A Stem Canker Disease of Tomato Caused by Alternaria alternata f. sp. lycopersici

R. G. Grogan, K. A. Kimble, and Iraj Misaghi

Department of Plant Pathology, University of California, Davis 95616.

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ABSTRACT

A. alternata f. sp. *lycopersici* was shown to be the cause of a serious stem canker disease of fresh market tomatoes. The fungus is a distinct pathotype capable of primary infection of leaves, stems, and fruit of susceptible tomato cultivars. Tomato was the only host demonstrated and only 25% of 265 tomato cultivars were susceptible. Resistance, probably inherited as a single dominant factor, has provided effective control.

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A stem canker disease of fresh market tomatoes (*Lycopersicon esculentum* Mill.) caused serious damage in recent years, especially in San Diego County of southern California. This disease has been called "Fusarium crown rot" or "Fusarium-induced root rot" and attributed to a form of *Fusarium oxysporum* different from the classical wilt organism (2, 5). Its etiology has been uncertain because of susceptibility of field-resistant cultivars in greenhouse inoculations, and failure to induce the disease by inoculation with *Fusarium*. (R. M. Endo, *personal communication*). In this report we present evidence that stem canker is caused by a virulent pathotype of *Alternaria alternata* (Fr.) Keissler = *A. tenuis* Auct. specifically pathogenic to certain cultivars of tomato.

Description of the disease.—Dark-brown-to-black cankers with concentric zonation occur on stems near the soil line or aboveground; large cankers are often associated with wounds produced by pruning petioles. The cankers enlarge slowly and by harvest stems are girdled and plants die. Small tan-to-brown lesions are interspersed between the larger lesions (Fig. 1-A). Tissues beneath the cankers exhibit a brown dry rot especially in the pith area (Fig. 1-B). The xylem in the canker is discolored and brown discontinous streaks may develop in the pith adjacent to the primary xylem 4-7 cm above and below the cankers.

Foliar symptoms comprise epinasty, inward rolling and angular necrotic spotting of topmost leaflets or, in later stages, complete necrosis of leaflets on one or both sides of the midrib (Fig. 1-D and E).

Percentage of diseased plants increases with time and by harvest 90% or more may be affected.

Serious damage from stem canker has occurred only on certain cultivars. Of the cultivars used for fresh market in southern California, Grandpak, 428VF, 6339VF, 6343VF, VFN Bush, and Earlypak 7 are susceptible, whereas 6718VF and various derivatives of the cultivar Ace (viz., Royal Ace, Ace 662, Ace 55, Cal Ace and H-11) are resistant. Because of its suitable horticultural characteristics and resistance to stem canker, 6718 VF has been grown on a major portion of the acreage during the past 2 years.

METHODS AND RESULTS.—*Isolation from diseased samples.*—Chips of tissue obtained from the margins of cankers were plated on water agar or PDA (potato-dextrose agar). Also spores were transferred directly to PDA. A small-spored long-chained *Alternaria* spp. grew from about 95% of the tissues and spores; in many isolations this was the only fungus obtained.

Inoculation.—Inoculation of Improved Pearson tomato plants with 12 randomly selected isolates of the fungus were made by applying spores and mycelium, scraped from colony surfaces, to petiole stubs left after clipping off leaves. Plants were covered for 48 hours with plastic bags. Consistent infection with all isolates was

evidenced by progressive invasion from petiole stubs into the stems. After 3-5 days, collapsed darkened areas had developed at points of inoculation and terminal leaflets had interveinal angular spots (Fig. 1-C and D). Within 8-10 days, stems were girdled and the plants died. Similar inoculations on nearly mature plants with green fruit resulted in somewhat slower development of stem cankers, necrotic angular spotting of terminal leaves, and premature ripening of fruit. Several green fruit inoculated through superficial wounds into the carpel wall were almost completely rotted after 8 days, and rotted surfaces became blackened by dense sporulation. Isolation from diseased plants yielded cultures of *Alternaria* sp. similar in all respects to isolates used for inoculation. The fungus was not recovered from stem streaks 7-10 cm from inoculated sites nor from necrotic terminal leaves indicating that these symptoms are induced by a translocated toxin discussed in a preliminary report (3).

Inoculation of Earlypak 7 and other susceptible cultivars by spraying noninjured 15-20 cm plants with spore suspensions containing approximately 2.5×10^5 spores/ml followed by 48 hours of incubation in a mist chamber at 21-24 C resulted in necrotic leaf, petiole, and stem spotting within 7-8 days (Fig. 1-E). Within 10-15

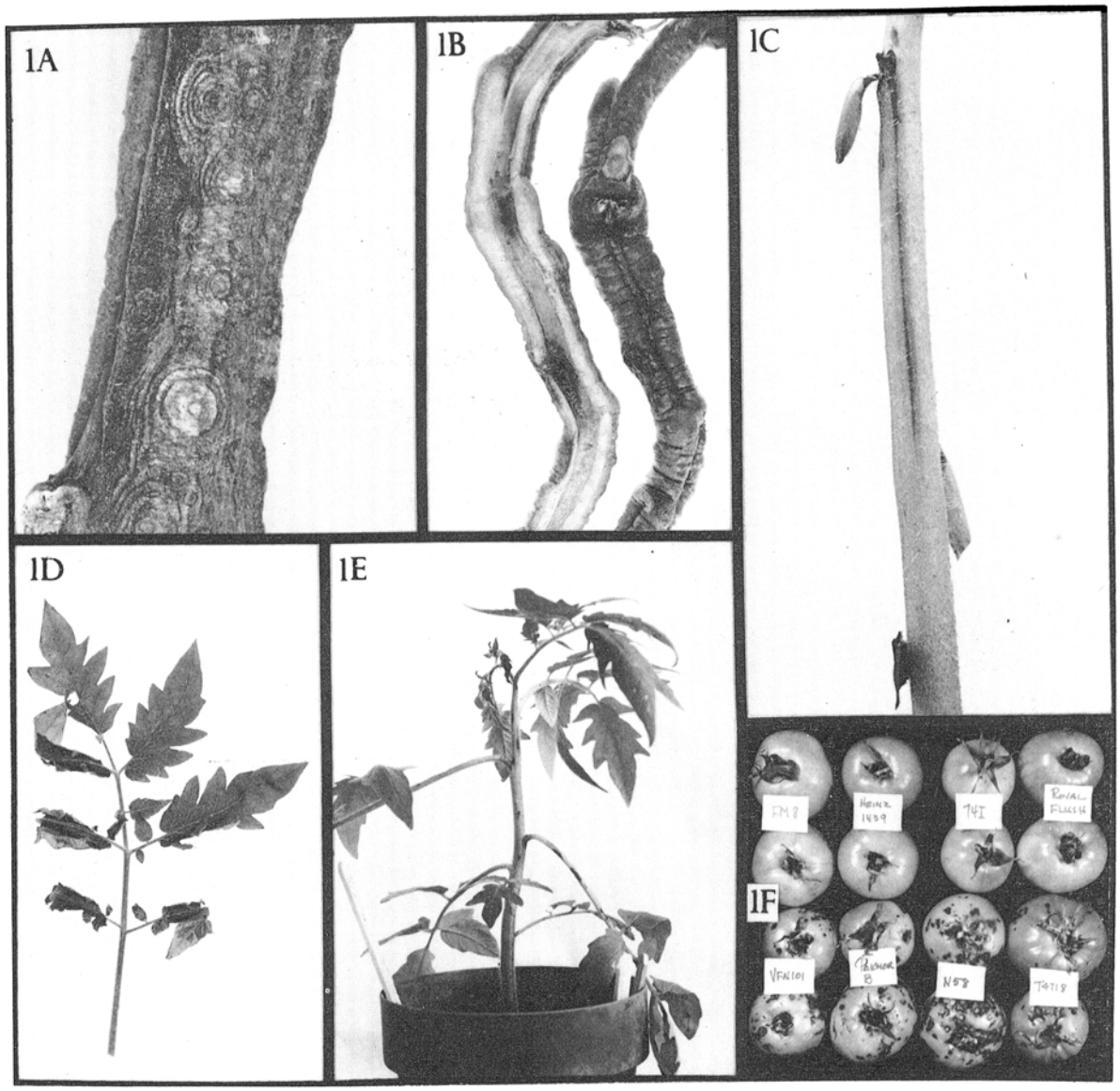

Fig. 1-(A to F). Symptoms of Alternaria stem canker on tomato. **A)** Naturally infected stem with large concentrically ringed lesions and many smaller lesions. **B)** Longitudinal section of a cankered stem showing discolored pith and surface view of cankers. **C)** Discoloration of stem caused by toxin resulting from inoculation of petiole stub at point immediately above attached leaflet. **D)** Toxin-induced leaf symptoms from greenhouse inoculated plant with stem cankers. **E)** Stem and petiole lesions, petiole epinasty and toxin-induced symptoms on upper leaves resulting from spray inoculation of intact plant with a spore suspension. **F)** Differential spotting on fruit from resistant or susceptible varieties induced by spray inoculation of uninjured fruit with a spore suspension; top row L to R resistant FM8, Heinz 1439, 741 and Royal Flush; bottom row L to R susceptible VFN101, Pakmore B, N58, and T4718.

days stem spots enlarged, stems were girdled and the plants died. Similar inoculations of Ace, 6718VF, and other field-resistant tomato cultivars have produced no symptoms. Furthermore, inoculations of excised stem sections of resistant Ace or 6718VF by spraying with a spore suspension followed by incubation in closed moist plastic boxes for 6-8 days produced no obvious damage, whereas inoculations of similar tissues from susceptible cultivars resulted in tissue discoloration and sporulation of Alternaria over the entire inoculated surfaces. Similar inoculations of detached green fruit from susceptible or resistant cultivars resulted in severe spotting of fruit from susceptible cultivars but none developed on fruit from resistant cultivars (Fig. 1-F).

Description of the fungus.—Cultures on PDA under fluorescent lights are at first fluffy and off-white, but become dusky neutral gray (9) with an off-white border within 48 hours. After the colony extends over the entire plate sporulation is abundant and the colony becomes appressed and nearly black. Colonies on CMA (cornmeal agar) are dark-brown, sparse, and appressed, but with slightly raised concentric bands with intense sporulation.

The fungus has maintained its original capacity for sporulation and pathogenicity on susceptible tomato

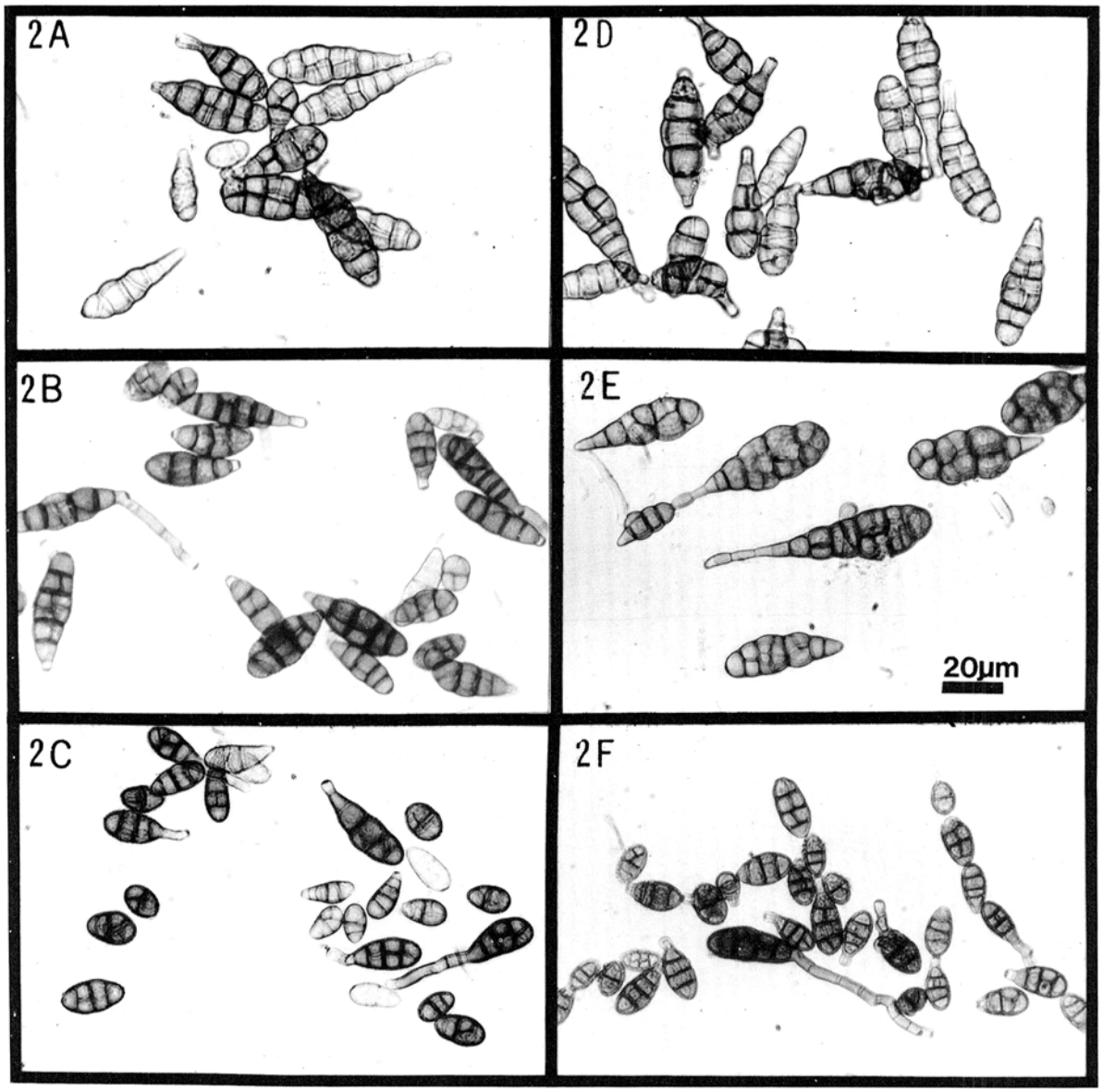

Fig. 2-(A to F). Differences in spore morphology of *Alternaria alternata* f. sp. *lycopersici* (A, B, C, and D) and 'saprophytic' *A. alternata* from ripe rotted tomato fruit (E and F) produced naturally or in vitro on several substrates. **A)** from naturally infected tomato stem, **B)** from propylene-oxide-sterilized tomato stem segments in vitro. **C)** from cornmeal agar and **D)** from stem canker produced by inoculation in greenhouse. **E)** from naturally infected ripe rotted tomato fruit. **F)** same as E but from cornmeal agar. All spores are same relative magnification.

TABLE 1. Morphological comparisons of *Alternaria* isolates from tomato stem canker and ripe tomato fruit rot with published descriptions of *A. alternata*, *A. tenuis*, and *A. tenuissima*[a]

		Isolate or source of data and source of spores								
		Alternaria alternata						*A. tenuis*		*A. tenuissima*
		f. sp. *lycopersici*[b]			(saprophytic)[c]		Simmons (10)	Groves & Skolko[d]	Neergaard[e]	Wiltshire
Measurements		Tomato stem from field	Propylene-oxide-sterilized tomato stems in vitro	Cornmeal agar	Black rotted ripe tomato fruit from field	Cornmeal agar	Neotype specimen from unidentified pithy stem	Malt agar in vitro	2-4 media in vitro	Petals of *Dianthus* (?) and *Ranunculus* (?)
Spore body:										
length:	Range	18-50	18-45	10-30	18-55	7-36	(10) 18-47[f]	-	7-70	-
	Mean	32.3	29.7	19.8	36.2	19.4	30.9	-	25.7	-
	S.D.	2.8	1.5	1.6	3.3	1.5	-	-	-	-
width:	Range	7-18	7-13	7-13	7-20	5-11	(5)7-18	(7) 10-16 (20)[f]	6-22	13-20
	Mean	12.4	9.8	9.5	14.4	8.5	12.6	-	11.2	-
	S.D.	2.8	1.5	1.6	3.3	1.5	-	-	-	-
Beak length:										
	Range	2-20	1-5	1-4	5-40	1-3	up to 25	-	1-58	1/3 of spore length
	Mean	6.8	2.2	2.0	13.4	1.0	-	-	5.0	-
	S.D.	6.0	1.5	1.3	10.9	1.0	-	-	-	-
Spores:										
% with beaks		72	79	62	73	61	-	-	80	-
total length		18-68	18-48	10-34	18-90	7-38	-	(16)20-50(70)	7-72	33-103
Conidiophores										
size range width × length		3-5×5-62	3-5×7-56	2-5×3-40	3-6×3-65	3-4×3-45	4-6×20-46	3-6 × "very variable"	3-6×5->125	4-5×50-60
no. septa		1-5	1-6	1-5	1-6	1-5	1-3	-	1-6	4-5 (?)

[a] *A. tenuis*, sensu lato Groves & Skolko (4) and Neergaard (8); *A. alternata* (Fr.) Keissler (10); *A. tenuissima* (Kunze ex Pers.) Wiltshire (12).
[b] Based on measurement of 100-150 randomly selected spores mounted in lactophenol.
[c] Saprophytic implies capability for attack of ripe tomato fruit only.
[d] Isolates cultured from various seeds.
[e] Average of means in Neergaard's table No. 5 (8).
[f] Numbers in parentheses in this column refer to "extreme" measurements taken from the cited references; they are not literature citations.

plants after subculturing for several months on PDA or CMA.

A comparison of spores and conidiophores from field and greenhouse stem cankers and in vitro cultures on CMA and propylene-oxide-sterilized tomato stems showed that its measurements agree quite well with published descriptions of *A. alternata* (Fr.) Keissler (11) and with *A. tenuis* sensu lato Groves and Skolko (4) and Neergaard (8) (Table 1). The stem-canker organism was also similar in morphology to *A. alternata* from rotted ripe tomato fruits collected near Davis, California (Table 1). Both organisms produced larger spores with longer beaks on natural substrates than on CMA or propylene-oxide-sterilized tomato stems in vitro (Fig. 2). Chains of spores produced in vitro were longer averaging eight-ten or more vs. only three-four per chain on the natural substrates. The published description of *A. tenuissima* (12) indicates that its spores are considerably larger in both width and length (Table 1).

Therefore, we have concluded that the stem canker fungus is identical morphologically with *A. alternata* = *A. tenuis,* but recommend that it be designated *A. alternata* (Fr.) Keissler f. sp. *lycopersici* because of its specific and primary pathogenicity on certain cultivars of tomato. A culture deposited in the American Type Culture Collection has been assigned the accession number ATCC 28329.

Survival of the fungus in field soil.—Rapid spread and widespread distribution of stem canker indicate that inoculum is airborne. However, the greater percentage of diseased plants in second-year plantings and the delay in disease development resulting from soil fumigation (A. O. Paulus, *personal communication*) suggested that inoculum also may be soil-borne. To test this possibility sections of cankered tomato stems were buried in a field in San Diego County, California. Samples retrieved at about monthly intervals were tested for presence of *A. alternata* by thoroughly washing under running tap water followed by incubating bits of tissue from cankers on acidified PDA. Isolates of *A. alternata* recovered by this procedure were inoculated to susceptible tomato plants to ascertain pathogenicity. The pathogen was recovered from each monthly sample for 13 months which is sufficient time for carry-over from one growing season to the next.

Effect of age of plants and spore concentration on infection.—New Improved Pearson tomato plants about 25 cm tall were sprayed with spore suspensions containing approximately 1.2×10^6, 6.0×10^5, or 3.0×10^5 spores/ml followed by 36 hours of incubation at 21-24 C in a mist chamber; all plants were infected and eventually killed.

Inoculation of seed by wetting with spore suspensions containing approximately 2.5×10^5 spores/ml prior to planting caused no reduction of emergence or apparent infection after emergence. Seedlings in the cotyledonary stage similarly inoculated with a spore suspension were not visibly infected but plants in the second true leaf stage and at various stages of development up to 6 weeks old (oldest tested in this experiment) were severely infected, indicating that young seedlings of susceptible cultivars are refractory to infection but later become susceptible.

Testing for resistance.—We have tested numerous cultivars for resistance by spraying unwounded plants 10-12 cm tall with spore suspensions containing approximately 2.5×10^5 spores/ml followed by 36-48 hours of incubation in a mist chamber prior to removal to an open greenhouse bench for disease development. Susceptible cultivars develop leaf spots, petiole and stem cankers resulting in death of all plants. The cultivar 6718VF, which is the F_1 hybrid of resistant × susceptible cultivars (not named in deference to wishes of the seed company) is completely resistant, but 75% of its selfed progeny were resistant and 25% susceptible indicating that resistance is controlled by a single dominant gene.

The cultivars used in various crosses to produce the stem canker-resistant cultivar Ace (Rutgers, J. T. D., Pritchard, Master Marglobe, Improved Garden State, and Victor provided from the Campbell Soup Company seed bank by S. J. Warnock) are all resistant to the Alternaria. It thus appears that resistance in Ace is not unique, but by happenstance it was horticulturally suitable for fresh market tomato production in southern California. Of 265 cultivars or breeding lines from various sources 203 (76.6%) were completely resistant, 53 (20.0%) completely susceptible, and nine (3.4%) were mixed populations of resistance and susceptible plants. Of 40 processing cultivars screened, 28 were resistant and 12 susceptible including two mechanical harvest types, VF 145-21-4 and VF 145-7879, that are favored by many growers and comprise a large portion of the cannning tomato acreage in northern California. There are no reports, however, that the disease occurs naturally in canning tomatoes in northern California, and we did not detect it in canning tomatoes in 1974.

Overall results from test inoculations suggest that susceptibility is derived from the cultivar Pearson which has been used in crosses by plant breeders to produce fresh-market and canning tomato types for California. In contrast, cultivars bred for fresh-market production in Florida, which do not have Pearson in their parentage; i.e., Florida MH1, Manalucie, Homestead Elite, Indian River, Immokallee, Floradel, Manapal, and Walters, are resistant.

Host range test inoculations.—Several potential hosts of Alternaria were inoculated by spraying whole intact plants or comparable plants with freshly excised petioles with a spore suspension containing approximately 2.5×10^5 spores/ml followed by incubation for 72 hours in a mist chamber. Final observations were made 15 days later when similarly inoculated Pearson tomato plants had developed girdling stem cankers. No symptoms were evident on four inoculated plants each of *Nicotiana multivalvis* Lindl.; *N. glutinosa* L.; *N. tabacum* 'Havana 425', and 'X-73'; *Nicandra physalodes,* (L.) Gaertn.; *Capsicum annuum* L. 'Yolo Wonder', and 'Pimento'; *C. frutescens* 'Tabasco'; *Solanum aviculare* Forst.; *S. nigrum* L.; *S. melongena* L. 'Black Beauty'; *S. tuberosum* L. 'Targhee'; *Datura meteloides* DC.; *D. stramonium* L.; *D. stramonium* var. *tatula* (L.) Torr; *Physalis floridana* Ryab.; *P. ixocarpa* Brot. 'Tomatillo'; *Salpiglossis sinuata* Ruz. & Pav; *Cucumis sativus* L. 'National Pickling'; and *Cucurbita pepo* L. 'Small Sugar Pumpkin'.

Although this range of test hosts is limited, the results indicate that the host range of the stem canker fungus is restricted and possibly limited to certain cultivars of tomato. This contrasts with a rather extensive host range of *A. solani* in the Solanaceae and the ability of *A. tomato*

to cause disease in eggplant, potato, horse nettle, and susceptible tomato cultivars (11).

Tests for pathogenicity of various isolates of Alternaria.—Isolates of small-spored chained *Alternaria* spp., presumably *A. alternata,* obtained from various sources have been tested for pathogenicity on stem-canker susceptible New Improved Pearson plants. Only one of 15 isolates (from Ventura County, California) from ripe tomato fruit was pathogenic to tomato foliage and stems and none of 11 isolates from cherry, apricot, peach, or prune was pathogenic.

An isolate labeled *A. tenuissima* isolated from blueberry in North Carolina by R. D. Milholland, four isolates labeled *A. longipes* from tobacco leaf spots received from E. G. Simmons also were nonpathogenic on tomato.

Thus it seems clear that the tomato stem canker *Alternaria* is a distinct pathotype and clearly different from other isolates of *A. alternata.*

Inoculation of Ace 55, 6718VF, and Earlypak 7 in field plots.—Inoculations of Ace 55 and 6718VF (resistant) and Earlypak 7 (susceptible) were made in a field plot near Chula Vista, California, during the spring of 1974 to determine the effect of time-of-pruning and inoculation on disease development and severity.

Inoculations were made by spraying the lower two-thirds of the plants with a spore suspension on either 28 February or 21 March; results were recorded on 21 March and 6 May. (Each treatment comprised three five-plant replications, but is reported as totals because of nearly identical results among replications). The specific treatments involving different dates of pruning and inoculation were as follows: (i) pruned and inoculated on 28 February; (ii) not pruned, inoculated 28 February; (iii) pruned 28 February and 21 March, inoculated 21 March; (iv) pruned 28 February and 21 March, not inoculated; (v) pruned only on 21 March, not inoculated.

All 30 Earlypak plants in treatments i and ii were infected by 21 March as evidenced by dark-brown to black lesions on the stems, but none had toxin-induced foliage symptoms or were dead. On 6 May, however, 5 of 15 plants in treatment i had toxin symptoms in the upper leaves and 13 of 15 were dead or nearly so and in treatment ii, 7 of 15 had toxin symptoms and 7 of 15 were dead or nearly so. Thus, pruning prior to inoculation was not requisite for infection and apparently had little effect on time required for symptom development or on symptom severity.

In treatment iii that was inoculated 21 days later, all 15 plants had stem lesions, but none had toxin symptoms or severe damage on 6 May. Furthermore, by 6 May infection had spread to the uninoculated control treatments, iv and v, and 7 of 15 and 14 of 15 of the respective plants had small lesions, but none was obviously damaged otherwise.

No symptoms of Alternaria stem canker developed on any inoculated plants of either Ace 55 or 6718VF.

Ten samples of stem cankers were taken at random from infected plants on 6 May, and five tissue chips from each were cultured on acidified PDA. Recovery of *Alternaria,* apparently in pure culture, was 100%; i.e., 50 from 50 chips plated. Inoculations with these isolates showed them to be pathogenic on susceptible Earlypak 7, but not on resistant Ace 55.

Essentially identical results were obtained in two similar field test plots; one located about 32 miles north of San Diego, California, inoculated on 6 May 1974 and another in Ventura County near Carpinteria, California, inoculated on 1 July 1974.

DISCUSSION.—Repeated isolations of *Alternaria* from naturally occurring stem cankers, production of the full disease syndrome by inoculation in both greenhouse and field, completion of Koch's postulates and the complete agreement of natural disease occurrence with resistance or susceptibility of various cultivars in field and greenhouse inoculations seem ample evidence that the stem canker disease of tomato is caused by *A. alternata* f. sp. *lycopersici* instead of *F. oxysporum* as previously reported (2, 5).

We have found no reports of a similar disease of tomato with differential cultivar resistance caused by a small-spored-chained *Alternaria* sp. except that by Douglas in 1922 from southern California (1). His *Alternaria* sp. appeared similar in morphology to *A. alternata* and induced spotting of leaves and green fruit which clearly distinguishes it from the essentially saprophytic pathotypes of *A. alternata* that are only capable of causing rot of ripe tomato fruit (6, 7). However, no mention was made of stem-cankering which is a major symptom of the stem canker disease. Most cultivars reported by Douglas to differ in susceptibility to leaf spotting were unobtainable for testing and no cultures of his organism are available. However, we found that the cultivar Trophy that he reported susceptible to leaf spotting was resistant to stem canker.

We considered the possibility that stem canker is a variant expression of the nail head spot disease described by Weber (11). However, the nail head spot organism is distinctly different morphologically and the syndromes of the two diseases, host range and cultivar response to the respective organisms are also different. Although the cultivars Marvel and Marglobe are resistant to both organisms, the numerous other cultivars resistant to the stem canker pathogen make this of doubtful significance. Furthermore, the cultivar John Baer, reported very susceptible to nail head, is resistant to stem canker.

The stem canker disease presently is controlled in San Diego County, California by use of resistant cultivars such as 6718VF that are horticulturally acceptable fresh-market types. Hopefully this resistance will continue effective so stem canker will become a rare and possibly extinct disease as has the nail head spot disease in Florida (R. E. Stall, *personal communication*).

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