Infectivity of Conidia of *Peronospora tabacina* After Freezing and Thawing

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ABSTRACT

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Conidia of *Peronospora tabacina*, the incitant of tobacco blue mold, attached to infected leaves or suspended in aqueous suspensions remained infective for more than 3 mo at −20 C. Conidia on sporulating lesions survived after six repeated cycles of freezing (−20 C) and thawing (25 C), whereas conidia suspended in water survived after two such cycles. Conidia on sporulating lesions stored at 5 C (100% RH) survived more than 34 but less than 57 days. The possible epidemiologic implications of this feature of the pathogen are discussed.

Whether the catastrophic epidemic of blue mold on tobacco (incited by *Peronospora tabacina* Adam) of 1979 in North America will recur in summer 1980 is a matter of speculation. Whether the 1979 severe epiphytotics resulted solely from the unusually favorable environmental conditions, from the appearance of a new highly virulent race of the pathogen, or from both is not yet known.

One factor that may affect recurrence is overwintering of the pathogen. This study was undertaken to examine the survivability of conidia of *P. tabacina* after storage at low temperatures and after repeated exposures to freezing and thawing.

MATERIALS AND METHODS

Burley tobacco plants (cvs. Judy's Pride, Ky14, Ky16, Burley 21) were sprayed with conidial suspensions of a local isolate of *P. tabacina* collected in late August 1979 at Georgetown, KY. After spraying, plants were held at 22–24 C in a moist chamber for 24 hr and then transferred to a greenhouse. On the seventh day after inoculation, when chlorotic lesions developed, plants were placed in moisture-saturated atmosphere at 20 C in darkness for 24 hr to induce sporulation. About 100 leaves with sporulating lesions were detached, placed in groups of five in paper bags which were put in plastic bags, and kept in a freezer at −20 C until used. Some leaves were kept in moist petri dishes at 5 C. Other leaves were first kept for 48 hr at 5 C; then conidia were brushed into glass distilled water with a camel's hair brush, washed over an 8-μm Millipore filter with an equal volume of water, and resuspended (5×10^4 conidia/ml) in glass distilled water. Aliquots of 10 ml were placed in plastic vials and frozen at −20 C at a rate of about 2 C/min. Ten days after first being frozen, some leaves with attached conidia were thawed at 25 C and the conidia tested for germination and infectivity. Other leaves received one to five additional cycles of freezing and thawing on subsequent days. Samples of frozen detached conidia were thawed at either about 0.2 C/min (2 hr) or 6 C/min (5 min) and tested for germination and infectivity. Other samples received one to five additional cycles of freezing and thawing on subsequent days.

After a final cycle of freezing and thawing, attached conidia were collected from leaves using the same procedure described for freshly produced conidia.

Thawed conidia and freshly produced conidia were tested for germination and infectivity. For germination, 30-μl droplets were placed in depressions on glass slides, incubated at 15 C for 24 hr, and examined microscopically. For infection, 90 droplets of conidial suspension (30 μl/droplet) were placed singly on the upper leaf surface of three tobacco plants. Plants were then treated as previously described to produce symptoms.

Table 1. Effect of duration and temperature of storage on infectivity of *Peronospora tabacina* conidia to tobacco plants

Treatment of conidia	Successful infections[a] (%)
Freshly detached	59
Attached to leaves and frozen at −20 C for 3 mo	62
Attached to leaves and stored at 5 C for 7 days	85
Attached to leaves and stored at 5 C for 34 days	20
Attached to leaves and stored at 5 C for 57 days	0
Detached and stored in aqueous suspensions at −20 C for 3 mo	77

[a]90 infection sites.

RESULTS

Because a positive correlation was noted between germinability and infectivity in all experiments, only the results of infectivity are presented. Tables 1 and 2 show that conidia attached to conidiophores on leaf surfaces of tobacco survived for more than 34 days if kept at 5 C (100% RH) but more than 3 mo if kept at −20 C. Attached conidia kept at −20 C retained infectivity for six repeated freezings and thawings. Reduction in infectivity was noticed only after three cycles of that procedure; no further reduction occurred up to the sixth cycle, at which about 25% of infectivity was retained. Detached conidia frozen in distilled water also retained infectivity for more than 3 mo but were much more sensitive to freezing and thawing regardless of the rate of warming. A total loss of viability occurred after three cycles of freezing and thawing. One freezing-thawing cycle had no deleterious effect on infectivity.

DISCUSSION

Our results demonstrated that conidia of *P. tabacina* survived for more than 3 mo at −20 C and survived repeated exposures to freezing and thawing.

Table 2. Infectivity of repeatedly frozen and thawed conidia of *Personospora tabacina* to tobacco

Freezing-thawing cycles	Successful infections (%)[a]		
	Conidia attached to tobacco leaf	Detached conidia in aqueous suspensions thawed at a rate of: 0.2 C/min	6 C/min
1	51	47	29
2	48	7	9
3	18	0	0
4	14	0	0
5	24	0	0
6	18	0	0

[a]90 infection sites.

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Survival at 5 C in moisture-saturated air was poor at 34 days and nil at 57 days. Hill (3) found that at 5 C and 30–40% RH, attached conidia showed 15% germination after 90 days and less than 1% after 131 days. Survival to repeated freezing and thawing was better in conidia attached to the leaf and frozen in air than in detached conidia suspended in water. Mazur (4) ascribed the hyper-resistance of spores frozen in air compared with those in water to the lower water content in cells that may eliminate deleterious intracellular ice formation. In our experiments, warming the conidial suspensions at a slow or rapid rate resulted in little difference in survivability. This is in accordance with Mazur (4), who concluded that warming rate exerts a large effect on survival of yeast cells only when cooling is rapid (10–100 C/min), not when it is slow (0.5–10 C/min).

Freezing leaves with sporulating lesions is a common technique for preserving fungal pathogens of the Peronosporales. *Pseudoperonospora cubensis* survived for 6 mo on squash leaves stored at −18 C (5), and Cohen (*unpublished*) found that *P. tabacina* survived for more than 6 mo on tobacco leaves at −18 C. Bromfield and Schmitt (1) reported that conidia remained infective after 25 mo of storage in 15% DMSO at −180 C. However, to the best of our knowledge, *P. tabacina* is the only oomycetous fungus reported that retains infectivity after repeated exposures to freezing and thawing. Detached conidia or sporangia of *Phytophthora infestans, Pseudoperonospora cubensis,* and *Sclerospora sorghi* in aqueous suspensions were killed after a single cycle of freezing-thawing (Cohen, *unpublished*).

Much has yet to be studied on the factors affecting the cryosurvival of *P. tabacina* in its conidial, mycelial, and oosporial stages, both in the laboratory and in nature. (2). Nevertheless, the data presented in this paper indicate that the pathogen survives at −20 C for at least 3 mo, resists repeated freezing and thawing, and retains infectivity after storage at 5 C for 34 days. These data, and the fact that the pathogen was recovered from infected leaves collected in central Kentucky in mid-January (Nesmith, *personal communication*), provide strong indications that the pathogen may overwinter in the northern regions of the United States where tobacco is grown. The possibility that the 1979 blue mold epiphytotic was attributable to the appearance of a new race, hyperresistant to freezing damage, should be investigated further.

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