RIPENING OF EUPENICILLIUM OCHROSALMONEUM ASCOSTROMATA ON SOIL

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ABSTRACT

Immature ascostromata of *Eupenicillium ochrosalmoneum*, a citreoviridin-producing fungus, that were recovered from dried corn ears at harvest ripened to form ascospores when incubated (25 °C) on moist nonsterile soil. Percentages of ascostromata containing ascospores were recorded for the following incubation intervals: 2 wk = 0%; 4 wk = 72%; 6 wk = 82%; 8 wk = 87%. Immature ascostromata harvested from 2-, 3-, 4-, and 5-wk-old cultures grown on autoclaved corn kernels were also capable of ripening on soil, their rates of ripening being similar and independent of the time of harvest. Burial of immature ascostromata in soil prevented ripening. The temperature range favorable to ascospore formation (20–30 °C) was narrower than that for ascostroma production (20–37 °C) or vegetative growth (15–37 °C). The dispersal of immature ascostromata onto field soils during corn harvest, with their subsequent ability to form ascospores on soil, suggests that the ascostroma may be an important source of inoculum in the disease cycle of *E. ochrosalmoneum*.

Key Words: *Eupenicillium ochrosalmoneum*, ascostromata, soil, corn.

MATERIALS AND METHODS

Ascostroma production. — Ascostromata produced *in vivo* were obtained from corn (DeKalb XL-12) grown to maturity in a controlled environment room (photoperiod 14 h; temperature 30 ± 1 °C day/20 ± 1 °C night; humidity 82 ± 5%) in the Biotron, University of Wisconsin, Madison. Seven ears (1 ear/plant) were each wound-inoculated 21 da after silk with 30 sterile wooden toothpicks (6 rows of 5 toothpicks each) dipped in a conidial suspension of *E. ochrosalmoneum* and inserted through the husk. Inoculum was prepared from colonies of *E. ochrosalmoneum* NRRL 6568 incubated for 7 da at 25 °C on Czapek’s agar slants. Conidia were suspended in 0.01% Triton-X-100, filtered through sterile glass wool, and adjusted to 10⁶/ml. Mature ears were harvested approximately 5 wk after wounding (12% moisture), husked, and the wounded kernels were removed. Ascostromata were confined to wounded kernels and formed primarily beneath the kernel pericarp as previously described by Wicklow *et al.* (1982). The dry kernels were gently shaken in vials to detach ascostromata.

Ascostromata were also produced on autoclaved, whole corn kernels to determine the ca-
pacity of immature ascostromata to ripen on soil when harvested at different times during their 4–6 wk period of maturation. Kernels were first soaked 24 h at 25 C in distilled water, then wounded at the germ and autoclaved in glass Petri dishes (10 kernels/plate) containing a double layer of Whatman No. 1 filter paper to which 8 ml of distilled water had been added. Kernels were inoculated at the wound with one drop of a 10⁶ conidia/ml suspension of *E. ochrosalmo­ neum* NRRL 6568 and incubated at 25 C in sealed plastic bags. At weekly intervals, lots of 50 kernels each were vigorously shaken in a jar containing 250 ml sterile 0.01% Triton-X-100. Detached ascostromata and kernels were allowed to settle and the conidial suspension was decanted. The procedure was then repeated with sterile distilled water before filtering through a Whatman No. 1 filter paper after a final rinse. Kernels were removed and the free ascostromata were air-dried until they became separated.

**Incubation on soil.**—Ascostroma ripening of *E. ochrosalmo­ neum* was investigated by incubating ascostromata on soil. Nonsterile soil from a corn field near Tifton, Georgia was first adjusted to a 14–17% water-carrying capacity (wt wt basis). Plastic medicine cups (30 cc) were filled with the soil and 300–500 ascostromata were scattered over the soil surface. In burial experiments, ascostromata were covered with approximately 1 cm of soil. Relative humidity was maintained at 100% by incubating soil cups at 25 C in a desiccator jar containing distilled water. To study the effects of temperature on ascospore formation, soil cups were placed in deep Petri dishes (3 cups/dish) containing 30 ml water and incubated in sealed plastic bags. Thirty autoclaved corn kernels, prepared as previously described, and 10 plates of Czapek yeast autolysate agar (CYA) (Pitt, 1979) were also inoculated and similarly incubated to determine the effects of temperature on ascostroma production and vegetative growth.

Soil moisture was measured by oven-drying soil cups at 100 C to a constant weight, both at the start of the incubation period and subsequently in soil cups sacrificed for ascostromata. In all cases the soil maintained its water-carrying capacity during incubation.

**Examination of ascostromata.**—At given intervals, ascostromata were removed from the soil surface of a cup to determine whether they had ripened and their viability. Ascostromata were surface-sterilized in 0.25% sodium hypochlorite for 2.5 min, rinsed twice for 1 min with sterile distilled water, and pipetted onto sterile filter paper. One hundred ascostromata were crushed and plated on potato dextrose agar with streptomycin (25 mg/liter) and tetracycline (1.25 mg/liter). Plates were incubated for 10 da at 25 C.

**RESULTS AND DISCUSSION**

Ascostroma of *E. ochrosalmo­ neum* recovered from dried Biotron corn ears at harvest showed no evidence of asci or ascospores. Although ascospores were not present after 2 wk of incubation at 25 C on moist nonsterile soil, 72, 82, and 87% of the ascostromata had formed ascospores after 4, 6, and 8 wk, respectively. Ascostromata contained relatively few ascospores after 4 wk incubation, whereas at 8 wk, most ascocarps were completely filled with ascospores.

The near-synchronous ripening of ascostromata produced in preharvest corn suggests that their formation in wounded kernels must have occurred within a restricted interval during ear maturation.

Immature ascostromata harvested from cultures grown on autoclaved corn kernels also
formed ascospores when incubated on moist soil. Ascostromata from 2- and 3-wk-old cultures did not contain asci or ascospores at harvest (FIG. 1). However, after 2 wk of incubation on soil, ascospores were detected in 9% (harvested from 2-wk-old cultures) and 39% (harvested from 3-wk-old cultures) of the ascostromata examined. Approximately 90% of these ascostromata had formed ascospores after 4 wk of incubation on soil. Apparently the immature ascostromata recovered from dried corn grown in the Biotron were not as far advanced in their maturation as those from 2- and 3-wk-old cultures on autoclaved corn kernels since, in the former, no ascospores were detected after 2 wk soil incubation.

Brefeld (1874) in his description of ascocarp development in “Penicillium glaucum” similarly demonstrated the capacity of detached immature ascostromata to form ascospores. To obtain all stages of ascostromata development using mixed cultures, he found it necessary to first grow the fungus for several weeks, when sow the immature ascostromata on fresh substrate to observe ascus and ascospore formation. The description of ascocarp development by Brefeld (1874) and others (Emmons, 1935; Stolk and Samson, 1983) supports the view that ascocarps of Eupenicillium species are more appropriately referred to as ascostromata rather than cleistothecia (Malloch, 1981; Subramanian and Rajendran, 1981).

Percentages of ascostromata containing ascospores in 4- and 5-wk-old cultures were similar to those for ascostromata harvested after 2 and 3 wk but incubated two additional weeks on moist soil (FIG. 1). This suggests that ascostromata detached from the parent mycelium ripened at a similar rate as ascostromata in situ. Viability tests provided additional evidence for the independent nature of ascostroma ripening. Nearly 50% of the immature ascostromata from 3-wk-old cultures failed to form colonies after plating on PDA, but continued to mature and eventually became filled with ascospores. Only after the immature ascostroma was first crushed or the peridial wall of the mature ascostroma ruptured, releasing ascospores, did colonies form on the agar surface. Immature ascostromata from 3-wk-old cultures failed to form ascospores when buried in soil for 16 wk. The specific factor(s) associated with soil burial that inhibit ascostroma ripening are not known. It is possible that burial of immature ascostromata through field cultivation...
might reduce numbers of potentially fertile ascostromata, since only 41% were viable after laboratory burial and none formed ascospores when reincubated on the soil surface.

To further understand the effects of environmental factors on ascostroma ripening, immature ascostromata were incubated at a range of different temperatures and these data were compared with the effects of similar temperatures on ascostroma production and vegetative growth. When immature ascostromata (obtained from 3 wk growth at 25 C) were placed on soil, ascospores formed only in those ascostromata incubated at 20–30 C (Table I). Ascospore formation was evident after 1 wk at 25 C and 30 C, with the highest rate of maturation at 30 C; ascospores were not present in ascostromata incubated at 20 C until 8 wk. During the 16 wk incubation on soil ascostromata remained 95–100% viable at all temperatures except 45 C, where all were dead within 2 wk. Temperatures favorable to growth of E. ochrosalmoneum ranged from 15–37 C, with an optimum at 30 C. Maximum ascostroma production occurred at 30–37 C; ascostromata did not form at 15 C. Concurrent with increasing numbers of ascostromata at higher temperatures was a decrease in ascostroma size, with the average diameter of ascostromata (n = 20) from corn kernels ranging from 426 µm (330–495 µm) at 20 C to 201 µm (165–264 µm) at 37 C. Ascostromata incubated on soil did not form ascospores at 37 C (Table I) despite abundant ascostroma production at that temperature. When these ascostromata were returned to 25 C at the end of the incubation period (16 wk), they remained viable but did not form ascospores. Ascostromata harvested from 3 wk cultures grown at 37 C and then incubated on soil at 37 C also failed to form ascospores, so physiological adaptation to elevated temperatures was not a prerequisite for ascospore formation. Tschanz et al. (1976) similarly reported that higher temperatures optimal for perithecia production in Gibberella zeae (Schw.) Petch often resulted in small, infertile ascoscarps.

Eupenicillium ochrosalmoneum ascostromata and citreoviridin production, but not ascospores, have been reported from standing corn in the vicinity of Tifton, Georgia (Wicklow and Cole, 1984; Wicklow et al., 1984). During December, January, and February the mean daily maximum temperature at Tifton is below 20 C (National Climatic Data Center, 1984), so it is likely that ascostromata cease ripening during winter months. However, formation of ascospores probably occurs in the spring since 13% of the ascostromata from the 15 C soil incubation (16 wk) formed ascospores after 6 wk at 25 C.

Although penicillia have been frequently reported to infest preharvest cereals (Caldwell et al., 1981; Hill and Lacy, 1984; Mislivec and Tuite, 1970), little is known about the life cycles of these fungi in nature. The majority of species assigned to Eupenicillium are considered soil organisms (Pitt, 1979; Stolk and Samson, 1983). Eupenicillium ochrosalmoneum is the first reported species of Eupenicillium whose life cycle involves the colonization of preharvest cereals (Wicklow et al., 1984). This study demonstrated that: (1) immature ascostromata of E. ochrosalmoneum from preharvest corn ripen to form ascospores when incubated on moist soil; (2) despite a minimum period of 4–6 wk required for ascospore formation, ascostromata separated from the parent mycelium during most of the period will form ascospores; (3) ascostromata do not ripen when buried in soil; (4) the temperature range favorable to ascospore formation is narrower than that for ascostroma production or vegetative growth. An important facet in the life cycle of E. ochrosalmoneum that deserves further study is the role of ascospores in the fungal disease cycle.

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LITERATURE CITED


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