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The effect of benomyl on tobacco leaf necrosis induced by Thielaviopsis basicola[1]

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Abstract

Tobacco leaf discs inoculated with a standardized suspension of endoconidia of *Thielaviopsis basicola* (Berk. & Br.) Ferr. were floated on tap water and on different concentrations of benomyl suspension. Lesion rating was 0.5, 0.25 and trace on 0.5-, 1- and 5-ppm benomyl concentration, respectively, as compared with 4.0 for the check. No lesions were formed on 10- and 50-ppm concentrations. Benomyl protected tobacco leaf discs systemically against lesion formation when applied before the penetration of the germ tubes. It also suppressed the development of mycelium on the necrotic spots.

The bearing of these results on the relation between leaf and root necrosis, is discussed.

Introduction

Black root rot caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr. is one of the most serious diseases of flue-cured tobacco in Canada. The soil-borne pathogen forms black necrotic lesions on the main and lateral roots. Szirmai (1940) found that myceliumof *T. basicola* caused necrotic lesions when applied, under humid conditions, to the injured surface of tobacco leaf. He attributed the formation of these lesions to tobacco veinal necrosis virus which, he thought, was associated with the fungus. Hecht and Bateman (1964) showed that virus-free *T. basicola* cultures induced necrosis on tobacco leaves and stems. A relationship was established between leaf and root necrosis of tobacco by *T. basicola* (Gayed, 1969). The present paper reports on the effect of the fungicide, benomyl, on the incidence and severity of tobacco leaf necrosis induced under laboratory conditions.

Materials and methods

The fungicide

The systemic fungicide Benlate benomyl (DuPont), previously known as Fungicide 1991, is a 50% wettable powder of the active ingredient 1-(butylcarbamoyl)-2-benzimidazole carbamic acid methyl ester.

The pathogen

The Harrow strain of *T. basicola* isolated from diseased tobacco roots and kept on frozen silica was subcultured on potato dextrose agar (PDA). Suspensions of 500,000 endoconidia per ml of water were used as inoculum for tobacco leaf discs.

[1] Contribution 53, Delhi Research Station.

Inoculation technique and disease assessment

Seedlings of tobacco (*Nicotiana tabacum* L.) variety 'Hicks Broadleaf' were grown in the greenhouse for 8–10 weeks. Detached leaves were placed with their upper epidermis on the rough surface of a masonite sheet. A 4-cm metallic borer fitted with a foam rubber pad protruding from its sharp end was used to cut leaf discs (Gayed, 1969). Pressing while rotating the borer through 45° and back to its original position cut a leaf disc and uniformly injured its upper surface. Two discs were cut from comparable parts of the lamina. For each treatment three leaves of similar position on the stem were tested. The injured surface was uniformly wetted with a standardized suspension of *T. basicola* endoconidia. The discs were floated on tap water or 0.5-, 1-, 5-, 10-, or 50-ppm suspension of benomyl with the inoculated surface facing upward. The dishes, each containing three leaf discs, were covered and placed in diffused light at room temperature. After 4 days, the necrotic lesions formed were rated on a scale between 0 for no lesions and 10 for total coverage of disc with lesions. Results of two trials were averaged.

Bioassay

Roots of two tobacco seedlings at the 4-leaf stage were cleaned and placed in either water or 50-ppm benomyl suspension. Tops of similar seedlings were cut at the transitional region and 1–2 cm of their cut ends were similarly treated as the roots. The seedlings and tops were kept in diffused light at room temperature. After 1 week, cross sections were cut at each node and 8 mm diameter discs were cut from the centre of each leaf. Both stem sections and leaf discs were transferred to PDA plates, each seeded with 2.5 million endoconidia. The plates were incubated at 25 °C for 7 days. The formation of a clear zone around the disc denoted the diffusion of material inhibitory to *T. basicola* growth. The experiment was repeated twice.

Microscopic studies

Inoculated leaf discs were floated in tap water for 0, 3, 6, 12, 24 and 36 h. The water in duplicate tests was replaced carefully with a 50-ppm benomyl suspension without touching the upper surface of the discs. Treated discs were observed at the end of each

Fig. 1. Tobacco leaf discs inoculated with *T. basicola* endoconidia were floated on a 50-ppm benomyl suspension 6, 12, 24 and 36 h after inoculation. Leaf lesion rating was taken 4 days after inoculation. The control (C) was floated on tap water.

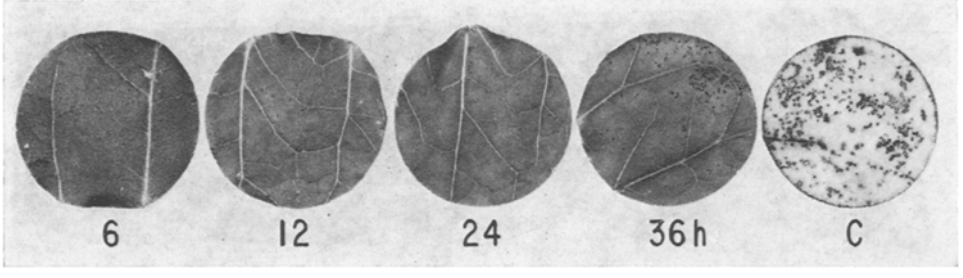

Fig. 1. Schijfjes tabaksblad werden 6, 12, 24 en 36 uur na inoculatie met endoconidiën van T. basicola op een 50-ppm benomyl suspensie gelegd. De verhouding van het aantal lesies werd 4 dagen na inoculatie bepaald. De controle (C) werd op leidingwater gelegd.

time interval for comparison with the untreated discs. At these intervals, the germination of endoconidia on the upper epidermis of a treated and a check disc was examined using the same techniques described by Hecht and Bateman (1964) and Fokkema (1968). Lesions formed on the remaining discs were rated 4 days after inoculation.

Results

Effect of benomyl concentration
Lesion rating of leaf discs floated on 0-, 0.5-, 1-, 5- 10- and 50-ppm benomyl suspensions was 4, 0.5, 0.25, trace, 0 and 0, respectively. Thus the severity of necrosis was reduced by concentrations between 0.5 and 5 ppm whereas 10- and 50-ppm concentrations completely inhibited the necrotic reaction. In the case of leaf discs floated on water, the tissue between the necrotic lesions became chlorotic (Fig. 1C), gradually turned brown and disintegrated within 15 days. In contrast, the lesion-free discs floated on 10-ppm benomyl remained green and intact for several weeks.

Bioassay
After 7 days incubation, the stem sections and leaf discs cut from seedlings and seedling tops dipped either in water or benomyl suspension and transferred to PDA seeded with *T. basicola* endoconidia, were examined. Stem sections cut at the four nodes as well as leaf discs cut from leaves of either benomyl-treated seedlings or treated tops were surrounded with a neutral zone denoting the systemic effect of the fungicide. Stem sections and leaf discs of the untreated plants showed no zone of inhibition.

Microscopic studies
Germination of *T. basicola* endoconidia on tobacco leaf discs floated on water was observed 6 h after inoculation and penetration of the epidermis by the fungus was most likely between 12 and 24 h. The morphological features of the germ tube and tissue penetration were similar to those previously described (Hecht and Bateman, 1964). The brown lesions could be seen by the naked eye 2 days after inoculation and they extended from the upper to the lower epidermis of the leaf; 5 days later, the mycelium of *T. basicola* was clearly visible on the upper surface of the lesions and endoconidiophores producing endoconidia were formed. Discs floated on benomyl 3 and 6 h after inoculation were free from lesions whereas those floated on benomyl 12 and 24 h had a few lesions while more lesions were formed on the 36 h after inoculation treatment (Fig. 1). As penetration takes place within 24 h after inoculation it is probable that the leaf necrosis occurred before the benomyl became effective in the 36 h after inoculation treatment. Mycelium development on these lesions was much suppressed compared with the mycelium formed on lesions of untreated discs.

Discussion

Szirmai (1940) and Hecht and Bateman (1964) produced leaf necrosis on intact leaf under greenhouse conditions. In the present investigation, a simple laboratory technique was used to produce necrosis on detached tobacco leaf discs. The factors involved in the formation of these lesions are not clearly understood, however, a relationship was established between root and leaf necrosis induced by *T. basicola* on

tobacco (Gayed, 1969).

Benomyl at low concentrations reduced the severity of leaf necrosis and leaf discs floated on 10 and 50 ppm did not develop any lesions. The systemic action of the fungicide protected the leaf tissue against necrosis before the penetration of the germ tubes took place. It also suppressed mycelial development on necrotic lesions after their formation. Benomyl also was effective in reducing the severity of black root rot disease of tobacco under controlled conditions in a growth chamber and in the field (unpublished). Such agreement in the effectiveness of the fungicide on the development of leaf and root necrosis supports the previous findings that root and leaf lesions might be the result of the same chemical reaction (Gayed, 1969).

Since 1-(butylcarbamoyl)-2-benzimidazole carbamic acid methyl ester breaks down in water rapidly into the fungitoxic derivative carbamic acid methyl ester (Clemons and Sisler, 1969) it is feasible that the latter compound may also be effective against tobacco leaf necrosis and black root rot caused by *T. basicola.*

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Samenvatting

Het effect van benomyl op tabaksbladnecrose veroorzaakt door Thielaviopsis basicola

Schijfjes tabaksblad dreven op leidingwater met verschillende concentraties benomylsuspensie. De bladeren waren geïnoculeerd met een gestandaardiseerde endoconidiënsuspensie van *Thielaviopsis basicola.* De verhouding van de lesies in de schijfjes op water met resp. 0.5, 1 en 5 ppm benomyl was 0.5, 0.25 en zeer weinig, vergeleken met 4.0 bij de controle. Er werden geen lesies gevormd op een benomylconcentratie van 10 en 50 ppm. Benomyl beschermde de schijfjes tabaksblad systemisch tegen lesievorming, als het vóór het binnendringen van de kiembuizen werd toegediend. Het onderdrukte ook de myceliumontwikkeling op de necrotische plekken.

De betekenis van deze resultaten voor de relatie tussen blad- en wortelnecrose wordt besproken.

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