Relationship Between *Phoma macdonaldii* and Premature Death of Sunflower in North Dakota

P. A. DONALD, Former Graduate Research Assistant, J. R. VENETTE, Associate Professor, and T. J. GULYA, Adjunct Professor, USDA ARS, Department of Plant Pathology, North Dakota State University, Fargo 58105

ABSTRACT

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Isolations from prematurely dead field-grown sunflowers yielded six potentially pathogenic fungal genera. *Phoma macdonaldii* was the single most commonly isolated organism from these plants. Koch's postulates were completed in the greenhouse with two isolates of *P. macdonaldii*. Evaluation of 370 field-grown plants showed a perfect association between presence of girdling lesions on naturally infected plants and 185 plants with premature death. *P. macdonaldii* was isolated from the girdling lesions.

The term "premature ripening" was first used in association with sunflower (*Helianthus annuus* L.) in 1950 (10) to describe a wilt and stalk rot of unknown etiology. Gulya et al (4) list synonyms of premature ripening; however, premature death better describes the symptomatology because sunflower plants die at any growth stage, often before seed set. Sunflower premature death is most often characterized by loss of plant vigor during midsummer to late summer followed by senescence and death of the plant a few weeks before normal maturity. Symptoms of premature death are difficult to distinguish from normal senescence, especially in early-maturing varieties. Leaves wilt and become necrotic, stalks turn dark brown to black, and plants die. Scattered individual plants die, but most often, premature death occurs in circular spots in the fields, especially fields with a history of premature death. All plants in a field may die prematurely in severe cases. Generally, prematurely dead plants have small heads, reduced seed yield, and seed low in weight and oil content. In North Dakota, prematurely dead plants can yield 65% less seed than plants without symptoms (1). Fungi in the genera *Phoma* (4), *Alternaria* (4), *Fusarium* (7), *Phymototrichum* (7), *Phomopsis* (5), and *Macrophomina* (13) have been suggested as contributing to premature death or causing it in different sunflower-growing regions of the world. Sunflower stem-infesting insects, *Apion occidentale* and *Cylindrocopturus adspersus*, also may act as vectors for some pathogenic fungi associated with premature death (3,13). This paper documents the relationship between *Phoma macdonaldii* Boerema and premature death of sunflowers in North Dakota.

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MATERIALS AND METHODS

Isolations. Each year in August and September from 1981 to 1985, five to 10 prematurely dead sunflower plants were collected from each of 20 commercial fields in eastern North Dakota. The stalks were cut about 60 cm above the soil line after plants were removed from the soil. The basal stalk portions, with roots, were bagged individually, kept in a cooler, and transported to the laboratory cold room (4 C) within 8 hr of collection. Isolations of fungi from the margins of necrotic tissue on roots and stems were made within 24 hr of collection. Affected tissue was excised, surface-disinfested in 95% ethanol, and flamed. Subsamples were aseptically plated onto acidified potato-dextrose agar (APDA). Isolates were incubated at 25 ± 5 C under fluorescent light (50 μE m^{-2} s^{-1}) with a 12-hr photoperiod. Fungi grew within 7 days, and hyphal tips were subcultured on APDA. Repeated hyphal-tip transfer was used to ensure purity. Fungi were identified by microscopic observation through use of Riddell preparations (8), and comparisons were made with known cultures. Pure isolates were retained in a collection on PDA slants at 22 C.

Pathogenicity tests. Twenty-seven fungal isolates were tested for pathogenicity. These isolates were representative of the total collection of 632 isolates from diseased field-grown plants. Ten plants were inoculated with each fungal isolate by immersing roots and lower stems up to the cotyledons in an inoculum suspension. The inoculum was prepared by growing fungi on PDA in glass culture plates (100 × 15 mm) until mycelial growth covered the agar surface. The mycelium and PDA from a single plate were blended with 100 ml of sterile distilled water (SDW) to form a suspension (12). Sunflower (cultivar Interstate 894) seedlings at the V4 (four-leaf) (11) growth stage that had been grown in pasteurized sand were removed from the sand, rinsed in tap water, immersed for 2 min in the fungal suspension, and transplanted singly into clay pots (1-L capacity) containing a mixture of pasteurized Glyndon sandy loam, sand, peat, and compost (3:1:1:1). Ten control plants were inoculated with SDW. Pots were arranged in a completely random design on the greenhouse bench, and plants were grown to maturity at 25 ± 5 C with a 16-hr photoperiod.

From the 27 isolates, one *F. moniliforme* Sheldon, one *A. alternata* (Fr.) Keissl., and two *P. macdonaldii* isolates were selected for further tests. In each of two trials, 10 seedlings were inoculated as previously described with each fungal isolate except *P. macdonaldii* isolate 1. Twenty and 10 seedlings were inoculated with *P. macdonaldii* isolate 1 in the first and second trial, respectively. Ten seedlings immersed in SDW were used as controls. Contamination from the bench or other pots was minimized by placing each pot in a plastic saucer and covering each pot with a sheet of plastic containing a small slit for the sunflower stalk. Plants were fertilized monthly with 20-20-20 NPK fertilizer (Peters Fertilizer Products, Allentown, PA) applied through a siphon mixer. Plants that senesced (2) when control plants were still green were considered prematurely

dead. Plants were harvested when controls senesced, about 2.5 mo after inoculation. Cross sections of the affected stem tissue from the base of each prematurely dead plant were plated on APDA to determine if any fungal pathogen could be consistently reisolated. Roots were examined for root development and disease symptoms. Shoot dry weight was recorded after plants were dried for 1 wk at 38 C in a forced-air dryer.

Natural field infections. The association between premature death and complete girdling of sunflower stalks was evaluated in the field. In 1984, a total of 85 unaffected and 85 prematurely dead plants from five fields were examined for stem-girdling lesions. In 1985, 100 unaffected and 100 prematurely dead plants from two fields were evaluated for stem-girdling lesions. Tissue from the margins of girdling lesions on a representative sample of 25 prematurely dead plants were plated on PDA to detect pathogenic organisms.

RESULTS AND DISCUSSION

Isolations. A total of 632 fungal isolates were obtained from dead and dying field-grown sunflower plants. Ninety-nine percent of the isolates were from six potentially plant-pathogenic genera. Forty-eight percent of the isolates were from the genus *Fusarium*, representing at least six species, and the largest number of isolates per species was 73. *P. macdonaldii* made up 27% of the isolates and was isolated from both roots and stalks but predominantly from lower stalks. *A. alternata*, found in 20% of the isolations, was predominantly from stalks. Other species of *Alternaria* were probably present, but *A. alternata* rapidly overgrew the other fungal species and genera, making isolation difficult. One species each of *Rhizoctonia, Nigrospora,* and *Pythium* (2, 2, and 0.2%, respectively) was isolated from roots. Nine unidentified isolates represented three morphologically distinct fungi.

Pathogenicity tests. The dip inoculation technique was chosen for pathogenicity tests because premature death had the characteristics of a root or lower stem problem. Most of the isolated fungi, except perhaps *A. alternata*, are root- or lower stem-inhabiting organisms (6), and the technique caused sufficient infection to test fungal pathogenicity at the seedling level. The toothpick inoculation technique (14) was tried but was found impractical with young seedlings. Twenty-two of the 27 fungal strains isolated from roots and stalks of dying field-grown sunflower plants had no effect on greenhouse-grown sunflower seedlings. The initial test showed that four isolates of *P. macdonaldii* and one isolate of *F. moniliforme* caused premature death. When retested, neither *A. alternata* nor *F. moniliforme* caused premature death, *P. macdonaldii* isolate 1 produced premature death on 47% of the plants, and *P. macdonaldii* isolate 2 produced premature death on 100% of the plants (Table 1). *P. macdonaldii* isolates 3 and 4 were not retested. All plants inoculated with isolates 1 and 2 of *P. macdonaldii* had black lesions at the base of the stalk, but only those that had lesions girdling the stalk died early. *P. macdonaldii* isolate 2 significantly reduced shoot dry weight, whereas *P. macdonaldii* isolate 1 did not. The root systems of the girdled plants were poorly developed and necrotic. Isolations from basal stalk tissue of prematurely dead plants resulted in reisolation of *P. macdonaldii* that was identical in characteristics to the inoculum. Therefore, Koch's postulates with *P. macdonaldii* were completed.

Because other fungi have been associated with symptoms of sunflower premature death, it is probable that sunflower premature death is a complex problem analogous to potato early death (9), where a number of organisms interact under different environmental conditions to cause the malady. Some of these fungi (*Phymatotrichum, Phomopsis,* and *Macrophomina*) associated with sunflower premature death in other parts of the world occur rarely, if at all, on sunflower in North Dakota because of the cool climate. The contribution of other fungi such as *F. moniliforme* may be highly dependent on specific environmental factors. No experiments were conducted to see if other organisms (i.e., *Fusarium*) aggrevated or accentuated the effect of *P. macdonaldii* infection.

All of the pathogens isolated in this study, with the exception of *Alternaria*, are root- and lower stem-infecting pathogens (6). One method of inoculation is not likely to be ideal for all these pathogens, and the dip technique was chosen as a compromise to expose both stems and roots to the inoculum. Stem lesions and root necrosis were seen in the *P. macdonaldii*-inoculated plants, but it is not known whether root symptoms were due to direct root infection, to basal stem infection progressing into the roots, or to basal stem girdling inhibiting nutrient transport into the roots and essentially starving the roots to death.

Natural field infections. In 2 yr of field sampling, the relationship between premature death and Phoma girdling was established. None of the unaffected plants were girdled by *P. macdonaldii*, whereas 100% of the dead sunflower plants were girdled by *P. macdonaldii*. Some of the nonprematurely dead plants had basal *Phoma* lesions, but the lesions never completely girdled the stem.

The isolation of *P. macdonaldii* from field-grown, naturally infected commercial sunflowers showing premature death symptoms and completion Koch's postulates in the greenhouse combined with a perfect association between Phoma girdling and premature death in the field gives evidence that *P. macdonaldii* is a causal agent of premature death of sunflower in North Dakota. We encourage use of the term Phoma girdling to describe this aspect of premature death.

Table 1. Effect of dip inoculation of different fungal isolates on greenhouse-grown sunflower premature death and shoot dry weight

Treatment[y]	Plants (no.)	Plants showing premature death (%)	Shoot dry weight (g)
Control (sterile distilled water)	20	0	27.9 a[z]
Fusarium moniliforme	20	0	31.3 a
Alternaria alternata	20	0	30.3 a
Phoma macdonaldii 1	30	47	27.5 a
P. macdonaldii 2	20	100	3.4 b

[y]Plants were dip-inoculated at the V4 growth stage and grown for 3 mo in the greenhouse.
[z]Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

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