

Infectivity of Conidia of *Peronospora hyoscyami* After Storage on Tobacco Leaves

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ABSTRACT

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Conidia of the blue mold fungus *Peronospora hyoscyami* were stored, either attached to infected leaves or detached and settled on healthy tobacco leaves, at various temperatures and relative humidities (RH) and assayed for infectivity to tobacco plants. Attached conidia retained a high level of infectivity after 113 days at 15 ± 3 C when stored at about 20% RH but lost infectivity within 9 days when stored at about 90% RH. Detached conidia retained infectivity for a relatively short period—9 days at 10 C and 2 days at 30–33 C.

Blue mold, caused by *Peronospora hyoscyami*, has become a major disease of tobacco in North America and Latin America in recent years. Estimates of crop losses caused by the disease in the United States and Canada were \$250 million in 1979 and more than \$84 million in 1980 (4). The economic impact of the disease declined in 1982 and 1983 because of the use of metalaxyl, but blue mold still

threatens tobacco production because of the possible buildup of metalaxyl-resistant strains of the fungus (1).

The fungus produces oospores in leaves and stems of tobacco, but researchers have been unable to infect tobacco plants with oospores in Germany (7), Canada (11), and Israel (Y. Cohen, *unpublished*). The participation of oospores in epidemics is probably slight.

Longevity of conidia was studied in detail in Europe (8) and Australia (5,6,14) to determine their role in epidemiology of the disease. In a previous study conducted in Kentucky (2), we showed that conidia of *P. hyoscyami* attached to sporophores on leaves of tobacco retained infectivity to tobacco plants for more than 3 mo when stored at -20 C and for more than 34 days when stored at 5 C

and 100% relative humidity (RH). Conidia suspended in water survived six repeated cycles of freezing (-20 C) and thawing (25 C).

This study, conducted with an Israeli isolate of the fungus, was undertaken to determine the infectivity of conidia, either attached to infected leaves or detached and allowed to settle on intact, healthy tobacco leaves, after storage at above-freezing temperatures.

MATERIALS AND METHODS

Inoculum production. The highly susceptible tobacco (*Nicotiana tabacum* L.) cultivar Ky 16 was used for inoculum increase, conidial storage, and infectivity tests. Plants were grown in 15-cm-diameter plastic pots to the seven- to eight-leaf stage in the greenhouse. An isolate of *Peronospora hyoscyami* (unidentified race) was collected in 1977 in a plant bed in Maalot (upper Galilee) and maintained by repeated inoculations on tobacco plants in growth chambers.

Conidia required for storage experiments were produced as follows: Tobacco plants were sprayed to runoff on adaxial leaf surfaces with a conidial suspension (about 5×10^4 conidia per milliliter) and incubated in a dew chamber (Percival, model I-60D) at 17 C in the dark for

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about 20 hr. Plants were then moved to a cabinet (25 C) (Conviron, Winnipeg, Canada) illuminated 12 hr/day with VHO fluorescent lamps at an intensity of $150 \mu\text{E m}^{-2} \text{s}^{-1}$. On the seventh day after inoculation, plants were illuminated continuously for 24 hr (12), then placed in the dew chamber for 20 hr to allow fungal sporulation. Under the conditions described, the fungus sporulated profusely on abaxial surfaces of leaves. For storage experiments of attached conidia, leaves 5, 6, and 7 (from stem base), in which the fungus sporulated heavily, were detached and placed on the bench (minimal night temperature 10 C, maximal day temperature 20 C, 40–60% RH, light intensity $50 \mu\text{E m}^{-2} \text{s}^{-1}$, in still air), abaxial surface upward, for 48 hr to allow leaves to dry to about 70–75% water content. At this stage, conidia were tested for infectivity (time zero) and leaves were transferred to the desired storage conditions.

Storage conditions. Two experiments were run in uncontrolled environmental conditions, both of them with fungus-sporulating leaves stored in the open on the bench. The first experiment was from December 1981 through April 1982 and the second was from December 1982 through April 1983.

Other experiments were conducted at a variety of storage conditions with

different combinations of temperature and relative humidity. In these experiments, one layer of fungus-sporulating leaves was placed in sealed plastic boxes ($60 \times 30 \times 30 \text{ cm}$) in which various RH levels were maintained with the aid of silica gel granules and water. Boxes were placed at various temperatures in the dark and opened every few days for about 20 sec for sampling. Changes in RH caused by opening the boxes were minimal.

Infectivity tests. In preliminary experiments, stored dried conidia were not consistently infective when suspended in tap or distilled water (6,14) and they were not consistently infective when gradually hydrated (9) on sporophores before suspension in water. We therefore used the following technique to assess infectivity of stored conidia: Pieces of detached, fungus-sporulating leaves of a known area were pressed several times against and rubbed over a known area of attached healthy leaves until most conidia were removed. Usually, more rubbing was required for leaves stored in higher than in lower RH. The number of conidia transferred in this procedure to the inoculated leaves, as determined with the aid of collodion imprints taken from them, ranged between 10^6 and 10^8 per 100 cm^2 leaf area. Usually a piece of about 20

cm^2 of stored leaf was used to inoculate a leaf surface of about $100\text{--}150 \text{ cm}^2$. Three to six leaves in one or two plants were inoculated in each assay. Inoculated plants were placed in a dew chamber at 17 C for 20 hr in the dark, then transferred to a cabinet (25 C) illuminated 12 hr/day at $150 \mu\text{E m}^{-2} \text{s}^{-1}$ until symptoms developed. Percentage infected leaf area was assessed visually on the seventh day after inoculation in the manner described before (12).

Experiments with detached conidia. Two (or three) intact fungus-sporulating eight- to nine-leaf plants were placed together with 12 (or 18) healthy plants in a growth cabinet calibrated to $22 \text{ C} (\pm 0.2)$ and 60–70% RH for 2–4 hr in either darkness or light ($150 \mu\text{E m}^{-2} \text{s}^{-1}$). Wind velocity (laminar flow) in chamber was 1 ms^{-1} . A conidial deposition of about $6,000/\text{cm}^2$ was counted on Vaseline-coated glass slides placed in chambers for 2 hr. At the end of the dispersal period, three plants were placed in a dew chamber to obtain infectivity at time zero and nine other plants were transferred to a cabinet calibrated to the desired environmental conditions for various periods. At the end of the incubation period plants were placed in a dew chamber at 17 C for 20 hr and then at 25 C for 7 days, when percentage infected

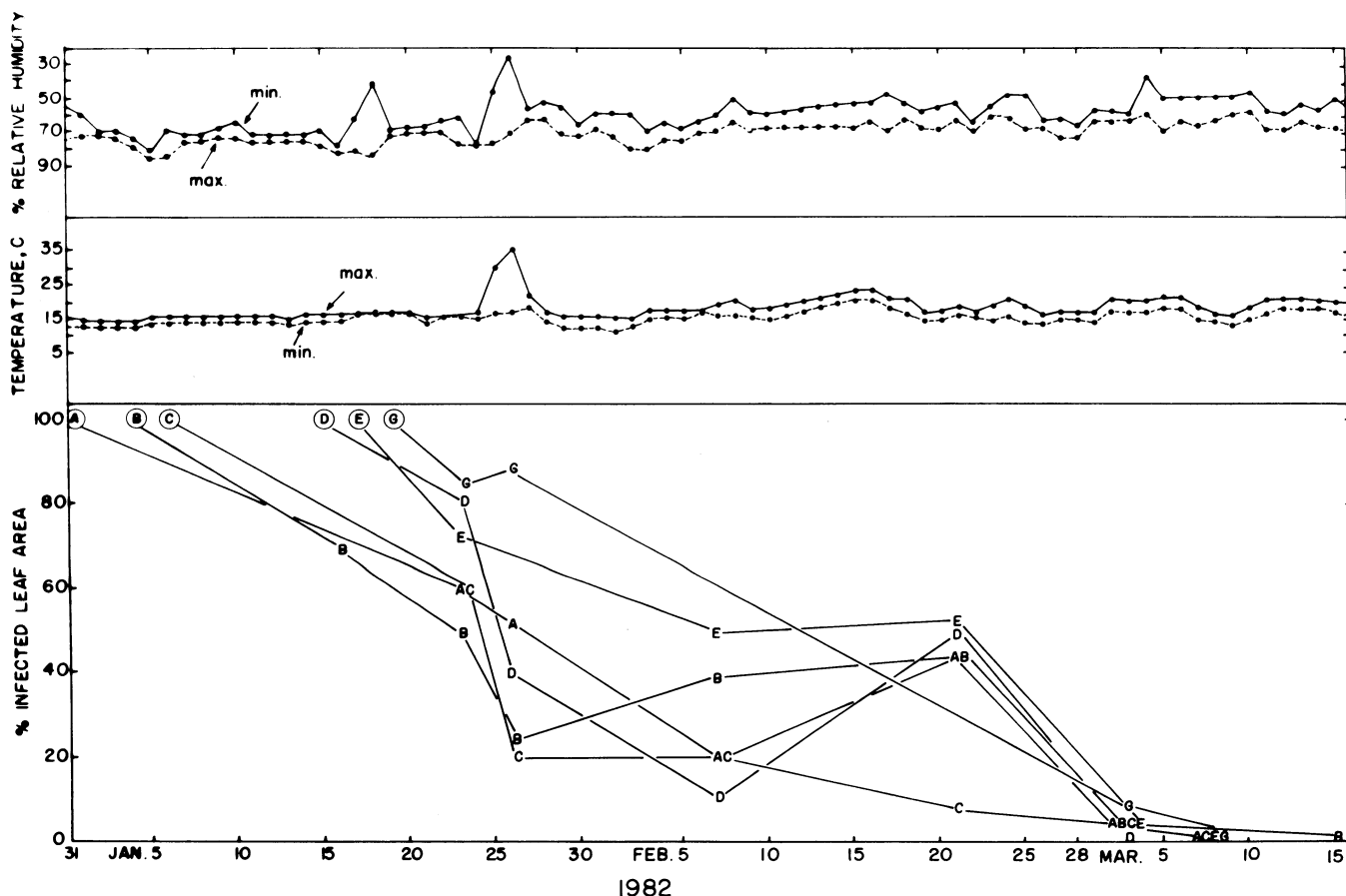


Fig. 1. Infectivity to tobacco plants (cultivar Ky 16) of conidia of *Peronospora hyoscyami* attached to tobacco leaves as a function of time. Experiment was conducted with six batches of fungus-sporulating leaves (indicated by letters) that were left on the bench under the conditions (temperature and relative humidity) indicated. Each infectivity assay was done with three conidia-bearing leaves, each inoculated onto two healthy plants (three leaves in each). For variability in infectivity levels, see Materials and Methods.

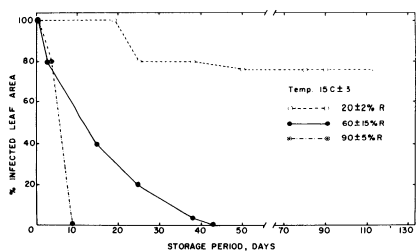


Fig. 2. Infectivity to tobacco plants (cultivar Ky 16) of conidia of *Peronospora hyoscyami* attached to tobacco leaves after storage at 15 ± 3 C at three relative humidities as a function of time. Experiment was conducted as described in Figure 1. For variability in infectivity levels, see Materials and Methods.

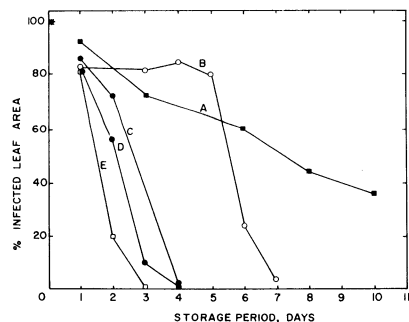


Fig. 3. Survivability (as assayed by infectivity to tobacco plants, cultivar Ky 16) of dispersed conidia of *Peronospora hyoscyami* settling on intact tobacco leaves at various temperatures as a function of time. (A) 10 C, 75–90% RH; (B) 22 C, 60–70% RH; (C) 27 C, 38–50% RH; (D) 27–29 C, 70–100% RH; and (E) 30–33 C, 55–90% RH. For variability in infectivity levels, see Materials and Methods.

leaf area was assessed visually.

In some experiments, conidia were detached by artificial rubbing of detached fungus-sporulating leaves on healthy intact leaves. In such experiments, fungus-sporulating leaves were treated and conidia were transferred to healthy plants, as described before. Inoculated plants (10^6 – 10^8 conidia per 100 cm^2) were incubated in growth chambers calibrated to the desired conditions and removed after various periods to a dew chamber to induce infection and then to a 25 C cabinet for symptom production.

Statistical analysis. The overall variability in conidial infectivity is expressed as the standard deviation (\pm SD) of the mean for leaves exhibiting increasing amounts of disease. In Figures 1–3, \pm SD values for leaves showing 0–20, 21–40, 41–60, 61–80, and 81–100% infected leaf area were 8, 19, 34, 34, and 18%, respectively.

RESULTS

Infectivity of attached conidia stored in uncontrolled environments.

Conidia attached to tobacco leaves remained infective to tobacco plants for as long as 113 days in 1982. During that storage period (December 1981–April 1982), temperatures ranged between 10 and 22 C and RH between 40 and 60%. In 1983 (Fig. 1), conidia remained infective from early January to early March (about 62–70 days). Conidia lost half of their infectivity in about 1 mo. Temperature during the 1983 experiment ranged between 9 and 34 C and RH between 30 and 85% (Fig. 1).

Infectivity of attached conidia stored in controlled environments. The RH in which leaves were stored had a strong influence on infectivity of conidia (Fig. 2). At 15 ± 3 C and of about 90% RH, conidia lost infectivity within 9 days, and at 45–75% RH, within 43 days. Under dry conditions (20% RH), minor reduction in infectivity was recorded after 113 days (longer periods not tested).

Temperature during storage had a much smaller effect on infectivity of attached conidia, especially under dry conditions. Thus, conidia stored at 20% RH at either 10 or 30 C retained similar high infectivity after 45 days (longer periods not tested), whereas conidia stored at 60–80% RH at 30 and 10 C lost infectivity within 1–4 and 13–14 days, respectively.

Infectivity of detached conidia. The infectivity of conidia dispersed by wind and allowed to settle on leaves and then kept in growth chambers is shown in Figure 3. Conidia remained infective for relatively short periods. These were longer at lower than at higher temperatures. At 10, 22, 27, 27–29, and 30–33 C, conidia were still infective after 10, 7, 4, 3, and 2 days, respectively. Infectivity declined very rapidly when plants were inoculated with many spores by rubbing. Decline of infectivity was somewhat slower in conidia settling on tobacco plants incubated in low RH than in high RH. Decline in infectivity was somewhat lower for conidia settling on abaxial (lower) surfaces than on adaxial surfaces.

DISCUSSION

Unlike in other studies, we have assayed conidial longevity of *P. hyoscyami* by testing their infectivity to tobacco plants rather than by testing their germinability in vitro. Conidia of *P. hyoscyami* showed prolonged longevity when kept attached to leaves and stored at low RH but not when dispersed and settled on green, healthy tobacco leaves. The rapid loss of infectivity of dispersed conidia may be attributed to the high relative humidity prevailing on the leaf surface of the green leaves on which conidia were settling. The loss of infectivity of spores stored on leaves was

similar to the loss of infectivity when spores were stored in a desiccator (6,14).

Kröber (8) showed that survival of conidia of *P. tabacina* is longer under cool, dry conditions than under warm, moist conditions, with moisture having a more pronounced effect than temperature. Conidial survivability on fungus-sporulating detached leaves in the field was 9 days even in the summer and also if buried in dry soil for several months. Kröber (8) concluded that *P. tabacina* can survive in Germany by its conidia. Patrick and Singh (10) reported that conidia mixed with soil (not specified if dry or wet) and kept at –4, 4, and 18 C were still viable after 7, 6, and 1 days, respectively.

It appears that conidia of *P. hyoscyami* show the highest survival capacity compared with other downy mildew pathogens such as *Peronospora parasitica*, *Peronospora farinosa* (8), *Pseudoperonospora cubensis* (3), and *Phytophthora infestans* (9,13).

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