Fungicide Control of Albinism in Citrus Seedlings Caused by *Alternaria tenuis*

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ABSTRACT

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Alternaria tenuis was isolated from albino but not from healthy citrus seedlings. Seed coats of ungerminated seeds from commercial seed lots that produced a large percentage of albino seedlings were also infected with *A. tenuis*. Surface-inoculation of freshly extracted, air-dried seeds with *Alternaria* isolates obtained from albino seedlings resulted in a high incidence of albinism after storage for 4 days at 4.5 C. Infected seeds stored for 64 days at 4.5 C showed extensive decay caused by *A. tenuis* and reduced germination. Application of the fungicide thiram, but not chlorothalonil, immediately after inoculation of seeds effectively controlled *A. tenuis* and prevented albinism.

Albinism of citrus seedlings can be a serious problem in Florida citrus nursery seedbeds. Diseased seedlings often die or become stunted, depending on the extent of chlorophyll deficiency. Incidence of albinism varies widely, but in 1981, as many as 50% of the seedlings in some commercial seedbeds were either albino or virescent seedlings. Albinism of citrus seedlings in California has been reported to be associated with seed infection by either *Aspergillus flavus* (3) or *Alternaria tenuis* (5). The disease has been controlled effectively in California when a fungicide seed protectant such as thiram or 8-hydroxyquinoline sulfate was applied to seeds after extraction (6,7). The purpose of this study was to identify the fungus causing albinism of citrus seedlings in Florida in 1981 and evaluate the fungicides thiram and chlorothalonil for control of the disease.

MATERIALS AND METHODS

Albino and healthy citrus seedlings (about 10 days old) were collected from a commercial seedbed. Tissue from seed coats, radicles, and cotyledons was removed, surfaced-sterilized in a 1% sodium hypochlorite solution for 1 min, rinsed, and placed on cornmeal agar (CMA) plates, which were incubated at 23 C and examined after 5–7 days for mycelial growth and sporulation. Ungerminated seeds were collected from 33 lots of cultivars sour orange (*Citrus aurantium*), Carrizo citrange (*Poncirus trifoliata* × *C. sinensis*), and Milam (unknown parentage). Seeds were surface-sterilized, rinsed, placed on water agar plates, and examined for *Alternaria* after 5 days.

Seeds of Carrizo citrange were removed from undecayed fruit by hand reaming, enzyme-treated with pectinase (1), rinsed, and air-dried. Seeds were divided into lots of 500 and placed in polyethylene bags. *Alternaria* inoculum was prepared by adding mycelia of *A. tenuis* to sterilized citrus seeds (moist), then incubating them in a flask at 23 C for 21 days, when all seeds were covered with nonsporulating mycelia. Fifty of these seeds were added to each lot of 500 seeds of the inoculated treatments. Treatments consisted of seeds uninoculated and inoculated with *A. tenuis* with and without thiram or chlorothalonil applied immediately after inoculation. Wettable powder formulations of the fungicides (1 g/100 g of seeds) were applied to completely cover the seeds.

Seed lots of each treatment were stored at 4.5 C for either 4, 64, or 124 days before planting in a sterilized peat-soil mix. Each treatment was replicated three times with 50 seeds each. Numbers of green and albino seedlings were recorded biweekly after seedling emergence. Both albino and healthy seedlings were examined for *Alternaria* by culturing seed parts on CMA.

RESULTS AND DISCUSSION

Seed coats, radicles, and cotyledons from albino seedlings collected from the commercial seedbed were infected with *A. tenuis*. *Alternaria* was not present on any seed parts from healthy seedlings. At least 50% of the ungerminated seeds from 10 of 33 seed lots were infected with *Alternaria* (Fig. 1). These same *Alternaria*-infected seed lots had previously produced a high incidence of albinism. *Aspergillus flavus* was not isolated from any albino or healthy seedlings.

Inoculations of seeds with the *Alternaria tenuis* isolates obtained from the seed coats of albino seedlings followed by incubation at 4.5 C for 4 days produced a high incidence of albinism (Table 1). After 64 days of storage at 4.5 C, germination of inoculated seeds was substantially reduced due primarily to decay caused by *A. tenuis*. The seed lot used for this experiment was apparently infected with *Alternaria*, as evidenced by 51.7% albinism in the uninoculated, nonfungicide treatment after 64 days of storage. Again, *A. tenuis* was isolated from the seed coats of albino seedlings but not from healthy seedlings. Albino seedlings were not produced by inoculated or uninoculated seeds treated with thiram. Said and Ryan (7) reported similar results for thiram. Chlorothalonil used for control of *Alternaria* species on other hosts appeared to give control on the seeds planted 4 days after inoculation but was ineffective on seeds stored for longer periods.

Infection of commercially harvested citrus seeds probably occurs during the extraction process. It is a common practice to chop the fruit into small pieces, mix with water, and let stand for several days to promote peel degradation (4). The degradation process is primarily

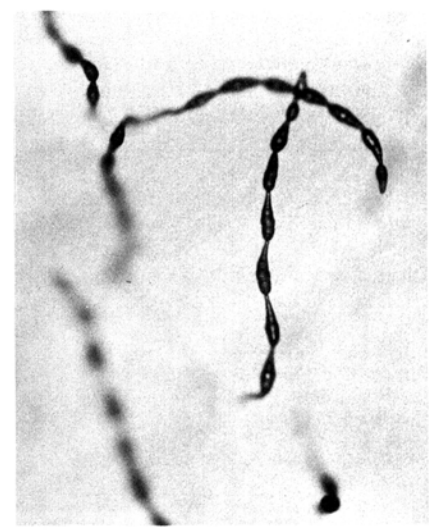

Fig. 1. Chains of spores produced by *Alternaria tenuis* on the surface of naturally infected sour orange (*Citrus aurantium*) seeds.

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Table 1. Incidence of albinism in Carrizo citrange (*Poncirus trifoliata* × *Citrus sinensis*) seedlings caused by *Alternaria tenuis* isolated from seed parts of albino citrus seedlings

		Days in storage[c]					
		4		64		124	
Fungicide[a]	Inoc.[b]	Germination (%)	Albinism (%)	Germination (%)	Albinism (%)	Germination (%)	Albinism (%)
None	No	72.6 ± 2.8[d]	1.9 ± 1.9	24.6 ± 3.3	51.7 ± 1.7	32.0 ± 4.7	58.9 ± 6.0
Thiram 70S	No	90.6 ± 1.8	0	70.0 ± 2.3	0	68.7 ± 7.7	0
Chlorothalonil 75W	No	97.3 ± 1.8	0.7 ± 0.7	39.3 ± 0.6	38.1 ± 7.2	15.3 ± 5.2	69.4 ± 2.8
None	Yes	92.0 ± 8.0	55.3 ± 15.3	2.0 ± 2.0	100.0	1.3 ± 1.3	100.0
Thiram 70S	Yes	94.0 ± 2.9	0	68.0 ± 6.1	0	72.0 ± 4.6	0
Chlorothalonil 75W	Yes	97.0 ± 0.7	6.7 ± 6.7	6.6 ± 1.3	58.3 ± 8.3	11.3 ± 4.4	96.7 ± 3.3

[a] Fungicide applied as a powder (1 g/100 g seed) immediately after inoculation.
[b] Seeds were inoculated with *Alternaria tenuis*.
[c] Seeds stored at 4.5 C in plastic bags after inoculation.
[d] Standard error of the mean.

the result of pectolytic enzymes produced by an array of microorganisms. As a result, seeds may become infected with *Alternaria*, which is ubiquitous on the fruit (2). This method of seed extraction is being replaced by a procedure that uses commercial pectinase and requires only several hours for extraction (1). Even enzyme-extracted seeds, however, should be treated with a seed protectant such as thiram or 8-hydroxyquinoline sulfate (7) to ensure control of *Alternaria*.

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