Effect of Flooding and Temperature on Incidence and Severity of Safflower Seedling Rust and Viability of Puccinia carthami Teliospores

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ABSTRACT

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Soil infested with *Puccinia carthami* was flooded under different time-temperature regimes in controlled-environment chambers. The incidence and severity of rust on safflower seedlings decreased in soils following flooding under increasing temperature and time. Rust was completely controlled in soil flooded for 4 and 7 days at temperatures maintained at a constant 36 or 39 C. Flooding at day-night temperature regimes varying from 36 to 26 C and 39 to 29 C for 4 and 7 days controlled rust in all but the 36-26 C, 4-day regime. Incidence and severity of rust were markedly reduced after soil was flooded for 7 days at 30 and 33 C and progressively decreased with prolonged flooding at 12, 18, and 24 C. Disease incidence in the field was significantly lower with flooding than without. Viability of teliospores was reduced when they were submersed in water or dispersed on agar at 18 to 39 C for 2-14 days. Spores were not viable after a minimum treatment of 4 days in water or on agar at 36 and 39 C. Loss of viability was slower as exposure temperature decreased. When spore suspensions in water at 30 and 33 C for 4 days were aerated, a small increase in germination occurred in subsequent assays.

Rust caused by *Puccinia carthami* Cda., is an important disease of safflower (*Carthamus tinctorius* L.) in the western United States. Seedling rust, initiated by sporidia from germinating soil- or seedborne teliospores, reduces stand (7) and may preclude monoculture of safflower. Seed treatment controls seedborne rust spores (6) but does not protect seedlings from infection by soilborne spores. Growers experienced in monoculture of safflower employ postharvest flooding of safflower fields to control soilborne rust spores. Although temperature and flooding duration required to control safflower rust have not been established, an arbitrary postharvest flood period of 7 days, during which maximum daytime air temperature is 39 C or above, gives control in the San Joaquin Valley. It is assumed that inoculum would not be reduced by germination of teliospores during flooding if soil and water temperature was unfavorably high for germination (2). Presumably control of rust results from unfavorable effects of flooding and temperature on the viability of the soilborne spores.

This study evaluated different soil flooding time and temperature regimes in controlled-environment chambers for efficacy in rust control. Control also was attempted by flooding naturally infested soil in the field. The effect of moisture and temperature on the viability of teliospores was studied in the laboratory.

MATERIALS AND METHODS

Controlled-environment chamber and greenhouse tests.—Soil was artificially infested with teliospores on safflower leaves collected in the field. The leaves were crushed and mixed with air-dried, steamed loam soil at a ratio of 1:227 (w/w) for each experiment. A layer of infested soil 3.5 cm deep was placed on air-dried noninfested steamed soil 5 cm deep in each 1-liter glazed crock. For flood treatments the soil in crocks was saturated with water, allowed to drain, and flooded after crock drain holes were plugged. A 4-cm water level was maintained above the soil surface. When soil was saturated and drained but not flooded, plastic bags were loosely fitted over the crocks to maintain the soil in a wet state. Dry soil contained in similar crocks served as a control. Crocks were placed in controlled-environment chambers set for a 14-hr day length. Temperatures were set at 6-degree intervals from 18 to 30 C and at 3-degree intervals from 33 to 39 C and were constant ±1 C, except as otherwise indicated. The treatments, ranging in length from 2 to 28 days, were arranged to end on the same day. Flooded soil was drained and all crocks were placed in the greenhouse at an ambient temperature of 21 to 24 C and allowed to dry for 14 days.

Seeds of cultivar Nebraska 10 were planted (10/pot) 3 cm deep in both treated and control crocks and water was added to saturate the soils. They were placed in controlled-environment chambers at 21 C to slow seedling emergence and enhance teliospore germination. The crocks were returned to the greenhouse 1 wk after the seedlings emerged. The plants were observed for the development of rust pustules on hypocotyls and cotyledons. Disease severity was rated on a scale of 0 to 3 as the plants were removed from soil 2-3 wk after seedling emergence.

Field flood test.—Soil infested with *P. carthami* was

flooded after harvest in 1974 in a field at Davis. An area about 30m × 6m was divided into four plots each enclosed with soil levees 50 cm high. Two of these were flooded and the water maintained 10 cm deep for 7 days from 5 to 11 September. Two plots were not flooded. Air temperatures and soil temperatures of flooded and nonflooded soil were recorded daily. Surface water was drained from flooded plots at the end of the seventh day. Levees were opened at one side to avoid ponding during subsequent seasonal rains. The soil was tilled on 1 March 1975, and Nebraska-10 safflower seed was planted in four 5.5-m-long rows in each plot. The plants were observed for rust symptoms after emergence and were harvested and examined for rust 2 mo after planting.

Laboratory tests.—The effects of submersion in water, temperature, and duration of the various treatments on subsequent germination of spores were studied. A mixture of rust spores and small fragments of safflower leaves was suspended in sterile distilled water (50 mg/80 ml) in 100-ml sterile glass beakers. Ten μliters of Tween-20 (polyoxyethylene sorbitan monolaurate) wetting agent were added to each beaker. After the spores had settled out of suspension, leaf residue was decanted and the water volume adjusted to 80 ml. Two beakers containing submersed spores for each time-temperature treatment were stored for 2-14 days at 6-degree intervals from 18 to 30 C and 3-degree intervals from 33 to 39 C. After the treatments were completed the water was decanted to about 10 ml. The concentrated spore suspension was pipetted to a 150-ml sterile beaker and washed three times by adding and decanting sterile distilled water. Five ml of a final 80-ml suspension was pipetted to each of two dishes of water agar. The water was decanted after 3-5 min. The dishes with spores were incubated at 24 C for 7 days above safflower seedlings as described (3) to stimulate germination. The water was decanted from the remaining spore suspension. The spores were dried for 12 hr at 30 C and then were dispersed on agar and incubated for germination as above. Germination was recorded after 7 days for 200 spores scored in each of two dishes per treatment. Nontreated spores served as germination controls. Final percentage germination was adjusted to account for spores that had germinated (up to 18% at 18 and 24 C) while submersed in water.

Teliospores collected from rusted leaves were washed and dried as described (3). The spores were dispersed on water agar and in empty dishes and given the same time-temperature treatments as the submersed spores above. Nontreated spores served as germination controls. After treatment, the spores were incubated over safflower

TABLE 1. Incidence and severity of rust *Puccinia carthami* on safflower seedlings, cultivar Nebraska 10, after exposure of artificially infested soil to different moisture-temperature regimes for 7 days[a]

Temperature (± 1 C)	Percentage rust and rust rating per soil treatment					
	Flooded		Wet[b]		Nonflooded (dry)	
	(%)[c]	rating[d]	(%)	rating	(%)	rating
18	100	3	100	3	100	3
24	100	2	100	3	100	3
30	15	1	100	3	100	3
33	10	1	100	3	100	3
36	0	0	15	1	100	3
39	0	0	0	0	100	3

[a]Safflower seeds were planted in soil 14 days after treatment was terminated.
[b]Soil was saturated and drained at start of experiment. Water was not added during experiment.
[c]Mean percentage of rusted seedlings of 40 seedlings in two experiments with two replications per experiment.
[d]Disease severity rating: 0 = no rust pustules; 1 = one to four pustules on cotyledons and/or hypocotyl; 2 = five to ten pustules on cotyledons and hypocotyl, with moderate twisting and elongation of hypocotyl; 3 = more than ten pustules on cotyledons and hypocotyl, with severe twisting and elongation of hypocotyl.

TABLE 2. Incidence and severity of rust *Puccinia carthami* on safflower seedlings, cultivar Nebraska 10, after flood treatments of artificially infested soil at different time-temperature regimes[a]

Duration of flood (days)	Percentage rust and rust rating per flood temperature (± C)					
	12 C		18 C		24 C	
	(%)[c]	rating[d]	(%)	rating	(%)	rating
0[b]	100	3	100	3	100	3
7	100	3	100	3	100	2
14	70	2	22	1	16	1
21	46	2	8	1	0	0
28	28	1	6	1	0	0

[a]Safflower seeds were planted in soil 14 days after treatment was terminated.
[b]Artificially infested nonflooded dry soil control.
[c]Mean percentage of rusted seedlings of 40 seedlings in two experiments with two replications per experiment.
[d]Disease severity rating: 0 = no rust pustules; 1 = one to four pustules on cotyledons and on hypocotyl; 2 = five to ten pustules on cotyledons and hypocotyl, with moderate twisting and elongation of hypocotyl; 3 = more than ten pustules on cotyledons and hypocotyl, with severe twisting and elongation of hypocotyl.

seedlings at 24 C. After 7 days, 200 spores were scored in each of two dishes per treatment. Final germination percentage was adjusted to account for spores that had germinated (up to 18% at 18 and 24 C) while exposed to time-temperature treatments on agar.

RESULTS

Environmental chamber and greenhouse tests.—Rust infection of the seedlings in flooded and nonflooded soils was evident by the development of light-green to cream-colored spermagonia on hypocotyls and cotyledons 4-7 days after seedling emergence. Rust pustules developed when seedlings were 10-14 days old. Elongation and twisting of hypocotyls was more pronounced with increased rust severity.

Rust was controlled in soil flooded for 7 days in environmental chambers when the soil temperature during flooding was 36 or 39 C (Table 1). Rust incidence and severity were markedly reduced but not controlled completely by flooding at 30 and 33 C. Control of rust in soil flooded for 7 days at 36 and 39 C prompted an evaluation of 2- and 4-day flood periods at the two temperatures. Control of rust was complete in soil flooded for 4 days but in soil flooded for only 2 days at 36 and 39 C the incidence of rust was 40 and 45%, respectively, compared to 100% in the controls.

In a 4-day flood treatment during which day temperatures were 36 or 39 C and night temperatures were 26 or 29 C, respectively, rust was controlled only in soil flooded in the 39/29 C day/night regime. Incidence of rust was 100% in nonflooded soil that was exposed to the same temperature regimes.

The incidence of rust on seedlings after soil was flooded for 7 days at 18 and 24 C was comparable to that in nonflooded soil (Table 1). However, in soils flooded for 14 to 28 days at 12, 18 and 24 C the incidence and severity of seedling rust decreased progressively with increased time and temperature (Table 2).

Field flood test for rust control.—Average high and low extremes of air temperatures during a 7-day flood period were 36 C (range 35-38 C) and 14 C (range 10-21 C). The soil temperature extremes at a 10-cm depth in flooded soil were 26 C (range 22-30 C) and 17 C (range 14-19 C). Temperature extremes in nonflooded soil were 35 C (range 33-36 C) and 26 C (range 26-28 C). Seedling emergence was completed 10 days after planting. A total of 1,190 plants from flooded soil and 1,248 plants from nonflooded soil were examined for rust. Rust pustules present were primarily on the hypocotyls at or slightly above the soil line. Although rust was not completely eliminated by flooding, the lower incidence in flood-treated soil (3.3%) than nonflooded soil (9.8%) was significant.

Laboratory studies on teliospore viability.—The percentage of germinated teliospores decreased in assays following submersion in water under increasing time-temperature regimes (Table 3). Germination percentage was the same whether or not the submerged spores were dried before they were assayed for germination. Spores were not viable after submersion for a minimum of 4 days at 36 and 39 C. When air was bubbled in water that contained submerged spores during 4-day treatments at 30 and 33 C, germination in subsequent assays increased 26 and 8%, respectively, compared to no germination after nonaerated treatments at the same temperatures.

Likewise, the percentage of germination decreased in assays after spores on agar were exposed to increasing time-temperature regimes (Table 4). Spores did not germinate after exposure on agar to 36 and 39 C for 4 days. Loss of viability of spores exposed on agar to 18 to 33 C for 7 days (Table 4) was slower in comparison to that of spores submerged in water under the same time-temperature regimes (Table 3).

TABLE 3. Percentage germination[a] of *Puccinia carthami* teliospores following submersion in water under different time-temperature regimes

Duration of submersion (days)	Germination of teliospores following submersion at temperatures of					
	18 C (%)	24 C (%)	30 C (%)	33 C (%)	36 C (%)	39 C (%)
0[b]	91	91	92	90	91	90
2	85	78	48	24	20	2
7	63	30	19	2	0	0
14	39	24	14	1	0	0

[a]Mean percentage of spores germinated after 7 days of incubation at 24 C in three experiments. Two hundred spores were scored in each of two replications per experiment.

[b]Spores exposed dry to the same temperatures as submersed spores.

TABLE 4. Percentage germination[a] of *Puccinia carthami* teliospores following exposure on agar to different time-temperature regimes

Duration of exposure (days)	Germination of teliospores following exposure to temperatures of:					
	18 C (%)	24 C (%)	30 C (%)	33 C (%)	36 C (%)	39 C (%)
2	89	89	87	69	35	1
7	88	79	59	18	0	0

[a]Mean percentage of spores germinated after 7 days of incubation at 24 C. Two hundred spores were scored in each of two replications per experiment. Germination in nontreated control was 89%.

DISCUSSION

These results support the observations that postharvest flooding at 39 C or above for 7 days is needed to control seedling rust of safflower in the field. The good control with only 4 days of flooding at 39 C suggests that rust perhaps could be controlled in the field by flooding for less than 7 days if temperatures are high enough. However, where temperature fluctuates, as in the air and flooded soils in the field test at Davis, longer flooding periods are needed for good control. Low minimum air temperatures and constant water flow that affected the temperature of flooded soil in the relatively small plots were believed to have been limiting factors for rust control. Seedling rust is not a problem in fields in the Sacramento Valley following natural flooding of the land in winter or early spring. Seasonal flooding, usually for several weeks at low temperatures, should reduce rust

inoculum in soils of flooded fields based on the decrease in rust incidence obtained from prolonged flooding at lower temperatures (Table 2), and the fact that as many as 18% of the teliospores germinated while submersed in water at such temperatures. However, where planting is delayed by spring flooding, rust may be limited in the field if soil temperature is unfavorably high (above 27 C) for teliospore germination during seedling emergence.

Control of safflower rust by flooding is related to the effects of flooding and temperature on subsequent germination of teliospores, notwithstanding the fact that other factors and sources of inhibition exist in the soil that may affect germination (4, 5). Effects of a soil environment on germination of teliospores were largely avoided as soil was not used in laboratory studies, yet viability of spores submersed in sterile water was affected. A higher percentage of spore germination after submersion treatments in water supplemented with aeration compared to germination after nonaerated treatments suggests an effect of oxygen on viability during submersion. However, spores on agar exposed to the same temperatures as spores submersed in water without aeration showed a similar loss of viability. It was noted, however, that spores on agar at 18 to 33 C lost viability more slowly than did spores submersed at the same temperatures.

High temperature and humidity are known to increase metabolism and thus affect the longevity of spores (1). Subsequent germination of teliospores of *P. carthami* that were dry when exposed to temperatures unfavorably high for germination (30 to 39 C), compared to that of wet spores exposed to the same temperatures indicates that the adverse effects on spore viability is due to interactions of moisture and temperature. Some other fungi lost viability completely when spores were exposed for long periods to temperatures above their maximum for germination (1).

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