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The bait leaf method for determining soil infestation with tobacco rattle virus-transmitting trichodorids

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Abstract

A rapid bait leaf method, using detached leaves of *Nicotiana tabacum* 'White Burley', for determining infestation of soil samples with TRV-transmitting trichodorids is briefly described. Bait leaf infection may already occur three hours after leaf burial and is apparently associated with trichodorid adherence to the leaf surface. Virus could always immediately be detected by inoculation onto attached 'White Burley' leaves with homogenates from bait leaves that had been buried for two days and more. After burial for shorter periods bait leaves first required additional exposure to light (for three days) to permit virus multiplication. Chances to detect viruliferous trichodorids were highest after keeping the bait leaf in soil for three days followed by a three-day incubation under light.

Introduction

Tobacco rattle virus (TRV) is a soil-borne virus transmissible by trichodorids. In previous experiments the effect of temperature on the transmission of the virus (Van Hoof, 1975) and of soil moisture content on nematode activity in connection with TRV infection (Van Hoof, 1976) was studied. In these experiments soil infestation with viruliferous nematodes was determined with a bait leaf method, using detached leaves of *Nicotiana tabacum* 'White Burley'. The technique had preference over the bait plant method in being readily applicable at different temperatures and in not changing the moisture content of the soil samples.

The present paper describes the method and reports on a further improvement.

Material and methods

The technique. Soil to be tested is sieved and mixed. Plastic boxes measuring 16 × 28.5 × 9 cm are filled with the mixture and leaves of 'White Burley' tobacco are buried in the soil. The boxes are kept at 15°C. After a certain period of time the leaves are collected, rinsed and either immediately tested for virus or after three days on wet filter paper at 20°C under light. Infectivity is then determined by inoculation of leaf homogenates of each bait leaf onto two leaves of an intact 'White Burley' plant. This is necessary because on the detached bait leaves no symptoms or vague yellow spots or rings develop (Fig. 1).

Fig. 1. Right: Detached 'White Burley' bait leaf after three days in infested soil followed by a three-day incubation under light. Left: Reaction of 'White Burley' leaf still attached to the plant seven days after inoculation with bait leaf homogenate.

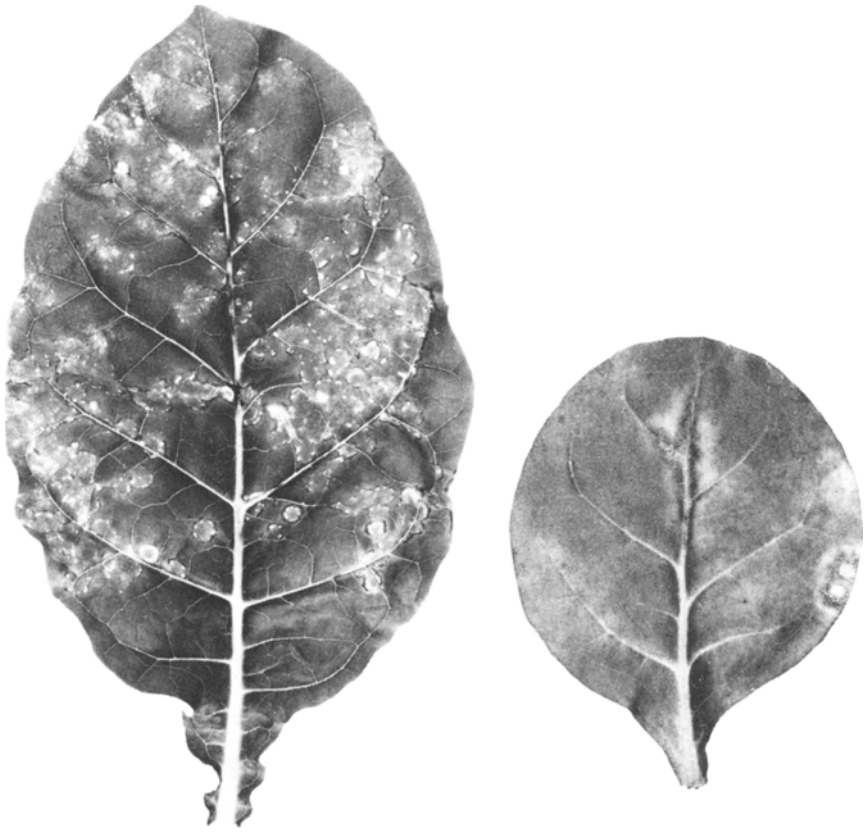

Fig. 1. Rechts: Afgeplukt vangblad van 'White Burley', nadat het drie dagen in besmette grond verbleef en drie dagen geïncubeerd werd onder licht. Links: Reactie van 'White Burley'-blad, dat nog aan de plant bevestigd was, zeven dagen na inoculatie met fijngemaakt vangblad.

Present experiments. Soil was sampled from an infested field at Overloon containing 120 trichodorid individuals (*Paratrichodorus pachydermus* and *Trichodorus viruliferus*) per 500 g, and its moisture content adjusted to 18% by addition of water.

In the first experiment, repeated three times, twelve boxes were filled and stored at 4°C, and placed at room temperature one day before testing. On each of six successive days, twelve 'White Burley' leaves were buried in the soil of each of two boxes, which were then kept at 15°C. On the seventh day all bait leaves were collected and rinsed. Some were immediately tested for virus content. Others were placed on wet filter paper at 20°C for three days and exposed to 12 h periods of light (at an intensity of 30 lx) alternated by 12 h periods of darkness.

The bait leaves had been taken from glasshouse-grown 'White Burley' plants, one leaf from each plant. The plants were then transferred to a growth chamber at 20°C.

Three days later the two oldest leaves of each plant were used to test the homogenized bait leaf for presence of virus. After inoculation, the plants were placed under wet cheese cloth to prevent inoculation damage. Local lesions were counted eight days later.

In the second experiment the bait leaves were buried for periods of $^3/_4$, $1^1/_2$, 3, 6, 12 and 24 h, after which they were incubated for three days under light as ascribed above. The tobacco plants used in this experiment were kept in the glasshouse only.

Numbers of nematodes, possibly adhering to the bait leaves, were determined by counting them in the rinsing water.

Results

The results of the first experiment are presented in Table 1a. They show that virus can be demonstrated in bait leaves after one day in infested soil and immediate testing. A maximum virus content was observed, however, in bait leaves that had been buried for a number of days. In trial I this was after 5 days, in the trials II and III of this experiment after 4 days. In duplicate tests where the bait leaves were kept under 12 h light per day for three days prior to testing, the maximum virus contents were reached after different bait periods (Table 1b). In trial I high numbers were reached after 1 day already. In trial II and III maxima were reached after 3 days. The local-lesion data of Table 1a and b are also graphically recorded in Fig. 2.

The results of the experiment on short bait periods are given in Table 2. These show that in periods as short as 3 h a bait leaf can become infected with TRV. After this time three trichodorid nematodes were recovered from the washing water. After 12 and 24 h bait periods, 6 and 8 trichodorids, respectively, were recovered.

Table 1. Virus content of bait leaves after 1 to 6 days in infested soil. The bait leaves were tested immediately (a) or after an additional period of 3 days under light (b).

Trial	Number of days					
	1	2	3	4	5	6
a: I	6[1] 19[2]	9–659	11–1621	10–1461	12–4292	12–2361
II	7–46	8–384	11–2729	11–4165	11–3201	11–1522
III	0– 0	9–367	11–1195	10–1826	10–1528	11–1571
Total	13–65	26–1410	33–5545	33–7452	33–9021	34–5454
	1 +3	2 +3	3+3	4 +3	5+3	6 +3
b: I	12[1]–3423[2]	10– 2876	12– 2777	10– 3167	12– 4024	10– 1506
II	11– 470	10– 3782	12– 5753	12– 5725	12– 4196	11– 4017
III	12–2034	12– 5333	12– 5545	12– 4749	11– 4537	11– 4584
Total	35–5927	32–11991	36–14075	34–13641	35–12757	32–10107

[1] Number of bait leaves infected with TRV out of 12 tested.
[2] Number of local lesions on 12 'White Burley' plants.

Tabel 1. Het virusgehalte van vangbladeren na 1 tot 6 dagen in besmette grond. De vangbladeren werden direct getoetst (a), of nadat ze drie dagen onder licht verbleven (b).

Fig. 2. The course of the virus concentration in bait leaves after varying numbers of days in infested soil.

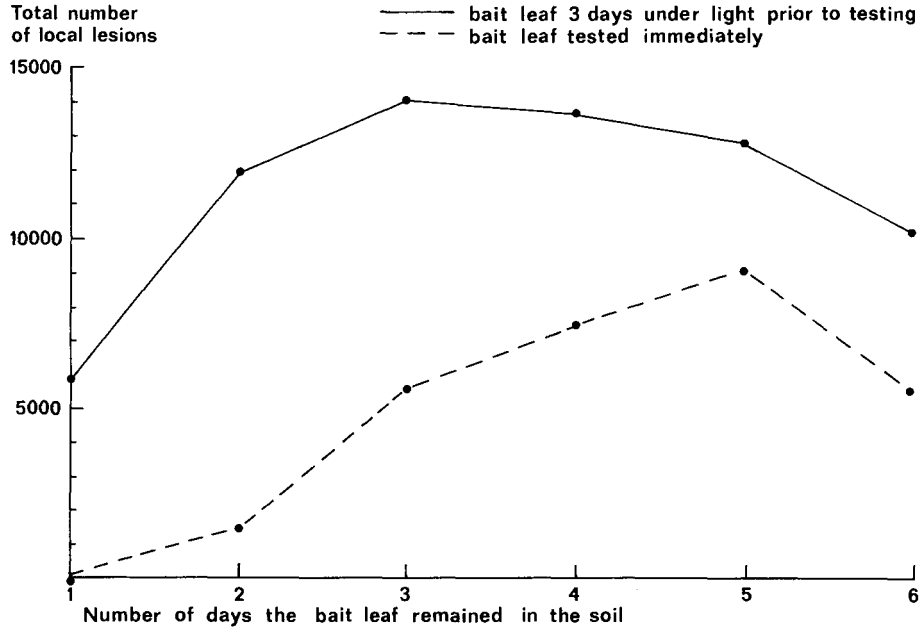

Fig. 2. Het verloop van de virusconcentratie in vangbladeren na verschillende aantallen dagen in besmette grond.

Table 2. TRV infection and trichodorid infestation of bait leaves after short periods in infested soil.

	Time of exposure to infection in soil					
	3/4 h	1 1/2 h	3 h	6 h	12 h	24 h
Number of infected leaves[1]	0	0	3	2	6	10
Number of trichodorids[2]	0	0	3	0	6	8

[1] Out of 12 leaves tested after 3 additional days under light.
[2] Recovered from equal samples of washing water.

Tabel 2. TRV-infectie en bezetting met trichodoriden van vangbladeren na korte perioden in besmette grond.

Discussion

The results show that one day and even three hours in infested soil may be sufficient for a bait leaf to become infected with TRV. However, the virus content is too small to be detected immediately in the bait leaf homogenate. After incubation of rinsed bait leaves for three days under 12 h light per day prior to homogenizing, a maximum

amount of detectable virus was found in bait leaves after a three-day bait period in infested soil. Two more days did not yield higher amounts of virus. Another additional day led to a decrease. This may have been due to beginning bait leaf decay.

After incubation under light, the virus concentration in bait leaves mostly was much higher than in the non-incubated ones, especially after short periods in infested soil. Then, this undoubtedly is due to time needed for multiplication in the nematode-inoculated leaves. Lower concentrations in leaves tested immediately after longer periods in infested soil may be caused by leaf detioration beginning before highest virus concentration has been reached. Another explanation is that virus multiplication is favoured by incubation in light and at a temperature (20°C) higher than that in the soil (15°C).

The results of the bait leaf method are further affected by the quality of the bait leaves used, depending on original growing conditions. Bait leaves from tobacco plants grown in a growth chamber at high light intensity and in strong air currents, gave poor results possibly due to increased cuticle thickness. More succulent plants from the glasshouse were apparently easier for nematodes to feed upon.

Bait leaf infection with TRV apparently is associated with trichodorid adherence to the leaf surface. With increasing bait duration the numbers of nematodes adhering to the leaf surface increased. The total numbers of nematodes adhering to the bait leaves, however, were low (Table 2).

The present data show that, with a three-day incubation of rinsed bait leaves under light prior to testing for infectivity on 'White Burley' leaves, a three-day bait period in soil to be tested for TRV infestation is recommendable.

Samenvatting

De vangbladmethode voor het bepalen van de besmetting van grond met tabaksratelvirus-overbrengende trichodoriden

Een snelle methode wordt beschreven voor het vaststellen van de besmetting van grond met trichodoriden, die TRV overbrengen. Vangbladeren – afgesneden bladeren van *Nicotiana tabacum* 'White Burley' – werden in de grond begraven (Fig. 1). De kortste tijd, waarin reeds virusoverdracht werd geconstateerd, was drie uur. Infectie van het vangblad bleek gecorreleerd te zijn met hechting aan het blad van nematoden (Tabel 2).

De hoogste virusconcentratie werd verkregen, indien het vangblad drie dagen in de besmette grond verbleef, gevolgd door een incubatie van drie dagen onder licht (Tabel 1 en Fig. 2).

References

Hoof, H. A. van, 1975. The effect of temperature on the transmission of tobacco rattle virus in tulips by *Trichodorus*, using the 'bait leaf' method. Nematologica 21: 104–108.

Hoof, H. A. van, 1976. The effect of soil moisture content on the activity of trichodorid nematodes. Nematologica 22: in press.

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