104-2111.

Rivard, C. L. O'Connell, S., Peet, M. M., Louws, F. J. 2010. Grafting Tomato with Interspecific Rootstock to Manage Diseases Caused by Sclerotium rolfsii and Southern Root-Knot Nematode. Plant Disease. 94(8):1015-1021.

Roberts, P.D., French-Monar, R. D., and McCarter, S. M. 2014. Compendium of Tomato Diseases: 43-44. American Phytopathological Society, St. Paul, MN.

Schwarz, D., Öztekin, G. B., Tüzel, Y., Brückner, B., Krumbein, A. 2013. Rootstocks can enhance tomato growth and quality characteristics at low potassium supply. Scientia Horticulturae. 149:70-79.

Smith, A. M. 1972. Drying and wetting sclerotia promotes biological control of Sclerotium rolfsii sacc. Soil Biol. Biochem. 4: 119-123.

Sutton, K. F., Lanini, W. T., Mitchell, J. P., Miyao, E. M., and Shrestha, A. 2006. Weed controls, yield, and quality of processing tomato production under different irrigation, tillage, and herbicide systems. Weed technology. 20:831-838.

Swett, C. L., and Nunez, J. 2017. Southern Blight Cliff Notes-2017. Sacramento Valley Agronomy Notes. (1):1-6.

Xu, A., Gleason, M. L., Mueller, D. S., Esler, P. D. 2008. Overwintering of Sclerotium rolfsii and S. rolfsii car. delphinii in Different Latitudes of the United States. Plant Disease. 92:719-724.

Table 2.1. List of cultivars and treatments evaluated in the 2018 and 2019 greenhouse experiments.

Name	*Type*	*2018*	*2019*	*Control*[a]
1892 HMX	processing	N/A	Y	
19406 UG	processing	Y	Y	
273 BQ	processing	Y	Y	2018, 2019
2756 SV	processing	Y	Y	
311 AB	processing	Y	Y	
319 DRI	processing	Y	Y	2018, 2019
3887 HMX	processing	Y	N/A	2018
4885 HMX	processing	Y	Y	
4909 HMX	processing	Y	Y	
5635M	breeding line	N/A	Y	
5707M	breeding line	N/A	Y	
5719M	breeding line	N/A	Y	
5737M	breeding line	N/A	Y	
5876M	breeding line	N/A	Y	
5913M	breeding line	N/A	Y	
6366 SUN	processing	Y	Y	
6415N	processing	Y	Y	
6416N	processing	Y	Y	
6428N	processing	Y	Y	2018, 2019
HZ 1310	processing	Y	Y	
HZ 1428	processing	Y	Y	2018, 2019
HZ 1662	processing	Y	Y	2018, 2019
HZ 2401	processing	Y	Y	
HZ 4707	processing	Y	Y	
HZ 5608	processing	Y	Y	
HZ 8504	processing	Y	Y	
Maxifort	rootstock	Y	N/A	
Multifort	rootstock	Y	N/A	

[a] Six and five cultivars were evaluated as controls in 2018 and 2019 respectively. The rate of inoculum in the control treatments was 10 sclerotia per 100cm^3 of soil.

Table 2.2. Fixed effects analysis of cultivar and inoculum on severity of southern blight of processing tomato in the greenhouse.

Year	*Effect*[a]	*Num DF*	*Den DF*	*F Value*	*Pr > F*[b]
2018	Inoculum	1	2526	2.74	0.0980
	Cultivar x Inoculum	25	2526	4.70	<.0001
	Inoculum 0	5	2526	0.00	1.0000
	Inoculum 10	20	2526	5.87	<.0001
	Date	13	2526	17.26	<.0001
2019	Inoculum	1	1734	0.22	0.6375
	Cultivar x Inoculum[c]	25	1734	12.28	<.0001
	Inoculum 0	4	1734	0.00	1.0000
	Inoculum 10	21	1734	14.61	<.0001
	Date	10	1734	14.4	<.0001

[a] Due to the treatment design including only 6 of 19 cultivars as non-inoculated controls in 2018 and including only 5 of 25 cultivars as non-inoculated controls in 2019, the experiment was evaluated as a nested model (disease_severity = inoculum inoculum*cultivar)

[b] Disease severity was assessed on a 0 to 7 rating scale, and was analyzed with the GLIMMIX procedure in SAS 9.4 using the multinomial model with the cumulative logit link function, with block as a random effect.

[c] The cultivar*inoculum interaction was investigated with the ‘slice’ option to examine the effect of cultivar within each inoculum level.

Table 2.3. Fixed effects analysis of severity of southern blight of processing tomato influenced by cultivar and grafting in the grafted greenhouse experiment of 2018 and 2019.

Year	*Effect*[a]	*Num DF*	*Den DF*	*F Value*	*Pr > F*[b]
2018	Cultivar	1	161	0.14	0.7049
	Graft	2	161	53.56	<.0001
	Cultivar × Graft[c]	1	161	0.04	0.8366
	Date	4	161	0.82	0.5164
2019	Cultivar	1	265	0	0.9908
	Graft	2	265	5.23	0.0059
	Cultivar × Graft	2	265	0.13	0.8804
	Date	5	265	2.06	0.0708

[a] Due to the treatment design including only 6 of 19 cultivars as non-inoculated controls, the experiment was evaluated as a nested model (disease_severity = inoculum inoculum*cultivar)

[b] Southern blight severity was rated on a 0 to 7 scale and analyzed with the GLIMMIX procedure in SAS 9.4 using the multinomial model with the cumulative logit link function, with block as a random effect.

[c] The cultivar*graft interaction was investigated with the 'slice' option to examine the effect of cultivar within each inoculum level.

Table 2.4. Fixed effects analysis of incidence of southern blight of processing tomato in the grafted field experiment in 2018 and 2019.

Year	*Effect*	*Num DF*	*Den DF*	*F Value*	*Pr > F*[a]
2018	Graft	2	116	232.93	<.0001
	Cultivar	1	116	8.79	0.0037
	Cultivar × Graft[b]	2	116	4.41	0.0143
	HZ 5608	2	116	160.65	<.0001
	HZ 8504	2	116	85.83	<.0001
	Date	3	116	10.76	<.0001
	Graft × Date	6	116	0.96	0.4559
	Cultivar × Date	3	1	0.34	0.8161
	Cultivar × Graft × Date	6	1	0.71	0.7185
2019	Graft	2	120	163.26	<.0001
	Cultivar	1	120	1.76	0.1866
	Cultivar × Graft	2	120	0.18	0.8356
	Date	3	120	2.45	0.0670
	Graft × Date	6	120	1.95	0.0788
	Cultivar × Date	3	1	0.03	0.9879
	Cultivar × Graft × Date	6	1	0.01	1.0000

[a] Southern blight severity was rated on a 0 to 7 scale and analyzed with the GLIMMIX procedure in SAS 9.4 using the multinomial model with the cumulative logit link function, with block as a random effect.

[b] The cultivar*graft interaction was investigated with the 'slice' option to examine the effect of cultivar within each inoculum level.

Table 2.5. Influence of cultivar and grafting treatment on southern blight incidence in field trials.

Year	Cultivar[a]	Graft	Average Incidence[b]
2018	HZ 5608	None	21.5 a
	HZ 5608	Standard	3.1 b
	HZ 5608	Tall	2.1 b
2018	HZ 8504	None	13.3 a
	HZ 8504	Standard	2.6 b
	HZ 8504	Tall	1.4 c
2019[c]		None	18.5 a
		Standard	2.1 b
		Tall	1.1 b

[a] Slicing evaluations were done separately for each cultivar.

[b] Within each year or cultivar, means followed by the same letter are not significantly different.

[c] The effect of grafting alone was significant.

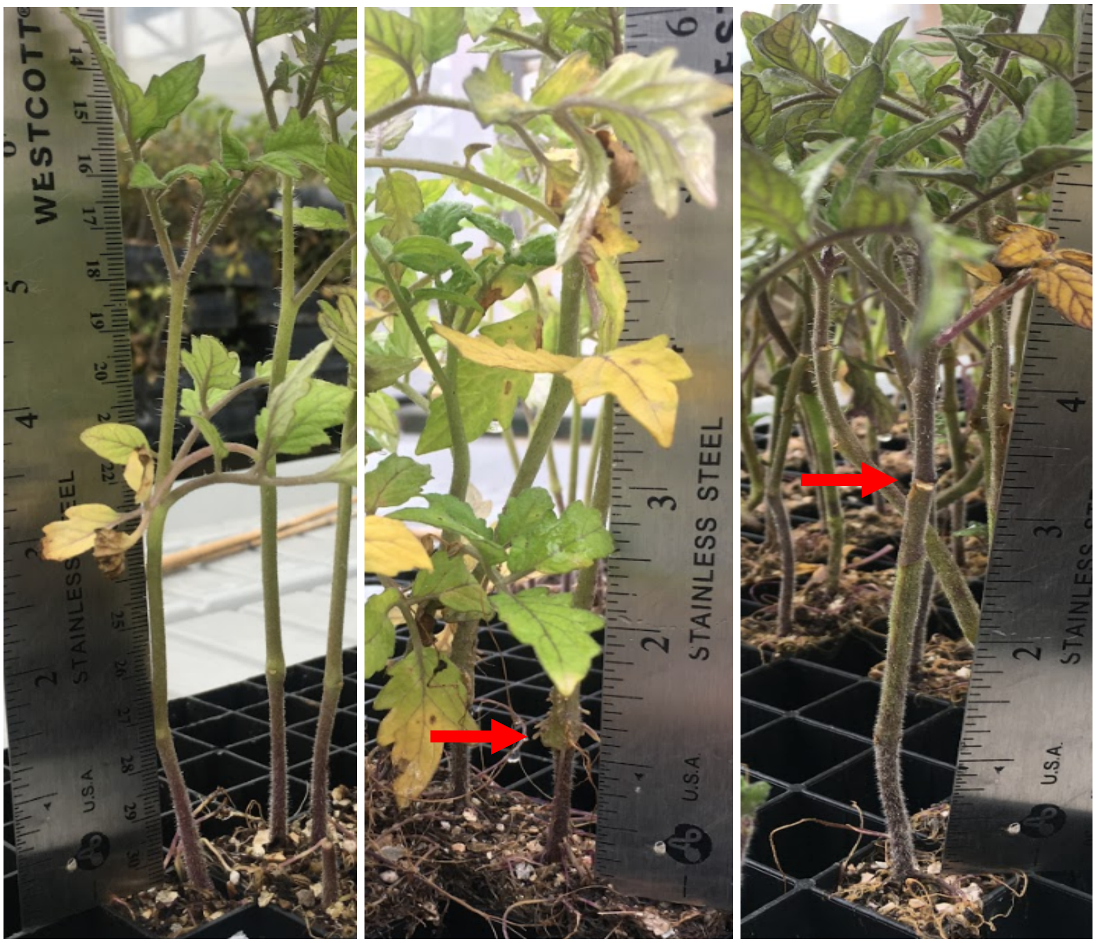


Figure 2.1. Examples of the nongrafted transplant (left), standard grafted transplant (middle), and tall grafted plant (right). The red arrow indicates the height of the standard graft union at approximately 2.54 cm and at approximately 7.62 cm for the tall grafted union.

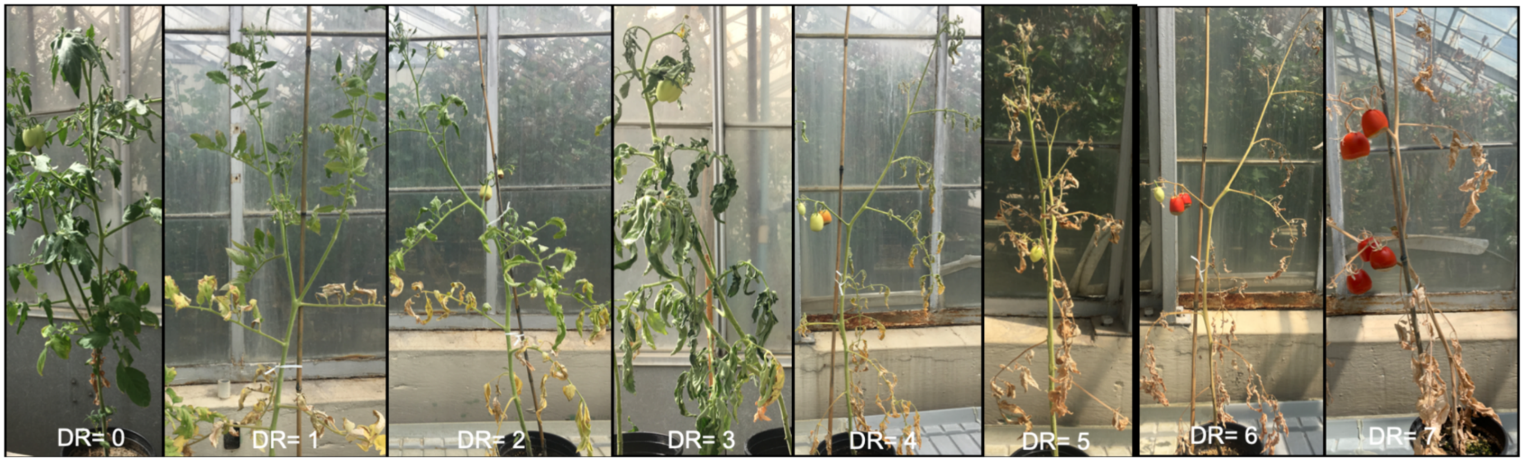


Figure 2.2. Examples of the 0 to 7 rating scale used to assess severity of southern blight of processing tomato in the greenhouse. From left to right ratings 0, 1, 2, 3, 4, 5, 6, and 7.

Figure 2.3. Examples of colonized tomato stems by *A. rolfsii* in the greenhouse (top images) and symptoms of southern blight observed on processing tomato in the field. White mycelia and white sclerotia growing around a tomato stem in the greenhouse (top left). Mature tan to reddish brown sclerotia around a dried tomato stem (top right). Image of a wilting tomato plant due to southern blight (bottom left) and image of a collapsed and dead tomato plant due to southern blight (bottom right). Image on bottom right photo credit to Alexander I. Putman.

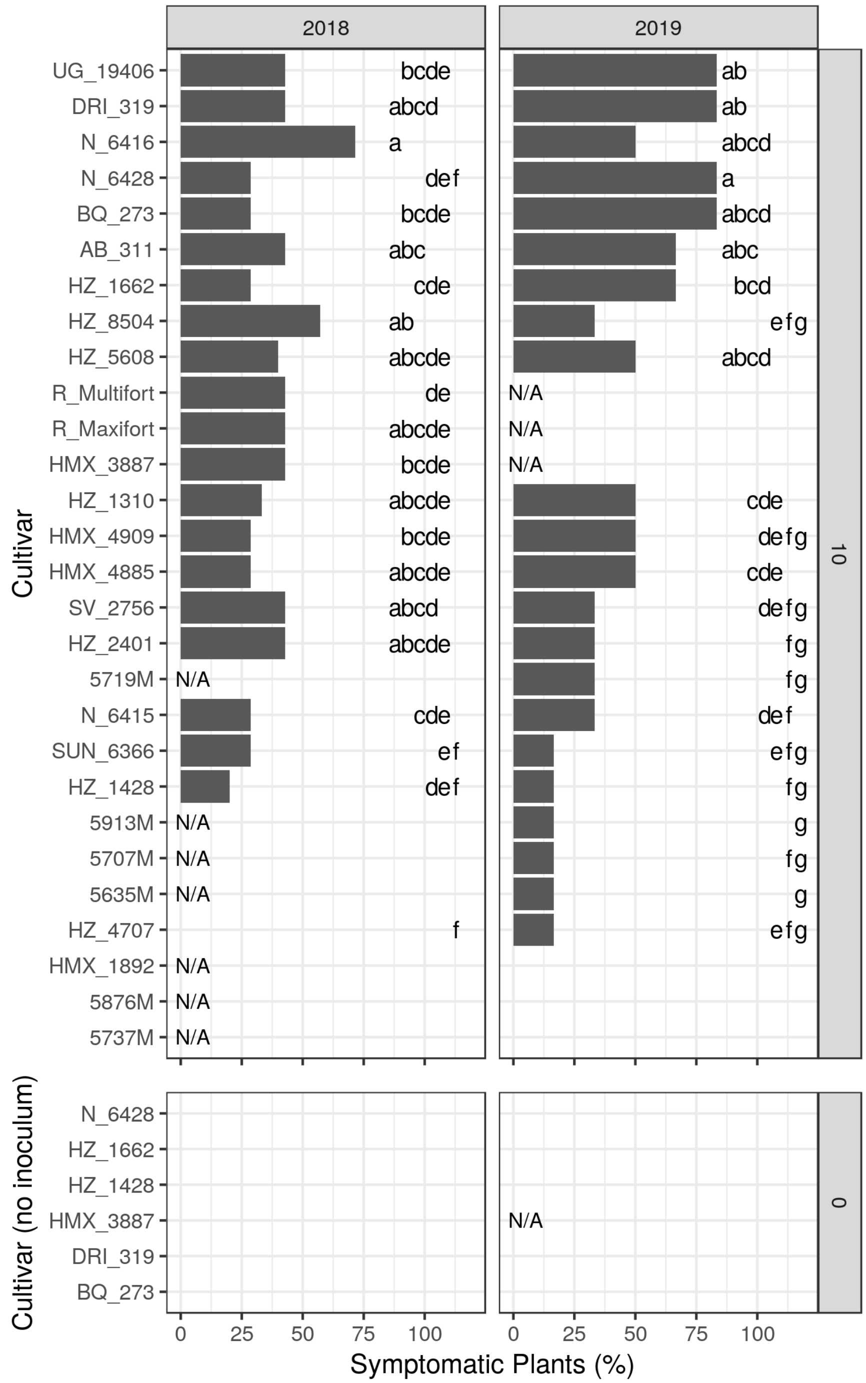


Figure 2.4. Percent symptomatic plants on the last rating date in the 2018 and 2019 cultivar evaluation studies in the greenhouse. **Top panels,** cultivars evaluated in soil inoculated with 10 sclerotia per 100 cm^3 soil. **Bottom panel**, cultivars evaluated in non-inoculated soil. Statistical analysis was based on all rating dates. Within each year or cultivar, means followed by the same letter are not significantly different.

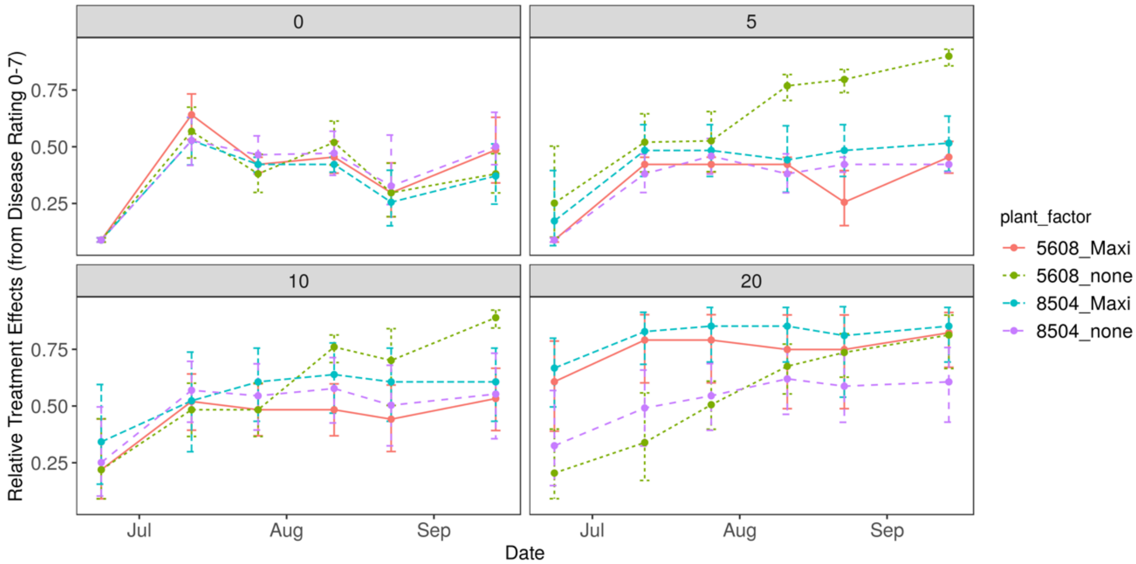


Figure 2.5. Relative treatment effects and 95% confidence intervals of disease severity under four inoculum levels of 0, 5, 10, and 20 sclerotia per 100 cm^3 soil from the 2017 greenhouse study. **Solid red line**, HZ 5608 grafted to Maxifort rootstock; **dotted green line**, non-grafted HZ 5608; **dashed blue line**, HZ 8504 grafted to Maxifort; **dashed purple line**, non-grafted HZ 8504.

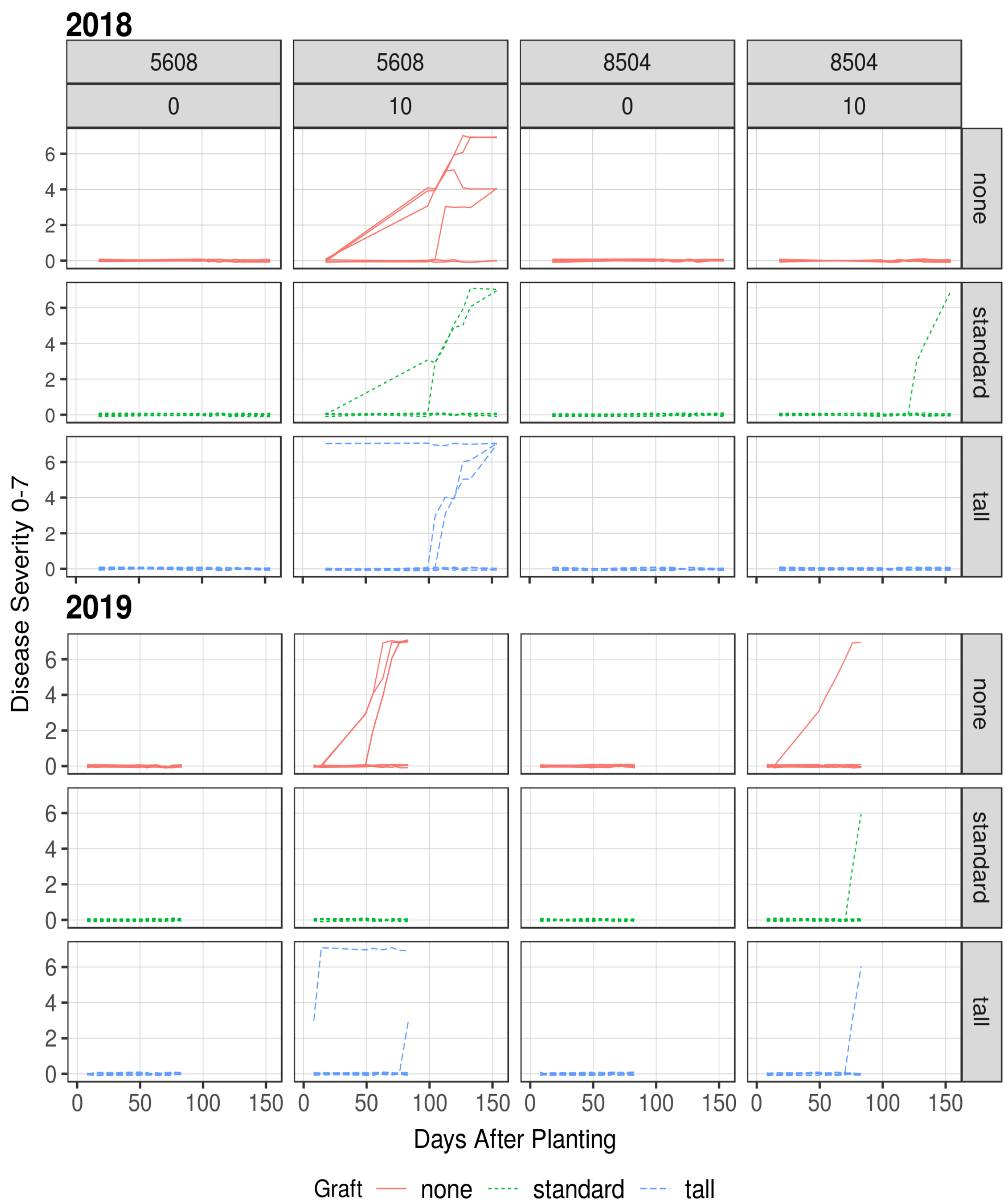


Figure 2.6. Disease severity of processing tomato cultivars HZ 5608 and HZ 8504 under two inoculum levels (0 and 10 sclerotia per 100 cm^3 soil) from the 2018 and 2019 greenhouse graft studies. Data was collected using a 0 to 7 rating scale; 0 = healthy and no disease, and 7 = completely wilted and dry, dead. Each line represents a replicate pot.

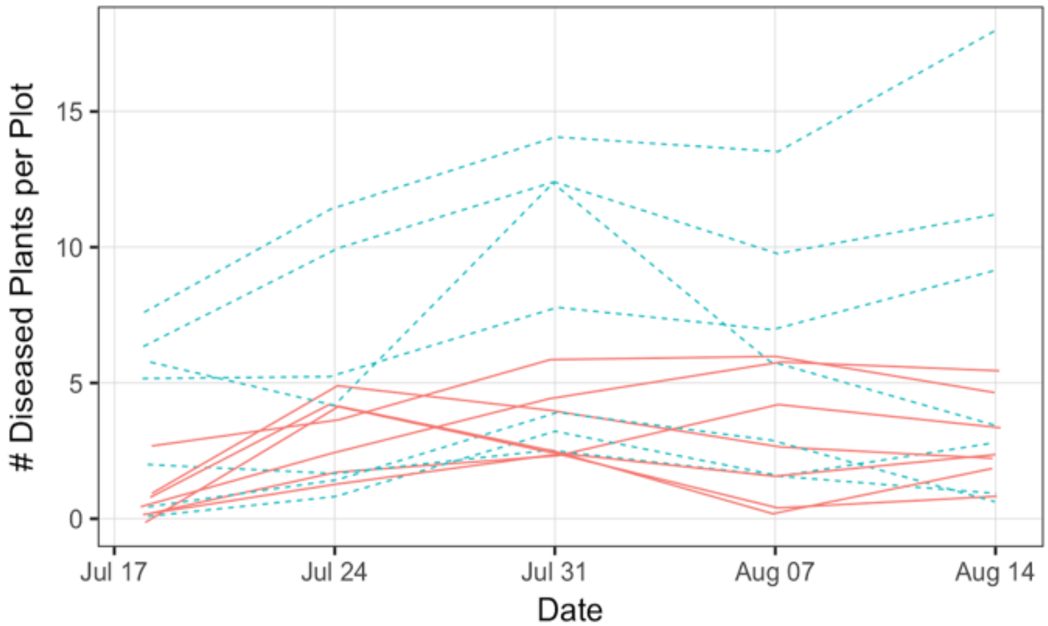


Figure 2.7. Incidence of southern blight of processing tomato cultivars HZ 5608 and HZ 8504 alone (dotted blue line) or grafted to Maxifort rootstock (solid red) in the 2017 field study. Each line represents the mean incidence of four 15.4 m segment within each of seven replicate plots.

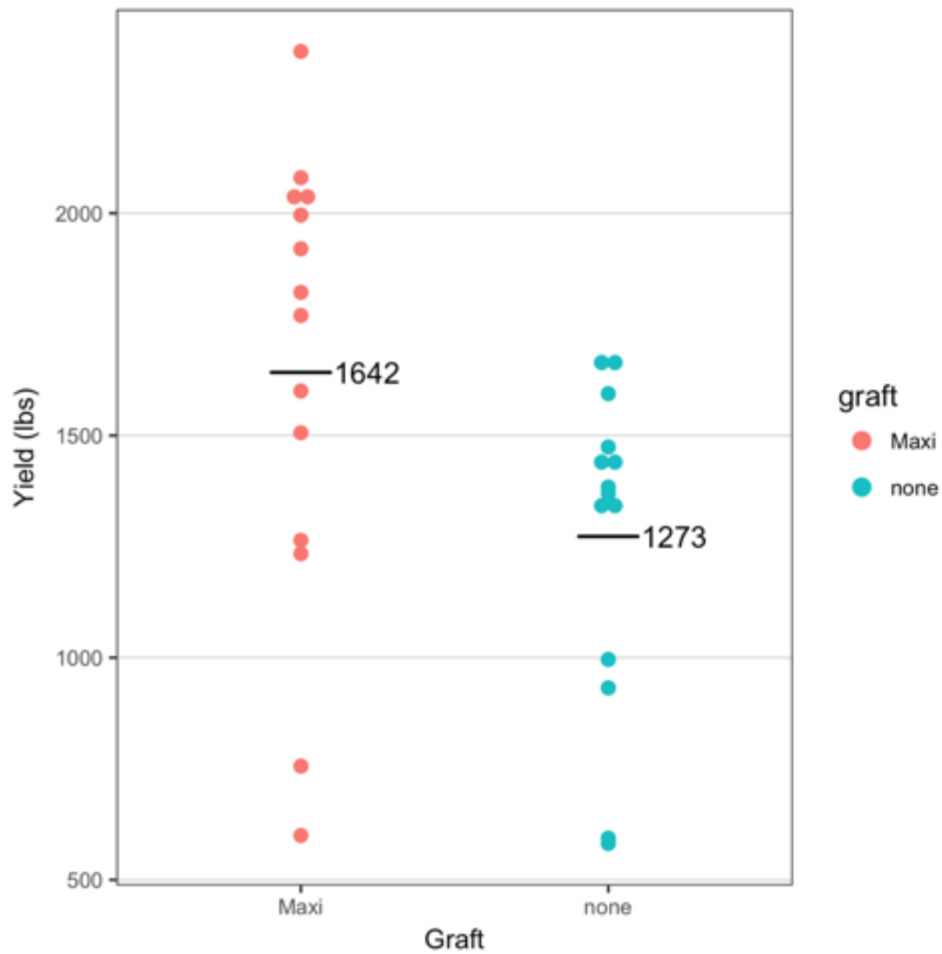


Figure 2.8. Yield per plot of processing tomato either non-grafted (**none**) or grafted to Maxifort rootstock (**Maxi**) collected in the field in October 2017. Each data point represents one of seven replicate plots across two cultivars (HZ 5608 and HZ 8504), and numbers and horizontal bars indicate average yield of each grafting treatment across replicate plots and cultivars.

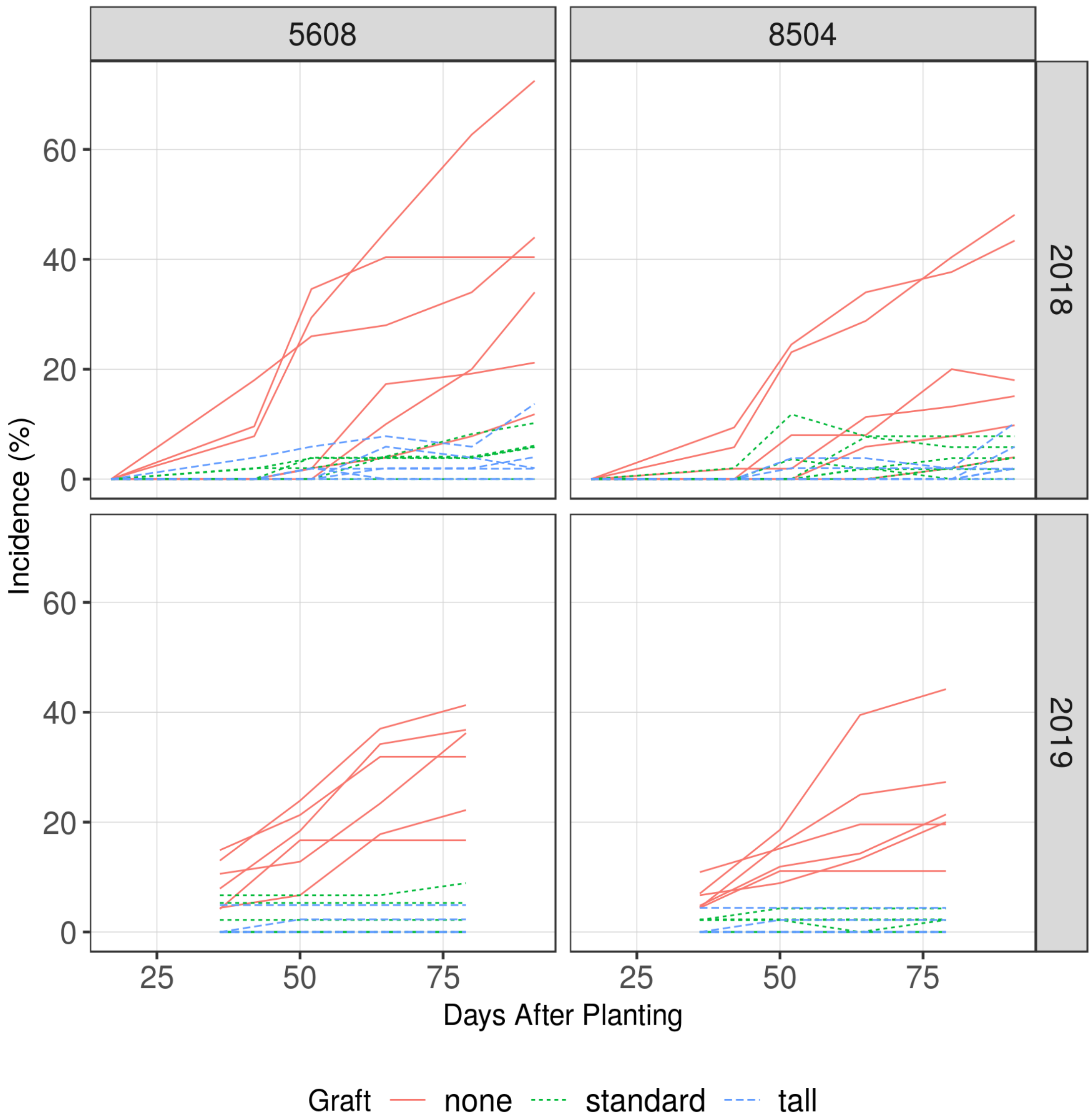


Figure 2.9. Incidence of southern blight of processing tomato cultivars HZ 5608 and HZ 8504 as influenced by grafting treatments: non-grafted (**red solid lines**), grafted to Maxifort rootstock at a standard height (**dotted green lines**), or grafted to Maxifort rootstock at a tall height (**dashed blue lines**) in the 2018 and 2019 field studies. Each line represents a replicate plot. Incidence was assessed for each plant by visually determining the presence or absence of southern blight symptoms and tracking the same plant across all rating dates.

GENERAL CONCLUSIONS

Currently, in California fresh market raspberry production there are no management practices commonly used to manage cane Botrytis. The twine treatment is the current experimental method of leaf removal practiced by growers in Ventura County due to its labor efficiency. Because it is an aggressive practice, we hypothesized that a practice that causes less wounding would improve cane Botrytis management. Across all three experiments there were no significant differences in cane Botrytis severity between the twine and manual treatments. We found leaf removal methods can be applied for certain cultivars and row spacings but the direction of the effect when compared to the no removal control may vary with context.

Host plant resistance is the most sustainable option in managing soilborne disease (Gullino et al. 2003), but like other crops it is believed there is little resistance to southern blight within commercial processing tomato cultivars. However, previous work has found some resistant breeding lines in the tomato germplasm. Our results suggest that some commercial cultivars may have a similar level of resistance to the resistant breeding lines. Grafting is another option for management of soilborne diseases. Disease control by grafting has already shown to be a beneficial alternative to the soil fumigant methyl bromide in Asia and much of Europe (King et al. 2008). Although grafting drastically reduced southern blight in our studies, the approach of grafting for management of southern blight may not be the best application. The use of resistant cultivars is a better and accessible approach for California processing tomato growers.