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INVESTIGATIONS OF CURRANT VIRUSES IN THE NETHERLANDS

II. FURTHER OBSERVATIONS ON SPOON LEAF VIRUS, A SOIL-BORNE VIRUS TRANSMITTED BY THE NEMATODE *LONGIDORUS ELONGATUS*[1]

Onderzoekingen betreffende bessevirussen in Nederland
II. *Verdere waarnemingen betreffende lepelblad, een grondvirus, dat wordt overgebracht door de nematode* Longidorus elongatus

F. A. VAN DER MEER
Institute of Phytopathological Research (I.P.O.), Wageningen

Spoon leaf virus (SLV) of red currants spreads very slowly in the field. All common Dutch red currant varieties are susceptible as well as some raspberry varieties and a number of weed species. The virus is soil-borne and is transmitted by the nematode *Longidorus elongatus.* In cross-protection tests SLV shows a close relationship to raspberry ringspot virus but there are some phytopathological differences between the two viruses. Soil treatment with DD gives a good control of the virus. Planting material can be indexed reliably by means of sap inoculation on *Chenopodium quinoa.*

INTRODUCTION

The symptoms of spoon leaf virus (SLV) and some data on the mode of transmission were mentioned in an earlier publication (VAN DER MEER, 1960). Although we had not then succeeded in obtaining infection with the aid of infected soil, field observations had indicated the likely presence of a wingless vector. Moreover we surmised a relation between SLV and other viruses which had previously been described. In the present paper these two points are further discussed. Some data on the control of SLV are also included.

FIELD OBSERVATIONS ON THE SPREAD OF SLV

Observations and experiments on the spread of SLV were carried out in a currant garden at Blokker, a village in the old fruit-growing centre "De Bangert" near Hoorn in the Province of North-Holland. This garden was planted in 1890. Like most of these old gardens in the Bangert area there is a topcrop of various varieties of apple, pear and plum. Every year the whole plantation is checked and a number of senile currant bushes removed and replaced by two-year-old bushes usually grown from cuttings taken from bushes in the same plantation. The result is a most heterogeneous plantation with bushes of all ages and sizes. Although originally planted with another variety, like most Bangert gardens, the plantation in question now consists exclusively of plants of the variety 'Fay's Prolific', which variety was introduced into the Netherlands by Messrs. ZOGGERE at Aalsmeer (seed catalogue 1890). In the

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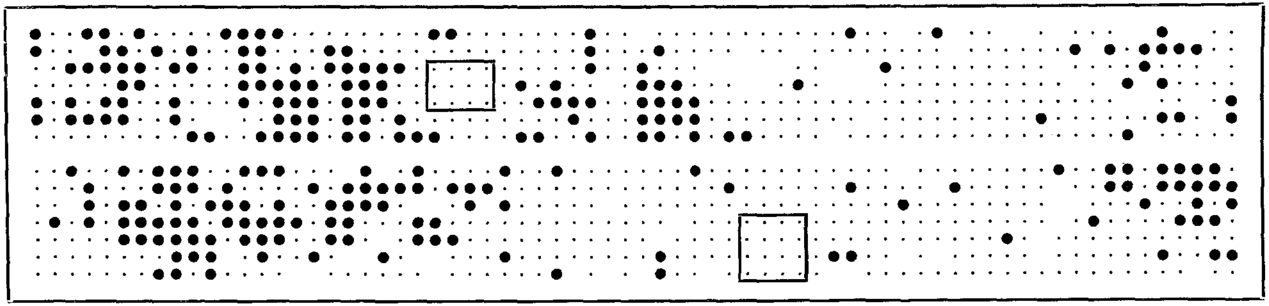
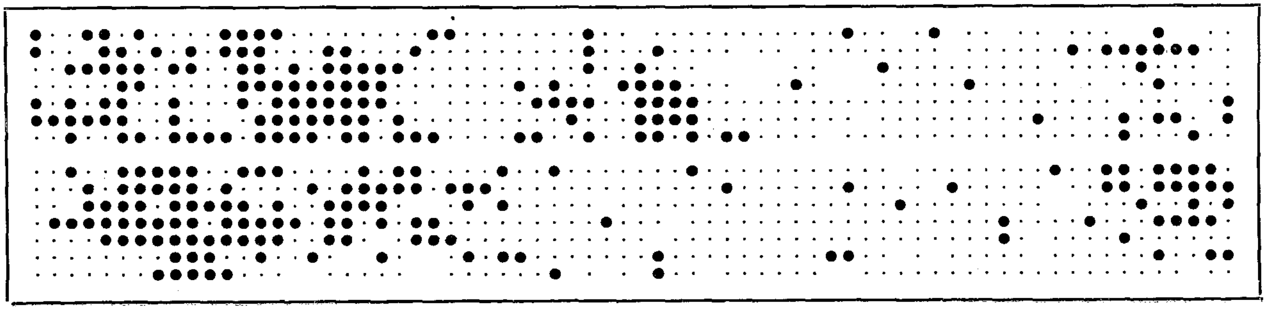


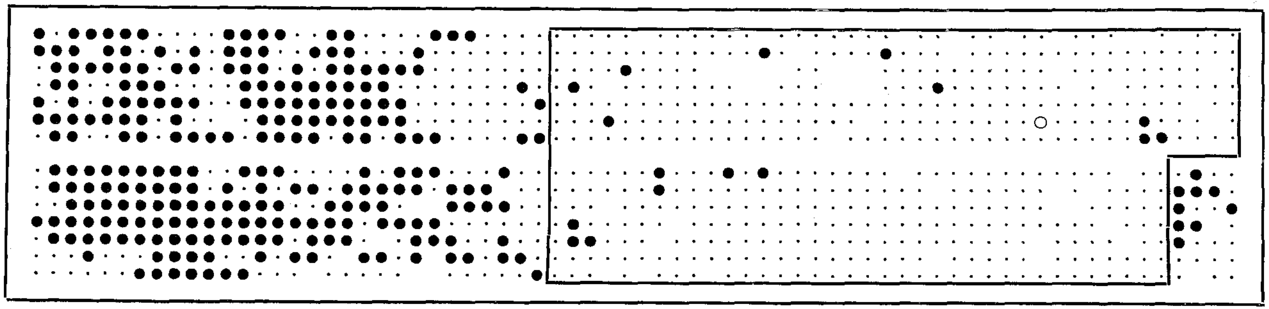
Situation in 1959. Outlined parts: kind of places considered to be not infected in weed testing experiments (Table 2).

Toestand in 1959. Omlijnde gedeelten: beschouwd als gezonde plaatsen bij het toetsen van onkruidsoorten (tabel 2).



Situation in 1961.

Toestand in 1961.



Situation in 1964.
Toestand in 1964.

FIG. 1. Part of a mapped plantation.
Gedeelte van een in kaart gebrachte aanplant.

● = Red currant with spoon leaf
Rode bes met lepelblad

· = Healthy red currant
Gezonde rode bes

○ = Newly-infected bush on DD-treated place
Herinfectie op een met DD behandelde plaats

Outlined part: Diseased plants removed in autumn 1961; places treated with DD and replanted with healthy bushes in March 1962.
Omlijnd gedeelte: Zieke planten verwijderd in najaar 1961; de open plaatsen behandeld met DD en heringeplant met gezond materiaal in maart 1962.

Right part: Diseased plants replaced as in outlined part, but not treated with DD.
Rechtse gedeelte: Zieke planten vervangen als in omlijnd gedeelte, doch zonder DD-behandeling.

garden there are 2800 red currant bushes, 42 % of which were diseased when our investigation started in 1959. From that time only bushes which were free from SLV were used for replanting. These yearly replantings were carried out by the owner of the garden, who removed the senile bushes without knowing our data, judging the bushes only by their appearance at a time when no leaves were present to show symptoms.

Fig. 1 shows a part of the plantation with a relatively high proportion of healthy bushes. The disease occurs in small patches which have increased little during a period of five years. On the other hand there are a number of solitary diseased plants in the healthy parts of the plantation. These plants, often 10–15 years old, were probably diseased at the time of planting. On account of the obscure history of the garden it is impossible to make a reliable estimate of when the outbreak first began and how it developed during the period before 1959. However, if we compare the present situation in this and in other Bangert gardens with data from people who have been concerned with the Bangert currant culture for many years (MAARSE, 1926, 1938; HOUTMAN, unpublished information) we can conclude that SLV spreads very slowly.

From the data of the mapped plantation part of which is shown in Fig. 1, we estimate a spread of eight or ten percent in five years. An exact percentage cannot be given because it is not known which bushes were newly planted in the years immediately before 1959 and whether they replaced SLV-infected bushes or SLV free ones. The state of health of the preceding bushes is of importance because we observed that newly planted bushes which replaced SLV-infected plants were far more likely to become infected than plants replacing healthy ones (Table 1). Moreover newly planted bushes do not show symptoms before

TABLE 1. Health situation of replanted currant bushes at three inspection dates. Number of bushes showing symptoms over number of bushes replanted.
Gezondheidstoestand van heringeplante struiken op drie waarnemingsdata. Aantal struiken met symptomen tegen totaal aantal heringeplante struiken.

	Experimental replants in infected area in Nov. 1959 at:		Scattered replants by the owner in Nov. 1959 at:		Scattered replants by the owner in Nov. 1960 at:	
	Diseased site[1]	Healthy site[2]	Diseased site	Healthy site	Diseased site	Healthy site
July 1960	0/73	0/19	0/64	0/47	—	—
July 1961	31/73	2/19	22/64	3/47	0/83	0/59
July 1962	70/73	15/19	36/64	8/47	22/83	1/59
	Zieke plaats[1]	*Gezonde plaats*[2]	*Zieke plaats*	*Gezonde plaats*	*Zieke plaats*	*Gezonde plaats*
	Herinplantingsproef in een infectiehaard in nov. 1959		*Verspreid geplant door de eigenaar in nov. 1959*		*Verspreid geplant door de eigenaar in nov. 1960*	

[1] A diseased site means a place where a SLV infected bush had been removed.
Met een zieke plaats wordt bedoeld een plaats waar een zieke struik gerooid is.
[2] A healthy site means a place where a SLV free bush had been removed.
Met een gezonde plaats wordt bedoeld een plaats waar een gezonde struik gerooid is.

the second year after planting, which is also evident from Table 1. Some of the new infections found in 1960 and 1961 are therefore due to reinfection at already infected sites and should not be interpreted as real spread of the disease.

Besides some data about the owner's replantings in 1959 and 1960, Table 1 shows the results of experimental replanting in a badly infected part of the garden (92 bushes) which was thoroughly rogued in September 1959. In these replantings reinfection took place very rapidly. From the data of the owner's replantings it appears that every year more SLV plants are removed than SLV free ones, although in 1959 the percentage of diseased bushes in the whole plantation was still no more than 42. This indicates that the senility of the bushes removed each year is partly due to SLV infection, and confirms our observation that diseased plants grow less well than healthy plants.

Only a proportion of the newly infected bushes showed mosaic before developing SLV (VAN DER MEER, 1960). This is in accordance with the results of the soil infection experiments (Table 2 last column). All bushes with mosaic developed SLV after some time, whereas the reverse did not occur. In some cases a bush displayed mosaic during two successive growing seasons. In such instances it was always an older bush, part of which developed mosaic in one year while the remaining part did so in the following year.

SUSCEPTIBILITY OF VARIOUS RED CURRANT VARIETIES AND OF GOOSEBERRY

In 1959 a number of currant bushes were dug up from a badly infected part of the garden, and young healthy bushes of the following varieties were planted in their places: 'Duitse Zure' ('Prince Albert'), 'Maarse's Prominent', 'Erstling aus Vierlanden', 'Jhr. van Tets', 'Rondom' and 'Fay's Prolific'. Already during the summer of 1961 symptoms of SLV were seen on 'Fay's Prolific', 'Maarse's Prominent' and 'Jhr. van Tets'. The other three varieties were still symptomless in 1962. However, sapinoculation with leaf extract on *Chenopodium quinoa* proved that most of the plants of 'Duitse Zure' and 'Erstling aus Vierlanden' were infected with SLV. 'Rondom' appeared to be free from virus at that time, but in the summer of 1963 most plants of this variety showed vague SLV symptoms and presence of the virus was confirmed by sap inoculation on *C. quinoa.* The varieties 'Duitse Zure' and 'Erstling aus Vierlanden' were again symptomless in 1963. The infected plants of the varieties 'Jhr. van Tets', 'Fay's Prolific' and 'Maarse's Prominent' showed some reduction of growth and productivity. On the other varieties SLV appeared to have little or no effect.

In a previous publication (VAN DER MEER, 1960) it was mentioned that a virus resembling SLV had been isolated from a gooseberry bush with badly ripening and irregularly shaped fruits. MAAT (1965) found this virus to be serologically closely related to SLV. However, so far we have not succeeded in proving a causal relation between the isolated virus and the symptoms just mentioned. Sap inoculation on *C. quinoa* showed that gooseberries of the variety 'Whitesmith' may be infected with SLV without being in any way distinguishable from bushes which are free from this virus.

Raspberries

In the spring of 1959 seven young plants of each of the following raspberry varieties were planted in an infected part of the garden: 'Lloyd George', 'Malling Landmark', 'Walfried', 'Radboud', 'Preussen' and 'Norfolk Giant'. In the spring of 1961 14 plants of the variety 'Malling Jewel' were added. From 1961 all plants were tested twice a year by means of inoculation with leaf extract on *C. quinoa* in which species SLV causes a local as well as a systemic necrosis. This was done in May and in August or September. SLV was not detected until May 1962, when it was found in one plant of the variety 'Norfolk Giant' and in two plants of the variety 'Walfried'. In the latest test in August 1963 only one new infection was found, again in the variety 'Walfried'. The infected 'Norfolk Giant' plant was a newly planted cane taken from one of the other plants in the garden in November 1961 to replace a plant which was killed by accident. It developed very badly, but was still alive in the autumn of 1963. The only reaction of the variety 'Walfried' to SLV was a slight reduction in growth. In June 1964 the virus was detected in four 'Malling Jewel' plants of which only two showed a faint line pattern in some leaves (Fig. 2 B). Otherwise there was no difference at all between infected and non-infected plants of this variety. At the same time one more plant of the variety 'Norfolk Giant' appeared to be infected. It showed circular yellow blotches on the leaves, as well as leaf deformation and a reduced growth of the young shoots (Fig. 2 A).

Fruit trees

In 1961 we tested 50 fruit trees from the Bangert garden, most of them older than ten years. No SLV was found in any of the 35 apples, 7 pears and 8 plums tested. However, we did find another virus, or possibly several other viruses, in apples, pears and plums. We did not go further into the identity of these viruses.

Weeds

Table 2 gives a survey of the occurrence of SLV in the roots of a number of weeds found in the Bangert garden. The presence of virus was demonstrated by means of sap inoculation on *C. quinoa*. In many plants a virus (or several viruses) was (were) demonstrated which produced only local symptoms on *C. quinoa*. Infection experiments on a number of hosts proved that the virus (or viruses) in question was (were) neither SLV nor any other known ringspot virus. The identity of this virus (or these viruses) was not further investigated. In a number of other cases we obtained systemic as well as local symptoms on *C. quinoa*. Infection experiments with these isolates on a number of hosts yielded symptoms identical with those of SLV. Serological experiments by MAAT (1965) confirmed this diagnosis. There appeared to be a definite relation between the occurrence of SLV in red currants and the occurrence of the virus in weeds. In places where the currants were healthy the virus was not present in the weeds (Table 2). Fig. 1 shows the places that were considered to be not infected in connection with this examination of weeds. In spite of the most careful tests we never found virus symptoms on the weeds listed in Table 2 in those places.

TABLE 2. The occurrence of SLV in weeds.
Het voorkomen van lepelblad in onkruiden.

Species	Near infected or healthy currant bushes	Number of plants tested	Number of plants with SLV	Only local symptoms on *C. quinoa*
Cardamine flexuosa	infected/*ziek*	46	15	20
Cardamine flexuosa	healthy/*gezond*	40	0	4
Veronica agrestis	infected/*ziek*	36	8	3
Veronica agrestis	healthy/*gezond*	16	0	0
Stellaria media	infected/*ziek*	35	18	13
Stellaria media	healthy/*gezond*	8	0	8
Polygonum persicaria	infected/*ziek*	31	10	6
Polygonum persicaria	healthy/*gezond*	12	0	0
Chenopodium spec.	infected/*ziek*	25	1	5
Chenopodium spec.	healthy/*gezond*	8	0	0
Taraxacum officinale	infected/*ziek*	45	0	5
Senecio vulgaris	healthy/*gezond*	36	0	19
Ranunculus spec.	infected/*ziek*	19	0	3
Euphorbia peplus	healthy/*gezond*	17	4	2
Potentilla anserina	infected/*ziek*	4	0	0
Sonchus spec.	healthy/*gezond*	12	0	8
Solanum nigrum	infected/*ziek*	10	0	10
Epilobium spec.	healthy/*gezond*	3	0	0
Scutellaria galericulata	infected/*ziek*	2	0	0
Poa annua	healthy/*gezond*	22	0	2
Soort	*In de omgeving van zieke of gezonde besse-struiken*	*Aantal getoetste planten*	*Aantal planten met lepel-blad*	*Alleen lo-kale vlek-ken op* C. quinoa

LABORATORY EXPERIMENTS ON THE MODE OF TRANSMISSION OF THE VIRUS

Soil transmission experiments

The field observations yielded strong indications that infection takes place via the soil, and therefore a number of soil transmission experiments was carried out in an insect-proof glasshouse. The results varied considerably. As "bait plants" we used currant seedlings as well as turnips, sugar beet, cucumber, spinach, chickweed and *C. quinoa*. Except for the currants the experimental plants were all sown in the infected soil. The currants were planted in this soil in the cotyledon stage. The soil used in the experiments had been collected in the vicinity of diseased currant bushes. At the end of the experiment the roots of the bait plants were tested on *C. quinoa*, in order to establish whether or not infection had taken place. In the roots of cucumber and turnips no virus could be demonstrated. All the other species gave positive results, but in most cases the infection percentage was very low. Of the herbaceous hosts used as bait plants *C. quinoa* gave the best average results. The currant seedlings sometimes yielded even higher infection percentages. These seedlings were not always available and therefore *C. quinoa* was used as bait plant in most experiments.

In some experiments the various layers of the soil were kept separate. In all cases infection was practically confined to the top 20 cm. Table 3 presents the results of one such experiment. As will be seen in this case there was even a marked difference between the 0–10 cm layer and the 10–20 cm layer.

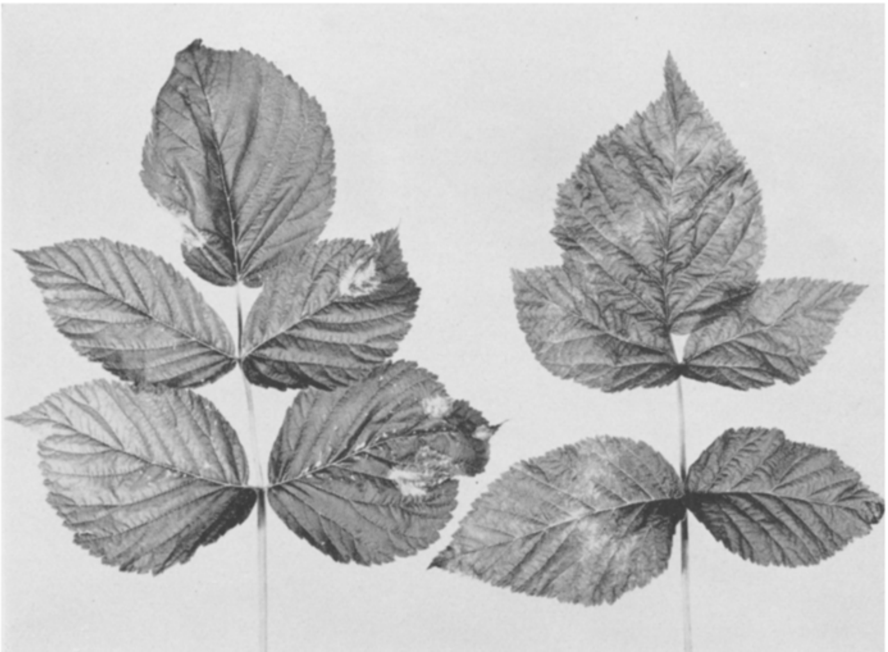
TABLE 3. The occurrence of virus in various layers of the soil as determined by its presence in bait plants; in parentheses the number of bait plants in each experiment.
Het voorkomen van het virus in de verschillende grondlagen aangetoond met behulp van vangplanten. Tussen haakjes het aantal planten van elke proef.

Layer	Spinach (roots)	Spinach (leaves)	Beet (roots)	Currant (leaves)	Number of currant plants displaying mosaic
0–10 cm	13 (80)	0 (50)	4 (150)	26 (30)	10
10–20 cm	2 (80)	0 (50)	5 (150)	17 (30)	6
20–30 cm	0 (80)	0 (50)	1 (150)	0 (30)	0
30–40 cm	0 (80)	0 (50)	2 (150)	0 (30)	0
40–50 cm	1 (80)	0 (50)	0 (150)	1 (30)	0
Steamed soil *Gestoomde grond*	0 (80)	0 (50)	0 (150)	0 (30)	0
Laag	*Spinazie (wortels)*	*Spinazie (bladeren)*	*Biet (wortels)*	*Bes (bladeren)*	*Aantal bessen met mozaïek*

During the investigation we found that the chance of infection of the bait plants is greatly increased by the presence of a "virus source". As virus source we used an older SLV-infected *C. quinoa*. A comparative experiment was carried out with 30 pots of infected soil. The soil was collected on 20 February 1963. One half of the pots was provided with a virus source, whereas the other half was not. About 25 seeds of *C. quinoa* were sown in each pot. When the roots of the young plants were tested in May it was found that infection had occurred only in the pots with a virus source. The infection percentage per pot varied from 10 to 40. In pots with sterilized soil, also provided with a virus source, all young plants remained healthy.

Transmission experiments with nematodes

With the aid of the SEINHORST (1962) sieving method we determined that the infected soil contained considerable numbers of *Longidorus elongatus* de Man. *Longidorus macrosoma* Hooper also occurred in almost all samples, but usually in smaller numbers. Infection experiments were carried out with both species, in the following way: the handpicked nematodes were added to pots filled with nematode-free soil, in which about 20 seeds of the experimental plant had been sown a few days before. At first various spinach varieties were used, but their low infection percentages, as well as the results of the soil infection experiments, led us to change over to *C. quinoa* in the later experiments. In these experiments a virus source (an older *C. quinoa*) was planted in the pots before the *C. quinoa* seeds were added. The older plant was inoculated with SLV before the seeds germinated. At first the roots were tested on *C. quinoa* 5–7 weeks after the nematodes had been added. The results were poor, and therefore the duration of the experiments was extended to 12–15 weeks. These later experiments convincingly proved *Longidorus elongatus* to be the vector of SLV. No transmission could be



A B

FIG. 2. Symptoms of SLV on raspberry. A. 'Norfolk Giant'; B. 'Malling Jewel'.
Symptomen van lepelbladvirus op framboos.

effected with *L. macrosoma*. The results of one of the later experiments are given in Table 4. Summarizing we may conclude that the infection percentage was increased by using larger numbers of nematodes, by addition of a virus source, or by prolonging the duration of the experiments. Even when all these conditions were favourable, however, *L. elongatus* proved a most inefficient vector.

TABLE 4. Tests on the transmission of SLV by *Longidorus elongatus* and other eelworms to potted plants of *C. quinoa*.
Overbrenging van lepelblad door Longidorus elongatus *op* Chenopodium quinoa *in potten.*

Species	Number of pots	Number of eelworms per pot	Number of plants infected over number of plants tested
L. elongatus (larvae/*larven*)	4	140	3/30
L. elongatus (adults/*volwassen*)	6	65	6/43
L. macrosoma	5	180	0/90
Tylenchorynchus dubius	2	60	0/9
All eelworms from 500 g of soil *Alle aaltjes uit 500 g grond*	2	not counted *niet geteld*	1/13
All eelworms from 500 g of soil except *Longidorus* spec. *Alle aaltjes uit 500 g grond uitgezonderd* Longidorus *spec.*	6	not counted *niet geteld*	0/129
Aaltjessoort	*Aantal potten*	*Aantal aaltjes per pot*	*Aantal geïnfecteerde planten tegen aantal getoetste planten*

IDENTITY OF THE VIRUS

Serology

In a previous publication (MAAT *et al.*, 1961) it was pointed out that SLV is closely related to the Scottish raspberry ringspot virus (RRV) (CADMAN, 1956). Further details on this relationship and on the serological relationship with other ringspot viruses are discussed by MAAT (1965).

Cross-protection experiments

In order to ascertain whether the various isolates from weeds, red currants, gooseberries, and from soil transmission experiments were identical, a number of cross-protection experiments were carried out on *Nicotinia rustica*. These plants were inoculated for the first time in the four leaf stage. About ten days later the first systemic symptoms appeared. These symptoms were confined to some four leaves, and after that the plants produced normal green leaves. However, these symptomless leaves did contain the virus, as could easily be proved by means of inoculation on *C. quinoa*. As soon as there were three or four symptomless leaves, these leaves were used for re-inoculation with the homologous or a heterologous isolate. For comparative purposes a number of plants of the same age were inoculated simultaneously for the first time. In these experiments all virus isolates from the Bangert, which included isolates from all weed species listed in Table 2 as well as some spinach isolates from the soil transmission experiments, showed complete mutual protection. In later experiments some isolates from the Bangert were compared with two isolates from Eckelrade-diseased cherry (PFAELTZER, 1959), one tomato black ring virus (TBRV) isolate from the strawberry variety 'Red Gauntlet' (MAAT *et al.*, 1961) and one Scottish RRV isolate, kindly supplied by Dr. C. H. CADMAN. Table 5 summarizes the results of these experiments, in which all isolates from the Bangert completely and mutually protected against the Scottish RRV isolate. The isolates from the Bangert and the Scottish RRV isolate together gave only partial protection against the two cherry isolates. This became apparent from the type of symptoms which appeared after the second inoculation, viz. light grey spots differing widely from the symptoms on comparable leaves of plants of the same age which had been inoculated for the first time. No protection occurred between the strawberry virus isolate and all the other isolates.

TABLE 5. Cross-protection experiments with some isolates of ringspot viruses. Data represent the results of the second inoculation (November 1961); first inoculation October 1961.
Premunitieproeven met enkele isolaties van kringvlekkenvirussen. De gegevens vermelden het resultaat van de tweede inoculatie in november 1961. De eerste inoculatie vondplaats in oktober 1961.

Virus isolates	From the Netherlands						Isolates from Scotland		Control plants[1]
	SLV isolates				Eckelrade isolates				
	Red currant	Goose-berry	Spinach	*Poly-gonum*	Cherry 1	Cherry 2	RRV	TBRV	
Red currant/*Rode bes*		–	–	–	+	+	–	++	++
Gooseberry/*Kruisbes*	–		–	–	+	+	–	++	++
Spinach/*Spinazie*	–	–		–	+	+	–	++	++
Polygonum persicaria	–	–	–		+	+	–	++	++
Cherry 1/*Kers 1*	+	+	+	+		–	+	++	++
Cherry 2/*Kers 2*	+	+	+	+	–		+	++	++
RRV	–	–	–	–	+	+		++	++
TBRV	++	++	++	++	++	++	++		++
Virus-isolaties	*Rode bes*	*Kruis-bes*	*Spinazie*	Poly-gonum	*Kers 1*	*Kers 2*	*RRV*	*TBRV*	*Controle-planten*[1]
	Lepelbladisolaties				*Eckelrader-isolaties*		*Isolaties uit Schotland*		
	Isolaties uit Nederland								

[1] Control plants were of the same age and inoculated only in November.
De controle-planten waren even oud en alleen in november geïnoculeerd.
++ = Symptoms normal for the strain involved.
Normale symptomen voor de betrokken isolatie.
\+ = Mild, somewhat different symptoms.
Zwakke en afwijkende symptomen.
– = No symptoms.
Geen symptomen.

Sap inoculations on red currant seedlings

In April 1961 sap inoculations were carried out on red currant seedlings with all isolates listed in Table 5, except for the Scottish RRV isolate. The seedlings were inoculated with sap from *N. rustica*. About three weeks after inoculation the first systemic symptoms appeared as a yellowish mosaic. Six weeks after inoculation all currant seedlings were tested by subinoculation to *C. quinoa.* From this it appeared that infections were obtained only with isolates connected with SLV, the percentages varying from about 30 to 60. We did not succeed in obtaining symptoms with the isolates from cherry and strawberry. All infected plants first produced about four leaves with mosaic, and then normal green ones again. Two months after inoculation all infected seedlings were planted in the open; some of them developed clear SLV symptoms in the same year. Due to circumstances beyond our control no data could be collected in 1962. In July 1963 clear SLV symptoms were found only on those plants that had been inoculated with the isolate from red currant or the isolate from gooseberry. In the groups infected with the other isolates no definite SLV symptoms appeared; the shape of the leaves sometimes hardly differed from those of healthy plants.

In May 1964 the RRV isolate was inoculated to 40 red currant seedlings. Only one of them became infected as shown by subinoculation to *C. quinoa.* At the same time nine out of sixteen red currant seedlings became infected after inoculation with SLV. Four weeks after inoculation the RRV infected plant showed mosaic and severe leaf deformation. Later it regenerated and produced normal leaves again.

Symptoms in Petunia *and tomato*

HARRISON (1961) states that in *Petunia* SLV causes systemic symptoms consisting of bright yellow rings or mosaic patterns, followed by "almost complete bleaching" of the shoots. Sap inoculations on *Petunia* with about 20 isolates from the Bangert consistently confirmed this observation. However, the term "almost complete bleaching" does not seem to be quite appropriate in our case; we would prefer "gold-coloured yellowing". The isolates from the Bangert cause a similar type of yellowing in tomato. In a previous publication (VAN DER MEER, 1960) this phenomenon was called "chlorosis". The Scottish RRV according to HARRISON (1961), as well as in our own experiments, does not cause yellowing of *Petunia* and tomato. The same was found by Miss H. J. PFAELTZER for the isolates from cherry, which was confirmed by the results of our experiments.

CONTROL

Indexing

In March 1961 110 red currant bushes from a severely infected garden were tested by means of sap inoculation on *C. quinoa.* Before being ground, the leaves were mixed with a 4% nicotine solution, in the proportion 1:10. Seventy-two bushes were found to be virus infected, in the remaining 38 cases the test plants did not react. Although field observations in June 1961 proved this test to be fully reliable, in later experiments it was found to be particularly important to perform the tests early in spring because the reliability decreases considerably when performed later than May. A reliable test method is a most important aid in selecting healthy plant material.

Treatment of the soil with a nematicide

In October 1960 a field experiment was started in a badly infected part of a red currant plantation near Hoorn. Before treatment the soil contained about

200 *L. elongatus* per 500 g soil. In this experiment nine plots were involved, three of which were treated with Shell DD at the rate of 800 l per ha. In March 1961 the field was planted with 200 red currant seedlings per plot.

In May 1962 the first SLV symptoms were observed in the untreated plots. By August 1962 the percentage of infected seedlings in these plots varied from 25 to 85%. In the nematicide-treated plots no infection had occurred at all. On account of the density of the crop, many plants had to be removed in December 1962. Of the remaining plants 104 out of 156 had become diseased in the untreated plots by June 1963, whereas all 67 plants in the treated plots had remained healthy.

In a part of the plantation mentioned earlier all diseased plants were dug out in October 1961. Before replanting with healthy bushes each place was treated with DD. In June 1964 only one out of 76 replanted bushes (planted on DD-treated sites) was found to be infected.

CONCLUSIONS

Field observations in an old Bangert red currant plantation during 1959–1960 and 1961 proved that there was natural spread of SLV. By "spread" we mean that the diseased patches in the plantation increased in size. This increase could not be measured exactly, because no data were available of the bushes replanted immediately before 1959, but the observations showed the disease to spread very slowly, only a few percent per year. The slow spread of the virus was also demonstrated by the fact that notwithstanding the age of the plantation and the use of partially diseased replanting material for many years, the percentage of diseased plants in 1959 was not higher than 42.

Although more factors may be involved the slow spread of the disease is probably connected with the inefficiency of the vector *Longidorus elongatus*, which was observed in our transmission experiments. The distribution and spread in the field much resembles that of RRV (CADMAN & HARRIS, 1952) except that the spread of RRV is much more rapid than that of SLV.

LISTER (1960), CADMAN & LISTER (1960) and CADMAN (1963) found that in various weeds tomato black ring virus and RRV are seed-borne and although the data on RRV are few this fact is used as an explanation of the general incidence and the local increase of these viruses. In our case we found a clear relation between the occurrence of the virus in weeds and its presence in red currants (Table 2) but this does not prove whether the disease spreads via the red currant or via the weeds or their seeds. We did not investigate whether SLV is seed-borne because the local spread is sufficiently explained by the use of diseased plant material and by the relative short distance between the bushes (100–120 cm), which is easily bridged by the roots of the surrounding plants. Also the general incidence of the virus in the Bangert area can be explained by the use of diseased plant material.

In serological and cross-protection experiments RRV and SLV were found to be very closely related; nevertheless there are marked phytopathological differences, viz. the difference in symptoms on *Petunia* and tomato, the poor susceptibility of raspberries to SLV as compared with their susceptibility to RRV (CADMAN & HARRIS, 1952; CADMAN, 1956) and the poor susceptibility of currant seedlings to RRV. In a previous publication (VAN DER MEER, 1960) it was mentioned that there are also differences between the various SLV isolates, viz. as regards their symptoms on currants and on a number of herbaceous hosts, and these have been confirmed by later investigations. For example some isolates cause very severe necrotic ringspot symptoms on tobacco whereas others show only a few faint chlorotic rings on the same host. In the field some infected red currant bushes show small and almost round leaves which are often curled downwards. Other infected bushes, however, show only very mild symptoms and are sometimes hardly different from healthy plants. A conclusive identification of viruses cannot be based on serological data only; phytopathological characteristics also have to be taken into consideration.

The symptoms shown by sensitive varieties such as 'Jhr. v. Tets,' 'Fay's Prolific' and 'Maarse's Prominent' may sometimes be so vague that they might be overlooked in visual selection. Therefore the indexing method described will be of practical importance for the selection of healthy plant material. In the case of varieties which generally show vague symptoms such as 'Rondom' as well as tolerant varieties such as 'Duitse Zure' and 'Erstling aus Vierlanden', the importance of an adequate indexing method is quite obvious.

Most encouraging results were obtained in controlling SLV by means of nematicides. This method of control should render it possible to reform the Bangert currant gardens. In places where there are only a few diseased plants, a treatment of single plant infected sites may be sufficient (compare Fig. 1).

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SAMENVATTING

Uit veldwaarnemingen in een oude Bangertse bessetuin te Blokker bleek dat de infectiehaarden van lepelblad zich uitbreiden. De uitbreiding verloopt langzaam en bedroeg in de genoemde tuin van 1959 tot 1964 slechts 8 tot 10 procent (fig. 1). Proeven en waarnemingen toonden aan dat herinplanting in de infectiehaarden binnen korte tijd leidt tot herinfectie (tabel 1). Alle gangbare Nederlandse besserassen zijn vatbaar voor lepelbladvirus evenals sommige frambozerassen (fig. 2) en een aantal onkruidsoorten (tabel 2). In appels, peren en pruimen kon het virus niet worden aangetoond. Uit premunitieproeven (tabel 5) bleek, dat lepelbladvirus nauwer verwant is met „raspberry ringspot"-virus (RRV) dan met de RRV-stam van Eckelraderzieke kers. Er werd geen verwantschap gevonden tussen het lepelbladvirus en „tomato black ring"-virus (TBRV). Lepelbladvirus blijft in de grond achter en blijkt voornamelijk gelocaliseerd te zijn in de bovenste grondlaag ter dikte van 20 cm (tabel 3). Het virus wordt overgebracht door *Longidorus elongatus*, een aaltje, dat in de Bangert algemeen voorkomt. Infectie met lepelbladvirus kan worden voorkomen door een grondbehandeling met DD (800 l per ha). Selectiemateriaal kan op eenvoudige en betrouwbare wijze getoetst worden door middel van sapinoculatie op *Chenopodium quinoa*.

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