The Effect of pH on Spore Germination, Growth, and Infection of Strawberry Roots by *Fusarium oxysporum* f. sp. *fragariae*, Cause of Fusarium wilt of Strawberry

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Abstract

Previous work has shown that raising the pH of acidic soil to near neutrality can reduce the incidence of Fusarium wilt. The basis for this effect has not been established. The present study assessed effects of pH on spore germination, growth, and infection of strawberry roots by *Fusarium oxysporum* f. sp. *fragariae*, the cause of Fusarium wilt of strawberry. There was not a significant effect of pH (5 versus 7) on the rate of spore germination at either 20 or 25°C for any of the three tested isolates (one representative of each clonal lineage of *F. oxysporum* f. sp.

fragariae found in California). Likewise, pH did not have a significant effect on fungal growth at 20°C. At 25°C, two isolates grew faster at pH 7 than at pH 5. Growth of the third isolate was unaffected by the difference in pH. For the strawberry cultivar Albion, the frequency of root infection was significantly higher for plants grown in acidified soil (near pH 5) than for plants grown in soil near neutrality. The higher frequency of root infection in acidified soil was associated with a lower level of microbial activity, as measured by hydrolysis of fluorescein diacetate.

Management of soilborne diseases is most effectively achieved through the use of genetically resistant cultivars. Where this resource is not available, management is heavily reliant on measures that reduce the abundance and/or efficacy of pathogen inoculum in soil. Crop rotation can contribute to a reduction in pathogen inoculum by providing an interval without an opportunity for pathogen reproduction (e.g., Scott et al. 2012). However, economic considerations may favor growing the same crop in successive years. For strawberries, this practice has been sustained through the use of preplant fumigation to reduce pathogen populations in soil (Wilhelm and Paulus 1980). However, regulatory restrictions and cost considerations have limited the availability and effectiveness of soil fumigation, increasing the need for other measures to maintain pathogen inoculum densities below damaging levels.

This issue is of great importance to the production of strawberries (*Fragaria* × *ananassa* Duchesne ex Rozier) in California, where soilborne pathogens have become increasingly problematic (Koike and Gordon 2015; Koike et al. 2016). This includes *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *fragariae* Winks & Williams, the cause of Fusarium wilt, which is now found in all major strawberry production areas in California. Fusarium wilt of strawberry was discovered in Australia (Winks and Williams 1965) and soon thereafter was reported in Japan (Okamoto et al. 1970). This disease was confirmed to occur in California in 2008 (Koike et al. 2009), in fields where altered fumigation practices failed to fully treat the soil (Gordon et al. 2016). A major gene for resistance to Fusarium wilt was recently identified in the University of California breeding population (Pincot et al. 2018); however, most commonly grown cultivars are susceptible.

Management of wilt diseases caused by *Fusarium oxysporum* can benefit from a reduction in the efficacy of inoculum, as has been reported to occur in suppressive soils (Alabouvette 1999; Bonanomi et al. 2010). Soil suppressiveness may occur naturally but can also

be enhanced by measures such as the adjustment of soil pH to 7.0 or close thereto, which has been shown to reduce damage caused by Fusarium wilt in a number of crops (Everett and Blazquez 1967; Fang et al. 2012; Gatch and du Toit 2017; Jones and Woltz 1967). Among the factors that may contribute to this effect are reduced access to micronutrients (Jones and Woltz 1970) and heightened competition from other soil microbes. A better understanding of the basis for an effect of pH might allow for an enhancement of the benefit it provides or alternative means to achieve the same effect.

The present study was undertaken to assess effects of pH that could explain a reduction in severity of Fusarium wilt of strawberry. The specific objectives were to quantify effects of pH on: 1) spore germination in vitro, 2) radial growth in vitro, 3) the frequency of root infection, and 4) the overall level of microbial activity in soil based on hydrolysis of fluorescein diacetate.

Materials and Methods

Spore germination. Three isolates of *F. oxysporum* f. sp. *fragariae* were included in spore germination experiments (GL 1059, GL 1080, and GL 1315) to represent each of the three clonal lineages known to occur in California (Henry et al. 2017). Isolates were grown on potato dextrose agar (PDA) under continuous fluorescent light at room temperature. Spore suspensions were obtained from fully colonized plates by depositing sterile distilled water on the surface of a culture, which was scraped with the edge of a sterile, glass microscope slide. The resulting suspension was filtered through two layers of sterile cheesecloth. The density of spores in the suspension was quantified microscopically using a hemocytometer and adjusted with sterile water to a density of 50 spores per microliter. The spore suspension was used to inoculate plates of PDA amended with 0.03% streptomycin sulfate, which were adjusted to either pH 5.0 ± 0.1 or 7.0 ± 0.1, by addition of 10% H₃PO₄ or 2 M KOH, respectively. One microliter of spore suspension (approximately 50 spores) of one of the three isolates was placed in each of four equally sized sectors on each of 12 plates per pH level. Plates were incubated in darkness, with half in an incubator set for 20°C and the other half incubated at 25°C. Spore germination was evaluated after 12, 14, and 16 h of incubation at 20°C, and after 8, 10, and 12 h at 25°C. Spores were stained with aniline blue (250 ml 85% lactic acid, 500 ml glycerol, 1.25 g aniline blue per liter of water) and examined microscopically at 40× to quantify spore germination. Spores were counted as germinated where the length of the germ tube was equal to or greater than the long dimension of the spore from which it emerged. Spore germination rate was calculated as the number of

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germinated spores divided by the total number of spores in a sector. The average of four sectors on a plate (=technical replications) constituted a biological replication, of which there were two for each isolate at each pH per time point. The full experiment was conducted twice.

Radial growth. Isolates GL 1059, GL 1080, and GL 1315 were each cultured on a plate of 2% water agar. Four-millimeter-diameter plugs, removed from the growing margin of a colony with a sterilized cork borer, were used to inoculate 9-cm-diameter plates of PDA amended with 0.03% streptomycin sulfate. The pH of PDA was adjusted to either 5.0 ± 0.1 or 7.0 ± 0.1 , as described above. A single agar plug was placed at the center of each plate. Each isolate was placed on 32 plates, 16 plates with PDA adjusted to pH 5.0 and 16 adjusted to pH 7.0. All plates were incubated in darkness, with half of the plates at each pH incubated at 20°C and the remaining plates incubated at 25°C. After 1, 3, 5, and 7 days of incubation, colony diameters were measured on two plates of each isolate at each pH. Colony diameter recorded for each plate was the average of measurements taken on two perpendicular axes. Plates were discarded after measurement, so subsequent measures were taken on plates not previously removed from the incubator. The full experiment was conducted twice.

Root infection. Soil used in root infection assays was obtained from a commercial strawberry field in Ventura County, CA with a history of Fusarium wilt. After collection, soil was passed through a 1.3-cm² sieve and stored at 4°C for approximately 8 months until used. Field soil was combined with fine white sand (<http://www.gohasties.com/product/fine-white-sand/>), which was sterilized by autoclaving twice, 24 h apart, to achieve a 60:40 blend of sand:field soil. This was done to ensure adequate drainage. The density of inoculum of *F. oxysporum* f. sp. *fragariae*, determined using soil dilution plating as described by Henry et al. (2017), was 191 ± 73 colony forming units per gram of soil. Composition of the soil was 88%

sand, 6% silt, and 6% clay, with less than 0.5% organic matter. The initial pH was 6.1.

Treatments were intended to achieve a pH of approximately 5.0 (=acidified soils) by the addition of 10% H₂SO₄, or a pH of approximately 7.0 (=neutral soils) by the addition of 0.5 M NaOH. pH was measured using an Oakton meter (Cole-Parmer, catalog code: UX-35613-20) with a spear-tip pH electrode (Cole-Parmer, catalog code: SK-59002-24). Soil was brought to field capacity with deionized water, and the electrode was inserted directly into the soil. The average of four measurements determined if additional acid or base was needed to reach the target pH. Prior to each set of measurements, the meter was calibrated using pH standards 4.0, 7.0, and 10.0. The sand:soil mix was dispensed into 3.8-liter pots, four for each of the two pH levels. Three strawberry crowns were placed at the center of each pot. Plants used in these experiments were obtained from a low-elevation nursery and were stored at -2°C prior to use.

Prior to planting, roots were held under running tap water to remove any adherent soil and then immersed in dilute nutrient water (described below), which was adjusted to the pH corresponding to that of the soil in which plants would be grown. Roots were trimmed to a length of approximately 6 cm to prevent contact with the bottom of the pot, so new roots would emerge only where they would be fully in contact with soil. After placement in pots, plants were maintained in a controlled environment chamber with a 12-h photoperiod and day/night temperatures of 28/20°C. Plants were supplied with dilute nutrient water (NH₄-N at 0.513 mg/liter, NO₃-N at 60.33 mg/liter, P at 6.11 mg/liter, K at 63.45 mg/liter, Ca at 3.7 meq/ml, Mg at 2.1 meq/ml, and Na at 0.06 meq/ml) as needed to maintain soil near field capacity. Nutrient water added to acidified and neutral soils was adjusted to 4.95 ± 0.01 and 7.16 ± 0.00 , respectively. This experiment was conducted twice using the cultivar Albion and twice using the cultivar San Andreas, which are known to be susceptible and resistant to Fusarium wilt, respectively (Gordon et al. 2016).

Fig. 1. The germination percentage of spores of *Fusarium oxysporum* f. sp. *fragariae* after incubation at 20°C for 12, 14, and 16 h on potato dextrose agar adjusted to either pH 5 or pH 7. Means represented by the height of each bar were computed from all replications of three isolates (GL 1315, GL 1080, and GL 1059) in two experiments ($n = 12$). Error bars correspond to $2 \times$ the standard error of the mean.

Between 14 and 19 days after an experiment was initiated, roots were sampled from two pots of each treatment, and the remaining four pots were sampled the following day. Prior to removal of roots, the pH of soil in each pot was measured at three locations separated by at least 3 cm (=technical replications). The average of these three measurements represented the value for each pot (=biological replication). Crowns with roots attached were separated from the soil matrix by hand after inverting a pot in a plastic tub. Any roots in contact with the side of the pot were excluded. After a gentle rinse under running water, primary roots with young feeder roots attached were separated from the crown and placed in a flask containing sterile 1% sodium hexametaphosphate, which serves to disperse soil aggregates into their component particles (Kettler et al. 2001). This flask was placed on a rotary shaker set at 50 rpm for 15 min, after which liquid was drained from the flask and replaced with fresh, sterile, 1% sodium hexametaphosphate. The flask was returned to the shaker for an additional 15 min, followed by a third rinse using the same procedure, and a final 15-min rinse in sterile water. Thereafter, roots were floated in sterile water, and root tips, 0.4 to 2.5 cm in length, were placed on plates of Komada's selective medium (KM) (Komada 1975), and incubated under continuous fluorescent light at room temperature (22–24°C).

A minimum of 30 root tips was assayed from each pot. After 5 to 7 days, root tips from which a fungal colony emerged were scored as infected. Colonies were identified as *F. oxysporum* based on colony morphology, and those provisionally identified as *F. oxysporum* f. sp. *fragariae* were stab-transferred to fresh plates of KM for direct comparison with a known pathogenic isolate of *F. oxysporum* f. sp. *fragariae* (GL 1080). After 8–10 days of incubation under continuous fluorescent light at room temperature, colonies identified as the pathogen based on colony morphology were tested for the presence or absence of a diagnostic DNA sequence (Suga et al. 2013), as

described by Henry et al. (2017). The proportion of root tips confirmed to be infected by *F. oxysporum* f. sp. *fragariae* was computed for each pot (=biological replication).

Microbial activity. Fluorescein diacetate (FDA) hydrolysis assays were performed as described by Schumacher et al. (2015). For each pot (=biological replicate), four 2-g soil samples were taken at the time roots were sampled. Each 2-g sample was placed in a 50-ml glass flask (four flasks in total) containing 15 ml of 60 mM potassium phosphate buffer (pH = 7.6). Soil was suspended by stirring/grinding with a glass rod. Fluorescein diacetate (0.2 ml of a 1 mg/ml solution in acetone) was added to each of three flasks, and 0.2 ml chloroform was added to the fourth. Flasks were covered with Parafilm and incubated at room temperature on a rotary shaker (100 rpm) for 1 h. The reaction was stopped by addition of 0.2 ml chloroform to the three flasks that originally received fluorescein diacetate; 1.5 ml was transferred from each flask to 2-ml tubes and centrifuged at 14,000 rpm for 2 min, after which 1 ml of the supernatant was transferred to a sterile cuvette. The flask that did not originally receive fluorescein diacetate was used to blank absorbance at 490 nm on a Genysis 10 UV-VIS spectrophotometer. Absorbance at 490 nm was recorded for three technical replicates, and an average of the three readings was taken as a measure of FDA hydrolysis activity for that sample (=biological replication).

Data analysis. All analytical procedures were conducted using SAS version 9.1 (SAS Institute, Cary, NC). Radial growth rates were computed as the slope of the line relating colony diameter to time of measurement using linear regression (Proc reg). The significance of differences in growth rate (mm/day) was assessed using analysis of variance (ANOVA) as implemented in Proc Mixed. In these analyses, pH and isolate were fixed effects, and replication, block (repetition of the experiment), and all interaction terms were coded as random effects. The significance of random effects was evaluated

Fig. 2. Radial growth rate of three isolates of *Fusarium oxysporum* f. sp. *fragariae* (GL 1315, GL 1080, and GL 1059) at 20°C on potato dextrose agar adjusted to either pH 5 or pH 7. Means represented by the height of each bar were computed from all replications in two experiments ($n = 4$). Error bars correspond to 2× the standard error of the mean.

with a χ^2 test to compare residual log likelihood values obtained with and without the random term included in the model. Only random effects that improved the fit of the model were retained. Spore germination data were also analyzed using Proc Mixed, as described above, but germination proportions were arcsine (square root) transformed prior to analysis. The same transformation was applied to frequency of root infection data, which were analyzed using the mixed procedure as described above, with cultivar and pH as fixed effects. Measures of fluorescence from FDA hydrolysis tests were analyzed as described for root infection data but without transformation of the data.

Results

Spore germination. After 12 h of incubation at 20°C, spore germination, across all isolates, ranged from 40 to 78% at pH 5 and from 43 to 81% at pH 7. Based on a three-way ANOVA of germination data for all three isolates, no random effects (block, replication, pH*block interaction, isolate*block interaction, pH*isolate interaction, and isolate*block*pH interaction) were significant ($P \geq 0.4493$). The effect of pH on spore germination was not significant ($P = 0.1020$), but the effect of isolate was significant ($P < 0.0001$), with mean germination rates ranging from a low of 47% (GL 1059) to a high of 75% (GL 1315). Analysis of data separately by isolate showed the effect of pH not to be significant for any of the three isolates ($P \geq 0.2078$). Because there was no interaction between isolate and pH, data were pooled across isolates to compute mean germination rates at pH 5 and pH 7 (Fig. 1).

After 14 h at 20°C, rates of spore germination across isolates ranged from 74 to 87% at pH 5 and from 64 to 88% at pH 7. ANOVA revealed no random factors to be significant ($P \geq 0.6547$), and the effect of pH on spore germination was not significant ($P =$

0.0831). The effect of isolate on spore germination was significant ($P < 0.0001$), but when data were analyzed separately by isolate, none of the three showed a significant effect of pH on spore germination ($P \geq 0.1627$). After 16 h at 20°C, there was not a significant effect of pH ($P = 0.4610$) or isolate ($P = 0.0722$) on spore germination. Similar results were obtained for spores incubated at 25°C, with no significant effects of pH on the rate of spore germination for any of the three isolates at any time point (analysis not shown).

Radial growth. At 20°C, regression showed colony diameters to have a strong linear relationship with time of measurement. Regression coefficients (R^2), across isolates and pH levels, were ≥ 0.9980 . The effect of pH on the slope of the line relating colony diameter to time (=growth rate in mm/day) was examined using a three-way ANOVA, with data for all isolates included. The results revealed the isolate*block*pH interaction to be significant ($P = 0.0001$), whereas all other random terms (block, replication, pH*block interaction, isolate*block interaction, and pH*isolate interaction) were not ($P \geq 0.2206$). With nonsignificant terms removed from the model, results of ANOVA indicated that main effects of pH ($P = 0.8865$) and isolate ($P = 0.2037$) were not significant. Mean growth rates were similar across all three isolates (Fig. 2).

Regression of colony diameter at 25°C on day of measurement gave R^2 values ≥ 0.9977 across all isolates and pH levels. Analysis of growth rate data at 25°C showed all random terms not to be significant ($P \geq 0.1108$). Main effects of isolate ($P = 0.0480$) and pH ($P < 0.0001$) were significant. To better understand the basis for a significant effect of pH, growth rate data were analyzed separately by isolate. The results showed no random terms to be significant for GL 1059 ($P \geq 0.4795$), GL 1080 ($P \geq 0.2367$), or GL 1315 ($P \geq 0.4795$). With data pooled across blocks, ANOVA revealed the effect of pH to be significant for GL 1059 ($P = 0.0494$) and GL 1315 ($P < 0.0001$), but not

Fig. 3. Radial growth rate of three isolates of *Fusarium oxysporum* f. sp. *fragariae* (GL 1315, GL 1080, and GL 1059) at 25°C on potato dextrose agar adjusted to either pH 5 or pH 7. Means represented by the height of each bar were computed from all replications in two experiments ($n = 4$). Error bars correspond to 2 $\times$ the standard error of the mean.

for GL 1080 ($P = 0.0937$). Where the effect of pH was significant, growth rate was higher at pH 7 than at pH 5 (Fig. 3).

Root infection. At the time roots were harvested, pH ranged from 5.12 ± 0.04 to 5.38 ± 0.04 in acidified soils and from 6.55 ± 0.04 to 7.02 ± 0.07 in neutral soils (Table 1). A minimum of 30 root tips was assayed for each replication of each treatment, and totals for each experiment ranged from 240 to 344 for acidified soils and from 120 to 300 for neutral soils (Table 1). Considering all fungi that emerged on KM, the percentage of root tips infected ranged from $13 \pm 2\%$ to $59 \pm 7\%$ in acidified soils and from $9 \pm 3\%$ to $30 \pm 9\%$ for neutral soils (Table 1). The percentage of fungi growing from root tips identified as *F. oxysporum* (inclusive of strains other than *F. oxysporum* f. sp. *fragariae*), based on colony morphology on isolation plates, ranged from 58.2 to 83.4%, across cultivars and pH levels. Only 9.6% of those colonies were identified as *F. oxysporum* f. sp. *fragariae* based on detection of a diagnostic DNA fragment.

Data on the proportion of root tips infected by *F. oxysporum* f. sp. *fragariae* were analyzed using a three-way ANOVA to include both repetitions of experiments with both strawberry cultivars. This analysis showed all random terms and interactions not to be significant ($P \geq 0.6547$). With random effects removed from the model, a two-way ANOVA revealed the effect of pH on frequency of infection of root tips to be significant ($P = 0.0066$), whereas the effect of cultivar was not significant ($P = 0.2089$). Although the overall effect of pH was significant, if data for the two cultivars were analyzed separately, the effect of pH was significant for Albion ($P = 0.0061$) but not for San Andreas ($P = 0.2799$). On average, a greater proportion of root tips was infected at pH 5 than at pH 7 for both cultivars (Fig. 4).

To test the generality of a pH effect on root infection, the proportion of root tips infected by *F. oxysporum* f. sp. *fragariae* was regressed on the proportion of tips infected by all other fungi. Values for each replicate in all four experiments were included, for a total of 32 data points. The two measures of root colonization were highly correlated ($R^2 = 0.4771$, $P < 0.0001$), indicating that the pH effect on root infection is not specific to the pathogen causing Fusarium wilt of strawberry.

Microbial activity. The activity of enzymes hydrolyzing fluorescein diacetate (=FDA hydrolysis activity), as measured by absorbance at 490 nm, was greater in neutral than in acidified soil for both Albion and San Andreas (Fig. 5). In a three-way ANOVA of FDA hydrolysis data, the cultivar*pH*block interaction was significant ($P = 0.0482$). With the three-way interaction retained but other random terms removed from the model, the effect of cultivar on FDA hydrolysis activity was not significant ($P = 0.6504$), whereas the effect of pH bordered on significance ($P = 0.0544$). Data were also analyzed separately by cultivar. For Albion, replication, block, and the pH*block interaction were all not significant ($P \geq 0.2942$). With random terms removed from the model, ANOVA showed the effect of pH on FDA hydrolysis activity to be significant ($P < 0.0001$). In ANOVA of data for San Andreas, the block*pH interaction was

significant ($P = 0.0007$), so data were analyzed separately by block. Replication was not a significant source of variation in either block ($P > 0.9999$), whereas pH was a significant source of variation in block one ($P = 0.0002$) but not in block two ($P = 0.0855$).

Discussion

A number of studies have reported that elevating the pH of acidic soil can reduce damage caused by Fusarium wilt. For example, Jones and Overman (1971) found incidence of Fusarium wilt of tomato to be reduced by amending soil with lime to increase pH from 6.0 to 7.0, and that disease incidence was further reduced at pH 7.5. Similarly, Fang et al. (2012) reported Fusarium wilt of strawberry to be significantly less severe in soil at pH 6.7 than at pH 5.2. The incidence of Fusarium wilt of spinach was reduced and yield was higher following successive annual applications of limestone to raise soil pH from below 6.0 to over 7.0 (Gatch and du Toit 2017). Similar effects of pH on Fusarium wilt have been reported for numerous other crops (Woltz and Jones 1970).

The mechanisms responsible for pH-mediated reductions in disease incidence are not fully understood, but a number of possibilities have been considered. As noted by Garrett (1938), soil pH could influence the activity of secreted enzymes and, if outside the optimal range, limit their efficacy in accessing nutrients and in gaining entry to a plant root. Evidence for an effect of pH on growth was presented by Sherwood (1923), who reported biomass of *F. oxysporum* f. sp. *lycopersici* to be maximal in media with a pH ranging from 2.8 to 5.0. In contrast, two isolates of *F. oxysporum* f. sp. *fragariae* grew faster at pH 7.0 than at pH 5.0 when incubated at 25°C (Fig. 3), whereas at 20°C the effect of pH on growth was not significant. Sherwood (1923) measured biomass in liquid culture, as opposed to radial growth on a solid medium, which complicates explication of the apparent discrepancy in results. However, it is fair to say that growth on a solid substrate is more representative of how filamentous fungi grow in soil than is growth in a liquid medium.

The rate of spore germination differed among isolates of *F. oxysporum* f. sp. *fragariae*, but in no case was there a significant effect of pH on spore germination, at either 20 or 25°C. However, because the basal medium in these experiments (PDA) was well endowed with nutrients, our findings do not preclude an effect based on availability of specific nutrients. An element of particular interest in this regard is calcium. Upward adjustment of soil pH has usually been accomplished by addition of limestone and/or hydrated lime, which also increases the concentration of calcium. Whereas calcium concentration in soil could affect a plant's ability to respond to a pathogen, Everett and Blazquez (1967) reported that calcium alone did not have an effect on disease incidence commensurate with what resulted from an increase in soil pH to 7.0 or above, from more acidic conditions. Our findings support that conclusion because pH adjustments that affected the frequency of root infection did not involve addition of calcium.

Table 1. The effect of soil pH on frequency of infection of strawberry root tips by *Fusarium oxysporum* f. sp. *fragariae* and other fungi

Cultivar ^a	Block ^b	Soil pH ^c		Root tips ^d	Infected ^e	<i>F. o. fragariae</i> ^f
		Initial	Final			
Albion	1	4.84 ± 0.03	5.13 ± 0.03	240	59 ± 7%	5.8 ± 2.0%
Albion	2	4.93 ± 0.03	5.26 ± 0.05	240	27 ± 1%	5.4 ± 0.8%
Albion	1	7.16 ± 0.02	6.55 ± 0.04	120	30 ± 9%	2.5 ± 1.6%
Albion	2	7.05 ± 0.01	6.93 ± 0.07	163	18 ± 2%	1.1 ± 1.1%
San Andreas	1	4.93 ± 0.03	5.38 ± 0.04	344	34 ± 4%	7.6 ± 1.6%
San Andreas	2	4.90 ± 0.05	5.12 ± 0.04	330	13 ± 2%	4.3 ± 1.6%
San Andreas	1	7.00 ± 0.04	7.02 ± 0.07	300	19 ± 2%	4.2 ± 1.5%
San Andreas	2	7.09 ± 0.03	6.67 ± 0.04	240	9 ± 3%	4.4 ± 2.7%

^a Strawberry cultivars Albion and San Andreas are susceptible and resistant to Fusarium wilt, respectively.

^b Blocks one and two refer to repeats of the experiment.

^c Initial pH was measured in the full mass of soil before it was dispensed into pots, and final pH was measured in each pot prior to sampling of roots.

^d The number of root tips sampled.

^e The percentage of root tips from which a fungal colony emerged on Komada's selective medium.

^f The percentage of all root tips infected by *Fusarium oxysporum* f. sp. *fragariae*.

Other minor nutrients that have been considered are iron, manganese, and zinc, all of which are less available in soil near neutrality than under more acidic conditions. Gatch and du Toit (2012) found that supplementing a defined liquid medium with Fe, Mn, or Zn significantly increased biomass and sporulation of *F. oxysporum* f. sp. *spinaciae*, the cause of Fusarium wilt of spinach. To explore a potential role of micronutrients in mediating an effect of soil pH on Fusarium wilt of tomato, Jones and Woltz (1970) amended pH-adjusted soil with lignosulfate metal complexes, which make micronutrients available at and above pH 7.0. A combination of manganese and zinc negated the effect of elevating soil pH on incidence of Fusarium wilt, whereas a mixture of manganese, zinc, and iron did not. Subsequent studies reinforced the importance of micronutrients as factors influencing development of Fusarium wilt. For example, Gatch and du Toit (2012) reported that adding Fe, Mn, and Zn to soil naturally infested with *F. oxysporum* f. sp. *spinaciae* negated the suppressive effect of limestone on severity of Fusarium wilt of spinach.

The ability of a fungus in soil to acquire the nutrients required to support growth and infection of a plant root will always be influenced by competition with other microbes, particularly bacteria. In an extensive review of the literature, Bonanomi et al. (2010) found that measures of microbial activity (such as FDA hydrolysis) were the best predictors of suppressiveness of soil to disease. Accordingly, the challenge posed by diminished availability of micronutrients at pH 7.0 or above will likely be heightened by increased bacterial activity in this pH range, relative to more acidic conditions. Consistent with this expectation, our data show that for the cultivar Albion, increased frequency of root infection by *F. oxysporum* f. sp. *fragariae* under acidic conditions was associated with a reduction in microbial activity, relative to what is observed in soil near neutrality (Fig. 5). Additional support for the importance of microbial activity is the observation that the effect of pH on severity of Fusarium wilt of strawberry was greater when plants were grown in field soil than in

sterilized potting mix, wherein bacterial abundance would be much lower than in field soil (Islas 2012).

As with Albion, the frequency of infection of San Andreas roots was higher in acidified than in neutral soil, but in this case the effect of pH was not significant. It is possible that a larger sample size and/or higher infection rate would have revealed a difference that was significant. The lack of significance may be attributable, in part, to a less consistent increase in microbial activity in experiments with San Andreas. However, whereas the effect of pH on microbial activity was significant in one of the two San Andreas experiments (Fig. 5), the effect of pH on root infection was not significant in either of the two experiments (analysis not shown). Thus, there was not a demonstrable correspondence between elevated microbial activity and a reduced rate of root infection for San Andreas.

San Andreas and Albion differ in susceptibility to Fusarium wilt, owing to the presence of a major gene for resistance in San Andreas (Pincot et al. 2018), but this seems unlikely to explain a differential effect of pH on root infection. Overall, there was not a significant difference in the frequency with which roots of the two cultivars were infected by *F. oxysporum* f. sp. *fragariae* (Fig. 4). This finding is consistent with evidence that resistance to Fusarium wilt, conferred by a major gene, is expressed only after the pathogen has reached the xylem parenchyma (Mes et al. 2000), and does not influence the initial infection process (Gordon 2017; Gordon et al. 1990). Nevertheless, it remains possible that other host-related factors contributed to a difference in the response to pH.

A reduction in the frequency of root infection, such as we have observed, can lower the risk of damage from Fusarium wilt. Although a single infection of the root cortex might lead to colonization of the xylem and systemic spread of the pathogen, most infections fail to achieve this, even on a susceptible host (Gordon 2017). Consequently, many infections are typically required before a plant becomes symptomatic. Therefore, reducing the rate of infection should lower the probability that disease will occur. This may result

Fig. 4. The proportion of strawberry root tips infected by *Fusarium oxysporum* f. sp. *fragariae*. Root tips were taken from plants growing in soil that was adjusted to a pH of either 5.0 or 7.0 (approximately). Means represented by the height of each bar were computed from all replications in two experiments ($n = 8$) for each of two cultivars: Albion and San Andreas. Error bars correspond to $2 \times$ the standard error of the mean.

in a reduced impact of *Fusarium* wilt if soil inoculum levels are close to the threshold required to cause disease. However, the higher the inoculum density in soil, the less likely an effect of pH, of the magnitude we have documented, will influence disease severity in a susceptible cultivar.

Overall, our findings support the hypothesis that pH-mediated reductions in incidence and severity of *Fusarium* wilt reflect a reduced frequency of root infections. This effect may be attributable to greater activity of microbes that compete with the pathogen and/or antagonize spore germination and subsequent fungal growth. If so, the benefit of elevating soil pH might also be achieved (or enhanced) by

other means, such as amending soil with organic materials that support bacterial growth (Bonanomi et al. 2010). If cost of materials is not a barrier, the latter approach could be superior in situations where the buffering capacity of soil makes pH adjustment impractical or where an elevated pH requires foliar applications of micronutrients to prevent a deficiency in the crop (Gatch and du Toit 2017). Furthermore, although a pH close to neutrality may reduce damage from *Fusarium* wilt, it can enhance the risk of damage from other pathogens, such as *Verticillium dahliae* (Dutta 1981), whereas elevating microbial activity in soil by other means may be more broadly suppressive of soilborne pathogens.

Fig. 5. Absorbance at 490 nm, as a measure of FDA hydrolysis activity in soil adjusted to a pH of either 5.0 or 7.0 (approximately), in which strawberry cultivars Albion or San Andreas were grown. Means represented by the height of each bar were computed from four replications for each cultivar in each of two experiments. Error bars correspond to 2× the standard error of the mean.

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