Phytophthora capsici Zoospore Infection of Pepper Fruit in Various Physical Environments

Charles L. Biles[1], Benny D. Bruton[2], Marisa M. Wall[3] and Melinda Rivas[1]

1. Biology Department, East Central University, Ada, Oklahoma 74820
2. USDA, ARS, SCARL, P.O. Box 159, Hwy. 3, Lane, Oklahoma 74555
3. Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003

Received: 1995 Mar 24; Revised: 1995 Jun 30

***Phytophthora capsici* is a pathogen on several economically important crops including pepper. The fungus attacks the roots, stems, leaves and fruit of the plant. Experiments were conducted to determine environmental conditions that may affect infection of pepper fruit by zoospores of *P. capsici*. Two techniques were used to inoculate the mature green pepper fruit. First, the blossom end of the fruit was submerged in an aqueous suspension of zoospores. This technique attempted to simulate dispersal of zoospores by flood irrigation. The second technique involved placing water drops of the zoospore suspension on the fruit. Disease incidence and severity were greater for the first technique than for the second technique. In addition, 100% of fruit were infected when immersed in the zoospore suspension for 10 min. Disease incidence and lesion length were greatest at 27 °C. Drop sizes of 10, 50, and 100 µl with identical zoospore content (5000 zoospores/drop) did not significantly affect lesion size. Disease incidence, however, was significantly greater when a 100-µl drop was used for inoculation, compared to the 10- and 50-µl drops. These data suggest that the most severe infection occurs when peppers are in contact with a large volume of inoculum (i.e. flood irrigation), in contrast to a small droplet with the same concentration of zoospores.**

INTRODUCTION

Chile peppers (*Capsicum annuum* L. long, green type) are widely grown in the southwestern United States. With the demand for Mexican/Spanish cuisine, pungent pepper production is increasing annually (*5*). Mature green peppers (green chile) are consumed fresh or processed for canning. Red fruits are dehydrated for use as chile powder or paprika.

In pepper-growing areas of the Southwest, *Phytophthora capsici* (Leonian) is a soilborne fungal pathogen that severely limits production (*3, 5*). The pathogen, *P. capsici*, has a broad host range attacking tomato, eggplant, cucumber, watermelon, pumpkin, squash, cocoa, macadamia, and peppers (*12-16*). On peppers, Phytophthora blight can occur on the roots, stems, leaves, and fruit (*2,19*). The pathogen infects fruits during prolonged periods of heavy rainfall and high humidity, especially when plants are over-crowded or over-fertilized with nitrogen (*19*). Rate of plant infection was shown to increase during the months of high rainfall in southern New Mexico (*3*). During this time (July and August) fields that do not have adequate drainage are often flooded. Infected fruits initially show water-soaked lesions and eventually shrivel and rot. The fruits turn white, and the interior of the pods are heavily colonized by the white mycelium of *P. capsici*. Similar conditions for infection and symptomology have recently been reported in Korea (*11*).

The purpose of this research was to further investigate environmental and physical factors that may affect the infection rate of *P. capsici* on peppers. Temperature range, zoospore concentration, time of exposure to inoculum, and drop size were variables investigated in regard to infection of the pepper fruit.

METHODS

Plant material and inoculum. Peppers (cv. New Mexico ‘Joe Parker’) were planted in a field plot on 1 May, 1993, south of Las Cruces, New Mexico. Experiments were repeated in 1994 in which the peppers were grown in field plots located in Lane, Oklahoma at the South Central Agricultural Research Laboratory, U.S.D.A., Agricultural Research Service. At both locations, standard practices were used in growing and maintaining the crop. Mature green peppers (*2*) were harvested in August and kept for no more than 1 week at 4-6 °C before being

used in experiments. Only undamaged peppers that were uniform in size and color were selected for use in the experiments described.

To induce zoospore production, the procedure described by Ristaino (*16*) was used. *P. capsici* (NM 6012) was grown on V-8 agar petri plates for 1 week. The agar with fungal hyphae was then cut into 0.5-cm squares and placed in a sterile petri dish with sterile distilled water. The solution was replaced with fresh water every three days. After 1 week, the sporangia were given a cold shock (1 h at 4-6 °C), followed by a 1-h equilibration at room temperature. The zoospores were separated from the solution by filtering through 2 layers of cheesecloth and the suspension calibrated using a hemacytometer. Zoospores were applied to the fruit within 30 minutes of calibration. Preliminary studies indicated that zoospores under the described conditions encysted within 2-3 h (unpublished data).

Experimental procedures. The first study undertaken was to determine the optimum range of zoospores for infection and disease development using the fruit-submersion technique. Concentration of zoospores ranged over 0-10^4/ml of deionized-distilled water. In the fruit-submersion technique, pepper fruit were individually placed in a 50-ml beaker at room temperature (25 °C). Total water volume was 25 ± 1 ml. The inoculum was introduced into the beaker until approximately 2.5-cm of the blossom end of a vertically positioned fruit was submerged. The fruit remained in the zoospore suspension for 2 h, then was removed and placed in a humidity chamber (92% RH) for 6 days. Lesion length was quantified as a measure of disease severity. This experiment was repeated 3 times with 7 replications per treatment. Standard errors of the mean were calculated to distinguish difference between treatments. This method of statistical analysis was used in each of the following experiments.

In a subsequent study, pepper fruits were submerged in zoospore suspensions, as previously described, for 10 min., 1, 2, 4, 6, and 8 h to determine optimum zoospore exposure for infection and disease development. On the basis of the previous study, data indicated that 10^3 zoospores/ml was insufficient for optimum infection and 10^4 zoospores/ml exceeded the threshold for distinguishing environmental effects on fruit infection. Therefore, the inoculum concentration selected was 5×10^3 zoospores/ml. The fruits were placed in a humidity chamber after the various inoculation times and evaluated for disease incidence and severity after 6 days at 25 °C. Each fruit represented a replication. Standard errors of the mean were calculated to distinguish differences between inoculum concentrations.

In order to compare the water-droplet technique with the fruit-submersion method, water droplets (100 μl) containing different concentrations of inoculum (0-10^4 zoospores per drop) were applied to individual fruits and placed in humidity chambers as described previously. Water droplets containing either 0, 10, 10^2, 10^3, or 10^4 zoospores per drop were introduced onto the fruit surface (midrib, flat surface of exocarp, one zoospore drop per fruit) and the fruit placed into humidity chambers for 6 days at room temperature (25 °C). Disease incidence (% infection) and disease severity, determined as lesion length, were measured 6 days after inoculation.

The effect of water droplet volume on zoospore infection was tested using 10-, 50-, and 100-μl droplets with a zoospore content of 5×10^3 zoospores per drop. Each drop, regardless of size, contained the same number of zoospores. Disease incidence and severity were determined at day 6.

Temperatures at 15, 25, 27, 30, and 35 °C were tested to determine the optimum temperature for infection using the water-droplet method. Water droplets (100 μl) containing 5×10^3 zoospores per drop were applied to the fruit surface and placed in humidity chambers. The humidity chambers were then placed in environmental chambers set at either 15, 25, 27, 30, or 35 °C. The fruit were left in the temperature chambers for 6 days and then scored for disease incidence and severity.

RESULTS and DISCUSSION

With the fruit-submersion technique (Fig. 1) disease incidence and severity increased as zoospore concentration increased. The fruit-submerged peppers inoculated with 10^4 zoospores/ml had a significantly higher incidence (100% ± 0%) than

those treated by the droplet method (50% ± 7.5%) (data not shown). Disease severity (lesion length) was also greater by the fruit-submersion inoculation method when compared to droplet method (Fig. 1). With both methods, infection was observed at concentrations as low as 10 zoospores/ml or 10 zoospores per drop of inoculum, depending on method.

Disease severity was not significantly different among 10-, 50-, and 100-μl drops, although an increasing trend was observed from 10- to 100-μl inoculum drop (Fig. 2). In contrast, disease incidence was 34% ± 6%, 21% ± 4%, and 17.5% ± 3.5% for the 100-, 50-, and 10-μl zoospore drop treatments, respectively, demonstrating a significant increase in incidence between the 50- and 100-μl zoospore droplets. There was no difference between the 10- and 50-μl drop treatments in regard to disease incidence. These data again indicate that increased water volume enhances infection.

Fruits exposed to a larger volume of inoculum, not necessarily a higher concentration, incur a higher level of infection. These results suggest that greater opportunity for *P. capsici* infection may occur during flood irrigation when compared to rain-splashed dispersal of inoculum. This may be due to the increased probability of the zoospores to chemotaxically find wounds (cracks) in the cuticle surface, encyst, and infect. *P. capsici* does not require a wound to penetrate, but wounding does enhance infection (*2*). The zoospore droplet has a limited number of zoospores that can migrate to the infection site, whereas the submerged fruit has enhanced access to potential infection sites. Other workers have also found that splash dispersal of *P. capsici* soilborne inoculum is not important in disease development of aerial plant parts until late in the season (*18*). Little research has been conducted on the spread of zoospores of *P. capsici* in the field due to flooding, irrigation, and splashing rain (*6*). Ristaino (*17*) did observe that disease incidence caused by *P. capsici* became greater when water applied to a pepper field increased (increased irrigation) or after rainfall. Dispersal of *P. capsici* inoculum (type of inoculum: oospores, sporangia, zoospores, was not specified) also occurred within rows in surface water (*18*). Zoospores of other *Phytophthora* species are also spread by rain splashing and in irrigation water (*9, 10*).

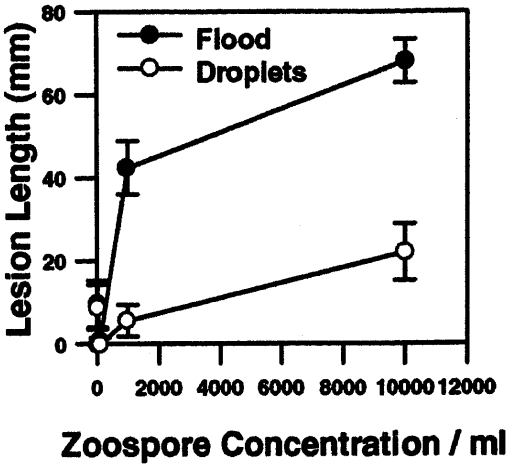

Figure 1. Disease severity (lesion length) of *Phytophthora* fruit rot of chile peppers at different inoculum levels. Two inoculation techniques were used; fruit submersion (fruit were submerged in a water suspension with 0, 10, 10^2, 10^3, or 10^4 zoospores/ml), and the droplet method. Vertical bars represent the standard error of the mean.

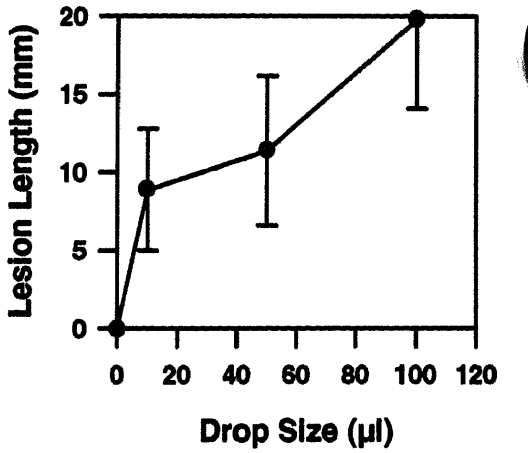

Figure 2. Disease incidence and severity of *Phytophthora* fruit rot of chile pepper inoculated with zoospore droplets of different sizes. All droplets contained 5×10^3 zoospores. Vertical bars represent standard error of the mean.

A 10-min exposure to zoospores of submerged fruit resulted in 80% disease incidence (data not shown) and a mean lesion length of 37 mm (Fig. 3). Maximum disease incidence (90-100%) and disease severity (80 mm) occurred with a 4-h exposure time to zoospores. A 4-h exposure time did not produce results significantly different from a 2-hr exposure time. Increasing exposure to 8 h did not significantly increase disease incidence or severity when com-

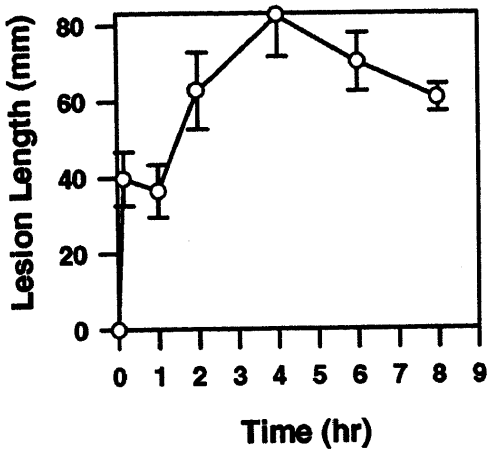

Figure 3. Disease severity of *Phytophthora* fruit rot of chile peppers submerged in a *P. capsici* zoospore suspension (5×10^3 zoospores/ml) for different lengths of time. Vertical bars represent the standard error of the mean.

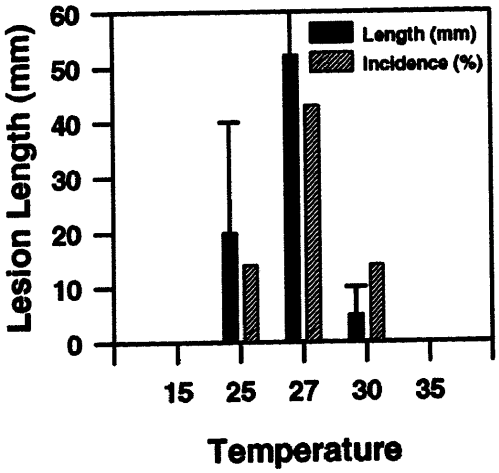

Figure 4. Disease incidence and severity of chile peppers inoculated with a 100-μl drop of zoospore suspension (5×10^3 zoospores per drop) and kept at different temperatures for 6 days. Vertical bars represent the standard error of the mean.

pared to 4 h. Zoospores of *P. parasitica* have been detected in furrow-irrigated tomatoes as early as 1 h after irrigation begins (*10*). Bernhardt and Grogan (*1*) showed that short periods of soil saturation (5-6 h) resulted in zoospore release from *P. capsici* sporangia. This suggests that infection in the field occurs quickly (within a few hours) and the longer the water stays in the furrow the greater the probability of infection. One control recommendation for this disease is to drain fields quickly to avoid infection of roots, leaves, and fruit (*19*). Another recommendation is to irrigate every-other row to reduce the amount of water in the rows (*3*). Hwang and Kim (*11*) have suggested several cultural and chemical control measures for *P. capsici*. Chemical control of oomycetes, which includes *Phytophthora* sp., has been well documented. However, consistent control of *P. capsici* with chemicals has not been achieved (*3*). Long periods of saturation of soils are well known to increase the incidence and severity of other *Phytophthora* diseases (*8, 11*).

Optimum temperature for infection of pepper fruits was 27 °C. Incidence at 27 °C was 28% and lesion length was 35 mm (disease severity). Infection did not occur at 15 or 35 °C (Fig. 4). On agar plates, New Mexico isolates of *P. capsici* grew at 15 to 40 °C (*4*). Variation in growth rate among the strains was greatest at 35 °C. A few of these strains appeared to be better adapted to either warm or cool temperatures. However, optimum temperature for infection of fruit does not appear to coincide with optimum mycelial growth.

ACKNOWLEDGMENTS

This research was partially funded by the New Mexico Agricultural Experiment Station and East Central Oklahoma State University. We thank Tresa Jo Morgan, Diann Baze, Anthony Dillard, Kevin Blackstone, Gaye Faubion, and Heather Palmer for technical support.

REFERENCES

1. Bernhardt, E.A. and Grogan, R.G., Effect of matric potential on the formation and indirect germination of sporangia of *Phytophthora parasitica, P. capsici,* and *P. cryptogea. Phytopathology* **72**, 507-511 (1982).

2. Biles, C.L., Wall, M.M., Waugh, M., and Palmer, H., Relationship of *Phytophthora* fruit rot to fruit maturation and cuticle thickness of New Mexican-type peppers. *Phytopathology* **83**, 607-611 (1993).

3. Biles, C.L., Lindsey, D.L., and Liddell, C.M., Control of *Phytophthora* root rot of chile peppers by irrigation practices and fungicides. *Crop Protection* **11**, 225-228 (1992).

4. Biles, C.L., Liddell, C.M., and Faubion, G.F., *Phytophthora capsici* strain characterization in southern New Mexico.

Phytopathology **81**, 1192 (1991).

5. Bosland, P.W., Bailey, A.L., and Iglesias-Olivas, J., *Capsicum pepper varieties and classification*. Cooperative Extension Service Circular 530, New Mexico State University, Las Cruces, NM (1990).

6. Bowers, J.H. and Mitchell, D.J., Effect of soil-water matric potential and periodic flooding on mortality of pepper caused by *Phytophthora capsici. Phytopathology* **80**, 1447-1450 (1990).

7. Cohen, Y., and Coffey, M.D., Systemic fungicides and the control of oomycetes. *Ann. Rev. Phytopathol.* **24**, 311-338 (1986).

8. Duniway, J.M., Role of physical factors in the development of Phytophthora diseases, in *Phytophthora: Its Biology, Taxonomy, Ecology and Pathology*. (Erwin, D.C., Bartnicki-Garcia, S., and Tsao, P.H., Eds.). American Phytopathology Society Press, St. Paul, MN (1983) pp. 175-187.

9. Gregory, P.H., Some major epidemics caused by *Phytophthora.* in *Phytophthora: Its Biology, Taxonomy, Ecology and Pathology*. (Erwin, D.C., Bartnicki-Garcia, S., and Tsao, P.H., Eds.). American Phytopathology Society Press, St. Paul, MN (1983) pp. 271-278.

10. Hoy, M.W., Ogawa, J.M., and Duniway, J.M., Effects of irrigation on buckeye rot of tomato fruit caused by *Phytophthora parasitica. Phytopathology* **74**, 474-478 (1984).

11. Hwang, B.K. and Kim, C.H., Phytophthora blight of pepper and its control in Korea. *Plant Disease* **79**, 221-227 (1995).

12. Kreutzer, W.A., Bodine, E.W., and Durrell, L.W., Cucurbit diseases and rot of tomato fruit caused by *Phytophthora capsici. Phytopathology* **30**, 972-976 (1940).

13. Kunimoto, R.K., Aragaki, M., Hunter, J.E., and Ko, W.H., *Phytophthora capsici*, corrected name for the cause of Phytophthora blight of macadamia racemes. *Phytopathology* **66**, 546-548 (1976).

14. Leonian, L.H., Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. *Phytopathology* **12**, 401-408 (1922).

15. Polach, F.J., and Webster, R.K., Identification of strains and inheritance of pathogenicity in *P. capsici. Phytopathology* **62**, 20-26 (1972).

16. Ristaino, J.B., Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* **80**, 1253-1259 (1990).

17. Ristaino, J.B., Influence of rainfall, drip irrigation, and inoculum density on the development of Phytophthora root and crown rot epidemics and yield in bell pepper. *Phytopathology* **81**, 922-929 (1991).

18. Ristaino, J.B., Larkin, R.P., and Campbell, C.L., Spatial and temporal dynamics of *Phytophthora* epidemics in commercial bell pepper fields. *Phytopathology* **83**, 1312-1320 (1993).

19. Shannon, E., *Chile disease control*. Cooperative Extension Service Guide H-219, New Mexico State University, Las Cruces, NM (1989).