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The Effect of Temperature on Disease Severity and Growth of *Fusarium oxysporum* f. sp. *apii* Races 2 and 4 in Celery

Sukhwinder Kaur,[1] Radwan Barakat,[2] Jaskirat Kaur,[1] and Lynn Epstein[1,†]

[1] Department of Plant Pathology, University of California, Davis, CA 95616-8680, U.S.A.
[2] Department of Plant Production & Protection, College of Agriculture, Hebron University, Hebron, Palestine
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ABSTRACT

Fusarium oxysporum f. sp. *apii* race 4, which is in *F. oxysporum* species complex (FOSC) Clade 2, causes a new Fusarium wilt of celery. We compared *F. oxysporum* f. sp. *apii* race 4 with race 2, which causes Fusarium yellows of celery and is in FOSC Clade 3. Optimal temperatures for celery yield are 16 to 18°C. Soil temperatures in California celery production areas can range up to 26°C, and the maximal rate of hyphal extension of *F. oxysporum* f. sp. *apii* races 2 and 4 in culture are 25 and 28°C, respectively. Here, we compared the effect of temperatures from 16 to 26°C on growth of *F. oxysporum* f. sp. *apii* races 4 and 2 in two celery cultivars: Challenger, which is resistant to *F. oxysporum* f. sp. *apii* race 2 and susceptible to race 4; and Sonora, which is susceptible to both *F. oxysporum* f. sp. *apii* races 2 and 4. Based on linear regressions, as temperature increases, there is an increase in the log of *F. oxysporum* f. sp. *apii* race 4 DNA concentration in celery crowns and in the reduction in plant height. Based on logistic regressions, as temperature increases, the incidence of vascular discoloration increases in celery with either *F. oxysporum* f. sp. *apii* race 2 or 4 infection. In both cultivars, temperatures of 22°C and above resulted in a significantly ($\alpha = 0.05$) greater concentration of *F. oxysporum* f. sp. *apii* race 4 than race 2 in planta. The concentration of *F. oxysporum* f. sp. *apii* race 2 in crowns in 'Challenger' is temperature-independent and comparatively low; consequently, 'Challenger' is, at least partly, resistant rather than tolerant to *F. oxysporum* f. sp. *apii* race 2.

Keywords: *Apium graveolens* var. *dulce*, celery, climate change, cultural control, *Fusarium oxysporum* f. sp. *apii*, *Fusarium oxysporum* species complex, host–pathogen interactions, plant pathogen, quantitative PCR, temperature

In 2017, California produced 94% of the 710,000 tons of celery (*Apium graveolens* var. *dulce*) that were grown in the United States (https://www.agmrc.org/commodities-products/vegetables/celery). The two most important soilborne diseases of celery (*A. graveolens* var. *dulce*) in California are caused by *Fusarium oxysporum* f. sp. *apii* race 2 (Subbarao and Elmer 2002), which was first observed around 1959 (Otto et al. 1976), and *F. oxysporum* f. sp. *apii* race 4, which was first observed in 2013 (Epstein et al. 2017; Henry et al. 2020). Both strains of *F. oxysporum* f. sp. *apii* were first observed in California.

F. oxysporum f. sp. *apii* has four described races, but only races 2 and 4 are agronomically important in California (Epstein et al. 2017). *F. oxysporum* f. sp. *apii* race 2 has been agronomically important in celery-production areas in the United States, Canada, and Argentina (Cerkauskas and Chiba 1991; Lori et al. 2016; Subbarao and Elmer 2002). *F. oxysporum* f. sp. *apii* race 2 is now defined as virulent on cultivars such as Tall Utah 52-70R Improved but avirulent on the cultivar Challenger (Epstein et al. 2017; Henry et al. 2020). Cultivar Sonora was introduced in 1997 and remains a popular, nonhybrid variety in areas where *F. oxysporum* f. sp. *apii* race 2 does not impact production, i.e., although it is more resistant to *F. oxysporum* f. sp. *apii* race 2 than the 'Tall Utah' lineage (Quiros 2002), it is moderately susceptible. The Tall Utah cultivars are no longer grown commercially in California. After a breeding effort to introgress celeriac (*A. graveolens* var. *rapaceum*) gene(s) into celery (Orton et al. 1984b), cultivars such as Challenger were introduced starting in 1999 and are now a major pillar for control of *F. oxysporum* f. sp. *apii* race 2. However, as of this writing, celery cultivars are susceptible to *F. oxysporum* f. sp. *apii* race 4. *F. oxysporum* f. sp. *apii* race 4 is in *F. oxysporum* species complex (FOSC) Clade 2, as are *F. oxysporum* f. sp. *apii* races 1 and 3 (Epstein et al. 2017; Henry et al. 2020). *F. oxysporum* f. sp. *apii* race 1 is not virulent on contemporary cultivars (Subbarao and Elmer 2002) and *F. oxysporum* f. sp. *apii* race 3 is not virulent on Challenger (Epstein et al. 2017; Henry et al. 2020). *F. oxysporum* f. sp. *apii* race 2 is in FOSC Clade 3 (Epstein et al. 2017; Henry et al. 2020).

Although both *F. oxysporum* f. sp. *apii* races 2 and 4 cause stunting in the field and a characteristic orangish-brown discoloration in the vascular tissue in the roots and crown, the two pathogens cause some different symptoms. *F. oxysporum* f. sp. *apii* race 2 causes the disease Fusarium yellows of celery, which can cause a pronounced leaf chlorosis in lower leaves, stunting, and a decrease in yield and quality in susceptible cultivars (Subbarao and Elmer 2002). *F. oxysporum* f. sp. *apii* race 4 causes a more severe disease called Fusarium wilt of celery to differentiate it from Fusarium yellows. *F. oxysporum* f. sp. *apii* race 4 most frequently causes a severe stunting and can cause death in younger plants (Henry et al. 2020). In cases of plant death, the symptoms progress from stunting, sometimes with lower leaf chlorosis, to wilting of the entire plant, and death. Compared with *F. oxysporum* f. sp. *apii* race 2, in contemporary California-grown cultivars, *F. oxysporum* f. sp. *apii* race 4 often produces a water-soaked lesion around the vasculature in the crown and to a lesser extent in the larger roots. Particularly in commercial production in the field, the water-soaked lesions may lead to rotting. In severe cases in both the field and the greenhouse, the vascular discoloration from *F. oxysporum* f. sp. *apii* race 4 may extend from the roots and crown into the petioles, which is generally limited to the roots and crown with race 2. After an *F. oxysporum* f. sp. *apii* race 4 infection, roots are frequently sloughed off or rotted. *F. oxysporum* f. sp. *apii* race 4 also may induce suckering and the production of adventitious roots from crowns.

†Corresponding author: L. Epstein; lepstein@ucdavis.edu

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*The *e-Xtra* logo stands for "electronic extra" and indicates there are supplementary materials published online.

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Celery has an optimum air temperature between either 16 and 18°C (Rubatzky et al. 1999) or 16 and 21°C (Smith 2021). *Fusarium* spp. are generally considered to have an optimum growth temperature at ~25°C (Nelson et al. 1989) to 28°C (Fravel et al. 1996). In 1935, presumably using *F. oxysporum* f. sp. *apii* race 1 isolates, which are polymorphic (Epstein et al. 2017), Ryker (1935) reported that the temperature optimum for *F. oxysporum* f. sp. *apii* growth in culture was ~28°C. There are multiple examples of FOSC formae speciales (e.g., f. sp. *ciceris* in chickpea, *cepae* in onions, *conglutinans* in cole crops, *lactucae* in lettuce, *lycopersici* in tomatoes, *medicaginis* in alfalfa, and sp. *melonis* in melons) in which disease severity in susceptible varieties increases as temperatures increase to ~22 to 28°C (Bosland et al. 1988; Jelínek et al. 2019; Navas-Cortés et al. 2007; Scott et al. 2010). In the case of FOSC f. sp. *conglutinans* yellows and wilt on crucifers, and f. sp. *ciceris* on chickpeas, higher temperatures also decreased expression of resistance (Bosland et al. 1988; Landa et al. 2006).

In California, celery is primarily grown on the south coast (where *F. oxysporum* f. sp. *apii* race 4 was first observed) with transplanting from early August to April for harvest from November to mid-July and the central coast (in which *F. oxysporum* f. sp. *apii* race 4 was first detected in 2019) with transplanting from March to September for harvest from late June to late December (Daugovish et al. 2008). Both areas can have average daily soil temperatures in the range of 21 to 25°C from July through September (Supplementary Fig. S1A), i.e., temperatures in the range of 21 to 25°C can occur either during earlier transplantation or earlier harvests in Ventura County on the south coast and either during later transplantation or later harvests in Monterey County on the central coast. Growers are constrained in when they can grow celery because there are legally mandated "celery-free" periods for the control of the western celery mosaic virus: January is the celery-free month in Monterey County; and 15 July to 4 August is celery-free period in Ventura County (CFDA 1998). Anecdotally, the most severe disease from *F. oxysporum* f. sp. *apii* race 4 in Ventura County has occurred when transplanting has occurred in August through mid-September; many growers in Ventura County prefer this time for transplanting because of the economics of producing for the Thanksgiving holiday market.

To aid growers in managing *F. oxysporum* f. sp. *apii*, our first objective was to determine the effect of soil temperatures from 16 to 26°C on disease severity and growth of both *F. oxysporum* f. sp. *apii* races 4 and 2 in two open-pollinated celery cultivars: Sonora, which is susceptible to both races 2 and 4; and Challenger, which is resistant to *F. oxysporum* f. sp. *apii* race 2 and is susceptible to *F. oxysporum* f. sp. *apii* race 4. Because a vascular discoloration-based disease severity score has been used to select for germplasm that is resistant to *F. oxysporum* f. sp. *apii* race 2 (Orton et al. 1984b; Quiros et al. 1993), and our longer-term research goal is to identify germplasm that is resistant to *F. oxysporum* f. sp. *apii* race 4, our second objective was to examine the association among the disease severity score, the growth of *F. oxysporum* f. sp. *apii* in celery crown tissue, and the reduction in plant height. Finally, the quantitative PCR estimates of *F. oxysporum* f. sp. *apii* DNA concentration in celery crowns was used to infer information about the mechanism of the Challenger–*F. oxysporum* f. sp. *apii* race 2 incompatible interaction.

MATERIALS AND METHODS

Soil temperatures in celery production areas with both *F. oxysporum* f. sp. *apii* races. Average daily soil temperatures were obtained from the California Irrigation Management Information Systems (CIMIS; cimis.water.ca.gov). The Camarillo (Supplementary Fig. S1A) and King City (Supplementary Fig. S1B) locales were selected for the following reasons: they have intensive celery production; *F. oxysporum* f. sp. *apii* races 2 and 4 are now present in both areas; and CIMIS maintains the sites to provide the highest quality data for their daily reference evapotranspiration calculation. CIMIS soil temperature sensors (Fenwal Electronics UUT51J1; Milford, MA) are buried 15 cm below the soil surface under established irrigated turfgrass.

Isolates. As described in Epstein et al. (2017), after virulence testing and multilocus DNA sequencing of 77 *F. oxysporum* f. sp. *apii* race 2 and 11 *F. oxysporum* f. sp. *apii* race 4 isolates, two strains, 207.A and 274.AC, were selected as representatives of the clonal populations of *F. oxysporum* f. sp. *apii* races 2 and 4, respectively. Strain 207.A was isolated in 2010 from celery from Santa Maria, California with symptoms of Fusarium yellows and strain 274.AC was isolated in 2013 from celery from Camarillo, California with symptoms of Fusarium wilt (Epstein et al. 2017). Strains were stored as described in Epstein et al. (2017). Whole genome assemblies of the isolates are available in GenBank (https://www.ncbi.nlm.nih.gov/) as JAAOOO000000000 and JAAOOQ000000000 for *F. oxysporum* f. sp. *apii* races 2 and 4, respectively (Henry et al. 2020).

Production of celery seedlings and inoculum. Celery cultivars Sonora and Challenger were germinated and grown for transplantation in cells 3.8 × 3.8 (at the top) × 5.7-cm high plug trays with PRO-MIX HP mycorrhizae medium (Premier Horticulture Inc., Quakertown, PA). Before transplanting, University of California at Davis greenhouse soil (UCDGM) was diluted (1:3 vol/vol) as a UCDGM/perlite mix and used to fill the lower 405 cm^3 of 6-cm upper diameter × 25.4-cm high Deepot tubes (Stuewe & Sons, Tangent, OR). The plugs were transplanted 2 months after seeding.

Inoculum was grown on hydrated, autoclaved millet seed at 22°C for 8 to 10 days under fluorescent lights as described in Henry et al. (2020). Inoculum was diluted (1:15 vol/vol) into the UCDGM/perlite mix (DI). Uninfested control soil was the UCDGM/perlite mix without millet seed. After a 1-cm-thick layer (28 cm^3) of the DI was placed over the lower layer, a transplant plug seedling (3.5 × 3.5 at the top × 4.7-cm deep) was positioned on top of the DI layer in the center and each plug was surrounded by 37 cm^3 DI. In total, each 40-cm^3 plug was surrounded on the bottom and sides by a total of 65 cm^3 of DI; the DI contained 40 mg wet weight (wt) of infested millet seed/cm^3 DI, which is equivalent to 26 mg of dry wt infested millet seed/cm^3 DI. The plants were then moved into the growth chambers that are described below.

Trials in growth chambers. Three separate E7/2 (Conviron, Winnipeg, Canada) growth chambers at the University of California at Davis Controlled Environment Facility with a 16-h photoperiod at either 16, 20, and 24°C, or at 18, 22, and 26°C, were used concurrently. There were two independent trials at each of the two sets of temperatures. Nighttime temperatures were set at 2°C lower than the daytime temperatures. Lights were turned on and off to simulate a 1-h sunrise and sunset. Each chamber had the same fluorescent and incandescent bulbs for a photosynthetic active range that simulates natural light. Each chamber had a growth area of 7,618 cm^2 and a growth height of 63 cm.

In each growth chamber, there was a two-factor experiment, in a completely randomized design with two cultivars (Sonora and Challenger) and three *F. oxysporum* f. sp. *apii* treatments (uninfested; infested with *F. oxysporum* f. sp. *apii* race 2; and infested with *F. oxysporum* f. sp. *apii* race 4). Within a chamber, each plant was irrigated by a separate dripper and received the same amount of water; the soil mix allowed all plants to be well-watered without over-watering. We avoided cross contamination of soil treatments by drip irrigation, spatial separation of the plant tubes in their racks, and insect control.

Planting in 6.4-cm diameter tubes with a well-watered perlite/soil mix allowed the soil temperatures to reflect the programmed chamber temperatures. There were five replicate plants of each treatment for each of the two trials. Trials were terminated after 35 days in the growth chamber.

Scoring of plant height and disease severity. After plants were removed from their tubes and roots and crowns were washed, the height of each plant was measured as the length from the top of the crown at the soil line to the tip of the most distal leaf blade.

Plant disease severity was scored for typical symptoms of *F. oxysporum* f. sp. *apii*-induced vascular discoloration on a 0-to-5 severity scale: 0, asymptomatic; 1, some discoloration in the lateral root vasculature; 2, some discoloration in the main root vasculature; 3, discoloration of <1/4 of the crown vasculature; 4, discoloration of >1/4 of the crown vasculature; and 5, plant dead.

Isolation of DNA from celery crown tissue and *F. oxysporum* f. sp. *apii*-infested millet grain. Crown tissue was harvested for estimation of *F. oxysporum* f. sp. *apii* races 2 and 4 concentration. For quantitative PCR (qPCR), the harvested and washed celery crowns were lyophilized and stored at −80°C. After manually rubbing off any remaining soil, the crown pieces were placed between two pieces of weighing paper, and pounded two or three times with a hammer. The smashed crown was placed in a 50-ml centrifuge tube with five, 5-mm-diameter stainless steel balls. The tissue was then pulverized by shaking at 1,240 rpm for 1 min in a Geno/Grinder 2010 (SPEX, Metuchen, NJ). After hand-shaking the tubes, the samples were again shaken at 1,240 rpm for 1 min. A 50-mg sample was purified with a Zymo Research *Quick*-DNA Fecal/Soil Microbe Miniprep Kit (Irvine, CA) using manufacturer's recommendations except for the 50-mg samples with 750 µl of Bashing-Bead Buffer (Zymo Research), the kit beads, and 0.5% (vol/vol) β-mercaptoethanol, which were vortexed at 3,200 rpm on a Benchmark BenchMixer (Edison, NJ) with a horizontal adapter for microfuge tubes first for 1 min to make sure that the powder was suspended, and then for 30 min. Total DNA was quantified with a Qubit dsDNA BR Assay Kit (Invitrogen/Life Technologies, Carlsbad, CA) in a Qubit 2.0 fluorometer (Invitrogen/Life Technologies). For an external standard for quantification, DNA from pure cultures of *F. oxysporum* f. sp. *apii* races 2 and 4 was purified as described in Kaur et al. (2017) and quantified using the Qubit fluorometer.

Primers and fluorescently labeled probe for qPCR of *F. oxysporum f. sp. apii* in celery crowns. Preliminary experiments indicated that PCR primers for single-copy *F. oxysporum* f. sp. *apii* DNA with SYBR Green detection (Epstein et al. 2017) were insufficiently sensitive to quantify the comparatively lower concentrations of *F. oxysporum* f. sp. *apii* race 2 in crown tissue, particularly in the incompatible cultivar Challenger (data not shown). To select multicopy target sequences for PCR amplification, we used the Geneious Prime v.2020.2.2 software (www.geneious.com) to select transposon DNA (Henry et al. 2020) that was present in *F. oxysporum* f. sp. *apii* race 2 but not *F. oxysporum* f. sp. *apii* race 4, and vice versa. Primers and probe (Table 1) were designed with Integrated DNA Technologies (IDT) PrimerQuest (Coralville, IA).

To bioinformatically determine the specificity of the primers/probe, we used the tool Primer-BLAST (NCBI, Bethesda, MD) on the nucleotide collection and the Whole Genome Shotgun Submissions (WGS; NCBI) database for *Fusarium*; as of 28 May 2021, the WGS database included 535 assembled *F. oxysporum* strains and 485 other strains in *Fusarium* spp. We considered >90% identity on >90% of the sequence on all three primers/probe as a putative sequence match. To confirm the primers/probe putative matches, we searched for matches of the FoaR2-MC1 and FoaR4_MC2 amplicons with the program BLASTn (NCBI) in the WGS database.

Each PCR reaction in 20 µl contained 1× PrimeTime Gene Expression Master Mix (IDT), 0.5 µM of each primer, 0.15 µM of the probe with a 5′ FAM reporter dye, a ZEN quencher (IDT), and a 3′ Iowa Black quencher (IDT), and 0.2 µg of bovine serum albumin. Optimal annealing temperatures were determined empirically. The efficiency of the *F. oxysporum* f. sp. *apii* races 2 and 4 with FoaR2-MC1 and FoaR4_MC2, respectively, was determined using a dilution series of purified *F. oxysporum* f. sp. *apii* races 2 and 4 DNA from six trials (Table 1; one example is shown in Supplementary Fig. S2).

For each sample, there were two reactions with 10 ng of total DNA from celery crowns, and one reaction with 3 ng of total DNA. Calculations of femtograms of *F. oxysporum* f. sp. *apii* DNA/ng celery DNA from both quantities demonstrated that all the 10-ng samples were free of PCR inhibitors, which might have produced artifactual results. The PCR program on a Bio-Rad CFX (Hercules, CA) was 3 min at 95°C, and 41 cycles of 15 s at 95°C, 30 s at 56°C, and 60 s at 72°C. All runs included no-template controls and a serial dilution of DNA from pure cultures of *F. oxysporum* f. sp. *apii* races 2 and 4.

As shown on the left axis of Figure 1B, the *F. oxysporum* f. sp. *apii* concentration is shown as a mass, i.e., log (femtograms of *F. oxysporum* f. sp. *apii* DNA/ng celery DNA). To convert mass to the number of *F. oxysporum* f. sp. *apii* cells per 1,000 celery cells, as shown on the right axis of Figure 1B, we used published genome sizes; the diploid celery genome has 6,665,155,014 bp per nucleus (Song et al. 2021) and the haploid *F. oxysporum* f. sp. *apii* races 2 and 4 have 64,759,272 and 67,371,990 bp per nucleus, respectively (Henry et al. 2020). Thus, ignoring organelle DNA, one celery cell nucleus contains the mass of DNA of 99 and 103 *F. oxysporum* f. sp. *apii* races 2 and 4 cells, respectively.

The primers and probes were also used to quantify the inoculum. Samples of the millet grain from four replicate flasks of either the uninfested controls or infested with either *F. oxysporum* f. sp. *apii* race 2 or race 4 were lyophilized and ground in liquid N_2. DNA was purified and quantified as were the celery crowns. Based on the Qubit fluorometer, there was no detectable DNA in the previously autoclaved millet grain controls. For each infested sample, there were two qPCR reactions with 0.3 ng of DNA, and one reaction with 0.03 ng of total DNA. Calculations of femtograms of *F. oxysporum* f. sp. *apii* DNA/ng celery DNA from both quantities demonstrated that all the 0.3 ng samples were free of PCR inhibitors.

Analysis of the effect of temperature on the three response variables: *F. oxysporum* f. sp. *apii* DNA concentration, reduction in plant height, and disease severity. In all plant studies, the uninfested control plants were evaluated via qPCR to demonstrate that treatments were not contaminated with either *F. oxysporum* f. sp. *apii* races 2 or 4; we only detected pathogen (race 4) DNA in one

TABLE 1. PCR primers and probe for multicopy template DNA for quantitative PCR estimation of *Fusarium oxysporum* f. sp. *apii* races 2 and 4 biomass in celery crowns[a]

Specificity	Name	Primer or probe	Sequence (5′3′)	Amplicon size, bp	Efficiency, % ± SEM[b]
F. oxysporum f. sp. *apii* race 2	FoaR2-MC1	Forward	tcttcggaccctaggcttatag	153	91 ± 3
		Reverse	aggtttaggttcaggcttcag		
		Probe[c]	ATATGGACG/ZEN/TTGCAGGCCCTACC		
F. oxysporum f. sp. *apii* race 4	FoaR4_MC2	Forward	gggtacgtggatagtaggtaca	107	96 ± 3
		Reverse	cgaagcaagcattaaaggagaag		
		Probe[c]	AGGCGGGCT/ZEN/TCAAAGATGTCGTTA		

[a] The protocol is designed for monitoring *F. oxysporum* f. sp. *apii* in growth chamber and greenhouse experiments. No amplification was detected in celery crowns of plants that were grown in uninfested soil.
[b] SEM, standard error of the mean.
[c] PrimeTime 5′ nuclease probes were purchased from IDT (Coralville, IA). The probes have a 5′ 6-FAM fluorescent reporter dye and two quencher dyes: an internal ZEN and a 3′ Iowa Black forward quencher (IABkFQ).

uninfested replicate in one trial. Except for the inclusion of the height of the uninfested controls in the calculation of the height reduction in the temperature trials, the uninfested controls were not included in the statistical analyses because their inclusion would have falsely decreased the variance estimates. All samples in each replicate were processed as a group to minimize the impact of potential confounders of, for example, any day-to-day variation in either processing DNA or performing qPCR. Linear regressions of the *F. oxysporum* f. sp. *apii* races 2 and 4 DNA standards were used to estimate the log (femtograms of *F. oxysporum* f. sp. *apii* race 2 or race 4 DNA/ng total DNA). After this value was detransformed, femtograms of fungal DNA was normalized to nanograms of celery DNA with the following equation: (fg of fungal DNA/[1 – ng of fungal DNA]).

F. oxysporum f. sp. *apii* concentration and plant height reduction data were originally examined in a mixed model with "trial" as a random effect; when $P > 0.05$, "trial" and nonsignificant interaction terms were removed from the model. Temperature, *F. oxysporum* f. sp. *apii* race, and cultivar were analyzed as fixed effects. To determine the effect of temperature on the four *F. oxysporum* f. sp. *apii*–celery interactions in planta, log femtograms of fungal DNA/ng celery DNA and reduction in plant height variables were analyzed by linear regression with temperature as an independent variable, and when indicated, contrast analysis, and Tukey's honestly significant difference (HSD) test.

To determine the effect of temperature on vascular discoloration in each of the four *F. oxysporum* f. sp. *apii*–celery interactions, we reclassified the ordinal disease score as a binomial variable (either an asymptomatic score of 0 or having vascular discoloration with a score ≥1). The responses were then analyzed by logistic regression with temperature as an independent continuous variable.

Data were analyzed in JMP Pro v.15 (SAS Institute Inc., Cary, NC). In the text, means ± standard error of the mean are shown.

Optimal temperatures for *F. oxysporum* f. sp. *apii* growth in celery extract agar. To prepare celery extract, 200 g of celery petiole segments were autoclaved in 1 liter of water. After the suspension was filtered through cheesecloth, the filtrate was autoclaved with agar (20 g/liter). Just before pouring into Petri dishes, chloramphenicol was added for a final concentration of 500 µg of chloramphenicol/ml. After incubation of a hyphal plug at the indicated temperature, the colony extension was measured daily starting at the 3rd day and ending on the 7th day. The average millimeter of radial hyphal extension per day on two axes of three replicates from three independent trials was determined. For analysis of the temperature of maximal hyphal extension, the square root of growth rate at temperatures <35°C were fitted to a Ratkowsky square-root, four-parameter model (Pietikäinen et al. 2005; Ratkowsky et al. 1983) employing the software suite "R" with the 'optim' function (R Core team 2020). The maxima for each trial and race were calculated in "R" with the 'optimize' function. The Ratkowsky maxima for each race and trial were initially analyzed in a mixed model with "trial" as a random effect and "race" as a fixed effect using the software JMP Pro v.15.

Pairwise correlations between vascular discoloration, *F. oxysporum* f. sp. *apii* concentration, and plant height. To better understand whether the disease severity ratings are a reasonable marker for selecting germplasm with potential resistance to *F. oxysporum* f. sp. *apii* race 4, we further analyzed the dataset from the growth chamber trials to determine to what extent the three response variables were correlated. Because the disease severity score only has six possible values, we analyzed all pairwise correlations for each of the two cultivars × two *F. oxysporum* f. sp. *apii* race combinations by the nonparametric Spearman's rank correlation ρ. Data were analyzed in the software JMP Pro v.15. We note that the trials in the growth chamber were designed so that each plant was an independent replicate.

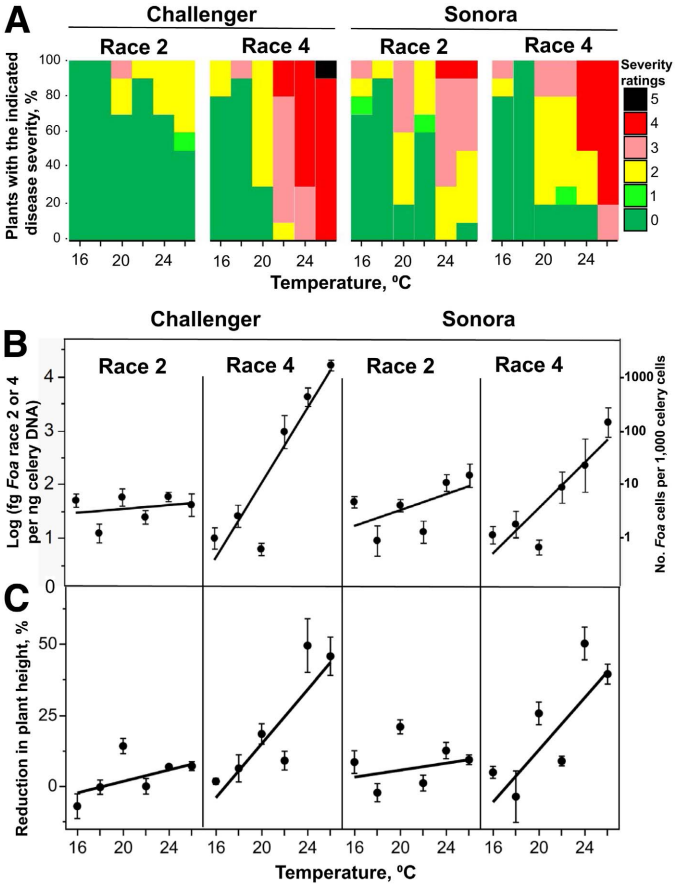

Fig. 1. The effect of growth chamber/soil temperature on the interaction of *Fusarium oxysporum* f. sp. *apii* (*Foa*) races 2 and 4 in celery cultivars Challenger and Sonora. Two-month-old plants were transplanted into either uninfested soil (not shown) or infested soil and incubated for 35 days. **A,** Percentage of plants (n = 10) with the disease severity scores shown on the right, rated from 0 (asymptomatic) to 5 (dead). **B,** On the left axis, the concentration of either *F. oxysporum* f. sp. *apii* race 2 or *F. oxysporum* f. sp. *apii* race 4 DNA on a log scale using real-time quantitative PCR with primers for multicopy template DNA. On the right axis, the approximate number of *Foa* cells per 1,000 celery cells. **C,** The reduction in plant height of plants grown in infested soil compared with the uninfested controls. One 'Challenger' plant at 26°C in *F. oxysporum* f. sp. *apii* race 4-infested soil died; that plant is excluded from the measures in B and C. Error bars are 1 standard error of the mean of five replicate plants from each of two independent trials. 'Challenger' is resistant to *F. oxysporum* f. sp. *apii* race 2 and 'Sonora' is susceptible. Both cultivars are highly susceptible to the new *F. oxysporum* f. sp. *apii* race 4.

RESULTS

Soil temperatures in celery production areas with both *F. oxysporum* f. sp. *apii* races. Soil temperatures typically exceed 21°C in Ventura County during transplanting from August to mid-September or during a harvest after mid-June (Supplementary Fig. S1A), and in Monterey County, between July and September (Supplementary Fig. S1B). In Camarillo in Ventura County, in the 9-year period between 2011 and 2019, the average daily soil temperature exceeded 21°C on 67 to 100% (median = 96%) of the days in the 45-day period from August 5th through September 18th.

Primers and fluorescently labeled probes for qPCR of *F. oxysporum* f. sp. *apii* in celery crowns. PCR primers and a probe were designed to amplify multicopy template DNA for *F. oxysporum* f. sp. *apii* races 2 and 4 (Table 1). The primers and probe are sufficiently specific for assays in the greenhouse and growth chamber; even with a PCR program with 41 cycles, there was no amplification (i.e., no quantification cycle value) with either primer pair and their probe from celery crowns that were grown in uninfested soil, with the exception of one replicate that was contaminated with *F. oxysporum* f. sp. *apii* race 4. In addition, in celery

that was grown in soil infested with *F. oxysporum* f. sp. *apii* race 2, there was no amplification from crowns with the *F. oxysporum* f. sp. *apii* race 4 primers and probe, and in celery that was grown in soil infested with *F. oxysporum* f. sp. *apii* race 4, there was no amplification from crowns with the *F. oxysporum* f. sp. *apii* race 2 primers and probe (data not shown). Based on the *F. oxysporum* f. sp. *apii* race 2 assembly (Henry et al. 2020), there are 99 exact copies of the FoaR2-MC1 primer/probe and 57 copies of a sequence with a single basepair deletion in the 7th bp of the forward primer. The *F. oxysporum* f. sp. *apii* race 4 assembly predicts there are 56 exact copies and one copy with a single nucleotide polymorphism (SNP) of the FoaR4_MC2 primers/probe. The *F. oxysporum* f. sp. *apii* races 2 and 4 multicopy amplicons are in the database GenBank as accessions MW222152 and MW222153, respectively.

The FoaR2-MC1 and FoaR4_MC2 primers/probe are quantitative (R^2 of *F. oxysporum* f. sp. *apii* races 2 and 4 standard DNAs > 99% and efficiency = 91 ± 3% and 96 ± 3% for races 2 and 4, respectively; Supplementary Fig. S2). The primers and probe also are extremely sensitive; the linear range of the standards for *F. oxysporum* f. sp. *apii* races 2 and 4 includes only 0.85 and 2.2 fg of total fungal DNA, respectively. Based on a genome size of *F. oxysporum* f. sp. *apii* races 2 and 4 of 64.8 and 67.4 Mbp (Henry et al. 2020), each *F. oxysporum* f. sp. *apii* races 2 and 4 nucleus and cell contains 42 and 44 fg of DNA, respectively, and consequently one can quantify an estimated 2 and 5% of a single *F. oxysporum* f. sp. *apii* races 2 and 4 cell, respectively, or as few as three template DNA molecules/reaction.

Based on 1,020 *Fusarium* spp. whole-genome assemblies in GenBank, including 535 *F. oxysporum* assemblies, the *Foa*R4_MC2 primers/probe are also predicted to amplify three strains that are closely related to *F. oxysporum* f. sp. *apii* race 4 (Henry et al. 2020): *F. oxysporum* f. sp. *apii* race 3, with 53 copies of the amplicon, and f. sp. *coriandrii* strains GL306, with 45 exact copies and two copies with one SNP and strain 3-2, with 43 exact copies and two copies with one SNP. Based on whole-genome sequenced FOSC in GenBank, the FoaR4_MC2 primers/probe would also amplify one DNA sequence in *F. oxysporum* f. sp. *fragariae* BRIP62109a. In addition to amplifying *F. oxysporum* f. sp. *apii* race 2, *Foa*R2-MC1 primers/probe would amplify multiple copies in *F. oxysporum* f. sp. *lini* strain 39, and f. sp. *conglutinans* strains FGL03-6, Fo5176 and race 1, and a single copy of *F. oxysporum* f. sp. *spinaciae* strains MF15 and MF42, *Fusarium* sp. NRRL 66894, *F. secorum* NRRL 62593, and *F. beomiforme* NRRL 25174.

Quantification of inoculum. Quantification of the *F. oxysporum* f. sp. *apii* races 2 and 4 in the millet grain inoculum indicated that the DNA concentrations were not significantly different (P = 0.75, 2-sample t test). Inoculum contained 205 ± 22 ng *F. oxysporum* f. sp. *apii* race 2 DNA and 192 ± 35 *F. oxysporum* f. sp. *apii* race 4/mg dry wt millet grain, which, based on the *F. oxysporum* f. sp. *apii* genome size (Henry et al. 2020), has a cell density of $(4.9 \pm 0.3) \times 10^6$ *F. oxysporum* f. sp. *apii* race 2 and $(4.4 \pm 0.8) \times 10^6$ *F. oxysporum* f. sp. *apii* race 4/mg dry wt millet grain ($P_{\text{2-sample } t \text{ test}}$ = 0.59). In the growth chamber plant tubes, the transplant plugs were cupped by uninfested soil or by 65 cm^3 of inoculum that contained either $(1.3 \pm 0.1) \times 10^8$ *F. oxysporum* f. sp. *apii* race 2 or $(1.1 \pm 0.2) \times 10^8$ *F. oxysporum* f. sp. *apii* race 4 cells/cm^3 DI.

Effect of temperature on three indicators of celery–*F. oxysporum* f. sp. *apii* interactions: growth of *F. oxysporum* f. sp. *apii* in planta; vascular discoloration-based disease severity; and reduction in plant height. To determine the effect of soil temperature on Fusarium yellows caused by *F. oxysporum* f. sp. *apii* race 2 and Fusarium wilt caused by *F. oxysporum* f. sp. *apii* race 4, transplants of cvs. Challenger and Sonora were grown in either uninfested soil (not shown) or in infested soil in tubes at six temperatures from 16°C to 26°C in two-degree intervals (Fig. 1). We quantified two celery responses: a vascular discoloration-based disease severity score (Fig. 1A) and reduction in plant height (Fig. 1C); and one *F. oxysporum* f. sp. *apii* response, i.e., its concentration in celery crown (Fig. 1B).

Because the disease severity ratings are not a continuous response variable, we used logistic regression to examine the effect of temperature on a binomial version of the vascular discoloration score. Because the log fungal concentration and height reduction were continuous and reasonably linear, we used the slopes of linear regressions to examine the effect of temperature on these variables. For the continuous variables, with mixed models with "trial" as a random effect, "trial" was not significant (Wald P values = 0.64 and 0.49 for *F. oxysporum* f. sp. *apii* concentration and height reduction, respectively). Consequently, the experiments were deemed consistent and the results were pooled.

One-hundred-nineteen of the 120 (99%) uninfested plants had no detected *F. oxysporum* f. sp. *apii* DNA and a vascular discoloration score of 0 (data not shown); a single Challenger control at 26°C was apparently cross contaminated because it had a symptomatic vascular discoloration score and was positive for *F. oxysporum* f. sp. *apii* race 4 DNA. Of the plants in infested soil, the *F. oxysporum* f. sp. *apii* concentration was only below the detection range in one Challenger plant in *F. oxysporum* f. sp. *apii* race 4-infested soil at 16°C; this plant was assigned a *F. oxysporum* f. sp. *apii* race 4 concentration of log = 0. Based on the computation of the detransformed 95% confidence intervals (CI values) of femtograms of *F. oxysporum* f. sp. *apii* DNA/ng celery DNA for each of 24 infested treatments, there was always a clear separation of the uninfested controls and the infested treatments, which never included a 0-value.

With *F. oxysporum* f. sp. *apii* race 4 in both the susceptible cvs. Challenger and Sonora, the fungal concentration in crowns, the disease severity, and the reduction in plant height were all highly temperature-dependent, i.e., significantly ($P \leq 0.0001$) greater as temperature increased (Table 2). With *F. oxysporum* f. sp. *apii* race 2 in the susceptible cultivar Sonora, fungal concentration (P = 0.006) and disease severity (P = 0.0003) increased with temperature, but a decrease in height was not temperature-dependent (P = 0.19). In the incompatible *F. oxysporum* f. sp. *apii* race 2–cultivar Challenger interaction, fungal concentration was not temperature-dependent (P = 0.37), but reduction in plant height (P = 0.009) and disease severity (P = 0.006) were temperature-dependent. Analyses of the linear regressions on temperature indicate that *F. oxysporum* f. sp. *apii* race 4-infected plants are affected to a greater extent by temperature than race 2-infected plants. That is, using the five replicates from each of two trials (n = 10), in a least-squares fit of slopes of log *F. oxysporum* f. sp. *apii* concentration on temperature, cultivar × race (P = 0.0013), race (P < 0.0001), and cultivar (P = 0.03) were significant. Using a Tukey HSD test with α = 0.05, *F. oxysporum* f. sp. *apii* race 4 in Challenger had the steepest growth slope as a function of temperature, *F. oxysporum* f. sp. *apii* race 4 in Sonora had a lower growth slope, and *F. oxysporum* f. sp. *apii* race 2 had the lowest growth slope in either Sonora or Challenger. Similarly, based on a comparison of slopes of height reduction as a function of temperature, temperature has a larger effect on the *F. oxysporum* f. sp. *apii* race 4 infections than it does on the *F. oxysporum* f. sp. *apii* race 2 infections (P < 0.0001). *F. oxysporum* f. sp. *apii* race 4 reduced plant height an average of 5.6% per degree centigrade versus 1.3% per degree centigrade for race 2, which is equivalent to an ∼4× greater impact of *F. oxysporum* f. sp. *apii* race 4 than race 2. We note that there was no decrease in plant height of the uninfested controls within the tested 16 to 26°C (Supplementary Fig. S3). Indeed, in a linear regression of height on temperature of the controls in uninfested soil, the slope was positive and significant for both Challenger (slope = 0.95 ± 0.24 cm per degree centigrade, R^2 = 0.27, P = 0.0002) and for Sonora (slope = 0.60 ± 0.27 cm per degree centigrade, R^2 = 0.11, P = 0.029).

In an analysis of cultivar and race at 18°C, there were no significant (α = 0.05) differences between either the reduction in plant

height ($P_{F\ test}$ = 0.59) or the concentration of either *F. oxysporum* f. sp. *apii* race 2 or race 4 ($P_{F\ test}$ = 0.53) for Challenger or Sonora. The detransformed 95% CI value was from 9 to 76 fg of *F. oxysporum* f. sp. *apii* race 4 DNA/ng Challenger DNA. At temperatures between 22 and 26°C, there was significantly (α = 0.05) more *F. oxysporum* f. sp. *apii* race 4 than *F. oxysporum* f. sp. *apii* race 2 DNA in planta. The detransformed 95% CI value was from 190 to 4,713 fg of *F. oxysporum* f. sp. *apii* race 4 DNA/ng Challenger DNA at 22°C, from 1,739 to 10,284 fg of *F. oxysporum* f. sp. *apii* race 4 DNA/ng Challenger DNA at 24°C, and from 9,675 to 27,563 fg of *F. oxysporum* f. sp. *apii* race 4 DNA/ng Challenger DNA at 26°C. In contrast, in the incompatible *F. oxysporum* f. sp. *apii* race 2 interactions with Challenger from 22 to 26°C, the detransformed 95% CI value had a maximum estimate of 123 fg of *F. oxysporum* f. sp. *apii* race 2 DNA/ng Challenger DNA. In the compatible *F. oxysporum* f. sp. *apii* race 2 interactions with Sonora from 22 to 26°C, the detransformed 95% CI value increased, but only to a maximum 458 fg of *F. oxysporum* f. sp. *apii* race 2 DNA/ng Challenger DNA. That is, in a Tukey's HSD test of log (fungal concentration/ng plant DNA; α = 0.05) at 26°C, there were four groups: Challenger–*F. oxysporum* f. sp. *apii* race 4 with the highest concentration; Sonora–*F. oxysporum* f. sp. *apii* race 4 with a lower concentration; and both compatible and incompatible *F. oxysporum* f. sp. *apii* race 2 with the lowest concentration. Thus, in all three compatible interactions, our data are consistent with the hypothesis that increasing temperature causes more fungal growth and as a result, an increase in the disease severity rating and, with an exception of Sonora–*F. oxysporum* f. sp. *apii* race 2, a reduction in plant height, i.e., stunting. In the incompatible interaction of Challenger and *F. oxysporum* f. sp. *apii* race 2, temperature did not have a significant effect on the *F. oxysporum* f. sp. *apii* concentration in crown tissue.

Optimal temperatures for *F. oxysporum* f. sp. *apii* growth in celery agar. To determine if temperatures that are better for growth of *F. oxysporum* f. sp. *apii* in planta are optimal for growth of *F. oxysporum* f. sp. *apii* in vitro, we determined the temperature for maximal hyphal extension of *F. oxysporum* f. sp. *apii* races 2 and 4 in celery extract agar. The temperature for maximal growth rate was estimated from a fitted Ratkowsky model (Pietikäinen et al. 2005; Ratkowsky et al. 1983; Smits et al. 1998). In a mixed model with "trial" as a random variable, "trial" was not significant for the maximum (Wald P = 0.91), indicating that results were reproducible. Based on an analysis of variance of the Ratkowsky predicted temperature for maximal growth rate in the three trials, race was highly significant (P = 0.008). *F. oxysporum* f. sp. *apii* races 2 and 4 have maximal growth rates at 25.2 ± 0.2°C and 27.7 ± 0.5°C, respectively. Estimates of the 95% CI values for maximal hyphal extension were from 24.1 to 26.2°C and from 25.7 to 29.1°C for *F. oxysporum* f. sp. *apii* races 2 and 4, respectively (Fig. 2). There were no significant differences between the maximal growth rate of the two races at their optimal temperatures (P = 0.33). Estimates of their maximal growth rates were 5.3 ± 0.2 and 5.5 ± 0.03 mm per day for *F. oxysporum* f. sp. *apii* races 2 and 4, respectively. Based on the Ratkowsky model, in culture, the linear growth rate of *F. oxysporum* f. sp. *apii* races 2 and 4 at 24°C was 42 and 56% greater, respectively, than at 18°C. To conclude, similar temperatures are conducive to growth of *F. oxysporum* f. sp. *apii* races 2 and 4 in vitro and in planta.

Pairwise correlations among the three response variables in the three compatible interactions versus the incompatible one. In both susceptible cultivars Challenger and Sonora with *F. oxysporum* f. sp. *apii* race 4, all pairs of response variables were highly significantly correlated (P < 0.0001) with ρ = 0.65 to 0.85. Notably, although the disease severity score is an indicator of the plant's response, it was highly significantly (P < 0.0001) correlated with fungal concentration in the crown (ρ = 0.85 for Challenger–*F. oxysporum* f. sp. *apii* race 4 and ρ = 0.65 for Sonora–*F. oxysporum* f. sp. *apii* race 4). In the susceptible Sonora–*F. oxysporum* f. sp. *apii* race 2 interaction, fungal

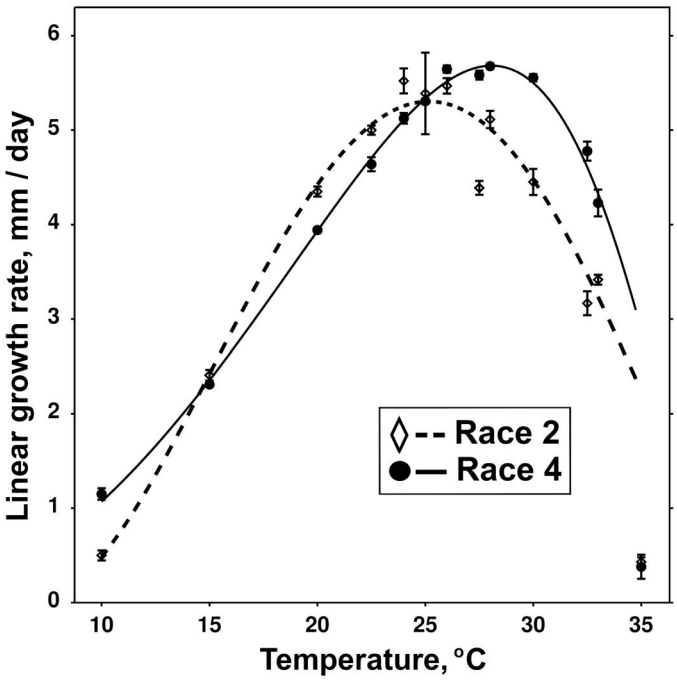

Fig. 2. Hyphal extension rate of *Fusarium oxysporum* f. sp. *apii* races 2 and 4 on celery extract agar as a function of temperature. Error bars show ± standard error of the mean from three independent trials, each with three replicates. The curves are fitted Ratkowsky models (Ratkowsky et al. 1983) for all temperatures <35°C.

TABLE 2. The effect of temperature on celery cultivars Sonora and Challenger exposed to either *Fusarium oxysporum* f. sp. *apii* race 2 or 4: the log concentration of fungal DNA in celery crowns, the extent of stunting, and a nominal assessment of an ordinal vascular discoloration-based disease severity score[a]

		Linear regressions				Logistic regressions	
		Log (fg of *F. oxysporum* f. sp. *apii* race 2 or race 4 DNA/ng plant DNA)		Reduction in plant height, %		Binomial vascular discoloration-based disease severity[c]	
Cultivar	*F. oxysporum* f. sp. *apii* race	Slope ± SEM[b]	*P* value	Slope ± SEM	*P* value	Rate of change of log odds ± SEM	*P* value
Challenger	4	0.4 ± 0.03[d]	<0.0001	4.2 ± 0.7[e]	<0.0001	0.91 ± 0.24	0.0001
Sonora	4	0.2 ± 0.04	<0.0001	4.6 ± 0.8	<0.0001	0.55 ± 0.14	<0.0001
Challenger	2	0.02 ± 0.02	NS[f] (0.37)	1.0 ± 0.4	0.009	0.35 ± 0.13	0.006
Sonora	2	0.07 ± 0.02	0.006	0.6 ± 0.5	NS (0.19)	0.38 ± 0.10	0.0003

[a] All values have temperature as the independent variable. Data are shown in Figure 1. n = 10, except where indicated as NS.
[b] SEM, standard error of the mean.
[c] Plants were classified as either asymptomatic (score 0) or symptomatic (scores 1 to 5). χ^2 P values for the temperature parameter are shown.
[d] No *F. oxysporum* f. sp. *apii* race 4 DNA was detected in one sample of Challenger at 16°C; this sample was assigned a log value = 0.
[e] One Challenger plant grown in *F. oxysporum* f. sp. *apii* race 4-infested soil at 26°C died; this plant was excluded from the analyses of fungal DNA concentration and reduction in plant height.
[f] NS, not significant (P > 0.05), with P value given in parentheses.

concentration, disease severity, and reduction in plant height were significantly ($P < 0.049$) correlated ($\rho = 0.3$ to 0.56), with a statistically strong correlation between the disease severity score and either the concentration of *F. oxysporum* f. sp. *apii* race 2 ($P < 0.0001$) or the reduction in plant height ($P = 0.0002$), and a weak correlation between reduction in plant height and *F. oxysporum* f. sp. *apii* race 2 concentration ($P = 0.049$). In the incompatible Challenger–*F. oxysporum* f. sp. *apii* race 2 interaction, there was no significant correlation between fungal concentration in the crown and either disease severity ($P = 0.051$) or height reduction ($P = 0.10$); disease severity and height reduction were significantly correlated ($\rho = 0.44$, $P = 0.002$).

DISCUSSION

Here we show that in soil temperatures of 22 to 26°C, the new *F. oxysporum* f. sp. *apii* race 4 grows significantly more in planta than *F. oxysporum* f. sp. *apii* race 2. This observation, at least partly, explains why *F. oxysporum* f. sp. *apii* race 4 is a more virulent pathogen than *F. oxysporum* f. sp. *apii* race 2 in disease assays in greenhouses that are maintained at 27 to 29°C (Henry et al. 2020) and in the field, particularly in August plantings in Ventura County, California. Similarly, a highly virulent strain of FOSC f. sp. *phaseoli* achieved 16× more biomass in bean root crowns at 7 dpi than a weakly virulent strain (Niño-Sánchez et al. 2015). We note that *F. oxysporum* f. sp. *apii* races 2 and 4 are in different clades within the FOSC (Epstein et al. 2017), i.e., are in different species, are comparatively divergent organisms, and appear to be incapable of exchanging DNA via conidial tube anastomosis (Henry et al. 2020). Thus, while both *F. oxysporum* f. sp. *apii* races are pathogenic on celery and to a lesser extent on cilantro, and are in a bonafide monophyletic group (Geiser et al. 2013), they are quite different, particularly in their accessory genomes (Henry et al. 2020).

Although both *F. oxysporum* f. sp. *apii* races 2 and 4 have similar growth rates in culture in the 22 to 26°C range (Fig. 2), and the growth of both races in planta in the three compatible interactions is temperature-sensitive (Table 2), there are differences in the extent of the temperature effect in planta. In a comparison of slopes of linear regressions of the log concentration of *F. oxysporum* f. sp. *apii* in celery crowns on temperature, *F. oxysporum* f. sp. *apii* race 4 in Challenger had the steepest slope, *F. oxysporum* f. sp. *apii* race 4 in Sonora had a significantly lower slope, and *F. oxysporum* f. sp. *apii* race 2 in either Sonora or Challenger had the lowest slopes. Indeed, the slope of *F. oxysporum* f. sp. *apii* race 2 in Challenger was not significantly different from 0, i.e., there was no evidence that the growth of race 2 in Challenger is affected by temperature, although reduction in plant height and disease symptoms were temperature-dependent. In contrast to *F. oxysporum* f. sp. *apii* races 2 and 4, celery is a cool-weather crop with an optimum between 16 and either 18 or 21°C (Rubatzky et al. 1999; Smith 2021). Thus, temperatures that are optimum for *F. oxysporum* f. sp. *apii* races 2 and 4 may be temperatures of either mild heat-stress and/or diminished immune response in celery. Regardless of whether soil temperatures in the 22 to 26°C range cause some heat stress in celery, the temperature-dependent increase in *F. oxysporum* f. sp. *apii* race 4 biomass at these temperatures (Fig. 1B) is very large. The detransformed mean of *F. oxysporum* f. sp. *apii* race 4 concentration at 24°C was 163× of that mean at 18°C. We also note that based on comparisons of *F. oxysporum* f. sp. *apii* concentration in plants in the 16 to 20°C range, the larger increase in *F. oxysporum* f. sp. *apii* race 4 versus race 2 at high temperatures cannot be explained by different levels of initial inoculum; there were no significant differences in *F. oxysporum* f. sp. *apii* concentration in the inoculum at the start of the experiment or in celery crowns after 35 days at 18°C. FOSC effectors play a critical role in disease outcomes (van Dam et al. 2017) and we postulate that gene expression of some *F. oxysporum* f. sp. *apii* race 4 effectors are temperature-dependent.

This is the first documentation that temperatures above the optimum for celery increase the severity of disease caused by *F. oxysporum* f. sp. *apii* races 2 and 4. In accordance with our results, grower experience in Ventura County with *F. oxysporum* f. sp. *apii* race 4 has been that plantings in August have greater disease severity and economic losses than plantings in late September to early October when temperatures are cooler. Based on our results, we recommend that growers in Ventura County delay transplantation in the late summer or early fall until average soil temperatures have dropped to ≤ 21°C (Supplementary Fig. S1). Additional research is required to determine the impact of temperatures above 21°C on crop debris; hypothetically, these temperatures might increase colonization of postharvest celery debris, and might conceivably either increase or decrease survival of the *F. oxysporum* f. sp. *apii* race 4 inoculum in the debris. Growers in Monterey County could transplant in either March or late September to avoid the typical warmer soil temperatures above 21°C from July to early September.

Here we show (Table 3) that there is a highly significant correlation between the ordinal vascular discoloration score, which is a manifestation of the plant's response (Davis et al. 1953), and *F. oxysporum* f. sp. *apii* race 4 growth in the crown of Challenger and Sonora. That is, vascular discoloration is positively correlated with fungal colonization in the crown of the hypervirulent *F. oxysporum* f. sp. *apii* race 4 and two susceptible hosts. Overall, the correlation between the vascular discoloration score and *F. oxysporum* f. sp. *apii* race 2 growth is also highly significant for the susceptible Sonora, but not significant for the resistant Challenger. Interestingly, phenolic-conjugates and possibly other compounds

TABLE 3. For each plant that was grown in infested soil, the correlation and *P* value of the *Fusarium oxysporum* f. sp. *apii* race 2 or 4 concentration in the crown by quantitative PCR, the vascular discoloration-based disease severity score, and the height reduction of a plant in infested soil compared with the uninfested controls[a]

Celery cultivar	*F. oxysporum* f. sp. *apii* race in the soil	Variable 1	Variable 2	Spearman's rank correlation	*P* value
Challenger	Race 4	*F. oxysporum* f. sp. *apii* concentration	Disease severity	0.85	<0.0001
Challenger	Race 4	*F. oxysporum* f. sp. *apii* concentration	Height reduction	0.68	<0.0001
Challenger	Race 4	Disease severity	Height reduction	0.72	<0.0001
Sonora	Race 4	*F. oxysporum* f. sp. *apii* concentration	Disease severity	0.65	<0.0001
Sonora	Race 4	*F. oxysporum* f. sp. *apii* concentration	Height reduction	0.69	<0.0001
Sonora	Race 4	Disease severity	Height reduction	0.75	<0.0001
Challenger	Race 2	*F. oxysporum* f. sp. *apii* concentration	Disease severity	0.25	NS[b] (0.051)
Challenger	Race 2	*F. oxysporum* f. sp. *apii* concentration	Height reduction	0.25	NS (0.10)
Challenger	Race 2	Disease severity	Height reduction	0.44	0.002
Sonora	Race 2	*F. oxysporum* f. sp. *apii* concentration	Disease severity	0.56	<0.0001
Sonora	Race 2	*F. oxysporum* f. sp. *apii* concentration	Height reduction	0.30	0.049
Sonora	Race 2	Disease severity	Height reduction	0.52	0.0002

[a] Data are shown in Figure 1. $n = 60$ except for Challenger-*F. oxysporum* f. sp. *apii* race 4, in which $n = 59$ because of one dead plant at 26°C. *F. oxysporum* f. sp. *apii* concentration and height reduction are continuous response variables. Disease severity (0, asymptomatic to 5, dead) is an ordinal response variable.
[b] NS, not significant ($P > 0.05$), with *P* value given in parentheses.

that are associated with vascular discoloration (Davis et al. 1953) are positively associated with both compatible and incompatible interactions. There is increasing evidence that plant pathogenic FOSC and other fungi with necrotrophic and hemibiotrophic lifestyles have usurped the host's apoptosis or programmed cell death, which provides a mechanism for resistance against biotrophs, and used it as a method to kill the host cell and facilitate the pathogen's necrotrophy (Kabbage et al. 2017).

This is, perhaps arguably, only the second article to examine the Challenger–*F. oxysporum* f. sp. *apii* race 2 incompatible interaction. In an electron microscopic examination of roots from a susceptible celery infected with *F. oxysporum* f. sp. *apii* race 2 in comparison with the *F. oxysporum* f. sp. *apii* race 2-resistant celeriac parent of cultivar Challenger, Jordan et al. (1989) observed 3-to-5× more electron-opaque bodies in the paravascular contact cells in the incompatible celeriac cultivar than in a susceptible celery (Tall Utah 52-70R), and only a few in the uninfected controls; a susceptible *F. oxysporum* f. sp. *apii* race 2 celeriac cultivar was included as a control. The electron-opaque material was positive for phenolics and polysaccharides. Importantly, tonoplast disruption/phenolic release was spatially associated with death of *F. oxysporum* f. sp. *apii* hyphae. Based on multiple investigations primarily with FOSC f. sp. *vasinfectum* in cotton, Beckman (2000) argued that the xylem's vessel-associated parenchyma cells (synonyms, vessel-associated cells, paravascular contact cells [VAC]) are a key to a plant's success in an incompatible FOSC interaction. In this scenario, infection with either a FOSC or other specific biotic and abiotic triggers induces VAC to store phenolics in their vacuoles. In the tomato–*F. oxsporum* f. sp. *lycopersici* pathosystem, the tomato resistance gene *I-2*, which encodes for a classical nucleotide binding-leucine rich repeat-type resistance protein (Ori et al. 1997), is expressed in the vessel-associated parenchyma cells (Mes et al. 2000). To sum up, despite the overall association of vascular discoloration and disease severity, the literature provides evidence that celery roots have vessel-associated parenchyma cells that are induced by *F. oxysporum* f. sp. *apii* race 2 to form phenolics, and that in the case of the progenitor of the *F. oxysporum* f. sp. *apii* race 2-resistant cultivar Challenger, the release of those phenolics is associated with limiting growth of *F. oxysporum* f. sp. *apii* race 2.

Incorporation of resistance into germplasm is the best method of control of diseases caused by the FOSC. Resistance to FOSC can be classified as either cases of "classical resistance" in which pathogen growth is limited particularly within the xylem (Bani et al. 2018; Pouralibaba et al. 2017), or from tolerance in which the pathogen grows but the host is less affected by toxins, effectors, etc. (Pagán and García-Arenal 2018). Although the term "tolerance" is often used synonymously with reduced susceptibility, here we have the data on pathogen growth per se. Our data indicate that the Challenger–*F. oxysporum* f. sp. *apii* race 2 interaction is at least partly based on classical resistance because pathogen concentrations remained low (at 24°C daytime, the detransformed 95% CI value was 37- to 93-fg race 2 DNA/ng Challenger DNA), i.e., at 35 days posttransplantation, the compatible interaction had a detransformed mean concentration that was 72× higher at 24°C. We note that *F. oxysporum* f. sp. *apii* races 2 and 4 can be isolated from presymptomatic tissue and some plants infected by *F. oxysporum* f. sp. *apii* race 2 in particular seem to remain completely asymptomatic. That is, as in numerous other FOSC–host interactions (Husaini et al. 2018), host resistance occurs after entry into the host cortex.

Here, we continued to characterize the susceptibility of the cultivar Challenger to *F. oxysporum* f. sp. *apii* race 4 (Epstein et al. 2017; Henry et al. 2020), primarily because Challenger has the best documentation for resistance to *F. oxysporum* f. sp. *apii* races 1, 2, and 3 and is available for use in a public breeding program. In the incompatible *F. oxysporum* f. sp. *apii* race 2 interaction in a Challenger ancestor, resistance appears to depend on one major and one quantitative gene that were introgressed from a celeriac (Orton et al. 1984a).

We used fluorescent probes with two quencher dyes and selected multicopy amplicons of transposable elements (Henry et al. 2020) that are in *F. oxysporum* f. sp. *apii* race 4 but not race 2, and vice versa. Based on the program RepeatMasker (v.4.0.8; https://www.repeatmasker.org/), the FoaR2-MC1 amplicon is part of a rolling circle/Helitron, a DNA transposon. Based on the online tool Repbase (https://www.girinst.org/repbase/; Kohany et al. 2006), the FoaR4_MC2 amplicon is part of a Gypsy Long Terminal retrotransposon with 0.8 similarity to *F. poae* Maggy_FPo-1 (Vanheule et al. 2016). We empirically demonstrated that these amplicons were sufficiently specific for these particular growth chamber experiments, and bioinformatically demonstrated that these *F. oxysporum* f. sp. *apii* races 2 and 4 amplicons are only present in 0.6% (3/535) and 1.3% (7/535) of the *F. oxysporum* assemblies in the database GenBank. Nonetheless, caution should be used in a field application because celery plants infected with a pathogenic race frequently contain at least one nonpathogenic FOSC strain (Epstein et al. 2017), and diverse FOSC strains can grow in cortical cells of nonhosts (Henry et al. 2019). Thus, although these primers/probe for multicopy repetitive elements are not recommended for quantification of *F. oxysporum* f. sp. *apii* biomass from the field without clear evidence that only the intended target strains are being amplified, they have allowed the quantification of *F. oxysporum* f. sp. *apii* races 2 and 4 concentration in greenhouse and growth chamber studies from ~1 *F. oxysporum* f. sp. *apii* cell/1,000 host cells to >1 *F. oxysporum* f. sp. *apii* cell/celery cell.

We utilized celery crowns to estimate *F. oxysporum* f. sp. *apii* growth for several reasons: our observations of severity scores over time (data not shown) are consistent with the hypothesis that infection of the crown vasculature is a critical stage in the disease process; collection of the single crown per plant is easier and less subjective than root, particularly fine root collection; and celery crowns have fewer PCR inhibitors than either roots or any dead tissue (data not shown). By comparing *F. oxysporum* f. sp. *apii* concentration estimates using both 3 and 10 ng total DNA from each celery crown sample, we confirmed that our quantification was not compromised by interference from PCR inhibitors. Nonetheless, researchers interested in using similar real-time PCR assays especially regarding survival of inoculum from crop debris should routinely monitor for potential interference from PCR inhibitors; internal standards may be useful for such studies (Bilodeau et al. 2012).

Here, we demonstrated that the virulence of *F. oxysporum* f. sp. *apii* race 4 in celery is highly temperature-dependent. Within the optimal range for celery growth between 16 and 18°C, *F. oxysporum* f. sp. *apii* races 2 and 4 have similar growth in planta, and cause similar and comparatively limited symptoms. However, at 22 to 26°C, compared with *F. oxysporum* f. sp. *apii* race 2, race 4 achieves significantly higher concentrations in planta and causes significantly more stunting. We conclude that in compatible interactions with both *F. oxysporum* f. sp. *apii* races 2 and 4, the data are consistent with the hypothesis that increased *F. oxysporum* f. sp. *apii* growth may cause the symptoms of stunting and vascular discoloration; that in contrast to the compatible interactions, in the incompatible interaction, *F. oxysporum* f. sp. *apii* race 2 concentration in cultivar Challenger crown tissue is not significantly correlated with either a reduction in height or a higher disease severity score; and that, particularly with *F. oxysporum* f. sp. *apii* race 4, a low vascular discoloration-based disease severity score appears to be a reasonable marker in screens for resistant germplasm.

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