Rhizopus Soft Rot of *Euphorbia trigona*

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ABSTRACT

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Rhizopus stolonifer was consistently isolated from a rapidly advancing soft rot of *Euphorbia trigona*. Symptoms were reproduced on healthy *E. trigona* by inoculating propagation wounds with sporangiospores of *R. stolonifer*. Plants wounded and immediately inoculated developed symptoms within 24 hr at 16, 21, and 27 C, but no symptoms developed at 32 C. Resistance to infection at 32 C could not be directly related to the rate of wound tissue formation by the host or to adverse effects of temperature on spore germination or mycelial growth of the fungus. Nine other species of *Euphorbia* were tested for susceptibility to Rhizopus soft rot. Dicloran (Botran 75W) applied to *E. trigona* propagation wounds before inoculation completely controlled Rhizopus soft rot.

In the spring of 1978, a soft rot of propagated cuttings and mother plants of *Euphorbia trigona* became a serious problem in some California nurseries. *E. trigona* is propagated by rooting terminal shoot cuttings taken from the fleshy stem.

On both mother plants and cuttings, symptoms were always associated with wounds created during propagation. Diseased cuttings failed to root, and infected mother plants could become completely rotted in 7 days.

Laboratory and greenhouse experiments were undertaken to 1) identify the causal organism, 2) determine the environmental factors that influence infection and pathogenesis, 3) evaluate the effectiveness of cultural and chemical controls, and 4) determine the susceptibility of other *Euphorbia* species to soft rot.

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MATERIALS AND METHODS

Isolations were made from diseased *E. trigona* by placing small pieces of tissue removed from the edge of soft rot lesions onto potato-dextrose agar (PDA). Plates were incubated at 24 C and examined for growth every 24 hr. Pure cultures of fungi recovered from these isolations were stored on PDA slants at 4 C pending pathogenicity testing.

For pathogenicity tests and inoculation studies, *E. trigona* plants were wounded by making a cross-sectional slice through the fleshy stem, simulating commercial propagation methods. Spore suspensions (0.5 ml per plant) containing approximately 5×10^3 spores per milliliter were placed on the cut surfaces. Plants were incubated in a greenhouse at 27 C.

This inoculation procedure was modified once *R. stolonifer* had been identified as the causal organism. *R. stolonifer* isolates were grown on PDA at 24 C for 48 hr before inoculation, and 5–10 sporangia were placed on the cut stems of plants.

To determine the effect of temperature on symptom development, *E. trigona*

plants grown in constant temperature chambers at 16, 21, 27, and 32 C were wounded and inoculated with *R. stolonifer* as described above. Symptom development was monitored daily for 7 days by measuring the advance of the soft rot lesion down the stem from the inoculation point.

To study the effect of temperature on diseased plants, we inoculated eight *E. trigona* plants at 27 C and allowed the soft rot to progress for 3 days. Half of the plants were then moved to a 32 C chamber, the other half remaining at 27 C. Symptom development on the two sets of plants was compared 4 days after the plants were separated (7 days after inoculation).

We also studied the influence of temperature on the rate of callus accumulation at wound sites on *E. trigona*. Plants were grown at 21, 27, and 32 C for 7 days to allow them to adapt physiologically to these temperatures. Three plants at each temperature were wounded as in the inoculation procedure. At 3-hr intervals, free-hand tangential sections were taken through the surface of wounds, immersed in aniline blue (2), and examined under a compound microscope for the blue stain indicating callus accumulation.

To evaluate temperature effects on mycelial growth of *R. stolonifer*, we removed mycelial disks 13 mm in diameter from PDA cultures of the fungus before sporulation. Disks were transferred to fresh PDA and exposed, at three-degree intervals, to temperatures from 21 to 39 C. The diameter of colony growth from the disks was measured after 24 and 48 hr. Sporangiospore suspensions from 48-hr PDA cultures of *R. stolonifer* seeded onto fresh PDA were exposed to the same range of temperatures to evaluate the influence of temperature on spore germination. Plates were examined for growth after 24, 48, and 72 hr.

A total of 10 Euphorbia species (*E. grandicornis*, *E. ingens*, *E. lactea*, *E. lathyrus*, *E. leuconeura*, *E. mammillaris*, *E. nivula*, *E. splendens*, *E. tirucalli*, and *E. trigona*) were tested for susceptibility to Rhizopus soft rot. Plants were grown in a greenhouse at 27 C before and after inoculation. Three plants of each species were each wounded at three sites, inoculated with *R. stolonifer*, and examined for symptom development 1 wk later.

Six fungicides (Table 1) were tested for their ability to control Rhizopus soft rot on *E. trigona*. The cut surfaces of wounded plants were sprayed to runoff with the test fungicides, allowed to dry for 5–10 min, and then inoculated with 5–10 sporangia. Plants wounded and sprayed with water before inoculation served as controls. For each treatment, two stems on each of four plants were wounded, sprayed, and inoculated. Inoculated plants were held in a greenhouse at 27 C for 7 days.

Table 1. Effectiveness of fungicides for controlling *Rhizopus stolonifer* rot of *Euphorbia trigona*

Fungicide[a]	Rate (g a.i./L)	Percentage of plants infected[b]
Dicloran (Botran 75W)	1.2	0
Captan (Orthocide 50W)	1.2	25
Chlorothalonil (Daconil 2787 75W)	1.4	75
Benomyl (Benlate 50W)	0.3	100
Mancozeb (Fore 80W)	1.4	100
Tribasic copper sulfate (53W)	2.5	100
Untreated		100

[a] Fungicides were applied with an air-pressurized garden sprayer.
[b] Percentage values based on eight stems on four plants inoculated and grown at 24 C.

RESULTS

R. stolonifer was consistently isolated from *E. trigona* with soft rot symptoms. When inoculated and grown at 16, 21, or 27 C, *E. trigona* developed soft rot symptoms within 24 hr. Lesions enlarged at similar rates (an average 1.7 cm/day) at these three temperatures. Soft rot symptoms did not appear in plants inoculated at 32 C. Plants held at 27 C were susceptible to infection for up to 4 hr after being wounded.

When diseased plants were moved from 27 to 32 C, soft rot symptoms did not progress beyond the area already infected. When these plants were returned to 27 C, symptom development did not resume, even though the pathogen could be recovered from infected plant parts. Soft rot lesions on plants left at 27 C continued to enlarge at a rate of 1.6 cm/day.

No differences were observed in the rate of callus accumulation at 21, 27, and 32 C. Sections taken through the wound surface after 9 hr showed accumulation of callus three to four cell layers deep below the wound at all three temperatures.

After 24 and 48 hr, the increase in colony diameter of *R. stolonifer* was greatest when mycelial disks were incubated at 27 C. Colony diameter did not increase at 36 or 39 C. Ninety to 95% of sporangiospores germinated within 3 hr on PDA at temperatures from 24 to 33 C. Spores incubated at 36 C germinated but produced limited hyphae that grew a few millimeters in 24 hr. Spores exposed to 36 C for 24 hr resumed normal growth when the incubation temperature was lowered to 27 C. Spores failed to germinate after 24 hr at 39 C and would not germinate when the incubation temperature was lowered to 27 C.

E. trigona, *E. lactea*, *E. mammillaris*, *E. ingens*, *E. lathyrus*, and *E. leuconeura* developed soft rot after being wounded and inoculated with *R. stolonifer*; *E. nivula*, *E. tirucalli*, *E. splendens*, and *E. grandicornis* did not. The susceptibility of *E. frankiana* was difficult to evaluate because wounded, uninoculated stems collapsed as rapidly as inoculated stems, even though *R. stolonifer* could not be recovered from withered, uninoculated plants.

Of the fungicides tested (Table 1), only dicloran (Botran 75W) gave complete control of Rhizopus soft rot. Captan (Orthocide 50W) controlled soft rot in six of the eight inoculated *E. trigona* stems, and chlorothalonil (Daconil 2787 75W) controlled soft rot on two of the eight inoculated stems. Benomyl (Benlate 50W), tribasic copper sulfate (53W), and mancozeb (Fore 80W) failed to control the disease.

DISCUSSION

R. stolonifer was identified as the organism causing soft rot of propagated cuttings of *E. trigona*. The fact that soft rot symptoms did not develop when *E. trigona* was wounded and inoculated at 32 C suggests that high temperature either adversely affected the fungus or increased the plant's capacity to resist infection. Neither hypothesis could be clearly proven.

Sporangiospores germinated and mycelial colonies grew in vitro on PDA at temperatures above 33 C. Although these data generally agree with Weimer and Harter (6) and with the maximum temperature for spore germination calculated by Halisky et al (1) (max 33 C), they were higher than those reported for mycelial growth (max 30.7 C). Our data also concur with reports by Miller et al (3) that exposing sporangiospores to temperatures above 35 C adversely affected later germination and growth of these spores at 24 C.

Srivastava and Walker (4) showed that in vitro growth of *R. stolonifer* was not correlated with the development of the fungus on sweet potato. The optimal temperature for disease development on sweet potato was 20 C, while optimal growth on PDA occurred at 28 C. The discrepancy was explained by the fact that pectic enzyme production by the fungus on sweet potato (optimum 20 C) was not correlated with growth of *R. stolonifer* on PDA. The repression or inactivation of pectolytic enzymes at 32 C would explain cessation of soft rot symptoms when infected *E. trigona* were moved from 27 to 32 C.

Increasing temperatures did not appear to accelerate wound tissue formation in *E. trigona*, as measured by staining for callus accumulation. Nine hours after wounding, no great differences were observed in callus accumulation among plants grown at 24, 27, and 32 C. Weimer and Harter (5) reported that under laboratory conditions, cork formation was only partially effective in preventing *E. stolonifer* infections on sweet potatoes. They were unable to demonstrate cork formation on wounded

potatoes under storage conditions but reported that the formation of a hardened latex crust did prevent infections in storage. *E. trigona* produced copious latex when wounded, and in our studies, the latex dried and hardened more rapidly at 32 C than at lower temperatures.

Similarly, the six *Euphorbia* species susceptible to soft rot have wide, fleshy stems that remained moist with latex for several hours after wounding when grown at 27 C. The resistant species have narrow, woody stems and produced little latex when wounded; the cut surfaces dried rapidly. This hardening of plant latex probably contributes to the resistance of these species and to the resistance observed in *E. trigona* grown at 32 C.

Rhizopus soft rot of *E. trigona* was controlled by treating the cut surface of stems with dicloran or captan. These experiments suggest that cultural practices for controlling soft rot should include maintaining mother plants continuously at 32 C and holding freshly cut propagation pieces at 32 C for 24 hr before planting.

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