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Wilt of Hardy Chrysanthemum Caused by a New Race of *Fusarium oxysporum* f. sp. *chrysanthemi*

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ABSTRACT

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A severe natural outbreak of chrysanthemum wilt caused by *Fusarium oxysporum* was observed in a nursery at the Agriculture Canada Research Station, Morden, Manitoba, Canada, in 1979. Pathogenicity tests using six differential chrysanthemum cultivars showed that this wilt pathogen (DAOM 175160) is different from the reported chrysanthemum wilt pathogens, *F. oxysporum* f. sp. *chrysanthemi* and *F. oxysporum* f. sp. *tracheiphilum* race 1. This new race is designated *F. oxysporum* f. sp. *chrysanthemi* race 2.

Key words: hardy chrysanthemum, *Chrysanthemum morilifolium*, Fusarium wilt, *Fusarium oxysporum*, physiological race.

INTRODUCTION

Toop (8) reported that *Fusarium* wilt of chrysanthemum (*Chrysanthemum morilifolium* Ramat.) was caused by *Fusarium oxysporum* f. sp. *callistephi* (Beach) Snyd. & Hans. However, other workers reported that the causal organism was not *F. oxysporum* f. sp. *callistephi*, but was either *F. oxysporum* f. sp. *tracheiphilum* (E. F. Smith) Snyd. & Hans. race 1 Armst. & Armst. (1) or *F. oxysporum* f. sp. *chrysanthemi* Litt., Armst. & Armst. (2). *F. oxysporum* f. sp. *chrysanthemi* was pathogenic to the chrysanthemum cv. Encore and Yellow Delaware, but *F. oxysporum* f. sp. *tracheiphilum* race 1 was pathogenic to the cowpea (*Vigna sinensis* (Torner) Savi) and the chrysanthemum cv. Encore, but not cv. Yellow Delaware (2).

In Canada, *Fusarium* wilt of chrysanthemum caused by *F. oxysporum* Schlecht was reported in British Columbia (6), Ontario (4), and Quebec (5). Gordon (6) isolated *F. oxysporum* from basal stems of diseased chrysanthemums collected from Manitoba in 1938, but whether the disease was *Fusarium* foot rot or *Fusarium* wilt was not reported.

In 1979, a severe outbreak of *Fusarium* wilt of chrysanthemums was observed in a nursery of about 0.5 hectare at the Agriculture Canada Research Station, Morden, Manitoba, Canada. All the cultivars and lines in this field were hardy chrysanthemums which survive well under the Canadian prairie winter conditions (7). *F. oxysporum* was isolated from roots and basal stems of wilted plants. This study was undertaken to determine the forma specialis and race designation of the *F. oxysporum* pathogen isolated from wilted chrysanthemum in Manitoba by comparing its pathogenicity with *F. oxysporum* f. sp. *chrysanthemi* and *F. oxysporum* f. sp. *tracheiphilum* race 1 on differential chrysanthemum cultivars.

MATERIALS AND METHODS

Disease in the field

During September, 1979, ten experimental lines of hardy chrysanthemum in a breeding nursury at the Morden Research Station were inspected for wilt symptoms. The number of wilted plants in each line was recorded. Stem samples of diseased plants were plated on potato dextrose agar (PDA) to isolate *Fusarium* organisms using aseptic techniques. A representative isolate of *F. oxysporum* (DAOM 175160) was used in the pathogenicity tests.

Pathogenicity tests

Growth of plants. Five hardy cultivars of chrysanthemum, Morden Eldorado, Morden Delight, Morden Canary, Morden Gaiety, and Susan Brandon (from the Agriculture Canada Research Station, Morden, Manitoba, Canada) and one non-hardy cultivar, Yellow Delaware (from Yoder Canada Ltd., Leamington, Ontario, Canada) were used as differential hosts. Stolons from each cultivar were planted in pots containing Cornell peat-lite

mix (3) and the plants were maintained in a greenhouse and used as stocks. Cuttings were taken from new growth of the stock plants, dipped in Seradix No. 1 (a rooting powder for soft wood cuttings from May & Baker Canada Inc., Mississauga, Ontario), and planted in Cornell peat-lite mix, one plant per pot (12-cm diam.). The plants were grown under a 16-hr photoperiod at 25 C for one month and then used for the inoculation tests.

Seeds of three cowpea lines, 147076, 170857 and 211110, were obtained from the USDA Quarantine Center, Beltsville, Maryland, USA. They were planted in Cornell peat-lite mix and kept under 16-hr photoperiod for 2 wk and then used for the inoculation test.

Preparation of inoculum. Three isolates of chrysanthemum wilt fusaria, including *F. oxysporum* f. sp. *chrysanthemi* (ATCC 52422), *F. oxysporum* f. sp. *tracheiphilum* race 1 (ATCC 16608), and one isolate of *F. oxysporum* (DAOM 175160) from Manitoba, Canada, were compared for pathogenicity to the six cultivars of chrysanthemum (Table 3). Isolate DAOM 175160 *F. oxysporum* was also tested for pathogenicity to the three cowpea lines. Spore suspensions (macro- and micro-conidia) of *F. oxysporum* were prepared from each isolate. After 14 days on potato dextrose agar (PDA) in Roux flasks at room temperature (20–22 C) under continuous lighting the cultures were washed with sterile water. The inoculum concentrations of DAOM 175160 for the three inoculation tests on chrysanthemum and cowpeas (Table 2) were 9.3×10^6, 8.9×10^6, and 3.4×10^6 spores/ml, respectively. The inoculum concentrations of DAOM 17560, ATCC 52422 and ATCC 16608 for the two experiments on six chrysanthemum cultivars (Table 3) ranged from 7.1×10^5 to 1.5×10^7 spores/ml.

Inoculation and disease rating

Five pathogenicity tests were conducted, including three experiments to test the pathogenicity of *F. oxysporum* DAOM 175160 to chrysanthemum cultivars, Yellow Delaware, Morden Eldorado and Morden Delight, and cowpeas (Table 2) and two experiments to compare *F. oxysporum* DAOM 175160 with isolates of the other two known races, ATCC 52442, and ATCC 16608, on the six chrysanthemum cultivars (Table 3). For each of the first three tests (Table 2), there were five inoculated and five uninoculated plants for each cultivar or line; for the last two tests (Table 3), there were 14–15 inoculated and 14–15 uninoculated plants for each cultivar.

For each inoculation test, plants of chrysanthemum or cowpeas were removed from pots and their roots washed in running tap water to remove Cornell peat-lite mix. They were inoculated with *F. oxysporum* by dipping the roots for 10 min in a spore suspension (500 ml for 10 to 15 plants) and transplanted in Cornell peat-lite mix at one plant/pot. For the controls, plant roots were soaked in sterile water for 10 min and transplanted in Cornell peat-lite mix. Plants were kept at 32 C, 16-hr photoperiod, watered daily for 32 days, and assessed for wilt using a scale of 0 (highly resistant, no wilting), 1 (resistant, slight wilting on some leaves), 2 (moderately resistant, most leaves wilted), 3 (susceptible, leaves severely wilted but green) and 4 (highly susceptible, leaves brown and plants dead). The severity of wilt of each cultivar was determined by calculating the disease index (D.I.) using the formula: $D.I. = \Sigma\ (nw)/T$, where n = number of plants in each wilt rating, w = wilt rating, *i.e.*, 0 to 4, and T = total number of plants. At 44 days after inoculation, the basal stems of chrysanthemums were harvested, surface disinfected for 45 sec in 70% ethanol and plated on PDA. After one week at room temperature plates were examined for the presence of the inoculated isolate of *F. oxysporum* by comparing the cultural characteristics of the recovered organisms with those of the known isolates.

RESULTS

In 1979, a total of 695 plants from 10 hardy chrysanthemum lines in the nursery at the Morden Research Station were inspected. The incidence of wilting varied greatly among the lines, ranging from no symptoms in the line 7517 × 7755 to 67% of wilted plants in the line 7778 × 7777 (Table 1). *Fusarium oxysporum* Schlecht was isolated from taproots and basal stems of wilted plants and, through application of Koch's postulates, the fungus was proven to be the causal agent for the wilt of hardy chrysanthemums. A single hyphal tip culture of the pathogen (DAOM 175160) was deposited in the culture collection at the Center for Land and Biological Resources Research, Agriculture Canada, Ottawa, Ontario, Canada.

F. oxysporum (DAOM 175160) isolated from hardy chrysanthemums in Manitoba did not cause wilt symptoms on the three cowpea lines, 211110, 170857, and 147076, the non-hardy chrysanthemum cv. Yellow Delaware or the hardy chrysanthemum cv. Morden Delight, but it caused severe wilting on the hardy chrysanthemum cv. Morden Eldorado (Table 2). No wilting was found in any of the

TABLE 1. Incidence of Fusarium wilt in nursery-grown lines of chrysanthemums at the Morden Research Station, Manitoba, in 1979

Line	No. of plants inspected	Fusarium wilt (%)
7778 × 7777	21	67
7778 × 7744	35	54
7736 × 7778	124	53
7778 × 7743	19	32
7736 × 7777	120	28
7549 × 7778	28	18
7746 × 7778	233	15
7809 × 7778	44	9
7727 × 7778	48	6
7517 × 7755	23	0

TABLE 2. Pathogenicity of *Fusarium oxysporum* (DAOM 175160) from Manitoba on chrysanthemum cultivars and cowpea lines

Plant	Cultivar or line	Wilt index[1]		
		Test 1	Test 2	Test 3
Chrysanthemum[2]	Morden Eldorado	4.0	4.0	3.8
Chrysanthemum[2]	Morden Delight	0	0	0
Chrysanthemum[3]	Yellow Delaware	0.3	0	0
Cowpea[4]	211110	0	0	0
Cowpea[4]	170857	0	0	0
Cowpea[4]	147076	0	0	0

1. Wilt index: 0 = no wilt, 4 = wilted and dead; based on 5 inoculated plants/cultivar or line.
2. Hardy chrysanthemum from Research Station, Agriculture Canada, Morden, Manitoba.
3. Non-hardy chrysanthemum from Yoder Canada Ltd., Box 370, Leamington, Ontario.
4. Cow pea seeds from USDA, BARC/East, Beltsville, MA 20705, USA.

non-inoculated plants.

Inoculation tests on six chrysanthemum cultivars showed that the pathogenicity of *F. oxysporum* (DAOM 175160) differed from *F. oxysporum* f. sp. *chrysanthemi* (ATCC 52422) and *F. oxysporum* f. sp. *tracheiphilum* race 1 (ATCC 16608) (Table 3). The cultivar Yellow Delaware was highly susceptible to ATCC 52422 resulting in severe wilting of plants, but it was highly resistant to DAOM 175160 and ATCC 16608 with few or no wilt symptoms being evident (Fig. 1, Table 3). The cultivars Morden Eldorado (Fig. 2) and Morden Canary were highly susceptible to DAOM 175160, but were highly resistant to ATCC 52422 and ATCC 16608. Both Morden Gaiety and Susan Brandon (Fig. 3) were highly resistant to DAOM 175160, but were highly susceptible to ATCC 52422 and ATCC 16608. Morden Delight was resistant to all three isolates of *F. oxysporum*.

Reisolations from basal stems of the inoculated plants showed that infection of plants occurred readily even in commercial cultivars with wilt resistance to DAOM 175160, ATCC 52422 or ATCC 16608 (Table 3). For example, the cultivars Yellow Delaware and Morden Gaiety were highly resistant to wilt caused by DAOM 175160, but more than 87% of the plants were infected 44 days after inoculation (Table 3).

DISCUSSION

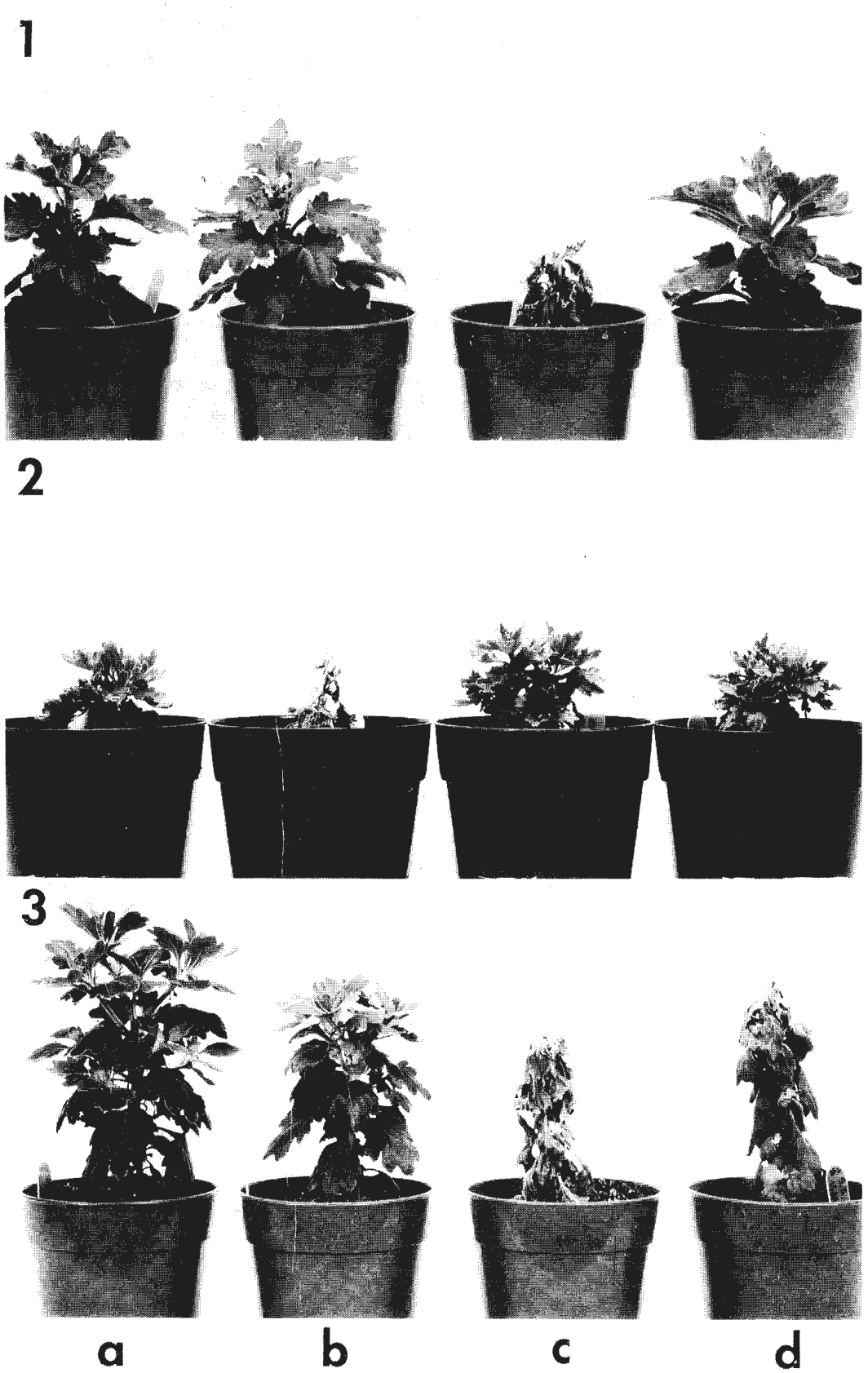
Based on the reactions of the six differential chrysanthemum cultivars in this study (Table 3), it is concluded that the chrysanthemum isolate of *F. oxysporum* DAOM 175160 from Manitoba is different from *F. oxysporum* f. sp. *chrysanthemi* (2), and *F. oxysporum* f. sp. *tracheiphilum* race 1 (1). Consequently, this new wilt pathogen in chrysanthemum was designated *F. oxysporum* f. sp. *chrysanthemi* race 2. The difference in pathogenicity among the three isolates of *F. oxysporum* tested (Table 3) suggests that resistance to *F. oxysporum* races is carried on more than one gene or different alleles of the same gene.

F. oxysporum was recovered from plants which showed no wilt symptoms (Table 3). This indicates a cultivar may be susceptible to infection by *F. oxysporum* but remain

TABLE 3. Pathogenicity of three isolates of *Fusarium oxysporum* (DAOM 175160, ATCC 52422, and ATCC 16608) on six differential cultivars of chrysanthemum

Cultivar[1]	Experiment	DAOM 175160		ATCC 52422		ATCC 16608	
		W.I.[2]	Infection[3] (%)	W.I.	Infection (%)	W.I.	Infection (%)
Yellow Delaware	1	0.9	100	3.9	100	0	47
	2	0.4	100	3.6	100	0	29
Morden Eldorado	1	3.9	80	0.1	73	0	0
	2	4.0	100	0.6	80	0	0
Morden Delight	1	0.4	93	0	7	0	13
	2	0.1	100	0	33	0	40
Morden Canary	1	4.0	93	0.2	67	0	60
	2	4.0	100	0.5	73	0	53
Morden Gaiety	1	0.2	87	3.4	100	3.8	100
	2	0.4	93	2.9	100	3.5	100
Susan Brandon	1	0.5	100	4.0	100	3.6	100
	2	0.3	100	4.0	100	3.3	100

1. All winter hardy cultivars except cv. Yellow Delaware which is a non-hardy cultivar.
2. Wilt index: 0 = no wilt, 4 = wilted and dead; based on 14 to 15 inoculated plants/cultivar.
3. Based on isolation of basal stems at 44 days after inoculation.



Figs. 1–3. Fusarium wilt of chrysanthemum cultivars Yellow Delaware (Fig. 1), Morden Eldorado (Fig. 2) and Susan Brandon (Fig. 3) 32 days after inoculation. **a,** uninoculated controls; **b,** *Fusarium oxysporum* f. sp. *chrysanthemi* race 2 (DAOM 175160); **c,** *F. oxysporum* f. sp. *chrysanthemi* (ATCC 52422); and **d,** *F. oxysporum* f. sp. *tracheiphilum* race 1 (ATCC 16608). Note severe wilting on Yellow Delaware due to ATCC 52422 (Fig. 1c), on Morden Eldorado by DAOM 175160 (Fig. 2b) and on Susan Brandon by ATCC 52422 (Fig. 3c) and ATCC 16608 (Fig. 3d). No wilt symptoms developed on the controls (Figs. 1a–3a).

tolerant to wilt.

The natural outbreak of Fusarium wilt in Manitoba in 1979 indicates that the disease can be serious on hardy chrysanthemums in western Canada. Of the six commercial chrysanthemum cultivars tested, Yellow Delaware, Morden Delight, Morden Gaiety and Susan Brandon are highly resistant to this newly discovered race of *F. oxysporum* f. sp. *chrysanthemi*. This study suggests that by growing resistant cultivars of hardy chrysanthemums, growers can avoid wilt caused by this new race of *Fusarium oxysproum* f. sp. *chrysanthemi* in western Canada.

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摘　　要

黃鴻章[1], Phillippe, L. M[2]., Marshall, H. H[2]., Collicutt, L. M[2]., and Neish, G. A[1]. 1992. *Fusarium oxysporum* f. sp. *chrysanthemi*新生理小種二號引起之菊花萎凋病. 植病會刊 1:57-61. (1. 加拿大農業試驗所, Lethbridge, Alberta, Canada, 2. 加拿大農業試驗所, Morden, Manitoba, Canada.)

1979年加拿大農業試驗所在 Morden, Manitoba 地區的試驗場苗圃所種植之菊花，發生很嚴重的萎凋病。使用六種不同的菊花栽培品種當判別植物，來測試分離菌株之病原性。結果顯示，引起當地菊花萎凋之病原菌的病原性與 *F. oxysporum* f. sp. *chrysanthemi*及 *F. oxysporum* f. sp. *tracheiphilum*生理小種一號不盡相同。因此，將本病原菌定名爲 *F. oxysporum* f. sp. *chrysanthemi* 生理小種二號。

關鍵字：菊花 (*Chrysanthemum morilifolium*)、萎凋病、*Fusarium oxysporum*、生理小種。