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# NOTCHED LEAF IN *GLADIOLUS* SPP., CAUSED BY VIRUSES OF THE TOBACCO RATTLE VIRUS GROUP[1,2]

*Kartelblad in* Gladiolus *spp., veroorzaakt door virussen van de ratelvirusgroep*

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Symptoms of notched leaf in gladiolus are described in this paper. The disease can occur either in patches in the field (only on sandy or light sandy loam soils) or in plants scattered throughout a field planted with a certain stock (independent of soil type). Experiments showed that this disease is caused by tobacco rattle virus (TRV), which is transmitted by *Trichodorus pachydermus* Seinhorst and *T. similis* Seinhorst. Gladiolus corms planted in infested soil show a more or less serious reduction of growth and sometimes typical symptoms of notched leaf. In experiments these notched leaf symptoms could be reproduced in the first season only when *Trichodorus* from infested soil was able to infect the young sprouts on top of the corms. If infection took place through the roots, the growth of the plants was not or only slightly retarded and the typical notched leaf symptoms appeared in the offspring in the following season. At least two serologically different strains of TRV could be isolated from affected gladiolus. These serotypes were not found to be typical for a certain location as in some cases both were isolated from the same infested field. In fields where potatoes showed stem-mottle and/or spraing, gladiolus was affected by notched leaf.

## INTRODUCTION

Notched leaf is a disease of gladiolus that has been known in the Netherlands for at least thirty years. Sometimes affected plants may be found scattered throughout a field, whereas in other cases they occur in patches in which the plants show more or less typical symptoms of the disease.

The cause of this deviation has long been unknown. Some growers considered it to be due to damage to the young sprouts, e.g. by mechanical cleaning of the corms (BALKENENDE, 1954) or by the treatment with naphthalene to control *Taeniothrips simplex* Morrison during the storage period. In preliminary experiments in the Laboratory for Flowerbulb Research at Lisse, however, the symtoms of notched leaf could not be reproduced by mechanical damage or by chemical treatments of the corms and the young sprouts.

In 1960 VAN DER WANT (unpublished results) isolated a virus by inoculating sap from diseased gladiolus leaves on 'White Burley' tobacco plants. The symptoms on the test plants closely resembled those of tobacco rattle virus (TRV). Electron-microscopical investigations of dip-preparations both from infected gladiolus and tobacco leaves showed the presence of rod-shaped virus particles of about the same type as those of tobacco rattle. At that time SOL & SEINHORST (1961) proved that TRV can be transmitted by *Trichodorus pachydermus* Seinhorst.

[1] Accepted for publication 18 November, 1966.

[2] A preliminary report on this subject has been published in Nematologica 10 (1964): 69–70.

More detailed work was started to investigate whether TRV is the cause of notched leaf in gladiolus and whether this virus is transmitted to the host by *Trichodorus* spp.

## SYMPTOMS AND OCCURRENCE OF THE DISEASE

Plants with typical symptoms show seriously distorted growth and notches along the margin or along the veins of one or more leaves, in most cases combined with chlorotic or brown necrotic stripes and spots (Fig. 1). The flower stem and bud occasionally stop growing and show deformations and necroses as well. These plants are very susceptible to infection by *Botrytis gladiolorum* Timmermans and often die prematurely. In infected stocks, besides these symptoms, less serious deviations from normal growth can often be observed; even a crumpling of the leaves between the veins may indicate that the plant is not healthy.

In a crop growing from cormels the symptoms are less conspicuous and it is almost impossible to recognize the disease in the field. The corms and cormels themselves show no definite symptoms. However, because growth can be seriously impaired, the size of the corms from infected plants at harvest time is much less than that of comparable healthy ones.

In the field, plants with typical notched leaf symptoms are often found scattered throughout a certain stocks, neighbouring plantings of the same or other cultivars originating from other stocks, often being quite healthy. This form of occurrence can be found on all types of soil where gladiolus is grown. BEIJER (unpublished results) found by microscopical observation of corms of an infected stock before planting that the symptoms can be seen already in the young leaves. Apparently such stocks have become infected in some previous season.

Besides the scattered occurrence of notched leaf in gladiolus fields, on sandy or light sandy loam soils the disease may occur in definite patches (Fig. 2). Sometimes the young sprouts may not even succeed in emerging, while in other cases growth is retarded and plants are distorted. Various grades of symptom expression may be found. Some of the plants may show typical notched leaf, whereas in others only chlorotic and necrotic stripes and spots are visible. Both the roots formed by the mother corm and the contractile roots show excessive side branching and brown necrotic spots and areas (Fig. 3).

In the "patch" form of the disease, different stocks and cultivars, independent of their origin, may be affected in the same field. This would suggest that the disease may be soil-borne.

## EXPERIMENTS AND OBSERVATIONS

### *Material and methods*

In all attempts to transmit TRV from gladiolus to other hosts mechanically with extracts from leaves, roots or corms, toxic effects on the inoculated plants were observed and infection was often inhibited. With gladiolus leaves the following method gave best results. Small pieces of leaf tissue (ca. 1 g) from areas showing symptoms were immersed in 2 ml 1/15 M phosphate buffer pH 7 and thoroughly sliced with a razor blade. After adding another 2 ml of buffer, the mixture was macerated in a mortar and passed through cheese cloth. All opera-

tions were carried out in a cold room (8 °C) as quickly as possible, all fluids and utensils being previously cooled at 0 °C. During extraction of roots the temperature conditions were less critical. The suspensions thus obtained were inoculated onto the leaves of test plants after dusting with carborundum powder.

Attempts to obtain infectious extracts from gladiolus corms have never been successful.

In the infection experiments the presence of TRV was best determined by inoculating leaves of 'White Burley' tobacco. Positive results, deduced from characteristic symptoms, were confirmed by electron-microscopical observations of dip-preparations made from the same test plants.

In order to show the presence of *Trichodorus* spp. in different soils, samples of 500 g were washed and the nematodes collected by methods as described by SEINHORST (1962). At first sampling was carried out by means of a soil sampler with a diameter of only 17 mm, as commonly used for this purpose; 80 subsamples were thoroughly mixed and 500 g was taken for further investigation. Much better results (up to 90% more surviving *Trichodorus* individuals) were achieved by using a metal cylinder with a diameter of 12 cm and 25 cm in length. Three samples per $m^2$ were taken and carefully mixed before weighing 500 g. Apparently the *Trichodorus* nematodes are easily killed by rough treatment of the soil. Usually the samples were taken from the upper 20 cm of soil.

Additional special techniques will be described later with the presentation of the results.

*Isolation of TRV from diseased gladiolus plants*

Experiments were started by inoculating sap collected from roots and leaves of diseased gladiolus plants from several stocks on 'White Burley' tobacco. Plants taken from diseased patches and those occurring scattered throughout the field were investigated separately; in the former case both plants with and without the typical notched leaf symptoms were taken. Symptoms on the test plants and electron micrographs of dip-preparations (BRANDES & WETTER, 1959), made from infected gladiolus and tobacco leaves, indicated that the virus isolated was of the tobacco rattle type.

The results of these experiments are given in Table 1. From roots of plants growing in diseased patches TRV could be isolated easily, especially when the plants showed the typical symptoms of notched leaf. Isolation of the virus from the leaves was more difficult, possibly partly owing to toxic effects of the extracts on tobacco leaves. No TRV could be isolated from the roots of diseased plants appearing scattered in a field, and the number of positive infections from leaf sap inoculations was much lower than from diseased plants taken from patches. Attempts to increase the number of infections with sap from roots and leaves by means of phenol extraction (SÄNGER & BRANDENBURG, 1961) were not successful.

*The presence of* Trichodorus *spp. and TRV in the soil and the occurrence of notched leaf in patches*

In several cases where patches with notched leaf in gladiolus fields were observed, the soil was sampled for the presence of *Trichodorus* spp. In addition, test plants known to be susceptible to TRV ('White Burley' tobacco, spinach, gladiolus) were planted or sown in a number of these fields. After several weeks

TABLE 1. Isolation of TRV from roots and leaves of gladiolus plants either with specific and non-specific notched leaf symptoms occurring in patches in the field, or from plants with specific symptoms scattered throughout the stock.

*Isolatie van TRV uit wortels en bladeren van gladioleplanten met typische kartelbladsymptomen, verspreid in de partij voorkomend of pleksgewijs optredend in het veld.*

| Appearance of the disease in the field (total number of plants investigated) | | Number of 'White Burley' tobacco plants showing symptoms of tobacco rattle virus after inoculation with sap from: | |
|---|---|---|---|
| | | Roots | Leaves |
| In patches / *Pleksgewijs* | | | |
| With notched leaf / *Met kartelblad* | (22) | 20 | 9 |
| With non-specific notched leaf / *Zonder typisch kartelblad* | (60) | 19 | 2 |
| Total | (82) | 39 | 11 |
| Notched leaf plants scattered throughout the stock / *Kartelbladplanten, verspreid in de partij voorkomend* | (27) | 0 | 4 |
| *Voorkomen van de ziekte (totaal aantal onderzochte planten)* | | *Aantal 'White Burley'-tabaksplanten met ratelsymptomen na inoculatie met sap van:* | |
| | | *Wortels* | *Bladeren* |

the presence of TRV in the roots of these plants was checked by sap-inoculation on 'White Burley' tobacco leaves.

From the results summarized in Table 2 it can be concluded that there is a correlation between the occurrence of notched leaf in patches and the presence of *Trichodorus* spp. and TRV. In Koegras on sandy loam soil no symptoms in gladiolus could be observed, however; this phenomenon will be dealt with in the discussion. Since a great number of soil samples from this field was thoroughly investigated and only *T. similis* was found, this strongly suggested that apart from *T. pachydermus* this species also is able to transmit TRV (see also *Infection experiments*). In two samples from Stavenisse only *T. teres* Hooper could be found, indicating that this species also can be a vector of the virus. At about the same time VAN HOOF (1964b) published results which confirmed this supposition.

Soil samples were also examined from four gladiolus fields on sandy soils with high percentages of plants with notched leaf scattered throughout the stock. In none of these *Trichodorus* spp. were found. From the roots of 'White Burley' tobacco and spinach planted in these soils no TRV could be isolated.

In one of the fields just mentioned 70–80 per cent of the plants were affected, whereas another stock of the same cultivar from the same grower did not show any symptoms. It appeared that in the year before the grower had split a stock of cormels of this cultivar into two parts, which were grown on different fields. In one of these fields both *Trichodorus pachydermus* and TRV were shown to be

TABLE 2. Correlation between the occurrence of *Trichodorus* spp. in different soils and the infection of roots of three plant species by TRV.

*Verband tussen het voorkomen van* Trichodorus *spp. in verschillende grond en de infectie van de wortels van drie plantesoorten door TRV.*

| Location | Mean number of nematodes per 500 g soil (total number of samples) | Number of plants with TRV shown to be present in the roots (total number of plants) | | |
|---|---|---|---|---|
| | | Tobacco | Spinach | Gladiolus |
| Lisse A (1961) | 219 (20)[3] | 6 (6) | 5 (10) | 6 (10) |
| Lisse A (1962) | 221 (26)[3] | 11 (34) | 7 (11) | 3 (6) |
| Lisse B (1961) | 279 (7)[3] | 5 (5) | | 5 (5) |
| Lisse B (1962) | 149 (52) | 15 (16) | 2 (11) | 31 (84) |
| Hillegom (1961) | 58 (12) | | | 7 (12) |
| Koegras[1] (1962) | 103 (28)[4] | 20 (21) | 1 (6) | 6 (21) |
| Voorhout (1962) | 108 (28)[2] | 12 (20) | 2 (5) | |
| Stavenisse[1] (1964) | 21 (2)[5] | 4 (4) | | |
| Total | | 73 (106) 69% | 17 (43) 40% | 58 (138) 42% |
| *Plaats* | *Gemiddeld aantal aaltjes per 500 g grond (totaal aantal monsters)* | *Tabak* | *Spinazie* | *Gladiool* |
| | | *TRV aangetoond in de wortels (totaal aantal planten)* | | |

[1] Sandy loam soil, the other locations sandy soil / *Zavelgrond, de overige percelen zandgrond*
[2] Mainly *T. pachydermus* / *In hoofdzaak* T. pachydermus
[3] Both *T. pachydermus* and *T. nanus* / *Zowel* T. pachydermus *als* T. nanus
[4] *T. similis* only / *Alleen* T. similis
[5] *T. teres* only / *Alleen* T. teres

present, whereas both were absent from the other field. Assuming that TRV is the cause of notched leaf, these observations would indicate that in cases where there is a scattered occurrence of diseased plants, infection of the corms has taken place in the foregoing season.

*Field experiments on the progress of notched leaf in successive growing seasons*

In 1962 healthy corms of cultivar 'Picardy' (4–6 cm circumference) were planted on soils known to be infested with *Trichodorus* spp. and TRV (see Table 2: Lisse A, Lisse B and Koegras). Corms of the same stock were planted on steam-sterilized sandy soil as a check. In 1963 and 1964 the offspring from the infested fields was planted on steam-sterilized sandy soil to be sure that appearance of the disease in these seasons could be attributed only to infection in 1962. In 1963 corms and cormels harvested from plants without symptoms in 1962 were planted separately. In 1964 only corms were planted, both from plants with and without notched leaf symptoms in 1963. The observations made in three successive years are summarized in Table 3.

In 1962 in two fields (Lisse A and Lisse B) 7% and 2% of the plants showed specific symtoms of notched leaf. The growth of most of the other plants, however, was strongly retarded, as is usually found when the disease occurs in

TABLE 3. The progress of notched leaf in the progeny of gladiolus cv. 'Picardy', planted in naturally infested soil in 1962. In subsequent years the corms and cormels were planted in steam-sterilized soil.

*Het verloop van de aantasting door kartelblad in een partij gladiolen cv. 'Picardy' die in 1962 in natuurlijk besmette grond werd geplant. In de daaropvolgende jaren werden de knollen en kralen in gestoomde grond geplant.*

| | 1962 | 1963 | 1964 |
|---|---|---|---|
| Soil type<br>*Trichodorus* | Percentage of notched leaf (total number of plants) | Percentage of plants with notched leaf symptoms (total number of plants) from corms (= a) and from cormels (= b), harvested from plants without symptoms in 1962 | Percentage of plants with notched leaf symptoms (total numbers of plants), selected from corms of plants with (+) and without (−) symptoms of notched leaf in 1963 |
| Lisse A<br>Sandy soil/*Zand*<br>*T. pachydermus* + some *T. nanus* | 7 (1250) | (a) 35 (200)<br>(b) 0[1] | (−) 16 (126)<br>(+) 82 (51)<br>(−) 3 (157) |
| Lisse B<br>Sandy soil/*Zand*<br>*T. pachydermus* + some *T. nanus* | 2 (350) | (a) 70 (150)<br>(b) 0[1] | (−) 19 (43)<br>(+) 22 (77)[3]<br>(−) 2 (131) |
| Koegras<br>Sandy loam soil/*Zavel*<br>*T. similis* | 0 (300) | (a) 30 (175)<br>(b) 0[1] | (−) 4 (137)<br>(+) 25 (44)[3]<br>(−) 2 (130) |
| Check<br>Sandy soil/*Zand*<br>Steam-sterilized/*gesteriliseerd door stoom* | 0 (750) | (a) 3[2] (200)<br>(b) 0[1] | (−) 0 (100)<br>(−) 0 (100) |
| *Grondsoort*<br>Trichodorus | *percentage kartelblad (totaal aantal planten)* | *percentage planten met kartelblad (totaal aantal planten) van knollen (= a) en van kralen (= b) van planten zonder symptomen in 1962* | *percentage planten met kartelblad (totaal aantal planten) van knollen, verzameld van planten met (+) en zonder (−) kartelblad symptomen in 1963* |

[1] 75 g of cormels were planted.
*Van de kralen werd steeds 75 g geplant.*

[2] This light infection is probably due to reinfestation of the soil along the margin of the field in 1962.
*Deze lichte infectie is waarschijnlijk toe te schrijven aan herbesmetting van de grond langs de rand van het proefveld.*

[3] The growth of most of the plants without notched leaf was retarded, indicating that they were not quite healthy.
*De groei van de meeste planten was vertraagd, hetgeen erop wijst dat ze niet geheel gezond waren.*

patches in the field. In contrast, in the other infested field (Koegras), no deviations could be observed in the crop, although growth was not as good as in the steam-sterilized soil which served as a check.

In 1963 the progeny of corms from plants without conspicuous symptoms in both fields in Lisse showed a high number of notched leaf (35 and 70%), scattered throughout the stock. In the progeny from Koegras, where no devia-

tions had been observed in 1962, 30% of the plants grown from corms showed disease symptoms.

In 1964 corms harvested from plants both with and without symptoms gave varying numbers of plants with notched leaf.

The cormels formed by plants in infested soil did not show clear symptoms in 1963 and only a very low percentage of the progeny in 1964 showed notched leaf (2–3%).

From the results of these and many other analogous field experiments, it may be concluded that infection by notched leaf occurs in soils where both *Trichodorus* spp. and TRV are present. In the first year symptoms may occur in patches in the field, which can be very conspicuous in a crop grown from corms. In a crop grown from cormels, and sometimes also from corms, the absence of conspicuous symptoms makes it impossible to recognize the presence of the disease in the season of infection. The appearance of symptoms in plants from corms in the second and third year is rather unpredictable, since part of the progeny of diseased plants may be free from symptoms, and healthy looking plants may yield offspring showing notched leaf in the next season.

Plants from cormels, either harvested from plants in infested soil or from symptom-bearing plants in the year after infection, do not show conspicuous symptoms when grown in non-infested soil. The corms from such a crop, however, may or may not be seriously affected; in some experiments (not mentioned in Table 3) 30% of the plants showed notched leaf in the second year after infection.

The infection of plants seems to be highly dependent on climatic and other conditions that are as yet poorly understood. For instance in 1963 cormels were planted on many fields known to be heavily infested (e.g. Lisse A, Lisse B and Koegras), but the percentages of infection in the offspring proved to be very low (from 0 to 8%).

Viruses of the tobacco rattle type are known to cause various symptoms in potatoes e.g. stem mottle and spraing (literature reviewed by VAN HOOF, 1964a). It therefore seemed worth while to investigate whether on infested soils where gladioli were known to exhibit notched leaf symptoms (Lisse A, Lisse B and Koegras, see Table 3), potato varieties susceptible to TRV would develop typical symptoms.

The tubers of potato varieties 'Erdgold' and 'Bevelander' grown in these fields in 1962 were planted the next season on a heavy clay soil at Wageningen known to be free from TRV and *Trichodorus*. This material showed the typical symptoms described for stem mottle. In Table 4 the observations made on the two potato varieties are listed together with those made on the gladiolus plants. From these data it may be concluded that there is a striking correlation between the occurrence of stem mottle in potatoes and notched leaf in gladiolus.

Furthermore, when gladiolus corms were planted in three fields where potatoes always developed more or less marked symptoms of spraing, the progeny showed a high percentage (from 20 to 50%) of plants with notched leaf symptoms in the next year. In only one of the fields a significant number of gladiolus plants (28%) had shown distinct symptoms of notched leaf in the season of growth.

Early-browning in peas has been shown by BOS & VAN DER WANT (1962) to be caused by a soil-borne virus, pea early-browning virus (PEBV). This virus is

TABLE 4. The occurrence of notched leaf in gladiolus and of stem-mottle in potato after planting in soil infested with *Trichodorus* spp. and TRV in 1962 and in the progeny in 1963, planted in uninfested soil.

*Het optreden van kartelblad in gladiolen en van stengelbont in aardappelen op grond besmet met* Trichodorus *spp. en TRV in 1962 en in de nateelt in 1963, geplant op onbesmette grond.*

| Experiment field (see Table 3) | Gladiolus Percentage notched leaf | | Potato Percentage stem-mottle | |
|---|---|---|---|---|
| | 1962 | 1963 | 1962 | 1963 |
| Lisse A | 7 | 35 | 12 | 57 |
| Lisse B | 2 | 70 | 10 | 56 |
| Koegras | 0 | 30 | 0 | 19 |
| Check | 0 | 3 | 0 | 2 |
| | *1962* | *1963* | *1962* | *1963* |
| *Proefveld (zie tabel 3)* | *Gladiool Percentage kartelblad* | | *Aardappel Percentage stengelbont* | |

serologically distantly related to TRV (MAAT, 1963). Peas of the susceptible variety 'Rondo' were sown on four fields known to be infested by TRV and *Trichodorus* spp., where gladioli were showing notched leaf symptoms. No symptoms of early browning were observed in the pea plants. We have not yet had the opportunity to plant gladiolus corms in soil infested with the PEBV isolate. In the meantime PEBV in the Netherlands has been shown to be transmitted by *T. pachydermus* and *T. teres* (VAN HOOF, 1962) whereas a related form of this virus in England is transmitted by *T. viruliferus* Hooper (GIBBS & HARRISON, 1964).

*Infection experiments*

The observations discussed in the foregoing paragraphs provided much evidence that TRV is the cause of notched leaf in gladiolus and that *Trichodorus pachydermus*, *T. similis* and possibly other *Trichodorus* species are vectors of the virus.

Elaborate mechanical inoculation experiments with sap from diseased gladiolus plants and from 'White Burley' tobacco infected with TRV (isolated from diseased gladiolus plants) were carried out. Roots and sprouts of two susceptible gladiolus cultivars, either previously dusted with carborundum or punctured with a needle, were inoculated in different stages of development. No symptoms of notched leaf were observed in any of the plants, either in the first or in the second year, and no TRV could be shown to be present in the roots of these plants by inoculation of sap on 'White Burley' tobacco.

In preliminary experiments the role of *T. pachydermus* and *T. similis* in transmission of TRV was investigated in the following way. Eighty selected individuals of these species from infested soil (Lisse A and Koegras) were added to the root system of each of 10 'White Burley' tobacco plants growing in soil free from nematodes. In 8 and 4 plants respectively, TRV was shown to be present after six to eight weeks. Plants growing in the same soil, to which no nematodes had been added, remained healthy.

Next it was investigated whether *T. pachydermus* is able to transmit TRV to the roots of gladiolus plants. Eighty nematodes collected from infested soil were added at the root base of each of 14 corms at planting time. After eight weeks, root sap from the plants was inoculated on 'White Burley' tobacco. TRV was shown to be present in the roots of 11 plants, whereas from 29 control plants, to which no eelworms had been added, no virus could be isolated. The roots of the infected gladiolus plants showed small, necrotic areas and excessive side branching. No symptoms could be observed in the leaves of these plants and growth was not retarded.

In another experiment corms were planted either in infested soil containing nearly 80 *T. pachydermus* per 500 g, or in the same soil in which all nematodes had been killed by grinding in a ball-mill, but to which 80 *T. pachydermus* had been added at the corm root base at the moment of planting. All experiments were performed in such a way that contact between growing roots of different plants was prevented (SOL, 1963). All plants were raised in a greenhouse under optimal conditions for growth. The progeny was grown the following year in steam sterilized soil. The results of this experiment are summarized in Table 5.

TABLE 5. The occurrence of typical notched leaf in gladiolus cv. 'Picardy' (circ. 4–6 cm), in 1963, after infection of the roots of the mother plants by TRV in 1962, using *T. pachydermus* from infested soil as a vector. In 1962 no symptoms of notched leaf could be observed in the plants.

*Het optreden van kartelblad in gladiolen in 1963 (cv. 'Picardy' 4–6 cm), nadat in 1962 de wortels van de moederplanten waren geïnfecteerd door TRV met* T. pachydermus, *afkomstig van besmette grond, als vector. In 1962 werden geen kartelbladsymptomen in de planten waargenomen.*

| Source of infection | Season 1962 | | Season 1963 | |
|---|---|---|---|---|
| | Total number of plants | Number of plants with TRV shown to be present in the roots, in the leaves or in both | Total number of plants | Number of plants with notched leaf symptoms |
| Naturally infested soil[1] *Natuurlijk besmette grond*[1] | 15 | 6 | 14 | 6 |
| Same soil, but eelworms killed; at planting time 80 *T. pachydermus* per corm added at the corm root base *Dezelfde grond, doch aaltjes gedood; bij het planten 80* T. pachydermus *per knol toegevoegd bij de wortelkrans* | 16 | 5 | 16 | 5 |
| Steam-sterilized soil *Gestoomde grond* | 38 | 0 | 36 | 0 |
| *Infectiebron* | *Totale aantal planten* | *Aantal planten waarin ratelvirus werd aangetoond in wortels, bladeren of beide* | *Totale aantal planten* | *Aantal planten met kartel-bladsymptomen* |

[1] This soil contained nearly 80 *T. pachydermus* and some *T. nanus* per 500 g soil.
*Deze grond bevatte ca. 80* T. pachydermus *en enkele* T. nanus *per 500 g grond.*

In the first season no symptoms of notched leaf could be observed. In 11 out of 31 plants – growing in untreated soil or in soil to which eelworms had been added – the presence of TRV could be demonstrated in the roots, in the leaves or in both by sap-inoculation on 'White Burley' tobacco. In the second season 11 out of 30 plants grown from corms showed specific symptoms of notched leaf (Fig. 4). Check plants remained healthy. Cormels both from infected and check plants were planted in the second season also. Growth of the former was far less and in some plants TRV was shown to be present in the leaves.

In the experiments just described no conspicuous symptoms could be observed in gladiolus plants in the season in which infection occurred. Apparently the sites of infection were the young developing roots. In order to explain the occurrence of the disease in patches in the field as is often observed under natural conditions (pg. 34), experiments were made in 1962 and 1963 to determine whether *T. pachydermus* and *T. similis* could transmit TRV to the young sprouts of the corms and if so, whether the symptoms would develop already in the first season.

Gladiolus corms were tightly enveloped in a tube of polyethylene sheet fixed with adhesive tape and wax, in order to prevent migration of eelworms put on top of the corm to the root base and vice versa (Fig. 5). The corms werd planted in small pots filled with steam-sterilized soil. Thirty males of *T. pachydermus* (from soil "Lisse A") or *T. similis* (from soil "Koegras") were placed in moist, steam-sterilized soil, either around the young sprout or at the root base of each corm. After four weeks at 15 °C the soil with nematodes was removed and the

TABLE 6. The influence of the site of infection by TRV on development of notched leaf symptoms in gladiolus plants.
Thirty *T. pachydermus* or *T. similis* eelworms from infested soil were brought either around the young sprouts on top of the corm or added to the soil at the base of the corm; cultivars 'Atlantic' and 'Jenny Lind'.
*De invloed van de plaats van infectie door TRV op de ontwikkeling van kartelbladsymptomen in gladioleplanten.*
*Dertig aaltjes* T. pachydermus *of* T. similis, *afkomstig van besmette grond, werden òf om de jonge knoppen op de knol gebracht òf aan de grond om de wortelkrans toegevoegd.*

| Vector | Site of infection | Number of plants | |
|---|---|---|---|
| | | total | with notched leaf |
| *T. pachydermus* | sprout/*spruit* | 18 | 10 |
| | roots/*wortels* | 18 | 0(3[1]) |
| | untreated/*onbehandeld* | 18 | 0 |
| *T. similis* | sprout/*spruit* | 34 | 19 |
| | roots/*wortels* | 34 | 0(2[1]) |
| | untreated/*onbehandeld* | 22 | 0 |
| Total | sprout/*spruit* | 52 | 29 |
| | roots/*wortels* | 52 | 0(5[1]) |
| | untreated/*onbehandeld* | 40 | 0 |

| *Vector* | *Plaats van infectie* | *Aantal planten* | |
|---|---|---|---|
| | | *totaal* | *met kartelblad* |

[1] Slight symptoms of notched leaf, only visible in the latest formed leaves after removal of the leaf-sheaths.

*Lichte symptomen van kartelblad in de jongste bladeren, alleen zichtbaar na het afpellen van de schedebladeren.*

FIG. 1. Gladiolus plants with typical notched leaf symptoms.

*Gladioleplanten met kenmerkende kartelblad-symptomen.*

FIG. 2. "Patch" infection of gladioli in a field infested with TRV and *Trichodorus* spp.

*Pleksgewijze aantasting van gladiolen in een met TRV en* Trichodorus *spp. besmet veld.*

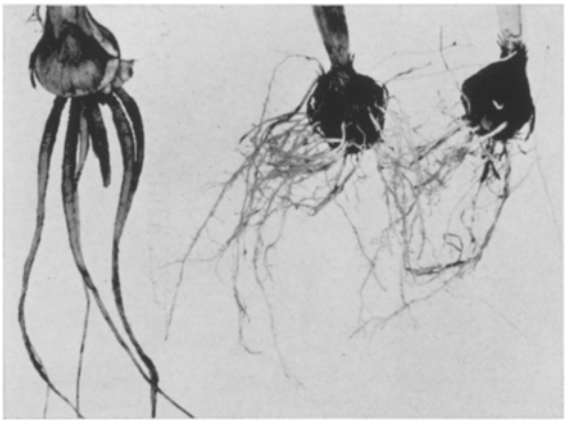

FIG. 3.
Left: Normal development of contractile roots of gladiolus plants, grown on steam-sterilized soil.
Right: Abnormal side branching of the roots from gladiolus plants, grown in soil infested with TRV and *Trichodorus* spp.

*Links: Normale ontwikkeling van de trekwortels van gladioleplanten, opgegroeid in gestoomde grond. Rechts: Abnormaal vertakte wortelgroei van gladioleplanten, gegroeid in grond geïnfecteerd met TRV en* Trichodorus *spp.*

FIG. 4. Gladiolus plants showing notched leaf symptoms after root infection in the preceding season with TRV transmitted by *T. pachydermus*.

*Gladiolen met kartelblad-symptomen na wortelinfectie in het voorgaande seizoen met TRV door* T. pachydermus.

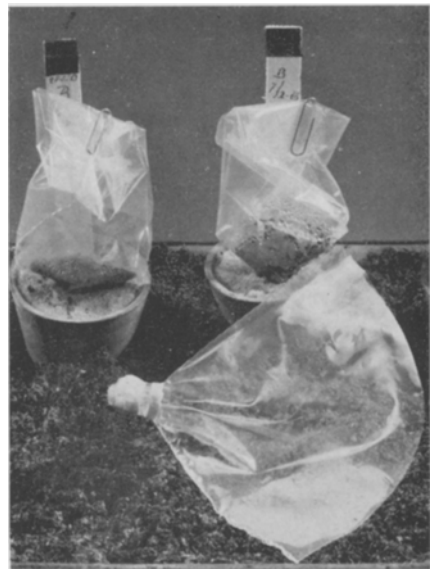

FIG. 5. Method used in the infection experiments to prevent migration of the eelworms from the sprout to the roots and vice versa.

*Methode bij de infectieproeven om de migratie van de nematoden van de spruit naar de wortels en vice versa te verhinderen.*

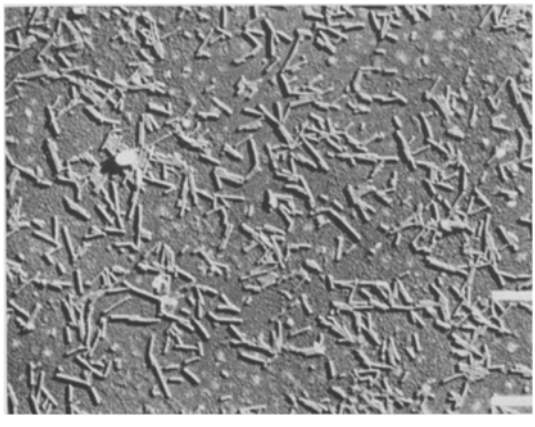

FIG. 6.
Purified TRV, isolated from a gladiolus leaf, infected in the sprout by *T. similis*; shadow cast with gold-palladium, electron microscope Philips E.M. 100. Magnification: × 20,000.

*Gezuiverd TRV, geïsoleerd uit een gladioleblad, geïnfecteerd in de spruit door* T. similis. *Geschaduwd met goud-palladium, elektronenmicroscoop Philips E. M. 100. Vergroting: 20.000 ×.*

sprouts or rootbases were thoroughly washed. The plants were then replanted in steam-sterilized soil in a greenhouse under optimal conditions for growth. During the growing period notched leaf symptoms appeared in 29 out of 52 plants which were infected via the sprouts (Table 6). In the case of infection through the roots no symptoms could be seen during the growing period. However, removal of successive leaf-sheaths at the end of the experiment revealed that in 5 plants out of 52 there were slight notched leaf symptoms in one or more of the youngest leaves. In none of 40 check plants symptoms of the disease could be observed. The experiments with *T. pachydermus* and *T. similis* gave essentially similar results.

These experiments indicate that the development of symptoms in the leaves in the year of infection must be ascribed to infection having taken place through the young sprouts. When no symptoms are visible in the year of infection, but the offspring proves to be diseased in the following year, this is due to infection having taken place through the roots.

In some fields infested with TRV *Trichodorus nanus* Allen was found (Table 2). Several attempts to transmit TRV by means of this species to tobacco and gladiolus plants were unsuccessful.

## IDENTIFICATION OF TRV ISOLATED FROM GLADIOLUS

The results of these experiments have demonstrated that notched leaf in gladiolus is caused by viruses of the tobacco rattle type being transmitted by different *Trichodorus* species. The relation between isolates of the virus from gladiolus and isolates from other host plants formed a further point of research. These investigations have not yet been concluded, but some preliminary results will be mentioned here.

### *Serological identification*

The serological relationship was investigated between TRV causing notched leaf and the TRV described by MAAT (1963), who kindly supplied us with an antiserum (A TRV-T) made against an isolate, originated from tobacco plants grown on a soil sample from Lisse. Some isolates of TRV from diseased gladiolus plants or from tobacco planted in infested soil where gladioli became infected were inoculated on 'White Burley' tobacco leaves. Preparations made from these leaves were tested against the antiserum from MAAT by the micro precipitin reaction under paraffin oil (VAN SLOGTEREN, 1955). These reactions were negative.

An antiserum (A TRV-Gl) was prepared according to the method described by MAAT (loc. cit.) against TRV isolated from a gladiolus plant with notched leaf symptoms infected in the sprout by *T. similis* (see Table 6). Twenty four isolates from gladiolus with notched leaf symptoms from eight different localities have been tested with the two antisera now available and with MAAT's antiserum against PEBV (A PEBV) (MAAT, loc. cit.). Eleven isolates showed a positive serological reaction with A TRV-Gl, six with A TRV-T and three with both A TRV-Gl and A TRV-T. Under the test conditions none of the isolates reacted with A PEBV and four isolates did not react with any of the antisera used. Apparently, different serotypes of TRV are able to cause notched leaf in gladiolus.

*Electron microscopy*

In electron-micrographs of dip-preparations made from leaves of 'White Burley' tobacco infected with the isolate also used for preparing A TRV-Gl, measurements of the virus particles were made. The gross morphology of these particles resembled those described for other isolates of TRV (Fig. 6). The diameter was ca. 20 mμ. All preparations showed the bimodal distribution, characteristic for TRV. The normal length of the short and long components, using the length of tobacco mosaic virus particles (300 mμ) as a standard, was established as 115 and 195 mμ respectively, the ratio being 1:1.7.

*Host plant reactions*

Five isolates of TRV – originating from 'White Burley' tobacco or from gladiolus grown on three different infested soils: Lisse A, Lisse B and Koegras (Table 3) – were transmitted by sap-inoculation from 'White Burley' tobacco to leaves of the following host plants: *Chenopodium amaranticolor*; *Vigna unguiculata* cv. 'Black'; *Pisum sativum* cv. 'Eroïca'; *Nicotiana tabacum* cv. 'White Burley'.

The symptoms on the plants were similar to those described by BOS & VAN DER WANT (1962) for TRV. No differences in symptom expression between the isolates could be observed. Afterwards it was shown that the isolates tested were all serologically closely related to the TRV isolate from gladiolus against which the antiserum A TRV-Gl had been prepared.

## DISCUSSION

The occurrence of notched leaf in gladiolus in the Netherlands varies greatly from year to year. This is true both in the year of infection and for the progeny of infected plants in the following year. Sometimes the crop grown in a field known to be heavily infested shows distinct symptoms, whereas in another year notched leaf seems to be almost absent from the same field. This applies also to the progenies of such crops in the following year. This would indicate that the conditions during, and possibly also before, cultivation of the gladioli may play an important role in the infection.

The experiments described have proved that the disease is caused by tobacco rattle virus and that *Trichodorus pachydermus*, *T. similis* and possibly other *Trichodorus* species act as vectors of this virus. It is thus plausible to assume that environmental conditions affecting the nematodes are partly responsible for this unpredictable occurrence of the disease. Though the ecology of these nematodes is still poorly understood, some factors that probably influence the rate of infection of gladioli by TRV may be mentioned.

The *Trichodorus* species under consideration are most often found in sandy or light sandy loam soils and infection of gladioli has been observed in these soil types only. It is known (SEINHORST, 1961) that the *Trichodorus* population is usually most numerous in a layer 20–60 cm below soil surface. This proved to be true also in our experimental field in Koegras, where the soil water table was at more than 1 m depth. In this field first-year symptoms have never been observed. In the two fields in Lisse, with a high soil water table (at about 40 cm depth), most specimens of *Trichodorus* are found in a layer from 0 to 10 cm below soil surface and here first-year symptoms, usually occurring in patches,

were often found. In the fields previously mentioned, where gladioli were grown on soil in which potatoes developed spraing symptoms (pg. 39) the soil water table was again very low. When one of these fields was planted with gladioli after deep-ploughing, during which the subsoil was brought to the top, many plants showed symptoms in the first year.

Our experience with different methods of soil sampling has shown that *Trichodorus* is easily killed by rough handling and by mixing of soil (pg. 35). This was confirmed by KUIPER (personal communication), who also found that heavy roto-tilling of the soil diminished the number of *T. teres*.

Of great importance for the rate of infection by TRV in infested soils are probably the rapid changes in the nematode population density during the course of the year and the high vertical mobility of these eelworms in the soil (KUIPER, loc. cit.). Probably these are greatly influenced by weather conditions (e.g. rainfall), the level of the soil water table and the soil structure.

Crop rotation is a further factor that may influence the rate of infection with TRV. The effect of a preceding crop on the occurrence of notched leaf in gladiolus was illustrated by the results of an experiment by KUIPER (loc. cit.), who found 1 to 8 per cent of gladiolus plants showing symptoms in the first year when grown after tulips, daffodils, irises, carrots, leek, beans and *Tagetes* sp., but 25 per cent diseased when the gladiolus crop followed cauliflower.

The results of our experiments indicate that serious symptoms of notched leaf may occur already in the first season if infection by TRV takes place directly through the young sprout soon after planting (pg. 42). Infection through the roots does not cause the typical notched-leaf symptoms, but manifests itself as a more or less serious retardation of growth. Probably the translocation of TRV in gladiolus proceeds slowly. Assuming that the specific symptoms in the leaves are formed only if TRV is present in the leaf tissue at a very early stage of development, it becomes clear why infection directly into the young sprouts on top of the mother corm should lead to the appearance of notched leaf symptoms in the first season.

After infection through the roots the virus apparently reaches the vegetative point only after the development of the leaves has passed the critical stage for symptom expression. This may explain why in some cases where infection had taken place in the roots (pg. 43), one or two small inner leaves showed slightly notched margins after removal of the outer leaves. In plants which do not form a flower stalk, formation of leaves continues throughout the growing season. Apparently only those leaves formed at the end of this period were reached by TRV while still in such an early stage of development that necroses in the tissues could lead to notched leaf. This hypothesis gains support from the fact that from plants growing on infested soil TRV could often be isolated from symptomless leaves.

It is very probable that the development of symptoms in the progeny of infected plants is also largely influenced by environmental conditions. Of three stocks of corms harvested from three different fields infested by tobacco rattle virus (Table 3, 1963), a hundred corms of each were forced at higher temperatures in a greenhouse. The percentages of plants showing notched leaf in the greenhouse were 22, 21 and 16, whereas the percentages in the open field were 35, 70 and 30 respectively. This points to a temperature influence on symptom

expression in plants grown from infected corms. However, TRV could also be isolated from leaves of plants without symptoms.

It therefore seems possible that a high temperature may stimulate leaf development to such an extent that virus translocation is insufficient to reach the young leaves in a stage suitable for symptom expression.

So far two different serotypes of TRV causing notched leaf in gladiolus have been differentiated (pg. 43). In experiments which will be discussed in a following paper both serotypes were also found among isolates of TRV from tulip and tobacco. On the other hand a few isolates tested from potato plants with stem-mottle (mentioned in Table 4) reacted only with the antiserum prepared against the TRV isolates from gladiolus (A TRV-Gl), see pg. 43.

It seems as if different hosts can be infected by two or more strains of TRV, with serologically different characteristics. Apparently the genetical structures determining biological properties, such as symptom expression in and infectivity towards certain hosts, are not necessarily antigenic and therefore need not to be correlated with the antigenic composition. Both serotypes have been found in different localities throughout the country and in several cases could even be isolated from the same infested field. So our results do not support the recent suggestions of HARRISON & WOODS (1966), that different serotypes of TRV have been developed by geographical isolation over a long period.

## SAMENVATTING

Kartelblad in gladiolen kenmerkt zich door slecht uitgegroeide, misvormde planten, waarbij langs de randen en de nerven van de bladeren necrotische strepen en karakteristieke kartel- en zaagranden optreden (fig. 1). De planten komen meestal niet tot bloei. Alle overgangen tussen normale planten en die met de beschreven symptomen komen voor.

In de knollen en kralen van de aangetaste planten zijn geen kenmerkende symptomen waar te nemen. In het gewas te velde kunnen deze aangetaste planten, in variërende percentages, verspreid tussen de gezonde planten van een partij voorkomen, terwijl aangrenzende partijen geheel gezond kunnen zijn. In deze gevallen, die op alle grondsoorten worden aangetroffen, is de partij één of meer seizoenen eerder geïnfecteerd. In andere gevallen worden planten als zojuist beschreven pleksgewijs in het gewas aangetroffen (fig. 2). Dergelijke aantastingen komen uitsluitend voor op de lichtere gronden en hierbij wordt eveneens een abnormale ontwikkeling van het wortelstelsel waargenomen (fig. 3).

In proeven kon worden aangetoond dat de ziekte veroorzaakt wordt door virussen van de ratelvirusgroep (zie tabel 1 tot en met 6). In de grond aanwezige aaltjes van het geslacht *Trichodorus* (*T. pachydermus* Seinhorst, *T. similis* Seinhorst en mogelijk nog andere soorten) brachten het virus op de waardplant over (tabellen 2, 5 en 6).

In het seizoen waarin infectie plaatsvond, ontstonden uitsluitend kenmerkende kartelblad-symptomen indien aaltjes, afkomstig van besmette grond, de mogelijkheid werd gegeven jonge, nauwelijks uitgegroeide spruiten aan te tasten (fig. 5, tabel 6). Infectie in de wortels veroorzaakt geen of slechts een geringe groeistoornis, maar in de nakomelingschap van deze planten komen in het volgende seizoen de typische symptomen van kartelblad voor (fig. 4, tabel 3, 5 en 6).

Op een aantal gronden waar aardappelen waren geïnfecteerd door stengelbont of kringerigheid, werden gladiolen door kartelblad aangetast. In proeven waar gladiolen en aardappelen door TRV op dezelfde percelen waren geïnfecteerd, bleek een opmerkelijke overeenkomst in het verloop van kartelbladaantasting bij gladiolen en stengelbontaantasting bij aardappelen te bestaan (tabel 4).

Bij het onderzoek van verschillende TRV-isolaties uit gladiolen met kartelblad-symptomen en uit andere waardplanten die afkomstig waren van met TRV en *Trichodorus* sp. besmette gronden, konden tenminste twee verschillende serotypen van het TRV worden aangetoond. Isolaties van het ene serotype bleken nauw verwant te zijn met een TRV-isolatie uit tabak 'White Burley', waartegen een antiserum is vervaardigd door MAAT (1963). Vertegenwoordigers van het andere serotype reageerden alle serologisch positief met een antiserum bereid tegen een TRV-isolatie uit gladiool. Beide serotypen bleken niet specifiek te zijn voor bepaalde plaatsen, daar beide verspreid door het land blijken voor te komen en in sommige gevallen uit eenzelfde perceel konden worden geïsoleerd. In een volgende publikatie zal hierop nader worden ingegaan.

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