

Effects of salinity stress on the development of *Phytophthora* root rots

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Root rots caused by species of *Phytophthora* are economically important diseases affecting a wide range of agricultural and ornamental crops. Although diseases caused by these fungi can sometimes be suppressed by fungicide treatments or use of resistant cultivars, in many situations they cannot be effectively controlled. These difficulties have prompted considerable research into conditions favoring the survival, reproduction, and spread of *Phytophthora* spp. in soil and their infection of plant roots.

In recent years, researchers have found that exposure of plants to environmental stress can significantly increase the occurrence or severity of *Phytophthora* root rots. Stresses to plant roots that are important under California conditions, and that have been found to increase susceptibility to disease, include those resulting from cycles of drought, heat, oxygen deficiency, and salinity. In some cases, stress has been shown to break down genetic resistance in plants, negating years of breeding effort. Although the mechanisms by which stress increases disease severity are not yet known, recent work with salinity stress has provided some information.

In these experiments, we exposed hydroponically grown chrysanthemums to salinity stress by adding solutions of sodium chloride or sodium chloride and calcium chloride to the nutrient solutions in which the plants were growing. The solutions were adjusted to electrical conductivities of approximately 10 or 20 dS/m (about 6,400 or 12,800 mg/L). After 24 hours, we returned the plants to nonsaline nutrient solution and inoculated both stressed and nonstressed plants by introducing zoospores of *Phytophthora cryptogea* into the containers of fresh solution.

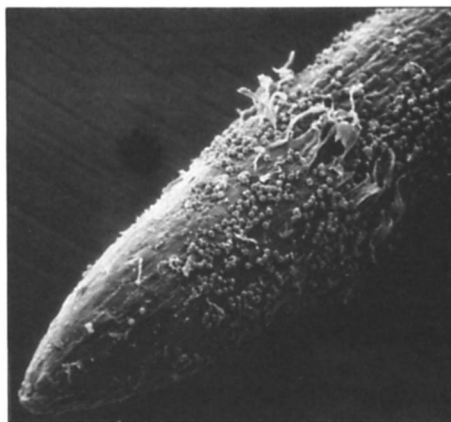
Although the salinity treatments themselves caused no significant damage to the roots, severe root rot developed in stressed roots within seven days of inoculation. Inoculation of nonstressed roots resulted in very little disease. Microscopic examination of roots revealed

that massive numbers of zoospores had become attached to the surface of salt-stressed roots, while relatively few were attached to nonstressed roots. Zoospores are attracted to sugars and amino acids that leak from roots, and it is believed that salinity-induced changes in root cell membranes increased the leakage of these substances, stimulating zoospore attraction and attachment to the roots.

Attraction of zoospores is not the only factor that can increase disease severity. Plants generally resist pathogen attack by a variety of methods, many of which involve active metabolic processes. One important effect of stress could be the suppression of these defense mechanisms, and recent experiments have provided evidence that this does occur in some cases. When hydroponically grown chrysanthemums were inoculat-

Although salt itself caused no significant damage, it lowered chrysanthemum roots' disease resistance. Roots grown in nutrient solution and exposed to (photo at right, top to bottom): no salt; 6,400 mg/L salt; and 12,800 mg/L. Roots on left were all inoculated with *Phytophthora cryptogea* zoospores before exposure to stress; roots at right were not inoculated.

Microscopic examination revealed numerous zoospores attached to salt-stressed root (photo below), but relatively few to nonstressed roots.



ed so that uniform numbers of zoospores were attached to roots, plants subsequently exposed to salinity stress still developed the most severe symptoms of root rot. Microscopic examinations revealed that *P. cryptogea* invaded and colonized stressed roots much more rapidly and to a greater extent than nonstressed roots. Resistance reactions, which were evident in the nonstressed roots, were greatly reduced in those exposed to salinity stress.

Although much of this research was done on hydroponically grown chrysanthemums, more recent work has used tomato and citrus in both greenhouse and field experiments. Tomato plants were grown to a prebloom stage in pots of soil and irrigated during the final five to seven days with solutions of sodium chloride and calcium chloride to gradually raise the electrical conductivity of the soil solution to 8 dS/m (about 5,100 mg/L). Plants stressed in this manner developed severe root and crown rot, and many died after inoculation with *P. parasitica*, whereas nonstressed plants developed only moderate root rot and no crown rot, and very few died. When stress was delayed until after bloom set, a similar increase in disease severity occurred, but only at much higher salinity levels (about 10,200 to 12,800 mg/L, or 16 to 20 dS/m). Although these experiments used greenhouse- and laboratory-



grown plants, we obtained similar results in a small field experiment at Davis.

In a recent field study with citrus trees on Troyer citrange rootstock in saline soils of the Coachella Valley, we found a strong positive correlation between the severity of *Phytophthora* root rot and the level of salinity. Although greenhouse studies indicated that salinity stress could alter rootstock susceptibility to *Phytophthora*, they also revealed that root growth nearly ceased under chronic salinity stress. Thus, rootstocks exposed for long periods to salinity levels of 3 to 4 dS/m (1,900 to 2,500 mg/L) were unable to replace roots decayed by *Phytophthora*.

While all the *Phytophthora* isolates we have worked with so far are fairly tolerant of salinity, an isolate of *P. parasitica* that we recovered from citrus soils of the Coachella Valley had the greatest tolerance. It survived and reproduced in soil at salinity levels equal to or greater than that of full-strength sea water. Thus, we expect that many *Phytophthora* spp. remain active in soils at salin-

ity levels that would severely stress most crop plants. This clearly is the case in the Coachella Valley and probably accounts for the severity of *Phytophthora* root rot in some citrus groves. These relationships between salinity and root diseases may occur in other crops elsewhere, but they may be overlooked, or the symptoms of chlorosis, wilt, and death of plants simply confused with direct salt injury.

It is still unknown precisely how environmental stresses and root pathogens interact in disease. However, the knowledge that these interactions occur, and information on the levels of stress that can trigger changes in plant susceptibility, should help researchers and growers devise cultural practices to minimize losses, and could help breeders detect and deploy genes providing more stable disease resistance.

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action of the membrane molecules with water. (The term "imbibition" is applied to the rapid uptake of water by dry seeds when they are placed in a moist environment.) Some membrane changes probably occur during imbibition, as the amount of water around the cell membranes increases. However, the exact nature of these changes is not certain, since small differences in membrane molecular composition profoundly affect the membrane's response to changes in hydration. The presence of salt during imbibition is quite likely to influence these changes in membrane structure.

In our studies, we have used the electron microscope to examine changes in the seed cell membranes during the early phases of germination. The technique of freeze-fracture electron microscopy was used, because it is especially suitable for study of membrane structure. In this method, frozen tissue is broken open in a vacuum chamber, and an extremely thin layer of platinum and carbon is deposited on the broken surface. The tissue is then dissolved away, leaving the thin platinum-carbon surface replica, which is examined in the microscope. Since the fracturing process frequently breaks open the frozen membranes, the platinum-carbon replica shows the interior structure of these membranes (fig. 1).

Small particles are usually visible on the fractured surface. They are membrane protein molecules that were buried in the membrane interior before it was broken open. The number of these protein particles per unit area is characteristic of the kind of membrane being examined. Changes in either the density or size of these particles reflect developmental changes occurring in the membrane, which may be the result of normal physiological processes or of environmental influences.

We have used this technique to study membranes of primary root (radicle) cells in cowpeas germinated in both water and salt solutions. The membrane appears normal in micrographs of dry seeds, and there is no evidence that it is modified (fig. 2). However, the number of protein particles is unusually high, and some of them appear to be clumped together as multiparticle aggregates.

When seeds are imbibed in water, the appearance of the membrane changes somewhat. In seeds that were imbibed in water for 17 hours, the number of particles per unit area of membrane declined to about half the density in dry membranes, apparently because of membrane expansion during imbibition (fig. 1). In other words, the total number of parti-

Effects of salt on cell membranes of germinating seeds

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Salt stress inhibits growth throughout the plant life cycle, but seed germination is generally the most sensitive stage. Salt stress mimics water stress in many ways, leading to the suggestion that the principal effect of salt is osmotic. However, since some salts are more inhibitory than others, specific toxic effects must also be involved. Although sodium chloride is among the less toxic salts, it is one of the most common and thus one of the most troublesome to agriculture.

The mechanisms by which salt inhibits seed germination are not known, but there is good reason to suspect that cell membranes are the sites for primary or secondary salt effects. Sodium chloride interferes with a wide variety of membrane functions, including permeability, transport of both organic and

inorganic solutes, and secretion. Salt sometimes causes structural alterations. Salt stress induces changes in membrane lipid composition in some plants and also causes release of membrane proteins in root cells. These structural modifications may be especially important, since many salt-tolerant species achieve their resistance by isolating salts from sensitive cellular processes through membrane compartmentation.

The interaction of salts with cell membranes during germination is complicated by the dramatic changes occurring in the seed during this time. The transition from a dry to hydrated state as the seed absorbs water during imbibition has a potentially profound effect on seed membranes, since membrane structure depends heavily on the inter-