Characterizing cultivar resistance and pathogen-seed interactions for *Fusarium falciforme* (FSSC 3+4), an emerging vine decline and stem rot pathogen of California processing tomato

By

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ABSTRACT

California produces over 97% of the processing tomatoes in the United States. An emerging threat to processing tomato production is *Fusarium falciforme*, a soilborne fungal pathogen that causes severe stem rot and premature vine decline. Early season vine decline predisposes fruit to sunburn and subsequent fruit rot, reducing fruit quality and causing severe yield losses. This pathogen has been reported in the top tomato-producing counties in California. At the time this study was initiated, no known management options were available for this disease.

Some economically important soilborne diseases have been successfully managed by deploying tolerant and resistant cultivars. In order to develop a cultivar-based management strategy, commercial processing tomato cultivars were screened for resistance to *F. falciforme*. Complete resistance was not observed in this study; however, commercial cultivars varied in susceptibility to *F. falciforme*. Tolerant cultivars — N6428, HM4909, H8504, HM58841, and H1776 — wherein yield was less affected by *F. falciforme*, were identified. Several of these cultivars had extended field holding traits (that enables fruit to retain quality in the field an extended time) which might contribute to improved performance. Conversely, susceptible cultivars — HM3887, H5608, N6416, H9663, and H1310 — that had statistically significant yield reductions and economically significant impacts in fruit quality were identified and should be avoided when *F. falciforme* is known to occur in a field. Disease phenotypes that corresponded to susceptibility were high incidence of early season vine decline (5 weeks pre-harvest) and vine decline at harvest. Foot / stem rot incidence and severity did not correspond to yield performance, as 80-100% of plants developed rot across all cultivars.

In addition, we evaluated the consistency of cultivar performance across years and sites having different inoculum pressure to determine how variable environmental, and other factors impact disease severity. Of the nine cultivars under study, we observed that only N6428 consistently performed well across sites and years, emphasizing the importance of assessing the stability of field tolerance over growing seasons and locations. To test the hypothesis that resistance to the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici* race 3 (*I3* resistance gene), and the Fusarium crown and root rot pathogen, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Frl* resistance gene), contributes to *F. falciforme* tolerance, we included cultivars with the *I3* gene (F3 cultivars) and the *Frl* gene (Fr cultivars) in our variety screenings. F3 cultivars ranged in susceptibility to *F. falciforme*, indicating that the F3 resistance gene did not contribute to tolerance.

Fusarium falciforme symptoms, including foliar chlorosis, necrotic flecking and sometimes leaf deformity, affect the entire plant, which might mean that this is a systemically colonizing pathogen. To test the hypothesis that *F. falciforme* can systemically colonize tomato plants, we evaluated seeds from artificially infected plants for colonization. We did not observe *F. falciforme* colonization at a detection threshold of 0.0002%, indicating that *F. falciforme* is not seedborne. As these and parallel studies indicate that *F. falciforme* may produce phytotoxic secondary metabolites, we assessed the impact *F. falciforme* infection had on seed viability (germination rates) across cultivars. We observed that the highly susceptible cultivar, HM3887, had reduced seed viability; however, there was a wide range in seed response to *F. falciforme*. Together, these results indicate that *F. falciforme*-infected tomato seeds do not provide a means of pathogen dispersal, although there may be impacts of the pathogen on tomato seed viability.

In conclusion, *F. falciforme* is a pathogen of significant impacts to both yield and seed viability; howe

INTRODUCTION

California produces over 95% of the processing tomatoes (*Solanum lycopersicum*) in the United States, with an estimated production of 10.89 million metric tonnes in 2018 (USDA, 2019). Decades of tomato production have allowed for the appearance of diverse soilborne pathogens, some of which can reduce crop yields. Economically-important soilborne pathogens include *Fusarium oxysporum* f. sp. *lycopersici* (causal agent of Fusarium wilt), *Fusarium oxysporum* f. sp. *radicis-lycopersici (*causing Fusarium crown and root rot), and *Fusarium noneumartii* (previously named *Fusarium solani* f. sp. *eumartii*, causal agent of Fusarium foot rot) (Romberg and Davis, 2007; Sandoval-Denis *et al.,* 2019).

Fusarium solani was first described as a pathogen of fresh market tomatoes in Australia in 1975 (Vawdrey and Peterson, 1988). Diseased plants exhibited symptoms such as tap root girdling, crown rot, wilting, and reduced yields; however, plants rarely died (Vawdrey and Peterson, 1988). Similar symptoms were reported in California processing tomatoes grown in Yolo County since 1984 (Cucuzza *et al.*, 1991). The disease had minor impact, with highest incidences of 5% (Cucuzza *et al.*, 1991). Taxonomic classification of the *Fusarium solani* species continues to change. The causal agent of the Fusarium foot rot pathogen was previously known as *Fusarium solani* f. sp. *eumartii* (Romberg and Davis, 2007), but was renamed *Fusarium noneumartii*, and now resides in the *F. falciforme* clade of the *Fusarium solani* species complex (FSSC) (Sandoval-Denis *et al.,* 2019).

In 2017, tomato plants with severe stem rot, root rot, and vine decline were observed in fields in Yolo County, California. *Fusarium falciforme* (FSSC 3 +4) was consistently recovered from diseased plant tissue (identified using BLAST analysis in Fusarium ID 1.0 and GenBank) and Koch's postulates were completed to confirm pathogenicity (Helpio and Swett, 2019).

Diverging from Fusarium foot rot symptomology, wherein impacts were of minor importance, reported symptoms of *F. falciforme* were severe stem rot and premature vine decline. Based on anecdotal reports by stakeholders over the last 3 years, this new Fusarium disease is emerging as a significant driver of premature vine decline and associated yield loss in California's top tomato-producing counties, i.e., Yolo, Fresno, Kings, Kern, San Joaquin, Sutter, and Colusa.

When this study was initiated, there were no known methods for managing *F. falciforme* in tomato. The use of tolerant and resistant cultivars is an effective management strategy for many soilborne pathogens (i.e., Fusarium crown and root rot and Fusarium wilt) (Ozbay and Newman, 2004; Catanzariti *et al*., 2015). Therefore, **objective 1.1 of this study was to evaluate selected commercially available processing tomato cultivars for response to *F. falciforme*.** To determine how consistent the cultivar response to *F. falciforme* was over a range of field conditions, **objective 1.2.1 was to evaluate the consistency of commercial cultivar performance across years under *F. falciforme* pressure.** Pathogen pressures vary across naturally infested tomato fields in California; therefore, **objective 1.2.2 was to evaluate consistency of cultivar performance at low and high pathogen pressures**.

Previous studies have shown that many fungal pathogens con contaminate / infect seeds as a means of dispersal and as a source of primary inoculum. For example, *Fusarium oxysporum* f. sp. *lycopersici* colonizes tomato seeds via the vascular system (Perveen and Ghaffar, 1995), whereas *Fusarium oxysporum* f. sp. *radicis-lycopersici* primarily colonizes tomato seed via direct contact with infected plant debris (Menzies and Jarvis, 1994). The concern that *F. falciforme* could be seedborne led to **objective 2.1, which assessed *F. falciforme* colonization on tomato seeds from fruit of artificially inoculated plants.** Moreover, because species in the FSSC, such as *F. virguliforme* (a causal agent of soybean sudden death), are known to produce

phytotoxic secondary metabolites that cause systemic foliar symptoms such as chlorosis, leaf deformity, and necrosis (Brar *et al*., 2011; Hartman *et. al*., 2015). Secondary metabolites produced by *Fusarium solani* in certain hosts, such as *Striga spp.* and *Ipomoea spp.,* can also inhibit seed germination (Abbas and Boyette, 1996; Ahmed *et al*., 2001). Parallel studies conducted by Del Castillo *et al*. (in progress) indicate that *F. falciforme* is not systemically colonizing affected foliar tissue, and thus whole plant symptoms may be due to systemically translocated phytotoxins. If so, this pathogen could have a negative impact on seed viability, which is important for the seed industry, which does not allow greater than 3% nonviable seed in seed lots (C. Swett pers. comm.); therefore **objective 2.2 of this study was to assess the impact of *F. falciforme* on tomato seed germination.**

MATERIALS AND METHODS

Objective 1. Evaluating commercial cultivar performance under *Fusarium falciforme* pressure

1.1 Evaluation of selected commercial processing tomato cultivars for response to *F. falciforme*

Experimental design

The primary objective of this experiment was to evaluate whether cultivars varied in response to *F. falciforme*, tolerance being defined as a type of response in which plants develop disease symptoms, but yield is not significantly affected, with significance defined by either statistical or economic analyses. This study was conducted at the UC Davis Armstrong Plant Pathology Research Station (GPS: 38.522426, -121.757284, soil type: Yolo clay loam) from May 7th through September 13th of 2019. The experiment was set up as a randomized complete block design with the two different pathogen treatments (non-inoculated, or inoculated) in different fields, separated by ~300m. Each field contained four blocks, divided into 13 cultivar subplots. Cultivars were randomly assigned to subplots within a block and demarcated by a fallow buffer. The cultivars selected were chosen from the top 30 commercially grown cultivars from the previous growing season based on PTAB, 2018 reports, seed company consultation, and consultation with the California Tomato Research Institute (Z. Bagley) (**Table 1, 2**). One buffer row was planted on each side of the field and in the front and back ten feet of each row.

Pathogen treatment

In 2019 transplants were grown at the UC Davis greenhouse complex by double seeding 72-cell trays (with the exception of H8504 which was single seeded). Seedlings were grown for five weeks in the greenhouse (21-35°C; 12:12 L:D) and then were hardened off in a lath house for one week before transplanting into the field (**Table 3**). As plants were not thinned before

transplanting; thus, there were two plants per plug with the exception of H8504, which was single seeded.

To prepare inoculum, cultures of *F. falciforme* isolate CS109 were grown on potato dextrose agar for 5-7 days under 24-hour light at 23°C. Spores and mycelium were suspended in sterile 0.5% KCl and filtered through two layers of sterile cheesecloth. Spore concentrations were determined using a hemocytometer (Hausser Scientific Bright-Line) and adjusted to 10^5 spores / µl by the addition of sterile 0.1% water agar. Trays of tomato transplants assigned to planted in the infested plot were dip inoculated with *F. falciforme* one day prior to planting by submerging up to the plant crown for one minute and left overnight to drain.

Planting and field maintenance

Transplant plugs were manually planted in 150 cm wide beds at 20 cm spacing in 6.5 m subplots (~32 plugs per cultivar subplot). All plugs had two plants and were not thinned, except for H8504, which had one plant per plug. Plants were irrigated with subsurface drip tubing buried ~10-15 cm deep on a calendar basis until 21 days pre-harvest, when the water was cut to encourage ripening (**Table 3**). Plants were fertilized via subsurface drip with a starter fertilizer (10-34-0) at a total of 187 L / ha applied over four weeks (46 L / ha / week). Starting eight weeks after planting, urea ammonium nitrate (UAN 32-0-0) was applied weekly at 75 L / ha for five weeks (total of 398 L / ha). At the start of fruit set, potassium thiosulfate (KTS 0-0-25) was applied at a low rate (140 L / ha). Insecticides were applied through the subsurface drip system during the growing season. Whiteflies were controlled using imidacloprid (as Advise Four) at the label rate. Soil nutrient composition for each field was analyzed in August of 2019, 5 weeks pre-harvest and is presented in **Table 4**. For weed management, a pre-emergent herbicide application

of oxyfluorfen (as Goal 2XL at 1.17 L / ha) was applied via the subsurface drip system 15 weeks pre-planting followed by hand weeding over the duration of the trial.

Disease evaluations

We determined *F. falciforme* disease incidence for all cultivar subplots throughout the season. We collected biweekly canopy data from 5 m (25-plug) monitoring plots within the cultivar subplots starting nine weeks after transplanting to monitor disease development until harvest. Depending on the cultivar, initial foliar symptoms included necrotic flecking, chlorosis, and sometimes deformity (**Fig. 1**). In some cases, these symptoms would progress to where entire branches were necrotic but retained a green stem (early whole plant decline). Eventually, stems would dry out and the plant would collapse and die (advanced whole plant decline) (**Fig. 2**). For those planted plugs which had two plants, data were recorded on whether one or both plants were in decline. Throughout the growing season, plants exhibiting vine decline were checked for presence of crown rot by making a small (~ 3 cm) epidermal cut at the crown. If there was evidence of rot, indicated by an external crown lesion or decline, plants were exhumed and evaluated for foot, crown, and stem rot (**Fig. 3)**. We stopped digging up plants six weeks before harvest to not impact yield. We evaluated percent of plants in each plot exhibiting advanced whole plant decline, foot, crown, and stem rot. To confirm *F. falciforme* infection, we also conducted tissue isolations from a subset of representative diseased plants by plating disinfested (30 seconds in 70% ethanol, 2 minutes 20% sodium hypochlorite) stem sections on *Fusarium* selective medium (FSM), as described (Swett and Gordon, 2017; Aegerter and Gordon, 2006).

Yield and fruit quality assessment

We collected yield data 125 days after planting from 2 m harvest plots in 2019 (~ 10 plugs). Fruit was sorted into red marketable, green unmarketable, or damaged. The damaged fruit category consisted of fruit with sunburn, rot, or blossom end rot (**Fig. 4**). Fruit from each category was combined and weighed to determine total yield (kg / 2 m harvest plot). We analyzed the effect of pathogen inoculation on total fruit yield and the percent total fruit with damage.

Statistical analysis

We conducted analysis of variance (ANOVA) and Tukey's pairwise means comparison to evaluate differences in pathogen treatment (non-inoculated vs. inoculated) for each cultivar. Response variables analyzed were total fruit biomass, total marketable yield, percent of green fruit, and percent damaged fruit, percent of the plants with decline symptoms five weeks before harvest, percent of plants with decline symptoms at harvest, and percent of plants with crown and stem rot symptoms at harvest. Analyses were conducted in RStudio 1.4.1717 and the Rcmdr package 2.7-1 (RStudio PBC, Boston MA). Percentage data were arcsine square root transformed prior to analysis. As disease was not observed in the non-inoculated field, these plants were excluded from analyses of decline and rot incidence.

1.2 Evaluating consistency of commercial cultivar response under variable *F. falciforme* pressure and across years

1.2.1 Evaluating consistency of the response of nine commercial cultivars to *F. falciforme* in 2019 and 2020

Experimental design

The purpose of this study was to evaluate field stability of tolerance traits across two years, comparing performance of nine cultivars in 2019 and 2020. These studies were conducted at the UC Davis Armstrong Plant Pathology Research Station from May through September of 2019 and repeated during the same months in 2020. This experiment was set up in a randomized complete block design. There were four blocks, in which the cultivar subplots were randomly assigned. Nine cultivars of the 13 cultivars tested in 2019 were tested again in 2020 to evaluate performance over time, including a highly susceptible cultivar (HM3887) as a positive control (**Table 1**).

Pathogen treatment, planting and field maintenance

Transplant care and pre-plant inoculation was conducted as described above for the 2019 cultivar response trial. In 2019, inoculated transplants were placed in a field not previously infested with *F. falciforme*. In 2020, transplants were produced by Westside Transplant, LLC (Winters, CA). Plants were double seeded in 338-cell trays and grown in a greenhouse for five weeks before hardening off for one week prior to transplant (**Table 3**). Plants were thinned to one plant per plug before planting. In 2020, inoculated transplants were placed in the same field as the 2019 trial. In addition, diseased tissue from the 2019 trial was incorporated into the soil post-harvest, resulting in higher inoculum pressure and soil nitrogen levels.

Field maintenance was conducted as described above for the 2019 trials (**Table 3**). In 2020, a higher rate (374 L / ha) of potassium thiosulfate (0-0-25) was applied via the subsurface drip system over three weeks after fruit set. Imidacloprid (as Macho 4.0 or Advise Four) was applied at a total of 1.4 L / ha, and 0.52 L / ha respectively, and chlorantraniliprole (as Coragen) was applied once at 0.56 L / ha. Soil nutrient composition for each field was analyzed in August of 2019 (before the end of the field study) and in November of 2020 (after harvest, and before incorporation). Of note, as the 2020 trial was conducted in the same field as 2019, there was a nutrient increase in 2020 trials, due to residual soil nitrogen (**Table 4**). Weeds were managed by hand weeding over the duration of both trials. In 2020, the pre-emergent herbicides trifluralin (as Treflan HPF at 2.34 L / ha) and oxyfluorfen (as Goal 2XL at 0.58 L / ha), were applied 14- and 1-weeks pre-planting, respectively. In 2019, plants were harvested 125 days after planting, 21 days after water was stopped (**Table 3**). In 2020, wildfire smoke cover from mid-August through September slowed soil drying and fruit ripening. We isolated from a subset of these plants to establish that *F. falciforme* was the only pathogen associated with disease symptoms, as described above.

Yield and fruit quality assessment

Yield data was collected from a 2 m harvest plot in 2019 (~10 plants at 20.3 cm spacing) and a 2.7 m harvest plot in 2020 (~8 plants at 30.5 cm spacing). In 2019, fruit was sorted into red marketable, green, and damaged. The unmarketable category consisted of fruit with sunburn or blossom end rot. Fruit from each category was combined and weighed to determine biomass (kg).

Statistical analysis

To evaluate differences in performance between years, we conducted ANOVA and Tukey's means comparison, as implemented in RStudio 1.4.1717 and Rcmdr package 2.7-1 (RStudio PBC, Boston MA). Yield response variables analyzed were: total fruit biomass, total marketable yield, percent green fruit, and percent damaged fruit. All percentage data were arcsine square root transformed prior to analysis. Where response variables lacked a significant year x cultivar interaction, data were combined for analysis of cultivar differences (n = 8).

1.2.2 Evaluating consistency of the response of commercial cultivars at low and high *F. falciforme* pressure in 2020

Experimental design

The study aimed to evaluate consistency of plant response (yield, and incidence of vine decline and foot / stem rot) across 13 commercial cultivars under variable pathogen pressure (**Table 1**). We conducted these studies at the UC Davis Armstrong Plant Pathology Research Station from May through September 2020. Treatments were inoculated plants planted in an artificially infested field (high pathogen pressure) and non-inoculated plants planted in a naturally infested field (low pathogen pressure); these fields were ~230m apart. *Fusarium falciforme* infection in plants in both fields was confirmed based on isolations from a subset of plants, as described above. Each field was set up as a randomized complete block design which contained four blocks (n = 4) with 13 cultivar plots (4.5m). However, some cultivars did not have all four blocks due to poor establishment and decline early in the season. In the low-pressure field, HMPL1 and HMPL2 had only two blocks (n = 2), and H1776, HM4909, HM5235, and UG4014 had three blocks (n = 3). In the high-pressure field, the cultivar SVTM9012 had only three blocks (n = 3).

Inoculation, planting, disease evaluations and yield assessment

Transplant care, inoculation and field maintenance occurred as described for the 2020 trials (**Table 3**). In the high-pressure field, plants were inoculated one day prior to planting as described in 1.1. Soil nutrient composition of each field was analyzed in July 2020, 9 weeks pre-harvest and is presented in **Table 4**. We monitored canopy disease development within the cultivar subplots starting nine weeks after transplanting in a 4.5 m monitoring plot (15 plants) until harvest. Harvest data was collected from a 2.7 m subplot within each monitoring plot (~8 plants within the previously mentioned monitoring plot) 125 days after planting. We determined percent of plants in each plot exhibiting foot / crown and stem rot. All fruit was harvested, sorted, and weighed as red marketable fruit, green fruit, and damaged fruit, as described above. We sub-divided the damaged fruit into three categories: sunburned fruit (shoulder or whole face bleached), rotten fruit (severe insect damage, watery rot / loss of firmness and / or presence of mold), and fruit with blossom end rot.

Statistical analysis

To evaluate differences in performance between a low, naturally occurring inoculum level and a high, artificially established inoculum level, ANOVA was conducted to determine effect of pathogen treatment for all cultivars combined and cultivar x site interactions. In addition, we conducted a pairwise means comparison using student's T-test to evaluate differences of the two sites for each cultivar. Response variables analyzed were: percent of plants with rot at harvest, total biomass, total marketable yield, percent green fruit, and percent damaged fruit. All percentage data were arcsine square root transformed prior to analysis in RStudio 1.4.1717 and Rcmdr 2.7-1 (RStudio PBC, Boston MA).

Objective 2. Evaluating the impact *Fusarium falciforme* on tomato seed

2.1 Assessing *F. falciforme* colonization of tomato seed

Experimental design

This trial was conducted in September 2020 at the UC Davis Armstrong Plant Pathology Research station. This experiment was replicated in two *F. falciforme*-infested fields (fields 1 and 2, respectively), ~0.70 km apart, set-up as a completely randomized design. Transplant care and pre-plant inoculation was described above for 2019 trial and field maintenance as described for 2020 trials. We evaluated seed colonization in two cultivars: HM3887, highly susceptible to *F. falciforme,* and HM58841 which is tolerant based on the 2019 cultivar response trials. In addition, the tolerant cultivar, HM4909, was examined in field 1 (not replicated). We collected fruit from plants that were in decline (more than half of the canopy collapsed or completely dead) (**Fig. 2**). Branches that were still alive had symptomatic leaflets with necrotic lesions and / or chlorosis. We collected 25-30 red marketable fruit (**Fig. 4A**) from 10 randomly selected plants / cultivar / field on September 7 and October 12 from fields 1 and 2, respectively. Fruit was bulked per plant and 100 seeds / plant were analyzed for a total of 1000 seeds / cultivar / field.

Seed extraction and viability assay

We surface disinfested fruit with 70% ethanol before cutting the fruit in half and extracting the pulp and seeds. The pulp and seed mixture was fermented overnight in ambient conditions (20 ± 3°C) and then washed with deionized water until the seeds were clean. Any underdeveloped or necrotic seeds that floated or mixed with the pulp were discarded during the washing process. The seeds were dried at room temperature and then stored in 7.62 x 15.24 cm manilla envelopes at 4°C before use.

Fusarium falciforme *seed colonization assay*

To assay for fungal colonization, 100 seeds per plant were randomly selected and surface disinfested by washing in 5% sodium hypochlorite for 30 seconds followed by rinsing in sterile deionized water (Swett and Gordon, 2017). Seeds were plated onto *Fusarium* selective medium (FSM) in aseptic conditions (using a laminar flow hood) and monitored for fungal growth for seven days (Aegerter and Gordon, 2006). All emerging colonies were sub-cultured onto potato dextrose agar (PDA) amended with tetracycline to identify. To identify all selected representative colonies, DNA was extracted using PrepMan Ultra Sample Preparation Reagent (Life Technologies, Carlsbad, CA). The translation elongation factor 1 alpha (EF1-α) gene was amplified by PCR (Bio-Rad T100 Thermal Cycler, Hercules, CA) using the primers EF-1 / EF-2 (O'Donnell *et al*., 1998), whereas other fungi were identified via amplification of the internal transcribed spacer (ITS) using the primers ITS1 and ITS4 (White *et al*., 1990; Moumni *et al*., 2020). Amplicons were purified using ExoSAP-IT (Applied Biosystems, Carlsbad, CA) and sequenced by Eurofins Genomics LLC (Louisville, KY). BLAST analyses in *Fusarium* ID and Genbank were used to identify isolates to species.

2.2 Assessing the impact of *F. falciforme* on tomato seed viability

2.2.1 Assessing the impact of *F. falciforme* on tomato seed viability across 16 commercial cultivars

Experimental design

This trial was conducted in 2019 and used the same fields, as described in the 2019 cultivar trial, above, with the addition of three cultivars (see Appendix A). Seed viability was evaluated from tomato plants grown in a non-inoculated field and an artificially inoculated field and we screened 16 commercial processing tomato cultivars. In September, we collected 25 red

marketable fruit (**Fig. 4A**) from randomly selected plants within each cultivar plot / block / field for seed extraction. Fruit collected from plants in the inoculated field were showing symptoms of decline (**Fig. 2**).

Seed extraction and viability assay

Fruit was surface disinfested with 70% ethanol before cutting the fruit in half and extracting the pulp and seeds. The pulp and seed mixture from the 25 fruit / cultivar was fermented overnight in ambient conditions (20 ± 3°C). After fermentation, the seeds were thoroughly washed with deionized water until seeds were clean, after which they were dried in a laminar flow hood. After the seeds were dry (~ 1 g), they were stored in 7.62 x 15.24 cm manilla envelopes at 4°C before use.

Seeds were sent to HM Clause (Davis, CA) for seed viability assays. Fifty seeds / cultivar / field were randomly chosen and placed on damp germination paper (4 reps / cultivar). The seeds were kept in moist chambers at 20-30°C and were evaluated for germination after 7 and 10 days. *Fusarium falciforme* infection in plants from both fields was confirmed based on isolations from a subset of plants as described above

2.2.2 Assessing the impact of *F. falciforme* on tomato seed viability in a repeated trial

Experimental design

This study was conducted at the UC Davis Armstrong Plant Pathology Research Station in September 2020. We evaluated two cultivars in two replicated fields inoculated with *F. falciforme*. Field 1 was the same as described above for the high inoculum load cultivar trial. Field 2 was inoculated and planted at the same time and was located ~150m away. In both fields, cultivars were planted into four blocks in similar-sized subplots; we randomly selected diseased plants from across each field for analysis. An additional cultivar, HM4909, was evaluated in

field 1. *Fusarium falciforme* infection was confirmed based on isolations from a subset of plants from both fields as described above. In both fields, we conducted isolations from a subset of plants (~10%) as described above to establish whether *F. falciforme* was present and whether other pathogens were also present.

Fruit collection and seed extraction

Fruits were collected from plants exhibiting vine decline (more than half of the branches were collapsed or dead) (**Fig. 2**). We collected 25-30 fruit from each of 10 plants / cultivar / field. Fruits were surface disinfested with 70% ethanol and the pulp and seeds from the fruit from one plant were extracted and fermented in ambient conditions (20 ± 3°C) overnight. The seeds were washed with deionized water to remove pulp and dried overnight in ambient conditions. Any under-developed or necrotic seeds that floated or were mixed with the pulp were discarded during the washing process. Seeds were stored in 7.62 x 15.24 cm manilla envelopes at 4°C before use in the viability assay.

Seed viability assay

To assay seed viability, we randomly selected 100 seeds per plant and surface disinfested by washing in 5% sodium hypochlorite for 30 seconds followed by rinsing with sterile deionized water (Swett and Gordon, 2017). In total, we analyzed 100 seeds / plant for a total of 1000 seeds / cultivar / field. Seeds were plated using sterile technique onto *Fusarium* selective medium (FSM) and monitored for germination over 14 days (Aegerter and Gordon, 2006). A seed was considered germinated if the hypocotyl, roots, and / or cotyledons emerged.

Table 1. Commercial tomato cultivars included in field trials to evaluate response to *Fusarium falciforme* in 2019 and 2020

	Trial		
Objective [z]	**1.1**	**1.2.1**	**1.2.2**
Year [y]	**2019**	**2019, 2020**	**2020**
Cultivar Number [x]	***F. falciforme* cultivar response**	**Cultivar consistency across years**	**Cultivar consistency under low and high *F. falciforme* pressure**
1	H8504	H8504	H8504
2	HM3887	HM3887	HM3887
3	H5608	H5608	H5608
4	H1776	H1776	H1776
5	HM58841	HM58841	HM58841
6	HM58801	HM58801	HM58801
7	N6428	N6428	N6428
8	HM5235	HM5235	HM5235
9	HM4909	HM4909	HM4909
10	N6416		N6434
11	H1428		HMPL1
12	H1310		HMPL2
13	H9663		SVTM9012
14	AB0311[w]		SVTM9016
15	DRI0319 [w]		SVTM9025
16	SVTM8011 [w]		UG4014

[z] Objective 1.1: Evaluating commercially available processing tomato cultivars for tolerance to *Fusarium falciforme.* Objective 1.2.1: Evaluating consistency of commercial cultivars across years. Objective 1.2.2: Evaluating consistency of commercial cultivars under low and high pathogen pressure in 2020.

[y] 2019 Inoculated field: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. 2019 Non-inoculated field: Plants were directly transplanted into a field with no history of *F. falciforme*. 2020 Inoculated field / High Pressure: Plants were dip inoculated with *F. falciforme* at transplanting for two consecutive years. Low Pathogen Pressure: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*.

[x] HM3887 was used as a susceptible positive control for all trials.

[w] See **appendix A.**

Table 2. Agronomic and disease resistance information for cultivars examined across two years of field trials

Cultivar [z]	Years Tested	Fusarium Wilt Resistance [y]	Fusarium Crown and Root Rot Resistance [x]	Field Holding (EFH) [w]	Maturity [v]	Vine Size
H8504	2019, 2020	F2	No	Yes	Late	Med/Lrg
AB0311	2019	F2	No	No	Late	Med/Lrg
SVTM8011	2019	F2	No	No	Late	Large
N6416	2019	F1	No	No	Early	Medium
HM3887	2019, 2020	F2	No	No	Late	Large
H1428	2019	F2	No	Yes	Late	Med/Lrg
DRI0319	2019	F2	No	No	Late	Large
H5608	2019, 2020	F2	No	No	Late	Large
H9663	2019	F2	No	No	Late	Large
H1776	2019, 2020	F2	No	Yes	Late	Med/Lrg
HM58841	2019, 2020	F2	No	Yes	Late	Large
H1310	2019	F3	No	Yes	Late	Large
HM58801	2019, 2020	F3	No	Yes	Late	Large
N6428	2019, 2020	F3	No	Yes	Late	Med/Lrg
HM5235	2019, 2020	F3	No	No	Late	Large
HM4909	2019, 2020	F2	Yes	No	Late	Large
UG4014	2020	F2	No	No	Early	Large
N6434	2020	F3	No	Yes	Late	Med/Lrg
SVTM9016	2020	F3	No	Yes	Late	Large
SVTM9012	2020	F3	No	No	Late	Large
SVTM9025	2020	F3	Yes	Yes	Late	Med/Lrg
HMPL1	2020	-	-	-	-	-
HMPL2	2020	-	-	-	-	-

[z] HMPL1 and HMPL2 were not commercially available at the time of this study, therefore no agronomic data was available.

[y] Fusarium wilt resistance (*I3* gene conferring resistance to *Fusarium oxyspoum* f. sp. *lycopersici*)

[x] Fusarium crown and root rot resistance (*Frl* gene conferring resistance to *Fusarium oxyspoum* f. sp. *radicis-lycopersici*)

[w] EFH Extended field holding capacity

[v] Early: 117 days or less to maturity; Late: 118 to approximately 145 days to maturity

Table 3. Timing of agronomic practices and data collection for 2019 and 2020 trials

Event	Dates, 2019	Dates, 2020
Pre-emergent herbicide		
Trifluralin as Treflan HFP	none	2/3/20
Oxyfluorfen as Goal 2XL	1/22/19	5/1/20
Transplants seeded	3/22/19	3/27/20
Transplants inoculated	5/8/19	5/13/20
Fields planted	5/7/19-5/9/19	5/12/20 - 5/15/20
Hand weeding starts	6/1/19	6/1/20
Fertilizer application		
Starter fertilizer (10-34-0)	5/29/19 - 6/26/19	6/10/20 - 6/23/20
Urea ammonium nitrate (UAN 32-0-0)	7/1/19 - 8/5/19	6/23/20 - 7/24/20
Potassium thiosulfate (KTS 0-0-25)	7/28/19	7/24/20 - 8/14/20
Insecticide application		
Imidacloprid as Macho 4.0 or Advise Four	8/9/19	7/15/20 - 8/14/20
Chlorantraniliprole as Coragen	none	8/14/20
Soil nutrient data collection	8/6/19	7/17/20
Canopy data collection		
~8 weeks preharvest	7/15/19	7/15/20
~6 weeks preharvest	7/22/19	7/29/20
~4 weeks preharvest	8/6/19	8/14/20
~2 weeks preharvest	none	8/28/20
Irrigation cut	8/19/19	8/24/20
Harvest data collection	9/9/19 - 9/13/19	9/14/20 - 9/18/20
Fruit collection for seed studies	10/3/19	8/3/20 - 9/8/20

Table 4. Results of field soil nutrient analysis for the *Fusarium falciforme* tolerance trial, conducted at the UC Davis Plant Pathology Research Station in 2019 and 2020

Year [z]	Field [y]	Trial Objective	pH [x]	EC (ppm) [w]	Nitrate Nitrogen (N) (ppm) [v]	Ammonium Nitrogen (N) (ppm) [v]	Phosphorus (P) (ppm) [u]	Potassium (K) (ppm) [t, s]	Calcium (Ca) (ppm) [t]	Magnesium (Mg) (ppm) [t]
2019	Non-Inoculated	1.1	6.9	0.3	4	3	8	175	2600	2050
2020	Inoculated	1.1, 1.2.1	7	0.4	9	3	6	160	2200	1260
	Inoculated (High); Field 1	1.2.2, 1.2.1, 2.2.2	-	-	41.2	-	21.1	347.5	-	-
	Naturally infested (Low)	1.2.2	-	-	16.2	-	7.3	226.5	-	-
	Field 2	2.2.2	-		14.8		10	223		

[z] In 2019, soil nutrient analysis was conducted by Perry Lab (Watsonville, CA). In 2020, soil nutrient analysis was conducted by the UC Davis Analytical Lab (Davis, CA).

[y] Field- 2019: Inoculated: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Non-inoculated: Plants were directly transplanted into a field with no history of *F. falciforme*. 2020: Inoculated / High Pathogen Pressure / Field 1: Plants were dip inoculated with *F. falciforme* at transplanting for two consecutive years. Naturally infested / Low Pathogen Pressure: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Field 2: plants were dip inoculated with *Fusarium falciforme* and planted into a field with inoculated with *Fusarium falciforme* and fumigated the previous year.

[x] pH was measured by saturated paste method.

[w] Electrical conductivity (EC) measured on the extract from the saturated paste.

[v] 2019: Ammonium and nitrate analyzed with a Timberline Ammonia Analyzer. 2020: Soil nitrate and extractable ammonium by flow injection analyzer method [SOP 312.3.].

[u] 2019: Phosphorus determined with molybdate complex. 2020: Extractable phosphorus analyzed using the Olsen method [SOP 340.3].

[t] 2019: Potassium, calcium and magnesium analyzed by AA spectrophotometer.

[s] 2020: Exchangeable potassium analyzed via displacement method [SOP 360.4].

RESULTS

Objective 1. Evaluating commercial cultivar performance under *Fusarium falciforme* pressure

1.1 Evaluation of selected commercial processing tomato cultivars for response to *F. falciforme*

Preliminary studies in 2018 indicate that while cultivars vary in disease severity and yield losses under pathogen pressure, no cultivars were completely resistant to *F. falciforme* and all develop some level of foot / stem rot and / or vine decline. Similarly, results of the 2019 trials revealed, 88%-100% of plants developed rot (foot, crown and / or stem rot) across all cultivars (**Table 5**). Given the high incidence of disease, this study aimed to evaluate *F. falciforme* tolerance in commercial cultivars by yield analyses. We define tolerance as when plants develop disease symptoms, but yield is not significantly affected. Significance can be defined by either statistical or economic thresholds. Thus, we compared yield performance based on the biomass of total fruit, red marketable fruit as well as percent damaged fruit.

To assess whether different yield metrics (total yield, total marketable red fruit, percent green fruit and percent damaged fruit) were influenced by *F. falciforme*, we conducted pairwise means comparisons using ANOVA. The effect of cultivar was significant for total fruit ($P = 0.004$), marketable red fruit ($P = 0.001$) and damaged fruit ($P < 0.001$), revealing differences in performance in the inoculated and non-inoculated field. In assessing tolerance, i.e., a lack of a significant reduction in yield between inoculated and non-inoculated treatments, most cultivars revealed some degree of tolerance based on statistical analyses (**Table 6**); HM3887 and H9663 were the exception, with significantly lower total fruit biomass under *F. falciforme* pressure. All

other cultivars were not significantly different and can be considered to show varying degrees of tolerance.

Growers face an economic threshold in which the field is abandoned if there is greater than a 40% yield reduction in marketable fruit (C. Swett pers. comm.). Based on this economic threshold, H5608, N6416, H9663, and HM3887 would have had economically significant reductions in marketable yield (range between 43% and 62% reduction) (**Table 7**) and H1428, HM58801, and HM5235 were less susceptible cultivars but still sustained a 33-38% yield reduction. In contrast, cultivars H8504, HM4909, H1776, HM58841 and N6428 were the most tolerant, ranging between 0% and 16% reduction in marketable yield (**Table 7**). Of note, HM58841 marketable yield was ~2 kg higher in the *F. falciforme* inoculated field than the non-inoculated field (**Table 6**).

Fusarium falciforme did not statistically influence the percent of damaged fruit overall ($P = 0.087$). However, there is an economic cut-off of ~3% damaged fruit, over which canneries will reject the load (C. Swett pers. comm.). Using this threshold as an indicator of economic impact, there were several cultivars which, in the *F. falciforme* treatment, had a 3% or greater increase in damaged fruit, compared to the non-inoculated field. Cultivars with an economically significant increase in damaged fruit included H1310, H9663 and N6416. All other cultivars did not have economically significant increases in percent damaged fruit (**Table 8**).

In combining all analyses from the 2019 trials, we define highly tolerant cultivars as those with consistently excellent yield performance, with less than 30% reduction in marketable fruit (to avoid borderline cases), no increase in damaged fruit, and with no statistically or economically significant differences between the *F. falciforme* and non-inoculated fields. Based on this analysis, H8504, HM4909, H1776, HM58841 and N6428 are all considered highly

tolerant (**Table 9**). Cultivars with moderate tolerance were those that typically did not suffer either statistically or economically significant impacts from *F. falciforme* but had moderate performance (with 33-38% yield reduction), which was close to that of poor performers (see below). H1428, HM58801, and HM5235 are all moderately tolerant based on this definition (**Table 9**). In contrast, susceptible (non-tolerant) cultivars typically suffered statistically and / or economically significant impacts under *F. falciforme* pressure based on one or more response variables. H1310, H5608, N6416, H9663, and HM3887 were not tolerant based on this definition.

To address interest from the breeding sector in assessing whether cultivars with resistance to either Fusarium wilt (F3 cultivars) or Fusarium crown and root rot (Fr cultivars) were resistant or tolerant to *F. falciforme*, we assessed if cultivar response (tolerance vs. susceptibility) to *F. falciforme* inoculation segregated by resistance status. Among the top performing cultivars (listed above), three are not F3 (H8504, HM4909, HM58841) and three are not Fr lines (H8504, HM58841, N6428). However, HM4909, the only Fr line included in this study, was classified as highly tolerant. Among the worst performers were two F3 cultivars (H1310, N6416) but none are Fr lines (**Table 2**).

In 2019, three Seminis cultivars, AB0311, DRI0319, and SVTM8011, were included in the cultivar response trial but were planted three weeks later and harvested prematurely (at the same time as the rest of the trial). Results are summarized in **appendix A**. Due to early harvest, there was a high percentage of green fruit (up to 48%), with more green fruit in the non-inoculated field ($P = 0.002$). Only DRI0319 did not have any yield reductions under *F. falciforme* pressure, indicating potential for tolerance.

To enable breeding efforts and to better understand phenotypes associated with tolerance performance, we evaluated disease severity among the same 13 cultivars to determine whether certain phenotypes corresponded with tolerance. Severity was quantified based on incidence of vine decline at different stages of crop development and incidence of rot from the foot (underground stem) into the crown (moderate progression) and the stem (advanced progression). Consistent recovery of *F. falciforme* from rotten stem lesions confirmed that rot symptoms were caused by *F. falciforme*.

Overall, cultivars did not differ significantly in vine decline incidence at five weeks preharvest (P = 0.209), vine decline at harvest (P = 0.103), rot development in foot, crown, or stem tissue (looking at percent of plants with any rot type) (P = 0.668), incidence of crown rot (P = 0.651), or stem rot (P = 0.907) in which all cultivars developed these symptoms to some degree (**Table 5**). This result was consistent with preliminary studies, indicating that commercially available cultivars evaluated to date do not show complete resistance to *F. falciforme.*

Under ideal conditions, tomato vines would be healthy five weeks preharvest, with dense canopies to covering ripening fruit. However, under *F. falciforme* pressure, premature vine decline and canopy collapse exposes the fruit and leads to sunburn and rot (**Fig. C**). At five weeks preharvest, we observed a range of incidence of vine decline, 1% to 17%, among the cultivars tested (**Table 5**). Cultivars with the greatest amount of vine decline were H1310, H9663, and N6416, with > 15% of plants dead and were considered susceptible (non-tolerant). In contrast, cultivars with < 5% decline were H1776, H5608, H8504, HM5235, and HM58841 (**Table 5**). The vines of these cultivars remained intact, despite having varying levels of foliar symptoms, such as necrotic flecking, chlorosis, and sometime leaf deformity (**Fig. 1**). These

cultivars were also rated as highly or moderately tolerant in the earlier study. Although statistically significant differences were not detected in vine decline incidence at five weeks preharvest (**Table 5**), vine decline incidence was 47% lower in the cultivars that were previously deemed tolerant than the susceptible cultivars (**Table 9**). At harvest, vine decline was 20% lower in the top-ranking cultivars.

Stem / crown rot caused by *F. falciforme* is unlikely to provide a phenotype for assessing tolerance. Regardless of cultivar, 87.5% to 100% of plants in the plot developed crown and / or stem rot. Nine cultivars had 100% incidence of rot within the plot. Incidence of crown rot slightly varied among cultivars and rot development ranged from 64.7% to 92.5% of plants within the plot (**Table 5**).

Incidence of stem rot, an indication of advanced *F. falciforme* progression, had the greatest variation among the cultivars tested, ranging from 24.4% to 73.3% (**Table 5**). H8504 and HM5235 had the lowest stem rot incidence (< 40% of plants). Cultivars with moderate incidence of stem rot (40% to 60% of plants) were H1776, H5608, H9663, HM3887, HM4909, HM58801, HM58841 and N6416. Cultivars with the highest incidence of stem rot were H1310, H1428 and N6428, all having < 60% incidence (**Table 5**).

1.2 Evaluating consistency of commercial cultivar response under variable *F. falciforme* pressure and across years

1.2.1 Evaluating consistency of the response of nine commercial cultivars to *F. falciforme* in 2019 and 2020

This trial aimed to evaluate consistency of performance of tolerance traits across years comparing performance of nine cultivars in 2019 and 2020. To evaluate yield performance, we compared production of total fruit biomass, red marketable fruit, percent green fruit as well as percent damaged fruit (**Table 10A, 10B**). The effect of year was significant for total fruit ($P < 0.001$), marketable red fruit ($P < 0.001$), percent green fruit ($P < 0.001$), and damaged fruit ($P < 0.001$), reflecting differences in yield performance between 2019 and 2020.

Comparing years, in 2020 there was significantly greater total fruit biomass ($P < 0.001$) and red marketable fruit ($P < 0.001$) than in 2019 overall. Among all the cultivars, the average amount of fruit produced in 2019 was 18.1 kg ± 1.0 kg, compared with 60.3 kg ± 2.2 kg produced in 2020. In 2019, the best performing cultivars, with over 20 kg of total fruit produced, were H8504, HM58841 and N6428. In 2020, the top performers were H1776, H5608, HM58801 and N6428; all of which produced > 60 kg of fruit. Thus, N6428 consistently was a top performer in both years (**Table 10A**).

The percent damaged fruit was higher in 2019 compared with 2020, with 14.4% ± 1.5% and 7.8% ± 1.1%, respectively ($P < 0.001$) (**Table 10B**). Cultivars that consistently had > 10% damaged fruit across both years were HM3887, HM4909, HM5235, and HM58841 (**Table 10B**). In contrast, H1776 and H8504 had < 10% damaged fruit in both years. Although there was a significant effect of year on the amount of green fruit ($P < 0.001$), a significant cultivar x year interaction was not observed ($P = 0.220$), allowing both years' data to be combined (**Table 11**).

Between years, cultivar did not influence the percent of green fruit produced (P = 0.975), averaging 13.1% ± 1.6% of the total yield (**Table 11**).

1.2.2 Evaluating consistency of the response of commercial cultivars at low and high *F. falciforme* pressure in 2020

This trial evaluated the consistency of plant performance (yield and foot / stem rot incidence) of the 13 cultivars between two sites: an artificially infested field with high *F. falciforme* pressure and a naturally infested field with low-pressure. Overall, there was significant variation in total fruit (P = 0.025) and marketable red fruit between fields (P < 0.001) (**Table 12**). Plants in the low-pressure field produced an average of 55.0 kg ± 2.0 kg, whereas those in the high-pressure field averaged 61.7 kg ± 2.0 kg. Green fruit yield was also significantly greater in the low-pressure field for all but two cultivars (H5608 and HMPL1) (P < 0.001) (**Table 12**). H5608 and HMPL1 green fruit development was not differentially affected by field and these two cultivars had the lowest green fruit levels in the low-pressure field.

Varying the *F. falciforme* pressure did not influence the percent of damaged fruit across all cultivars combined (P = 0.360) (**Table 12**). However, cultivar HMPL2 had significantly more damaged fruit in the high- vs. the low-pressure field (P < 0.05). The percent of damaged fruit that was sunburned was significantly greater in the high-pressure field (P < 0.001), with 19.9 and 8.7% sunburn in the high- and low-pressure fields, respectively. Similarly, the percent fruit with blossom end rot was also higher from the high pathogen pressure field (P = 0.012) (**Table 13**).

Foot / stem rot symptoms were observed on all cultivars in both fields at consistently high levels (> 80% of the plants). Plants in the low pathogen pressure field ranged from 81.5% to 100% incidence of foot / stem rot, with an average of 92.6% ± 1.8%. The high-pressure field had

statistically similar levels of foot / stem rot (P = 0.590) as the low-pressure field, averaging 94.0% ± 1.7% rot incidence (**Table 14**).

Looking at cultivar performance in both fields, HMPL2 had the greatest percentage of damaged red fruit, differing from one cultivar (SVTM9025) in the low-pressure field and for eight cultivars in the high-pressure field (P < 0.05). There were no other statistically significant differences among cultivars, but based on economic threshold of 3% damaged fruit, only H1776 and SVTM9025 had less than 3% fruit damage (low-pressure field only). N6428 (high-pressure) and N6434 (low-pressure) had less than 4% damage and are considered borderline cases. There were no differences in cultivars based on total or marketable fruit; top performers included N6428, H1776, H5608, and HM58801, all had > 60 kg marketable red fruit per 2.7 m harvest plot. Taken together, this study indicates that N6428 and H1776 were the top performers based on yield and damaged fruit metrics, although SVTM9025 and N6434 were also strong performers with low fruit loss and moderate yields (> 50 kg / harvest plot).

Objective 2. Evaluating the impact of *Fusarium falciforme* on tomato seed

2.1 Assessing *F. falciforme* colonization of tomato seed

Because *F. falciforme* has systemic effects on plant growth (Del Castillo *et. al.,* in progress), this study was designed to determine if that the fungus was capable of systemic movement in the plant, leading to seed colonization. In 2020 we tested two cultivars, HM3887 and HM58841 in two *F. falciforme*-infested fields (field 1 and 2). In addition, a third cultivar, HM4909, was evaluated in field 1 only. We did not detect *F. falciforme* colonization of tomato seed at a 0.0002% detection threshold (**Table 15**). We did observe seed colonization by other fungi including non-pathogenic *Fusarium oxysporum*, *Aspergillus spp*., *Monocillium loricatum, Talaromyces spp. Chaetomium concolutum,* and *Collariella bosyrychodes,* indicating that our

assay detected fungal colonization. Overall, fungal colonization was greater in field 2 (0.3% ± 0.2%) compared with field 1 (0.1% ± 0.1%) and in HM58841 compared to other cultivars (**Table 15**).

2.2 Assessing the impact of *F. falciforme* on tomato seed viability

2.2.1 Assessing the impact of *F. falciforme* on tomato seed viability across 16 commercial cultivars

In these trials, the viability of seeds from commercially available tomato cultivars was evaluated to determine *Fusarium falciforme* impact. *Fusarium falciforme* infection was consistently recovered in a subset of plants, and no other pathogens were present (as part of analyses described above). Analysis of *F. falciforme* treatment effect on seed viability was based on a comparison to the baseline germination rates of non-inoculated plants, which was evaluated for all cultivars in a single replicated field trial in 2019. Baseline rates are reported in **Table 16** and ranged from 50-94%. In 2019, we tested 16 cultivars in a single field and observed a range in cultivar response to the *F. falciforme* treatment (**Table 17**). Seven cultivars had a reduction in seed germination ranging from 2.3% to 18.2% in *F. falciforme* infected vs non-infected plants. In contrast, eight cultivars had increased germination rates ranging from 2.7% to 31.0% when the plants were inoculated with *F. falciforme.* The germination rate of one cultivar, H9663, was unaffected when the pathogen was present.

2.2.2 Assessing the impact of *F. falciforme* on tomato seed viability in a repeated trial

In 2020, two cultivars, HM3887 and HM58841, were tested in two replicated inoculated fields (with the addition of HM4909 in field 1). Analysis of *F. falciforme* impact on seed viability was based on comparing viability of seeds collected from artificially inoculated tomato plants to baseline germination rates (of non-infested tomato plants), as in the previous trial. The three cultivars, HM3887, HM4909, and HM58841, range in their susceptibility to *Fusarium falciforme* from highly susceptible to tolerant, as reported in the 2019 *F. falciforme* cultivar response trial. In both fields, seeds collected from fruits of the highly susceptible HM3887 had reduced germination. HM58841, classified as tolerant in the 2019 trial, showed no difference in germination in field 1 and had a slight increase in field 2. Germination for HM4909, a highly tolerant cultivar, was only tested in field 1 and had a 54% reduction when compared to the baseline (**Table 18**).

Three cultivars, HM3887, HM58841, and HM4909 were tested in the same location in 2019 and 2020. HM3887 and HM4909 were not consistent across years, having a slight increase in germination in 2019 to a > 50% reduction (52.0% and 57.1%, respectively) in 2020. However, HM58841 varied slightly shifting from a +7.0% difference to
-1.2% difference in 2019 and 2020, respectively (**Table 19**).

Figure 1. (A) Necrotic flecking and chlorosis on a leaflet observed in *Fusarium falciforme* inoculated field. **(B)** Necrotic flecking on a tomato branch, indicating early signs of canopy decline. **(C)** Leaf deformity and severe chlorosis / bleaching.

Figure 2. Advanced canopy decline observed in *Fusarium falciforme* inoculated field.

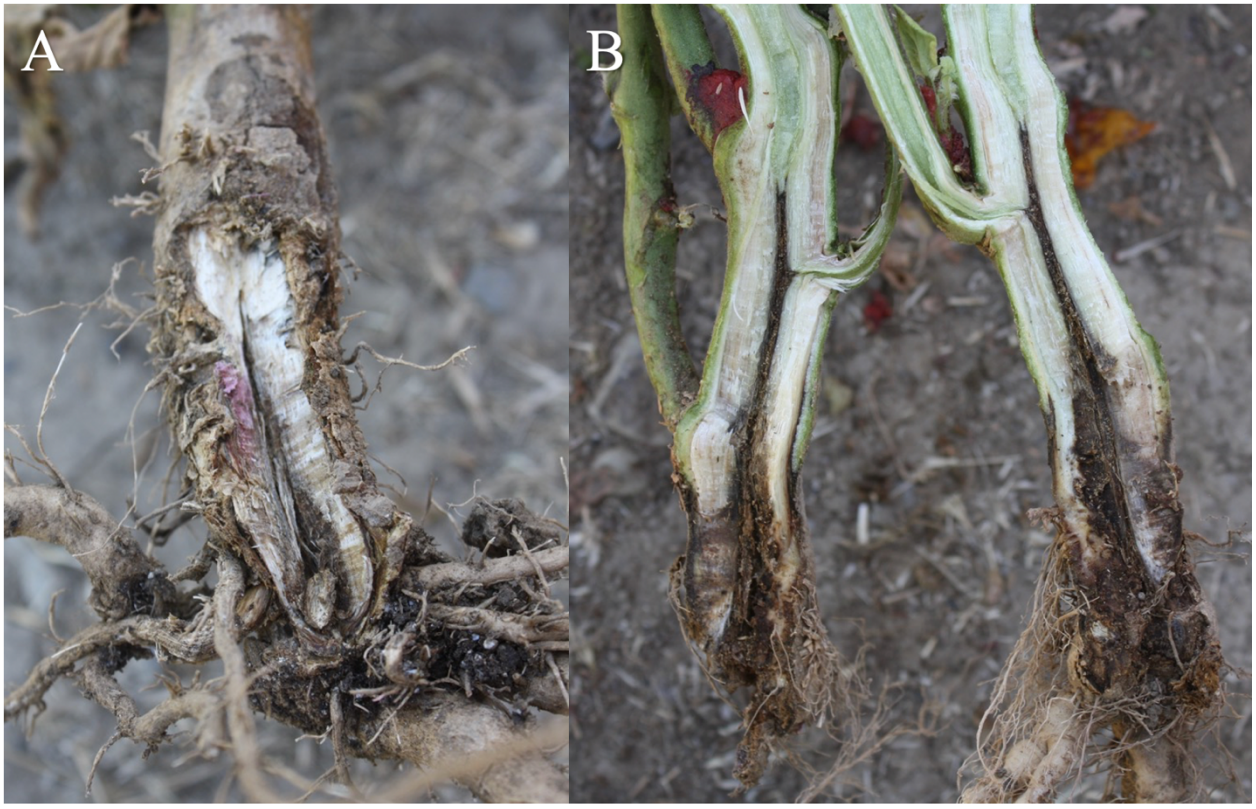

Figure 3. (A) Foot rot observed in the *Fusarium falciforme* inoculated field. **(B)** Plants exhibiting crown and stem rot in a field inoculated with *F. falciforme*.

Figure 4. (A) Marketable red fruit. **(B)** Green Fruit. **(C)** Damaged fruit with sunburn. **(D)** Damaged fruit with blossom end rot.

Table 5. Disease development in commercial cultivars grown in a *Fusarium falciforme*-treated field in 2019 [z]

	% Vine Decline Incidence		% Rot Incidence [w, v, u]		
Cultivar	**5 Weeks Preharvest [y]**	**Harvest [x]**	**Any Rot (Foot, Crown, and / or Stem)**	**Crown Rot**	**Stem Rot**
H1310	15.1 ± 2.4 a	72.1 ± 9.3 a	97.5 ± 2.5 a	87.5 ± 1.8 a	65.7 ± 12.8 a
H1428	7.0 ± 7.0 a	57.6 ± 9.8 a	100.0 ± 0.0 a	92.5 ± 7.5 a	73.3 ± 13.1 a
H1776	2.0 ± 1.2 a	45.8 ± 9.8 a	87.9 ± 12.1 a	83.1 ± 13.1 a	53.5 ± 20.9 a
H5608	1.0 ± 1.0 a	59.7 ± 20.5 a	100.0 ± 0.0 a	87.4 ± 7.9 a	58.6 ± 22.8 a
H8504	1.0 ± 1.0 a	28.3 ± 9.9 a	87.5 ± 12.5 a	71.3 ± 12.5 a	24.4 ± 4.0 a
H9663	17.0 ± 6.0 a	85.1 ± 5.3 a	100.0 ± 0.0 a	78.4 ± 13.2 a	56.4 ± 20.4 a
HM3887	10.0 ± 7.6 a	42.6 ± 17.3 a	92.7 ± 7.4 a	77.9 ± 11.1 a	52.4 ± 10.9 a
HM4909	10.0 ± 4.8 a	52.7 ± 15.2 a	100.0 ± 0.0 a	73.5 ± 13.5 a	54.6 ± 13.1 a
HM5235	3.0 ± 3.0 a	50.3 ± 8.1 a	100.0 ± 0.0 a	64.6 ± 14.1 a	37.2 ± 7.3 a
HM58801	7.1 ± 3.1 a	41.1 ± 9.1 a	100.0 ± 0.0 a	67.4 ± 5.6 a	57.9 ± 2.0 a
HM58841	2.0 ± 1.2 a	60.5 ± 6.0 a	100.0 ± 0.0 a	91.5 ± 7.1 a	56.8 ± 6.6 a
N6416	15.4 ± 5.9 a	78.1 ± 8.6 a	100.0 ± 0.0 a	92.4 ± 3.2 a	50.0 ± 21.5 a
N6428	10.0 ± 6.6 a	55.5 ± 18.9 a	100.0 ± 0.0 a	75.9 ± 19.3 a	63.0 ± 22.9 a
P-value cultivar	0.209	0.103	0.668	0.651	0.907

[z] Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*.

[y] Percent of 25 plants in decline 5 weeks preharvest ± standard error (n = 4).

[x] Percent of plants in decline at harvest ± standard error within a 2 m harvest plot (7-29 plants) (n = 4).

[w] Percent of plants with rot ± standard error within a 2 m harvest plot (7-29 plants); any rot includes foot, crown and / or stem rot (n = 4).

[v] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 4).

[u] ANOVA and means comparison conducted on arcsine-square root transformed data.

Table 6. Yield comparison of commercially available cultivar performance in the absence and presence of *Fusarium falciforme* foot rot and vine decline (2019) [z]

	Kg Fruit [y]		Percent of Total [x, w]	
	Total Fruit	Marketable Red Fruit	% Green Fruit [v]	% Damaged [u]
Non-Inoculated				
H1310	23.2 ± 0.5 abcdefgh	13.9 ± 1.4 abcde	26.6 ± 4.3 a	19.0 ± 2.9 abc
H1428	28.8 ± 0.9 fgh	17.1 ± 0.6 cde	26.5 ± 3.5 a	18.5 ± 3.7 abc
H1776	25.4 ± 1.3 defgh	14.2 ± 1.8 abcde	23.7 ± 3.0 a	27.5 ± 4.3 abcd
H5608	28.5 ± 2.8 fgh	17.9 ± 1.4 de	19.7 ± 4.4 a	20.7 ± 4.0 abcd
H8504	29.3 ± 2.4 gh	19.7 ± 2.0 e	20.8 ± 2.3 a	15.0 ± 3.8 abc
H9663	25.6 ± 1.3 defgh	12.9 ± 0.3 abcde	20.8 ± 3.0 a	36.0 ± 2.3 cd
HM3887	29.8 ± 3.3 h	17.2 ± 2.2 cde	17.3± 3.0 a	30.4 ± 3.7 cd
HM4909	25.0 ± 2.7 cdefgh	13.3 ± 1.4 abcde	26.4 ± 1.4 a	27.0 ± 5.5 abcd
HM5235	22.0 ± 2.2 abcdefgh	13.1 ± 2.3 abcde	17.9 ± 3.7 a	29.1 ± 2.2 bcd
HM58801	23.5 ± 1.6 bcdefgh	14.8 ± 0.4 abcde	16.6 ± 1.9 a	23.8 ± 2.7 abcd
HM58841	20.3 ± 1.9 abcdefgh	13.4 ± 1.6 abcde	12.9 ± 3.3 a	24.5 ± 3.6 abcd
N6416	23.1 ± 2.1 abcdefgh	14.7 ± 0.6 abcde	11.9 ± 2.1 a	26.6 ± 4.3 abcd
N6428	28.1 ± 1.9 efgh	18.4 ± 1.8 de	19.5 ± 2.0 a	19.0 ± 2.7 abc
Inoculated				
H1310	10.3 ± 0.6 a	6.8 ± 0.5 ab	10.3 ± 2.3 a	26.7 ± 2.7 abcd
H1428	17.3 ± 2.0 abcdefgh	10.6 ± 1.2 abcde	23.3 ± 2.9 a	20.0 ± 2.5 abc
H1776	16.7 ± 1.5 abcdefg	12.5 ± 1.5 abcde	17.2 ± 6.2 a	9.6 ± 3.1 ab
H5608	17.2 ± 2.3 abcdefgh	10.2 ± 1.6 abcde	24.2 ± 10.7 a	19.3 ± 4.8 abc
H8504	23.6 ± 0.6 cdefgh	16.7 ± 1.7 bcde	22.7 ± 7.2 a	8.5 ± 1.1 a
H9663	10.6 ± 2.3 ab	4.9 ± 1.2 a	15.8 ± 4.5 a	45.4 ± 4.1 d
HM3887	14.4 ± 3.3 abcd	9.1 ± 2.6 abcd	23.6 ± 3.2 a	26.4 ± 9.3 abcd
HM4909	17.3 ± 2.4 abcdefgh	10.9 ± 1.7 abcde	25.0 ± 9.2 a	14.8 ± 1.5 abc
HM5235	15.5 ± 1.4 abcde	8.7 ± 1.0 abcd	20.8 ± 7.9 a	26.9 ± 6.9 abcd
HM58801	16.0 ± 0.4 abcdef	9.3 ± 1.0 abcd	27.5 ± 7.9 a	19.7 ± 1.9 abc
HM58841	22.1 ± 6.4 abcdefgh	14.0 ± 5.1 abcde	22.3 ± 9.7 a	19.8 ± 6.9 abc
N6416	12.3 ± 2.5 abc	7.3 ± 1.7 abc	9.3 ± 7.2 a	34.4 ± 6.0 cd
N6428	20.3 ± 3.3 abcdefgh	15.3 ± 3.0 bcde	9.4 ± 0.4 a	17.6 ± 3.3 abc
P-value cultivar	0.004	< 0.001	0.140	< 0.001
P-value pathogen	< 0.001	< 0.001	0.341	0.087
P-value cultivar x pathogen	0.064	0.332	0.355	0.053

[z] Inoculated field: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Non-inoculated field: Plants were directly transplanted into a field with no history of *F. falciforme*.

[y] Average total fruit biomass harvested and the total biomass of marketable, red fruit ± standard error within the 2 m plot (n = 4).

[x] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 4).

[w] ANOVA and means comparison conducted on arcsine-square root transformed data.

[v] Percent green fruit out of total fruit (red, green, and damaged) ± standard error within the 2 m plot (n = 4).

[u] Percent of total red fruit that was damaged (sunburn, rot, or blossom end rot) ± standard error within the 2 m plot (n = 4).

Table 7. Percent reduction in yield of commercial cultivars grown in the *Fusarium falciforme*-treated field, compared to the non-inoculated field in 2019 [z]

	Percent Reduction [y]	
Cultivars [x]	**Total Fruit** [w]	**Marketable Red Fruit** [v, u]
H1310	55.6%	51.3% *
H1428	39.9%	38.3%
H1776	35.3%	12.2%
H5608	39.7%	43.2% *
H8504	19.4%	15.2%
H9663	58.6%	62.1% *
HM3887	51.7%	47.4% *
HM4909	31.0%	18.2%
HM5235	29.6%	33.3%
HM58801	32.0%	37.3%
HM58841	0.0%	0.0%
N6416	46.7%	50.5% *
N6428	28.0%	16.9%

[z] Inoculated field: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Non-inoculated field: Plants were directly transplanted into a field with no history of *F. falciforme*.

[y] If yield was not reduced, zero value was used to indicate no reduction.

[x] Cultivars were selected among the top 30 grown commercially available cultivars in California for 2018.

[w] Percent reduction in total fruit biomass = 1 – (Inoculated total fruit biomass / non-inoculated total fruit biomass).

[v] Percent reduction in marketable red fruit = 1 – (Inoculated marketable red fruit biomass / non-inoculated marketable red fruit biomass).

[u] * indicates that the cultivar exceeded the economic threshold of 40% yield reduction in marketable fruit which determines whether the field is abandoned (C. Swett, pers. comm.).

Table 8. Percent difference of damaged fruit among commercial cultivars grown in the *Fusarium falciforme*-treated field, compared to the non-inoculated field in 2019 [z]

	Percent Difference [y]	
Cultivars [x]	**Green Fruit** [w]	**Damaged** [v]
H1310	0.0%	7.8% [u]
H1428	0.0%	1.5%
H1776	0.0%	0.0%
H5608	4.5%	0.0%
H8504	1.9%	0.0%
H9663	0.0%	9.4% [u]
HM3887	6.3%	0.0%
HM4909	0.0%	0.0%
HM5235	2.9%	0.0%
HM58801	10.9%	0.0%
HM58841	9.5%	0.0%
N6416	0.0%	7.8% [u]
N6428	0.0%	0.0%

[z] Inoculated field: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Non-inoculated field: Plants were directly transplanted into a field with no history of *F. falciforme*.

[y] A decrease in green fruit or damaged fruit in the inoculated treatment compared to the non-inoculated treatment is denoted as a 0% difference.

[x] Cultivars were selected among the top 30 grown commercially available cultivars in California for 2018.

[w] Percent difference green fruit = (% non-inoculated green fruit - % inoculated green fruit).

[v] Percent difference damaged fruit = (% non-inoculated damaged fruit - % inoculated damaged fruit).

[u] Indicates that the cultivar exceeded the economic threshold of 3% increase in damaged fruit which determines whether the load is rejected (C. Swett, pers. comm.).

Table 9. Comparison of commercial cultivar performance under *Fusarium falciforme* pressure in 2019 [z]

	Performance Category		Yield Impacts		Disease Incidence		
Cultivar	***F. falciforme* Tolerance Classification** [y]	**Disease Severity** [x]	**Yield Reduction** [w]	**Damaged Fruit Increase?** [v]	**Vine Decline 5 Weeks Preharvest** [u]	**Vine Decline at Harvest** [t]	**Stem Rot Incidence** [s]
H1310	Susceptible	Severe	High	Yes	High	High	High
H1428	Moderately Tolerant	Severe	Moderate	No	Moderate	Moderate	High
H1776	Highly Tolerant	Moderate	Low	No	Low	Moderate	Moderate
H5608	Susceptible	Severe	High	No	Low	Moderate	Moderate
H8504	Highly Tolerant	Low	Low	No	Low	Low	Low
H9663	Susceptible	Severe	High	Yes	High	High	Moderate
HM3887	Susceptible	Moderate	High	No	Moderate	Moderate	Moderate
HM4909	Highly Tolerant	Severe	Low	No	Moderate	Moderate	Moderate
HM5235	Moderately Tolerant	Moderate	Moderate	No	Low	Moderate	Low
HM58801	Moderately Tolerant	Moderate	Moderate	No	Moderate	Moderate	Moderate
HM58841	Highly Tolerant	Severe	Low	No	Low	High	Moderate
N6416	Susceptible	Severe	High	Yes	High	High	Moderate
N6428	Highly Tolerant	Severe	Low	No	Moderate	Moderate	High

[z] Inoculated field: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Non-inoculated field: Plants were directly transplanted into a field with no history of *F. falciforme*.

[y] Tolerance classification- Highly tolerant: less than 30% reduction in yield and did not increase in the amount of damaged fruit under *F. falciforme*. Moderately tolerant: 33%-38% reduction in yield and did not increase in the amount of damaged fruit under *F. falciforme*. Susceptible: greater than 40% reduction in yield and greater than 3% damaged fruit under *F. falciforme*.

[x] Disease Severity- Low: less than 40% vine decline incidence and less than 40% stem rot incidence at harvest. Moderate: 40%-50% vine decline incidence and greater than 40%-60% stem rot incidence at harvest. Severe: greater than 50% vine decline incidence and greater than 60% stem rot incidence at harvest.

[w] Yield Reduction- Low: 0%-16% reduction in yield under *F. falciforme*. Moderate: 33%-38% reduction in yield under *F. falciforme*. High: greater than 40% yield reduction under *F. falciforme*.

[v] Damage fruit increase: “Yes” indicated that the cultivar had more than a 3% increase in damaged fruit under *F. falciforme* pressure, exceeding the economic threshold wherein a load is rejected.

[u] Vine decline incidence five weeks preharvest- Low: less than 5% vine decline. Moderate: 5%-15% vine decline. High: greater than 15% vine decline.

[t] Vine decline incidence- Low: less than 40% vine decline at harvest. Moderate: 40%-60% vine decline at harvest. High: greater than 60% vine decline at harvest.

[s] Stem rot incidence- Low: less than 40% stem rot at harvest. Moderate: 40%-60% stem rot at harvest. High: greater than 60% stem rot at harvest.

Table 10A. Comparison of cultivar response to *Fusarium falciforme*-treated fields in 2019 and 2020 [z]

	Kg Fruit [y, x]			
	Total Fruit		Marketable Red Fruit	
Cultivar	**2019**	**2020**	**2019**	**2020**
H1776	16.7 ± 1.5 a	64.3 ± 6.0 de	12.5 ± 1.5 a	60.2 ± 5.6 c
H5608	17.2 ± 2.3 a	67.5 ± 6.1 de	10.2 ± 1.6 a	62.1 ± 5.3 c
H8504	23.6 ± 0.56 abc	52.8 ± 6.6 de	16.7 ± 1.7 ab	47.2 ± 6.2 c
HM3887	14.4 ± 3.3 a	51.1 ± 4.3 cde	9.1 ± 2.6 a	42.2 ± 4.3 bc
HM4909	17.3 ± 2.4 a	54.6 ± 6.4 de	10.9 ± 1.7 a	46.7 ± 6.1 c
HM5235	15.5 ± 1.4 a	54.4 ± 13.0 de	8.7 ± 1.0 a	47.3 ± 13.6 c
HM58801	16.0 ± 0.4 a	70.7 ± 4.5 de	9.3 ± 1.0 a	63.1 ± 2.9 c
HM58841	22.1 ± 6.4 ab	49.4 ± 6.5 bcd	14.0 ± 5.1 a	43.7 ± 6.5 c
N6428	20.3 ± 3.3 a	77.5 ± 6.5 e	15.3 ± 3.0 a	65.2 ± 6.5 c
P-value cultivar	0.106		0.091	
P-value year	< 0.001		< 0.001	
P-value cultivar x year	0.064		0.114	

[z] Plants were artificially inoculated with *F. falciforme* at transplanting.

[y] Average total fruit biomass harvested and the total biomass of marketable, red fruit ± standard error within the 2 m (2019) and 2.7 m (2020) plots (n = 4).

[x] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 8 for both trials combined).

Table 10B. Comparison of cultivar response to *Fusarium falciforme*-treated fields in 2019 and 2020 [z]

	Percent of Total [y, x]			
	% Green Fruit [w]		% Damaged Fruit [v]	
Cultivar	**2019**	**2020**	**2019**	**2020**
H1776	17.2 ± 6.2 abcd	1.9 ± 0.5 ab	7.6 ± 2.0 abc	4.4 ± 0.8 abc
H5608	24.2 ± 10.7 bcd	3.5 ± 1.0 abcd	15.1 ± 4.7 abc	4.2 ± 1.4 ab
H8504	22.7 ± 7.2 abcd	3.5 ± 0.4 abcd	6.6 ± 1.1 abc	7.3 ± 1.4 abc
HM3887	23.6 ± 3.2 cd	6.0 ± 1.9 abcd	19.5 ± 6.0 bc	11.4 ± 4.8 abc
HM4909	25.0 ± 9.2 bcd	4.9 ± 1.4 abcd	11.3 ± 2.4 abc	10.1 ± 3.1 abc
HM5235	20.8 ± 7.9 abcd	1.4 ± 0.4 a	22.9 ± 7.0 c	15.3 ± 6.0 abc
HM58801	27.5 ± 7.9 d	5.8 ± 2.8 abcd	14.4 ± 2.3 abc	4.5 ± 0.8 abc
HM58841	22.3 ± 9.7 abcd	2.4 ± 0.7 abc	16.6 ± 5.9 abc	10.0 ± 3.5 abc
N6428	9.4 ± 0.4 abcd	13.4 ± 3.7 abcd	16.0 ± 3.1 abc	2.7 ± 0.6 a
P-value cultivar	0.828		0.018	
P-value year	< 0.001		< 0.001	
P-value cultivar x year	0.220		0.335	

[z] Plants were artificially inoculated with *F. falciforme* at transplanting for two consecutive years.

[y] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 8 for both trials combined).

[x] ANOVA and means comparison conducted on arcsine-square root transformed data.

[w] Percent green fruit out of total fruit (red, green, and damaged) ± standard error within the 2 m (2019) and 2.7 m (2020) plots (n = 4).

[v] Percent of total red fruit that was damaged (sunburn, rot, or blossom end rot) ± standard error within the plots (n = 4).

Table 11. Yields for processing tomato cultivars grown in a *Fusarium falciforme*-treated field in 2019 and 2020 (combined) [z]

	Kg Fruit [y]		Percent of Total [x, w]	
	Total Fruit	Marketable Red Fruit	% Green Fruit [v]	% Damaged Fruit [u]
Cultivar	**Years combined**	**Years combined**	**Years combined**	**Years combined**
H1776	40.5 ± 9.4 a	36.3 ± 9.4 a	9.5 ± 4.1 a	6.0 ± 1.2 a
H5608	42.4 ± 10.0 a	36.1 ± 10.2 a	13.9 ± 6.3 a	9.6 ± 3.1 a
H8504	38.2 ± 6.3 a	32.0 ± 6.5 a	13.1 ± 4.9 a	6.9 ± 0.8 a
HM3887	32.7 ± 7.4 a	25.7 ± 6.7 a	14.8 ± 3.7 a	15.4 ± 3.9 a
HM4909	35.9 ± 7.7 a	28.8 ± 7.4 a	15.0 ± 5.7 a	10.7 ± 1.8 a
HM5235	35.0 ± 9.5 a	28.0 ± 9.6 a	11.1 ± 5.2 a	19.1 ± 4.5 a
HM58801	43.4 ± 10.6 a	36.2 ± 10.3 a	16.7 ± 5.6 a	9.5 ± 2.2 a
HM58841	35.8 ± 6.7 a	28.8 ± 6.8 a	12.4 ± 5.9 a	13.3 ± 3.4 a
N6428	48.9 ± 11.3 a	40.2 ± 10.0 a	11.4 ± 1.9 a	9.4 ± 2.9 a
P-value cultivar	0.954	0.953	0.975	0.061

[z] Plants were artificially inoculated with *F. falciforme* at transplanting.

[y] Average total fruit biomass harvested and the total biomass of marketable, red fruit ± standard error within the 2 m (2019) and 2.7 m (2020) plots (n = 8).

[x] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 8 for both trials combined).

[w] ANOVA and means comparison conducted on arcsine-square root transformed data.

[v] Percent green fruit out of total fruit (red, green, and damaged) ± standard error within the plot (n = 8).

[u] Percent of total red fruit that was damaged (sunburn, rot, or blossom end rot) ± standard error within the plot (n = 8).

Table 12. Cultivar performance in two fields representing low and high levels of *Fusarium falciforme* pressure (2020)

	Kg Fruit [y]		Percent of Total [x, w]	
Cultivar [z]	**Total Fruit**	**Marketable Red Fruit**	**% Green Fruit [v]**	**% Damaged [u]**
Low Pathogen Pressure				
H1776	60.5 ± 3.9 a	41.6 ± 6.5 abc	30.3 ± 7.1 defg	2.5 ± 0.8 ab
H5608	72.1 ± 7.1 a	56.5 ± 7.1 abc	17.6 ± 2.8 bcdef	5.7 ± 0.2 ab
HM4909	51.7 ± 9.0 a	32.6 ± 5.9 abc	27.7 ± 6.6 cdefg	11.8 ± 6.9 ab
HM5235	55.3 ± 13.7 a	33.5 ± 8.9 abc	30.2 ± 5.1 defg	13.3 ± 4.3 abc
HM58801	49.3 ± 4.5 a	31.1 ± 3.6 abc	34.2 ± 2.9 efgh	4.8 ± 0.7 ab
HMPL1	51.0 ± 9.0 a	42.9 ± 5.7 abc	10.8 ± 3.4 abcde	5.0 ± 0.7 ab
HMPL2	52.7 ± 11.4 a	28.5 ± 10.4 abc	35.0 ± 7.3 defgh	20.1 ± 4.1 bc
N6428	54.4 ± 7.7 a	23.2 ± 5.8 a	57.0 ± 6.5 h	4.8 ± 1.8 ab
N6434	63.1 ± 6.5 a	32.2 ± 4.4 abc	47.4 ± 3.6 gh	3.6 ± 0.8 ab
SVTM9012	48.5 ± 5.0 a	31.3 ± 5.4 abc	32.1 ± 6.4 defg	6.4 ± 1.6 ab
SVTM9016	60.8 ± 6.1 a	32.6 ± 5.9 abc	40.0 ± 4.9 fgh	13.5 ± 2.6 abc
SVTM9025	45.1 ± 7.7 a	25.2 ± 5.7 ab	44.5 ± 5.0 gh	2.2 ± 0.5 a
UG4014	50.4 ± 7.5 a	35.9 ± 8.4 abc	28.0 ± 8.7 cdefg	4.8 ± 2.0 ab
High Pathogen Pressure				
H1776	64.3 ± 6.0 a	60.2 ± 5.6 abc	1.8 ± 0.5 a	4.5 ± 0.9 ab
H5608	67.5 ± 6.1 a	62.1 ± 5.3 bc	3.5 ± 1.0 ab	4.4 ± 1.5 ab
HM4909	54.6 ± 6.4 a	46.7 ± 6.1 abc	4.9 ± 1.5 ab	10.5 ± 3.0 abc
HM5235	54.4 ± 13.0 a	47.3 ± 13.6 abc	1.4 ± 0.4 a	15.5 ± 5.9 abc
HM58801	70.7 ± 4.5 a	63.1 ± 2.9 c	5.8 ± 2.8 ab	4.8 ± 0.9 ab
HMPL1	53.0 ± 4.7 a	44.7 ± 3.3 abc	4.5 ± 1.3 ab	11.1 ± 3.4 abc
HMPL2	52.3 ± 10.6 a	36.4 ± 10.2 abc	9.2 ± 3.3 abc	28.1 ± 9.6 c
N6428	77.5 ± 6.5 a	65.2 ± 6.5 c	13.4 ± 3.7 abcd	3.2 ± 0.7 ab
N6434	58.7 ± 8.9 a	51.7 ± 7.6 abc	6.0 ± 1.7 ab	6.0 ± 1.9 ab
SVTM9012	59.8 ± 8.4 a	51.2 ± 6.9 abc	3.3 ± 0.4 ab	11.0 ± 2.0 abc
SVTM9016	62.9 ± 7.8 a	57.7 ± 7.6 abc	3.6 ± 1.7 a	5.1 ± 1.4 ab
SVTM9025	61.6 ± 8.7 a	57.1 ± 8.4 abc	2.9 ± 0.7 a	4.6 ± 0.9 ab
UG4014	65.0 ± 8.0 a	59.4 ± 8.2 abc	2.2 ± 0.7 a	7.2 ± 2.7 ab
P-value cultivar	0.362	0.070	< 0.001	< 0.001
P-value pathogen	0.025	< 0.001	< 0.001	0.360
P-value cultivar x pathogen	0.692	0.279	0.005	0.410

[z] Low pathogen pressure field was naturally infested with *F. falciforme*. High pathogen pressure field was artificially infested by dip inoculating transplants with *F. falciforme* for two consecutive years.

[y] Average total fruit biomass harvested and the total biomass of marketable, red fruit ± standard error (n = 4).

[x] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 4).

[w] ANOVA and means comparison conducted on arcsine-square root transformed data.

[v] Percent green fruit out of total fruit (red, green, and damaged) ± standard error within the plot (n = 4).

[u] Percent of total red fruit that was damaged (sunburn, rot, or blossom end rot) ± standard error within the plot (n = 4).

Table 13. Cultivar performance in two fields representing low and high levels of *Fusarium falciforme* pressure (2020)

	Percent of Total Damaged Fruit [y, x]		
Cultivar [z]	**% Sunburn**	**% Fruit Rot**	**% Blossom End Rot**
Low Pathogen Pressure			
H1776	13.0 ± 6.7 a	59.4 ± 6.5 abcd	27.6 ± 3.5 abcd
H5608	2.5 ± 1.7 a	84.6 ± 1.8 cd	12.9 ± 2.1 ab
HM4909	7.6 ± 7.6 a	43.9 ± 17.2 abcd	48.5 ± 19.8 abcd
HM5235	6.5 ± 4.7 a	50.4 12.4 abcd	43.1 ± 15.0 abcd
HM58801	3.4 ± 3.4 a	63.9 ± 7.7 abc	32.7 ± 9.1 abcd
HMPL1	5.2 ± 5.2 a	59.9 ± 2.2 abcd	34.9 ± 7.3 abcd
HMPL2	4.7 ± 4.7 a	94.6 ± 5.4 d	0.7 ± 0.7 a
N6428	10.3 ± 6.6 a	31.9 ± 11.2 ab	57.8 ± 14.8 bcd
N6434	11.8 ± 5.8 a	48.5 ± 9.6 abcd	39.7 ± 3.9 abcd
SVTM9012	5.1 ± 5.1 a	68.0 ± 7.2 abcd	26.9 ± 2.8 abcd
SVTM9016	3.9 ± 2.2 a	19.4 ± 5.1 a	76.8 ± 6.1 d
SVTM9025	25.0 ± 17.7 a	41.1 ± 15.8 abc	33.9 ± 13.8 abcd
UG4014	14.0 ± 7.7 a	40.9 ± 2.7 abcd	45.2 ± 5.3 abcd
High Pathogen Pressure			
H1776	28.6 ± 14.3 a	52.6 ± 13.1 abcd	18.7 ± 2.8 abc
H5608	18.1 ± 4.8 a	54.3 ± 4.4 abcd	27.6 ± 3.1 abcd
HM4909	24.0 ± 5.0 a	57.3 ± 4.4 abcd	18.7 ± 2.2 abc
HM5235	29.1 ± 11.4 a	34.8 ± 10.0 abc	36.1 ± 5.6 abcd
HM58801	7.9 ± 3.4 a	69.6 ± 9.8 abcd	22.5 ± 7.0 abc
HMPL1	28.8 ± 11.1 a	40.2 ± 10.7 abcd	31.0 ± 11.2 abcd
HMPL2	13.6 ± 6.1 a	72.2 ± 14.3 bcd	14.2 ± 13.1 a
N6428	31.1 ± 15.0 a	38.4 ± 12.3 abc	30.4 ± 4.7 abcd
N6434	11.8 ± 3.5 a	69.5 ± 8.1 abcd	18.7 ± 5.8 abc
SVTM9012	11.0 ± 5.9 a	77.2 ± 8.5 bcd	11.8 ± 3.3 a
SVTM9016	8.0 ± 4.8 a	22.9 ± 1.6 ab	69.2 ± 6.0 cd
SVTM9025	27.4 ± 8.8 a	45.8 ± 8.9 abcd	26.8 ± 6.4 abcd
UG4014	19.1 ± 6.8 a	46.7 ± 4.8 abcd	34.2 ± 11.0 abcd
P-value cultivar	0.415	< 0.001	< 0.001
P-value pathogen	< 0.001	0.942	0.012
P-value cultivar x pathogen	0.982	0.252	0.366

[z] Low pathogen pressure field was naturally infested with *F. falciforme*. High pathogen pressure field was artificially infested by dip inoculating transplants with *F. falciforme* for two consecutive years.

[y] Percent of damaged fruit with sunburn, fruit rot, or blossom end rot ± standard error (n = 4).

[x] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison conducted on arcsine-square root transformed data ($n = 4$).

Table 14. Foot, crown and / or stem rot incidence at harvest across cultivars in two fields representing low and high levels of *Fusarium falciforme* pressure (2020)

	Percent Foot, Crown, and / or Stem Rot [z, y]	
Cultivar	**Low Pathogen Pressure** [x]	**High Pathogen Pressure** [w]
H1776	86.3 ± 8.3 a	87.5 ± 12.5 a
H5608	94.1 ± 3.4 a	100.0 ± 0.0 a
HM4909	81.5 ± 18.5 a	100.0 ± 0.0 a
HM5235	100.0 ± 0.0 a	87.5 ± 7.2 a
HM58801	96.4 ± 3.6 a	96.9 ± 3.1 a
HMPL1	100.0 ± 0.0 a	100.0 ± 0.0 a
HMPL2	85.7 ± 14.3 a	95.0 ± 5.0 a
N6428	100.0 ± 0.0 a	88.5 ± 7.9 a
N6434	96.9 ± 3.1 a	90.2 ± 6.1 a
SVTM9012	89.4 ± 7.1 a	84.9 ± 8.3 a
SVTM9016	96.4 ± 3.6 a	96.4 ± 3.6 a
SVTM9025	91.7 ± 4.8 a	87.5 ± 12.5 a
UG4014	100.0 ± 0.0 a	86.9 ± 5.1 a
P-value cultivar	0.817	
P-value pathogen	0.590	
P-value cultivar x pathogen	0.461	

[z] Percent of plants with foot, crown, or stem rot within the plot ± standard error (n = 4).

[y] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison conducted on arcsine-square root transformed data (n = 4).

[x] Low pathogen pressure field was naturally infested with *F. falciforme*.

[w] High pathogen pressure field was artificially infested by dip inoculating transplants with *F. falciforme* and planting in a field that had been previously infested by growing inoculated plants in 2019.

Table 15. Percent colonization of tomato seeds extracted from fruits of commercial cultivars grown in *Fusarium falciforme*-treated fields in 2020

Cultivar [z]	Field [y]	Total % Colonization [x]	% *Fusarium spp.* [w]	% *F. falciforme* [v]	% *F. oxysporum* (Non-pathogenic)	% Other [u]
HM3887	1	0.0%	0.0%	0.0%	0.0%	0.0%
	2	0.3% ± 0.2%	0.1% ± 0.1%	0.0%	0.1% ± 0.1%	0.2% ± 0.1%
	Average	0.2% ± 0.1%	0.1% ± 0.1%	0.0%	0.1% ± 0.1%	0.1% ± 0.1%
HM58841	1	0.4% ± 0.2%	0.2% ± 0.1%	0.0%	0.2% ± 0.1%	0.2% ± 0.1%
	2	0.3% ± 0.2%	0.0%	0.0%	0.0%	0.3% ± 0.2%
	Average	0.4% ± 0.2%	0.1% ± 0.1%	0.0%	0.1% ± 0.1%	0.3% ± 0.1%
HM4909	1	0.0%	0.0%	0.0%	0.0%	0.0%

[z] HM3887 is susceptible to *Fusarium falciforme,* HM58841 and HM4909 are highly tolerant to *F. falciforme* (**Table 9**).

[y] Fields 1 and 2 were artificially inoculated with *F. falciforme* at planting and grown in a field previously inoculated with *F. falciforme.*

[x] Percent total colonization was calculated as an average 100 seeds from ten plants per cultivar per field ± the standard error. In total, 1000 seeds were evaluated for each cultivar in each field.

[w] Percent *Fusarium* was calculated as the average percent colonization of ten plants per cultivar colonized with *Fusarium spp.* ± the standard error.

[v] Percent *F. falciforme* was calculated as the average percent colonization of ten plants per cultivar colonized with *Fusarium falciforme* ± the standard error.

[u] Percent other was calculated as the average colonization of fungi that were not *Fusarium spp.* ± the standard error. Fungal species recovered in this assay were: *Aspergillus spp*., *Monocillium loricatum, Talaromyces spp. Chaetomium concolutum,* and *Collariella bosyrychodes.*

Table 16. Baseline germination rates of seeds extracted from non-inoculated commercial processing tomato cultivars in 2019 [z]

Cultivars [y]	Baseline % Germination [x]
SV8011TM	90.5% ± 1.5%
DRI0319	82.0% ± 3.0%
AB0311	90.5% ± 0.5%
HM5235	88.0% ± 1.0%
H8504	88.5% ± 1.5%
N6416	88.5% ± 1.5%
H5608	87.5% ± 4.5%
H9663	91.0% ± 1.0%
HM4909	94.0% ± 2.0%
H1310	76.5% ± 5.5%
HM3887	85.0% ± 2.0%
HM58801	83.0% ± 5.0%
N6428	86.0% ± 6.0%
HM58841	86.5% ± 6.0%
H1776	69.5% ± 9.5%
H1428	50.0% ± 0.0%

[z] Plants were grown at the UC Davis Armstrong Plant Pathology Research Station in 2019 in a field with no history of *Fusarium falciforme*.

[y] Cultivars were selected among the top 30 grown commercially available cultivars in California for 2018.

[x] Baseline germination was calculated as an average of the percent germination of 50 seeds ± the standard error (n = 4).

Table 17. Germination rates of seed collected from fruit of commercial cultivars grown in a *Fusarium falciforme*-treated field in 2019

Cultivars [z]	% Germination- Inoculated [y, x]	% Difference [w]
SV8011TM	74.0% ± 15.0%	-18.23%
DRI0319	71.0% ± 15.0%	-13.41%
AB0311	81.0% ± 0.0%	-10.50%
HM5235	80.0% ± 5.0%	-9.09%
H8504	81.5% ± 4.5%	-7.91%
N6416	83.0% ± 7.0%	-6.21%
H5608	85.5% ± 10.5%	-2.28%
H9663	91.0% ± 1.0%	0.00%
HM4909	96.5% ± 1.5%	+2.66%
H1310	80.0% ± 9.0%	+4.58%
HM3887	88.5% ± 6.5%	+4.12%
HM58801	87.0% ± 8.0%	+4.82%
N6428	91.0% ± 1.0%	+5.81%
HM58841	92.5% ± 3.5%	+6.94%
H1776	76.0% ± 4.0%	+9.35%
H1428	65.5% ± 1.5%	+31.0%

[z] Cultivars were selected among the top 30 grown commercially available cultivars in California for 2018.

[y] Percent germination was calculated as an average of the percent germination of 50 seeds ± the standard error (n = 4).

[x] Plants were dip inoculated with *F. falciforme* and planted into a field with no history of *F. falciforme*.

[w] Percent difference = 1- (% Germination Inoculated / % Baseline Germination).

Table 18. Germination rates of seed collected from fruit of commercial cultivars grown in *Fusarium falciforme*-treated fields in 2020

Cultivar [z]	Field [y, x]	% Germination-Inoculated [w]	% Germination-Baseline [v]	% Difference [u]
HM3887	1	40.8% ± 6.6%	-	-52.0%
	2	82.1% ± 4.0%	-	-3.5%
	-	-	85.0% ± 2.0%	-
HM58841	1	85.5% ± 4.0%	-	-1.2%
	2	89.0% ± 2.8%	-	+2.9%
	-	-	86.5% ± 6.0%	-
HM4909	1	40.4% ± 5.4%	-	-57.1%
	-	-	94.0% ± 2.0%	-

[z] HM3887 is susceptible to *Fusarium falciforme,* HM58841 and HM4909 are highly tolerant to *F. falciforme* (**Table 9**).

[y] Field 1: plants were dip inoculated with *Fusarium falciforme* and planted into a field with inoculated with *Fusarium falciforme* the previous year.

[x] Field 2: plants were dip inoculated with *Fusarium falciforme* and planted into a field with inoculated with *Fusarium falciforme* and fumigated the previous year.

[w] Percent germination for the inoculated treatment was calculated as an average of the percent germination of seeds out of 100 ± the standard error (n = 10).

[v] Baseline germination was calculated as an average of the percent germination of 50 seeds ± the standard error (n = 4) in 2019.

[u] Percent difference = 1- (% Germination Inoculated / % Baseline Germination).

Table 19. Germination rates of seed collected from fruit of commercial cultivars grown in *Fusarium falciforme*-treated fields across years (2019 and 2020) in the same location [z]

Cultivar [y]	Year	% Germination- Inoculated [x, w]	% Difference [v]
HM3887	2019	88.5% ± 6.5%	+4.1%
	2020	40.8% ± 6.6%	-52.0%
	Average	64.6%	-24.0%
HM58841	2019	92.5% ± 3.5%	+6.9%
	2020	85.5% ± 4.0%	-1.2%
	Average	89.0%	+2.9%
HM4909	2019	96.5% ± 1.5%	+2.7%
	2020	40.4% ± 5.4%	-57.1%
	Average	68.4%	-27.2%

[z] Plants were artificially inoculated at transplanting for two consecutive years.

[y] HM3887 is susceptible to *Fusarium falciforme,* HM58841 and HM4909 are highly tolerant to *F. falciforme* (**Table 9**).

[x] Percent germination in 2019 was calculated as an average of the percent germination of seeds out of 50 ± the standard error (n = 4).

[w] Percent germination in 2020 was calculated as an average of the percent germination of ten 100-seed lots ± the standard error.

[v] Percent difference = 1- (% Germination Inoculated / % Baseline Germination).

DISCUSSION

Fusarium falciforme is a trans-kingdom pathogen, which was discovered causing disease in humans, sea turtles, and then more recently, in agricultural crops (Zhang *et al.,* 2006; Edupuganti *et al.*, 2011; Smyth *et al.,* 2019). *Fusarium falciforme* was first reported as a pathogen of tomato in Mexico in 2019 (Vega-Gutierrez *et al.*, 2019) and was identified as the causal agent of stem rot and vine decline in California processing tomatoes around the same time (Swett *et. al*., in prep.). When we began this study there were no known management methods for this disease. Anecdotal observations in grower fields indicated that cultivars may vary in tolerance based on some cultivar's ability to yield well despite high *F. falciforme* pressure (C. Swett pers. comm.).

Based on two years of field trials, we confirmed that cultivars vary in response and show yield was less affected by *F. falciforme* in some cultivars, such as N6428, HM4909, H8504, HM58841, and H1776. These cultivars may provide an initial platform from which to develop a cultivar-based management regime. There were several poor performers including HM3887, H5608, and N6416; and these should also be included in management recommendations as cultivars growers may want to avoid when planting tomato to a *F. falciforme*-infested field. In this study, tolerance was measured using both statistically significant yield differences between inoculated and non-inoculated fields as well as economic parameters. Using this approach allowed for the consideration of economically relevant differences, such as fruit quality thresholds in which harvests may be rejected, that would not be considered statistically significant. However, these research trials were conducted on small plots in comparison to grower fields. To address this, large-scale field trials across 3-4 sites in California have been initiated to annually screen cultivars for tolerance to *F. falciforme*.

In evaluating consistency in cultivar performance, we found that there was significant variation in performance between years and field sites, which had different *F. falciforme* pressures. The only exception to this was cultivar N6428, which consistently performed well across sites and years. This result reflects the importance that both inoculum loads as well as variable environmental conditions play on development of foot and crown rot caused by *F. falciforme*. For example, in 2020, there was continuous smoke cover for the last month pre-harvest; these conditions likely influenced ripening, perhaps making disease impacts on yield less obvious. The results of this study highlight the importance of assessing consistency of tolerance traits across growing seasons and locations. Statewide trials expanding this research are being conducted annually to screen additional cultivars for tolerance to *F. falciforme* and to assess stability of tolerance across sites in California. Downstream analyses of combined trials can augment the preliminary analyses run herein with ANOVA, using a more robust mixed model restricted estimate of maximum likelihood, which is more appropriate to data such as ours, with high levels of random noise (N. McRoberts, pers. comm.).

Disease phenotypes that corresponded with yield performance were early vine decline (5 weeks pre-harvest) and to a lesser extent, vine decline at harvest. Neither foot / stem rot incidence nor severity corresponded with yield performance, as 80-100% of plants developed foot / stem rot across cultivars, independent of tolerance classification. Although foot / stem rot incidence is not a good indicator of tolerance, presence of foot / stem may be a useful diagnostic symptom for this disease. The lack of interaction between stem rot incidence and yield performance may indicate the importance of the vine decline component of this disease and fruit quality, i.e., high incidence of vine decline early in the season exposes the fruit, increasing sunburn and fruit rot.

In some cultivars, the lack of a relationship between disease severity and yield may be due to extended field holding, a genetic trait that enables fruit to retain quality in the field for long periods of time. It is notable that, with one exception (H1310), all cultivars with extended field holding (H8504, H1428, H1776, HM58841, HM58801, N6428) were moderately or highly tolerant (**Table 9**). In some cases, such as for N6428, vine decline incidence was high, but yield was not strongly affected by the pathogen. Cultivar performance in cases like this likely reflect the extended field holding traits of the fruit. Both vine decline and extended field holding may be important characteristics capable of influencing yield performance. Breeding studies could be developed to test this hypothesis. Ultimately, cultivars with lower vine decline incidence and / or extended field holding could become key indicators rather than foot / stem rot incidence.

Results of our cultivar response trial revealed that all the cultivars used in this study were susceptible to *F. falciforme* to some degree. Other *Fusarium* diseases, such as Fusarium wilt (*I3* resistance gene, F3 cultivars) (Catanzariti *et al.,* 2015) and Fusarium crown and root rot (*Frl* resistance gene, Fr cultivars) (Fazio *et al.,* 1999), can be managed with single-gene dominant resistance. In theory, such resistance could be identified and deployed as an effective management tool for combatting *F. falciforme*. Although the results of our cultivar response trial failed to reveal evidence of single gene resistance, it is possible that resistance genes may be present in wild species. In a preliminary assessment, accessions of *Solanum pimpinellifolium* were not resistant (data not shown). However, there are dozens of other wild species, such as *S. habrochaites, S. chilense, S. peruvianum,* the source of resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Fazio *et al.,* 1999) and *S. pennellii,* the source of resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Catanzariti *et al.,* 2015). These wild tomato species may potentially provide a source of genetic resistance, meriting further investigation.

Another approach to find resistance involved testing the hypothesis that *I3* and / or *Frl* resistance is effective vs. *F. falciforme*. Results from our trials indicate that the *I3* gene did not provide resistance to *F. falciforme*, as several F3 cultivars were infected and performed poorly, such as H1310 and N6416. Only one Fr cultivar, HM4909, was assessed in these trials, and it was placed in the tolerant class. Whereas this finding provides preliminary support, the hypothesis that the *Frl* gene provides resistance to *F. falciforme*, further testing is needed. Future studies and further evaluation of Fr cultivars should be conducted to better resolve the role of the *Frl* gene in tolerance to *F. falciforme*.

It is important to note that in many California processing tomato fields, *F. falciforme* co-occurs with *Fusarium oxysporum* f. sp. *lycopersici,* the cause of Fusarium wilt, in addition to other pathogens (C. Swett pers. comm.). In a four-year survey of 108 fields with *F. falciforme* infestations, more than 25% also had Fusarium wilt present (Swett *et al*., in prep.). Although *I3* resistance does not appear to confer resistance to *F. falciforme*, several F3 cultivars performed well in the *F. falciforme* response trials, such as N6428 and HM5235. This result suggests that in cases where growers need to manage both diseases, a potential solution might be to plant cultivars that exhibit both resistance to Fusarium wilt and tolerance to *F. falciforme*. Additional studies to identify bone fide resistance to *F. falciforme* should focus on F3 cultivars, to provide co-management options for growers.

In the field, *F. falciforme* appears to induce systemic symptoms that affect the entire plant. For that reason, we hypothesized that this pathogen might be capable of systemic colonization, and if this was the case, then seed colonization might be an important means of pathogen spread. In this study we evaluated 5000 tomato seeds for *F. falciforme* infection, with a detection threshold of 0.0002%. We did not detect *F. falciforme* associated with tomato seed.

These results suggest that *F. falciforme* is not seedborne. However, it is possible that *F. falciforme* could contaminate seeds below the detection threshold of this study. The presence of whole-plant symptoms without systemic fungal colonization is consistent with other *F. solani*-pathosystems that produce phytotoxic secondary metabolites (toxins) (Brar *et al*., 2011; Pudake *et al*., 2013; Hartman *et al*., 2015). Due to the potential impacts of fungal toxins on seed viability, we examined the effects of *F. falciforme* on seed germination rates. The replicated study revealed that there was a trend in which *F. falciforme* reduced seed viability in highly susceptible cultivars, such as HM3887. However, there was a wide range in seed response across the study, meriting further investigation. If *F. falciforme* does influence seed viability, then the presence of *F. falciforme* in breeding fields could pose a problem when trying to increase seed numbers in breeding efforts.

In summary, this work demonstrates that selection of tolerant cultivars may provide a means to reduce yield loss of processing tomato caused by *F. falciforme* vine decline in the short term. Because growers often have constraints in cultivar selection due to contracts with processing companies, it will be important to identify as many cultivars with tolerance to *F. falciforme* as possible. Beyond this, breeding efforts to identify possible resistance early in breeding efforts can eliminate highly susceptible genotypes as well as reveal sources of resistance. The preliminary work with seeds suggests that a possible negative effect of *F. falciforme* on seed viability. Further work to characterize the potential for *F. falciforme* to produce toxins driving vine decline are needed. For example, if a toxin is involved, resistant lines such as those previously mentioned for the *F. virguliforme*-soybean system. In the latter case, multi-gene resistance to plant toxins have provided the primary means for disease management (Stephens *et al*., 1993; Brzostowski *et al*., 2014). The results of the seed infection component of

this study indicate that seed may not provide a means of spread, a critical finding for the tomato industry. However, however, impacts of *F. falciforme* on seed viability may have some importance to the breeding sector.

REFERENCES

1. Abbas, H. K. and C. D. Boyette. (1996). Control of morning-glory species using *Fusarium solani* and its extracts. *International Journal of Pest Management, 42*(4), 235–239. doi: 10.1080/09670879609372001
2. Aegerter, B. J., & Gordon, T. R. (2006). Rates of pitch canker induced seedling mortality among *Pinus radiata* families varying in levels of genetic resistance to *Gibberella circinata* (anamorph *Fusarium circinatum*). *Forest Ecology and Management*, *235*, 14-17. doi: 10.1016/j.foreco.2006.07.011
3. Ahmed, N. E., Sugimoto, Y., Babiker, A. G. T., Mohamed, O. E., Ma, Y., Inanaga, S., & Nakajima, H. (2001). Effects of *Fusarium solani* isolates and metabolites on *Striga* germination. *Weed Science*, *49*(3), 354–358. https://doi.org/10.1614/0043-1745(2001)049[0354:eofsia]2.0.co;2
4. Brar, H. K., Swaminathan, S., & Bhattacharyya, M. K. (2011). The *Fusarium virguliforme* toxin fvtox1 causes foliar sudden death syndrome-like symptoms in soybean. *Molecular Plant-microbe Interactions*, *24*(10), 1179–1188. https://doi.org/10.1094/mpmi-12-10-0285
5. Brzostowski, L. F., Schapaugh, W. T., Rzodkiewicz, P. A., Todd, T. C., and Little, C. R. (2014). Effect of host resistance to *Fusarium virguiliforme* and *Heterodera glycines* on sudden death syndrome disease severity and soybean yield. *Plant Health Progress* doi:10.1094/PHP-RS-13-0100
6. Catanzariti, A., Lim, G. T. T., & Jones, D. A. (2015). The tomato *I-3* gene: a novel gene for resistance to Fusarium wilt disease. *New Phytologist*, *207*(1), 106–118. https://doi.org/10.1111/nph.13348
7. Coleman, J. J. (2016). The *Fusarium solani* species complex: ubiquitous pathogens of agricultural importance. *Molecular Plant Pathology, 17*(2), 146-158. https://doi.org/10.1111/mpp.12289
8. Cucuzza, J., Watterson, J., & Bernhardt, E. (1991). Foot rot of tomato caused by *Fusarium solani* in California. *Plant Disease Back Issue Abstracts*, *76*(101). doi: 10.1094/PD-76-0101B.
9. Douriet-Angulo, A., Lopez-Orona, C. C., Lopez-Urquidez, G. A., Vega-Gutierrez, T. A., Tirado-Ramirez, M. A., Estrada-Acosta, M. D., Ayala-Tafoya, F., & Yanez-Juarez, M. G. (2019). Maize stalk rot caused by *Fusarium falciforme* (FSSC 3 + 4) in Mexico. *Plant Disease 103*(11). https://doi.org/10.1094/PDIS-05-19-1055-PDN
10. Fazio, G., Stevens, M. R., & Scott, J. W. (1999). Identification of RAPD markers linked to Fusarium crown and root rot resistance (*Frl*) in tomato. *Euphytica*, *105*(3), 205–210. https://doi.org/10.1023/a:1003497719705
11. Gordon, T. R., Kirkpatrick, S. C., Aegerter, B. J., Wood, D. L., & Storer, A. J. (2006). Susceptibility of Douglas fir (*Pseudotsuga menziesii*) to pitch canker, caused by *Gibberella circinata* (anamorph = *Fusarium circinatum*). *Plant Pathology*, *55,* 231–237.
12. Gupta, A. K., Choudhary, R., Bashyal, B. M., Rawat, K., Singh, D., & Solanki, I. S. (2019). First report of root and stem rot disease on papaya caused by *Fusarium falciforme* in India. *Plant Disease, 103*(10). https://doi.org/10.1094/PDIS-11-18-2118-PDN

13. Hartman, G. L., Chang, H.-X., & Leandro, L. F. (2015). Research advances and management of soybean sudden death syndrome. *Crop Protection*, *73*, 60–66. https://doi.org/10.1016/j.cropro.2015.01.017

14. Helpio, E. L., & Swett, C. L. (2019). First report of a new stem and crown rot disease of processing tomato in California caused by *Fusarium falciforme*. *Phytopathology, 109*(11).

15. Kandhare, A. S. (2015). Toxic efects of seed-borne fungi on seed health of chickpea. *International Journal of Innovative Science, Engineering & Technology*, *2*(2), 238-240.

16. Lee, H., & Sumner, D. A. (2015). Economics of downscaled climate-induced changes in cropland, with projections to 2050: evidence from Yolo County California. *Climatic Change*, *132*(4), 723–737. https://doi.org/10.1007/s10584-015-1436-9

17. Johnston-Monje, D., Loewen, S., & Lazarovits, G. (2017). Mycobiomes of tomato plants with vine decline. *Canadian Journal of Plant Pathology*, *39*(2), 184–200. https://doi.org/10.1080/07060661.2017.1325938

18. Pudake, R. N., Swaminathan, S., Sahu, B. B., Leandro, L. F., & Bhattacharyya, M. K.. (2013). Investigation of the *Fusarium virguliforme* fvtox1 mutants revealed that the FvTox1 toxin is involved in foliar sudden death syndrome development in soybean. *Current Genetics*, *59*(3), 107–117. https://doi.org/10.1007/s00294-013-0392-z

19. Romberg, M. K., & Davis, R. M. (2007). Host range and phylogeny of *Fusarium solani* f. sp. *eumartii* from potato and tomato in California. *Plant Disease*, *91*(5), 585-592.

20. Sandoval-Denis, M., Lombard, L., & Crous, P. W. (2019). Back to the roots: a reappraisal of *Neocosmospora*. *Persoonia-Molecular Phylogeny and Evolution of Fungi, 43*, 90-185. https://doi.org/10.3767/persoonia.2019.43.04

21. Smyth, C. W., Sarmiento-Ramirez, J. M., Short, D. P. G., Dieguez-Uribeondo, J., O'Donnell, K., & Geiser, D. M. (2019). Unraveling the ecology and epidemiology of an emerging fungal disease, sea turtle egg fusariosis (STEF). *PLoS Pathogens, 15*(5). https://doi.org/10.1371/journal.ppat.1007682

22. Sousa, E. S., Melo, M. P., Mota, M. J., Sousa, E. M. J., Beserra, Jr., J. E. A., & Matos, K. S. (2017). First report of *Fusarium falciforme* (FSSC 3 + 4) causing rot in lima bean (*Phaseolus lunatus* L.) in Brazil. *Plant Disease, 101*(11). https://doi.org/10.1094/PDIS-05-17-0657-PDN

23. Stephens, P.A., Nickell, C.D. and Lim, S.M. (1993). Sudden death syndrome development in soybean cultivars differing in resistance to *Fusarium solani*. Crop Science, 33. 63-66. https://doi.org/10.2135/cropsci1993.0011183X003300010009x

24. Swett, C. L., & Gordon T. R. (2015). Endophytic association of the pine pathogen *Fusarium circinatum* with corn (*Zea mays)*. *Fungal Ecology*, *13,* 120-129. https://doi.org/10.1016/j.funeco.2014.09.003

25. Swett, C.L. and Gordon, T.R. (2017), Exposure to a pine pathogen enhances growth and disease resistance in *Pinus radiata* seedlings. *Forest Pathology*, *47*(1), e12298. https://doi.org/10.1111/efp.12298

26. Tirado-Ramirez, M. A., Lopez-Orona, C. A., Velazquez-Alcaraz, T. de J., Diaz-Valdes, T., Velarde-Felix, S., Martinez-Campos, A. R., & Retes-Manjarrez, J. E. (2018). First report of onion basal rot caused by *Fusarium falciforme* in Mexico. *Plant Disease, 102*(12). https://doi.org/10.1094/PDIS-05-18-0757-PDN

27. Vawdrey, L. L., & Peterson, R. A. (1988). *Fusarium solani*, the cause of foot rot of tomatoes in Central Queensland. *Australasian Plant Pathology*, *17*(1), 24–25. https://doi.org/10.1071/APP9880024

28. Vega-Gutierrez, T. A., López-Orona, C. A., López-Urquídez, G. A., Velarde- Felix, S., Amarillas-Bueno, L. A., Martínez-Campos, A. R., & Allende- Molar, R. (2019). Foot rot and wilt in tomato caused by *Fusarium falciforme* (FSSC 3 + 4) in Mexico. *Plant Disease*, *103*(157). https://doi.org/10.1094/pdis-06-18-1001-pdn

29. Vega-Gutierrez, T. A., Tirado-Ramirez, M. A., López-Urquídez, G. A., Angulo-Castro, A., Martinez-Gallardo, J. A., López-Orona, C. A. (2019). *Fusarium falciforme* (FSSC 3 + 4) causing root and stem rot in papaya (*Carica papaya)* in Mexico. *Plant Disease 103*(10). https://doi.org/10.1094/PDIS-05-19-0917-PDN

30. Zhang, N., O'Donnell, K., Sutton, D. A., Nalim, F. A., Summerbell, R. C., Padhye, A. A., & Geiser, D. M. (2006). Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *Journal of Clinical Microbiology, 44*(6), 2186–2190. https://doi.org/10.1128/JCM.00120-06

31. Zhang, X., Li, L., Yang, J., Yan, H., & Liu, D. (2020). Rot on rhizomes and adventitious roots of *Dioscorea polystachya* caused by *Fusarium falciforme* in China. *Plant Disease, 104*(5). https://doi.org/10.1094/PDIS-12-19-2529-PDN

Appendix A. Yield comparison of commercially available cultivar performance in the absence and presence of *Fusarium falciforme* foot rot and vine decline (2019) [z]

	Kg Fruit [x]		Percent of Total [w, v]	
Cultivar [y]	**Total Fruit**	**Marketable Red Fruit**	**% Green Fruit [u]**	**% Damaged Fruit [t]**
Non-Inoculated				
AB0311	25.1 ± 3.0 b	16.5 ± 1.2 b	45.1 ± 9.0 a	11.0 ± 3.1 a
DRI0319	23.9 ± 1.5 b	16.7 ± 0.4 b	48.5 ± 13.9 a	23.2 ± 3.5 a
SV8011TM	26.8 ± 1.5 b	17.4 ± 1.1 b	41.0 ± 14.5 a	20.9 ± 2.6 a
Inoculated				
AB0311	13.7 ± 2.9 a	6.7 ± 1.9 a	16.3 ± 3.4 a	20.4 ± 3.9 a
DRI0319	18.8 ± 3.8 ab	8.4 ± 3.4 ab	15.6 ± 2.2 a	16.8 ± 3.0 a
SV8011TM	10.1 ± 4.3 ab	5.9 ± 3.0 a	20.3 ± 2.3 a	18.7 ± 1.1 a
P-value cultivar	0.627	0.884	0.995	0.226
P-value pathogen	< 0.001	< 0.001	0.002	0.778
P-value cultivar x pathogen	0.187	0.754	0.773	0.041

[z] Inoculated field: Plants were dip inoculated with *F. falciforme* at transplanting into a field with no history of *F. falciforme*. Non-inoculated field: Plants were directly transplanted into a field with no history of *F. falciforme*.

[y] These cultivars were planted three weeks later than the rest of the cultivars in the 2019 tolerance trial (objective 1.1); therefore, they were taken out of the dataset due to high amounts of green fruit.

[x] Average total fruit biomass harvested and the total biomass of marketable red fruit within the 2 m harvest plot, ± the standard error (n = 4).

[w] Means separated by different letters within columns are significantly different ($P \leq 0.05$) based on ANOVA and Tukey's means comparison (n = 4).

[v] ANOVA and means comparison conducted on arcsine-square root transformed data.

[u] Percent green fruit out of total fruit (red, green, and damaged) within the plot ± the standard error (n = 4).

[t] Percent of total red fruit that was damaged (sunburn, rot, or blossom end rot) within the plot ± the standard error (n = 4).