Neth. J. Pl. Path. 84 (1978) 185–204

Leek yellow stripe virus and its relationships to onion yellow dwarf virus; characterization, ecology and possible control

L. BOS, N. HUIJBERTS, H. HUTTINGA and D. Z. MAAT[1]

Research Institute for Plant Protection (IPO), Wageningen

Accepted 12 May 1978

Abstract

Since 1970 yellow stripe disease of leek (*Allium porrum*) has developed epidemically in the south-eastern part of the Netherlands coincident with increasing year-around cultivation of the crop. Many autumn and winter crops now become totally infected. Apparently similar attacks, first reported in Germany in 1937, are increasingly attracting attention in various European countries.

This paper describes the leek yellow stripe virus (LYSV) as a new potyvirus related to onion yellow dwarf virus (OYDV), which was so far incompletely described. LYSV is hardly infectious to onion (*A. cepa*) and shallot (*A. ascalonicum*) and OYDV behaves similarly on leek. The leek virus further differs from OYDV in not being infectious to *A. fistulosum* and in causing distinct local lesions on *Chenopodium amaranticolor* and *C. quinoa*.

The two viruses closely resemble each other in external symptoms in their respective hosts, in persistence of infectivity in expressed sap, and in particle morphology and length (LYSV 820 nm; OYDV 833 nm). Intracytoplasmic inclusion bodies slightly differ. Further biophysical characters of the two viruses, such as sedimentation coefficient (OYDV 143 S), buoyant density in CsCl (LYSV 1.326; OYDV 1.306, or 1.258 in Cs_2SO_4), and molecular mass of coat protein subunit (LYSV 34000; OYDV 30000 dalton), are characteristic of the potyvirus group, but do not assist in judging their relationships. Serologically they are only distantly related if at all.

The leek virus is not seed-borne. It is aphid-transmitted in the non-persistent manner and its main epidemic build-up is during late summer and autumn. The sole sources of infection are nearby leek crops. Awaiting the development of resistant leek cultivars, it is advised to avoid sowing leek seed beds and planting spring crops near overwintering leek, and to remove infected plants showing up during summer.

Introduction

A yellow stripe disease of leek (*Allium porrum*) has developed epidemically in the south-eastern parts of the Netherlands since ca. 1970. Each year disease incidence rapidly increases during autumn. Autumn and especially overwintering crops may be entirely infected, and infection may lead to complete failure of winter crops. Symptoms in leek, sap transmissibility to leek, light and electron microscopy, as well as data from the literature tentatively suggested that the disease be caused by onion yellow dwarf virus (OYDV) (Bos, 1972). Apparently, the disease rapidly spread simultaneously in Belgium (Verhoyen, 1973).

Infection of leek by OYDV had earlier been reported by Bremer (1937), Heinze (1952), Kupke (sometimes fields 100% infected, 1957) and Härdtl (1965) in Germany, by Grancini (1951) in Italy, by Cornuet (1959) in most vegetable growing areas in France, and by Novák (1959) in Czechoslovakia, but these reports were based mainly or exclusively on visual observation. More recently, Paludan (1977) reported a high

[1] The last two authors are responsible for biophysical virus characterization and serology, respectively.

percentage of infection of leek in Denmark, which he also ascribed to OYDV, and Štefanac (personal communication, 1977) found leek around Zagreb, Yugoslavia, almost completely affected by yellow stripe. In recent years the leek disease has also increased in East Germany (DDR) (Graichen, 1976).

However, soon after its discovery in the Netherlands, we found that the leek virus was not pathogenic to onion *(Allium cepa)* and that typical OYDV isolates did not cause symptoms in leek. We also became aware of the lack of information on the exact identity of OYDV, which so far had resisted purification (Spánik et al., 1961; Jermoljev et al., 1962; Głowinskowska, 1973). This prompted us to carefully study the leek virus and to further characterize OYDV. Detailed results are now reported. A few tentative results on the leek virus have been mentioned already by Bos (1976) and on its purification by Huttinga (1975). Some comparable results on the yellow stripe disease of leek in Belgium, also tentatively ascribed to OYDV, have meanwhile been published by Verhoyen (1973), Verhoyen and Horvat (1973) and Horvat and Verhoyen (1975a, b).

Another virus reported from naturally infected leek is the soil-borne tomato black ring virus (Calvert and Harrison, 1963; Graichen, 1975). For notes on additional viruses reported from other *Allium* species, see Havránek (1973), Graichen (1975) and Bos (1976).

Fig. 1. Yellow stripe symptoms in naturally infected leek plant.

Fig. 1. Geelstreepsymptomen in natuurlijk geïnfecteerde preiplant.

Fig. 2. Irregular yellow striping in leaf of naturally infected leek plant.

Fig. 2. Onregelmatige geelstreping in blad van natuurlijk geïnfecteerde preiplant.

Symptoms and incidence of the disease

The leek disease is characterized by yellow striping of the entire lamina but especially at its base (Fig. 1). The stripes are irregular and interrupted (Fig. 2). Entire leaves may also be yellow. Usually, affected plants are dryer and lower in weight than normal. Leaves are then slightly flaccid, the white stems are lustreless, and keeping quality of the harvested product is impaired. During summer, affected plants occur haphazardly and with low incidence. The disorder usually starts to attract attention during September. Autumn and winter crops may be 100% infected. Such crops especially suffer from early frosts and may soon thereafter completely succumb.

The disease especially occurs in centres of vegetable growing, where leek is intensively grown on small holdings and on small fields. It has more or less explosively developed with the introduction of the year-around cultivation of the crop with overwintering leek being harvested up to the middle of May. All cultivars of leek have been found affected in nature. In the Netherlands the disease is now considered one of the major problems in leek growing.

Materials and methods

Virus isolates. The leek virus was isolated several times. Since no appreciable differences were detected, most research was on the isolates Ap10, Ap22 and Ap23 all from cultivated plants from different parts of the country. Ap23 was from a nearly completely yellow plant. For comparison one virus isolate used was from shallot *(A. ascalonicum)* with yellow stripe and leaf curling (Aa21), four isolates were from onion with yellow dwarf symptoms (Ac19, Ac24, Ac28, and Ac41) and one from *A. scorodoprasum* (As26) of the Wageningen Botanic Garden. The onion isolates Ac24 and Ac28 were provided by Drs N. Paludan, Lyngby, Denmark, and M. Verhoyen, Louvain-la-Neuve, Belgium, respectively. The isolates were maintained in

plants in the glasshouse and in leaf material dried and stored over $CaCl_2$ at 4 °C. Propagation was in leek and onion, respectively.

Virus transmission in sap, host range tests and determination of persistence of infectivity in expressed sap were in the conventional ways, mostly using water as a diluent and carborundum (500 mesh) as an abrasive. Plants were grown and kept in an insectproof glasshouse at 18–22 °C. Assay and indicator hosts were leek plants, mostly cv. Goliath and Winterreuzen for the leek virus and onion plants cv. Rijnsburger and Noordhollandse Strogele for OYDV. Later *Chenopodium quinoa* was also used as a local-lesion host for the leek virus.

For *insect transmission* virus-free aphids were provided by the Entomology Department (IPO). They were first starved for 2 h and then given a 5-min virus acquisition period on virus-containing leaves in a petri-dish. This was followed by an inoculation access period on healthy test plants (4 aphids per plant), whereafter the aphids were transferred to another series of plants for 24 h and then killed with an aphicide.

Virus purification of the leek virus was as described earlier by Huttinga (1975). OYDV (Ac41) was purified likewise by molecular sieving on Sephadex G-200. However, instead of filtering on paper in a Büchner funnel before loading it onto the column, the OYDV suspension was centrifuged for 10 min at 8000 *g*. For antiserum production an additional purification was by equilibrium density-gradient centrifuging in CsCl. For this purpose 0.95 ml of virus suspension was mixed with 2 ml of CsCl solution (0.623 g/ml), and overlaid with paraffin oil. The CsCl gradients were centrifuged for 19 h at 5 °C at 30000 rpm in a Beckman SW41 rotor. The virus was recovered by puncturing the bottom of the tubes and collecting appropriate fractions. The CsCl was removed by dialyzing against buffer.

The sedimentation coefficient at infinite dilution was determined by the graphical method of Markham (1960) using a Spinco Model E ultracentrifuge with schlieren optics. The buoyant density in CsCl was determined using the method described by Maat et al. (1978). For the buoyant density in Cs_2SO_4 we used the same method, centrifuging mixtures of 1.9 ml of Cs_2SO_4 solution (0.535 g/ml) and 1.10 ml of virus suspension, and using the relation $\rho^{25} = 12.1200 n_D^{25} - 15.1662$ (Vinograd and Hearst, 1962). The molecular mass of the coat-protein subunit was determined as described before (Huttinga and Mosch, 1974).

Serology. For antiserum production rabbits were injected intravenously twice, with a one-week interval. Three or four weeks later they were injected with an emulsion of equal volumes of virus and Freund's incomplete adjuvant. Per injection the virus obtained from 250–500 g of plant material was administered.

The serological test method applied was the micro-precipitin test. To determine relationships purified virus preparations or plant extracts clarified by low-speed centrifuging and diluted with 0.5 M tris-citric acid buffer, pH 8, were used. Dilutions of antisera and purified virus preparations were made with 0.1 M tris buffer, pH 8.

Light microscopy was in epidermal leaf strips after staining with 1% phloxine and 1% methylene blue in Christie's solution and viewing in water.

Electron microscopy in crude sap and purified suspensions in water was after negative staining with 1 or 2% PTA, pH 6 to 6.5. For length measurements tobacco mosaic virus (TMV) was added as an internal standard by simultaneous chopping of plant material with the virus under study and a small piece of TMV-containing

'White Burley' tobacco leaf (Bos, 1975). Measurements were made from negatives with a binocular microscope at × 12.5 using a micrometer eyepiece.

Further details on special techniques will be given under Results.

Results

Host range and symptoms

Host range tests were with three to ten plants and important hosts were tested twice or more often. Back inoculations from inoculated and non-inoculated leaves were done with average samples at least two and three weeks after inoculation, respectively.

Results with two leek isolates, one shallot isolate and three onion isolates are

Table 1. Summary of host range tests.

Test species	Virus isolates					
	leek isolates		shallot isolate	onion isolates		
	Ap10	Ap22	Aa21	Ac19	Ac28	Ac41
Allium ascalonicum 'Noordhollandse Gele'	–*s[1]		S	S	S	S
Allium cepa 'Noordhollandse Bloedrode'	–*s	–*s	S	S	S	S
'Noordhollandse Strogele'	–*s	–*s	S	S	S	S
'Rijnsburger'	–*s	–*s	S	S	S	S
'Witte Lissabon'	–*s	–*s	S	S	S	S
Allium fistulosum	–*–	–*–	–*s	–*s	–*s	–*s
Allium porrum 'Brabantse Winter'	S	S	–*s	–*s	–*s	–*s
'Goliath'	S	S	–*s	–*s	–*s	–*s
'Luikse Winter'	S	S	–*s	–*s	–*s	–*s
'Siegfried'	S	S	–*s	–*s	–*s	–*s
'Winterreuzen'	S	S	–*s	–*s	–*s	–*s
Allium ramosum	–*–	–*–	–*–	–*–		–*–
Allium senescens	–*–	–*–	–*–	–*–		–*–
Allium tuberosum	–*–	–*–	–*–	–*–		–*–
Allium tuberosum × *ramosum*	–*–	–*–	–*–	–*–		
Chenopodium album	L–*					
Chenopodium amaranticolor	L–*	L–*	L–*	L–*	–*–*	–*–*
Chenopodium quinoa	L–*	L–*	L–*	L–*	–*–*	–*–*
Gomphrena globosa	l s*					
	– –	– –*	– –*	– –*	– –*	– –*
Nicotiana clevelandii	– –*	– –*	– –*	– –*	– –*	– –*
Nicotiana megalosiphon	– –*	– –*	– –*	– –*	– –*	– –*

[1] The two symbols represent the situation in inoculated and non-inoculated leaves, respectively.

l s = latent local and systemic infection as detected by back inoculation.

L S = local and systemic symptoms; in case of symptoms usually no back inoculation was made to prove presence of virus.

S = systemic symptoms, no special observations made on inoculated leaves.

– –* = no symptoms; systemic infection not tested by back inoculation.

–*– = no symptoms; local infection not tested by back inoculation.

Tabel 1. Samenvatting van de waardreeksproeven.

Fig. 3. Onion 'Noordhollandse Strogele' seven weeks after sap inoculation with onion isolate Ac41; right, three affected leaf pieces. Extreme left, healthy control plant.

Fig. 3. Ui 'Noordhollandse Strogele', zeven weken na sapinoculatie met het uie-isolaat Ac41; rechts, drie aangetaste bladstukken. Geheel links, gezonde controleplant.

summarized in Table 1. With the leek isolates symptoms as observed in the field could readily be reproduced in most inoculated plants of all leek cultivars tested. They showed up ca. 14 days after inoculation. Symptoms produced by the yellow isolate Ap23 did not differ from those of the other two. No clear symptoms were produced in any of the onion cultivars inoculated and in the shallot cultivar tested. Sometimes in onion plants a vague striping was observed, but such symptoms were not convincing. Results of back inoculation onto leek always were poor, and *C. quinoa* assay plants, if reacting, produced few local lesions only. In some instances, when inoculated onion plants were tested with the electron microscope, potyvirus-like particles could be detected.

Likewise, most of the onion plants of all four cultivars tested became readily infected with all three onion isolates and the shallot isolate, and symptoms that started to appear 14 days after inoculation were typical of onion yellow dwarf with leaf

Fig. 4. Local reaction of *Chenopodium amaranticolor* 39 days after inoculation with the leek isolate Ap 10.

Fig. 4. Lokale reactie van Chenopodium amaranticolor, 39 dagen na inoculatie met het prei-isolaat Ap 10.

Fig. 5. Local reaction of *Chenopodium quinoa* 22 days after inoculation with leek isolate Ap 10.

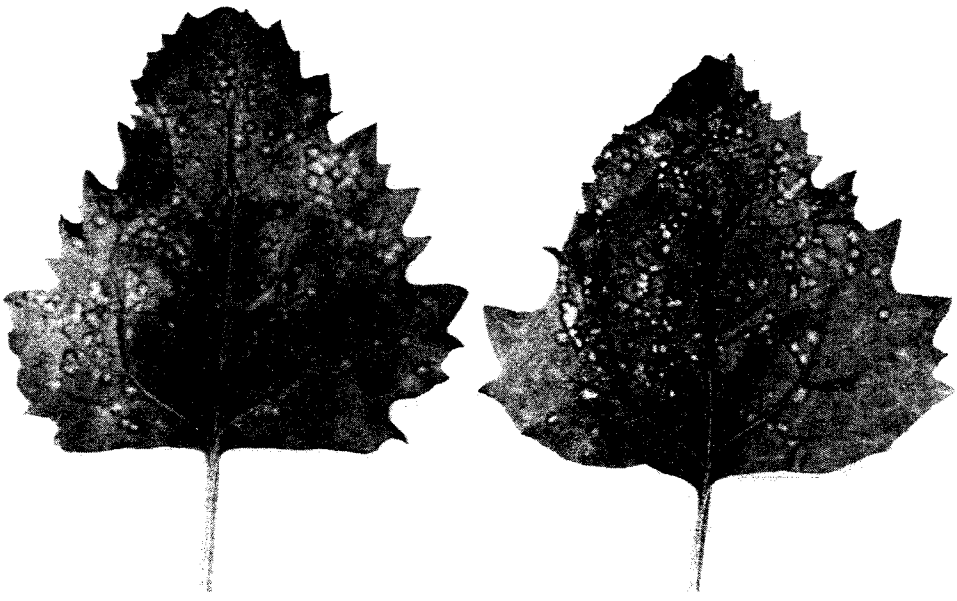

Fig. 5. Lokale reactie van Chenopodium quinoa, 22 dagen na inoculatie met het prei-isolaat Ap 10.

striping, flaccidity and growth reduction (Fig. 3). Similar symptoms were produced by the Danish isolate Ac24 and isolate As26 from *A. scorodoprasum* when tested on two of the onion cultivars. Strikingly, none of the non-leek isolates produced any symptom on any of the five leek cultivars, highly susceptible to the leek virus. When non-inoculated leek leaves were back inoculated onto onion, virus could be detected but usually reaction was late and in few plants.

A. ascalonicum could become infected with all isolates tested. Characteristic symptoms were produced with onion and shallot isolates only. Symptoms by the shallot isolates were weak, however.

In *A. fistulosum* no infection could be established with leek isolates, whereas all other isolates, including the one from *A. scorodoprasum*, did produce a symptomless systemic infection.

In both *Chenopodium* species tested the two leek isolates listed in Table 1 and Ap23 from a yellow leek plant all produced a characteristic but late local reaction. In *C. amaranticolor* ca. 3 weeks after inoculation tiny chlorotic lesions started to appear in yellowing leaves and later developed into distinct green rings (Fig. 4). Similarly, in *C. quinoa* local lesions usually appeared in about three weeks in senescent inoculated leaves as green rings (Fig. 5). Sometimes, however, they started as soon as 11 days after inoculation as small chlorotic spots. The onion isolates Ac28 and Ac41 never produced local nor systemic symptoms on both *Chenopodium* species. With Aa 21, Ac19 and Ac24 (from Denmark), however, many dry or necrotic local lesions were readily formed in some six days after inoculation. The first two isolates were later found to contain a carlavirus contaminant and Ac24 presumably did as well. The contaminant is present in all shallot plants tested so far and is now described as shallot latent virus (Bos et al., 1978).

None of the isolates could infect the two *Nicotiana* species listed. In one experiment a latent local infection was obtained with Ap10 in *N. debneyi*, and latent local as well as latent systemic infection with the same isolate in *Gomphrena globosa*, but the latter could not be confirmed in a second experiment. No infection was obtained with Ap10 in *Petunia hybrida*, Aa21 in *Phaseolus vulgaris* 'Bataaf', Ap10 in *Spinacia oleracea* and *Tetragonia expansa*, Ap22 and Ac19 in *Trifolium pratense* and *T. repens*, and Ap10 in *Zinnia elegans*.

During 1977 500 plants of highly sensitive 'Goliath' leek were planted around a shallot trial field with part of the plots severely affected by yellow dwarf. Although the OYDV rapidly spread to the free shallot plots, no symptoms were observed on any of the leek plants. Five out of six leek plants back inoculated onto *C. amaranticolor* and *C. quinoa* produced local lesions typical of the above-mentioned shallot latent virus normally absent in leek, and the sixth plant, when retested by electron microscopy, also contained particles characteristic of that virus.

In leek varietal field tests performed by the Trial Stations at Venlo and Breda (Messrs J. M. H. Derckx and C. J. Roelands, respectively) differences in disease attack were found. Dark coloured grower's selections were more resistant to the disease. Observations on farmer's fields also suggest differences in resistance. We have tested this further by inoculating twice in the glasshouse during June 1974 50 to 75 plants each of the six cultivars of apparently varying degrees of resistance and transplanting them to the open by mid October. Five of the cultivars, including a reasonably 'field resistant' one, were nearly 100% diseased by December 2. In the

sixth cultivar, a grower's selection, over 50% of the plants were still free of symptoms and most of the diseased ones showed weak symptoms only. Ten of the symptomless plants did contain the virus, however, when back tested onto *C. quinoa*, generally reacting with many local lesions.

Transmission experiments

Mechanical transmission. OYDV has been reported to be hard to transmit mechanically to onion (Louie and Lorbeer, 1946). Therefore in two experiments during 1974, using sap from infected leek (Ap10) diluted 1 : 5 (wt/vol), the leek cultivars Goliath and Winterreuzen were inoculated 1, 2 or 3 times with one- to two-day time intervals and thereafter grown at 13 to 17 °C, 10 to 22 °C or 20 to 25 °C. Most plants became readily infected after two inoculations and at the lowest temperature one inoculation was sufficient with both cultivars to obtain 100% disease. Thus, mechanical transmission in sap, obtained from diseased plants by grinding leaf tissue with tap water and using carborundum (500 mesh) as an abrasive, was easy from leek to leek.

In another experiment also performed during 1974 and at 20 to 25 °C three different grades of Carborundum and 7.5% Norit SX-1 were compared as abrasives. Inoculations were made onto 'Iglo' and 'Winterreuzen'. Results were as follows, Carborundum 500 mesh: 11/12 and 4/4 (for 'Iglo' and 'Winterreuzen', respectively); 120 mesh: 8/12 and 1/4; 80 mesh: 3/12 and 1/4; Norit SX-1: 5/12 and 0/4. Thus Carborundum 500 mesh proved superior.

Seed transmission in leek was tested by Mr J. M. M. van Bakel (IPO researcher, stationed at Research Station for Outdoor Vegetables at Alkmaar) because of its great potential ecological importance. In 1972 12 plants of 'Goliath' with striking symptoms in the field were transplanted to a glasshouse at Alkmaar. After flowering seeds were harvested and sown. Also 15 bulbils, which had developed in the inflorescences of the diseased plants, were planted in the glasshouse. No symptoms were detected in the resulting ca. 24 000 seedlings over a period of some months, nor were virus particles found with the electron microscope in three samples of slightly discolored plantlets. In contrast, 14 out of 15 plants raised from bulbils displayed clear yellow stripe symptoms.

In 1973 characteristically affected plants of 15 leek cultivars of a varietal test were transferred to the same glasshouse for seed production. From each cultivar some 1000 seedlings were later visually observed for symptoms for a period of two months and seven samples of plants with questionable chlorosis were tested on leek and *C. quinoa*. All examinations and tests were negative. The cultivars tested were: Alaska, Arctica, Van Beveren Blauw, Catalina, Legina, Libertas, Liekens I, Liekens II, Luwi, Malabar, Melwina, Siegfried, Vaes, Wila and Winterreuzen. Consequently, no indications of seed transmission in leek were obtained.

Insect transmission. With *Myzus persicae*, the only aphid species tested, transmission was readily obtained in 8 out of 24 leek plants inoculated for $^1/_2$ h immediately after acquisition with 4 aphids each. Thereafter infectivity was lost since none of the 24 plants exposed to feeding from $^1/_2$ h to 24 h after acquisition became infected.

Fig. 6. Course of attack by yellow stripe in leek field 'Herfstreus' at Groessen near Arnhem during 1975.

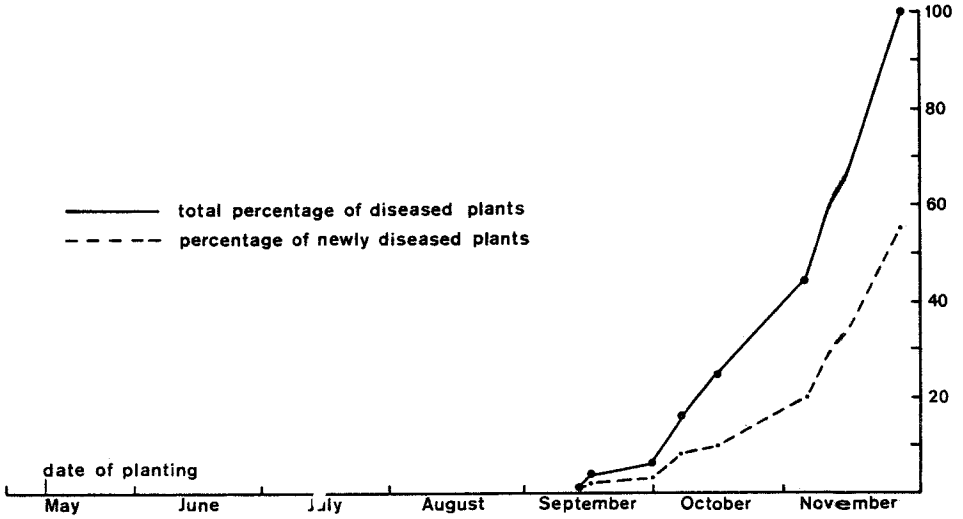

Fig. 6. Verloop van de aantasting door geelstreep in preigewas 'Herfstreus' te Groessen bij Arnhem in 1975.

To obtain more detailed information on the time of infection and the rate of natural virus spread at two locations during 1975 weekly observations were made on all 250 (first field) and 175 (second field) plants in each of two adjacent rows (500 and 350 plants in total) in two leek fields starting one week after planting. Observations were made by Mr B. J. Luimes and Mr M. de Witte of the State Agricultural Advisory Service.

The field at Groessen, east of Arnhem was in an area where much leek is grown all the year around, but it was some 500 m from the nearest leek crop. The cultivar Herfstreus was planted May 10. First symptoms were observed on September 12th and by November 25th practically all plants were affected. Increase was exponentially (Fig. 6). Distribution in the field was erratic.

The other field was of 'Goliath' and at Maarssen in a less open area with little leek. Planting had been on July 5th. The first two diseased plants were found on July 22nd and four weeks later 8 plants (2.4%) were affected. Thereafter no further spread was observed until the date of harvest (October 16th).

Rapid field spread of the virus in presence of sources of infection was also demonstrated in a field trial at Wageningen during 1975 with 20 small plots of 200 plants each of 'Goliath' and 'Winterreuzen' set up to determine yield reduction by the virus. The plants were planted July 8 on small plots, each separated by four rows of previously sown hemp *(Cannabis sativa)*, at that time ca. 2 m high. Half of the plots were planted with plants previously inoculated in the glasshouse with the leek virus (Ap10). By the end of August there was no visual difference between the plots with inoculated plants and those with non-inoculated plants. Then, one plot was completely cleared and replanted with healthy plants. Of these, in three months 48 out of 120 became diseased.

Persistence of infectivity

Sap from artificially infected leek plants (Ap10) was obtained by grinding with water 1:3 (w/vol.) and used for determining the persistence of infectivity. Infectivity was tested on whole plants of *C. amaranticolor*, two plants per treatment. At initial dilution 120 local lesions were produced. There was a rapid decline in infectivity between dilution 10 and 100, and at 1000 no infectivity was left. At heat treatment infectivity decreased between 45° and 60°C, with no infectivity left at 60°C. After one day of ageing 48 lesions were still produced but after two days only two vague local lesions resulted. In another test after three days a few local lesions were still formed on *C. quinoa* but no more after 4 days. Similarly, with the onion isolate Ac19 the ageing endpoint was between 3 and 4 days and with Ac41 between 2 and 3 days.

Purification

Purification of virus from field-grown leek with characteristic symptoms has already been described by Huttinga (1975). Buoyant density in CsCl of virus particles and molecular mass of the coat protein subunit were 1.326 g/cm^3 and 34000 dalton, respectively.

For the onion isolate Ac41 the purification method including CsCl centrifuging yielded highly purified virus preparations with particles characteristic of the poty-

Fig. 7. Leaf epidermis of leek with intracytoplasmic inclusion bodies (i) after natural infection by leek yellow stripe virus. (n = nucleus.) Bar represents 10 μm.

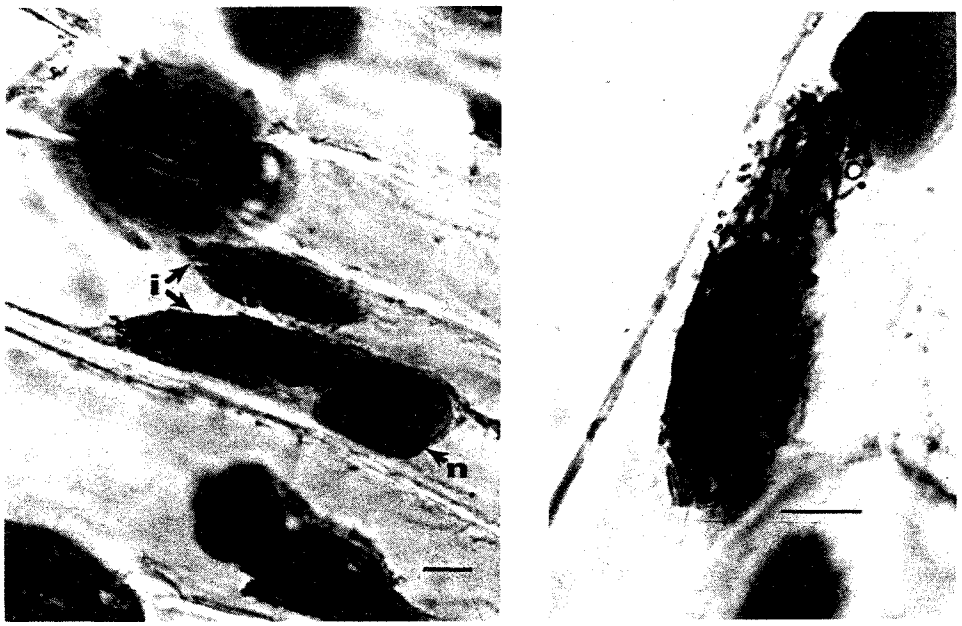

Fig. 7. Bladepidermis van prei met celinsluitsels (i) na natuurlijke infectie met preigeelstreepvirus; (n = kern.) Vergrotingsstaaf geeft 10 μm weer.

virus group. But many particles were broken and yield was low. The sedimentation coefficient at infinite dilution in 0.1 M tris-HCl pH 9 at 20 °C was 143 S. The buoyant density at 25 °C was 1.306 and 1.258 g/cm^3 in CsCl and Cs_2SO_4, respectively. The molecular mass of the coat protein subunit was 30 000 dalton.

Serology

The maximum titers for the antiserum to the leek virus purified by Huttinga (1975) were 4096 to the homologous virus and 1 to 4 to normal plant material. For the OYDV antiserum prepared to Ac41 these figures were 16 384 and 16.

Using purified virus preparations, the leek virus antiserum with a homologous titer of 4096 had a titer of only 4 to OYDV (Ac41). The OYDV antiserum then with a homologous titer of 1024 had a titer of 16 when tested with a leek virus preparation.

In tests with clarified extracts from diseased field-grown leek from the Netherlands and from Germany, the leek virus antiserum had a titer of 256 to both isolates and the OYDV antiserum had a titer of only 16 to both.

Fig. 8. Leaf epidermis strip of onion with intracytoplasmic inclusion bodies ca. 5 months after inoculation with onion yellow dwarf virus (Ac19). Bar represents 10 μm.

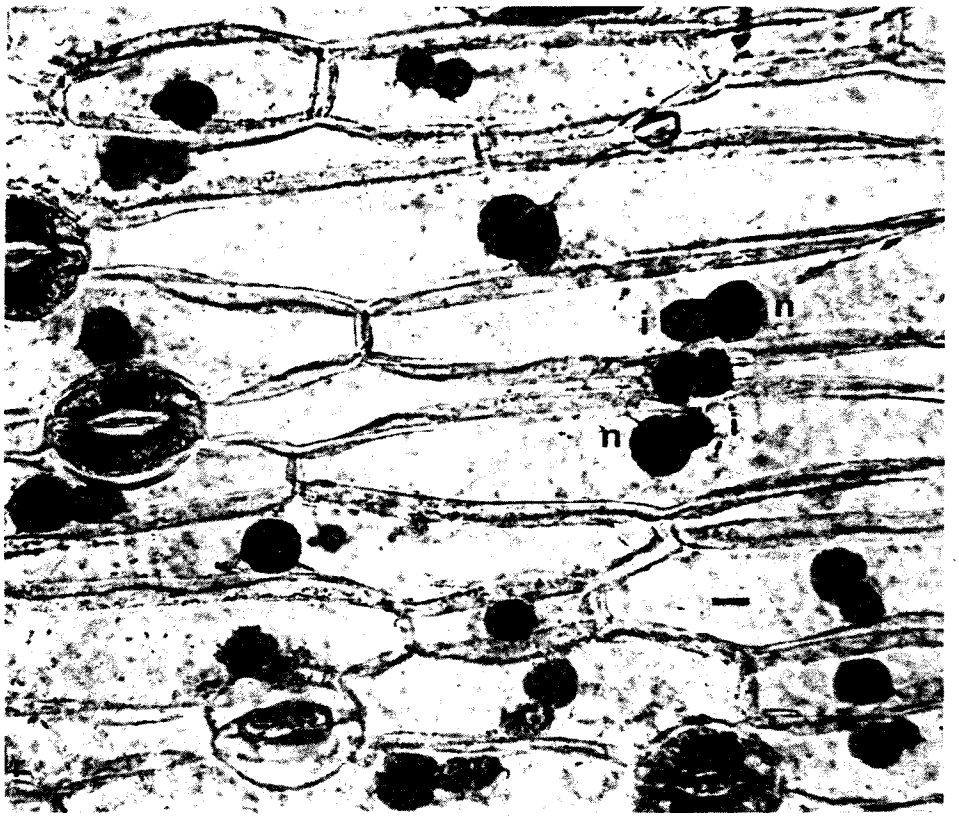

Fig. 8. Bladepidermis van ui met celinsluitsels ca. 5 maanden na inoculatie met uiegeelstreepvirus (Ac19). Vergrotingsstaaf geeft 10 μm weer.

Experiments with the two antisera and normal serum and clarified extracts from glasshouse-grown virus-free onions and onions infected with Ac19, Aa21, Ac24, As26, Ac28, and Ac41 showed strong non-specific reactions using undiluted plant extracts. With extracts diluted four times no reactions were obtained with normal serum and the leek virus antiserum, whereas the OYDV antiserum still reacted. Antiserum titers were from 256 to 4096 to the diluted extracts from infected plants and 16 to an extract from non-infected plants.

Inclusion bodies

Light microscopy of stained epidermal strips from leaves of naturally infected leek plants or from plants inoculated with isolate Ap10 readily revealed the presence of very characteristic intracytoplasmic inclusion bodies, often two per cell. They were mostly fibrous in structure (Fig. 7). The nuclei of affected cells appeared normal. Similar structures were observed with the other leek isolates studied in this respect. With the onion isolates Ac21 and Ac41 in onion intracytoplasmic inclusions were more rounded and granular or sometimes vacuolate or with globular structures (Fig. 8) but never fibrous.

Fig. 9. Electron micrograph of cluster of aggregated virus particles in crude sap of naturally infected leek. TMV particles (t) added as magnification standard. Bar represents 500 nm.

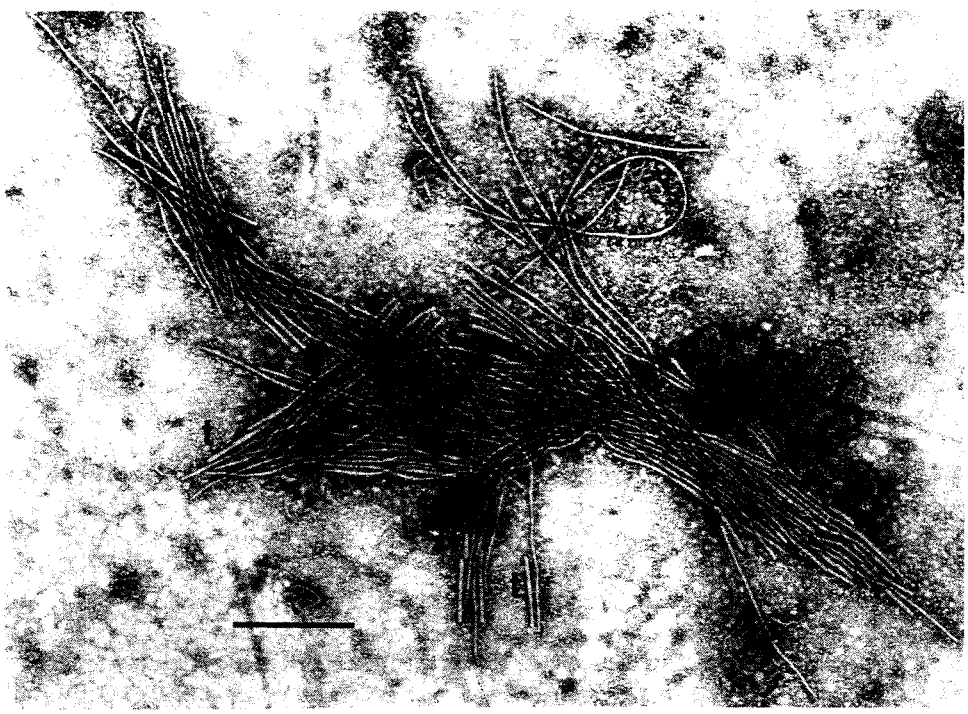

Fig. 9. Elektronenfoto van groep van samengeklonterde virusdeeltjes in ruw sap van natuurlijk geïnfecteerde prei. TMV-deeltjes (t) toegevoegd als vergrotingsstandaard. Vergrotingsstaaf geeft 500 nm weer.

Electron microscopy

In chop preparations with 1% PTA pH 7.0 flexous particles could always be readily detected with all isolates. Often extensive groups of intertwined particles and end to end aggregates occurred (Fig. 9). An electron micrograph of purified leek virus has been published by Huttinga (1975).

Measurements in crude sap preparations of 40 particles of the leek isolate Ap10 together with 31 particles of tobacco mosaic virus added as an internal standard revealed a relative particle length of 820 nm. Similar measurements of 70 particles of the onion isolate Ac41 together with 182 particles of tobacco mosaic virus yielded an average particle length of 833 nm.

Discussion

During our studies we soon detected that virus isolates from onion with characteristic yellow dwarf symptoms did not cause yellow stripe in leek, as generally assumed in the literature (see Introduction). Likewise, the leek isolates did not cause yellow dwarf disease in onion. Hence, in addition to conventional OYDV a second virus appeared to be involved, and OYDV itself required more precise characterization. The latter virus has been thought to be difficult to transmit mechanically (Louie and Lorbeer, 1966) and earlier attempts at purification (for literature, see Huttinga, 1975) were futile. This explains the lack of information on intrinsic properties of the virus.

In our experiments mechanical transmission did not pose any problem and difficulties in purification due to high contents of mucilage in *Allium* spp. was easily overcome by molecular sieving (chromatography) on Sephadex columns (Huttinga, 1975). Now purification was improved by additional equilibrium density-gradient centrifuging in CsCl, allowing the preparation of good antisera.

Host range tests (Table 1) confirmed that leek harbours an entity biologically clearly distinct from that in onion and shallot.

There were no obvious differences between the shallot isolate and the onion isolates studied although deviating shallot isolates have been reported (Brierley and Smith, 1946). The onion and shallot isolates apparently are true OYDV as biologically described in the literature (for a summary of characters, see Bos, 1976). This virus is hardly infectious to leek as also found by Havránek (1973) and Štefanac (1977).

No differences were detected among the many leek isolates tested in addition to the two of Table 1 studied in detail. Likewise a German isolate provided by Dr H. L. Weidemann, Institut für Viruskrankheiten BBA, Braunschweig, West Germany, could be distinguished from Dutch isolates neither biological nor serologically.

Although leek isolates could be introduced with difficulty into onion plants (see also Horvat and Verhoyen, 1975a), they never caused onion yellow dwarf and in onion reached low concentrations only. Apparently, onion plants are highly resistant to infection by the leek isolates and vice versa.

The leek isolates studied further differed from the OYDV isolates in not being infectious to Welsh onion *(A. fistulosum)* (which accords with Costa et al., 1971, but is in conflict with Brierley and Smith, 1946) and in causing local lesions in *C. amaranticolor* and *C. quinoa*. Likewise, Verhoyen (1973) and Verhoyen and Horvat (1973)

obtained such lesions in *C. quinoa* with a leek virus but not with a virus from onion. No lesions were produced on *C. amaranticolor* with Verhoyen and Horvat's leek virus, but susceptibility of their test plant 'selection' may have differed (cf. Van der Want et al., 1975). Verhoyen and Horvat (1973) also obtained local lesions on *C. quinoa* with viruses from shallot and garlic. We also did with the shallot isolate Aa21 and the Danish onion isolate Ac19, but their lesions appeared more rapidly and were smaller and more necrotic than those of the leek virus. They are now known to be caused by a shallot latent virus (Bos et al., 1978) omnipresent in shallots. It was also once detected in six leek plants growing near shallots at a shallot trial field.

The two groups of virus isolates distinguished by host specificity do resemble each other in nature of symptoms as well as in some other characters. They are similar in persistence of infectivity in expressed sap, and in particle shape and size. The intracytoplasmic inclusion bodies in leek (Bos, 1972; present study; Horvat and Verhoyen, 1975a and b) were striped or fibrous and elongate or spindle-shaped. In this respect they slightly differed from those of our onion isolates which were clearly rounded as earlier found for OYDV by Tate (1935) and McWhorter (1937). However, the OYDV inclusion depicted by Christie and Edwardson (1977) more resembles LYSV inclusions. There were only slight differences in biophysical properties between the two groups of isolates. These were characteristic of the potyvirus group, but do not seem reliable for classifying virus (isolates) within that group (Huttinga and Mosch, 1974). Likewise, within the group there is overlap in many other characters. The particle sizes we found for both leek and onion isolates (820 and 833 nm, respectively, with TMV as a magnification standard) agree with the length of the Belgian leek virus (815 nm with chrysanthemum virus B as a standard, Verhoyen and Horvat, 1973). They differ slightly from the normal length of OYDV (772 nm) as determined by Schmidt and Schmelzer (1964) after shadowcasting.

So far, serology of OYDV has been poor because of problems in purification. Antisera had low titres, viz. 1:8 (Jermoljev et al., 1962) and 1:32 (Van Slogteren, personal communication) and the antigens used were not further identified. With the latter antiserum a relationship between pepper veinal mottle virus and OYDV has been claimed (De Wijs, 1973).

Our antisera to virus purified from naturally infected leek with typical yellow stripe symptoms and to a typical onion isolate (Ac41) have good titers to homologous virus (maximum 4096 and 16 384, respectively) but they also react with normal plant antigens (titers 1 to 4 and 16, respectively). This means that the reactions of the OYDV antiserum with the leek virus and those of the leek virus antiserum with OYDV may have been due to reactions with normal plant antigens rather than with the heterologous virus. So the viruses, if at all, are only very distantly related serologically. Therefore, the leek isolates are now being described as a distinct leek yellow stripe virus (LYSV) related to OYDV in other than serological aspects.

Since OYDV is known to occur in *Narcissus pseudonarcissus* (Brierley and Smith, 1946) and to be able to infect *N. tazetta orientalis* and *N. odorus regulosus* (Henderson, 1953), OYDV and LYSV may be related to several other potyviruses of liliiflorous ornamentals and this should be further investigated. The differences between LYSV and OYDV, however, point to rather high host specificities. For a discussion of variation within the potyvirus group in connection with the cluster of viruses closely related to bean yellow mosaic virus, see Bos (1970) and Beczner et al. (1976).

Biological relationships to potyviruses not normally infectious to monocotyledonous plants are suggested by infectivity of LYSV to three *Chenopodium* species and possibly to *Gomphrena globosa*, as we have found, and production of local lesions in pea and common bean (perhaps *Vicia faba*) by OYDV and systemic symptoms in these and some other legumes by OYDV and a related isolate from *A. scorodoprasum* (Havránek, 1973). High host specificity of the *Allium* viruses is indicated, however, by our tests including a number of non-liliiflorae and by those of Verhoyen and Horvat (1973).

It is likely that most reports on the occurrence of yellow stripe in leek concern infections by LYSV. Consequently, the virus already may have a wide distribution. The first author has also personally observed the disease in leek in Poland in 1976, where it had not previously been reported.

Ecologically the LYSV behaves independently from OYDV. In the trial field where OYDV was rapidly and intensively spread from shallot to shallot, a highly LYSV susceptible leek cultivar remained free of yellow stripe and the OYDV could not be detected in the leek plants. Hence, under field conditions leek appears practically immune to OYDV, and the *Allium* species tested, even when infected by OYDV, do not act as sources of infection to leek. This seems to conflict with Verhoyen (1973) who often found severe attacks of leek near shallot and onion crops, but Horvat and Verhoyen (1975a) later reported that it was hard to get the virus from leek into onion. So far, no other wild or cultivated host of the LYSV has been detected other than leek itself.

Seed transmission, that plays an important role in the epidemiology of other potyviruses with limited host range (as with bean common mosaic and lettuce mosaic viruses), could not be detected for OYDV by several authors (see Bos, 1976) except by Härdtl (1962, 1972), who reported 6–29% seed transmission in onion 'Stuttgarter Riesen' on the basis of field observations. Admittedly, the virus could be detected in pollen from infected plants by Louie and Lorbeer (1965). Seed transmission of LYSV could not be detected in our studies in 15 leek cultivars when carefully examining ca. 40 000 seedlings in the glasshouse. This accords with Verhoyen (1973) who never detected infected seedlings grown from commercial seed samples and with those of Graichen (1976). Moreover, infection rates in early spring crops are nil or extremely low and the disease has never been observed in parts of the country with no previous or with little leek cultivation. It also is of low incidence in areas with scattered large-size fields. Incidence has rapidly increased with the introduction of year-around cultivation in regions where the crop is grown intensively on small vegetable holdings with small fields. In such areas, small groups of diseased overwintering plants are often found in private gardens near leek seed beds. In 1972 Verhoyen (1973) observed early infections on such seed beds. Low rates of infection in early crops apparently are due to low chances of transmission from overwintering sources because of sparse aphids in spring. Infection build-up in the field as at Groessen (Fig. 6) is typical. It suggests a very low inoculum potential early during summer. That aphid spread can be intensive early during summer with nearby sources of infection has been demonstrated by our barrier experiment at Wageningen.

Many aphid species are known to transmit OYDV in a non-persistent manner (e.g. Drake et al., 1933: over 50 species) and Verhoyen and Horvat (1973) success-

fully transmitted the leek virus with *Aphis fabae* and we did so with *Myzus persicae*. The role of aphids in spreading yellow stripe of leek is also obvious from disease spread in the field, even though usually aphids do not colonize on leek. As with OYDV, migrating probing insects may act as efficient vectors, however (see also Drake et al., 1933).

Control of aphids with insecticides is known to be of little help to reduce spread of nonpersistent viruses. Theoretically, oil spraying could reduce transmission by aphids, but costs are high, as is phytotoxicity, and mineral oils are not yet permitted on consumption crops in the Netherlands.

All leek cultivars tested so far are susceptible and sensitive to infection by LYSV, although there are differences. Research on resistance has meantime been started at the Institute for Horticultural Plant Breeding at Wageningen to support actual breeding for resistance by private breeders.

With OYDV in onion and shallot most control is by avoiding sources of infection. Since most infections are from vegetatively propagated shallots, certification of planting material of this crop is practised in many countries (e.g. Henderson, 1953). Other sources of overwintering may be volunteer plants, onion sets or onions replanted for seed production. Eradication of the disease in onion in the onion growing district near Christchurch, New Zealand, has been reported by strict prohibition of onion seed crops in the district, by destruction after harvesting of all stray, small, injured and otherwise worthless bulbs and by lifting and further prohibition of shallots (Chamberlain and Baylis, 1948).

Since overwintering leek crops are the only source of infection for LYSV, enforcement of a leek-free period would seem sufficient to completely eradicate the disease, but this may be impossible in an area with intensive leek cultivation in an extensive area on many small holdings and mixed with private gardens. Since initial spread in spring by aphids from overwintering plants is low, it is advised to avoid seed beds near overwintering crops or even a few overwintering plants in nearby private gardens at less than a few hundred meters. Plants showing infection during spring and summer should immediately be removed and destroyed.

Samenvatting

Preigeelstreepvirus en zijn verwantschap met uiegeelstreepvirus; karakterisering, ecologie en mogelijke bestrijding

Sinds ongeveer 1970 is in het Zuidoosten van Nederland in prei *(Allium porrum)*, ongeveer gelijktijdig met de opkomst van de jaarrondcultuur bij dit gewas, snel een geelstreepziekte (Fig. 1 en 2) naar voren gekomen waardoor thans vele herfst- en wintergewassen volledig worden aangetast. Overwinterende gewassen kunnen er zelfs geheel door mislukken. Gelijksoortige aantastingen, reeds in Duitsland bekend sinds 1937, trekken in toenemende mate de aandacht in talrijke landen.

In deze publikatie wordt het preigeelstreepvirus (LYSV) als een nieuw virus uit de aardappelvirus-Y-groep (potyvirusgroep) beschreven. Het is verwant aan het tot dusver onvoldoende gekarakteriseerde uiegeelstreepvirus (OYDV) (Fig. 3). De twee virussen zijn over en weer nauwelijks infectieus voor elkaars hoofdwaardplanten

(resp. prei en ui *(A. cepa)* plus sjalot *(A. ascalonicum)*). Het preivirus verschilt verder van het uievirus doordat het niet infectieus is voor grof bieslook *(A. fistulosum)* en op *Chenopodium amaranticolor* en *C. quinoa* duidelijke lokale lesies doet ontstaan (Tabel 1, Fig. 4 en 5).

De twee virussen lijken veel op elkaar in de in hun eigen waardplanten veroorzaakte uitwendige symptomen (Fig. 1, 2 en 3), in de bestendigheid van het infectievermogen in uitgeperst plantesap en in deeltjesmorfologie en -lengte (LYSV 820 nm; OYDV 833 nm). De cytoplasma-insluitsels (Fig. 7 en 8) verschillen enigszins. De overige biofysische eigenschappen, zoals de sedimentatiecoëfficiënt (OYDV 143S), zweefdichtheid in CsCl (LYSV 1,326; OYDV 1,306) en in Cs_2SO_4 (OYDV 1,258) en molecuulgewicht van de ondereenheden van de eiwitmantel (LYSV 34 000; OYDV 30 000 daltons), zijn karakteristiek voor de potyvirusgroep maar helpen niet bij de beoordeling van de verwantschap tussen de twee bestudeerde virussen. Serologisch zijn de twee virussen onderling niet of slechts ver verwant.

Het preivirus gaat niet over met zaad en de enige besmettingsbron voor nieuwe aanplanten zijn naburige oude preigewassen. De belangrijkste verspreiding door bladluizen vindt plaats gedurende nazomer en herfst (Fig. 6). In het beschikbare preirassenassortiment is geen bevredigende resistentie voorhanden. Tegengaan van verspreiding van het nonpersistente virus door bladluisbestrijding en met oliebespuitingen lijkt technisch en economisch niet effectief. Met resistentie-veredeling is een begin gemaakt. Voorlopig wordt geadviseerd om binnen een afstand van minstens 200 m tot zaaibedden en voorjaarsgewassen van prei geen overwinterende prei toe te staan en om gedurende voorjaar en zomer de aanvankelijk spaarzaam voorkomende zieke planten zo spoedig mogelijk en nauwgezet te verwijderen.

Acknowledgments

Miss M. P. Schor has ably assisted in most of the test plant work. We are greatly indebted to Mr J. M. M. van Bakel for performing the seed-transmission tests at Alkmaar and to Messrs B. J. Luimes and M. de Witte for making the field observations at Groessen and Maarsen. We thank Drs N. Paludan, Lyngby, Denmark and M. Verhoyen, Louvain-la-Neuve, Belgium, for kindly providing their isolates of OYDV, and Dr H. L. Weidemann, Braunschweig, Germany, for sending infected German leek plants.

References

Beczner, L., Maat, D. Z. & Bos, L., 1976. The relationships between pea necrosis virus and bean yellow mosaic virus. Neth. J. Pl. Path. 82: 41–50.

Bos, L., 1970. The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands, and the problem of variation within the potato virus Y group. Neth. J. Pl. Path. 76: 8–46.

Bos, L., 1972. Ernstige uitbreiding van uiegeelstreepvirus in prei. Gewasbescherming 3: 81–87.

Bos, L., 1975. The application of TMV particles as an internal magnification standard for determining particle sizes with the electron microscope. Neth. J. Pl. Path. 81: 168–175.

Bos, L., 1976. Onion yellow dwarf virus. C.M.I./A.A.B. Descr. Pl. Viruses 158: 4 pp.

Bos, L., Huttinga, H. & Maat, D. Z., 1978. Shallot latent virus, a new carlavirus. Neth. J. Pl. Path. 84: 227–237.

Bremer, H., 1937. Uber die bisher fälschlich 'Zwiebelrotz' genannte Gelbstreifigkeit an Zwiebelsamenträgern. Phytopath. Z. 10: 79–105.

Brierley, P. & Smith, F. F., 1944. The perennial tree onion a carrier of onion yellow dwarf virus. Phytopathology 34: 506–507.
Brierley, P. & Smith, F. F., 1946. Reaction of onion varieties to yellow dwarf virus and three similar viruses isolated from shallot, garlic and narcissus. Phytopathology 36: 292–296.
Calvert, E. L. & Harrison B. D., 1963. Outbreaks of tomato black ring virus in onion and leek crops in Northern Ireland. Hortic. Res. 2: 115–120.
Chamberlain, E. E. & Baylis, G. T. S., 1948. Onion yellow dwarf. Successful eradication. N.Z. Jl. Sci. Technol. A29: 300–301.
Christie, R. G. & Edwardson, J. R., 1977. Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stns. Monogr. Series 9: 150 pp.
Cornuet, P., 1959. Maladies à virus des plantes cultivées et méthodes de lutte. Inst. Natn. Rech. Agron. Paris, 440 pp.
Costa, A. S., Costa, C. L., Nagai, H. & Kitajima, E. W., 1971. Welsh onion, a source of the onion yellow dwarf virus (Port. with Engl. summ.). O Biologico 37: 157–159.
Drake, C. J., Tate, H. D. & Harris, H. M., 1933. The relationship of aphids to the transmission of yellow dwarf of onion. J. econ. Ent. 26: 841–846.
Głowinskowska, A., 1973. Purification of plant viruses by the methods of column chromatography and ultracentrifugation (Polish with Eng. summ.). Zesz. probl. Postep. Nauk roln. 142: 29–36.
Graichen, K., 1975. *Allium*-Arten als natürliche Wirte nematoden-übertragbarer Viren. Arch. Phytopath. PflSchutz Berlin 11: 399–403.
Graichen, K., 1976. Viruskrankheiten der Zwiebel und des Porrees vorbeugend bekämpfen. Gärtnerpost 6:7.
Grancini, P., 1951. Malattie da virus degli ortelggi il mosaico della cipolla. Flora 6:19 pp.
Härdtl. H., 1962. Untersuchungen über Verbreitung und Schaden der Gelbstreifigkeitsvirose bei Zwiebeln in der Pfalz. Z. PflKrankh. PflSch. 69: 587–597.
Härdtl, H., 1965. Gelbstreifigkeit bei Porree. Gesunde Pflanzen 17: 235–236.
Härdtl, H., 1972. Die Übertragung der Zwiebelgelbstreifigkeit durch den Samen. Z. PflKrankh. PflSch. 79: 694–701.
Havránek, P., 1973. Occurrence of viruses in the genus *Allium* and virus-free clones of common garlic (*Allium sativum*). Proc. 7th Conf. Czechosl. Pl. Virol., High Tatras 1971. Publ. House Slov. Acad. Sci., Bratislava: 133–136.
Heinze, K., 1952. Virusübertragungsversuche mit Blattläusen auf Dahlien, Zwiebeln, Wasserrüben und einigen anderen Pflanzen. Z. PflKrankh. PflSch. 59: 3–13.
Henderson, D. M., 1953. Virus yellows of shallots. Plant Path. 2: 130–133.
Henderson, W. J., 1935. Yellow dwarf, a virus disease of onions, and its control. Res. Bull. Iowa agric. Exp. Stn 188: 209–255.
Horvat, F. & Verhoyen, M., 1975 a. Inclusions virales dans le cytoplasme de cellules de poireaux et de *Chenopodium quinoa* infectés par le virus provoquant la 'striure du poireau'. Parasitica 31: 55–61.
Horvat, F. & Verhoyen, M., 1975 b. Inclusions in mesophyll cells induced by a virus causing chlorotic streaks on leaves of *Allium porrum* L. Phytopath. Z. 83: 328–340.
Huttinga, H., 1975. Purification by molecular sieving of a leek virus related to onion yellow dwarf virus. Neth. J. Pl. Path. 81: 81–83.
Huttinga, H. & Mosch, W. H. M., 1974. Properties of viruses of the potyvirus group. 2. Buoyant density, S value, particle morphology, and molecular weight of the coat protein subunit of bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus, and potato virus Y^N. Neth. J. Pl. Path. 80: 19–27.
Jermoljev, E., Čech, M., J. Pozděna, J. & Chod, J., 1962. The serodiagnosis of yellow dwarf (stripe) of onions and the chances of the checking of this disease. Rostl. Vyr. 8: 551–556.
Kupke, W., 1957. Die Gelbstreifigkeit, eine gefährliche Krankheit des Porrees. Rhein. Monatsschr. Gew.-, Obst- u. Gartenbau 45: 173.
Louie, R. & Lorbeer, J. W., 1965. An effective procedure for the mechanical transmission of onion yellow dwarf virus. Phytopathology 55: 129.
Louie, R. & Lorbeer, J. W., 1966. Mechanical transmission of onion yellow dwarf virus. Phytopathology 56: 1020–1023.
Maat, D. Z., Huttinga, H. & Hakkaart, F. A., 1978. Nerine latent virus: some properties and serological detectability in *Nerine bowdenii*. Neth. J. Pl. Path. 84: 47–59.
Markham. R., 1960. A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. 77: 516–519.

McWhorter, F. P., 1937. Cell inclusions in onion yellow dwarf. Phytopathology 27: 1627–1628.
Novák, J., 1959. Ein Beitrag zur Kenntnis der Viruskrankheiten der Zwiebelgemüse in der Tschechoslowakei (Czech. with German summ.). Sbornik Vysoké Školy Zemědelské v Praze. 1959; 287–309.
Paludan, N., 1977. Viroses in vegetables. Plantesygdomme Denmark 1976: 64–65.
Schmidt, H. B. & Schmelzer, K., 1964. Elektronenmikroskopische Vermessung des Zwiebelgelbstreifen-Virus. Phytopath. Z. 50: 191–195.
Špánik, V., Valenta, V. & Bystrický, V., 1961. An attempt at purification and electron microscopy of the onion yellow dwarf virus (Czech. with Engl. summ.). Biologia (Bratislava) 16: 615–618.
Štefanac, Z., 1977. Onion yellow dwarf virus in Yugoslavia (Eng. summ.). Acta Bot. Croat. 36: 39–45.
Tate, H. D., 1935. Intracellular abnormalities associated with yellow dwarf of onions. Iowa State Coll. J. Sci. 9: 677–683.
Verhoyen, M., 1973. La 'Striure chlorotique du Poireau'. II. Note concernant l'épidemiologie du virus. Parasitica 29: 35–40.
Verhoyen, M. & Horvat, F., 1973. La 'Striure chlorotique du Poireau'. I. Identification de l'agent causal. Parasitica 29: 16–28.
Vinograd, J. & Hearst, J. E., 1962. Equilibrium sedimentation of macromolecules and viruses in a density gradient. Progr. Chem. Org. Nat. Prod. 20: 372–422.
Want, J. P. H. van der, Boerjan, M. L. & Peters, D., 1975. Variability of some plant species from different origins and their suitability for virus work. Neth. J. Pl. Path. 81: 205–216.
Wijs, J. J. de, 1973. Pepper veinal mottle virus in Ivory Coast. Neth. J. Pl. Path. 79: 189–193.

Address

Instituut voor Plantenziektenkundig Onderzoek, Postbus 42, 6700 AA Wageningen, the Netherlands.