Heat Level in Chile Pepper in Relation to Root and Fruit Infection by Phytophthora capsici

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Abstract. Phytophthora blight, caused by Phytophthora capsici Leon., is a major plant disease that limits chile pepper (Capsicum annuum L.) production in New Mexico. Chile pepper producers in New Mexico report that Phytophthora blight symptoms appear to develop slower and its incidence is lower in hot than in nonhot chile pepper cultivars. There has been no previous systematic assessment of the relationship of chile pepper heat level to chile pepper response to P. capsici. Three hot (‘TAM-Jalapeno’, ‘Cayenne’, and ‘XX-Hot’) and two low-heat (‘NuMex Joe E. Parker’ and ‘New Mexico 6-4’) chile pepper cultivars were inoculated at the six- to eight-leaf stage with zoospores of P. capsici under greenhouse conditions. Additionally, detached mature green fruit from three hot (‘TAM-Jalapeno’, ‘Cayenne’, and ‘XX-Hot’) and one low-heat (‘AZ-20’) chile pepper cultivars were inoculated with mycelium plugs of P. capsici under laboratory conditions. When plant roots were inoculated, Phytophthora blight was slowest to develop on ‘TAM-Jalapeno’ in contrast to all other cultivars. All ‘TAM-Jalapeno’ plants showed wilting symptoms or were dead ~22 days after inoculation compared with 18, 15, 14, and 11 days for ‘NuMex Joe E. Parker’, ‘New Mexico 6-4’, ‘XX-Hot’, and ‘Cayenne’, respectively. When fruit were inoculated, lesion length ratio was significantly higher for ‘TAM-Jalapeno’ fruit than for ‘Cayenne’, ‘XX-Hot’, and ‘AZ-20’ fruit. Similarly, lesion diameter ratio was higher for ‘TAM-Jalapeno’ fruit than for fruit of other cultivars. Furthermore, mycelial growth on lesion surfaces was more extensive on ‘TAM-Jalapeno’ fruit than on fruit of other cultivars. Results from this study indicate that there is little or no relationship between heat level and chile pepper root and fruit infection by P. capsici.

Phytophthora blight, caused by Phytophthora capsici, constitutes a limiting factor to profitable production of many crops worldwide (Erwin and Ribeiro, 1996), including chile pepper (Sanogo, 2003, 2004; Sanogo and Carpenter, 2006). Phytophthora blight was first described by Leonian (1922) as a disease affecting roots, stems, leaves, and fruit of chile pepper. Within 5 d of inoculation, P. capsici can cause death of chile pepper plants, especially when plants are young. Infection of the plant stem is indicated by dark purplish lesions close to the soil surface. As the disease progresses, lesions rapidly coalesce and completely girdle the stem. Chile pepper fruit become infected during prolonged periods of heavy rain and high humidity in flooded and poorly drained fields. Infected fruit initially show small, water-soaked, dull green lesions that rapidly shrivel and rot (Biles et al., 1992). Severely diseased fruit are covered with a white powdery mat as a result of mycelium and sporangia production on the infected fruit surfaces. The most effective means for controlling Phytophthora blight are chile pepper cultivars that are resistant to P. capsici. Although genetic resistance has been identified in some chile pepper lines (Bosland and Lindsey, 1991), currently there are no commercial chile pepper cultivars that are resistant to P. capsici in all environments. At present, management of Phytophthora blight relies on reducing saturation conditions in field soil to minimize spread and reproduction of P. capsici.

Chile peppers (nonbell-type) are characterized by the presence of capsaicinoids in fruit, and capsaicin and dihydrocapsaicin account for more than 80% of the total capsaicinoids produced (Zewdie and Bosland, 2001). Capsaicin-related alkaloids evoke pain equivalent to pain evoked by heat on noninceptors, which are pain-sensing neurons (Caterina et al., 2000). Therefore, capsacin-evoked pain is best described in terms of burning or heat sensation. In this study, the term “heat” is used in lieu of “pungency”, which is a term commonly used in the literature on “hot” peppers. Heat level in chile pepper is expressed in Scoville heat units (Scoville, 1912). According to Bosland and Votava (2000), the following ranges of Scoville heat units (SHU) are used for classifying chile pepper products: 0 to 700 SHU (low heat), 700 to 3,000 SHU (mild heat), 3,000 to 25,000 SHU (moderate heat), 25,000 to 70,000 SHU (high heat), and greater than 70,000 SHU (very high heat).

Chile pepper producers in New Mexico and Arizona observed that Phytophthora wilt symptoms develop slower and its incidence is lower in hot than in low-heat chile pepper cultivars. On a farm in South Carolina, Keinath (2007) observed that disease incidence was higher on bell pepper than on the jalapeno type. Although no explanation for this observation was provided, it is possible that this differential incidence between the two pepper types is because jalapeno is a hot chile pepper type in contrast to bell pepper.

Information on the relationship of chile pepper heat level to diseases and plant pathogen populations is scanty, observational, and inconsistent across pathosystems. Blazquez (1976) reported that powdery mildew, caused by Oidiopsis sp., was less severe on most no-heat cultivars than on jalapeños. It has been observed that production of microcystic spores of Verticillium dahliae was less in jalapeño fields (Bhat et al., 2003) than in nonjalapeno fields, which may be attributed to the fact that jalapeno is a hot chile pepper.

There has been no previous systematic assessment of the relationship of chile pepper heat level to chile pepper response to P. capsici. Elucidation of this relationship may reveal features usable in breeding programs intended for developing resistance to P. capsici. In laboratory experiments, Beard (2006) showed that mycelial growth of P. capsici was reduced by 49% to 63% on media amended with Capsicum oleoresin varying in heat level from 100,000 to 300,000 SHU relative to nonamended media. These results suggest that infection and colonization of hot chile pepper tissue by P. capsici may be reduced compared with low-heat chile pepper tissue.

The hypothesis of this study was that the severity of Phytophthora blight would be greater in low-heat than in hot chile peppers.
The objectives of this study were to evaluate the effect of hot chile pepper cultivars on root and fruit infection by *P. capsici* and the development of Phytophthora blight.

**Materials and Methods**

**Inoculum preparation.** An isolate of *P. capsici* recovered from a field-infected chile pepper plant in New Mexico was used in this study. This isolate is one of the most highly virulent isolates routinely used in chile pepper breeding program (Bosland and Lindsey, 1991; Oelke et al., 2003). The isolate was maintained on water agar in 9-cm-diameter petri plates at room temperature (23 to 25 °C). The inoculum used in plant infection consisted of zoospores produced following methods outlined by Bosland and Lindsey (1991). A 1-cm mycelial plug of *P. capsici* grown on water agar was placed onto a 9-cm-diameter petri plate containing V8 juice agar and incubated in the dark at 25 °C. After 5 d, six mycelial plugs (1-cm diameter) were cut from the V8 juice agar culture. The plugs were placed in 20 mL sterile distilled water in 9-cm-diameter petri plates and maintained in the dark at 25 °C for 3 d to induce sporangia formation. The plates were then transferred to 10 °C for 60 min and returned to 25 °C for 90 min to trigger the release of zoospores. The contents of the petri plates were then passed through three layers of cheesecloth to remove agar plugs. The number of zoospores was counted using a hemacytometer, and inoculum concentration was adjusted to 3 × 10⁴ zoospores per milliliter.

**Plant production and inoculation.** In a greenhouse, seeds of three hot (‘TAM-Jalapeno’, ‘Cayenne’, and ‘XX-Hot’) and two low heat (‘NuMex Joe E. Parker’ and ‘New Mexico 6-4’) chile pepper cultivars (Table 1) were planted in sterilized Terra-Lite Metro Mix 300 (W.R. Grace & Co., Memphis, TN) in 128-cell flats. Heat levels for these cultivars were determined through high-performance liquid chromatography analysis or obtained from published reports (Bosland et al., 1996; Bosland and Votava, 1997). At the second true leaf stage, seedlings were individually transplanted into round plastic pots (with a diameter of 12 cm on top and 9.5 cm on the bottom, depth of 12 cm) filled with sterilized Terra-Lite Metro Mix 300. Seedlings were fertilized once every 2 weeks by adding 150 mL solution of 20–20–20 (N–P₂O₅–K₂O) fertilizer to each pot. Plants were inoculated at the six- to eight-leaf stage by dispensing 5 mL of inoculum on the soil in each pot at 2 cm away and around the stem. The addition of 5 mL of zoospore suspension of *P. capsici* yielded an inoculum level of 15 × 10⁴ zoospores per pot. For seedlings serving as controls (noninoculated), 5 mL of water was added to each pot.

The experimental design was a randomized complete block design. Each treatment (cultivar) was replicated 10 times with a pot being a replicate. The experiment was carried out in Nov. 2006 (Trial 1) and was repeated in December of 2006 (Trial 2). Plants were monitored daily and disease severity was recorded daily throughout the duration of the study on aboveground plant parts using a scale described by Sanogo (2004): 0 = no visible disease symptoms, 1 = stem necrosis with no girdling, 2 = stem necrosis with girdling, 3 = stem necrosis with less than 50% defoliation, 4 = stem necrosis with greater than 50% defoliation, 5 = wilted, and 6 = dead. Disease severity rating was recorded daily to a maximum of 34 d.

The number of days to reach a severity rating of 2 or greater and a severity rating of 5 or greater was determined and used in statistical analysis. Control plants were not included in the statistical analysis and were used to monitor the success of the inoculation procedure as well as to compare between healthy and diseased plants. Experiments were analyzed separately to assess whether findings were similar and generalizable across experiments. The SAS GLM procedure (version 8; SAS Institute, Cary, NC) was used to calculate F statistics, means, and SEs of means for number of days to reach a severity rating of 2 or 5 and for severity rating per day. Least significant differences were used to carry out pairwise comparisons among treatments at a significance level of 0.05. Residuals were checked for normality and the GENMOD procedure using the Poisson distribution was used if normality was not satisfied. Results were compared between the normal-based results (GLM) and Poisson-based results (GENMOD). In general, results were consistent between the two analyses so only normal-theory results are presented here.

**Fruit production and inoculation.** Seeds of three hot (‘TAM-Jalapeno’, ‘Cayenne’, and ‘XX-Hot’) and one low-heat (‘AZ-20’) chile pepper cultivars (Table 1) were planted in May 2006 in a field at the Leyendecker Plant Science Research Center, New Mexico State University. The center is located 14.5 km south of Las Cruces at an elevation of 1172 m and with a mean annual precipitation of 20.3 cm. Each cultivar was planted in four replicate plots, and the dimensions of each plot were 20 m in length and four rows wide with a row spacing of 1 m. Standard field practices were used in growing and maintaining the crop (Bosland and Walker, 2004). Mature green chile pepper fruit were harvested in Aug. (Trial 1) and Sept. 2006 (Trial 2) and were stored at 4 °C and inoculated within 1 week. Only undamaged fruit that were uniform in size (2.5 to 5.0 cm in diameter) and color were selected for inoculation. Field-grown fruit were used because of the difficulty of obtaining a high number of fruit under greenhouse conditions. These fruits were healthy and not infected by *P. capsici* because no control fruit develop any symptoms of Phytophthora blight or any other diseases during the course of the experiments. Fruit heat level and moisture content are shown in Table 1. Heat level was determined by using the improved high-performance liquid chromatography (HPLC) method described by Collins et al. (1995). Fruit were cut in small pieces and oven-dried at 60 °C for 2 d and ground to pass through a 1-mm screen. Capsaicinoids were extracted from fruit powder using acetonitrile. In this HPLC method, the concentration of capsaicinoids is expressed in part per million (ppm), which may be converted into SHU by multiplying by a factor of 15. The heat detection limit was found to be 3 ppm or 45 SHU (Collins et al., 1995). Harvested fruit of the four chile pepper cultivars were surface-sterilized by immersing in 1% sodium hypochlorite solution for 45 s, rinsed with sterile distilled water, and air-dried. Immediately after surface sterilization, fruit were randomly placed into two ethanol-disinfested plastic moist chambers (60 × 40 × 30 cm) and kept at 21 °C and a high relative humidity (100%) under a diurnal regime (12 h of white fluorescent light and 12 h of dark). A 1-cm mycelial plug was taken from a 3-d-old culture of *P. capsici* on V8 juice agar and placed centrally on each fruit. Inoculated fruit were observed daily for *P. capsici* infection. The inoculated nonwounded fruit were evaluated 5 d after inoculation by measuring three variables: 1) the water-soaked lesion length divided by the overall length of the fruit (lesion length ratio); 2) the water-soaked lesion diameter divided by the overall diameter of the fruit (lesion diameter ratio); and 3) the sporulation (mycelial growth) area divided by the total area of the lesion (mycelial growth ratio).

The experimental design was a generalized randomized complete block. Each chamber served as an experimental block. Each treatment (cultivar) was replicated five times within each block with a fruit of each cultivar being a replicate. The experiment was carried out in Aug. 2006 (Trial 1) and was repeated in Sept. 2006 (Trial 2). Fruit used in Trial 1 and Trial 2 were harvested from plots with different flowering events and therefore were considered as two independent populations of fruit. Heat and moisture levels were similar for the two populations of fruit harvested. Experiments

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**Table 1.** Heat level and fruit moisture content of chile pepper entries used in this study.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Scoville heat units (SHU)</th>
<th>Heat scale</th>
<th>Fruit moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ-20</td>
<td>800</td>
<td>NA</td>
<td>90.3</td>
</tr>
<tr>
<td>New Mexico 6-4</td>
<td>800</td>
<td>NA</td>
<td>90.3</td>
</tr>
<tr>
<td>NuMex Joe E. Parker</td>
<td>811</td>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>TAM-Jalapeno</td>
<td>11,870</td>
<td>7</td>
<td>90.0</td>
</tr>
<tr>
<td>Cayenne</td>
<td>16,242</td>
<td>8</td>
<td>87.6</td>
</tr>
<tr>
<td>XX-Hot</td>
<td>33,260</td>
<td>NA</td>
<td>88.8</td>
</tr>
</tbody>
</table>

*Measured high-performance liquid chromatography values (n = 2).
*BDL = below detection limit of 45 SHU.
*Stated heat level in a report by Bosland and Votava (1997).
*Relative heat scale (from 0 to 10) as defined by Bosland et al. (1996) with 0 = mildest and 10 = hottest.
*NA = not available.
Results

Root infection. The Cayenne cultivar had significantly higher disease severity during the first 14 d after inoculation than the other cultivars (Fig. 1). Chile pepper cultivars TAM-Jalapeño and NuMex Joe E. Parker had significantly lower disease severity during the first 20 d after inoculation in the trial conducted in Nov. 2006 (Fig. 1A). The same trend was observed with ‘TAM- Jalapeño’ in the second trial conducted in Dec. 2006 (Fig. 1B). The chile pepper cultivar TAM-Jalapeño had significantly lower disease severity compared with all other cultivars throughout the duration of the study (Fig. 1B).

In Trial 1, ‘Cayenne’ seedlings displayed stem necrosis with girdling (disease severity rating of 2 or greater) 10.7 ± 1.2 d from inoculation compared with 12.8 ± 1.2, 14.6 ± 1.2, 15.6 ± 1.2, and 19.5 ± 1.2 for ‘XX-Hot’, ‘New Mexico 6-4’, ‘NuMex Joe E. Parker’, and ‘TAM-Jalapeño’, respectively (P = 0.0001; Fig. 2A). All the ‘Cayenne’ plants displayed wilting symptoms or were dead (disease severity rating of 5 or greater) 12.8 ± 1.4 d after inoculation compared with 16.1 ± 1.4, 17.2 ± 1.4, 20.6 ± 1.4, and 22.1 ± 1.4 d after inoculation for ‘XX-Hot’, ‘New Mexico 6-4’, ‘Joe E. Parker’, and ‘TAM-Jalapeño’, respectively (P = 0.0002; Fig. 2A).

Similar results were observed in Trial 2. ‘Cayenne’ seedlings displayed stem necrosis with girdling (disease severity rating of 2 or greater) 9.2 ± 1.3 d from inoculation compared with 9.4 ± 1.3, 11.5 ± 1.3, 12.2 ± 1.3, and 18.5 ± 1.3 for ‘XX-Hot’, ‘New Mexico 6-4’, ‘NuMex Joe E. Parker’, and ‘TAM-Jalapeño’, respectively (P < 0.0001; Fig. 2B). All ‘Cayenne’ seedlings displayed wilting or were dead (disease severity rating of 5 or greater) 10.5 ± 1.5 d after inoculation compared with 12.4 ± 1.5, 13.9 ± 1.5, 16.4 ± 1.5, and 22.3 ± 1.5 for ‘XX-Hot’, ‘New Mexico 6-4’, ‘NuMex Joe Parker’, and ‘TAM-Jalapeño’, respectively (P = 0.0001; Fig. 2B).

Fruit infection. Lesion length ratio was significantly higher for ‘TAM-Jalapeño’ fruit than for ‘Cayenne’, ‘XX-Hot’, and ‘AZ-20’ fruit in both trials (Fig. 3). In the first trial (Aug. 2006, P < 0.0001), lesion length ratio was 1.00 ± 0.08 for ‘TAM-Jalapeño’ fruit compared with 0.56 ± 0.08, 0.36 ± 0.08, and 0.31 ± 0.08 for ‘Cayenne’, ‘XX-Hot’, and ‘AZ-20’, respectively. In the second trial (Sept. 2006, P < 0.0001), lesion length ratio was 0.99 ± 0.08 for ‘TAM-Jalapeño’ fruit compared with 0.46 ± 0.08, 0.34 ± 0.08, and 0.0 for ‘Cayenne’, ‘XX-Hot’, and ‘AZ-20’, respectively (‘AZ-20’ was excluded from the analysis because all values were zero). Chile pepper fruit responded similarly to P. capsici infection in both trials, except that lesion length ratio was significantly lower for ‘AZ-20’ fruit than for all other cultivar fruit in the second trial (Fig. 3).

Similar trends, but somewhat less pronounced, were observed for the lesion diameter and mycelial growth ratios (Figs. 4 and 5). Lesion diameter ratios for ‘TAM-Jalapeño’ fruit were the highest with values of 1.00 ± 0.01 and 0.93 ± 0.10 in Trials 1 and 2, respectively (P < 0.0001 in both trials), whereas lesion diameter ratios for ‘AZ-20’ fruit were the lowest with values of 0.25 ± 0.01 and 0.00 in Trials 1 and 2, respectively.

Varieties had significantly different mean mycelial growth ratio in Trial 1 (P < 0.0001) but not in Trial 2 (P = 0.1183). Mycelial growth ratios were 0.08 ± 0.03 and 0.00 for ‘AZ-20’ fruit in Trials 1 and 2, respectively, and 1.00 ± 0.03 and 0.15 ± 0.06 for ‘TAM-Jalapeño’ fruit in Trials 1 and 2, respectively.

Discussion

This study was conducted to validate field observations. The experiments were completed under greenhouse conditions to minimize the influence of confounding factors on plant response as may be encountered under field conditions. Results from this study did not support the hypothesis that severity of Phytophthora blight would be greater in low heat chile peppers than in hot chile peppers. There was no relationship between chile pepper heat level and root and fruit infection by P. capsici. The determinants of heat in chile pepper are the capsaicinoids that are produced in the placenta of fruit. No capsaicinoids are produced in the roots. Therefore, intuitively, it was expected that heat level would affect fruit infection and would have no bearing on root infection by P. capsici. However, because P. capsici infects both roots and aboveground plant parts, it was important to examine the development of Phytophthora blight not only on fruit, but also on roots. Whereas Phytophthora blight...
was slowest to develop on roots of ‘TAM-Jalapeño’, the disease was fastest on the fruit. Conversely, the disease was faster to develop on roots and slower on fruit of all other cultivars. It appeared that factors other than the level of capsaicinoids are involved in differential development of Phytophthora blight among chile pepper cultivars varying in heat levels.

This is the first systematic study on the relationship between chile pepper heat and development of Phytophthora blight. Therefore, there are no previous available results for comparison in this pathosystem. Furthermore, there is a dearth of information on other pathosystems. Blazquez (1976) reported that powdery mildew, caused by Oidiopsis sp., was less severe on most low-heat cultivars than on jalapeños. However, it is unlikely that heat was involved because leaves were assessed for disease severity, and no capsaicinoids are produced in the leaves.

A search of the literature beyond pepper plant pathogen systems revealed that there is also scarcity of information on the relationship of pepper heat and pepper interactions with pests such as insects. Larue (2006) showed that although infestation by European corn borers (Ostrinia nubilalis) tended to be less on hot versus sweet (low-heat) peppers, other factors such as canopy density may be involved by affecting egg desiccation and larval hatch on fruit.

The direct effect of heat level on mycelial growth of P. capsici has been assessed by Beard (2006) in laboratory experiments. Agar medium was amended with hot Capsicum oleoresin (6.6% capsaicin with 1,000,000 SHU) at concentrations varying from 15% to 30% aqueous solutions. After 6 d incubation at 25°C, mycelial growth was reduced by 49% to 83% on medium amended with Capsicum oleoresin relative to non-amended medium. Although the heat levels selected in the study by Beard (2006) are well above those measured in the fruit used in this study, from the results obtained, it may be inferred that infection of fruit from hot chile peppers by P. capsici would be reduced to a greater extent compared with fruit from low-heat chile peppers. However, this was not the case in this study. The fact that disease severity was lower on fruit of ‘AZ-20’, a low-heat chile pepper cultivar, than on ‘TAM-Jalapeño’, a hot chile pepper cultivar, suggests that factors other than heat level may be involved in fruit response to P. capsici. For example, cuticle thickness has been shown to be a factor in fruit resistance to P. capsici (Biles et al., 1993). Although not measured in this study, it is conceivable that fruit cuticle thickness may explain part of the results of this study. It also must be realized that the cultivars used in this study are not genetically similar; therefore, the response observed may be affected by genetic differences among cultivars, although the extent of this influence is not known.

The methods used in this study, including inoculum type and level, inoculation procedure, and detached fruit technique, were
similar to those used in other studies (Biles et al., 1993; Bosland and Lindsey, 1991). A difficulty encountered in using the detached fruit technique is ensuring collection of fruit that are of the same age. In this study, fruit of the same age and size were used to minimize variation among fruit harvested on different dates. Uniformity in fruit characteristics was indicated by the fact that fruit heat level and moisture were consistent among fruit within each cultivar across the two experiments conducted.

Although zoospores are commonly used in plant infection studies with *P. capsici*, fruit infection studies have been carried out using zoospores or mycelial plugs (Biles et al., 1993; Gevens et al., 2006). In this work, mycelial plugs were used to provide a readily infective inoculum and to circumvent the difficulty associated with placement of zoospore inoculum on the fruit surface. The merits of using mycelial plugs over zoospore inoculum have also been recognized in other studies (Gevens et al., 2006).

*Phytophthora capsici* is known to vary genotypically and phenotypically (Erwin and Ribeiro, 1996), and therefore virulence among isolates may be variable. In this study, the *P. capsici* isolate used is one of the most highly virulent isolates used routinely in the chile pepper breeding program for evaluating germplasms for disease resistance (Bosland and Lindsey, 1991; Oelke et al., 2003). The merit of using one of the most virulent isolates, as done in this work, is in ensuring evaluation of cultivars under the highest potential risk associated with the presence of *P. capsici*.

This study was limited to five cultivars, but the heat levels in the cultivars examined are representative of the spectrum of commonly encountered heat levels in chile pepper grown for fresh and processing market. Similar number of cultivars has been used in other work (Larue, 2006) with a comparable range of heat levels. Larue (2006) used five pepper entries (*C. annuum*) varying in heat level from 0 to 50,000 SHU to evaluate fruit infestation by European corn borer (*O. nubilalis*).

Exploration of the relationship of pepper heat to pest interactions with plant pathogens and pests is a novel area that merits further research. The significance of this study is three-pronged. First, from the perspective of chile pepper producers, this study indicates that there is no strong evidence of a relationship between chile pepper heat level and development of Phytophthora blight on chile pepper. Second, from the perspective of further research, it delineated a methodology for assessing the relationship between cultivar heat level and pathogenic virulence whereby evaluation of both fruit and root infection is taken into consideration. Third, from the perspective of contribution to the literature on Phytophthora blight, this study provides ecological information on behavior of *P. capsici* for comparison in future studies.

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