Survival of *Phytophthora infestans* in Seeds Extracted from Infected Tomato Fruits

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ABSTRACT

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Seeds were extracted from healthy and blighted tomatoes collected from three field-grown cultivars, processed as in a commercial operation, and the seeds plated on a selective medium. *Phytophthora infestans* usually was isolated (avg 91.7%) from discolored, freshly extracted, wet seeds but not from discolored seeds that had been dried in an oven (29.5–37.5 C) for 6 hr or air-dried for 72 hr following extraction. Fermentation for 24 hr eliminated the fungus from discolored seeds of cultivar 7718 but not from discolored seeds of cultivar Castle 1025 or those of an unidentified cultivar of cherry tomato. Treatment of wet, discolored seeds with either NaOCl (0.5% for 5 min) or 0.12 N HCl (pH 1.6, for 25 min) significantly reduced the frequency of isolation. In discolored seeds, hyphae were observed on and in the seed coat, in the remnants of the funiculus, and between the endosperm and the seed coat. When freshly extracted, wet or dried discolored seeds were planted in steamed UC soil mix or in field soil in the greenhouse, some of the seedlings emerging from wet seeds were infected (avg 26%), whereas seedlings emerging from dry seeds were healthy. Infected tomato fruits placed in the field on a wet soil surface or plowed under and kept continuously wet by irrigation occasionally gave rise to infected seedlings.

After an absence of 32 yr, the late blight disease of tomato caused by *Phytophthora infestans* (Mont.) de Bary suddenly reappeared in 1979 on tomato seedlings grown for transplants in plastic houses located in three widely separated locations in southern California. The sudden and simultaneous reappearance of the late blight disease in three different nurseries, production of tomato seeds in late blight areas outside of the United States, and the known susceptibility of tomato fruits to *P. infestans* suggested that the pathogen was seed-transmitted and was being reintroduced into southern California on infested or infected tomato seed.

Although tomato fruits are infected readily, transmission of *P. infestans* by tomato seed has not been studied thoroughly. Reed (8) observed that the pathogen was present in infected tomato seeds, but he failed to transmit the disease by planting infected seeds in the greenhouse or in the field. Boyd (1) also noted that the pathogen was present in the seed, but in contrast to Reed, he claimed that the infested or infected seeds gave rise to infected plants in the greenhouse and the field. The studies by Reed (8) and by Boyd (1) are difficult to interpret and to compare because the experimental procedures were not adequately described.

The following study was undertaken to determine the location of the fungus in seed, to investigate the factors affecting survival of *P. infestans* in or on tomato seed, and the conditions required for seed transmission.

MATERIALS AND METHODS

Histological studies. Microscopic studies of thin sections of seeds were made to determine the location of the pathogen. Light-colored seeds from healthy fruits and discolored seeds from infected fruits were fixed in Navashin's solution for 7 days, the seeds were dehydrated by using Johansen's method (4), infiltrated with paraffin, and sectioned with a rotary microtome into 12-μm slices. These were stained with safranin and counterstained with fast green. Four seeds from healthy fruits and 10 discolored seeds from infected fruits were used for the study.

Effect of seed-processing operation on viability of *P. infestans*. Only commercially processed tomato seeds are planted in California fields. These seeds are processed as follows: The fruit is ground and placed in large metal containers and allowed to ferment, usually for 24–36 hr, to free the pulp from the seed. The seeds are collected and soaked in either 0.12 N HCl at pH 1.6 for 25 min or in 0.5% NaOCl for 5 min (the NaOCl treatment is used less frequently because it reduces seed viability, but certain countries request the treatment). The seeds are then washed, centrifuged, and dried on perforated trays through which warm air (29.5–37.5 C) is passed for 6 hr. To study the effects of these procedures on the viability of the pathogen, healthy and field-infected mature tomato fruits of cultivars 7718, Castle 1025, and an unknown cultivar of cherry tomato were collected from different locations in the coastal area of southern California and used in these studies. Initial experiments established that untreated, moist, discolored seeds collected from infected fruits readily yielded *P. infestans*, while only a few of the moist nondiscolored seeds in infected fruits yielded the organism. For this reason, all subsequent research was performed with moist, discolored seeds harvested from infected fruits.

Freshly extracted, discolored seeds from infected tomato fruits were fermented in a beaker for 24–28 hr to remove the gel surrounding the seeds and repeatedly washed with tap water. Other freshly extracted discolored seeds were put in a beaker, covered with one layer of cheesecloth and washed for 15 min in running water (distilled). The seeds were then treated with 0.5% NaOCl for 5 min or HCl at pH 1.6 for 25 min, or were dried either on a laboratory bench (22 ± 1 C) for 72 hr or in a ventilated oven (29.5–37.5 C) for 6 hr.

Following each treatment, 20 discolored seeds were placed on each of five plates containing rye seed agar-A (2) supplemented with pimaricin, ampicillin, rifampicin, and pentachloronitrobenzene (5). The plates were incubated at 21 ± 1 C in the dark. Observations were made 1, 2, 5, and 14 days after plating, and the seeds yielding *P. infestans* were counted.

Seed transmission. *Greenhouse study.* Since moist, infected seeds yielded the fungus but dried, infected seeds did not, the former when planted should give rise to blighted seedlings whereas the latter should not. This possibility was evaluated in the greenhouse.

One hundred healthy tomato fruits of cultivar 7718 were collected at the mature green stage from a tomato field in Ventura County, southern California. Each fruit was injected at the stem end with 0.5 ml of a spore suspension (10^4 sporangia of *P. infestans* per milliliter); control fruits were inoculated with 0.5 ml of sterile,

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distilled water. The fruits were incubated in a growth chamber set at 19 ± 1 C and supplied with 8 hr of artificial illumination. After 2 wk, light-colored seeds were harvested from healthy fruits and discolored seeds from infected fruits. The moist, white seeds extracted from healthy fruits were left untreated, but the moist, discolored seeds were divided into three groups. One group was left untreated, the second was treated with NaOCl (0.5% for 5 min), and the third was air-dried (22 ± 1 C for 72 hr) on a laboratory bench. Samples of 100 seeds from each treatment were plated (20 seeds per plate), on the selective medium to measure levels of recovery of *P. infestans*.

Seeds from healthy and infected fruits were planted in steamed UC soil mix or in field soil in flats with 50 individual spaces (3 × 3 cm). Each treatment was represented by 100 seeds in a flat, with two seeds per space. The flats were incubated in a growth chamber with a day temperature (0800 hours–1700 hours) of 16.5 ± 1 C and a night temperature of 10 ± 1 C. These are the average day and night temperatures during the winter in the coastal area of southern California. The growth chamber was supplied with 9 hr (0800–1700 hours) of artificial illumination. The soil was kept moist by watering to avoid drying of the seeds. Seedlings were observed after emergence for symptoms of late blight and signs of the pathogen.

Field study. To verify our supposition that blighted seedlings may arise from infected seeds if the seeds are kept moist, the following field experiment was carried out. Apparently healthy and naturally infected tomato fruits of cultivar 7718 were collected from a tomato field in Orange County, southern California. Fifty "healthy" or 50 infected fruits were placed on the soil surface, or were plowed under (18 cm deep). The treatments were distributed in a randomized block, with each block being 3.5 × 3.5 m, with four replications per treatment. A similar plot was set up 10 m from the first but this plot was irrigated three times daily for 5 min with overhead sprinklers to keep the fruits and the seeds wet. Ten seedlings randomly collected at weekly intervals from each treatment block were examined for late blight, then they were surface-disinfested with 0.5% NaOCl for 1 min, rinsed in tap water, placed in moist chambers, and incubated at 21 ± 1 C in the dark. The seedlings were examined 1, 2, and 5 days later for signs of the pathogen.

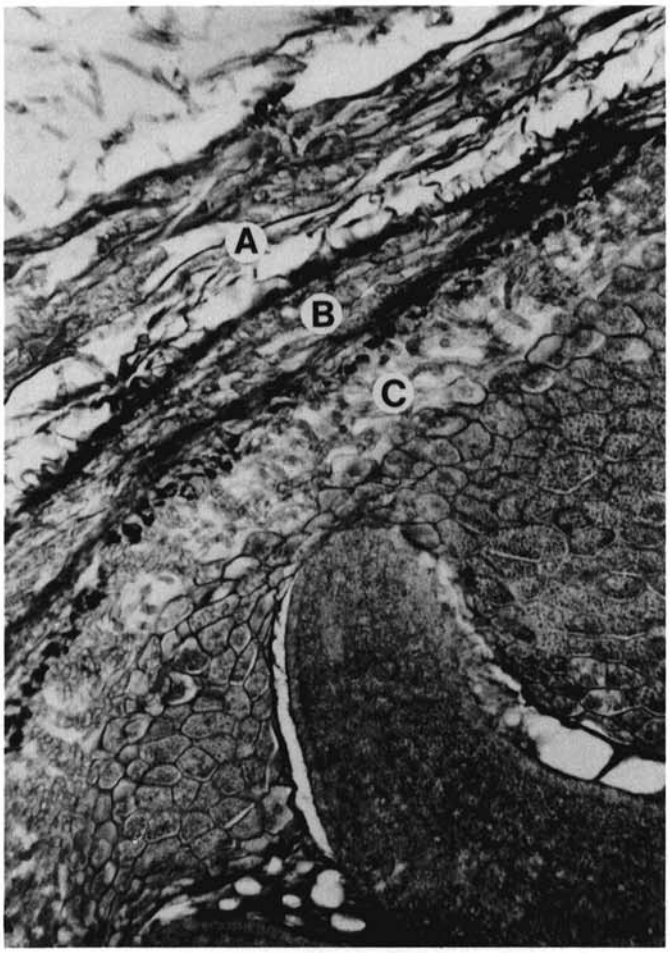

Fig. 1. Mycelia of *Phytophthora infestans* **A,** in the gel surrounding a seed, **B,** in the seed coat, and **C,** between the endosperm and seed coat of discolored seeds extracted from late blight-affected tomato fruit (×130).

RESULTS

Histological studies. *P. infestans* was present as a network of coenocytic mycelia in the gel surrounding the infected seeds, on and in the seed coat, in the remnants of the funiculus, and between the endosperm and the seed coat (Fig. 1). The embryo and the endosperm were apparently free of infection. Occasional sporangia were present on the seed surface. No dormant mycelia, or reproductive or resting spores were observed in infected tissues. Six of the 10 discolored seeds studied were internally and externally colonized and four were covered with external mycelia only. There were no hyphae present on or in seeds extracted from healthy fruits.

Effects of seed-processing operation. *P. infestans* was found on 91.7% of the wet, discolored seeds from infected fruits (Table 1). The various seed treatments significantly decreased the recovery of the pathogen from seeds of all three of the cultivars (Table 1). Fermentation eliminated the pathogen from seeds of cultivar 7718 and lowered recovery considerably from discolored seeds of Castle 1025 and of cherry tomato. Treatment with either NaOCl or with HCl reduced recovery significantly but did not eliminate the pathogen from the discolored seeds. Drying seed at either room temperatures (22 ± 1 C) for 72 hr, or in an oven (29.5–37.5 C) for 6 hr, completely eliminated the pathogen from discolored seeds extracted from infected fruits.

Seed transmission. *Greenhouse study*. When moist, discolored seeds extracted from infected fruits were planted in steamed UC soil mix or in field soil in the greenhouse, a fair number of the germinated seedlings developed late blight (Table 2). Less than 10% of seedlings from discolored seeds disinfested with NaOCl and planted in steamed UC mix developed late blight compared to less than 5% in field soil in the greenhouse. No late blight developed on seedlings from discolored seeds extracted from infected fruits if seeds were first air-dried and then planted in steamed UC soil or field soil.

Late blight lesions on which sporangia and sporangiophores were found were present mostly on the stems; sporulation was most

TABLE 1. Effect of various seed treatments on the recovery of *Phytophthora infestans* from discolored seeds extracted from blighted tomato fruit

Source of seed	Treatment	Recovery (%)[x] from cultivars: 7718	Castle 1025	Cherry tomato	Avg[y]
Healthy fruit	None, seed wet when plated	0	0	0	0 d
Blighted fruit	None, seed wet when plated	3	93	89	91.7 a
	Fermented, 24-28 hr, seed wet when plated	0	12	22	11.3 c
	NaOCl, 0.5%, 5 min, seed wet when plated	2	22	37	33.7 b
	HCl, 1% (pH 1.6), 25 min, seed wet when plated	4	38	10	30.7 b
	Air-dried, 22 C, 72 hr, seed dry when plated	0	0	0	0 d
	Oven-dried, 29.5–37.5 C, 6 hr, seed dry when plated	0	0	0	0 d

[x]Seed plated on rye seed agar (2) supplemented with pimaricin, ampicillin, rifampicin, and PCNB (5).

[y]Treatment averages with no letter(s) in common are significantly different on Duncan's multiple range test for $P < 0.01$.

abundant at the crown area. No lesions were observed on the roots of the infected seedlings.

Field study. Only one emerged plant out of 320 (0.3%) developed late blight when the infected fruit was placed on the soil surface, but when such fruits were placed on the soil surface and irrigated daily, 98 of 280 (35%) emerged seedlings developed late blight. When the infected fruits were plowed under, 18 plants of 280 (6.4%) emerged seedlings developed late blight but when such fruits were plowed under and the plots irrigated daily, 53 plants of 280 (18.3%) emerged seedlings developed late blight. The number of blighted tomato seedlings that emerged from apparently healthy tomato fruits placed on the soil surface (0.0%) or plowed under (1.1%), were very low compared to the number of seedlings emerging from seeds of infected fruits. Some of the apparently healthy fruits had latent infections when used because 10% of the fruit that had been placed on the soil surface developed late blight 1 wk after the initiation of the experiment. Since the winter of 1982–1983 was very wet, the soil remained moist most of the time.

Most of the lesions and sporulation on the infected seedlings, emerging from healthy and infected fruits in the field, were located mainly near the crown area of the stem. After 48 hr of incubation in moist chambers, the stems of the infected seedlings were covered with sporangiophores and sporangia typical of *P. infestans*.

TABLE 2. Emergence of blighted tomato seedlings from seeds from healthy or late blight-affected tomato fruits planted in steamed UC soil mix or in field soil and incubated in flats in a controlled environment chamber[x]

Treatment[y]	Infected seedlings (no.)		
	UC mix	Field soil	Avg[z]
Moist white seeds from healthy fruits	0	0	0 c
Moist discolored seeds from infected fruits	34	18	26 a
NaOCl-treated	7	4	5.5 b
Air-dried	0	0	0 c

[x]One hundred seeds were planted following a treatment. Temperature was regulated at 16 C during the day (0800 hours–1700 hours) and 10 C at night.

[y]Treatment details as in Table 1. *Phytophthora infestans* was recovered from 19% of moist, discolored NaOCl-treated seeds and from 57% of moist, discolored untreated seeds. It was not recovered from healthy or air-dried discolored seeds.

[z]Averages with no letter(s) in common are significantly different ($P < 0.01$) using pairwise treatment comparison on chi-square for numbers of infected and noninfected seedlings.

DISCUSSION

P. infestans not only colonizes the interior of the tomato fruit as coenocytic hyphae but also the gel surrounding the seeds, the surface of the seed, the space between the endosperm and the seed coat, and the remnants of the funiculus and the seed coat. Although both Reed (8) and Boyd (1) reported that *P. infestans* was present in tomato seed, this is the first report of the locus of seed infection.

Vegetative hyphae were observed colonizing the fruit and seed without the formation of oospores, chlamydospores, and dormant mycelia. The absence of oospores is not surprising since their formation requires the presence of both the A1 and A2 compatibility types (3) and only the A1 compatibility type is present in southern California (*unpublished*). Although Patrikayeva (7) has reported that *P. infestans* produces chlamydospores in agar media, their formation has not been verified by others either on culture media or in plants in the field. The lack of resistant structures and the susceptibility of vegetative hyphae of *P. infestans* to drying appears to explain why desiccation of infected or infested seed readily destroys the fungus. This conclusion agrees with the hypothesis of Neergaard (6) who divides seedborne fungi into two groups based on their tolerance to desiccation: tolerant (=xerotolerant) and intolerant (=hydrophilic). Neergaard also attributed fungal tolerance to seed desiccation to the formation of drought-resistant structures (e.g., chlamydospores, oospores, sclerotia, resistant conidia, dormant mycelia, etc.) and intolerance to the susceptibility of vegetative hyphae of pythiaceous fungi to an arid environment.

Our finding that seed transmission of *P. infestans* in tomato occurs only when the seed is kept moist may help to explain the two conflicting reports on seed transmission by Reed (8) and by Boyd (1). Reed reported that the fungus was not transmitted through tomato seed, whereas Boyd reported that it was. Since neither reports were detailed, we cannot be certain that retention or nonretention of hyphal viability is the factor responsible for the reported difference in seed transmission.

Since all commercial tomato seed is dried before being sold, commercial seed should not be a source of inoculum of the late blight fungus. This conclusion is strengthened by the fact that not only fermentation of the seed, but also treatment of the seed with NaOCl or with HCl considerably reduced the population of the fungus on the surfaces of tomato seed. This is important since commercial seedsmen discard tomato fruit that is obviously affected with late blight but fruit with latent infections and incipient rot may be overlooked and incorporated into the seed-processing operation. Such fruit would be in the early stages of colonization, and colonization of the seed would tend to be external rather than internal. Externally colonized seed would then be disinfested by both the fermentation process and the acid treatment.

Seed transmission of *P. infestans* in the field cannot, however, be eliminated as a source of inoculum since situations may arise in which the pathogen encounters moist conditions that prevent the desiccation and death of the fungus in or on the seed. This may occur, for example, when infected fruit falls on soil that is kept moist by rains, or infected fruit is plowed under and rains keep the infested or infected seed sufficiently moist to retain the viability of the mycelium. Infected or infested seed would germinate in 2–3 wk and give rise to some blighted seedlings, essentially as demonstrated in the field experiment reported in this paper.

This phenomenon was observed in four very weedy fields in San Diego County during the very wet winter of 1982–1983. Blighted seedlings were observed arising in clumps with infection and sporulation confined to the crown of the stems. This type of infection is typical of infections arising from infected or infested seed, whereas lesions that develop on all aerial organs of the plant are typical of aerial infections carried by windblown sporangia arising from distant sources. In contrast, the winter of 1983–1984 was extremely dry and seed transmission in the field was not observed.

We concluded that seed transmission of *P. infestans* does not occur in commercial tomato seed because the external hyphae are partially destroyed by fermentation and by acid treatment of the seed and both internal and external hyphae are destroyed by the routine seed-drying operation. The simultaneous reappearance of late blight in 1979 in three greenhouses cannot, therefore, be explained by seed transmission through the use of commercial seed. However, transmission of the fungus may occur in the field through infected or infested seed when the fruit and seed remain wet. Since blighted seedlings appear to survive for only a few weeks, their importance resides in the ability of the fungus to form infective sporangia on the stems when susceptible solanaceous host plants are present as volunteers, as weeds, or as newly planted transplants. These findings also have a bearing on latent blight-affected, commercial tomato fruits that are shipped into California during winter and early spring from Florida and Mexico. When such tomato fruits develop symptoms of late blight, they are picked out by the grocer and discarded into waste bins. The contents of these waste bins are then dumped at the local waste disposal facility where the blighted seeds, if they encounter moist conditions, may germinate to give rise to blighted seedlings. This then constitutes another method by which *P. infestans* may be introduced and perpetuated. Since the winter of 1979 was characterized by heavy and frequent rainfall, this method of introduction may explain the sudden reappearance of the disease in the three greenhouses in 1979. No direct evidence in support of this conjecture is advanced.

However, it was noted that the blighted greenhouse seedlings manifested aerial infections rather than stem base infections, indicating that the infections arose from secondary windblown sporangia rather than from infected or infested seed.

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