Aerobiology of *Claviceps purpurea* in Kentucky Bluegrass

S. C. ALDERMAN, USDA-ARS, National Forage Seed Production Research Center, Corvallis, OR 97331

ABSTRACT

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Airborne ascospores of *Claviceps purpurea* were monitored with volumetric spore traps in two fields of Kentucky bluegrass in Oregon during 1989 and 1990. A diurnal periodicity in ascospore release was observed, with peak catches between 1:00 and 6:00 a.m. Sporulation events (one or more consecutive days on which ascospores were trapped) generally began 2–3 days after rain and continued for 1–16 days. Cumulative number of ascospores trapped paralleled cumulative hours per day with rain. Ascospore release in the field was not correlated with temperature or relative humidity. Under controlled temperature conditions, however, ascospore release increased with rise in temperature from 5 to 20 C.

Additional keywords: *Poa pratensis*, pollen, spore trapping

Claviceps purpurea (Fr.:Fr.) Tul., causal agent of ergot, attacks about 400 species of grasses (2) and is an important pathogen of Kentucky bluegrass (*Poa pratensis* L.) in the Pacific Northwest (1). Ergot is controlled in Kentucky bluegrass in Oregon by postharvest field burning, although state legislation was passed in 1991 to phase out field burning by 1998. Development of alternative controls could be aided through an understanding of the environmental conditions regulating pathogen population dynamics.

C. purpurea overwinters as sclerotia, which must undergo a period of cold temperatures (0–10 C) for about 6 wk to break dormancy (15). Ascospores are produced during the spring through early summer (3,11,13,18). Infection occurs only during the period of flowering, since the ovary is the primary site of infection (12). Rainfall is an important factor in initiation of ascospores (13,17), but little quantitative data are available concerning the effect of rain or other environmental conditions on ascospore production and release. The objective of this study was to determine the effect of temperature, relative humidity, and rain on ascospore release in *C. purpurea*.

MATERIALS AND METHODS

Aerobiology. A 0.05-ha field of Kentucky bluegrass was located at Pure Seed Testing Research Farm in Woodburn, Oregon, and a 0.8-ha field was located at the O. M. Scott Research Farm in Gervais, Oregon. Both fields were maintained under recommended management practices (19).

Ascospore populations were monitored from mid-April to mid-June at each site during 1989 and 1990 with a 7-day recording volumetric spore trap (Burkard Manufacturing Co., Rickmansworth, England). The trap was placed in the center of each plot with the orifice approximately 42 cm above ground level. Air intake, adjusted to 10 L per minute, was checked twice a week. Spore trap tape was coated with a thin layer of silicone grease formulated for use with rotorods (Ted Brown Associates, Los Altos, CA). Tapes were divided into daily segments, and hourly partitions were gently demarcated by placing the tape on a template and gently touching the edge of a razor blade to the tape surface. Tapes were mounted on glass slides, and ascospores were stained with aniline blue (30 mg of aniline blue, 20 ml of deionized water, 10 ml of glycerol, and 10 ml of lactic acid) and covered with a cover glass. The number of ascospores trapped per hour was counted under a microscope at 300× and summed over 12:00 p.m. to 11:59 a.m. the next day to establish daily counts.

Identification of ascospores and pollen was based on comparisons with known ascospores of *C. purpurea* and pollen of Kentucky bluegrass placed on spore trap tape and processed the same as tapes from the Burkard traps. Ascospores were deposited on the tape by placing stromata from field-collected sclerotia in a block of water agar on the inside of a petri plate lid, directly over a prepared spore trap tape. Pollen collected from Kentucky bluegrass was dusted onto a spore trap tape. Only spores and pollen identical to those on the reference slides were counted.

Temperature, relative humidity (RH), and rainfall were monitored with a micrologger (Model CR21X, Campbell Scientific, Logan, UT) programmed to read sensors at 15-min intervals and record averages at half-hour intervals. Sensors included a temperature/RH sensor (Model 207, Campbell Scientific) and tipping bucket rain gauge (Model TE525, Campbell Scientific). At least once weekly, an aspirated wet-dry bulb thermometer (Model 566, Belfort Instrument Co., Baltimore, MD) was used to confirm accuracy of readings from temperature and RH sensors.

Incidence (percent heads infected) and severity (sclerotia per head) of ergot were assessed within 1 wk of harvest by visual inspection of 200 seed heads collected at random by someone walking a diamond-shaped pattern extending to the margins of each plot. The timing and duration of flowering were indirectly estimated by counting bluegrass pollen on Burkard spore tapes used for ascospore determinations.

Effect of temperature on spore release. To determine the effect of temperature on spore release, sclerotia of *C. purpurea* bearing mature stromata were placed on the surface of water agar in 8.5-cm-diameter petri plates. Petri plate lids, also containing a thin layer of water agar, were positioned over the plates to capture ejected ascospores, since preliminary studies indicated that nearly all ascospores were ejected upward. Plates were placed in chambers at 5, 10, 15, or 20 C with a 12-hr daily photoperiod. At daily intervals through 3 days, ascospores were counted under a compound microscope at 150×. Ascospores in each of five fields along each of two axes transversing the length and width of the release area were counted and spore density was determined. Since the area of spore deposition was ellipsoid, the area of each site of spore deposition was determined on the basis of the equation of the area of an ellipse and multiplied by spore density to determine ascospore number. The experiment was repeated.

Statistical analyses. Linear regression analyses were conducted to determine the relationship between spore release and each of four environmental variables (rain, average temperature, average RH, and hours per day of RH > 95%) at 0, 1, 2, 3, and 4 days preceding (lag) spore catches. Spore release was also related to rain, temperature, and RH averaged over 2 days or 3 days preceding spore release. For each combination, significance of the regression (r^2) and plots of

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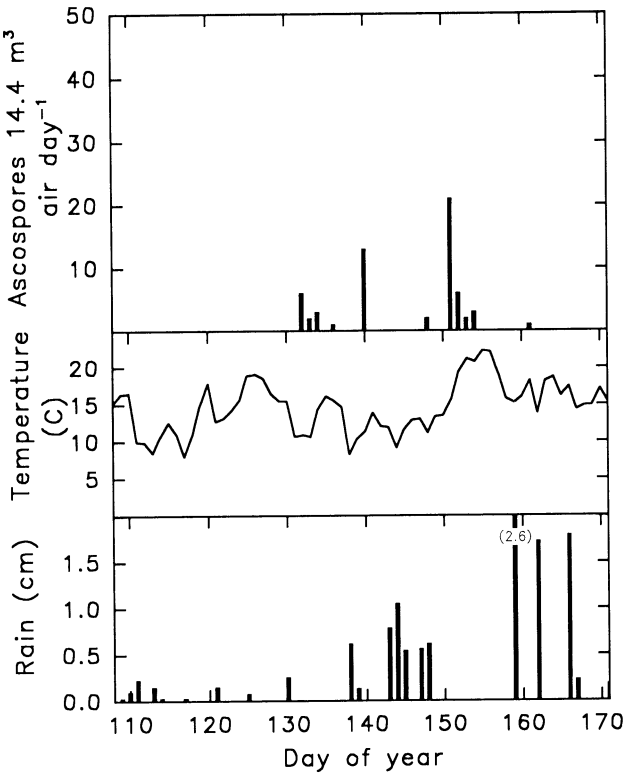

Fig. 1. Number of ascospores of *Claviceps purpurea* trapped, temperature, and amount of rainfall recorded in a field of Kentucky bluegrass at Gervais, Oregon, during 1989.

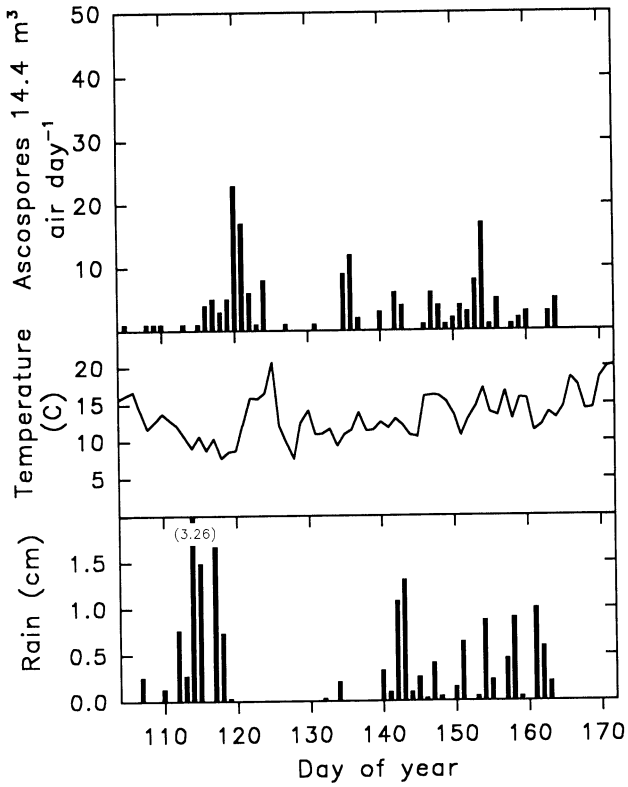

Fig. 2. Number of ascospores of *Claviceps purpurea* trapped, temperature, and amount of rainfall recorded in a field of Kentucky bluegrass at Gervais, Oregon, during 1990.

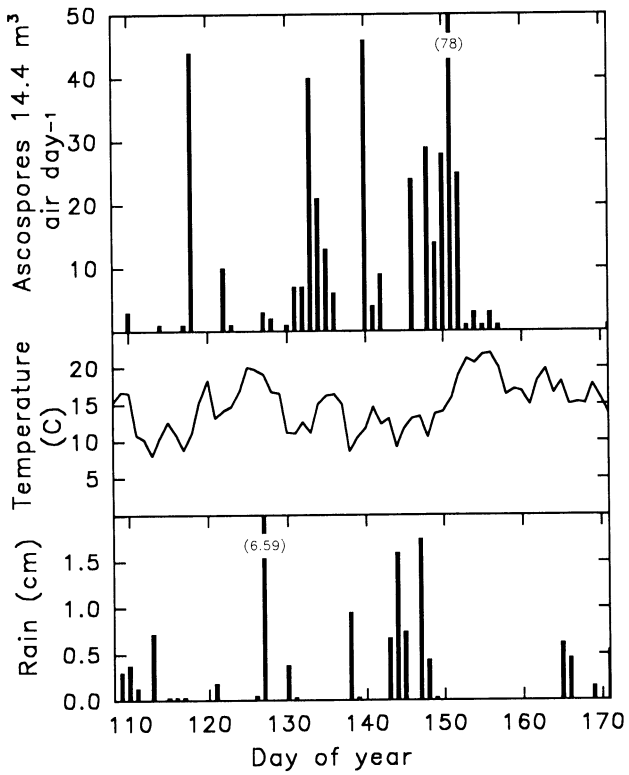

Fig. 3. Number of ascospores of *Claviceps purpurea* trapped, temperature, and amount of rainfall recorded in a field of Kentucky bluegrass at Woodburn, Oregon, during 1989.

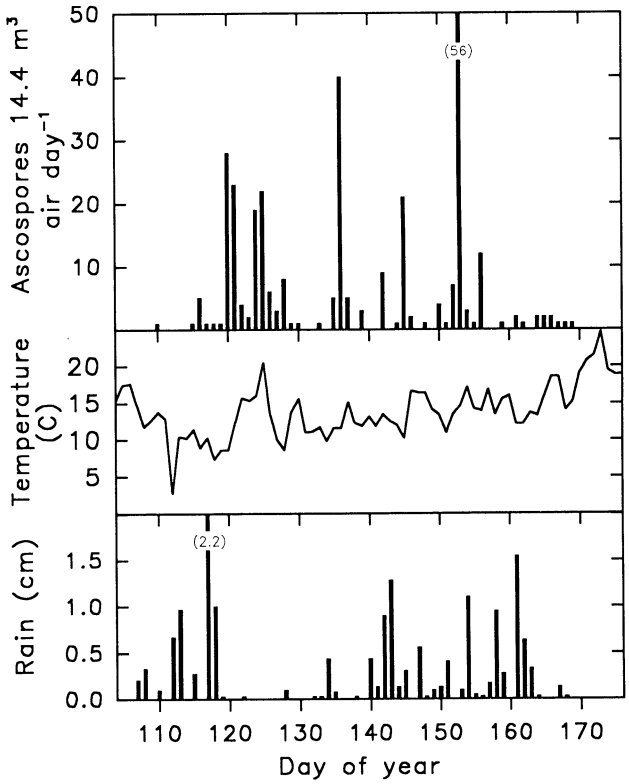

Fig. 4. Number of ascospores of *Claviceps purpurea* trapped, temperature, and amount of rainfall recorded in a field of Kentucky bluegrass at Woodburn, Oregon, during 1990.

residuals were evaluated.

Data also were analyzed in terms of sporulation events, defined as one or more consecutive days on which spores were trapped. Polynomial regression was used to relate the number of days following rain to initiation of a sporulation event. In a separate analysis, ascospore number on each consecutive day of a sporulation event was described using polynomial regression. Regression analyses were conducted using SigmaPlot (Jandel Scientific, San Rafael, CA).

RESULTS

At Gervais, ascospores were trapped on 11 days in 1989 (Fig. 1) and 39 days in 1990 (Fig. 2); rainfall occurred on 21 and 31 days in 1989 and 1990, respectively. At Woodburn, ascospores were trapped on 30 days in 1989 (Fig. 3) and 43 days in 1990 (Fig. 4); rainfall occurred on 24 and 40 days in 1989 and 1990, respectively.

Flowering, based on pollen counts, extended about 30 days (Fig. 5). Ascospores were trapped before and after flowering, except at Gervais in 1989, when spores were trapped only during the period of flowering. A diurnal periodicity in ascospore release was observed, with peak catches between 1:00 and 6:00 a.m. (Fig. 6). A periodicity was also observed in release of pollen of Kentucky bluegrass, with peak pollen catches between 6:00 and 10:00 a.m. (Fig. 7).

Most sporulation events were initiated 1–3 days following rain (Fig. 8). Events were generally not initiated the day of rain or more than 4 days following rain. Once initiated, sporulation events occurred over 1–16 days (Fig. 9). Cumulative rainfall duration paralleled cumulative number of spores trapped at each location (Fig. 10). Correlation coefficients of cumulative hours per day of rainfall and cumulative number of spores were 0.94 and 0.96 at Woodburn during 1989 and 1990, respectively, and 0.95 and 0.97 at Gervais during 1989 and 1990, respectively.

Cumulative number of spores released was not significantly correlated with cumulative RH, hours per day of RH $> 95\%$, or temperature. Number of ascospores trapped per day was not significantly correlated ($P = 0.05$) with rainfall, average RH, hours per day of RH $> 95\%$, or temperature. Under controlled temperature conditions, ascospores released per day increased with rising temperature from 5 to 20 C (Fig. 11). Similar results were obtained when the experiment was repeated. Cumulative number of ascospores trapped during the period of flowering was not correlated with percent heads infected or average number of sclerotia per infected head (Table 1).

DISCUSSION

Peak catches of ascospores occurred between 1:00 and 6:00 a.m., whereas peak catches of pollen occurred between 6:00 and 10:00 a.m., indicating a synchronization between flowering and airborne numbers of ascospores. In wheat (8) and barley (5–7), resistance to *C. purpurea* developed in ovaries following pollination. It is not known if a similar mechanism operates in Kentucky bluegrass, although the timing of flowering in Kentucky bluegrass would favor infection by *C. purpurea*, since ascospores would land on stigmas or ovaries prior to arrival of pollen.

A correlation between ascospore number during flowering and percent infection or number of sclerotia per head was not observed, although additional infection may have occurred through secondary spread. Following infection, a sugary exudate containing abundant conidia (honeydew) develops in infected ovaries within 5–10 days (4,10,18). Over a 30-day period of flowering, conidia potentially can initiate two or three secondary cycles. Honeydew may also be spread by insects (10,16) or through contact of healthy and infected seed heads, although neither means of transmission was quantified in this study.

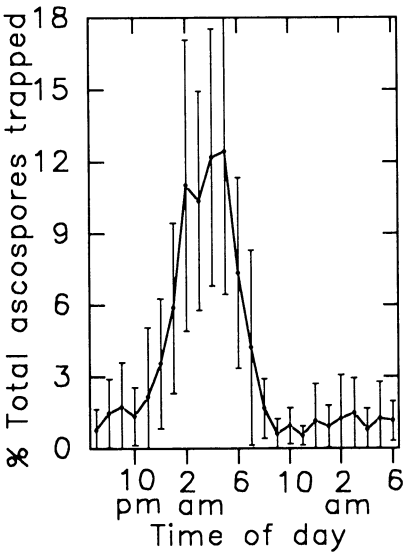

Fig. 6. Diurnal periodicity of release of ascospores of *Claviceps purpurea*. Each point represents mean and standard deviation calculated from each of two sites during 1989 and 1990.

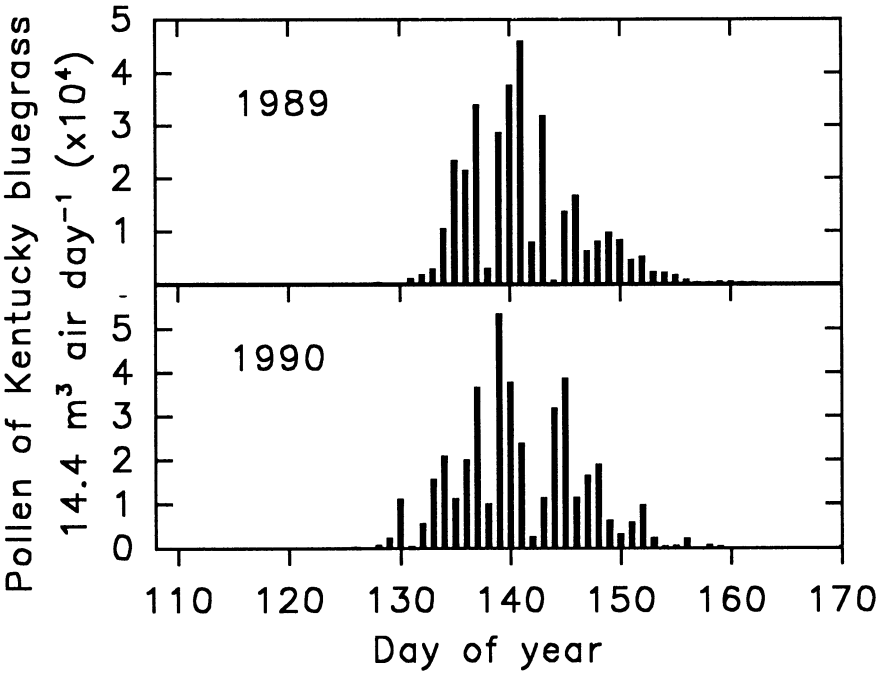

Fig. 5. Amount of pollen of Kentucky bluegrass trapped at Gervais, Oregon, during 1989 and 1990.

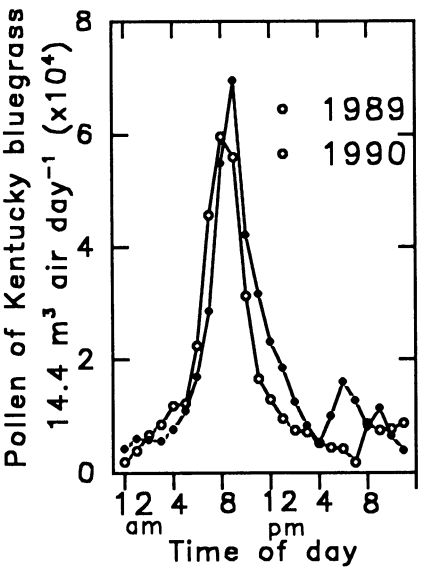

Fig. 7. Diurnal periodicity of pollen release in Kentucky bluegrass at Gervais, Oregon, during 1989 and 1990.

Ascospore release occurred throughout the period of spore trapping and extended before and after flowering in Kentucky bluegrass. Bretag and Merriman (3) and Cooke and Mitchell (6) observed ascospore release in *C. purpurea* over a 2-mo period, and Wood and Coley-Smith (18) observed germination of sclerotia of *C. purpurea* over a 5-mo period. The availability of ascospores in the early spring could support infection of early flowering grasses. Bretag and Merriman (3) reported that infection of ryegrass may provide conidial inoculum for infection of wheat, which flowers after the ryegrass. Infection of early flowering weed grasses in cereal crops was reported by Mantle and Shaw (14) to be important in the development of ergot in cereals. The significance of early-flowering weed grasses in the epidemiology of ergot in Kentucky bluegrass has not been determined.

I observed a strong diurnal periodicity in ascospore release. Conversely, Mantle and Shaw (13) reported no periodicity in their study of ascospore release from ergot in wheat, although their results were based on a single sporulation event over a 6-day period.

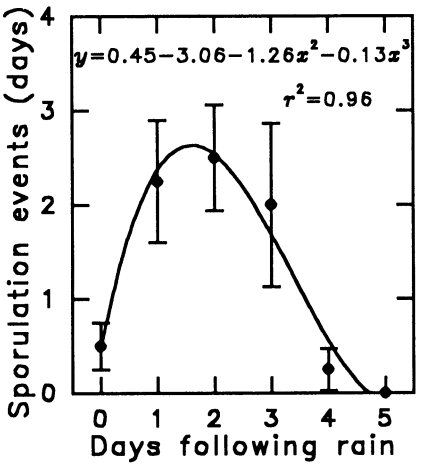

Fig. 8. Sporulation events of *Claviceps purpurea* in Kentucky bluegrass following rain. Data were pooled from the four sites. Each point represents mean and standard deviation calculated from each of two sites during 1989 and 1990.

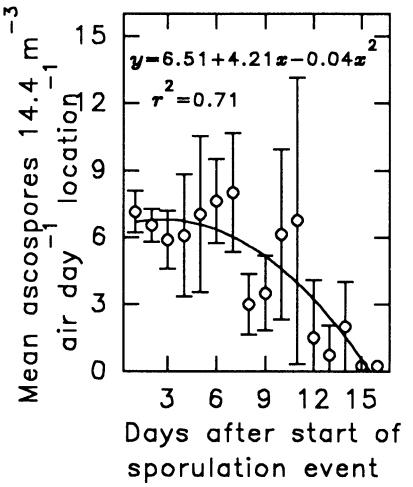

Fig. 9. Number of ascospores of *Claviceps purpurea* trapped during consecutive days of sporulation events. Each point represents mean and standard deviation calculated from each of two sites during 1989 and 1990.

Under controlled temperature conditions, ascospore number increased from 5 to 20 C, although considerable variation in numbers of ascospores released was observed at each temperature. Additional variability would be expected under field conditions and may account for the lack of correlation between temperature and ascospores trapped. Mitchell and Cooke (15) reported similar rates of germination of sclerotia of *C. purpurea* under controlled conditions at 10, 15, and 20 C, although ascospore release was not quantified.

In this study, a relationship between RH and spore release was not established. Vladimirsky (17) reported that optimal field conditions for stromata production were 12.1 C and high (>76%) RH. Hadley (9) reported that under controlled conditions, ascospore discharge was restricted at 100% RH but abundant at 77–94% RH.

Rainfall appeared to be the most important weather event in initiating spore release in *C. purpurea*. Once initiated, release was observed for up to 16 days. Longer periods of spore release may be possible under longer periods of rainfall or saturated moisture conditions at the soil surface or under crowns or plant canopy.

In the Pacific Northwest, most of the hectarage of Kentucky bluegrass is irrigated up to and through the period of flowering. Since infections occur only during or following flowering, it may be possible to reduce ascospore inoculum by reducing irrigation or frequency of irrigation during the period of flowering. The effect of timing of irrigation on severity of ergot in Kentucky bluegrass has not been established. In addition, effect of rainfall, mechanical transfer, and insect transmission on secondary spread of honeydew of *C. purpurea* has not been established. Additional quan-

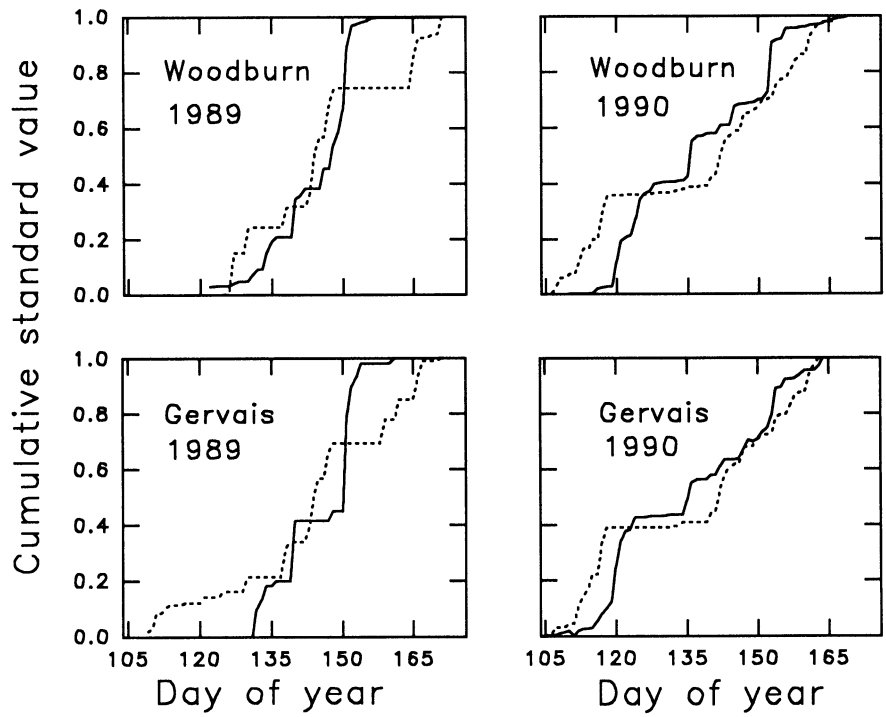

Fig. 10. Cumulative number of ascospores of *Claviceps purpurea* (solid line) and cumulative hours per day of rain (dotted line), standardized from 0 to 1 on the basis of maximum value, with respect to time at each of two sites during 1989 and 1990.

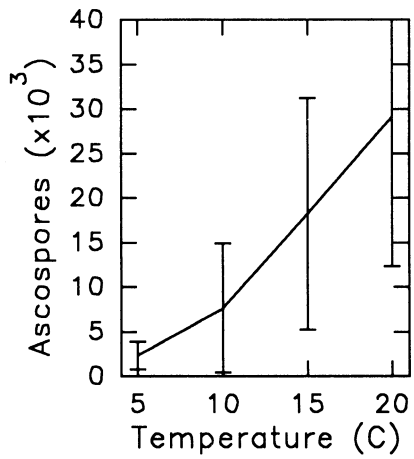

Fig. 11. Number of ascospore of *Claviceps purpurea* released at each of four temperatures. Mean and standard deviation are based on ascospore release from each of eight stromata at each temperature.

Table 1. Cumulative number of ascospores (day 130–155) of *Claviceps purpurea*, percent infection, and average number of sclerotia per head within 1 wk prior to harvest

Site	Year	Cumulative ascospores (no.)	Percent infection	Sclerotia per head (av. no.)
Woodburn	1989	357	26	4.79
	1990	161	16	11.65
Gervais	1989	59	2	1.00
	1990	84	22	4.80

titative research is needed on this phase of the life cycle.

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