Sunflower Yellows, a New Disease Caused by Phialophora sp.

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ABSTRACT

Yellows, a new systemic disease of sunflower, *Helianthus annuus*, was found in Manitoba, Canada, in 1968. Yellows is caused by *Phialophora* sp. Chlorosis and necrosis of the leaves, dwarfing, and sterility are the main symptoms. The pathogen was isolated from all parts of the plant except florets and seed.

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Sunflowers (*Helianthus annuus* L.) have been cultivated commercially in southern Manitoba since 1943. Annual production has varied from 1,200 to 24,000 hectares, with disease being a limiting factor. Sunflower diseases that are important in Canada are leaf mottle or wilt and downy mildew, which are caused, respectively, by the systemic, soil-borne pathogens *Verticillium dahliae* Kleb. and *Plasmopora halstedii. Sclerotinia sclerotiorum* causes wilt and root rot, but is not systemic. Each of these diseases has characteristic symptoms. In 1968, near Morden, Manitoba, disease was observed at flowering time in plots of inbred CM 144 growing in a disease nursery heavily infested with *V. dahliae.* However, since CM 144 was considered on the basis of previous observations to be highly resistant to *V. dahliae* (4), it was theorized that as a result of the excessive rainfall during the summer, plant vigor was decreased and resistance had broken down. That this theory was untenable was indicated when the same symptoms recurred in CM 144 in the same field in 1969 during an abnormally dry season. Subsequent studies, which are described herein, showed that the symptoms were those of a new sunflower disease caused by an unidentified species of *Phialophora.* The disease was called "yellows" in recognition of its main symptom, a pronounced and uniform systemic yellowing of leaves.

MATERIALS AND METHODS.—*Field studies.*—

Inbred CM 144, planted in plots in the disease nursery in the spring of 1969 and 1970, was observed for symptoms. Symptom severity on individual plants was classified each season. Plant material was sterilized with a 1:1 mixture of Javex (5.25% sodium hypochlorite) and 95% ethanol and incubated on 2% water agar (Bacto). Mycelium from fungal colonies that grew from the plant material was transferred to potato-dextrose agar (PDA, Difco) slants. In 1969, both normal and diseased plants were sampled, and isolations were made from the inside of tap roots, and from stems at 25 cm above the soil surface. In 1970, two leaves showing characteristic symptoms of yellows were collected from the upper portion of each of 30 plants. One petiole slice from near the base of each leaf was tested. In addition, 40 commercial sunflower fields in southern Manitoba were surveyed to determine the occurrence of yellows. Leaves and stems of at least six suspected plants were collected from each field.

Pathogenicity studies.—Sixteen isolates of *Cephalosporium acremonium* Corda and *Cylindrocarpon* spp. were included in initial pathogenicity tests, but none caused disease. Cultures of *Phialophora* sp. tested for pathogenicity included ten mass-transfer isolates, six single-spore isolates, and two isolates obtained from plants inoculated with different single-spore isolates. Inoculum was prepared by suspending in distilled water conidia from cultures grown on PDA or on 20% V8-juice agar. Inoculum concentration varied among experiments from 2×10^5 spores/ml to 3×10^6 spores/ml, but within experiments was similar for all isolates.

Four experiments were conducted in a growth cabinet maintained at 21-22 C and equipped with fluorescent lighting to provide at plant height 900–1,500 ft-c for an 18-hr day-length. Inbred lines CM 144, CM 162, and CM 204 planted in perlite were irrigated with water and grown until the cotyledons had fully expanded. The seedling roots then were washed free of perlite, immersed in inoculum for 5-7 min, trimmed slightly, and replanted in flats containing a 7-cm layer of perlite. Control plants were treated with distilled water. Control and inoculated plants were always in separate flats. Plants, after transplanting, were irrigated daily with 0.03% RX 15, a complete fertilizer (Garden Research Laboratories Ltd., Toronto).

In a single greenhouse experiment, seedlings of CM 204 growing in soil were inoculated, three plants/isolate, at the cotyledonary stage by hypodermic injection of inoculum into the hypocotyl. Control plants were injected with distilled water. The plants were kept at 21-27 C and illuminated by fluorescent lighting that provided a minimum light intensity of 1,500-2,000 ft-c for an 18-hr day-length.

RESULTS.—The first symptom of yellows in the field occurs as the plant approaches flowering. The leaves turn a dull, light-green color. Large areas of the leaf then turn a dull yellow color, usually starting at the apex and margin and extending inwards. Next, 5- to 10-mm long angular patches of interveinal tissue become necrotic. Leaf margins turn necrotic and necrosis progresses centripetally (Fig. 1). The bottom leaves on dying plants are dry and withered, whereas the upper leaves may still appear green. Diseased

Fig. 1. Typical symptoms of yellows on a field-grown plant.

plants are stunted; flower heads remain small and may be sterile.

Yellows is a rather inconspicuous disease, and symptoms might be confused with those due to nitrogen or to some other mineral deficiency, or to excess water. Yellows might also be confused with Verticillium wilt, although there are characteristic differences (5). Initial symptoms of Verticillium wilt consist of patches of bright yellow color, whereas entire leaves of yellows-affected plants assume a dull green color. In advanced stages of Verticillium wilt, the yellow areas turn brown except at the edge of the affected area, where the yellow color persists for a long time. When yellows is involved, necrosis is much less pronounced and the necrotic tissue is not surrounded by a yellow edge. In some years, sunflowers mildly affected with Verticillium wilt may have leaves that are almost entirely yellow, flaccid, and thin. Leaves affected with yellows remain much more turgid.

Yellows occurred in the disease nursery both in 1969 and 1970. In 1969, of 313 plants of CM 144 examined on 23 September, 20% had been prematurely killed and produced no seed, 29% expressed moderate to severe symptoms, 13% had mild symptoms, and 38% were normal. In 1970, of 141 plants inspected on 24 August, 7% had been prematurely killed, 33% had moderate to severe symptoms, 18% had mild symptoms, and 42% were normal. In 1969, tap roots of normal and of diseased plants had internally abundant microsclerotia of *V. dahliae* and were similar in appearance. On the other hand, fibrous roots of healthy and diseased plants were conspicuously different; those of plants with yellows were brown and the cortex was sloughed off, whereas those of healthy plants were white and apparently normal. Isolations made from both types of plants yielded a predominance of *Cephalosporium acremonium* and *Phialophora* sp., whereas fibrous roots of plants with yellows yielded mainly *Cylindrocarpon* spp. In 1970, *Phialophora* sp. was isolated in pure culture from 56 of 60 petiole slices. Two plants that had died prematurely yielded *Phialophora* sp. The fungus was isolated from tap roots, fibrous roots, pieces of stems sampled at various heights, leaf petioles taken from near soil level and from the top of plants, and from leaf veins and bracts. It was not isolated from florets or from 17 poorly developed or aborted seeds. *V. dahliae* was not isolated.

Yellows was found in one of 40 fields surveyed. It occurred in the cultivar Krasnodarets at a location about 35 miles from the site near Morden. About 4% of the plants were infected. *Phialophora* sp. was readily isolated from petioles and stems of all eight plants sampled.

Pathogenicity of Phialophora sp.—Leaf chlorosis and dwarfing of stems which occurred commonly on field-grown plants also occurred commonly on plants root-inoculated with each of 14 isolates of *Phialophora* sp. Other symptoms on root-inoculated plants were unequal growth and twisting of leaves, leaf necrosis, and premature collapse of cotyledons. Sterility, another symptom observed in the field, was caused by four other isolates on stem-inoculated plants grown to maturity. Vascular browning occurred in tap roots and extended throughout the stem into leaf traces. The fibrous root system of inoculated plants was always smaller than that of control plants. Stunting and death of plants were apparent as early as 10 and 15 days after inoculation.

The results of growth cabinet experiments 1-3 are given in Table 1; the data have been combined because isolates within experiments did not differ in pathogenicity. In experiment 2, CM 204 was more severely diseased than CM 144. In experiment 3, the mean fresh weights of roots and of shoots of inoculated plants were each reduced by 60%, and height was reduced by 45%. The greater reduction in weight would suggest that diseased stem and leaf tissue also has a reduced water content.

In the fourth growth cabinet experiment, the virulence of single-spore isolate 556-3 of *Phialophora* sp. was compared with that of a pathogenic isolate of *V. dahliae* on CM 162, which is highly susceptible to Verticillium wilt. Four weeks after inoculation, the 10 control plants were healthy and had a mean height of 10.4 cm. The 10 plants inoculated with *V. dahliae* were dead or dying, and had a mean height of 2.1 cm. Ten plants inoculated with *Phialophora* sp. were still growing, though all were severely diseased; their mean height was 4.7 cm. Verticillium wilt became apparent earlier, developed faster, and resulted in more necrosis than did yellows.

In the greenhouse experiment, pathogenicity data for isolates 21, 27, 554-2-65, and 556-1-90 were taken 87 days after inoculation. The control plants produced normal seed and had a mean height and head diameter of 60 and 7.3 cm, respectively. The inoculated plants were sterile, and had a mean height and head diameter of 48 and 3.6 cm, respectively.

Phialophora sp. was isolated from 158 of 206 tissue samples taken from petioles, hypocotyl, and stem pieces at various heights, and from fibrous and tap roots. Most samples were cross sections of aboveground parts; *Phialophora* sp. clearly originated from brown discolored vascular bundles associated with these samples. Bacterial contamination was common and might have prevented growth of the pathogen from some samples. *Phialophora* sp. was never isolated from control plants.

The pathogen.—The generic identity of the pathogen (*Phialophora* sp.) has been confirmed by S. J. Hughes, Plant Research Institute, Ottawa. *Phialophora* sp. on PDA characteristically produces phialides with collarettes (Fig. 2); phialides occur in fascicles or "bushes" (Fig. 3). On water agar, however, the fungus easily can be mistaken for *Cephalosporium* because the apex of phialides hardly differentiates. In fact, the *Phialophora* pathogen was identified incorrectly as *Cephalosporium* in initial work (1). Species identification of the sunflower pathogen awaits comparison with *P.* (=*Verticillium*) *cinerescens* (Wollenw.) Van Beyma, which is an important vascular pathogen of ornamental carnation, *Dianthus caryophyllus,* in Europe (3, 7), and has also been reported from the United States (2).

TABLE 1. Pathogenicity of 13 isolates of *Phialophora* sp. to sunflower[a]

Experiment[b]	*Phialophora* isolates	Cultivar	Total plants		Height (cm)			
					Control		Inoculated	
			Control	Inoculated	Range	Mean	Range	Mean, % of control
1	30, 46-49	CM 144	20	50	23-31	27.8	5-20	53
2	553-1,-2,-3	CM 144	10	50	19-27	23.7	3-19	54
	554-2, 556-1	CM 204	8	40	17-23	21.0	1-18	41
3	20, 26, 30	CM 204	6	18	40-50	46.3	5-43	55

[a]Plant heights were determined 30-49 days after inoculation by root immersion in a conidial suspension.
[b]Experiments conducted in a growth cabinet. Plants were kept at 21-22 C and received 900-1,500 ft-c for an 18-hr day-length.

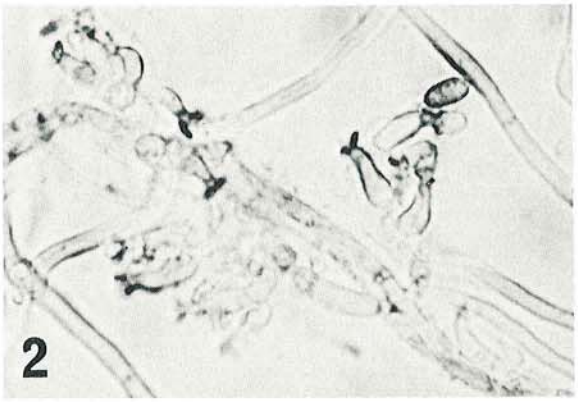
DISCUSSION.—Yellows of sunflower is caused by *Phialophora* sp. The pathogen was consistently associated with yellows. Original isolates and reisolates were morphologically similar, and symptoms of artifically inoculated plants were similar to those observed in the field. The pathogen is found in the vascular tissue throughout an infected plant which becomes systemically invaded. *Cephalosporium acremonium* and *Cylindrocarpon* spp. that were isolated commonly from plants with yellows are secondary invaders.

Little is known about the life cycle of the yellows pathogen. *Phialophora* sp. is soil-borne and overwinters in Manitoba, probably in infected debris. Attempts to isolate *Phialophora* sp. from seeds of diseased plants were unsuccessful, but the sample was small and the data are inconclusive. The Verticillium wilt pathogen also is systemic; Sackston & Martens (6) showed that the pathogen can be transmitted in sunflower seed.

Field observations in 1969 and 1970 indicated that 49 and 40%, respectively, of plants of inbred CM 144 were moderately or severely diseased or were killed. Inbred lines may be assumed to be inherently weak, and more prone to attack by pathogens than commercial cultivars which are cross-pollinated and much more vigorous. However, results of pathogenicity studies (J.A. Hoes, *unpublished data*) indicate that the cultivar Commander is highly susceptible to yellows. Therefore, this new disease could be economically important.

Yellows is not widespread. The disease was not found in 39 of 40 fields surveyed in the old established sunflower area of Manitoba. Yellows has been introduced only recently, or its distribution is restricted by as yet unknown ecological factors.

Plant vigor may be important in disease development. In 1968, when yellows was first observed, precipitation in July and August totalled 31.4 cm, which was more than twice the 47-year average of 13.4 cm (Morden Research Station Records). Incidence and severity of yellows coincided with poorly drained spots in which water collected. It may be that



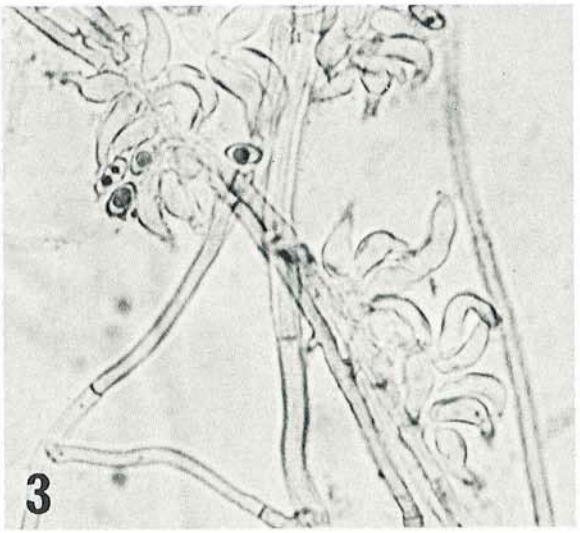


Fig. 2-3. *Phialophora* sp. on PDA. **2)** Phialides showing collarettes (× 1,000). **3)** Fascicles or "bushes" of phialides (× 1,000).

water per se is important in facilitating infection by *Phialophora* sp. However, vigor of plants growing in these spots would certainly have been reduced. In experiment 2, CM 204, which had a smaller root system and was less vigorous than CM 144 when the plants were inoculated, subsequently became more severely diseased than did CM 144.

Control of yellows probably is possible by breeding for resistance. This is suggested by the observation that rows of completely healthy sunflower cultivars occurred adjacent to severely diseased rows of CM 144.

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