

Susceptibility of Yellow Starthistle to Selected Plant Pathogens

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ABSTRACT

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Susceptibility of the widespread weed yellow starthistle (*Centaurea solstitialis*) to several plant pathogens was investigated. Starthistle plants developed symptoms from disease incited by *Fusarium oxysporum* f. sp. *carthami*, *Verticillium dahliae*, *Phytophthora* spp., *Botrytis cinerea*, and *Sclerotinia sclerotiorum*. Except with *B. cinerea*, diseases incited by the pathogens were frequently lethal to the plants. Alfalfa (AMV), lettuce (LMV), and turnip (TuMV) mosaic viruses were transitted to starthistle plants by aphids and mechanical inoculation. Mosaic, leaf abnormalities, and moderate to severe stunting were common symptoms induced by the viruses. Some plants infected with LMV or TuMV died in the rosette stage. TuMV had the most adverse effect on starthistle plants.

Yellow starthistle (*Centaurea solstitialis* L.) is a widespread annual weed that invades pastures, range and wastelands, roadsides and grainfields and is scattered throughout much of the United States with heavy infestations in California, Washington, Oregon, and Idaho (8). In Canada it occurs from Ontario to British Columbia (8). Starthistle is an introduced weed that appears to be relatively free of pathogens in the United States. Rust (*Puccinia* spp.) has been collected from starthistle in Turkey (8); therefore, rust is a naturally occurring pathogen on the weed in its center of origin or in certain other geographic areas. However, documentation of research on the rust-starthistle pathosystem is sparse, and information is virtually unknown regarding other pathogens of the weed.

Populations of starthistles at various locations in California were surveyed for diseases. The paucity of diseases observed in the surveys prompted a study with selected plant pathogens to determine if starthistle was susceptible to the plant pathogens and if any of the test organisms showed promise as possible biological control agents.

MATERIALS AND METHODS

Pathogens used in experiments with starthistle were *Fusarium oxysporum* f. sp. *carthami* Klis. & Hous. (*F. o. carthami*), *Verticillium dahliae* Kleb. (pathotypes T-1 and SS-4), *Phytophthora*

cryptogea Pethyb. & Laff., *P. drechsleri* Tucker, *P. parasitica* Dast., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Botrytis cinerea* Pers. ex Fr., alfalfa mosaic virus (AMV), lettuce mosaic virus (LMV), and turnip mosaic virus (TuMV). Pathogens were originally isolated from plants of safflower (*Carthamus tinctorius* L.) (2-7,9), except *S. sclerotiorum*, which was isolated from stems of diseased starthistle plants. Cultures of fungal pathogens were maintained on potato-dextrose agar (PDA) or lima bean agar (LBA). Experiments were conducted in the greenhouse and in controlled-environment chambers. Pathogenicity of *V. dahliae* was also assessed in the field. Average ambient day and night temperatures in the greenhouse were 28 and 21 C, respectively. The problem of inconsistent stands of plants resulting from erratic seed germination was resolved by planting pregerminated seed with the radical slightly developed or by transplanting 3- to 4-day-old seedlings.

Inoculum. A 6-mm disk from a culture of *S. sclerotiorum* growing on PDA was placed on each of two senescing leaves of 18 6-wk-old starthistle plants. The plants were enclosed in plastic bags for 5 days and kept at 24 C. Pots were placed in trays for subirrigation during the test.

A suspension containing 1×10^5 conidia per milliliter of *B. cinerea* in sterile distilled water was misted on 24 flowering seed heads of several starthistle plants. Moistened plastic bags were placed over the inoculated flower heads to maintain conditions favorable for infection. The bags were removed after 24-48 hr. Flower heads misted with sterile water only were treated similarly.

Soil was infested with *F. o. carthami* grown for 7 days on autoclaved wheat grain prepared as described previously (3). Infested grain was mixed with autoclaved loam (1.5 g/100 g) in plastic bags and placed in galvanized flats. Three

rows of 12 pregerminated seeds were planted in each of 10 flats and covered lightly with soil. Control flats contained autoclaved grain mixed with soil. Flats were placed in plastic pans to which water was added for subirrigation. Plants were observed for symptoms for 4-5 wk, then the tops were removed by cutting through the root-hypocotyl area and evaluated for vascular discoloration. Soil was then removed from individual flats, mixed, and placed in clean flats. Fresh inoculum was not added. Planting was repeated, and six seeds of a susceptible safflower cultivar were randomly placed in the soil of each flat to test viability of the inoculum. The experiment was repeated four times. Separate flats of soil were inoculated with conidia (250,000/ml) washed from 2-wk-old *Fusarium* cultures on grain. Conidia were pipetted in trenches followed by planting of germinated seeds. Flats were treated as before.

V. dahliae was grown on a medium consisting of autoclaved vermiculite, V-8 juice, and CaCO₃ (6). Autoclaved medium covered with foil in 400-ml glass beakers was inoculated with 12 pieces (2 × 2 mm) from a culture of *V. dahliae* growing on PDA. One to 2 wk later, 12 germinated starthistle seeds were planted on a layer of vermiculite that was spread over the inoculum and covered lightly with vermiculite. Ten milliliters of sterile distilled water was added per beaker. Plants growing in the beakers were kept under fluorescent lights (4,088 lux) in the laboratory at 24 C for 3 wk, transplanted to autoclaved loam in plastic pots and kept in a growth chamber under a 14-hr photoperiod (21,000 lux) for 3-4 wk, then transferred to the greenhouse. Day-night temperatures in the growth chamber were maintained at 24 ± 1 and 18 ± 1 C, respectively. Seeds were collected from mature plants and assayed for *Verticillium* on water agar or filter paper (4).

Four-day-old healthy seedlings were transplanted to *V. dahliae*-infested soil in a field plot in November. Eighty seedlings were planted at 24-in. intervals in a single bedded row. Sixty-nine plants that survived the winter season were evaluated for disease symptoms through the flowering stage.

Pathogenicity of *Phytophthora* species was evaluated from symptoms on stems and roots of 5- to 6-wk-old plants grown singly in 1-L glazed crocks containing sandy loam. Inoculum consisted of 1-wk-old cultures of *Phytophthora* growing on autoclaved V-8 juice-vermiculite medium.

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For surface-flooding, 3 g of inoculum mixed with 22 g of sandy loam was spread on the surface of the soil in each crock. The soil was saturated and drained before and after inoculum was added. After 24 hr at 21 C, the crocks' drain holes were plugged and the crocks were set in a water bath at 27 C for 24 hr. Water was added and maintained at a depth of 1 cm on the soil surface. The crocks were randomized on greenhouse benches after the flooding period, and the soil was flushed with water and drained. Plants were irrigated daily thereafter and observed for symptoms. Noninoculated plants received similar care. To obtain root infection, the inoculum (3 g/22 g of soil) was placed in holes extending through the root zone. Crocks were cared for as before, except during flooding, the crock drain holes were left open and the crocks were placed in plastic containers set in water baths. Water was added and maintained in the containers at a level that allowed flooding of the root zone.

Four-day-old plants were transplanted into crocks containing soil that was infested with inoculum as described previously at a rate of 10 g/100 g of soil. Twenty-four plants were planted for each *Phytophthora* species and a control group in noninfested soil. Crocks were placed in shallow plastic pans and irrigated daily. When plants were 7 wk old, soil was washed from roots of plants in infested and noninfested soil. Plants and roots were separated, dried, and weighed.

Turnip, lettuce, and alfalfa mosaic viruses were maintained in safflower plants. Leaves from infected plants were triturated in 0.01 M phosphate buffer, pH 7.0, with a mortar and pestle, and the extracted juice was rubbed on the first pair of leaves of 2- to 3-wk-old starthistle plants in 5-in. plastic pots. Twenty-three plants were inoculated for each virus. Groups of plants of the same age were inoculated by viruliferous aphids that were transferred from infected safflower plants. Inoculated plants were kept in a randomized design in the greenhouse and observed for symptoms. Groups of TuMV-infected plants were allowed to bolt, flower, and develop seed. Plant height, weight, branching, and number of flower heads of virus-infected and healthy plants were compared. Seeds were collected from infected plants and planted in the greenhouse. Plants were observed for mosaic symptoms.

RESULTS

Six of 18 starthistle plants inoculated with *S. sclerotiorum* died within 7–10 days. *S. sclerotiorum* was reisolated from crowns of dead plants.

Botrytis sporulated profusely on seed-head flowers within 24–48 hr under moist conditions. Infected heads became brown and the supporting stem turned yellow and necrotic 7–14 days after inoculation. Diseased seed heads did not produce seeds.

Starthistle plants were susceptible to isolates representing different races of *F. o. carthami*. As many as 23% of the plants growing in infested soil developed yellowing and necrosis of lower leaves within 2–4 wk. The disease was frequently lethal, with plants dying in the seedling or early rosette stage. Diseased plants that survived had vascular discoloration in the upper root and lower stem. Systemic symptoms were not observed in bolting plants that had symptoms earlier. Disease occurred in plants that were growing in soil containing *Fusarium*-infested grain or V-8-juice-vermiculite but not when only conidia were used as inoculum. Pathogenicity of *Fusarium* isolates did not differ significantly.

Disease incidence was not increased by four successive plantings of starthistle in the same *Fusarium*-infested soil. Infestation of soil with isolates of *Fusarium* from diseased starthistle plants did not alter disease incidence or severity.

The reaction of starthistle to *V. dahliae* pathotype SS-4 was mild and limited to the rosette stage. Yellowing of the lower leaves occurred in 16 of 43 plants in the greenhouse within 3–5 wk. Among 72 plants, T-1 was lethal to 33 (45%), and 23 (32%) showed yellowing of leaves. Symptoms from T-1 developed in plants in rosette, vegetative, and flowering stages. Six hundred and 1,200 seeds collected from SS-4- and T-1-infected plants in the greenhouse, respectively, did not yield *Verticillium* in laboratory assays.

Among 69 plants in a field plot infested with *V. dahliae* pathotype T-1, nine diseased plants died before or after flower bud development and 20 surviving diseased plants developed symptoms extending into the upper branches of the plant.

Roots and stems of starthistle plants were susceptible to *Phytophthora* spp. under flooded conditions. In two experiments with three *Phytophthora* spp., 61–66% of the plants inoculated in the root zone died and 61–77% died from stem infection. Plants showed wilting 24–48 hr after flooding followed by yellowing of leaves, leaf necrosis, and subsequent death. Plants that survived did not have extensive rotting of roots or stems. Mean dry weights of roots (3.16–3.66 g) and plants (3.94–4.21 g) grown in artificially infested soil were significantly reduced compared with those of roots (6.65 g) and plants (5.19 g) grown in noninfested soil.

Aphid transmission on of LMV, AMV, and TuMV to starthistle was 63, 51, and 21%, and mechanical transmission was 34, 31, and 39%, respectively. Systemic mosaic was a common symptom of each virus. Plants infected with AMV or LMV showed moderate stunting and fernlike leaf development on plants in the rosette stage. TuMV caused severe symptoms such as stunting, leaf distortion, reduced leaf size, and necrosis. Plant height (48.8 cm), plant dry weight (10.7 g), and root

dry weight (1.9 g) of TuMV-infected plants were significantly reduced compared with similar components (60.8 cm, 16.7 g, and 4.5 g, respectively) from healthy plants. The numbers of lateral branches formed and flower heads produced were not significantly reduced. Occasionally, TuMV and LMV were lethal to plants in the early rosette stage. Two hundred plants grown from seeds produced on TuMV-infected plants did not show mosaic or other symptoms of virus infection in the greenhouse.

DISCUSSION

Although some yellow starthistle plants affected by *S. sclerotiorum* were found in scattered areas in surveys, the weed for the most part appears free of diseases in the field. Because populations of starthistle have a paucity of diseases, the plants may be resistant to infection by plant pathogens in nature. However, susceptibility of starthistle to various plant pathogens in a manipulated environment casts some doubt on its resistance. The weed is vulnerable to attack under an environment that is favorable to the pathogen. During the seedling and rosette stages, which extend from fall into spring, rainfall and temperatures provide a favorable environment for leaf pathogens, yet leaf diseases have not been observed. During the winter season, cool ambient temperatures might preclude the activity of some plant pathogens. When the plants bolt in late spring and arid conditions prevail, root infection by soilborne pathogens could occur.

Little is known regarding the impact of *Phytophthora* spp. on starthistle in nature. Although starthistle was susceptible to the three *Phytophthora* spp. tested in this study, the activity of these and other *Phytophthora* spp. during the rainy season may be precluded by low soil temperature. During the dry season when starthistle plants are branching and flowering, temperatures are more conducive for *Phytophthora*; however, moisture becomes a limiting factor. Irrigation to enhance *Phytophthora* activity may not be economical or practical because of the terrain where starthistle grows.

F. o. carthami was usually lethal to infected young plants in the greenhouse, but disease development did not progress in plants that survived. Ideally, a forma specialis of *F. oxysporum* is needed that would affect plants beyond the seedling and rosette stages, thereby affecting reproduction.

V. dahliae has the potential to reduce seed production by infected plants, because it spreads systemically into the flowering branches.

Botrytis is not a likely candidate for use in biological control of starthistle because of moisture requirements for its establishment on seed-head flowers. Nevertheless,

when such conditions are afforded naturally or artificially, the pathogen can preclude seed development.

Yellow starthistle plants were susceptible to AMV, LMV, and TuMV in the greenhouse, but it is not known if the plants are infected by these or other viruses in nature. Weeds and wild plants are commonly infected with viruses (1); however, host preference of vectors could influence the role of starthistle plants as a virus host. Starthistle plants infected with TuMV would be less competitive in weed populations because of stunting and thus have reduced potential for normal development and seed yields.

In this study, starthistle plants were generally susceptible to pathogens in the

seedling and rosette stages. In preliminary studies on diseases of starthistle seedlings, the plants were vulnerable to preemergence and postemergence diseases caused by certain soilborne pathogens (J. M. Klisiewicz, *unpublished*).

Biological control with indigenous organisms depends to a large extent on their host-specificity. The pathogenic fungi used in this study are not host-specific and could attack other plants of recognized value.

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