Salinity Effects on the Susceptibility of Chrysanthemum Roots to *Phytophthora cryptogea*

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ABSTRACT

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Root systems of hydroponically grown chrysanthemums were exposed to salinity stress after inoculation with zoospores of *Phytophthora cryptogea*. Although inoculated with similar numbers of zoospores, roots subsequently exposed to 0.2 M NaCl and 0.01 M $CaCl_2$ in half-strength Hoagland's solution developed severe root rot symptoms, whereas roots that were not exposed to salinity stress developed only mild symptoms of disease. Roots exposed to nutrient solution containing 0.1 M NaCl and 0.005 M $CaCl_2$ developed symptoms of intermediate severity. When unstressed roots and roots exposed to the highest level of salinity stress were excised, placed in humidity chambers, and inoculated in a uniform manner, large differences were observed in the ability of *P. cryptogea* to colonize the tissues. After 48 hr of incubation, *P. cryptogea* was not cultured from tissue segments of unstressed roots taken 12 mm or farther from the point of inoculation, and the frequency of recovery in tissues closer to the point of inoculation was relatively low. By contrast, *P. cryptogea* was cultured with high frequency from tissue segments of stressed roots up to 12 mm from the point of inoculation, and with lower frequency up to 20–24 mm. When plants were exposed to salinity stress 4 or 12 hr after inoculation, severe disease resulted, but not if stress was delayed until 24 or 48 hr after inoculation. Thus, the host appeared to be susceptible to pathogen establishment only if stress occurred within 24 hr of inoculation. These data suggest that salinity stress alters the normal, active defense responses of the plant. The potential importance of salinity stress in the occurrence or severity of Phytophthora root rot is discussed.

Additional key words: Chrysanthemum morifolium, environmental stress, predisposition.

In an earlier paper (8), I reported that hydroponically grown chrysanthemums were predisposed to Phytophthora root rot by exposure to salinity stress prior to inoculation. Similarly, stresses resulting from drought (2,5) and prolonged soil flooding (2,7) can predispose various plant species to Phytophthora root rots. How roots are affected by stress and subsequently predisposed to disease is not known with certainty. In the case of salinity (8) and prolonged flooding (7), it was shown that greater numbers of zoospores attached to roots immediately following a stress event. This was attributed in the case of flooding stress, to an increased exudation of chemotactically active substances that stimulated zoospore attraction and encystment (7). A similar effect has been hypothesized to occur in drought stress predisposition (2,5), although there is no direct supporting evidence.

While stress-induced changes in root exudates could influence disease severity by altering zoospore attachment patterns and the number of initial infection sites, this is not the only effect that stress may exert on root tissues. The physiological processes that are activated in plant tissues following pathogen invasion, which serve to limit pathogen establishment or spread, also may be impaired by stress (1,10). Thus, in addition to possible increases in the numbers of initial infection sites on roots, stress also may interfere with normal resistance mechanisms in the tissue. In earlier experiments with salinity (8) and flooding (7) stresses, inoculum was not limiting, and it was not possible to evaluate whether increased disease was due solely to enhanced zoospore encystment on roots, or whether there were concomitant changes in tissue susceptibility.

This paper presents the results of a study undertaken to determine whether salinity stress alters the susceptibility of chrysanthemum root tissue to infection by *Phytophthora cryptogea*.

MATERIALS AND METHODS

Cuttings of *Chrysanthemum morifolium* 'Paragon' were rooted by suspending their cut ends in crocks of aerated half-strength Hoagland's (6) solution. After rooting, three plants were evenly spaced in each of 18 2-L ceramic crocks of fresh nutrient solution, and grown as previously described (8). Zoospore inoculum of *P. cryptogea* Pethyb. and Laff. (ATCC 46581) also was obtained by methods previously described (8). To determine whether salinity stress causes changes in tissue susceptibility, several methods were used to eliminate the potential for stress-induced changes in inoculum attraction or attachment to roots.

Postinoculation stress. After a 10- to 14-day growth period in the crocks of nutrient solution, the plants in nine crocks were inoculated by adding 4×10^6 motile zoospores to the solution in each crock. Plants in the remaining nine crocks were used as uninoculated controls. Inoculated plants were exposed to the zoospore inoculum for 4 hr to assure adequate time for cyst attachment and initial penetration of the root tissue. After the 4-hr inoculation period, three 3-cm terminal root segments were randomly collected from each inoculated plant. The root segments were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, and were set aside for light microscopic examination to determine relative numbers of attached cysts.

After the root systems were sampled, the infested nutrient solution was discarded and replaced with fresh nutrient solution. Both the inoculated and uninoculated groups of plants then were divided into three subgroups, with three crocks in each. One pair of subgroups was maintained in the nutrient solution to serve as unstressed controls, while the other two pairs were subjected to salinity stress by adding NaCl and $CaCl_2$ to the nutrient solution, bringing it to a final concentration of 0.1 M NaCl and 0.005 M $CaCl_2$, or 0.2 M NaCl and 0.01 M $CaCl_2$. Plants were held in the salinized nutrient solutions for 24 hr, after which the solutions were discarded and replaced with fresh half-strength Hoagland's solution. Plants were observed over the following 7- to 10-day period for symptoms of root rot. At the termination of each experiment, the root systems were harvested, dried at 95 C, and weighed. The entire experiment was repeated twice.

Colonization of excised roots. To further determine whether salinity stress results in increased tissue susceptibility, experiments were done to eliminate any possibility of secondary inoculum cycles that could contribute to disease severity within the crocks of nutrient solution. This was accomplished by growing plants in

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nutrient solution and exposing some to 0.2 M NaCl and 0.01 M $CaCl_2$ in nutrient solution for 24 hr as previously described. Four hours after the stressed plants had been returned to fresh nutrient solution, 25 5-cm-long terminal root segments were collected from both stressed and unstressed plants. The 25 root segments from each treatment were suspended in humidity chambers by resting the tip and cut end of each root on narrow strips of Plexiglas in petri dishes containing wet filter paper. The two Plexiglas strips in each humidity chamber were placed parallel to each other, ~4 cm apart, and five root segments were evenly spaced along their length.

Zoospore inoculum was obtained using methods previously described (8) and the zoospores were induced to encyst by vigorous agitation with a Vortex mixer. The concentration of zoospore cysts in the inoculum suspension was adjusted to 8×10^4/ml, and each root segment was inoculated by pipetting 10 μl of this suspension directly onto the root tip region (the terminal 4-mm portion of the root in contact with the Plexiglas support). The root segments were maintained in the humidity chambers for 48 hr at room temperature, after which each was removed from the chambers, immersed briefly in 0.5% NaOCl followed by a distilled water rinse, and cut into 4-mm-long segments, starting from the root tip. Each 4-mm segment was cultured on modified PV medium (4) and evaluated 48 hr later for the presence of *P. cryptogea*.

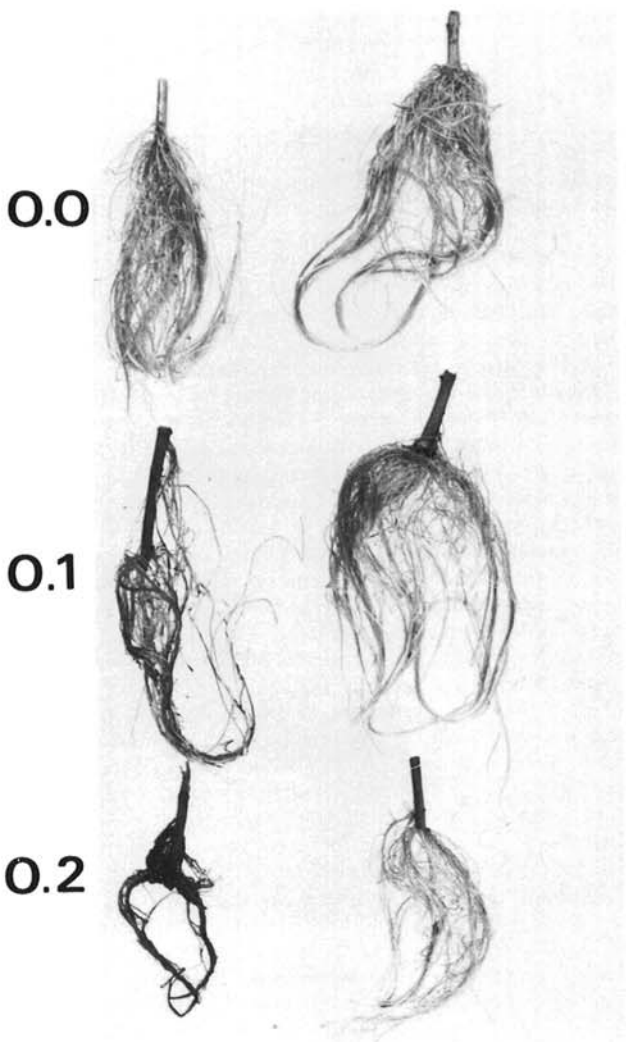

Fig. 1. Chrysanthemum roots grown in half-strength Hoagland's solution and exposed for 24 hr to (from top to bottom) no salt; nutrient solution amended with 0.1 M NaCl and 0.005 M $CaCl_2$; and nutrient solution amended with 0.2 M NaCl and 0.01 M $CaCl_2$. Plants on the left were inoculated with 4×10^6 zoospores of *Phytophthora cryptogea* 4 hr before application of the stress treatments, while those on the right were not inoculated.

Delayed application of stress. To determine if there was a critical time interval within which plants had to be exposed to stress to result in a significant increase in disease, additional experiments were done by using a modification of the postinoculation stress technique. In these experiments, plants were exposed to zoospore inoculum for 4 hr, as described previously, after which the infested nutrient solution was discarded and plants were returned to fresh solution. Separate groups of inoculated plants then were exposed to salinity stress (0.2 M NaCl with 0.01 M $CaCl_2$) for 24 hr, either 4 hr after the inoculation treatment (ie, immediately after the solution was changed), or at intervals of 12, 24, or 48 hr after inoculation. One group of inoculated plants was held in nonsalinized nutrient solution to serve as unstressed controls, while another group was exposed to the salinity treatment only, without inoculation.

All plants were observed for symptoms of root rot for 7 days following exposure to the salinity treatments, and then were harvested. At harvest, 100 root segments were collected randomly from among the plants comprising each treatment group, and cultured on modified PV medium (4). Individual root systems then were dried at 95 C and weighed. The complete experiment was repeated twice.

RESULTS

Postinoculation stress. Microscopic examination of root segments collected after the 4-hr inoculation treatments consistently showed that there were no significant differences in the number of zoospore cysts initially attached to roots of plants in different treatment groups. Although it was determined that roots had equivalent amounts of inoculum attached to them, disease severity was greatly increased by application of the salinity treatments. Lesions developed and spread rapidly on roots exposed to salinity stress, so that large portions of the root system were rotted at harvest (Fig. 1). Root necrosis was least severe in unstressed plants, most severe in plants exposed to the highest level of stress, and intermediate in plants exposed to the intermediate level of stress (Fig. 1). The severity of disease at the high level of stress was further evidenced in a comparison of the dry weights of harvested root systems (Fig. 2). As observed previously (8), uninoculated plants exposed to the high salinity treatments were slightly stunted, but otherwise did not appear to have been seriously damaged.

Colonization of excised roots. When excised roots were inoculated in petri dish humidity chambers, roots exposed to

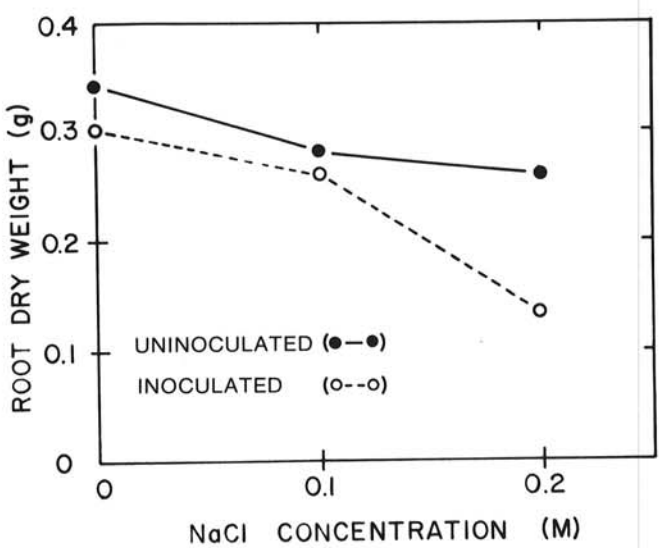

Fig. 2. Dry weights of chrysanthemum roots 10 days after a 24-hr exposure to half-strength Hoagland's solution containing no salt; 0.1 M NaCl and 0.005 M $CaCl_2$; or 0.2 M NaCl and 0.01 M $CaCl_2$. Inoculated plants were exposed to 4×10^6 zoospores of *Phytophthora cryptogea* 4 hr before application of the stress treatments (LSD = 0.1034 at $P = 0.05$).

salinity stress were far more susceptible to infection and colonization than unstressed roots. In unstressed roots, the frequency of recovery of *P. cryptogea* was relatively low in tissue segments 4 or 8 mm from the point of inoculation (Fig. 3), and at greater distances that pathogen was not detected at all. On the other hand, in roots exposed to salinity stress, *P. cryptogea* was recovered with high frequency from tissue segments near the point of inoculation, and with lesser frequency from segments up to 20 or 24 mm from the tip (Fig. 3).

Delayed application of stress. Severe root rot developed only on roots exposed to salinity stress within 12 hr of inoculation. This was evidenced by a significant decrease in root dry weight and an increase in the percentage of infected roots relative to unstressed controls (Fig. 4). On the other hand, stress applied 24 or 48 hr after inoculation did not significantly influence either measure of disease severity (Fig. 4).

DISCUSSION

In earlier experiments (8), salinity stress was shown to predispose chrysanthemums to severe root rot by *P. cryptogea*. In those experiments, roots were exposed to zoospore inoculum only after they had been subjected to various levels of salinity. One effect of salinity stress was to greatly increase the number of zoospore cysts that attached to roots. Although the mechanism underlying the increased attachment of cysts was not established, such an increase of inoculum at the root surface presumably could increase the number of successful infections within a stressed root system, and result in more severe disease. While the observed differences in inoculum attachment on roots are believed to be important to the overall phenomenon of predisposition, they made it difficult to determine whether the resulting extensive necrosis was influenced in any way by an increased susceptibility of the host. To address the question of increased susceptibility, experimental procedures were reversed from those previously reported (8), so that roots were exposed to salinity stress after pathogen attachment and initial penetration, rather than before. This technique eliminated any possibility of salinity-induced changes in inoculum attraction or attachment to roots. Even though microscopic examination confirmed that equivalent numbers of zoospores attached to roots following inoculation, moderate to severe decay developed only on root systems subsequently exposed to salinity stress. Roots maintained free of stress developed only mild symptoms of infection and necrosis (Figs. 1 and 2). In this respect, the results reported here resemble those previously reported (8).

While the postinoculation stress technique provided evidence that salinity stress increased the susceptibility of root tissue to severe disease, it did not totally eliminate the possibility of inoculum differences. Such differences could arise from secondary inoculum cycles within the crocks of nutrient solution. Although I have no direct evidence that secondary inoculum contributed significantly to the observed levels of disease, sporangia have been observed on infected roots and there is some evidence (T. J. Swiecki and J. D. MacDonald, *unpublished*) that they may form more quickly or in larger numbers on stressed roots. While this is believed to result from a more vigorous infection and colonization of stressed roots by the pathogen, it does raise the possibility that stressed roots were exposed to greater amounts of secondary inoculum than unstressed roots near the end of the experiments.

To eliminate the possibility of secondary infections, roots were excised from stressed and unstressed plants and placed in humidity chambers where their exposure to inoculum could be carefully controlled. In these experiments, only the tip and cut ends of each root were in contact with Plexiglas supports, while the rest of the root was suspended in water-saturated air between the supports. Once in place, each root was inoculated at the tip end with an equivalent number of zoospore cysts. Because the suspension technique essentially eliminated the possibility of secondary infection cycles, it was felt that the distance from the point of inoculation that the pathogen was recovered was a reflection of pathogen growth through the root tissue. The results (Fig. 3) clearly showed that the pathogen was better able to infect and colonize root segments that had been exposed to salinity. Further evidence of this ability is provided by histological studies of roots collected from intact plants at various times after inoculation (11), which showed that *P. cryptogea* successfully infected and colonized stressed chrysanthemum roots more rapidly and to a greater extent than unstressed roots.

The mechanism by which salinity stress increases susceptibility of roots to pathogen invasion is not understood, but may result from an impairment of normal host defense mechanisms. Plants respond to pathogenic invasion in numerous ways that function to block, slow, or prevent the pathogen from its successful establishment or spread in host tissue (1). Many of the defensive responses of plants involve biosynthesis of compounds that are

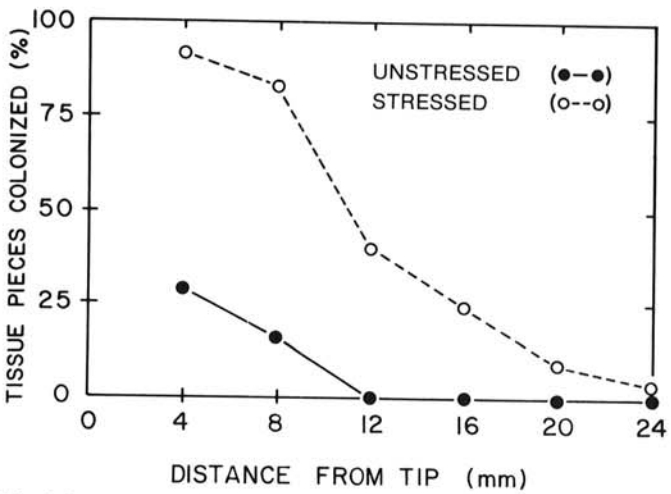

Fig. 3. Percentage of root pieces colonized by *Phytophthora cryptogea* 48 hr after inoculation. Twenty-five unstressed roots and 25 roots exposed for 24 hr to 0.2 M NaCl and 0.01 M $CaCl_2$ in half-strength Hoagland's solution were excised from hydroponically grown plants, placed in humidity chambers, and inoculated by pipetting ~800 zoospore cysts onto each root tip. After 48 hr, each root was cut into serial 4-mm segments and each segment cultured individually onto a selective medium. Each point represents the percentage of the total number of segments from which *P. cryptogea* was recovered.

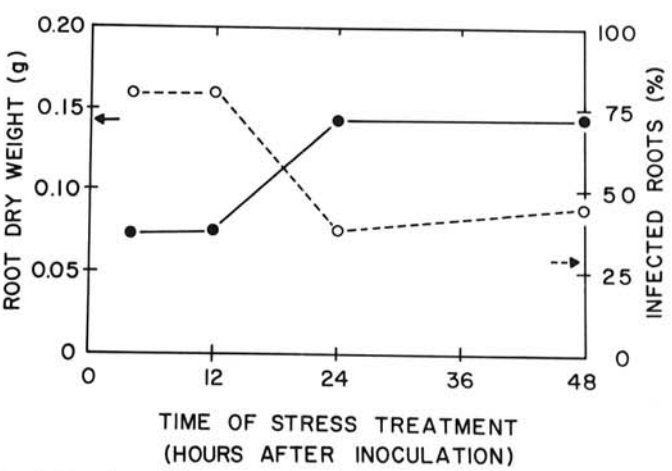

Fig. 4. Root dry weight and percentage of roots infected in hydroponically grown chrysanthemums inoculated with *Phytophthora cryptogea* and exposed to salinity stress at various times after inoculation. All plants were inoculated at time zero, and the root systems of subgroups were immersed in nutrient solution containing 0.2 M NaCl and 0.01 M $CaCl_2$ for 24 hr intervals commencing 4, 12, 24, or 48 hr after inoculation. Dry weights (solid line) and percentages of roots infected (broken line) were determined after 7 days and are plotted as a function of the time at which salinity treatments were initiated. Arrows indicate the dry weight and percent infection of unstressed plants (LSD for root dry weight = 0.0511 at $P = 0.05$).

toxic to the invading pathogen (1). One effect of physiological stress, which has been hypothesized following drought stress (10) and oxygen stress (J. M. Duniway, *unpublished*) is interference with the biosynthesis of compounds involved in normal resistance reactions. Salinity stress has many adverse effects on plants including changes in membrane permeability (3), the ultrastructure of organelles (9), and synthesis of DNA, RNA, and proteins (3). Chemical inhibitors of these processes have been reported to reduce plant resistance through inhibition of phytoalexin synthesis (1).

While there is no information to indicate how healthy chrysanthemum roots typically resist infection by *Phytophthora*, evidence suggests it may involve rapid biosynthesis of toxic compounds. This evidence comes, in part, from the delayed stress experiments which showed that exposure to stress within 12 hr of inoculation resulted in severe root rot, whereas stress episodes 24 or 48 hr after inoculation had no significant effect (Fig. 4). This strongly suggests that defensive mechanisms are activated in roots shortly after inoculation, and stress during that period may suppress the host response, allowing successful establishment of the pathogen. Stress after 24 hr did not significantly increase root rot, presumably because the tissue had already reacted to prevent pathogen establishment. This hypothesis is further supported by histological studies (11) showing a necrotic reaction in infected epidermal cells of unstressed roots within 24 hr of inoculation. This reaction was much less evident in stressed roots.

Under field conditions, plants grown in saline soil would be exposed to stress continually. While the degree of plant stress would probably fluctuate in intensity between irrigation cycles, it would not typically follow the single-exposure pattern employed in these experiments. Although single-exposure episodes of stress are outside the norm of what plants would encounter in the field, use of this technique has provided evidence that salinity stress can increase the susceptibility of chrysanthemum roots to pathogenic invasion. This effect presumably would occur in stressed roots concomitantly with the increased attachment of zoospore cysts described previously (8). Although the mechanisms underlying these two effects have yet to be identified, there is a clear hazard that plants grown in saline conditions can be predisposed to severe root infection by *Phytophthora* sp. and perhaps other pathogens as well.

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