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

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ABSTRACT

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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 chlorosis and leaf curling also developed on adjacent uninoculated leaves within 21 days of inoculation, indicating that the fungus was developing systemically in *N. repanda*. Plants did not die until 60 days after inoculation. Lesions (local and systemic) on *N. repanda* were capable of sporulating anytime between 7 and 60 days after inoculation. Virulence of *P. tabacina* to *N. repanda*, but not to Ky-14, was greatly affected by the host plant from which inoculum was obtained. When inoculum was harvested from Ky-14, blue mold on *N. repanda* was two to three times more severe than when the source of inoculum was from *N. repanda*. These data suggest that *N. repanda* could be an ideal host of *P. tabacina* and a significant inoculum source over a long period.

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).

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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 2×10^4/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 6×10^1, 2×10^2, 6×10^2, 2×10^3, 6×10^3, 2×10^4, and 6×10^4 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, $\leq$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 (2×10^2, 2×10^3, and 2×10^4/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 2×10^4 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

rated on the scale of 0–4. The experiment was repeated twice and the mean ± SD of six plants of each host per each of the four treatments is given.

Germination of sporangia of *P. tabacina* obtained from KY-14 or *N. repanda* was examined microscopically. Twenty microliters of sporangial suspension (500–600 sporangia) obtained from each host were incubated on depression slides at 19 C in the dark for 18 hr. The average percentage of spore germination was determined from eight replicates for each treatment six times during the serial passage study.

RESULTS

Symptomatology. Extensive chlorotic lesions and wilting were observed on Ky-14 plants on the seventh day after inoculation with a suspension of 2×10^4 sporangia per milliliter obtained from Ky-14. Extensive necrosis was present after an additional 3–4 days. Usually, plants died within 12–13 days of inoculation. In contrast, there were fewer chlorotic lesions on leaves of *N. repanda* than on KY-14 on the seventh day. These lesions became more obvious 10 days after inoculation. *N. repanda* leaves were also curled by that time, a condition absent on Ky-14. Within 14–21 days, chlorosis was extensive on all parts of the inoculated leaf. General chlorosis and leaf curling continued to develop on adjacent unsprayed leaves, and within 40–50 days, infection was evident in all leaves and the seed capsules. When plants were placed under moist and dark conditions, sporulation was profuse on inoculated and systemically infected plant parts. Necrosis on leaves was limited and plants did not die until 60 days after inoculation. Uninoculated control plants (sprayed with water) were free of symptoms of blue mold, with senescence only on the lowest leaves.

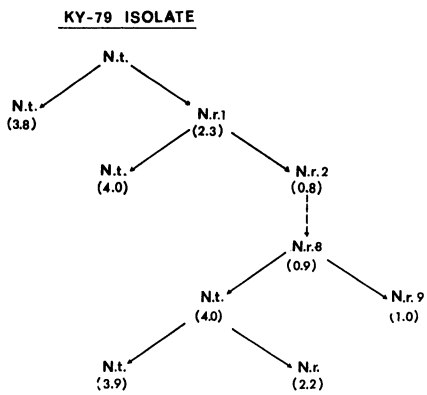

Fig. 1. Scheme for study of virulence of *Peronospora tabacina* during serial passages through *Nicotiana tabacum* (N.t.) and *N. repanda* (N.r.). Numbers following plant abbreviations are generation numbers; numbers in parentheses are disease severity ratings (scale of 0–4, where 0 = no lesions; 1 = 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 2×10^4 sporangia per milliliter.

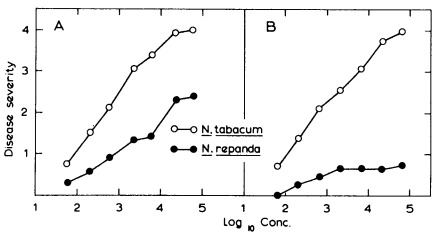

Fig. 2. Severity of blue mold on *Nicotiana tabacum* (cv. Ky-14) and *N. repanda* inoculated with various concentrations of sporangial suspensions of *Peronospora tabacina* (Ky-79 isolate). **(A)** Sporangia obtained from Ky-14 plants and **(B)** sporangia obtained from *N. repanda*.

Germination. Examination of sporangia obtained from KY-14 plants showed that 95% or more of them were of similar size (16 ± 1.7 μm long) with 80% germination. However, sporangia obtained from *N. repanda* were more variable in size and germination. About 75% of the sporangia obtained from locally infected leaves were similar in size to those obtained from KY-14. These sporangia had 72% germination. The other 25% were larger (24 ± 2 μm long) and did not germinate on depression slides. When inoculum was obtained from newly emerging leaves of systemically infected *N. repanda* plants, about 50% of the population of the sporangia was of the larger size and did not germinate. The other 50% were the smaller, more readily germinating type of spore.

Virulence. Disease was up to twice as severe on Ky-14 as on *N. repanda* when sprayed with sporangial suspensions of *P. tabacina* obtained from Ky-14 plants (Fig. 2A). The severity of blue mold on both hosts increased with increasing inoculum density. However, when Ky-14 and *N. repanda* were inoculated with sporangia obtained from *N. repanda*, disease severity on Ky-14 was four to six times greater than that observed on *N. repanda* (Fig. 2B). Virulence of the fungus obtained from Ky-14, as indicated by the severity of blue mold on *N. repanda*, was two to three times greater than that of the fungus obtained from *N. repanda* plants (Fig. 2A,B). The virulence of the pathogen to Ky-14 plants remained high regardless of the source of inoculum.

These observations were consistent during eight serial passages through *N. repanda* (Fig. 1). Greater disease severity was recorded on *N. repanda* when sporangia were harvested from Ky-14 than when sporangia were obtained from *N. repanda*. Disease severity, at the same inoculum concentration, observed on Ky-14 plants was similar with inoculum collected from either Ky-14 or *N. repanda*. This was always higher on KY-14 than that on *N. repanda*.

Changes in combinations of temperature (19 or 26 C) and duration of leaf wetness (20 or 48 hr) did not affect the differences in disease severity observed between the hosts. Disease ratings of 3.8–3.9 ± 0.3 and 0.9–1.1 ± 0.8 were recorded on Ky-14 and *N. repanda* plants, respectively.

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 is a consequence of 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* becomes systemic in *N. repanda*. Nesmith and Jones (11; *unpublished*) provided circumstancial evidence when they observed and photographed classical systemic colonization of *P. tabacina* on *N. repanda* in Texas. They were unable to verify conclusively that these symptoms resulted totally from systemic colonization.

N. repanda is capable of producing inoculum of *P. tabacina* over a long period of time. This is because the fungus systemically colonizes *N. repanda* and produces inoculum over most of the plant surface with only minimal initial inoculum required. Furthermore, *N. repanda* survives longer after infection and serves as a continuing source of *P. tabacina* for 8–9 wk after inoculation. The fungus can sporulate profusely on any infected part at any growth stage during this period. This could result in release of large amounts of inoculum during weather favorable for downy mildew. Further studies are needed to clarify the role of *N. repanda* in nature as a source of *P. tabacina* inoculum for blue mold epidemics.

ACKNOWLEDGMENT

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