Cladosporium herbarum Causes a Leaf Spot on Marshmarigold in Western North America

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Abstract

Caltha leptosepala ssp. howellii and Caltha leptosepala ssp. leptosepala (marshmarigold, Ranunculaceae) inhabit stream banks and wet meadows in the northern Rocky and Cascade mountain ranges of North America. Cladosporium herbarum (anamorphic Davidiellaceae) is a cosmopolitan saprophytic fungus and epiphyte that can invade senescing tissues of many plants, induce disease in ripe fruits of some plant species, and occupy other diverse environments. Instances of biotypes of C. herbarum specialized to cause disease on a given host are rare. This study reports a biotype of C. herbarum that caused disease on C. leptosepala. The name Cladosporium leaf spot is proposed for this disease.

Introduction

Little information is available concerning diseases associated with herbaceous plants in subalpine habitats. Yet these are important natural ecosystems valued for recreation, aesthetics, watershed, and wildlife. More information about plant diseases in natural ecosystems would increase the understanding of both the role of plant diseases in specific ecosystems and their efficient management. Knowledge of plant diseases and the pathogens that cause diseases in natural ecosystems can lead to a better understanding of their ecology and host specificity as well as contribute to the development of sound disease management strategies in agroecosystems (5,18). For example, Browning identified several characteristics of populations of the progenitors of cultivated wheat, barley, and oats in a natural ecosystem in the Fertile Crescent of southwest Asia that are applicable to disease management strategies in agroecosystems (5).

Marshmarigolds (Caltha spp.) are members of the buttercup family (Ranunculaceae) and inhabit stream banks and wet meadows in subalpine habitats of the Rocky and Cascade Mountains in western North America. Experimental results reported herein are pertinent to multiple occurrences of a plant disease observed by the first author on Caltha spp. in these habitats in western North America. This disease, a leaf spot, was observed at varying intensities and in five states from 1995 to 2007 on Caltha leptosepala ssp. howellii (Huth) P.G. Sm. (syn. Caltha biflora DC.) and Caltha leptosepala ssp. leptosepala DC. The first taxon has been utilized repeatedly in classification of plant communities in such habitats [e.g., (6,13)]. A history of species concepts in the Caltha leptosepala complex has been provided (14), and complete synonymies reflecting current taxonomic opinion are available online [e.g., (10,20)].
**Disease Symptoms and Intensity**

Disease symptoms on plants in natural habitats consisted of lesions on leaves and petioles that were brown to dark brown in color, with or without a chlorotic halo (Fig. 1). Shape of lesions was circular to oval, and on leaves they were generally 1 to 14 mm in diameter. Lesions sometimes coalesced to blight leaves. Infections were also observed as a blighted area along the midvein of leaves (Fig. 2). Disease symptoms were similar among all locations where the disease was observed.

Lesions developed on green, non-senescing leaves and petioles at each location. Incidence and severity of the disease appeared to reflect location and time of seasonal observations. Diseased plants were not randomly distributed, but were in clustered patterns at the various locations, and disease severity appeared to be greater during summers with relatively high rainfall than in summers with less moisture.

The disease was observed in several separated sites in the Gospel Hump Wilderness of ID and the Granite Basin of WY, where disease incidence and severity were always more severe where stands of *Caltha* were relatively dense. Disease incidence and severity increased during the season and became particularly severe in the Gospel Hump Wilderness, where in September of 1995 and 1997 (disease in this area was monitored several times both years) 98% of plants in a particular meadow were infected with 30 to 60% of the leaf area per plant blighted. The *Caltha* stand in this meadow was extensive (approximately 40 × 90 m in area) with leaves of adjacent plants touching throughout most of the stand. Much less disease (1 to 30% severity on 0.1 to 25% of the plants) was observed in other areas of the Gospel Hump Wilderness and the other seven collection locations where plant stands were smaller and less dense.

**Collection of Diseased Plants, and Isolation and Identity of the Pathogenic Agent**

Leaves and petioles of symptomatic plants of *C. leptosepala* ssp. *howellii* (Fig. 1) were collected from wet meadows and stream banks in the Gospel Hump Wilderness in Idaho Co., ID, in 1995 and 2003; upper Palisades creek in Bonneville Co., ID, in 1996; Mt. Rainier in Pierce Co., WA, in 1997; Hylaitve Lake in Gallatin Co., MT, in 2001; Odessa Lake in Larimer Co., CO, in 2001; and Coal creek near Wilson in Teton Co., WY, in 2005. Leaves and petioles of symptomatic plants of *C. leptosepala* ssp. *leptosepala* (Fig. 2) were collected from wet meadows in Granite Basin in Teton Co., WY in 2007. Plant samples were placed in sealed plastic bags, transported to the laboratory, washed in running water and surface-disinfested in 1% NaOCl for 1 min. Tissues were then excised from lesion margins and transferred to 2% potato dextrose agar (PDA). Plated plant tissues were incubated on a laboratory bench at 21 to 23°C for 3 to 10 days before subculturing of fungi. *Caltha* specimens were identified to species with keys and descriptions in Hitchcock and Cronquist (8).
A Cladosporium species was consistently recovered from lesion margins of symptomatic plants collected from seven regions in five states. Isolates were stored in pure culture on slants of PDA at 5°C or on pre-sterilized, colonized rye kernels at -15°C. Recovered isolates keyed to C. herbarum in published monographs (9,17) on the basis of conidiophore morphology and dimensions of conidia and conidiophores (Fig. 3). Geniculate-sinuous, nodulose to nodose ("knotty/gnarled" (17)) conidiophores (most frequently ca. 90 to 200 (-250) µm long, 5-6 µm wide at the nodes), catenate, verruculose conidia (most frequently ca. 5-17 × 4-7 µm), and ramoconidia ca. 15-21 µm long were typical of the species (9,17). Representative isolates were deposited with ATCC as indicated above.

Although other fungi were occasionally isolated from diseased tissues, none were consistently associated with the disease.

Isolates of C. herbarum maintained were from C. leptosepala ssp. howellii (Fig. 1) collected from the Gospel Hump Wilderness in Idaho Co., ID, in 1995 (isolate CL-1 = ATCC 201852); upper Palisades Lake in Bonneville Co., ID, in 1996 (CL-2 = ATCC 201853); Mt. Rainier in Pierce Co., WA, in 1997 (CL-3 = ATCC 201854); Mt. Rainier in Pierce Co., WA, in 1997 (CL-4 = ATCC 201855); Hyalite Lake in Gallatin Co., MT, in 2001 (CL-5); Odessa Lake in Larimer Co., CO, in 2001 (CL-7); Coal Creek, near Wilson in Teton Co., WY, in 2005 (CL-Coal Cr); and from C. leptosepala ssp. leptosepala (Fig. 2) from Granite Basin in Teton Co., WY, in 2007 (CL-GrBsn).

Confirmation of Pathogenicity, Including Comparison with Other Isolates of the Same Species and Other Species of Cladosporium

Pathogenicity trials were conducted on non-symptomatic C. leptosepala ssp. howellii plants collected from Gospel Hump Wilderness, ID, and transported to a greenhouse at Washington State University. Fourteen-day-old conidia of representative isolates CL-1, CL-2, or CL-3 (from three locations and two states) were aseptically transferred from PDA to water, adjusted to 1.5 × 10^6 conidia/ml, and 0.3 ml aliquots of the conidial suspensions were imbibed onto 1-cm² filter papers that were then placed on the adaxial surface of leaves. Young leaves were selected for inoculation and generally only one leaf per plant was inoculated. Each isolate was inoculated onto three leaves at two inoculation sites per leaf. Filter paper squares were placed about 2.5 cm apart near the center of a leaf. Three leaves on three separate plants were used as a non-inoculated control and received moistened filter paper squares without a spore suspension. Isolates of Cladosporium herbarum (ATCC 11281, ATCC 11282, and ATCC 76226 from species of Malus, Typha, and Beta, respectively) as well as isolates of C. cladosporioides (Fresen.) G.A. de Vries (ATCC 34668), C. oxysporum Berk. & M.A. Curtis (ATCC 76499), and C. sphaerospermum Penz. (ATCC 62723) from plants of Daucus, Lespedeza, and Helianthus, respectively, were analogously
cultured and used for inoculation using the same technique. Inoculated plants were placed in a mist chamber at 20 to 22°C for 24 h and then removed to a greenhouse. Each of three trials had three replicates per isolate plus non-inoculated controls. Plants of *C. leptosepala* ssp. *howellii* were inoculated several weeks after they had been collected to ensure the plants were well established after transplanting and that leaves were not latently infected with fungi. Leaves were inoculated that had expanded after the plants had been collected.

On plants inoculated with *C. herbarum* originally isolated from *Caltha* plants (isolates CL-1, CL-2, or CL-3), lesions began to be evident on inoculated leaves eight days after inoculation and were associated with the sites of inoculation. These lesions, of an appearance highly similar to those seen in the field, were observed on plants inoculated with each of the three isolates. A fungus matching descriptions (9,17) of *C. herbarum* was re-isolated from these lesions. Plants inoculated with the other isolates of *Cladosporium* (*Cladosporium* species other than *C. herbarum*, or isolates of *C. herbarum* from non-*Caltha* hosts) did not show lesions, nor did any of the non-inoculated control plants display lesions. *Cladosporium* leaf spot is a disease name often applied when leaf spotting occurs on a given plant species and is caused by a species of *Cladosporium* [e.g., (1,2,15,16)]. The name also seems apt for the leaf spots induced by *C. herbarum* on *Caltha leptosepala* as described in this study.

The causal agent of this plant disease appears to be a specialized biotype of *Cladosporium herbarum* (Pers. : Fr.) Link, a species widely documented as a leaf epiphyte. Although this species, which occupies many environmental niches, is documented as causing disease on senescing plant materials or ripe fruits (7,17), we know of only one other report in which a seemingly specialized biotype of *C. herbarum* was shown to cause disease on a given host, on yellow starthistle in Greece (4).

### Adaptation of Caltha spp. in their Natural Habitats

*Caltha leptosepala* ssp. *howellii* and *C. leptosepala* ssp. *leptosepala* flower during and soon after snow melt in the spring, and before the first leaves of a growing season are fully expanded. New leaves are then produced in the center of a crown whorl throughout the growing season. Seeds are produced shortly after flowering. Disease lesions were not observed until after flowering and the first leaves had fully expanded. The early flowering of *C. leptosepala* likely increases the probability of *C. leptosepala* producing viable seed during a short growing season and in the presence of a pathogen that progressively causes more disease during the growing season.

Stand density or crowding of the host is a characteristic of disease epidemics in agro-ecosystems (5). Stand density appeared to be a contributing factor for development of high disease intensities (incidence and severity) of *Cladosporium* leaf spot in stands of *Caltha* ssp. in the natural ecosystems observed in this study. Diseases caused by *Colletotrichum nupharicola* on *Nuphar lutea* (L.) Sm. (yellow pond-lily) and by *Macrosorpha scirpinfestans* E.G. Simmons & D.A. Johnson on *Scirpus acutus* Bigelow and *S. validus* Vahl (bulrush) were often severe in natural ecosystems when host plants were crowded (11,12).

### Implications for Further Research

Results of the above experiments confirmed that *C. herbarum* isolated from lesions of *Caltha leptosepala* ssp. *howellii* can induce a leaf spot on non-symptomatic plants of that taxon. Preliminary experiments indicated that disease was not induced when the non-host crops spinach and lettuce were inoculated under conditions analogous to the experiments described above (data not shown). However, the genus *Caltha* contains several species outside the *C. leptosepala* complex, including species widely cultivated as ornamentals (3,19). We do not assume that the *C. herbarum* isolated by us would prove pathogenic on all *Caltha* spp., and therefore we refrain from designating these isolates as 'f. sp. *calthae*', although that remains a possibility. Further experiments with other species of *Caltha* and other genera in the
Ranunculaceae, and possibly other plants, would better define the host range of this biotype of *C. herbarum*. Meanwhile, our report together with that of Berner et al. (4) raises the distinct possibility that host specialization within *C. herbarum* may be more frequent than previously documented.

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**Literature Cited**