

## A New Wilt Disease of Lettuce Incited by *Fusarium oxysporum* f. sp. *lactucum* forma specialis nov.

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### ABSTRACT

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A wilt disease was observed in the fall of 1990 in one commercial lettuce field (*Lactuca sativa* 'Empire') in Fresno County, California, and in the fall of 1991 in two nearby fields (cvs. Empire and Desert Queen). The disease is characterized by the death of some plants in the seedling stage, accompanied by a red streak through the cortex of the crown and upper root. Older affected heads show a tan-to-yellow tipburn with black streaks in the vascular system of some affected leaves, brown streaks in the vascular system of the crown, and a reddish brown discoloration of the cortex of the crown and upper root. *Fusarium oxysporum* was readily isolated from all affected plants, and in greenhouse inoculations this fungus caused a wilt disease of lettuce but not of any other crop species onto which it was inoculated. All pathogenic isolates belonged to the same vegetative compatibility group. Because of its apparent specificity for lettuce, the name *Fusarium oxysporum* Schlechtend.:Fr. emend. W.C. Snyder & H.N. Hans. f. sp. *lactucum* forma specialis nov. is proposed.

In the fall of 1990, plants showing symptoms of a wilt disease were observed in a commercial field of lettuce (*Lactuca sativa* L. 'Empire') near Huron, California (Fresno County). The problem was first noticed at thinning, when some seedlings wilted and died. At harvest, affected heads showed tipburn, sometimes accompanied by yellowing of leaves and brown or black streaks in the vascular tissue. Lettuce was not planted in this field in 1991; however, in the fall of 1991, identical disease symptoms were noted in two different fields of lettuce located approximately 6 km apart and 6 km from the field where the disease was originally found. There are no reports in the literature of a wilt disease of lettuce. The present study was undertaken to describe the disease, characterize the pathogen, determine its host range and specificity, and examine cultivar susceptibility.

### MATERIALS AND METHODS

**Isolation and preservation of the fungus.** Initial isolations of *Fusarium oxysporum* Schlechtend.:Fr. in 1990 were made from mature lettuce heads obtained at harvest time. Additional samples were collected in 1990 after the crop

residue was disked under, by removing plant material from the surface of the soil in each of six locations throughout the field. In 1991, symptomatic plants were collected from several areas in each of the two fields in which the disease was found. Isolations from field samples and from samples from greenhouse inoculations were made by washing the plants with tap water, removing and discarding the epidermis (except in isolations from hosts other than lettuce), cutting tissue which included the discolored vascular system or cortex into small pieces, and surface disinfecting the pieces in 0.525% sodium hypochlorite for 3 min. Pieces of tissue were placed on 2% water agar and incubated at 24 C, and the fungi that grew out into the agar were transferred to potato-dextrose agar (PDA). Isolates from field material were transferred to PDA amended with 200 mg/L each of penicillin G potassium and streptomycin sulfate. Plates were incubated at ambient temperature (approximately 25 C) under 12 hr/day fluorescent illumination (two 34W daylight tubes). Single-spore subcultures from these colonies were recovered and stored on dried filter paper at 4 C (4).

**Determination of vegetative compatibility groups.** Nitrogen nonutilizing (*nit*) mutants were produced as previously described (3,13) for three isolates of *F. oxysporum* (HL-1, HL-2, and HL-3) obtained from three lettuce heads collected in 1990 at harvest. Mutants were characterized as to *nit* designation and used as testers to determine if *F. oxysporum* recovered from other field isolations and from greenhouse inoculations

were of the same vegetative compatibility group (VCG).

**Production of inoculum.** Isolates were grown for 10 days on PDA plates with 12 hr of light per day, after which 40 ml of tap water was added to each plate, spores were scraped from the surface with a spatula, and the suspension was filtered through one layer of muslin. The concentration of spores was determined by hemacytometer and adjusted with tap water to  $5 \times 10^6$  spores per milliliter.

**Pathogenicity tests.** Seeds were planted in steamed river sand. Lettuce and tomatoes were planted in 10-cm<sup>2</sup> pots with several seeds per pot, and cotton and muskmelon were sown individually in 2.5-cm<sup>2</sup> planting cells. Plants were grown for 10-14 days and then inoculated by a root dip method (18). Roots were washed, trimmed to 5 cm, and soaked in a spore suspension for 10-20 min. Inoculated seedlings were then transplanted into steamed river sand in individual Cone-Tainers (tubes 1.5 in. wide, 5.5 in. long) and maintained in a greenhouse at approximately 25 C. Plants were observed periodically for disease development and given a final disease rating 3-4 wk after inoculation. Plants that were dead or yellow and severely stunted when compared with the controls were rated positive for wilt.

Pathogenicity tests of field isolates were performed with HL-1, HL-2, and HL-3. Eight plants each of lettuce cultivars Empire and Salinas were inoculated with each isolate. Control plants were prepared similarly but soaked in plain tap water instead of a spore suspension.

**Morphological and cultural characterization of the fungus.** For microscopic observation of spores and spore-bearing structures, fungal isolates HL-1, HL-2, and HL-3 were grown on carnation-leaf agar (CLA) (12) for 10-14 days under fluorescent illumination (12 hr/day) at approximately 25 C. Fungal structures were observed directly on the plates by placing a cover slip on the agar, and hyphae and spores were also observed in water mounts.

Gross cultural characteristics were determined with 10-14-day-old cultures of isolates HL-1, HL-2, and HL-3 grown on PDA under fluorescent illumination (12 hr/day) at approximately 25 C. The

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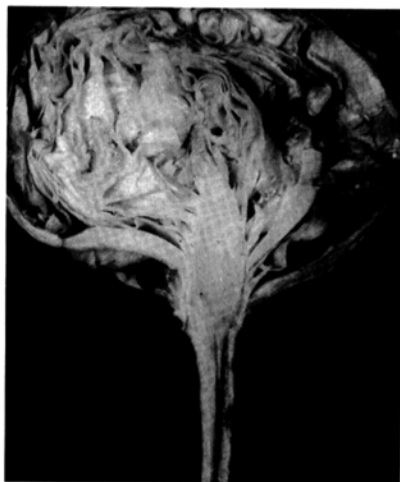


Fig. 1. Empire lettuce showing symptoms of infection by *Fusarium oxysporum*. Tipburn of the leaves and discoloration of the cortex of the crown and upper root are apparent.

effect of temperature on growth rate was determined by plating 5-mm squares of isolate HL-1 (from 7-day-old PDA cultures) onto 10-cm PDA plates. Cultures were grown for 6 days in incubators set at 8, 12, 16, 20, 24, 28, 32, and 36  $\pm$  1 C (four plates per temperature), after which the diameter of the colony was measured and 5 mm was subtracted for the width of the original agar block (14). The experiment was repeated four times at each temperature.

**Host range.** Crop species which are grown in the area of California where the lettuce wilt occurred and which are susceptible to various *Fusarium* wilt diseases were selected. Lettuce isolate HL-1 was used to inoculate lettuce cv. Empire, tomato cv. EP 7 (a breeding line susceptible to the three tomato races of *Fusarium* wilt), muskmelon cv. Topmark, watermelon cv. Chilean Black, and cotton cv. Acala SJ-2. Empire lettuce was inoculated with the following formae speciales of *F. oxysporum*, as were the known hosts of these pathogens: *F. o. lycopersici* races 1, 2, and 3 (tomato); *F. o. melonis* race 2 (muskmelon); *F. o. niveum* (watermelon); and *F. o. vasinfectum* (cotton). Ten plants per test were inoculated with  $5 \times 10^6$  spores per milliliter. Test plants were rated for disease 4 wk after inoculation.

**Cultivar susceptibility.** Lettuce cultivars typically grown in the affected area were chosen. Autumn Gold, Empire, Excell, Salinas, Vanguard, Vanguard 75, Vanmax, Viva, and Winterset were inoculated with isolate HL-1 as described above, at  $5 \times 10^6$  spores per milliliter. Plants were rated for disease 4 wk after inoculation. All cultivars were tested at least twice. Because of erratic germination in some of the cultivars, the number of plants in each replicate varied, but at least ten plants were tested per cultivar.

**Effect of inoculum concentration on disease severity.** Inoculum was grown as before and spore concentrations were

Table 1. Disease reactions of lettuce cvs. Empire and Salinas to isolates of *Fusarium oxysporum*

Cultivar	Disease reaction	Isolate		
		HL-1	HL-2	HL-3
Empire	Plants dead	8/8 <sup>a</sup>	2/8	0/8
	Plants wilted	0/8	6/8	7/8
	No reaction	0/8	0/8	1/8
Salinas	Plants dead	0/8	0/8	0/8
	Plants wilted	2/8	0/8	0/8
	No reaction	6/8	8/8	8/8

<sup>a</sup> No. of plants affected/total no. of plants inoculated.

adjusted with tap water to 0,  $5 \times 10^2$ ,  $5 \times 10^3$ ,  $5 \times 10^4$ ,  $5 \times 10^5$ , and  $5 \times 10^6$  spores per milliliter. Twenty Empire lettuce plants were inoculated with each spore concentration, and plants were rated for disease at weekly intervals for 7 wk.

## RESULTS

**Symptomatology.** This disease was first noticed in the field at thinning, when some seedlings appeared wilted or dead. Inner tissues of affected seedlings appeared red or brown. At harvest, affected heads showed tipburn, sometimes accompanied by yellowing of leaves and brown or black streaks in the vascular system. Some plants were stunted or did not form heads. Discolored vascular streaks in yellow leaves extended from the crown and were continuous with a red-brown discoloration in the cortex and vascular system of the crown and upper taproot (Fig. 1). In many cases symptoms were not visible on the outside of the crowns or roots.

**Isolation and pathogenicity.** *F. oxysporum* was readily isolated, generally in pure culture, from the red-brown cortical areas of the crowns and stems of the three heads of lettuce obtained at harvest from the field affected in 1990. In addition, *F. oxysporum* was obtained from the blackened vascular streaks in one of the heads. All 14 isolates tested from these heads were vegetatively compatible. After harvest, lettuce heads were collected from six evenly spaced areas of that field. The disease had been noted in only one of these areas. Of five lettuce heads collected from the diseased section, *F. oxysporum* of the same vegetative compatibility group (VCG) was obtained from four, but not from any of the 17 heads collected from the other sections of the field. No other fields exhibiting disease symptoms were located in 1990. In 1991, *F. oxysporum* of the same VCG was readily isolated from all 24 symptomatic plants collected from several areas of the two additional fields where the disease was found.

All three of the fungal isolates (HL-1, HL-2, and HL-3) used in the initial pathogenicity tests caused typical wilt symptoms on Empire lettuce. In these tests, cv. Salinas appeared resistant to the disease (Table 1). With the exception of one plant, inoculated Empire plants either



Fig. 2. Empire lettuce inoculated with *Fusarium oxysporum* isolate HL-1 (left and center) and noninoculated (right). Note blackened necrotic taproots of inoculated plants.

died or were severely stunted. Stunted Empire plants showed dark streaking of the vascular tissue extending from the taproot into the stem, and the root systems were much smaller than those of the controls (Fig. 2). In contrast, only two of the inoculated Salinas plants showed stunting, and the internal appearance of these plants was different from that of the infected Empire plants. Instead of a darkened vascular system, the affected Salinas plants showed diffuse brown streaking in the cortex of the underground area of the crown. Additionally, 10 of 16 of the Salinas plants which were not stunted showed similar diffuse brown streaking in the crown. Root systems did not appear to be stunted in any of the inoculated Salinas plants. *F. oxysporum* of the original VCG was reisolated from all five symptomatic Empire lettuce plants tested, and from the brown streaked areas of all eight Salinas lettuce tested, but not from six noninoculated plants. Fungal isolate HL-1 appeared to be the most virulent (Table 1) and was used for all further inoculations.

**Cultural characteristics of the fungus.** All isolates grew rapidly on PDA, producing white aerial mycelia and orange sporodochia. The underside of the colonies was peach colored with purple pigment diffusing into the agar starting at the center of the colony and pro-

gressing outward as the colony aged. Growth occurred in HL-1, the isolate used in the temperature studies, between 8 and 32 C, with maximum growth at 28 C (78 mm of growth in 6 days). At 8 and 32 C, there was 2 and 0.5 mm of growth in 6 days, respectively.

**Morphological characteristics of the fungus.** The cultural and morphological characteristics of this fungus corresponded closely to the type description (12). Most macroconidia were three-septate, but four-septate conidia were occasionally seen. Chlamydoconidia were present in abundance after 10 days on CLA, and were terminal or intercalary, mostly single but sometimes in pairs or clumps.

**Effect of inoculum concentration on disease severity.** Control plants and plants inoculated with  $5 \times 10^2$  spores per

milliliter showed no wilt throughout the course of the experiment. However, after 6 wk, 100% of the Empire plants inoculated with as few as  $5 \times 10^4$  spores per milliliter of HL-1 showed wilt symptoms. When a concentration of  $5 \times 10^6$  spores per milliliter was used, 100% of the plants showed wilt symptoms after 4 wk (Fig. 3). To avoid ambiguity in disease ratings, a concentration of  $5 \times 10^6$  spores per milliliter was used for further inoculations, and tests were rated 3-4 wk after inoculation.

**Host range.** None of the other crop species (tomato, muskmelon, watermelon, and cotton) inoculated with HL-1 showed symptoms of wilt, and lettuce inoculated with the other formae speciales (*F. o. lycopersici* races 1, 2, and 3; *F. o. melonis* race 2; *F. o. niveum*; and *F. o. vasinfectum*) showed no symp-

toms of wilt. In addition, all isolates caused typical wilt symptoms on all of their usual suscept except for *F. o. lycopersici* race 1 on tomato, which caused wilt in seven of the 10 plants inoculated.

Although all isolates of *F. oxysporum* caused wilt only on their respective hosts, these fungi did have other effects on "nonhosts." Fresh weights of muskmelon and watermelon inoculated with lettuce isolate HL-1 were significantly reduced compared to noninoculated controls. The average weight of noninoculated muskmelon was 7.174 g (SD 0.445), while the plants inoculated with HL-1 averaged 3.486 g (SD 1.819). Noninoculated watermelon averaged 7.169 g (SD 1.177), while inoculated plants averaged 4.412 g (SD 0.732). The fresh weight of Empire lettuce was significantly reduced when it was inoculated with the other formae speciales, except with *F. o. lycopersici* race 2 (Fig. 4). Plant species which were not suscept, but which were affected by the other formae speciales, generally looked normal above ground except that they were smaller than the controls. The crown below the soil line often showed corky, superficial lesions (Fig. 5). The root systems looked normal in color except for the corky lesions that sometimes extended down the taproot. If the lesions were extensive, the number of secondary roots was reduced compared to the noninoculated controls. When examined internally, none of these plants showed any of the symptoms characteristic of a wilt pathogen on a susceptible host (i.e., vascular browning accompanied by yellowing, wilting, stunting, and/or death of the plant). Nor was there any of the brown streaking of the cortex HL-1 caused in Salinas lettuce. HL-1 was reiso-

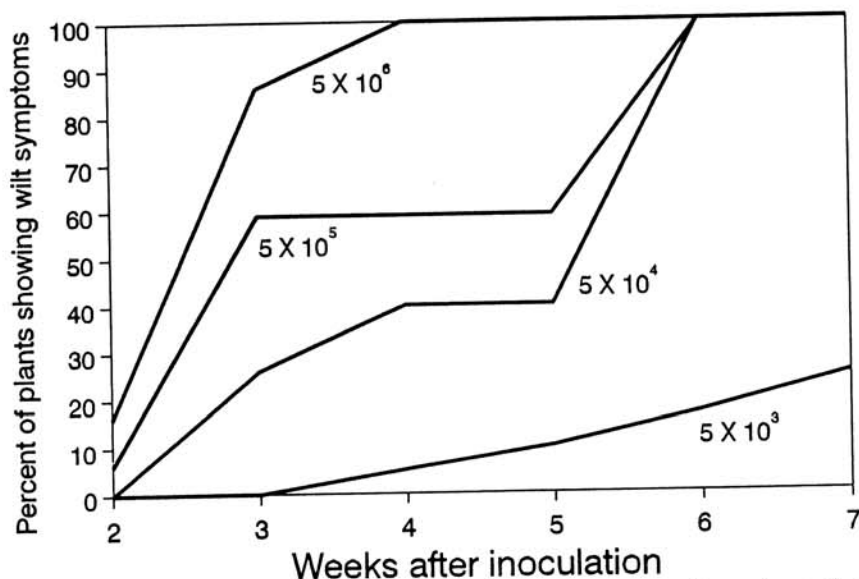


Fig. 3. Effect of concentration of spores of *Fusarium oxysporum* on wilt symptoms of cv. Empire lettuce at the indicated times after inoculation.

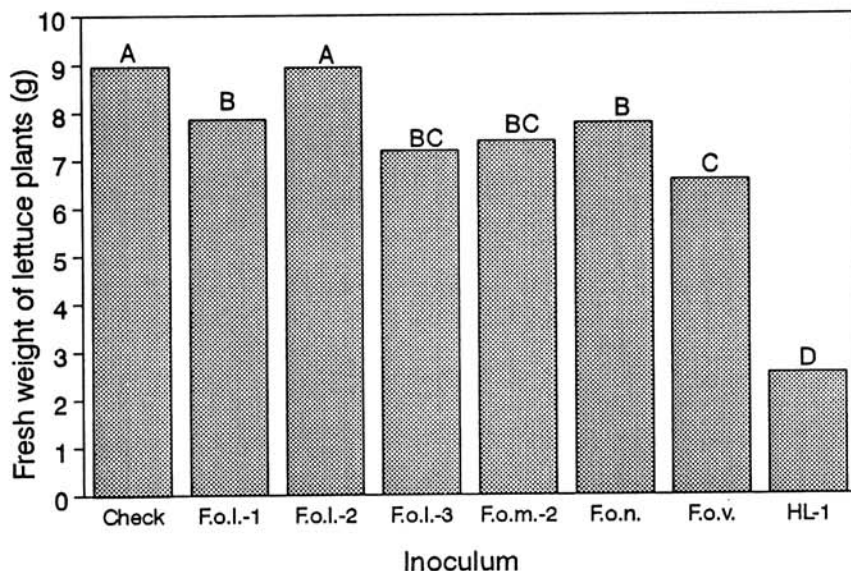


Fig. 4. Effect of inoculation with various formae speciales of *Fusarium oxysporum* on the fresh weight of cv. Empire lettuce. F.o.l.-1, -2, and -3 = *F. o. lycopersici* races 1, 2, and 3; F.o.m.-2 = *F. o. melonis* race 2; F.o.n. = *F. o. niveum*; and F.o.v. = *F. o. vasinfectum*. HL-1 is an isolate of *F. oxysporum* which causes wilt of lettuce.

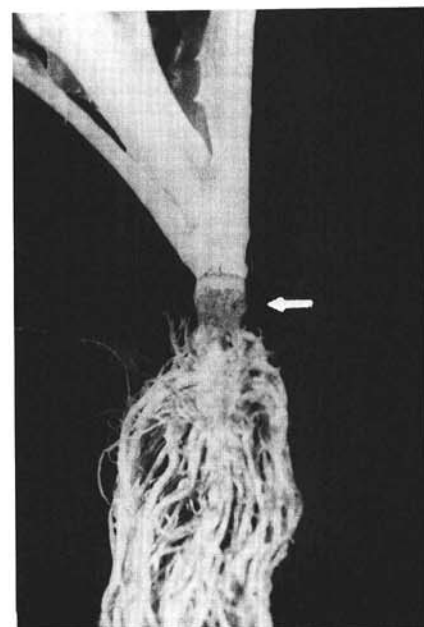


Fig. 5. Corky epidermal lesion on lettuce (arrow) caused by inoculation with *Fusarium oxysporum* f. sp. *melonis* (a pathogen of melon).

**Table 2.** Lettuce cultivar susceptibility to isolate HL-1 of *Fusarium oxysporum*

Cultivar	Plants tested (no.)	Plants in each disease category (%) <sup>a</sup>			
		0 <sup>b</sup>	1	2	3
Autumn Gold	10	40	20	20	20
Empire	48	25	11	31	34
Excell	24	29	4	54	13
Salinas	36	67	14	19	0
Vanguard	20	0	0	30	70
Vanguard 75	10	0	0	30	70
Vanmax	15	0	0	20	80
Viva	20	0	20	45	35
Winterset	20	0	20	20	60

<sup>a</sup> Plants were scored for disease 4 wk after inoculation.

<sup>b</sup> 0 = No disease, 1 = plants stunted compared to controls, 2 = plants severely stunted and yellow, and 3 = plants dead.

lated from surface-disinfested, unpeeled sections of the crowns of all hosts except for melon, and all formae speciales were reisolated from the surface-disinfested sections of Empire lettuce onto which they had been inoculated. The various formae speciales, including the lettuce pathogen, were confirmed by vegetative compatibility comparisons to be the original isolates used for inoculation.

**Cultivar susceptibility.** All lettuce cultivars tested showed some susceptibility to HL-1, with Salinas the most tolerant and Vanguard, Vanguard 75, and Vanmax the most susceptible (Table 2).

## DISCUSSION

Fusarium wilt of lettuce was found in three fields, one in 1990 and two in 1991, all within approximately 6 km of each other near Huron, California. In 1990, the disease was confined to a large area along approximately one-third of one edge of a field. One of the fields affected in 1991 showed two areas of approximately 10 × 30 m each in which the disease was most severe; one of these areas contained both of the cultivars of lettuce (Empire and Desert Queen) planted in that field. The other field affected in 1991 showed one large area in which the disease was most severe, and both of the fields affected in 1991 also showed scattered diseased plants throughout the rest of the crop. Both of these fields had grown prior crops of spring lettuce in which no disease was noted, indicating that cropping season may play a role in disease development. The spring crops were planted in November of the previous year and grown over the winter when temperatures were relatively cool, while in both 1990 and 1991 the fall lettuce was grown under relatively hot conditions.

*F. oxysporum* was readily isolated from the red-brown cortical areas of the stems and crowns of all affected plants tested from all three fields, and often from the darkened vascular streaks in the leaves. All isolates recovered were of the same VCG, suggesting pathogenicity, because VCG diversity is generally high in nonpathogenic and weakly pathogenic populations of *F. oxysporum* (5,8,13). Inoculations with this organism showed

it to cause wilt on lettuce seedlings, but not on tomato, muskmelon, watermelon, or cotton.

The means of introduction to the three affected fields and the means of spread of this disease are unknown; but as with other Fusarium wilt diseases, the causal organism might be spread by movement of infested soil, water, and/or seed. If the pathogen was introduced into these fields on seed, it was probably not the seed used to plant the crops in the seasons in which the disease was noted. Remnants of this seed were not available, but the fields involved belonged to three different growers and were planted for two different buyers (who provided the seed), which makes it unlikely that the same seed lot was used for all three fields. One of the fields affected in 1991 was planted with two different cultivars of lettuce, both of which showed the disease. Also, the fact that the disease occurred in large patches (in one case overlapping two lettuce cultivars) rather than scattered throughout the fields suggests introduction of the organism prior to the crops in which the disease was noted, or from a source other than seed. Tomatoes and melons were grown previously in these fields, and both are hosts to Fusarium wilt diseases. It is possible that the *F. oxysporum* which caused the wilt disease on lettuce arose from a wilt-causing *F. oxysporum* already present in the area; however, isolates obtained from lettuce were specific for lettuce in our tests and caused wilt on neither tomatoes nor melons nor any other plant species on which inoculations were attempted.

Regarding the effects of this *F. oxysporum* on other crop species, as well as the effects of the other formae speciales on lettuce, it appears that the ability to colonize "nonsusceptible" species is common among the wilt-causing formae speciales of *F. oxysporum* (9,10,11,17). Also, non-wilt-causing pathogenic and nonpathogenic isolates of *F. oxysporum* and other *Fusarium* species commonly colonize tissues of a wide range of plant species on which they cause no disease (1,7,9,15,16); and there is ample evidence for the formation of brown superficial lesions on the roots of various plant species by non-wilt-causing

*F. oxysporum* and other *Fusarium* spp. (1,6,7,15,16). The present study would indicate that the wilt-causing *F. oxysporum* formae speciales can, under certain conditions, cause the formation of superficial brown lesions on plant roots onto which they are inoculated; this capacity would appear to exist independently of their ability to cause wilt. In addition, the formae speciales of *F. oxysporum* used in this study caused stunting of some of the nonhosts onto which they were inoculated. The brown streaking of the cortex which HL-1 caused in Salinas lettuce appears to be typical of a resistant cultivar of a species which is susceptible to a wilt forma of *F. oxysporum* (11).

At present, this wilt disease of lettuce has been noted in only a small geographic area; however, the fact that two new fields were found in the second year of our observations and that three different growers are involved suggests a potential for spread. The disease in the most heavily affected areas of these fields was severe enough to preclude harvest.

We propose that the causal organism of Fusarium wilt of lettuce be designated *Fusarium oxysporum* Schlechtend.:Fr. emend. W.C. Snyder & H.N. Hans. f. sp. *lactucum* forma specialis nov. Isolate HL-1 is designated as the type culture of the pathogen, and cultures have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 (ATCC 76616).

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