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A rapid method for distinguishing the tulip and daffodil races of Ditylenchus dipsaci (Kuehn)

W. A. WINDRICH[1]

Instituut voor Plantenziektenkundig Onderzoek (IPO), Wageningen

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Abstract

Methods of inoculation with bulb and stem eelworms to obtain artificially infected tulip bulbs and leaves are described. Using these methods one can rapidly determine whether an infested crop of daffodils is attacked by the tulip or the daffodil race of *Ditylenchus dipsaci*.

Introduction

The tulip race of the stem nematode *Ditylenchus dipsaci* (Kuehn) attacks both tulip and daffodil, whereas the daffodil race only affects the latter. Stem nematodes found in daffodils may therefore belong to either of the two races.

The regulations for the control of stem nematodes in bulbs prescribe more severe actions against the tulip race than against the daffodil race. For a proper application of these regulations it is, therefore, necessary to be able to distinguish the tulip race of the stem nematode from other races. Whether stem nematodes in daffodils belong to the tulip race or not was originally determined by planting healthy tulip bulbs in pots with sterilized soil into which parts of daffodils infested with the stem nematode to be identified were mixed. If in due time symptoms of attack were found in the stems, leaves, flowers or bulbs of these tulips along with all developmental stages and eggs of the nematode, it could be assumed that the population belonged to the tulip race (Silver, pers.comm.). However, such an investigation took more time than was acceptable with respect to the period within which measures should be taken. It is also possible to infest tulip bulbs with tulip stem nematodes shortly after lifting by placing about 50 specimens in a moist piece of cotton wool on a wound made by removing a small piece of the cuticle of the outer bulb scale (Kooistra, 1965).

This investigation was initiated to develop and evaluate methods that might be used to distinguish the two races, including refinement of the method of Kooistra as well as leaf inoculations.

[1] Stationed at the Flower Bulb Research Centre, Lisse, The Netherlands.

Inoculation of tulip bulbs with the tulip race

With a slight modification of the method of Kooistra (1965) (e.g. use of moist sand instead of cotton wool) a high percentage of successful inoculations was obtained with the cv. 'Elmus' when bulbs were inoculated shortly after lifting (Fig. 1). If bulbs after inoculation were kept at 13 °C, symptoms became visible in about 8 days (after lifting bulbs are normally stored at 18 °C). The percentage of successful inoculations decreased with increase of the period between lifting and inoculation (Table 1). When bulbs were stored in moist peat at –2 °C just after lifting, the decrease in the number of successful inoculations was evident only after a much longer period (Table 2). Up to 100 % successful inoculations could be obtained with newly lifted bulbs of the cv. 'Rose Copland' and 'Copland's Record'.

A still better method of inoculation was to introduce an aqueous suspension of 50–100 nematodes under the epidermis of the outer bulb scale. For this reason the nematodes were first concentrated as described by Seinhorst (1945). The nematode suspensions were poured in drawn out glass tubes with a closed point. When the nematodes had settled in the narrow part, the tip was broken off while the top of the tube was kept closed with the thumb. By pressing with the thumb or letting in a little air, a very concentrated drop of nematode suspension can be transferred to a siliconized watch glass. The silicone prevents the spreading of water and the nematodes. If the suspension in the drop is sufficiently concentrated, 50–100 nematodes can be removed from it at a time on the tip of a sharpened bamboo splint or pig's hair mounted on a shaft. By making a small half circular cut in the epidermis of the outer fleshy scale of a bulb, a slip of about 10 mm^2 of the epidermis could be loosened from the underlying tissue while it remained attached on one side. The nematodes were then pushed under this close to where it was still attached to the bulb. The slip was then pressed gently on the underlying tissue again (Fig. 2.)

Bulbs inoculated in this way, were kept in a plastic bag after the wound whether or

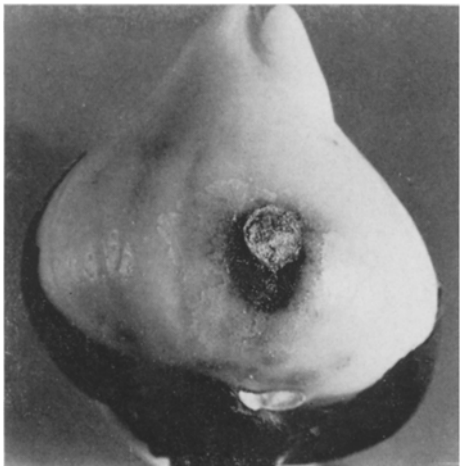

Fig. 1. Inoculation of a tulip bulb (variety 'Elmus') with the tulip race of the stem eelworm by use of the modified method of Kooistra (moist sand instead of cotton wool.) Characteristic symptoms of attack around the wound 4 weeks after inoculation.

Fig. 1. Inoculatie van een tulpebol, cv. 'Elmus', met het tulperas van het tulpestengelaaltje door toepassing van een variatie op de methode Kooistra (vochtig zand in plaats van watten). Karakteristieke symptomen van aantasting rond de wond, 4 weken na inoculatie.

Table 1. Influence of the lapse of time between date of lifting and date of inoculation on the percentage of successful inoculations with the tulip race of *Ditylenchus dipcasi* on normally stored (at 18 °C) bulbs of tulip variety 'Elmus'.

Date of inoculations	Number of inoculations	% successful inoculations after 23 days
3. 8.1966	50	78
11. 8	100	59
13. 9	200	42
23. 9	100	32
25.10	400	33

Tabel 1. De invloed van het tijdsverloop tussen rooitijdstip en inoculatiedatum op het percentage geslaagde inoculaties met het tulperas van Ditylenchus dipsaci bij normaal bewaarde (bij 18 °C) bollen van tulp cv. 'Elmus'.

Table 2. Influence of the lapse of time between date of lifting and date of inoculation on the percentage of successful inoculations with the tulip race of *Ditylenchus dipsaci* on bulbs of the tulip variety 'Elmus' stored at –2 °C.

Date of inoculations	Number of inoculations	% successful inoculations after 21 days
15.2.1967	100	43
14.4	50	16
2.5	50	0
16.5	50	0
7.6	50	0

Tabel 2. De invloed van het tijdsverloop tussen rooitijdstip en inoculatiedatum op het percentage geslaagde inoculaties met het tulperas van Ditylenchus dipsaci op bollen van tulp cv. 'Elmus', die bij –2 °C waren bewaard.

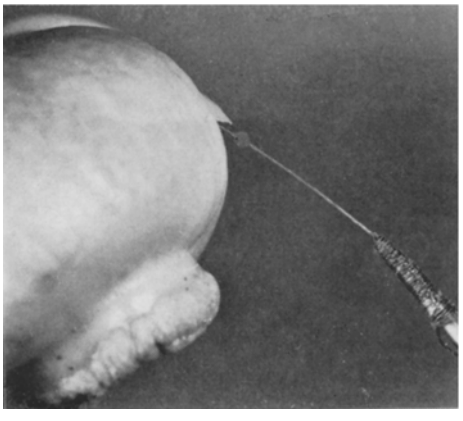

Fig. 2. Inoculation of a tulip bulb (variety 'Elmus') with the tulip race of the stem eelworm. A drop of water with 50–100 nematodes is introduced under a slip of about 10 mm² of the epidermis of the outer fleshy scale by means of a pig's hair.

Fig. 2. Inoculatie van een tulpebol (cv. 'Elmus') met het tulperas van het tulpestengelaaltje. 50–100 aaltjes worden in een druppel water met behulp van een varkenshaar onder de epidermis van de buitenste vlezige rok gebracht.

not was covered with sellotape. Moreover a number of inoculated bulbs was pressed with the inoculated side on moist silver sand.

The latter method gave the highest percentage of successful inoculations within 2 weeks (100% out of 50 inoculated bulbs). With this method one may expect a high percentage of successful inoculations of bulbs inoculated just after lifting as well as of bulbs stored at –2 °C just after lifting.

Inoculation of leaves

Leaves of various age were inoculated by introducing nematodes under the epidermis in much the same way as described in the second method of inoculation of bulbs, keeping the wound as small as possible. Symptoms of attack became visible within 2 days after inoculation, much faster than in bulbs. They could even be obtained with a single nematode (Fig. 3). The age of the leaves (inoculated just after emerging from the bulb until well after flowering) did not influence their susceptibility to attack.

Comparison of the reaction of bulbs and leaves to inoculation with the tulip race and with the daffodil race

The tulip race used in the experiments was obtained from infested tulips, the daffodil race from daffodils grown on a field where this crop had been heavily infested during 4 years but tulips remained healthy.

Two hundred bulbs of the cv. 'Elmus', 'Copland's Record' and 'Rose Copland' were inoculated with the daffodil race, immediately after lifting. Even 85 days later no symptoms of stem nematode attack had developed on any of these bulbs. Of 200 bulbs

Fig. 3. To the left: characteristic symptoms of attack by tulip stem eelworm after inoculation of the outer leaf of the tulip sprout with one male specimen. To the right: necrosis, caused only by making the wound.

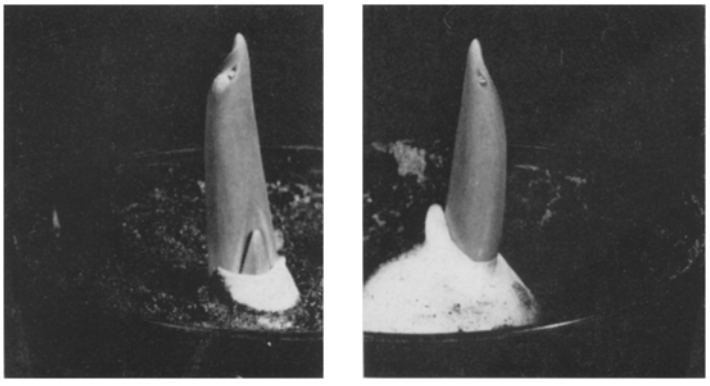

Fig. 3. Links: karakteristiek symptoom ten gevolge van aantasting met het tulpestengelaaltje na inoculatie van het buitenste blad van een tulpespruit met een manlijk exemplaar. Rechts: Geen inoculatie, alleen wondreactie (necrose).

Fig. 4. Inoculation of a tulip leaf with stem eelworms. To the left with eelworms of the tulip race, a joined area of attacked tissue is seen; to the right with eelworms of the daffodil race, only a limited area has become necrotic as a result of mechanical damage.

Fig. 4. Een tulpeblad geïnoculeerd met stengelaaltjes. Links met aaltjes van het tulperas; duidelijk is een aaneengesloten vlek van aangetast weefsel te zien. Rechts, met aaltjes van het narcisseras; slechts een kleine vlek zichtbaar, die necrotisch is geworden ten gevolge van de mechanische beschadiging.

of the same cultivars inoculated with the tulip race 80% showed symptoms within 14 days.

In March 1967, January 1968 and February 1969 a total of 42 inoculations were made on leaves with the tulip race and 42 with the daffodil race. Of the former 40 resulted in symptoms of attack, whereas none occurred in the latter where only a limited area became necrotic as a result of mechanical damage (Fig. 4).

According to field observations, tulip leaves are also susceptible to an attack by stem nematodes from onions (Kok et al., 1963; Windrich, 1968), however, in the case of daffodils, nine inoculations of leaves with stem nematodes from onions gave no symptoms and attack of daffodils by stem nematodes from onions has never been reported. Neither did stem eelworm isolated from *Muscari* spp. (Silver, 1967, pers. comm.) or hyacinth (van Slogteren, 1919) attack daffodil leaves and bulbs.

Therefore it can be decided whether a stem nematode population from daffodils contains nematodes belonging to the tulip race by inoculation of tulip bulbs or leaves.

Results can be obtained in 2 weeks with bulb inoculations, whereas 1 week is sufficient for a test with leaf inoculations.

Samenvatting

Een snelle methode om het tulperas en het narcisseras van het stengelaaltje Ditylenchus dipsaci (Kuehn) van elkaar te onderscheiden

Een methode om tulpebladeren en -bollen kunstmatig te infecteren met het stengelaaltje, *Ditylenchus dipsaci*, wordt beschreven.

Karakteristieke symptomen van aantasting ontstaan 8–14 dagen na inoculatie met het tulperas; inoculatie met het narcisseras levert geen symptomen.

Aangezien het tulperas zowel de tulp als de narcis kan aantasten en het narcisseras slechts de laatste, kan door middel van de beschreven methode in korte tijd worden uitgemaakt of een in narcis gevonden stengelaaltje wel of niet tot het tulperas behoort.

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