Host Range of *Alternaria alternata* f. sp. *cucurbitae* Causing Leaf Spot of Cucumber

DEMETRIOS JOHN VAKALOUNAKIS, Plant Protection Institute, Heraklio, Crete, Greece

ABSTRACT

Vakalounakis, D. J. 1990. Host range of *Alternaria alternata* f. sp. *cucurbitae* causing leaf spot of cucumber. Plant Dis. 74:227-230.

Sixty-two cultivated and weed species in 16 botanical families were artificially inoculated or exposed to natural infection in greenhouse experiments. Of these, 27 species of Cucurbitaceae were found to be susceptible to a forma specialis of *Alternaria alternata* that attacks cucumber. To differentiate this *A. alternata* pathotype from others, it is proposed that it be designated *Alternaria alternata* f. sp. *cucurbitae*.

During the 1979–1980 crop season, a severe leaf spot disease of cucumber (*Cucumis sativus* L.) was noticed on greenhouse crops grown in some plastic houses in the Sitia area of Lasithi, Crete, Greece, along the coastal strip between Koutsouras and Goudouras (9). Since then, the disease has spread to other areas, and during 1987–1988 it caused severe losses in most of the cucumber-growing areas in Crete.

Accepted for publication 31 July 1989.

Symptoms appear in late autumn, mainly on the leaves of the middle and upper part of the plants. Necrotic flecks, surrounded by chlorotic halos, appear on the leaf, and these enlarge to spots that may coalesce to form circular lesions up to 5 cm or more in diameter. The lesions bear black-brown conidiophores and conidia of the pathogen. Severely infected leaves become yellow, grow senescent, and die. No other part of the plant is affected. During the winter, relative humidity in the plastic houses is high and plant vigor is reduced due to fruit bearing and unfavorable microclimatic conditions (reduced illumination and average air temperature lower than 15 C). As a result, infection progresses rapidly throughout the crop and causes severe damage within a few days (10). The pathogen of this leaf spot disease of cucumber was identified as *Alternaria alternata* (Fr.) Keissler (10).

This report concerns experiments designed to determine the pathogen host range in cultivated and wild species and to characterize the strains of *A. alternata* that attack cucumber.

MATERIALS AND METHODS

Greenhouse experiments with artificially inoculated plants. Two experiments with artificially inoculated plants were carried out during the 1986–1987 and 1987–1988 crop seasons in greenhouses of the Plant Protection Institute, Heraklio, Crete. Fourteen young cucumber plants of the highly susceptible cultivar Pepinex 69 were planted in two rows in each experimental plot (4 × 2 m) to increase inoculum potential in greenhouses and facilitate possible secondary infections of potential hosts. Three days after planting the cucumbers, potential hosts were planted between the two cucumber rows. Weeds were allowed to grow in the experimental plots to determine whether they might become hosts of the pathogen. Experiments were arranged in randomized complete-block designs with four replicates of seven plants each per potential host. Plants were grown in sterilized soil according to local commercial procedures.

Isolate DJV 9 of *A. alternata* was obtained from a diseased cucumber plant in a commercial greenhouse near Koutsouras in the Sitia area of Crete and used for artificial inoculations. Inoculum was prepared by growing the fungus for 7 days on acidified potato-dextrose agar (APDA) containing 4.5 ml of 25% lactic acid in 1 L of PDA (final pH 4). The plates were kept at room temperature under Daylight fluorescent lamps to induce sporulation. Conidia were washed off with tap water and filtered through cheesecloth.

In the first experiment, potential hosts were planted on 17 October 1986. Artificial inoculations were performed on 27 November, 15 December, and 24 December 1986 using spore suspensions containing approximately 6×10^5 spores per milliliter (as estimated by hemacytometer counts). Final observations of disease development were made on 30 April 1987.

Potential hosts in the second experiment were all individuals from Cucurbitaceae species; they were planted on 7 October 1987. Artificial inoculations were performed on 9 December 1987, using spore suspensions containing 1.8×10^4 spores per milliliter, and again on 22 December, using suspensions containing 2.1×10^3 spores per milliliter. Final observations of disease development were made on 2 March 1988.

Greenhouse experiments with plants exposed to natural infection. Potential hosts from species belonging to several plant families were planted during the 1986–1987 crop season in plastic houses in which cucumbers were grown. These houses belonged to 14 growers in the Sitia and Ierapetra areas, Lasithi, Crete, where *A. alternata* had persistently caused severe losses of cucumber crops. In these greenhouses, growers planted the highly susceptible cucumber cultivar Brunex between 5 and 20 October 1986. Potential hosts were planted among the cucumber rows on 20 October according to a completely randomized design. Three replicate plantings were done for each host and greenhouse. Final observations of disease development were made on 7 and 8 April 1987.

In all experiments, sections were cut from the margins of lesions on infected plants, immersed in 0.5% NaOCl for 5 min, rinsed in sterile water, and then plated on APDA to confirm the presence of *A. alternata.* A leaf spot index (LSI) based on a 0–5 visual scale was used to score susceptibility to infection by the pathogen. The scale was based on the

Table 1. Symptoms of *Alternaria alternata* infection among artificially inoculated potential hosts in greenhouse experiments

Family	Species	LSI[a]
Compositae	*Lactuca sativa* L.	0
Cruciferae	*Brassica oleracea* L. var. *capitata*	0
Cucurbitaceae	*Benincasa hispida* (Thunb.) Cogn.	1-3
	Citrullus lanatus (Thunb.) Mansf.	3-4
	Cucumis africanus L.	0-1
	C. anguria L.	2-3
	C. dipsaceus Ehrenb.	1
	C. ficifolius A. Rich	1-2
	C. hardwickii Royle	1-3
	C. longipes Hook.	2-3
	C. melo L.	3-4
	C. pustulatus	3-4
	C. sativus L. (control plants)	5
	Cucurbita ficifolia Bouche	1
	C. foetidissima Kunth.	1
	C. lundelliana Bailey	1-2
	C. maxima Duch.	1
	C. mixta Pang.	2-3
	C. moschata (Duch.) Poir.	2-3
	C. palmata Wats.	2-3
	C. pepo L.	1-3
	C. sororia	2-3
	C. texana A. Gray	1
	Ecballium elaterium (L.) A. Rich	0-1
	Lagenaria leucantha Rusby var. *clavata* Makino	4-5
	L. siceraria (Mol.) Standl. subsp. *asiatica* (Kob.) Heiser	4-5
	L. vulgaris Ser.	4-5
	Luffa cylindrica Roem.	1-2
	Momordica charantia L.	2-3
Leguminosae	*Phaseolus indica* All.	0
Rosaceae	*Fragaria vesca* L.	0
Solanaceae	*Capsicum annuum* L.	0
	Lycopersicon esculentum Mill.	0
	Nicotiana tabacum L.	0
	Solanum melongena L.	0

[a] Leaf spot index, a 0–5 scale based on number and size of lesions and percentage of leaf area affected on the whole plant, where 0 = no symptoms; 1 = few necrotic flecks surrounded by chlorotic halos, 0–1% affected; 2 = many necrotic flecks and/or few necrotic spots surrounded by chlorotic halos, 1–8% affected; 3 = necrotic spots usually surrounded by chlorotic halos, 8–20% affected; 4 = small necrotic lesions usually surrounded by chlorotic halos (which may coalesce), 20–50% affected; and 5 = large necrotic lesions and leaf tissue collapse, 50–100% affected.

Table 2. Weed species observed in greenhouse experiments

Family	Species[a]
Amaranthaceae	*Amaranthus retroflexus* L.
	Gomphrena globosa L.
Chenopodiaceae	*Chenopodium album* L.
Compositae	*Aster squamatus* (Spr.) Hier.
	Erigeron canadensis L.
	Lactuca serriola L.
	Sonchus asper (L.) Hill.
Convolvulaceae	*Convolvulus arvensis* L.
Cyperaceae	*Cyperus longus* L. var. *badius* Desf.
Geraniaceae	*Erodium malacoides* (L.) Willd.
Graminae	*Setaria viridis* (L.) Beauv.
Leguminosae	*Glycyrriza glabra* L.
	Medicago polymorpha L.
	Melilotus indica (L.) All.
Oxalidaceae	*Oxalis corniculata* L.
	O. pes-caprae L.
Urticaceae	*Urtica urens* L.

[a] At least 30 plants of each species were observed. No weed species showed symptoms of infection by *Alternaria alternata* during the experiments.

number and size of lesions and the percentage of leaf area affected on the whole plant, where 0 = no symptoms, 0% of leaf area affected; 1 = few necrotic flecks surrounded by chlorotic halos, 0–1% affected; 2 = many necrotic flecks and/or few necrotic spots surrounded by chlorotic halos, 1–8% affected; 3 = necrotic spots usually surrounded by chlorotic halos, 8–20% affected; 4 = small necrotic lesions usually surrounded by chlorotic halos (which may coalesce), 20–50% affected; and 5 = large necrotic lesions and leaf tissue collapse, 50–100% affected. The mean LSI was determined when the LSI of control plants of *C. sativus* was close to 5.

Tests for pathogenicity of isolates of *A. alternata* obtained from various plant species. Nine isolates of *A. alternata* obtained from various sources (tomato, sunflower, pear, and citrus) were tested for pathogenicity on cucumber, melon, and watermelon. These tests were carried out in small greenhouses by mist-spraying 15 plants of each cucurbit species per isolate. Artificial inoculations with each isolate were carried out on 1 and 5 December 1987 and on 13 January 1988. Inoculum concentrations for all isolates and inoculations were approximately 6×10^5 spores per milliliter. Final observations were made on 26 February 1988.

RESULTS

Greenhouse experiments with artificially inoculated plants. Results of the two greenhouse experiments are given in Table 1. In the first experiment, five of 13 plant species tested belonging to the Cucurbitaceae were found to be susceptible to infection by *A. alternata*, with symptom severity varying among the species. Control plants of *C. sativus* were severely infected (LSI = 5). The remaining eight species from five different families showed no symptoms. In the second experiment, all 26 cucurbitaceous species tested showed slight to severe disease symptoms. Again, severity varied depending upon the species. None of the weed species present in either experiment (Table 2) showed any disease symptoms. Observations were made on at least 30 plants from each weed species.

Greenhouse experiments with plants exposed to natural infection. Results of these experiments are shown in Table 3. Of 22 plant species tested in all greenhouses, only five Cucurbitaceae species were susceptible to the pathogen. The remaining 17 species from six different families were symptomless.

In all trials, *A. alternata* was exclusively and consistently isolated from samples taken from cucurbitaceous species when pieces of infected tissue were plated out on petri dishes with APDA.

Tests for pathogenicity of isolates of *A. alternata* obtained from various plant species. None of nine isolates of *A. alternata* obtained from tomato, sunflower, pear, and citrus was pathogenic on cucumber, melon, or watermelon.

DISCUSSION

Of the 62 cultivated and weed species from 16 different botanical families that were artificially inoculated or exposed to natural infection in greenhouse experiments, 27 species of Cucurbitaceae had symptoms of infection by isolates of *A. alternata* that attack cucumber. Disease severity varied among the species. The most susceptible hosts included *C. sativus, C. melo* L., *Citrullus lanatus* (Thunb.) Mansf., *Lagenaria vulgaris* Ser., *L. siceraria* (Mol.) Standl. subsp. *asiatica* (Kob.) Heiser, and *L. leucantha* Rusby var. *clavata* Makino.

The susceptibility of melon (*C. melo*) and watermelon (*Citrullus lanatus*) to the pathogen is of particular concern in Crete. These crops are often grown in the same greenhouses as cucumbers, usually as a second crop in late winter (late January) when the greenhouse microclimate (cool and humid) favors disease development. Consequently, early infections (between January and April) of melon and watermelon may occur in the near future, particularly if severe *A. alternata* infection of the preceding cucumber cultures increases the inoculum levels in some greenhouses.

Alternaria infections similar to those described in this paper are very common on cucumber but are caused by *A. cucumerina* (Ellis & Everh.) J. A. Elliott (synonym *A. brassicae* (Berk.) Sacc. f. *nigrescens* Pegl.) (1,5) or *A. pluriseptata* Karst. & Har. (synonyms *A. cucurbitae* Let. & Roum., *Stemphylium ilicis* Tengwall, *Ulocladium cucurbitae* (Let. & Roum.) Simmons, *U. atrum* G. Preuss) (4,8). These fungi are distinct from *A. alternata* in conidium size and morphology (1,4,5,7,8,10). In *A. cucumerina*, conidia have obclavate or rostrate shapes and are solitary or, occasionally, in chains of two. The conidia have an overall length of 130–220 (av. 180) μm and width of 15–24 (av. 20) μm in the broadest part. Beaks (106–135 μm long) are often much longer than the bodies of the spores (1). In *A. pluriseptata*, conidia are obovoid or, most commonly, subspherical or sarciniform, with a length of 16.5–19.8 (av. 18.6) μm and width of 13.2–18.7 (av. 16) μm in the broadest part. Bases are broadly conical or round and apexes are broadly rounded. Conidia of *A. pluriseptata* are solitary or, very rarely, in chains of two through the production of a short conidiophore (false beak). True beaks are absent (7). In *A. alternata*, on the other hand, conidia are formed in long, often branched chains and frequently have a short conical or cylindrical beak. The length of this beak can be up to one-third the length of the conidium. Conidia are ovoid, obclavate, or ellipsoidal, with an overall length of 20–63 (av. 37) μm and width of 9–18 (av. 13) μm in the broadest part (1).

Table 3. Symptoms of *Alternaria alternata* infection among naturally infected potential hosts in greenhouse experiments

Family	Species	LSI[a]
Chenopodiaceae	*Beta vulgaris* L.	0
	Spinacia oleracea L.	0
Compositae	*Cichorium endivia* L.	0
	C. intybus L.	0
	Lactuca sativa L.	0
Cruciferae	*Brassica oleracea* L. var. *capitata*	0
	Raphanus sativus L.	0
Cucurbitaceae	*Cucumis sativus* L. (control plants)	3-5
	Cucurbita moschata (Duch.) Poir.	1-3
	C. pepo L.	1-3
	Lagenaria vulgaris Ser.	3-5
	Luffa cylindrica Roem.	1-3
	Momordica charantia L.	1-3
Leguminosae	*Phaseolus vulgaris* L.	0
	Vicia faba L.	0
Liliaceae	*Allium cepa* L.	0
Solanaceae	*Capsicum annuum* L.	0
	Lycopersicon esculentum Mill.	0
	Nicotiana tabacum L.	0
	N. glutinosa L.	0
	Solanum melongena L.	0
Umbelliferae	*Apium graveolens* L.	0
	Daucus carota L.	0

[a] Leaf spot index, a 0–5 scale based on number and size of lesions and percentage of leaf area affected on the whole plant, where 0 = no symptoms; 1 = few necrotic flecks surrounded by chlorotic halos, 0–1% affected; 2 = many necrotic flecks and/or few necrotic spots surrounded by chlorotic halos, 1–8% affected; 3 = necrotic spots usually surrounded by chlorotic halos, 8–20% affected; 4 = small necrotic lesions usually surrounded by chlorotic halos (which may coalesce), 20–50% affected; and 5 = large necrotic lesions and leaf tissue collapse, 50–100 affected.

The pathogen isolated in the greenhouse experiments is undoubtedly *A. alternata* (10) as reported by Groves and Skolko (3), Neergaard (6), and Simmons (7). However, it is clearly different from known formae speciales of *A. alternata* because of its pathogenicity to Cucurbitaceae species. Because of this, a subspecific forma specialis designation should be used for isolates of *A. alternata* causing leaf spotting of cucurbits. Accordingly, I propose to designate the fungus *Alternaria alternata* (Fr.) Keissler f. sp. *cucurbitae*, following the precedent of Grogan et al (2), who named the fungus causing a stem canker disease of tomatoes *A. alternata* f. sp. *lycopersici*. A culture of *A. alternata* f. sp. *cucurbitae* has been deposited at the Commonwealth Agricultural Bureaux International Mycological Institute (Kew, Surrey, England) and assigned the accession number IMI 325055.

ACKNOWLEDGMENTS

I thank W. R. Jarvis (Agriculture Canada, Harrow, Ontario) for his critical reading of the manuscript and Z. Kypriotakis (agronomist, Municipality of Heraklio) for his valuable assistance in the identification of weed species. I gratefully acknowledge the following for supplying seeds of cucurbitaceous species: National Institute of Agrobiological Resources (NIAR), Japan; the Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), Costa Rica; National Board for Plant Genetic Resources (NBPGR), India; Institute of Plant Introduction and Genetic Resources (IPBR), Bulgaria; Plant Introduction Station, Iowa State University, United States; Zentralinstitut fur Genetik und Kulturpflanzenforschung (ZIGuK), German Democratic Republic; and J. Staub, Department of Horticulture, University of Wisconsin, Madison, United States. I also thank ICI Hellas Co. for its financial support for the publication of this article.

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