Symptom Development and Disease Severity in *Nicotiana tabacum* and *N. repanda* Caused by *Peronospora tabacina*

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**ABSTRACT**


*Nicotiana tabacum* (cv. Ky-14) and *N. repanda* developed different symptoms when inoculated with *Peronospora tabacina* (Ky-79 isolate). On Ky-14, chlorotic lesions and sporulation developed rapidly within 6–7 days and plants died within 12–13 days of inoculation. On *N. repanda*, chlorosis and leaf curling (a symptom absent in Ky-14) occurred within 10–14 days of inoculation. Extensive and 6 X 10³ sporangia per milliliter. A glass chromatography sprayer was used and leaf curling (a symptom absent in Ky-4) occurred within 10-14days of inoculation. Extensive and 6 X 10³ sporangia per milliliter. A

During the 1940s, Wolf (19) reported that *Nicotiana repanda* Willd., a native tobacco in Texas, was infected with a downy mildew fungus. During the springs of 1983 and 1984, Nesmith and Jones (11) and Nesmith and Keeney (12) visited the region and observed that *N. repanda* growing naturally in Texas was infected with a downy mildew fungus that sporulated profusely. Preliminary experiments showed that the disease was caused by *Peronospora tabacina*.

*N. repanda* has been considered resistant to *P. tabacina* while the commercial cultivars of *N. tabacum* L. tested were susceptible (1,5). Studies on the reactions of seedlings of *Nicotiana* species to *P. tabacina* under glasshouse conditions showed that leaves of *N. repanda* produced only small lesions with light or medium sporulation (1,5).

Thus a conflict exists as to the reaction of *N. repanda* to this fungus. To resolve this issue, a study was conducted under controlled conditions on both *N. repanda* and *N. tabacum* to examine the reactions of these hosts to *P. tabacina*. In addition, change in virulence of the pathogen during serial passage through both hosts was examined. Preliminary reports on this study have been presented (13,14).

**MATERIALS AND METHODS**

**Plant material.** Seedlings of burley tobacco (*N. tabacum* cv. Ky-14) and *N. repanda* (accession 46, *Nicotiana* species collection, Oxford, NC) were grown in the greenhouse as described previously (15) except for the following changes. About 4 wk after planting, seedlings were transplanted to plastic flats (six seedlings per flat) containing Pro-Mix BX. Three days after transplanting, flats were watered to saturation with a 0.2% solution of 20-20-20 (NPK) fertilizer and 2 days later transferred to growth chambers (15) for preconditioning. The use of controlled-environment growth chambers to precondition plants resulted in uniform development of blue mold lesions on leaves inoculated previously with sporangia of *P. tabacina* (15).

**Pathogen and inoculation.** An isolate of *P. tabacina* (Ky-79) obtained in 1979 from plants in a field near Georgetown, KY, was maintained continuously on burley tobacco grown in the greenhouse or growth chamber. For the initial inoculation of both plant species, sporangia were obtained from freshly sporulating lesions on leaves of 7- to 12-wk-old Ky-14 plants 6-7 days after inoculation. Sporangia were gently brushed into a small quantity of distilled water collected on a filter (3-µm pore size) and resuspended in distilled water to a concentration of 2 X 10⁴/ml.

Disease development was evaluated after inoculation of the upper leaf surfaces of 7-wk-old plants of Ky-14 and *N. repanda*. Plants were sprayed uniformly with concentrations of 6 X 10¹, 2 X 10², 6 X 10², 2 X 10³, 6 X 10³, 2 X 10⁴, and 6 X 10⁴ sporangia per milliliter. A glass chromatography sprayer was used to apply the inoculum. Each concentration of inoculum was applied to three or four leaves per plant, using six plants of each species. After inoculation, plants were covered with plastic bags, sprayed lightly with distilled water, and incubated at 19 C for 20 hr in the dark. Plants were then uncovered and kept in a growth chamber (23 C, 60–70 µE s⁻¹ m⁻², 12 hr of light supplied by cool-white fluorescent light) for a minimum of 7 days. This experiment was repeated three times.

Relative disease severity was estimated visually as the chlorotic area of each inoculated leaf starting on the seventh day after inoculation, using a scale of 0–4, where 0 = no lesions; 1 = yellow lesions, ≤ 25% of leaf area chlorotic; 2 = yellow lesions, 25–50% chlorotic; 3 = yellow lesions, 50–75% chlorotic; and 4 = more than 75% of leaf area chlorotic.

To evaluate changes in virulence, sporangia were obtained separately from Ky-14- and *N. repanda*-infected plants in the manner described before. The sporangial suspensions (2 X 10³, 2 X 10⁴, and 2 X 10⁵/ml) were serially passed through Ky-14 and *N. repanda* plants (six plants per species) for eight generations. The scheme of this study is shown in Figure 1. Disease severity was evaluated as described in the previous experiment. The complete serial passage study was performed twice. Some interim steps were performed an additional two or more times.

In another experiment, the combined effects of temperature and duration of leaf wetness on the severity of blue mold on both hosts was examined. Ky-14 and *N. repanda* plants were inoculated with 2 X 10⁴ sporangia per milliliter (obtained from *N. repanda*), covered with plastic bags, and kept in the dark at combinations of either 19 or 26 C for 20 or 48 hr. Plants were then uncovered and incubated in a growth chamber (23 C, 70 µE s⁻¹ m⁻², 12 hr of light) for disease development. Seven days after inoculation, disease was

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obtained from tabacina sporangial suspensions of inoculated with various concentrations of inoculum concentration, observed on tabacum Fig. 2. Severity of blue mold on 728 Plant milliliter. fungus obtained from Ky-14, as indicated changes are not clear and should be inoculation with 2 75% lesions, numbers abbreviations are generation numbers; both hosts increased with increasing ences in proportions of spore types of repanda through Peronospora tabacina Fig. 1. Scheme for study of virulence of severe on Ky-14 as on N.t.N. 104 (Ky-79 isolate). (A) Sporangia collected from either Ky-14 or 'N. repanda. (cv. Ky-14) and N. repanda, (N.r.). Numbers following plant abbreviations are generation numbers; numbers in parentheses are disease severity ratings (scale of 0-4, where 0 = no lesions; 1 = yellow lesions, ≤25% of leaf area chlorotic; 2 = yellow lesions, 25-50% chlorotic; 3 = yellow lesions, 50-75% chlorotic; and 4 = more than 75% of the leaf area chlorotic) after inoculation with 2 × 10^8 sporangia per milliliter.

Fig. 1. Scheme for study of virulence of Peronospora tabacina during serial passages through Nicotiana tabacum (N.t.) and N. repanda (N.r.). Numbers following plant abbreviations are generation numbers; numbers in parentheses are disease severity ratings (scale of 0-4, where 0 = no lesions; 1 = yellow lesions, ≤25% of leaf area chlorotic; 2 = yellow lesions, 25-50% chlorotic; 3 = yellow lesions, 50-75% chlorotic; and 4 = more than 75% of the leaf area chlorotic) after inoculation with 2 × 10^8 sporangia per milliliter.

DISCUSSION
This study revealed differences in the reactions of N. tabacum (Ky-14) and N. repanda to P. tabacina under controlled environmental conditions. All commercial cultivars of tobacco (N. tabacum) grown in the United States are considered susceptible to blue mold. Ky-14 has been categorized as highly susceptible to the disease. Our data support these findings (18).

The light to moderate symptoms of blue mold on N. repanda 7 days after inoculation indicates that this species is less susceptible than N. tabacum. This supports the findings of Clayton (1) and Hill and Mandryk (5). In their studies, disease was rated 6-8 days after inoculation and N. repanda was categorized as resistant to blue mold. However, under certain other conditions, N. repanda is susceptible to blue mold. Both Wolf (19) and Nesmith and Jones (11) reported that N. repanda in the wild in Texas is susceptible to a downy mildew and that the pathogen sporulated profusely under favorable environmental conditions. In our study, the fungus sporulated abundantly on N. repanda, even when chlorotic lesions were not obvious; extensive chlorosis and sporulation developed with time.

We observed, during serial passage of P. tabacina through plants of Ky-14 and N. repanda, that the virulence of the pathogen to N. repanda was greatly affected by the host plant from which inoculum was obtained. It is unlikely that this change in virulence is due to differences in proportions of spore types of varying germinability obtained from each host, because increasing the concentration of inoculum to three times the standard test concentration did not affect the severity of disease on N. repanda plants (Fig. 2B). The reasons for these changes are not clear and should be investigated.

Reasons for changes in proportions of spore types of varying size and germinability upon passage through different hosts are not known. Schlitz (17) reported a similar variability in spore size but did not discuss germinability.

Most downy mildew species produce localized infections, usually in leaf tissue (3,4,6,9,16). However, many downy mildew fungi have been shown to grow systemically in their hosts. Systemic colonization by the blue mold fungus P. tabacina was macroscopically observed in tobacco plants after stem injection of young plants in the field (7) and greenhouse with a sporangial suspension of the fungus (2,8). Examination of P.
tabacina in systemically colonized Ky-14 plants showed that the fungus was confined to the vascular tissue of the stem; severe necrosis of all root tissues was also noted (10). Although detailed histological studies were not included in our study, a logical scenario is suggested. P. tabacina became systemic in N. repanda, then progressed upward through the stem tissue to infect leaves and other plant parts. To our knowledge, there is no previous report to indicate that P. tabacina survives longer after infection in systemically infected burley tobacco. (Abstr.) Phytopathology 74:815.


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LITERATURE CITED


