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Life History of *Elsinoë palicari*¹

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*Elsinoë palicari* Tiffany and Mathre is widespread on native *Panicum virgatum* L. in Iowa. In June *Sphaecoma* (imperfect stage) conidia develop from acervuli on overwintered stromata of wind-dispersed leaf litter and culms. Germ tubes penetrate between epidermal cells of young leaves, and 2 wk later tiny yellowish, subepidermal stromata are visible. Secondary conidia develop from acervuli on these stromata within 2 wk and are spread to adjacent leaves. Stromata become black, raised, shiny, ovoid-elongate patches, composed of thick-walled pseudoparenchymatous cells. Chlorosis or necrosis does not develop around the stromata. Stromata are visible on all leaves throughout the season. Acervuli, appearing as white spots in black stromata, open by irregular tears in the epidermis. Ascostromata develop on lower leaves infrequently from late July into September. They are tan-brown, raised, ovoid-elongate, softer, and more loosely structured than stromata. Ascii mature at different times. Ascosporae are discharged from the bitunicate ascii and germinate directly by budding or by germ tubes.


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In 1961, a leaf-spotting pathogen on *P. virgatum* was described as *E. palicari* by Tiffany and Mathre. The genus *Elsinoë* exhibits a simple, undifferentiated, innate, intra- or sub-epidermal ascomye. Members of the genus produce hyaline to yellowish ascosporae with three transverse septa and longitudinal septa in some cells in globose to elliptical bitunicate ascii (Luttrell, 1973).

The only other reports of *Elsinoë*, or of the closely related genus *Myriangium*, on a member of the Graminaceae are of *M. bambusae* by Rick on *Phyllostachys pubera* Munro in China (Tai, 1931) and *E. saccaria* by Lo on *Saccharum officinarum* L. from Taiwan (Lo, 1964) and Florida (Todd, 1960). All other known hosts for *Elsinoë* spp. are herbaceous or woody dicots. Most *Elsinoë* spp. are tropical or subtropical. However, temperate-zone diseases caused by *Elsinoë* spp. do occur (Brook, 1973; Jenkins, 1932; Jenkins et al., 1946; Jones, 1924; and Massey and Jenkins, 1953). Most studies have concerned life histories, symptoms, inoculations, and growth in culture with economically important hosts. *E. palicari* has never been observed on other hosts, including other species of *Panicum*.

There has been recent interest in *P. virgatum* because of its use as summer and fall forage. The purpose of this study was to elucidate a life cycle for *E. palicari* and to provide background information for assessing disease losses and selecting plant material in breeding programs.

**MATERIALS AND METHODS**

**Field Observations**

Leaf collections were made regularly from August 1981 to December 1984 at various sites (Fig. 1). Diseased leaves were cataloged and either pressed or killed and fixed. Leaves with ascostromata (stromata that consistently developed ascii and ascosporae) and stromata (stromata that did not usually develop ascii, but often formed acervuli) were placed in moist chambers at 4, 15, and 25°C for 3-5 days and observed at regular intervals for development.

Overwintering and spring development were observed by placing 100 leaves, with ascostromata or stromata from fall 1983 collections, in nylon mesh bags attached to stakes anchored on the soil surface in a reconstructed prairie near Ames, Iowa. Samples were removed the following March, May, and June, examined directly for conidia and/or ascosporae, and then placed in moist chambers at 4, 15, and 25°C for 3-5 days and observed for conidia or ascosporae.

Ascospore maturity was followed from August to October 1984 by removing 15-20 ascostromata from the lowest or next lowest leaf and observing ascosporae of 100 ascii from each sample. Ascosporae were considered mature if transverse septa were observed. This maturity information was used to determine persistence of the sexual system.

**Inoculation of *Panicum virgatum***

Isolates for inoculation were obtained from green infected leaves of *P. virgatum* collected at various sites. The leaves were dipped in 95% ethyl alcohol and immersed in 0.525% NaOCl for 5 min. Small pieces of leaves were placed on potato dextrose agar (PDA) amended with an 80 mg/l solution of streptomycin sulfate. Isolates were incubated in the dark or in laboratory light at 25°C and transferred to PDA every 4 wk. Cultures were initiated by spreading 0.3 ml of a conidial suspension over surfaces of PDA plates and then holding plates for 3 wk at 25°C. Conidia were harvested from plates with deionized water, concentrations determined with a hemacytometer and used as inoculum.

**Detached Leaves** — Leaves of plants grown from rhizomes of previously infected plants from Kalow State Preserve (IKP) in Pocahontas County and leaves of plants of the cultivar Blackwell (BP) grown from 1979 seed were detached, and 2 drops of a conidial suspension containing 25 × 10⁶ spores/ml of water were placed on

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each leaf. Leaves were placed in humidity chambers and incubated in laboratory light at 25 C. Half of the leaves from each source were inoculated on the adaxial surface and the remaining leaves were inoculated on the abaxial surface. Samples were removed periodically up to 15 days after inoculation, cleared in saturated chloral hydrate for 7 days, stained 20 min in 0.1% cotton blue in water, and mounted in polyvinyl alcohol-glycerin-lactic acid (PVLG) (Omar et al., 1979). Spore germination and early stromatal development were observed.

Greenhouse — Inoculated plants were sprayed with conidia suspended in deionized water until runoff. Control plants were sprayed with water. Plants were considered infected when a stroma was observed on any leaf.

In experiment one, inocula from isolates of E. panici obtained from six Iowa prairies were adjusted to 15 × 10^6 conidia/ml. Each inoculum was sprayed individually and in all possible pairs on 4-mo-old BP. There were 2 replicate pots per treatment. Plants were covered with plastic bags for 72 hr after inoculation, and moist treatment was continued each night for 6 wk by placing pots in a 1.6 m × 1 m × 1 m humidity box equipped with a wet sphagnum floor and a mister. Stromata were observed for sexual structures for 4 mo.

In experiment two, infection and disease development were compared among BP, IKP, and plants grown from rhizomes of previously uninfected Kalsow State Preserve plants (UKP). Plants were sprayed with 25 × 10^6 conidia/ml obtained from a Kalsow State Preserve isolate. Half of the plants from each source were covered with plastic bags for 72 hr. The remainder were left uncovered. Moist treatment for the covered plants was continued at night in the humidity box for 4 wk.

In experiment three, 17 × 10^6 conidia/ml were used to inoculate five pots of IKP and 2 pots of BP that had approximately equal numbers of leaves. All plants were covered with plastic bags for 72 hr immediately after inoculation, and also at night for 4 mo. Disease progression on emerging plants and their individual leaves was monitored and marked approximately every 10 days. Different tillering rates between IKP and BP resulted in unequal numbers of leaves assessed.

Spore Studies

Conidia — Conidial germination was observed in spore suspensions on slides in sterile moist chambers, on water agar (WA) or on PDA plates incubated at 25 C.

The effect of temperature on conidial germination was observed by preparing 0.3 ml of a 1 × 10^6 conidia/ml deionized water suspension from a 3-wk-old culture of a greenhouse resolate, spreading on 3- or 4-day-old WA plates and incubating in the dark at 5, 10, 15, 20, 25, and 30 ± 1 C. One hundred spores per plug were observed on each of three plugs, randomly cut with a #6 corkborer from three replicate plates from each temperature after 24, 72, and 120 hr. Spore germination and chlamydospore formation were noted.

Ascospores — To study ascospore discharge and germination, ascostromata were removed aseptically from leaves, added to 2 ml of sterile deionized water, and the suspension spread on WA slides placed in sterile moist chambers. Slides were held at 4 or 25 C and observations were made periodically for 12-48 hr.

RESULTS

Field Observations

Panicum virgatum infected with E. panici was collected at 43 Iowa sites in 26 counties (Fig. 1). Commercial varieties planted along roads were monitored for disease but were not observed to be infected with E. panici. E. panici was never found on any other host, including other species of Panicum.

Overwintering — Conidia were observed on stromata on overwintered P. virgatum residue in early spring of 1982-1984. Conidiophores in acervuli on 1983 culms and leaves of P. virgatum overwintered in mesh bags contained conidia from April to early June, 1984, or produced them when overwintered material was placed in moist chambers. Ascostromata produced in Fall 1983 were recovered occasionally from overwintered material next spring, but were less intact than stromata. No ascus or ascospores were present, but some conidia were observed.

Infection and disease progression — The first signs of infection on emerging leaves of P. virgatum at Doolittle Prairie were raised, translucent spots approximately 1 × 1 mm observed during mid-June to early July. Acervuli developed in these stromata after 1 wk. By mid-July, when inflorescences of P. virgatum plants were expanded, nearly 50% of leaves randomly collected were infected. Over 50% of leaves were infected by mid-August. Most stromata occurred on abaxial leaf surfaces, but moderate to heavily infected leaves had stromata on both surfaces (Fig. 2). Conidia were recovered from acervuli developed on stromata throughout the growing season. Lower leaves were infected first, and the fungus progressed slowly up the plant. By late summer or early fall, leaves, leaf sheaths, culms, the rachis, glumes, and lemmas were infected on heavily diseased plants. Some plants near but not in contact with diseased plants remained uninfected throughout the season. Disease patterns were similar during each year of the study.

Ascostromata were first observed on leaves collected from three prairies in August 1983. In 1984, ascostromata were observed on leaves collected from the same and from two additional sites (Fig. 1). Usually ascostromata developed separately, but occasionally were with larger asexual stromata.

Percentages of leaves with ascostromata collected from the Kalsow State Preserve from August 11 to October 5, 1984 are presented in Fig. 3. Percentages of #1 (lowest) and #2 (next to lowest) leaves with ascostromata remained considerably higher than for other leaves on the same plants. Similar patterns were seen from ascostromata collections at two other sites during the same period.

By August, ascostromata contained flattened eroded areas where mature ascus had released ascospores. Mature ascus were observed on leaves from Kalsow State Preserve from July 25 to September 28, 1984 (Fig. 4). Percentages of #1 leaves with functional ascostromata varied as the ascostromata matured and eroded. Number 2 leaves had similar patterns for comparable collection dates. A similar pattern of ascostroma, ascus, and ascospore development was observed at the two other sites during this period. The sexual system evidently continues to function from July to September as continued production of asci.
Establishment of *E. panici* on Inoculated Plants

**Detached Leaves** — Detached inoculated leaves were cleared to examine germination and early hyphal development. Typically, conidia germinated within 3 days after inoculation. Conidia germinated by short, thick germ tubes, which darkened and developed into irregularly torulose hyphae, the width occupying about half the width of an epidermal long cell. Hyphae grew parallel to the long axis of epidermal cells and between epidermal cell walls. Hyphae quickly modified into masses of blocky cells comprising the young stromata. Each cell measured approximately 2.5 × 2.5 µm. Most young stromata were located over vascular bundles and associated with silica cells or trichomes. Conidial germination and infection of abaxial and adaxial surfaces were similar and consistent on the two different sources of *P. virgatum*.

**Greenhouse** — **Experiment one.** Stromata were observed on the abaxial surfaces of leaves on 4-mo-old BP 14 days after inoculation with a concentration of 15 × 10⁶ conidia. Stromata developed on leaves inoculated with all six isolates and pairs of isolates, and conidia were produced in acervuli 2-3 wk after inoculation. With continued high humidity, new secondary infections developed after 1 mo on leaves that had emerged since inoculation. Stromata were examined periodically for sexual stages during the 4 mo after inoculation, but none was observed. Conidia were consistently present.

**Experiment two.** Typical stromata developed 3 wk after inoculation with 25 × 10⁶ conidia/ml on BP, IKP, and UKP receiving high humidity. Inoculated plants without humidity treatment were not diseased.

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Fig. 3. Percentages of #1 leaves (lowest) to #6 leaves (top) of *Panicum virgatum* with ascostromata of *Elsinoë panici* collected from Kalsow State Preserve, August-October, 1984.

occur, replacing those that had been present in upper eroded areas of ascostromata.
Table 1. Numbers of healthy and infected leaves occurring from secondary infections following inoculations with *Elsinoë panic* on *Panicum virgatum* Kalsow and Blackwell plants, February 5, 1984.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
</tr>
<tr>
<td>Kalsow</td>
<td>N</td>
</tr>
<tr>
<td>Blackwell</td>
<td>N</td>
</tr>
</tbody>
</table>

H = number healthy leaves  
D = number of diseased leaves  
N = new plants emerging; tagged  
M = missing data

**Experiment three.** All pots of IKP and BP developed stromata with conidia 17 days after inoculation with 17×10⁶ conidia/ml. Fifty-five days after inoculation, 5% of the newly emerged leaves of IKP were infected (Table 1). Emerging plants expressed disease approximately 10 days after emergence. Seventy days after inoculation, 17% of the leaves on IKP plants were diseased. The percentage of diseased leaves decreased for IKP plants as leaves emerged at a rapid rate. Ninety days after inoculation, 44% of leaves on IKP plants were infected. Leaves of BP plants showed no secondary infections until 119 days after inoculation, when disease had increased on BP and IKP leaves to nearly equal percentages.

In all greenhouse inoculations, the disease progressed slowly in individual plants and spread from plant to plant in the same pot. Leaves became infected at different rates, but lower leaves were infected before upper ones. Only after the disease was well established on abaxial surfaces were symptoms seen on the adaxial surface. Uninoculated IKP plants in adjacent pots remained uninfected throughout the experiment. Ascostromata were not observed.

**Spore Studies**

**Conidia** — Hyaline to slightly pigmented conidia from any source, such as field collections, cultures from diseased leaves, budding ascospores, or subcultures were heterogeneous in size and shape (Fig. 5). Most conidia were slightly elongate and uninucleate (Fig. 6). On diseased leaves, conidia were produced from flask-shaped conidiophores lining acervuli, but in culture, conidia were either produced infrequently from randomly occurring conidiophores (Fig. 7) or more often budded from germ tubes, chlamydospores, or conidia (Figs. 8, 9, 10). Conidia germinated by germ tubes or by budding. Germination by germ tubes was typically monopolar, but occasionally a germ tube would emerge from the side of a conidium, and bipolar germination occasionally was observed (Figs. 11, 12, 8).

Germination of conidia was extremely variable among experiments conducted to determine temperature effects (Fig. 13). Germination was highest at 5-15°C, declined at 20-25°C, and increased at 30°C. The same isolate, subcultured for each experiment, was used for the first three experiments. A different isolate was used for experiment four, and this may account for the relatively low percentage germination of this isolate. Because of the extreme variation among experiments, they were not combined as replications to satisfy conditions required for an analysis of variance.

Between 25-35°C, many conidia enlarged and became round with dense cytoplasm. These structures were interpreted as chlamydomospores formed by direct conversion of a conidium. These chlamydomospores were similar to detached terminal and intercalary chlamydospores formed in mycelial cultures.

**Ascospores** — Ascii from ascostromata collected August 17, 1984, from Doolittle Prairie spontaneously released ascospores in water mounts. Ascospores germinated readily in water by budding elongate conidia (Fig. 14). Most young buds were 3.3×3.3-4.4 µm, but grew to typical conidial size. One-septate ascospores germinated as readily as 2- or 3-septate ascospores. Each cell of the ascospore was capable of germinating, and sometimes some cells of a single ascospore produced germ tubes while other cells budded conidia. Conidia formed during germination continued to develop conidia by budding. Occasionally, ascospores still within an ascus would germinate by forming germ tubes which grew through the ascus walls.

**CONCLUSIONS AND DISCUSSION**

**Life Cycle**

*Panicum virgatum* plants in Iowa resume growth from early to late June. About the same time, when temperatures are favorable and during periods of high humidity and dew, conidia develop from acervuli in overwintered stromata in host debris. Because conidia are thin-walled spores, they probably do not survive the winter. No ascospores were observed from overwintered stromata or ascostromata; thus, conidia seem to be the primary inoculum.

During June, splashing rain and dew spread conidia to leaves of young *P. virgatum* plants where conidia germinate during cool (10-15°C) periods of high humidity or dew, usually during the night and early morning hours. Spores can germinate from 5 to 30°C. Germination at higher temperatures is important in establishing secondary infections later in the season.

Conidia germinate, producing hyphae that infect leaves and modify into stromata by 3 days after inoculation. Conidia may form resistant chlamydospores at 25-35°C. Conidia budded from chlamydospores at 30°C may increase inoculum when this temperature occurs in the field during the summer. Conidia germinate by either forming germ tubes or budding, but chlamydospores germinate only by budding elongate conidia.

One to two wk after leaf emergence, tiny, translucent microscopic stromata appear on leaves. Young stromata can develop acervuli and produce conidia within 1-2 wk. Thus, approximately every 4 wk, a new generation of conidia can develop and be available to infect new leaves. Conidia are disseminated by rain and dew.

Stromata increase in number as the season progresses. Colonization by *E. panic* is first observed on lower leaves and progresses up the plant, eventually involving all leaves. Stromata become black and firm as they mature, and by August, diseased leaves are obvious because of the numerous shiny black stromatal areas. Acervuli on stromata continue to produce conidia. Although no chlorosis or necrosis is associated with spots, some leaves occasionally display a red pigmentation around the periphery of the black stromata.

In addition to stromata, softer ascostromata containing asci and ascospores may develop, primarily on the lower two leaves of plants, from late July until the first frost. These two types of stromata differ in stromal morphology (Gabel, 1985), distribution among sites, predic-
tability of development any year in the field, and leaves colonized.

Shortly after release, ascospores germinate by germ tubes or by budding conidia. Thus, ascospores could be a source of inoculum in late summer or early fall. As early as August, many ascostromatal areas are eroding, accompanied by ascospore release from mature asci. Some ascostromata may overwinter and can produce conidia in the spring.

In fall, diseased leaves become part of the prairie litter or remain on the persistent culms. *E. pani*c does not colonize such dead host material. In spring, conidia produced from stromata on partially decomposed and scattered leaf debris are the source of primary inoculum. When spring burning of prairies as a management practice removes overwintering plant debris, the fungus is usually eliminated for the following growing season.

The life cycle of *E. pani*c is similar to that of other species of *Elsinoe*. Conidia of *E. corni* Jenkins and Bitanc. (Jenkins et al., 1953) and *E. ampelina* Sheir (Brook, 1973) served as primary inoculum. However, ascospores as well as conidia were primary inoculum of *E. veneta* (Burk.) Jenkins (Jones, 1924).

Optimum temperature for conidial germination was different for some species, for example 22-26°C for *E. veneta* (Jones, 1924) compared with 10-15°C for *E. pani*c.

Two species, *E. favetti*B. and Jenkins (Whiteside, 1975) and *E. brasiliensis* Bitanc. and Jenkins (Zeigler and Lozano, 1983) produce two types of conidia. Hyaline conidia germinate by germ tubes or budding and colored conidia only by budding. Colored conidia of these species could be similar in function to the chlamydospores of *E. pani*c.

Incubation periods of 6 days for *E. veneta* (Jones, 1924), 10-14 days for *E. brasiliensis* (Zeigler and Lozano, 1983), and 7-14 days reported here for *E. pani*c are similar. Brook (1973) reported a decrease of incubation period for *E. ampelina* with an increase in temperature.

In contrast with conidia of *E. ampelina* which initiated infections up to 10 m in 4 mo (Brook, 1973), conidia of *E. pani*c were not widely disseminated.

The sequence of infections of *E. veneta* on new cane growth (Jones, 1924) is very similar to the progression observed with *E. pani*c. *Elsinoe panici* does not elicit a chlorotic or necrotic response on its

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**Figs. 5-12. Elsinoë *panici* conidia.**

Fig. 5. Wet mount of heterogeneous conidia from culture (Bar equals 10 µm). Fig. 6. Elongate conidium with a single nucleus from a squash of field material, stained with Whittman's aceto-iron hematoxylin observed with phase-contrast optics (Bar equals 10 µm). Fig. 7. Wet mount of conidia formed from conidiophores in culture (Bar equals 10 µm). Fig. 8. Wet mount of elongate conidium with bipolar germ tubes budding elongate conidia (Bar equals 10 µm). Fig. 9. Wet mount of elongate conidia budding from chlamydospores cultured on potato dextrose agar at 35°C (Bar equals 0 µm). Fig. 10. Wet mount of elongate conidia budding from chlamydospores cultured on potato dextrose agar and observed with phase-contrast optics (Bar equals 10 µm). Fig. 11. Wet mount of monopolar germination of conidium in culture (Bar equals 10 µm). Fig. 12. Wet mount of conidium producing a germ tube (see arrow) from the median area (Bar equals 10 µm).
graminaceous host. Only a red discoloration was associated with stromata of E. saecularis on specimens (No. 541 and 522) on loan from the New York Botanical Garden Mycological Herbarium (NY). Myriangium bambusae specimens (NY) developed stromata with no associated chlorosis or necrosis. However, many species of Elsinoe attacking dicotyledons hosts produce necrotic areas (Jenkins, 1931a, 1932; Jenkins et al., 1946; and Massey and Jenkins, 1935).

Ascospores of E. panici germinate to form germ tubes or bud, functioning similarly to ascospores of M. bambusae (Tai, 1931), E. canavaliae Rac. (Jenkins, 1931a, b), E. veneta (Jones, 1924), and E. brasilienisi (Zeigler and Lozano, 1983) if microconidia, conidia, and sprout cells of these fungi are interpreted as similar structures.

Distribution and Persistence

A collection of diseased P. virgatum in Story County, Iowa, made by C.E. Bessey in 1882 was determined to be infected with E. panici (Tiffany and Mathre, 1961), documenting the disease in central Iowa over 100 years. Collections of E. panici on P. virgatum made during the late 1950s to early 1960s document the disease from Story, Pocahontas, Boone, and Webster counties in central and west-central Iowa (Tiffany and Mathre, 1961). Collections made during the present study document the disease from these same counties and confirm that E. panici frequently infects native P. virgatum throughout Iowa. P. virgatum is widely distributed in Iowa and is common in many prairie remnants (Pohl, 1966). The few collections of P. virgatum from northeastern Iowa examined in the present study were not diseased. Collections from north-central Nebraska (Keya Paha and Holt counties) and western South Dakota (Lawrence and Harding counties) indicate that the disease may be widespread.

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Gabel and Tiffany: Life History of Elsinoe panic

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