Severity of Verticillium Wilt, Plant Growth, and Spectral Reflectance Indices of Chile Pepper Under Periodic Flooding and No-flooding Conditions

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Abstract. Verticillium wilt, caused by *Verticillium dahliae*, is an economically important disease on chile pepper (*Capsicum annuum* L.), which is grown primarily under furrow and drip irrigation in New Mexico. Field observations by producers indicate that Verticillium wilt is more prevalent on chile pepper grown under drip irrigation than under furrow irrigation. To test these observations, chile pepper plants at the six- to eight-leaf stage inoculated or noninoculated with *V. dahliae* were subjected to either periodic flooding or no-flooding conditions in the greenhouse. Plants under periodic flooding were subjected to three events of 7 day flooding and two events of no flooding over a period of 5 weeks. Differences among treatments were assessed using disease severity, plant dry weight, and spectral reflectance-based physiological indices such as moisture stress index (MSI), chlorophyll-based difference index, water band index, and photochemical reflectance index (PRI). There was a significant interaction between flooding and inoculation with respect to disease severity ($P < 0.003$). In plants inoculated with *V. dahliae* and subjected to periodic flooding, the relative treatment effect (RTE) based on disease severity was increased from 40% to 130% relative to noninoculated plants subjected to no flooding. However, under conditions of no flooding, RTE increased by $\approx 200\%$ to 286% when plants were inoculated with *V. dahliae* relative to noninoculated plants. In general, periodic flooding and inoculation with *V. dahliae* were accompanied by a decrease in plant dry weight as well as in the level of spectral reflectance-based physiological indices. In noninoculated plants subjected to periodic flooding, plant dry weight was reduced by $\approx 45\%$ to 50% relative to noninoculated plants subjected to no flooding. In plants inoculated with *V. dahliae* and subjected to periodic flooding, reduction in plant dry weight was $\approx 46\%$ to 60% relative to noninoculated plants subjected to no flooding. Under conditions of no flooding, plant dry weight was reduced by $\approx 33\%$ to 52% when plants were inoculated with *V. dahliae* relative to noninoculated plants. Of all the spectral reflectance-based physiological indices, PRI was the most affected by flooding and inoculation. For noninoculated plants, the mean area under the curve for PRI (AUC-PRI) was decreased by $\approx 90\%$ under periodic flooding relative to no flooding. In plants inoculated with *V. dahliae* and subjected to periodic flooding, reduction in AUC-PRI was $\approx 93\%$ relative to noninoculated plants subjected to no flooding. Under conditions of no flooding, AUC-PRI was reduced by $\approx 93\%$ when plants were inoculated with *V. dahliae* relative to noninoculated plants. These results showed that chile pepper growth and physiology were affected similarly by flooding and inoculation with *V. dahliae*. However, severity of Verticillium wilt was greater under no-flooding than under periodic flooding conditions.

Chile pepper (*Capsicum annuum* L.) is a vegetable crop of great economic importance in the southwestern United States. Major states producing chile pepper (nonbell type) are New Mexico, California, Texas, and Arizona. In 2006, chile pepper production

in New Mexico accounted for $\approx 41\%$ of U.S. chile production with a value of ≈ 102 million dollars (USDA-NASS, 2007).

In New Mexico, chile pepper is produced under sprinkle, drip, and furrow irrigation. However, furrow irrigation is the most practiced irrigation method in the state (Skaggs et al., 2000). Drip irrigation is designed to supply water to plant root zone where it is most needed, thus conserving this resource. Furrow irrigation consists of flooding fields at varying intervals. Depending on soil moisture conditions, flooding may be performed at 7- to 10-d, 10- to 15-d, and 15- to 21-d intervals. After flooding events, saturated conditions may be established in soil for

3 to 7 d depending on soil types and environmental conditions. Furrow irrigation can be thought of as periodic flooding of fields.

Major constraints to chile pepper production in New Mexico are soilborne diseases, which are manifested by wilt symptoms after plant infection. *Verticillium dahliae* is a major soilborne fungal pathogen that causes wilt in several crops, including chile pepper (Pegg and Brady, 2002; Sanogo and Carpenter, 2006), in the United States and worldwide. *Verticillium dahliae* produces microsclerotia, which enable the pathogen to persist for at least 14 years in infested field soil (Wilhem, 1955). Additionally, this fungus survives in numerous weed species across many plant families (Sanogo and Clary, 2003; Woolliams, 1966). *Verticillium dahliae* affects the integrity of vascular tissues in the root and the stem. Plant infection by *V. dahliae* results in stunting, leaf chlorosis and necrosis, defoliation, vascular necrosis, wilting, and death (Pegg and Brady, 2002). After infection of pepper plants, symptoms of Verticillium wilt may appear within 3 to 6 weeks. In plants infected with *V. dahliae*, several physiological processes such as respiration, transpiration, and photosynthesis are affected, and therefore yield reduction may be incurred (Tomescu et al., 2000).

Verticillium dahliae and *Phytophthora capsici*, causal agent of Phytophthora blight, are the two most significant pathogens associated with chile pepper wilt in New Mexico (Sanogo and Carpenter, 2006). *Verticillium dahliae* and *P. capsici* may occur together in the same chile pepper production fields and in the same plants (Sanogo and Carpenter, 2006). The occurrence of both pathogens in the same fields represents a serious challenge to crop management. Previous studies on control of chile pepper wilt have shown that *P. capsici* could be reduced significantly by use of alternate-furrow irrigation in contrast to flood irrigation of every furrow (Biles et al., 1992) and by use of drip irrigation (Ristaino and Johnston, 1999; Xie et al., 1999). However, field observations by chile pepper producers using drip irrigation systems indicate that Verticillium wilt is increased in drip-irrigated compared with furrow-irrigated chile pepper fields. These observations were confirmed in a 3-year field survey in which Sanogo and Carpenter (2006) found that the incidence of plant infection by *V. dahliae* was $\approx 32\%$ greater under drip irrigation than under furrow irrigation.

The cause of increased Verticillium wilt on chile pepper grown under drip irrigation relative to furrow irrigation is not known. Field notes by chile pepper producers and field surveys are observational in nature, and as such they do not provide any systematic cause-and-effect relationship to be established between irrigation and development of Verticillium wilt. There has been no systematic study to characterize chile pepper infection by *V. dahliae* and development of Verticillium wilt under drip and furrow irrigation systems. It is important to conduct a systematic evaluation of the effect of

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irrigation on Verticillium wilt on chile pepper. Such a study will be useful for crop management from at least two perspectives. First, it will provide an ecological understanding of the behavior of *V. dahliae*. Second, it will provide a knowledge base for designing strategies to minimize Verticillium wilt in chile pepper production fields.

The hypothesis of this study was that Verticillium wilt increases on chile pepper grown under drip irrigation compared with chile pepper grown under furrow irrigation. Experimentally, furrow irrigation and drip irrigation were represented by periodic flooding and no-flooding conditions, respectively. The objectives of this study were to assess the effects of periodic flooding and no-flooding conditions on severity of Verticillium wilt, plant growth, and spectral reflectance-based physiological indices of chile pepper.

Materials and Methods

Inoculum preparation and plant production. An isolate of *V. dahliae*, recovered from a field-infected chile pepper plant, was used in this study. This isolate was maintained on water agar in 9-cm diameter petri plates. Inoculum consisted of conidia, which were produced by adding a 1-cm mycelial plug of *V. dahliae* grown on water agar to 100 mL of sterilized Czapek-Dox broth contained in a 250-mL Erlenmeyer flask. All flasks were placed on a rotary shaker and incubated at room temperature (23 to 25 °C) in darkness for 7 to 10 d, after which the contents of each flask were passed through three layers of cheesecloth to separate conidia from mycelial plugs and mats. The number of conidia in the filtered suspension was estimated with a hemacytometer and adjusted to a concentration of 4×10^6 conidia per milliliter.

The chile pepper cultivar 'AZ20', which is susceptible to *V. dahliae*, was used in this study. Seeds were planted in a greenhouse in six-cell plastic trays (with cell dimensions of 6, 4, and 6 cm in length, width, and depth, respectively) filled with sterilized Terra-Lite Metro Mix 360 (W. R. Grace & Co., Memphis, TN). At the fourth fully expanded true leaf stage, seedlings were transplanted into plastic pots (with a diameter of 10 cm on top and 7.5 cm on bottom and a depth of 9 cm) filled with sterilized Terra-Lite Metro Mix 360 at the rate of one seedling per pot. Seedlings were then fertilized by adding to each pot 150 mL solution of a 20%N–20%P₂O₅–20%K₂O fertilizer (Scotts Co., Marysville, OH) containing 3.94% ammoniacal nitrogen, 6.05% nitrate, 10.01% urea, 0.05% magnesium, 0.0068% boron, 0.0036% copper (chelated Cu), 0.05% iron (chelated Fe), 0.025% manganese (chelated Mn), 0.0009% molybdenum, and 0.0025% zinc (chelated Zn).

Effects of periodic flooding and no flooding on infection of chile pepper by Verticillium dahliae. At the six- to eight-leaf stage, potted plants were inoculated with 0, 5, and 10 mL of conidia suspension (4×10^6

conidia/mL) to generate three inoculum levels: 0, 2×10^7 , and 4×10^7 conidia per pot. Conidia suspension was dispensed onto the surface of the soil in each pot ≈ 2 cm away from the lower stem of each plant. For seedlings serving as controls (inoculum level = 0), no inoculum was dispensed on top of soil in each pot.

After inoculation, one set of plants were subjected to periodic flooding conditions in the greenhouse. Flooding conditions were established using methods outlined by Kuan and Erwin (1980) by placing pots containing plants on plant saucers (12.5 cm diameter and 3.5 cm depth), which were kept full of tap water (electrical conductivity = 0.5 dS/m). Periodic flooding consisted of 7 d of flooding followed by 7 d of no flooding. During the 7 d of no-flooding conditions, pots containing plants were removed from plant saucers, placed on the greenhouse bench, and watered as needed. Each potted plant was subjected to three events of flooding and two events of no flooding over a 5-week period. A second set of plants was maintained on the greenhouse bench, monitored daily, and irrigated as needed over the same 5-week period. No saucers were placed under potted plants in this second set and plants were irrigated by gently adding water on top of soil with a plastic beaker to avoid splash onto above-ground plant parts. Soil water potential was monitored in potted plants placed under both periodic flooding and no-flooding conditions using Watermark soil moisture sensors (Spectrum Technologies, Plainfield, IL).

The study was conducted as a 2×3 factorial experiment with six treatments generated from the combination of two flooding conditions (periodic flooding and no flooding) and three inoculum levels (0, 2×10^7 , and 4×10^7 conidia per pot). The six treatment combinations were evaluated in a split plot with flooding as whole plot factor and inoculation as subplot factor. Each treatment was replicated five times with a pot being a replicate. The entire experiment was completed twice. Expt. 1 was conducted from 18 Apr. to 23 May 2006, and Expt. 2 was carried out from 1 June to 6 July 2006.

Measurement of spectral reflectance-based physiological indices. The spectral reflectance in the 350 nm to 2500 nm range was measured on the two most fully expanded chile pepper leaves below the shoot apex using a portable field spectroradiometer, FieldSpec Pro FR (Analytical Spectral Devices, Boulder, CO), attached through a fiber-optic cable to the LI-COR 1800 integrating sphere (LI-COR 1800-12; Li-COR, Lincoln, NE). An integrating sphere is designed to collect all of the radiation reflected from or transmitted through a surface. The 1800-12 is an external integrating sphere, which means that the sample is held to the outside of the sphere with a small section of the sample acting as part of the sphere wall. The interior of the sphere is coated with barium sulfate to make a uniform diffuse reflector. An internal integrating sphere would have the sample entirely inside the sphere, generally near the

center. A measurement of reflectance involves comparing the wall illumination caused by a focused beam of radiation reflected from the sample material with that reflected from the white reference material. The two measurements were taken sequentially. The 1800-12 uses the same illumination for both sample and reference measurement by moving it from one port to the other between measurements. The integrating sphere was initialized following procedures given in the "RS3 Quick Reference for FieldSpec Pro." Each single leaf sample was scanned individually on the upper (adaxial) surface of the leaf, taking 50 spectra for each single leaf to reduce spectral and detector noise. The collected hyperspectral data were stored on a hard disk for subsequent viewing and analysis with software provided with the spectroradiometer (Analytical Spectral Devices, ViewSpecPro v.4.2). The measured reflectance spectra were used to calculate four physiological indices: moisture stress index (MSI), water band index (WBI), chlorophyll-based difference index (CBDI), and photochemical reflectance index (PRI). MSI is determined by the ratio of the reflectance at 1600 nm and reflectance at 820 nm (Hunt and Rock, 1989) and measures the plant water stress; the lower the value of MSI, the less stressed is a plant. WBI is the ratio between reflectance at 900 nm and reflectance at 970 nm (Gamon and Qiu, 1999; Penuelas et al., 1997) and measures the water content in the leaf; the higher the value of WBI, the higher the water content in the leaf. CBDI, which is the ratio of $(705 - 750)/(705 + 750)$ measures the total chlorophyll concentration; the higher the value of CBDI, the higher chlorophyll concentration in the leaf. PRI is the ratio of $(531 - 570)/(531 + 570)$ and measures the photosynthetic activity of the plant; the higher value of the PRI, the higher photosynthetic activity of the plant. Measurements of spectral reflectance were done at the end of each periodic flooding across all treatments. The area under the spectral reflectance index curve (AUC) was computed using the trapezoid method as follows:

$$AUC = \sum_{i=1}^{n-1} [(Y_{i+1} + Y_i)/2] \times [T_{i+1} - T_i],$$

where Y_i = spectral reflectance index value at the i th observation, T_i = time (in days) at the i th observation, and n = total number of observations. AUC was used in assessing differences among treatments.

Assessment of disease severity and plant biomass. Plants were monitored for disease symptoms. At the end of the third periodic flooding (5 weeks after inoculation), disease severity on aboveground plant parts was evaluated using a scale modified from Bejarano-Alcázar et al. (1996) and based on the percentage of foliage affected with chlorosis, necrosis, wilting, defoliation, and the presence of discoloration in vascular tissue in the stem: 0 = no symptoms; 1 = 1% to 33% foliage affected; 2 = 1% to 33% foliage

affected with vascular discoloration; 3 = 34% to 66% foliage affected; 4 = 34% to 66% foliage affected with vascular discoloration; 5 = 67% to 100% foliage affected; 6 = 67% to 100% foliage affected with vascular discoloration; and 7 = dead plant.

Each plant was severed at the crown and cut longitudinally to assess the presence of vascular discoloration. Roots of each plant were washed free of soil. Aboveground plant parts and roots of each plant were combined in a brown paper bag and placed in an oven at 65 °C. Plant dry weight was recorded after 48 h.

Statistical analysis. Data on the following variables were used: 1) disease severity ratings, 2) plant dry weight, and 3) the area under the spectral reflectance index curve (AUC). Data on plant dry weight and AUC were subjected to analysis of variance using Proc Mixed procedure in SAS (SAS Institute, Cary, NC). The natural logarithm transformation was applied to data on plant dry weight before analysis of variance. For each variable, data from individual trials were analyzed separately to assess consistency of results and homogeneity of error variances among experiments. Homogeneity of error variances was evaluated following procedures outlined by Gomez and Gomez (1984). When results were consistent among experiments and error variances were homogeneous ($P > 0.05$), data from all experiments were combined. Because the data consisting of disease severity ratings were ordinal, they were analyzed by nonparametric approaches using SAS macros F1_LD_F1 (experimental design with one whole plot factor and one subplot factor) and LD_CI (limits of confidence interval) as outlined by Brunner et al. (2002). These macros allow computation of relative treatment effects (RTE), 95% confidence intervals for RTE, and analysis of variance-type statistics for gauging the significance of main factors and the interactions among these factors. Means separation for plant dry weight and area under the reflectance index curve was based on the probability value of least squares means difference among experimental factors, which was obtained using the LSMEANS statement in SAS with the PDIF option. Presentation of data was completed following methods outlined by Gomez and Gomez (1984).

Results

Homogeneity of error variances was not indicated for disease severity ($P < 10^{-3}$) and plant dry weight ($P = 0.01$). Therefore, data were analyzed for each experiment separately for these variables. However, homogeneity of error variances was indicated for reflectance-based physiological indices, so data on these indices were pooled across both experiments.

Disease severity and plant biomass

Expt. 1. The first symptoms of infection were observed as leaf drop on chile pepper 'AZ20' in the no-flooding treatment at the

highest inoculum level by 18 d after inoculation. At 5 weeks after inoculation, severe wilting was observed in no-flooding treatment under both low and high inoculum levels. Conversely, plants subjected to periodic flooding displayed symptoms of slight leaf chlorosis and leaf scorching under both low and high inoculum levels. Noninoculated plants subjected to the no-flooding treatment displayed no perceptible symptoms. Conversely, slight chlorosis was visible on foliage of noninoculated plants subjected to periodic flooding.

As revealed by analysis of variance (Table 1), there was a significant interactive effect of flooding and inoculation ($P = 10^{-5}$) on disease severity. The RTEs computed for the various levels of flooding-inoculation treatments are shown in Table 2. RTE was 0.28 for noninoculated plants subjected to periodic flooding and for noninoculated plants subjected to no flooding. In plants inoculated with *V. dahliae* and subjected to periodic flooding, RTE increased by $\approx 40\%$ relative to noninoculated plants subjected to no flooding. However, under conditions of no flooding, RTE increased by $\approx 200\%$ when plants were inoculated with *V. dahliae* relative to noninoculated plants.

Means and SE of the means for plant dry weight are shown in Figure 1A. There was no significant interactive effect of flooding and inoculation (Table 5) on plant dry weight ($P = 0.3066$). However, there were significant main effects of flooding ($P = 0.0115$) and inoculation ($P = 0.0002$) on plant dry weight. When noninoculated plants were subjected to periodic flooding, plant dry weight was reduced by $\approx 45\%$ relative to noninoculated plants subjected to no flooding. In plants inoculated with *V. dahliae* and subjected to periodic flooding, reduction in plant dry weight was $\approx 56\%$ to 60% relative to noninoculated plants subjected to no flooding. Under conditions of no flooding, plant dry weight was reduced by $\approx 40\%$ to 52% when plants were inoculated with *V. dahliae* relative to noninoculated plants.

Expt. 2. Leaf drop was the first symptom observed on chile pepper 'AZ20' at low and high inoculum level at 21 d after inoculation.

Table 1. Analysis of disease severity ratings on chile pepper 'AZ20' in relation to inoculation with *Verticillium dahliae* and flooding treatments (Expt. 1).^z

Factor	ANOVA-type statistics ^y			
	Fn	df1	df2	P value
Flooding (F)	72.54	1	7.71	0.00003
Inoculation (I)	28.03	1.41	∞	<0.00001
F $\times$ I	14.99	1.41	∞	0.00001

^zThere were six treatments consisting of two flooding conditions (periodic flooding and no flooding) and three inoculum levels (0 , 2×10^7 , and 4×10^7 conidia per pot).

^yFn is an ANOVA-type statistic equivalent to an F value with estimated degree of freedom df1 (numerator) and df2 (denominator). The significance of main factors and interactions among these factors is gauged by the P values.

ANOVA = analysis of variance.

At 5 weeks after inoculation, severe defoliation, wilting, and death were observed in the no-flooding treatment under both low and high inoculum levels. Noninoculated plants subjected to no-flooding treatment displayed no perceptible symptoms. Defoliation was observed on plants subjected to periodic flooding treatment under both low and high inoculum levels, albeit at a limited extent. Slight defoliation was also visible on noninoculated plants subjected to periodic flooding.

There was a significant interaction of flooding and inoculation ($P = 0.0027$) on disease severity (Table 3). The RTEs computed for the various flooding-inoculation levels are shown in Table 4. RTE was 0.21 for noninoculated plants subjected to the periodic flooding treatment and for noninoculated plants subjected to no flooding. For plants inoculated with *V. dahliae* and subjected to periodic flooding, RTE increased by $\approx 130\%$ relative to noninoculated plants subjected to no flooding. Under conditions of no flooding, RTE increased by $\approx 286\%$ when plants were inoculated with *V. dahliae* relative to noninoculated plants.

Means and SE of the means for plant dry weight are shown in Figure 1B. There was no significant interactive effect of flooding and inoculation (Table 5) on plant dry weight ($P = 0.1767$). However, there were significant main effects of flooding ($P = 0.045$) and inoculation ($P = 0.0004$) on plant dry weight. In noninoculated plants subjected to periodic flooding, plant dry weight was reduced by $\approx 50\%$ relative to noninoculated plants subjected to no flooding. In plants inoculated with *V. dahliae* and subjected to periodic flooding, reduction in plant dry weight was $\approx 46\%$ to 50% relative to noninoculated plants subjected to no flooding. Thus, flooding effect was not significantly exacerbated by inoculation with *V. dahliae*. Under conditions of no flooding, plant dry weight was reduced by $\approx 33\%$ to 47% when plants were inoculated with *V. dahliae* relative to noninoculated plants. Thus, the effect of no flooding on plant dry weight in the presence of *V. dahliae* was comparable to the effect of periodic flooding on plant dry weight regardless of the absence or presence of *V. dahliae*. In essence, these results showed that flooding stress was equivalent to inoculation stress on plant growth.

Spectral reflectance-based physiological indices

As shown in Table 6, analysis of variance revealed significant main and interactive effects ($0.01 < P < 0.0010$) of flooding and inoculation treatments on AUC for PRI. Significant main effects were detected for flooding ($P = 0.0004$) and for inoculation ($P = 0.0038$) with respect to CDBI. However, there were neither significant main effects nor interactive effects ($P > 0.6$) of flooding and inoculation treatments on the AUC for MSI and WBI.

The mean area AUC for PRI in plants noninoculated was decreased by $\approx 90\%$ under

Table 2. Median, relative treatment effects (RTE), and 95% confidence intervals (CIs) for RTE of severity ratings on chile pepper 'AZ20' in relation to inoculation with *Verticillium dahliae* and flooding treatments (Expt. 1).

Flooding ^z	Inoculation ^y	Median ^x	RTE	95% CI for RTE
Periodic flooding	0	0	0.28	(0.246–0.326)
	20	1	0.42	(0.293–0.557)
	40	1	0.35	(0.246–0.481)
No flooding	0	0	0.28	(0.246–0.326)
	20	2	0.78	(0.748–0.801)
	40	7	0.89	(0.847–0.907)

^zPlants under periodic flooding treatment were subjected to 7 d of flooding followed by 7 d of no flooding over a 5-week period. Plants subjected to no-flooding treatment were monitored daily and irrigated as needed over the same 5-week period.

^yThere were three inoculum levels of 0, 2×10^7 (20 million), and 4×10^7 (40 million) conidia per pot, which were designated 0, 20, and 40, respectively.

^xDisease severity on aboveground plant parts was evaluated using a scale based on the percentage of foliage affected with chlorosis, necrosis, wilting, defoliation, and the presence of discoloration in vascular tissue in the stem: 0 = no symptoms; 1 = 1% to 33% foliage affected; 2 = 1% to 33% foliage affected with vascular discoloration; 3 = 34% to 66% foliage affected; 4 = 34% to 66% foliage affected with vascular discoloration; 5 = 67% to 100% foliage affected; 6 = 67% to 100% foliage affected with vascular discoloration; and 7 = dead plant.

Fig. 1. Effect of periodic flooding and no flooding and inoculation of chile pepper 'AZ20' with *Verticillium dahliae* at three inoculum levels on plant dry weight in (A) Expt. 1 and (B) Expt. 2. Columns are the means for each treatment and bars on the columns are the SES of the means. There were three inoculum levels of 0, 2×10^7 (20 million), and 4×10^7 (40 million) conidia per pot, which were designated 0, 20, and 40, respectively. For each experiment, there was no significant interaction between flooding treatment and inoculation ($P > 0.150$). Probability of least squares means difference (P -diff) between periodic flooding and no flooding was 0.0115 in Expt. 1 and 0.045 in Expt. 2. The P -diff values among inoculum levels (0, 20, and 40) were as follows in experiment 1: P -diff (0 and 20) = 0.0023; P -diff (0 and 40) < 0.0001; and P -diff (20 and 40) = 0.1775. The P -diff values among inoculum levels (0, 20, and 40) were as follows in Expt. 2: P -diff (0 and 20) < 0.0001; P -diff (0 and 40) = 0.0073; and P -diff (20 and 40) = 0.0476.

Table 3. Analysis of disease severity ratings on chile pepper 'AZ20' in relation to inoculation with *Verticillium dahliae* and flooding treatments (Expt. 2).^z

Factor	ANOVA-type statistics ^y			
	F _n	df1	df2	P value
Flooding (F)	39.29	1	7.71	0.00024
Inoculation (I)	26.09	1.66	∞	< 0.00001
F $\times$ I	6.58	1.66	∞	0.0027

^zThere were six treatments generated from the combination of two flooding conditions (periodic flooding and no flooding) and three inoculum levels (0, 2×10^7 , and 4×10^7 conidia per pot).

^yF_n is an ANOVA-type statistic equivalent to an F value with estimated degree of freedom df1 (numerator) and df2 (denominator). The significance of main factors and interactions among these factors is gauged by the P values.

ANOVA = analysis of variance.

periodic flooding relative to no flooding (Fig. 2). In plants inoculated with *V. dahliae* and subjected to periodic flooding, reduction in AUC-PRI was $\approx 93\%$ relative to noninoculated plants subjected to no flooding. Under conditions of no flooding, AUC-PRI was reduced by $\approx 93\%$ when plants were inoculated with *V. dahliae* relative to noninoculated plants. Thus, the effect of no flooding on PRI in the presence of *V. dahliae* was comparable to the effect of periodic flooding on PRI regardless of the absence or presence of *V. dahliae*.

The mean AUC for CBDI was reduced by $\approx 10\%$ in plants under periodic flooding relative to those under no-flooding conditions (Fig. 3). In plants inoculated with *V. dahliae*, the mean value of AUC-CBDI was reduced by $\approx 7\%$ to 10% relative to noninoculated plants. Thus, the effect of periodic flooding on AUC-CBDI was comparable to the effect of inoculation on AUC-CBDI.

In both Expts. 1 and 2, soil water potential varied from -0.003 to -0.005 MPa in pots under periodic flooding conditions and from -0.005 to -0.025 MPa in pots under no-flooding conditions. These values of water potential are well within those recorded with the same type of sensors in chile pepper fields

fluctuating between -0.006 and -0.017 MPa during furrow irrigation events. Average daily air temperatures in the greenhouse ranged from 24.4 to 28.2 °C in Expt. 1, and from 23.3 to 28.3 °C in Expt. 2 over the duration of each experiment.

Discussion

This study is the first known report that systematically examined the relationship of periodic flooding and no-flooding conditions to the development of *Verticillium* wilt on chile pepper. Periodic flooding was shown to be less conducive to *Verticillium* wilt compared with no-flooding conditions where soil moisture is below the saturation level. Results from this study support the hypothesis that *Verticillium* wilt increases under drip irrigation compared with furrow irrigation, which were experimentally represented by no-flooding and periodic flooding conditions, respectively. These results may help to explain field observations by chile pepper producers indicating an increased level of *Verticillium* wilt under drip irrigation compared with furrow irrigation.

The conditions imposed in this study were assumed to be representative of those encountered under a field environment in drip-irrigated and furrow-irrigated fields. The method of flooding was comparable to that used in other studies (Kuan and Erwin, 1980) and was assumed to mimic moisture level that would be found under field conditions. The scope of this study was limited to three periodic events during a 5-week period of active vegetative growth of chile pepper. However, it is recognized that more than three periodic flooding events may take place in production fields. Additionally, it must be realized that flooding duration may be less than the level selected in this work. Within the scope of this study, it was established that *Verticillium* wilt was enhanced under no-flooding conditions in contrast to periodic flooding.

Several methods of plant inoculation and types of inoculum have been used in plant infection studies with *Verticillium dahliae* across many crops. Plant inoculation methods included root-dip with or without root trimming (Bhat and Subbarao, 1999; Tsor et al., 1998), soil infestation (Riggs and Graham, 1995; Riley and Bosland, 1998), and stem injection (DeVay et al., 1997). In the root-dip method, plants may need to be uprooted, dipped in inoculum suspension, and transplanted. Plant response to infection may be affected as a result of wounding generated during uprooting and transplant shock. Stem injection may modify plant response by generating wounds and impede assessment of the interaction between host plant and pathogen at the host interface. Soil infestation was used in this study because this method does not affect the physiological integrity of plants.

Inoculum types used in several studies consisted of conidia (Bhat and Subbarao, 1999; Riggs and Graham, 1995; Tsor et al., 1998) and microsclerotia (Farley et al., 1970;

Table 4. Median, relative treatment effects (RTE), and 95% confidence intervals (CIs) for RTE of severity ratings on chile pepper 'AZ20' in relation to inoculation with *Verticillium dahliae* and flooding treatments (Expt. 2).

Flooding ^z	Inoculation ^y	Median ^x	RTE	95% CI for RTE
Periodic flooding	0	0	0.21	(0.141–0.344)
	20	3	0.55	(0.376–0.713)
	40	3	0.41	(0.301–0.533)
No flooding	0	0	0.21	(0.129–0.368)
	20	6	0.72	(0.644–0.774)
	40	7	0.90	(0.807–0.914)

^zPlants under periodic flooding treatment were subjected to 7 d of flooding followed by 7 d of no flooding over a 5-week period. Plants subjected to no-flooding treatment were monitored daily and irrigated as needed over the same 5-week period.

^yThere were three inoculum levels of 0, 2×10^7 (20 million), and 4×10^7 (40 million) conidia per pot, which were designated 0, 20, and 40, respectively.

^xDisease severity on aboveground plant parts was evaluated using a scale based on the percentage of foliage affected with chlorosis, necrosis, wilting, defoliation, and the presence of discoloration in vascular tissue in the stem: 0 = no symptoms; 1 = 1% to 33% foliage affected; 2 = 1% to 33% foliage affected with vascular discoloration; 3 = 34% to 66% foliage affected; 4 = 34% to 66% foliage affected with vascular discoloration; 5 = 67% to 100% foliage affected; 6 = 67% to 100% foliage affected with vascular discoloration; and 7 = dead plant.

Table 5. Analysis of plant dry weight measured on chile pepper 'AZ20' in relation to inoculation with *Verticillium dahliae* and flooding treatments.^z

Factor	P value ^y	
	Expt. 1	Expt. 2
Flooding (F)	0.0115	0.045
Inoculation (I)	0.0002	0.0004
F × I	0.3066	0.1767

^zThere were six treatments generated from the combination of two flooding conditions (periodic flooding and no flooding) and three inoculum levels (0, 2×10^7 , and 4×10^7 conidia per pot).

^yThe significance of main factors and interactions among these factors is gauged by the P values.

Table 6. Analysis of the area under the index curve for moisture stress index (MSI), water band index (WBI), chlorophyll-based difference index (CBDI), and photochemical reflectance index (PRI) measured on chile pepper 'AZ20' in relation to inoculation with *Verticillium dahliae* and flooding treatments.^z

Factor	P value ^y			
	MSI	WBI	CBDI	PRI
Flooding (F)	0.6336	0.9107	0.0004	0.0117
Inoculation (I)	0.8304	0.9895	0.0038	0.0008
F × I	0.9567	0.9883	0.4075	0.0160

^zThere were six treatments generated from the combination of two flooding conditions (periodic flooding and no flooding) and three inoculum levels (0, 2×10^7 , and 4×10^7 conidia per pot). Data from Expts. 1 and 2 were combined.

^yThe significance of main factors and interactions among these factors is gauged by the P values.

Riley and Bosland, 1998) of *V. dahliae*. Both types of propagules play significant roles in the life cycle of *V. dahliae* and can initiate plant infection. Whereas microsclerotia serve as survival structures, conidia have been shown to be the propagules responsible for population increases in microsclerotial-infested soil (Farley et al., 1970). Soil infestation with conidia was used in this study and the levels selected were based on previous studies (Riggs and Graham, 1995). The two levels of conidial inoculum used were comparable in terms of their effects on the

Fig. 2. Effect of periodic flooding and no flooding and inoculation of chile pepper 'AZ20' with *Verticillium dahliae* at three inoculum levels on photochemical reflectance index (PRI). Columns are the means for each treatment (combined data from two experiments) and bars on the columns are the SES of the means. There were three inoculum levels of 0, 2×10^7 (20 million), and 4×10^7 (40 million) conidia per pot, which were designated 0, 20, and 40, respectively. There was a significant interaction between flooding and inoculation ($P = 0.0160$); within each flooding treatment, columns with the same letter are not significantly different at $P = 0.05$.

magnitude of disease severity, plant dry weight, and spectral reflectance-based physiological indices.

Periodic flooding, as practiced in production fields using furrow irrigation, has not been previously examined with respect to the ecology of *V. dahliae*. In fact, there is a paucity of information on the relationship of periodic flooding to the development of Verticillium wilt on many crops. Several previous studies have focused on the effects of short-term and long-term continuous flooding on *V. dahliae* on various crops. Pullman and DeVay (1982) reported a decrease of $\approx 90\%$ in population densities of *V. dahliae* in cotton field soil after 17 weeks of flooding. Ioannou et al. (1977a) subjected tomato tissues infected with *V. dahliae* to 10, 20, and 40 d of continuous flooding and found a significant reduction in production of microsclerotia in tomato tissues only in

Fig. 3. Effect of periodic flooding and no flooding and inoculation of chile pepper 'AZ20' with *Verticillium dahliae* at three inoculum levels on chlorophyll-based difference index (CBDI). Columns are the means for each treatment (combined data from two experiments) and bars on the columns are the SES of the means. There were three inoculum levels of 0, 2×10^7 (20 million), and 4×10^7 (40 million) conidia per pot, which were designated 0, 20, and 40, respectively. There was no significant interaction between flooding treatment and inoculation ($P = 0.4075$). Probability of least squares means difference (P -diff) between periodic flooding and no flooding was 0.004. The P -diff values among inoculum levels (0, 20, and 40) were as follows: P -diff (0 and 20) = 0.015; P -diff (0 and 40) = 0.0013; and P -diff (20 and 40) = 0.3658.

20- and 40-d flooding treatments. Inhibition of microsclerotia production was attributed to decreased concentration of O_2 and increased concentration of CO_2 in flooded soils (Ioannou et al., 1977a).

The two experiments described in this study were in congruence with regard to the effects of periodic flooding and no flooding on Verticillium wilt development. In general, periodic flooding and inoculation with *V. dahliae* were accompanied by decreased plant dry weight and decreased levels in spectral reflectance-based physiological indices such as PRI and CBDI. However, it is not known why MSI and WBI were not significantly affected by flooding and inoculation treatments. Plant stress from periodic flooding was equivalent to plant stress from inoculation based on plant growth and spectral reflectance-based physiological indices. Despite the plant stress caused by periodic flooding, disease severity was greater under no flooding than under periodic flooding.

The mechanisms underlying the increase of Verticillium wilt under no-flooding conditions compared with periodic flooding are not known. Although changes in population density of *V. dahliae* were not assessed in this study, it is plausible that periodic flooding affected inoculum density of *V. dahliae* as reported under continuous flooding (Ioannou et al., 1977a; Pullman and DeVay, 1982). It is also conceivable that the activity of *V. dahliae* was impeded under periodic flooding as reflected in the fact that the decrease in plant dry weight in noninoculated plants was within the range recorded for plants inoculated with *V. dahliae*. Under no-flooding conditions, it is possible that short-term soil

moisture fluctuation enhanced population levels and activity of *V. dahliae*. Farley et al. (1970) have shown that conidia production by *V. dahliae* increased in soil infested with microsclerotia and subjected to repeated cycles of air-drying and remoistening with soil moisture fluctuation between 4% and 50% field capacity.

An important factor that was not addressed in this study is the contribution of soil microflora to the differential response of chile pepper to *V. dahliae* under periodic flooding and no-flooding conditions. Although such contribution may be negligible in this greenhouse study because sterilized soil was used in all experiments, it is plausible that under field conditions, soil microflora may affect population levels and activity of *V. dahliae*. Ioannou et al. (1977b) have reported that in saturated soil activity of antagonistic soil, microflora may increase, whereas in nonsaturated soil, antagonistic activity of soil microflora may be decreased.

In addition to validating field observations and field surveys on increased Verticillium wilt of chile pepper under drip irrigation compared with furrow irrigation, this study showed that plant growth and physiology were more reduced under periodic flooding than under no-flooding conditions. However, it is not known to what extent these results reflect changes in plant growth and physiology under field conditions. There are no previous field data available for comparison. This area merits further research.

The information emanating from this study should be useful to chile pepper producers in understanding the ecology of *V. dahliae* with respect to furrow irrigation and drip irrigation as represented here by periodic flooding and no flooding, respectively. It appears that furrow irrigation may alleviate the impact of Verticillium wilt in fields infested with *V. dahliae*. Recommendations to implement furrow irrigation for control of Verticillium wilt are to be gauged on whether a given production field is solely infested with *V. dahliae*. These recommendations may not be tenable in production fields with a history of Phytophthora blight. In such production fields, implementing furrow irrigation would be laden with the risk of increasing Phytophthora blight. Producers will have to determine whether Phytophthora blight or Verticillium wilt is the most significant problem they are facing

and apply information from this study and others to resolve the problem to their desired satisfaction.

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