European Journal of Plant Pathology **102:** 867–873, 1996.

Lettuce ring necrosis, caused by a chytrid-borne agent distinct from lettuce big-vein 'virus'

L. Bos and N. Huijberts
DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands

Accepted 10 July 1996

Key words: Olpidium brassicae, soil transmission, tobacco stunt virus

Abstract

Ring necrosis is a serious disease of lettuce (*Lactuca sativa)* with often coalescing necrotic rings and ring-like patterns on middle leaves of plants or groups of plants in glasshouses during winter. Affected leaves may decay and plants rapidly become unmarketable. The disease was shown to be soil-borne and transmitted by the zoospores of *Olpidium brassicae*. Symptoms in lettuce do not appear before seven weeks after inoculation via the soil. Additives to the inoculum and chilling of source leaves, inoculum buffer and utensils enabled mechanical transmission of a pathogenic agent to *Chenopodium quinoa, C. amaranticolor, Nicotiana benthamiana, N. clevelandii, N. hesperis*, and *N. occidentalis* but not to lettuce. The *Chenopodium* spp. reacted with local lesions, infection was symptomless in *N. clevelandii* and mostly so in *N. benthamiana*, but *N. hesperis* and *N. occidentalis* reacted with leaf spotting and plant stunting. With zoospores of an originally pathogen-free fungus culture further cultivated on the roots of cuttings from sap-inoculated plants of *N. clevelandii* and *N. occidentalis*, the agent could be transferred back to lettuce and the symptoms of ring necrosis be reproduced. The agent biologically resembles those of lettuce big-vein (LBV) and freesia leaf necrosis and the tobacco stunt virus. In lettuce it often occurs together with LBV 'virus' but differs in longer incubation period, type of symptoms and symptom appearance only during winter. It could be separated from a mixture with LBV 'virus' by serial transfer always selecting plants without LBV symptoms. So far cultural hygiene, including soil disinfection addressing the vector, is the main means of control.

Introduction

In the Netherlands in the 1980s a new disease of glasshouse-grown lettuce (*Lactuca sativa*), characterized by necrotic rings and ring patterns in leaves, hence called lettuce ring necrosis (LRN), increasingly attracted attention (Huijberts et al., 1983a), although it may already have occurred since the early 1960s (Huijberts et al., 1983b). Distribution in crops is usually spotwise, and up to 40% of the plants of single crops have been reported to become unmarketable. Similar symptoms have been mentioned by seed merchants to occur in Belgium, France, and Great Britain, and they have been reported from Belgium (Verhoyen et al., 1985). In southern France, the disease, called 'taches orangées', is causing severe losses in lettuce under plastic every winter (H. Lot, pers. comm. 1990).

Distribution patterns of diseased plants in crops and frequent occurrence together with lettuce big-vein (LBV) suggested transmission via soil, which was soon experimentally demonstrated when lettuce seedlings were grown in sterilized soil mixed with soil and root debris from diseased field-grown lettuce plants (Huijberts et al., 1983a). Resemblance with LBV suggested the involvement of the chytrid fungus *Olpidium brassicae* as a vector. Infectivity was found to survive storage of soil in plastic bags at 18 °C for at least 11 months (Van Dorst, 1983).

Soil-transmission experiments by Verhoyen et al. (1985) pointed to a possible difference between the agents of LRN and LBV, but their attempts to mechanically transmit and purify the LRN agent failed. Parallel and further to our earlier studies (Huijberts et al., 1990; Bos and Huijberts, 1990) and those of Vetten et al.

(1987) on LBV, we also tried to identify the cause of LRN and to study its possible relationships with LBV 'virus' (LBVV), which had been found related to, but different from, tobacco stunt virus (TStV) described in Japan (Vetten et al., 1987; Huijberts et al., 1990). Although associated with virus-like particles, the very labile agents of LBV and of the possibly related freesia leaf necrosis (Bouwen, 1994) remain shrouded in mystery. This paper summarizes our results obtained in the course of years, with emphasis on the mechanical transmission of a pathogenic agent, tentatively called LRN agent, and reinoculation of lettuce with it to confirm its causal involvement in LRN.

The disease

Symptoms usually do not occur until 7–8 weeks after planting. Numerous brownish necrotic rings up to 7 mm in diameter are produced all over the leaf surface or at the leaf base or leaf tip, where they may coalesce into ring-like patterns (Figure 1). Consequently, leaf parts may die or decay. Outer leaves, as a rule, remain normal as do the heart leaves of the lettuce head. Diseased heads are unmarketable (Figure 2) even when only few leaves are affected. Plant roots appear normal, but light microscopy of their epidermal cells or root hairs usually reveals large numbers of zoosporangia and resting spores of *O. brassicae*.

Diseased plants have been found in different parts of the country. They may be erratically distributed throughout a glasshouse crop but usually occur spotwise or are concentrated in large parts of a crop. Since slight necrosis already renders affected plants unmarketable, considerable yield loss may ensue. The symptoms may occur together with those of LBV in the same plant or separately in adjacent plants. LRN is commonly observed from the beginning of November through January, but sometimes as early as October.

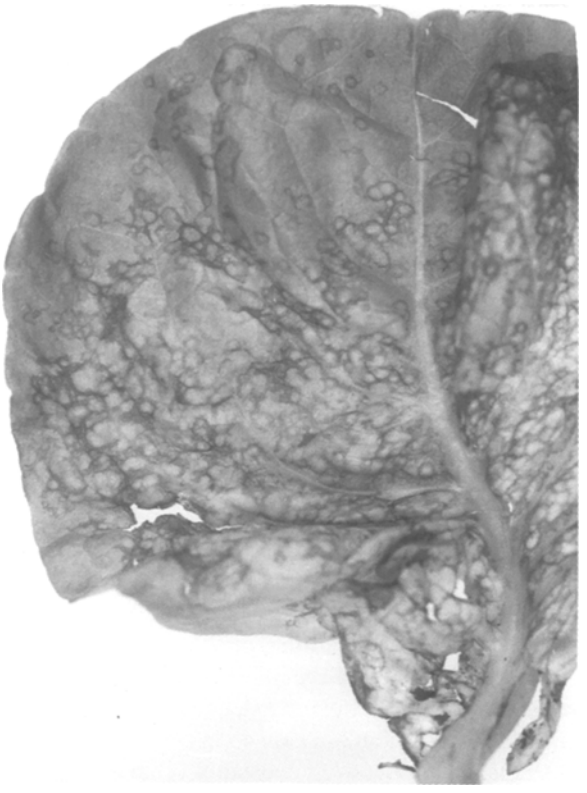

Figure 1. Necrotic ringspotting in lettuce 'Attractie' submitted to infection by planting in infested soil.

Figure 2. Severe damage by ring necrosis in lettuce 'Attractie', planted in infested soil; right, healthy control.

Materials and methods

Isolates

Since 1981, various pathogenic isolates were studied for orientation. More systematic experiments were done in 1989 and 1990 with isolate Ls247 obtained in December 1988 from a breeder's glasshouse in De Lier, Westland Glasshouse District. The isolates were propagated and maintained by planting lettuce seedlings in commercial potting soil mixed with soil and root debris from LRN-infected lettuce plants. Long-term preservation was in infested soil, air-dried and stored in plastic bags at 4 °C.

Test-plant species and accessions

Lettuce cv. Patty was used for maintenance of isolates and for indexing. The *Nicotiana occidentalis* and *N. hesperis* accessions were as used by Van Dijk et al. (1987).

Transmission

Transmission of the pathogenic agent via soil was as mentioned for routine maintenance of the agent, or by pouring zoospore suspensions from diseased plants containing $2 \times 10^5 - 10^6$ zoospores of *O. brassicae* per ml onto small peat blocks with the test seedlings. The seedlings together with the peat blocks were later transplanted into sterile soil in pots. The presence of the fungus in roots was ascertained by light microscopy of roots, mostly unstained, in water for the characteristic shape and light refraction of the resting spores.

Zoospore suspensions of the fungus were obtained by immersing roots of plants for 15 min in tap water, and zoospore concentrations were determined with a haemocytometer. A pathogen-free culture of *O. brassicae*, used earlier for studying LBV (Huijberts et al., 1990), originated from disease-free plants of *Solanum villosum* and was maintained on lettuce in a separate glasshouse compartment.

Mechanical transmission of the agent was in sap obtained by homogenizing infected leaves in 0.03 M potassium phosphate buffer, pH 7.0, containing sodium disulphite ($Na_2S_2O_5$, 2.5 g/litre), sodium diethyldithiocarbamate (DIECA, 5 g/litre), and activated charcoal (Norit, 70 g/litre) after refrigerator chilling of the source leaves, buffer solution, mortars and pestles as for LBV (Huijberts et al., 1990).

Results

Separation of LRN agent from LBVV

In the field, LRN is often associated with LBV. During serial transfers of Ls247 for a period of one year (1989) not all plants became LRN infected, and occasionally a few of the lettuce plants submitted to infection showed symptoms of LBV or LRN, either alone or in combination. Further transfer always was from plants with LRN symptoms only until LBV contamination was eliminated.

Transfer from lettuce

Regular and easy maintenance of LRN by growing healthy lettuce seedlings in infested soil readily demonstrated soil transmission of the LRN agent. The transmission in zoospore suspensions from diseased plants poured onto seedling-containing soil proved the involvement of *O. brassicae* as a vector. LRN or LBV never showed up in control plants when steam-sterilized potting soil was used.

Despite numerous attempts, mechanical transmission from lettuce to lettuce, or from any other plant species back to lettuce, was never accomplished. *Chenopodium quinoa* always reacted with local lesions after sap inoculation from diseased, but never from healthy lettuce plants. These lesions also showed up after inoculation from symptomless experimental plants hosting the agent (see below). *C. quinoa* was therefore considered the best indicator species to test for infection.

Artificial host range and symptoms

Test plant species found particularly useful for studying LBV (Huijberts et al., 1990) were tested for susceptibility and sensitivity to the LRN agent by sap and zoospore inoculation. The species and accessions used and the results from a number of tests are assembled in Table 1 (for sap and zoospore inoculations from lettuce) and Table 2 (for zoospore and soil inoculations from species other than lettuce). The results of mechanical back-inculations onto *C. quinoa* (Table 1, columns 3 and 7) suggested the species or accessions with symptoms and some of them without symptoms to be infected with the LRN agent. This assumption was further supported by the fact that transfer on 6-03-1990 of zoospores from *O. brassicae* cultures on *N. benthamiana, N. hesperis* 67A and *N. occidentalis* (Table 2) originally zoospore-inoculated on 8-02 from diseased lettuce (Table 1, col. 6), led to reproduction of symptoms in *N. occidentalis* accessions Japan and P1 (Table 2, col. 1, 3 and 7) and, more importantly, of LRN in 'Patty' lettuce (Table 2, col. 3).

For final proof of the actual causative involvement in LRN of the sap- and zoospore-transmissible agent that systemically infects *Nicotiana* spp. and causes local lesions in *C. quinoa*, aboveground cuttings were taken on 6-03-90 from the *Nicotiana* genotypes inoculated with sap one month earlier (Table 1, col. 1) and found infected thereafter (Table 1, col. 3). The infected cuttings were planted into sterile potting soil,

Table 1. Reaction of plant species and accessions to mechanical inoculation from leaves and zoospore inculation from roots of lettuce with Ls247; data assembled from different experiments in 1990

Manner of inoculation	Mechanical inoculation					Zoospore inoculation			
Plant species	8–02			7–05	31–05	8–02		7–05	31–05
and accession	Local	Syst.	B-in.[1]	Syst.	Syst.	Syst.	B-in.[1]	Syst.	Syst.
N. benthamiana	0/3[2]	0/3	++[3]	0/2	0/2	0/4	2/2	0/2	0/3
N. clevelandii	0/3	0/3	++	0/2	0/2	0/5	0/2	0/2	
N. hesperis									
67A	3/3	+	++	2/2		5/5[4]	2/2	0/2	
N. occidentalis									
Japan	3/3	2/3	++	0/2	0/2	4/5	0/2	1/2	3/3
P1	3/3	3/3	+	2/2	2/2	5/5[4]	0/2	2/2	2/2
37B	+	2/3	++	0/2		5/5	2/2	0/2	
C. quinoa	+			2/2	2/2				
L. sativa								6/10	10/14

[1] Results of back inoculation onto *Chenopodium quinoa*.
[2] Number of plants reacting with symptoms out of number of plants inoculated.
[3] Severity of rection as assessed by number of local lesions.
[4] See Figure 3.

Table 2. Reaction of plant species and accessions to zoospore and soil inoculation with Ls247 from roots of plant species other than lettuce. Figures printed in bold concern inoculations with zoospores and soil made 'viruliferous' on rooted cuttings from sap-inoculated *Nicotiana* spp. Data assembled in 1990

Inoculum sources	*N. ben.*	*N. cl.*	*N. hesp.*		*N. occ.*			*N. ben.* +
Dates of			67A		Japan		P1	*N. occ.*[4]
zoospore transfer	6-03[1]	**7-05**[2]	6-03[1]	18-06[3]	**7-05**[2]	**31-05**[2]	6-03[1]	18-06[3]
Plant species and accession								
N. benthamiana	3/3[5](?)	**0/3**	0/3 (?)	0/3	**0/3**		3/3	0/6
N. clevelandii		**0/3**		0/3	**0/3**	**0/3**		0/6
N. hesperis								
67A		**1/3**			**2/3**			
N. occidentalis								
Japan	2/3	**0/3**	2/3	1/3	**0/3**	**3/3**	1/3	0/6
P1	3/3(?)	**3/3**	3/3	3/3	**2/3**	**3/3**	3/3[6]	6/6
37B		**0/3**			**0/3**	**3/3**		
L. sativa		**1/10**	9/10	4/10	**2/10**	**10/14**		7/20

[1] Inoculation with zoospores from *Nicotiana* sp. originally inoculated with zoospores from infected lettuce.
[2] Inoculation with originally agent-free zoospores made viruliferous on rooted cuttings from sap-inoculated plants.
[3] Transmission in soil and root debris from plants infected after inoculation as mentioned under [2].
[4] Summation of results obtained with soil mixtures from rooted cuttings from sap-inoculated plants of *N. benthamiana* and *N. occidentalis* (Japan, P1, and 37B).
[5] Number of plants reacting out of number of plants inoculated.
[6] Severe reaction.

and five weeks later (on 12–04), agent-free zoospore supensions were added to the soil. Three weeks thereafter (on 3-05), the rooted plantlets were all still found infected when retested on *C. quinoa* and *N. occidentalis* P1. Zoospores taken four days later (on 7-05; Table 2, col. 2 and 5)) from the cultures of *N. clevelandii* and *N. occidentalis* Japan proved LRN-infected when transferred, but on *N. hesperis* A67, *N. occidentalis* P1 and lettuce only, and few lettuce plants became infected (1/10 and 2/10 only). However, when zoospores from

rooted *N. occidentalis* Japan were retested three weeks later (on 31-05; Table 2, col. 6), *N. occidentalis* Japan also reacted and 10/14 plants of lettuce did so. When soils from pots with rooted diseased *Nicotiana hesperis* and soil mixtures from pots with *N. benthamiana* and *N. occidentalis* Japan, P1 and 37B were replanted on 18-06 with *N. benthamiana, N. clevelandii, N. occidentalis* Japan and P1, and with lettuce, the latter two became clearly infected (Table 2, col. 4 and 8). In all these trials, the symptoms by the agent after its passage via the *Nicotiana* species (sap-inoculation of them followed by back-inoculations via zoospores from them) produced in the species other than lettuce were identical to those obtained in them after sap inoculation, and the symptoms reproduced in lettuce were identical to the LRN symptoms as observed in field-infected lettuce plants.

Utmost care was taken to check the supposedly LRN agent-free fungus culture used in these experiments for absence of the agent. Zoospores were transferred to lettuce plants, and these were examined for absence of symptoms, and were additionally mechanically back-inoculated onto *C. quinoa* and *N. occidentalis* P1 (five plants each, per test) for absence of reaction. The lettuce plants from which the agent-free zoospores were taken were also tested for absence of infection by sap-inoculation to *C. quinoa* and *N. occidentalis* while their root debris and soil remnants were used to grow plants of *N. clevelandii, N. hesperis* 67A, and lettuce. No indication of infection was obtained.

Lactuca sativa 'Patty' and 'Attractie'. Systemic necrosis did not show up before 7 weeks after inoculation via soil, but plants that reacted usually did so within 8 weeks (Figure 1). Symptoms were identical to those observed in nature. The rate of infection never was 100%.

Chenopodium quinoa always reacted when sap-inoculated from lettuce with LRN, but with local lesions only. They appeared about a week after inoculation and consisted of chlorotic or slightly necrotic spots, developing into green rings when leaves turned yellow.

C. amaranticolor sometimes reacted with numerous tiny chlorotic local lesions, but often did not, while reactions on *C. quinoa* were clear.

Figure 3. Symptoms caused by the agent of lettuce ring necrosis in *Nicotiana occidentalis* P1 (left) and *N. hesperis* 67A (right) 23 days after zoospore inoculation of surrounding soil; upper row, healthy controls.

Nicotiana benthamiana and *N. clevelandii* practically never showed any symptom (Tables 1 and 2) but at occasions some indistinct systemic veinal discoloration was observed (Table 2). All four accessions of *N. hesperis* and *N. occidentalis* tested, often reacted with symptoms upon mechanical as well as zoospore and soil inoculation, and *N. occidentalis* P1 always reacted in nearly all of the plants submittted to infection (Table 1). Local symptoms resulting from mechanical inoculation (Table 1, col. 1) appeared in some eight days. They consisted of diffuse chlorotic to mildly necrotic spots and were followed by systemic chlorotic to necrotic spotting, leaf malformation and varying degrees of plant stunting, which was severest for accessions P1 and 67A. In the first zoospore-inoculation trial (Table 1, col. 6), plants developing in inoculated soil showed distinct systemic symptoms (Figure 3) and all plants did so one month after inoculation. With *C. quinoa*, the agent could mostly be readily detected in non-inoculated tip leaves of plants of all *Nicotiana* spp. (Table 1, col. 3).

Discussion

LRN, like LBV, has never appeared in numerous lettuce plants grown as controls in steam-sterilized potting soil, and both have never spontaneously done so throughout the years in any experiment at IPO-DLO with lettuce in potting soil. Reproduction of the disease in lettuce plants grown in sterile soil mixed with soil and root debris from diseased plants, and transfer of the agent in zoospores of *Olpidium brassicae* released from diseased lettuce plants, proved the soil transmission of the disease and involvement of the soil-borne chytrid fungus as a vector. These data further corroborate our earlier preliminary results (Huijberts et al., 1983a) and those of Verhoyen et al. (1985). LRN survival in air-dry soil and root debris, as first demonstrated by Van Dorst (1983) after 11 months of storage, show internal survival of the agent in resting spores of the fungus.

Serial transfer of an LRN isolate (Ls247), and disappearance of LBV from the culture by selective propagation from plants showing only symptoms of LRN, have shown that the two diseases are caused by distinct entities, both vectored by the same organism, as already suggested by Verhoyen et al. (1985). In our earlier investigations on LBV (Huijberts et al., 1990), symptoms of LRN never emerged. Our isolate Ls247 was thus originally contaminated with LBV. This finding explains the frequent association of both diseases and their occurrence under similar cropping conditions. In lettuce they clearly differ in type and time of first appearance of symptoms. Chlorotic vein-banding, the characteristic initial symptom of LBV, first shows up 17 days after soil inoculation (Bos and Huijberts, 1990) in the youngest leaves, whereas the symptoms of LRN first appear 7–8 weaks after inoculation on leaves of intermediate age. This long period of incubation in lettuce plants may explain the exclusive occurrence of the disease during winter months when development of lettuce crops is slow and plants remain in the soil for longer periods of time than during summer. In glasshouse tests, when lettuce plants are kept for periods sufficiently long to allow symptom expression, symptoms of LRN are produced irrespective of temperature or light conditions.

The still enigmatic LRN agent seems to share several features with that of LBV. The latter most likely is a virus closely related to, but different from (Huijberts et al., 1990), the, as yet taxonomically ungrouped, tobacco stunt virus (TStV; Kuwata and Kubo, 1986) which has rod-shaped particles (Kuwata and Kubo, 1981). Such particles were also frequently but inconsistently found in plants with LBV (Kuwata et al., 1983; Kuwata and Kubo, 1984; Vetten et al., 1987; Huijberts et al., 1990). Our frequent endeavours to see such particles in plants with LRN have also failed, and this agrees with tentative experiences by Verhoyen et al. (1985). Their detection of a few elongated particles in purified extracts from lettuce is not convincing.

We have now succeeded in mechanical transmission and biological isolation of an infectious agent from LRN-diseased lettuce, and in proving it to be the incitant of LRN by reintroducing it into lettuce and reproducing characteristic LRN symptoms in lettuce. Symptoms of the LRN agent in *Nicotiana* spp., resembling those of LBV 'virus' and of TStV, further suggest a close relationship to these two viruses. Results obtained at IPO-DLO with freesia leaf necrosis, another soil-borne disease probably transmitted by *O. brassicae*, suggest its agent to be related also. Virus-like symptoms resembling those of LBV 'virus' could recently be induced with sap from diseased freesia on *C. quinoa*, *N. hesperis* 67A, and *N. occidentalis* P1 employing our techniques used for LBV, but during winter months only, and back-inoculations to freesia have also been unsuccessful so far. Results of purification of the freesia agent remain uncertain, although particles have been found resembling those of TStV and those associated with LBV 'virus' (Bouwen, 1994).

The group of likely related viruses and virus-like agents obviously remain difficult to study. Earlier promising results obtained with TStV and LBV 'virus' (Masri and Hiruki, 1983; Kuwata and Kubo, 1984, 1986; Vetten et al., 1987; Huijberts et al., 1990) have not had any follow-up. Our results with LRN obtained during many years of observation agree with and extend those obtained in Belgium (Verhoyen et al., 1985) and in southern France (H. Lot, pers. comm. 1990).

LRN could be confused with, but evidently differs from, a number of necrotic lettuce diseases. 'Lettuce ringspot' is another soil-borne disease of lettuce, described in Great Britain, but its spots are yellow and occur on lower leaves and the indication 'ringspot' more particularly derives from its having been ascribed to a strain of the easily sap-transmissible nematode-borne tomato black ring virus (Smith and Short, 1959). The related tobacco ringspot virus had slightly earlier been identified as the cause of lettuce calico, a disease also associated with necrotic flecks (Grogan and Schnathorst, 1955) and thereafter of a similar

but more severe ringspot disease of lettuce ('taches en anneaux de la laitue') occurring during winter in glasshouses in western France (Morand et al., 1973; Morand and Poutier, 1978). Internal rib necrosis and rusty brown discoloration causing extensive losses in California in 'Climax' crisphead lettuce is symptomatologically also different and was found to be caused by lettuce mosaic virus (Coakley et al., 1973). Such necrosis is far from rare in lettuce with lettuce mosaic virus (Bos et al., 1994).

The soil- and water-borne nature of LRN and LBV readily explains their coming to the fore as a consequence of modern, highly intensive, often continuous cropping, under irrigation or on substrate and in circulating nutrient solutions. Information on the LRN agent and its vector have at an early stage already led to practical advice for control with emphasis on cultural hygiene addressing the vector as by chemical soil disinfection or steam sterilization (Huijberts et al., 1983b). Since resting spores of *O. brassicae* were found to be readily spread in drainage water and thence in surface water, where they remained viable for at least seven weeks and still contained the agents of LBV and LRN, it was advised to exclusively use rain water or tap water for plant watering and/or to each four days add 20 ppm of Agral to water used for lettuce growing on substrate (Van Dorst, 1987; Van Amersfoort, 1987). Such care is especially needed on holdings commercially producing seedlings for distribution to lettuce growers (Van Amersfoort, 1987). Screening and breeding for reduced vulnerability to LRN, as promising for LBV (Bos and Huijberts, 1990), is also worth consideration to avoid large-scale use of chemical soil-disinfectants.

References

Bos L and Huijberts N (1990) Screening for resistance to big-vein disease of lettuce (*Lactuca sativa*). Crop Protection 9: 446–452

Bos L, Huijberts N and Cuperus C (1994) Further observations on lettuce mosaic virus in relation to lettuce (*Lactuca sativa*), and a discussion of resistance terminology. European Journal of Plant Pathology 100: 293–314

Bouwen I (1994) Freesia leaf necrosis: Some of its mysteries revealed. Acta Horticulturae 377: 311–318

Coakley SM, Campbell RN and Kimble KA (1973) Internal rib necrosis and rusty brown discoloration of Climax lettuce induced by lettuce mosaic virus. Phytopathology 63: 1191–1197

Grogan RG and Schnathorst WC (1955) Tobacco ring-spot virus – the cause of lettuce calico. Plant Disease Reporter 39: 803–806

Huijberts N, Blystad D-R and Bos L (1990) Lettuce big-vein: Mechanical transmission and relationships to tobacco stunt virus. Annals of Applied Biology 116: 463–475

Huijberts N, Bos L and Van Dorst HJM (1983a) Kringnecrose bij kassla, misschien veroorzaakt door een bodemvirus. Weekblad Groenten en Fruit 10 juni 1983: 43–44

Huijberts N, Bos L and Van Dorst HJM (1983b) Grondontsmetting belangrijk bij kringnecrose. Tuinderij 9 juni 1983: 33

Kuwata S and Kubo S (1981) Rod-shaped particles found in tobacco plants infected with tobacco stunt agent. Annals of the Phytopathological Society of Japan 47: 264–268

Kuwata S and Kubo S (1984) Properties of two *Olpidium*-transmitted viruses: tobacco stunt and lettuce big vein. (Abstract). 6th International Congress of Virology, Sendai, Japan: 45

Kuwata S and Kubo S (1986) Tobacco stunt virus. CMI/AAB Descriptions of Plant Viruses 313: 4

Kuwata S, Kubo S, Yamashita S and Doi Y (1983) Rod-shaped particles, a probable entity of lettuce big vein virus. Annals of the Phytopatological Society of Japan 49: 246–251

Masri SA and Hiruki C (1983) A new group of elongated plant viruses having a capsid protein of unusually high molecular weight (Abstract). Canadian Journal of Plant Pathology 5: 208

Morand JC and Poutier JC (1978) Les taches en anneaux de la laitue, une souche de 'tomato black ring virus'. Annales de Phytopathologie 10: 101–102

Morand J-C, Paulin J-P and Samson R (1973) Une nouvelle maladie sur laitue provoquée par un virus. Pépiniéristes Horticulteurs Maraîchers; la Revue Française de l'Horticulture Professionnelle 134: 19–21

Smith KM and Short ME (1959) Lettuce ringspot: A soil-borne virus diseases. Plant Pathology 8: 54–56

Van Amersfoort R (1987) Bobbelblad en kringnecrose bij sla. Al een zorg vanaf de opkweek. Weekblad Groenten en Fruit 24 augstus 1987: 30–31

Van Dijk P, Van der Meer FA and Piron PGM (1987) Accessions of Australian *Nicotiana* species suitable as indicator hosts in diagnosis of plant virus diseases. Netherlands Journal of Plant Pathology 93: 73–85

Van Dorst HJM (1983) Virus diseases in lettuce. Annual Report Glasshouse Vegetable Research Station, Naaldwijk 1982: 95–96

Van Dorst HJM (1987) Virusziekten bij sla. Het oppervlaktewater als besmettingsbron. Tuinderij 30 juli 1987: 23

Verhoyen M, Legrand G and Meunier S (1985) Recherches sur l'étiologie d'une nécrose annulaire de la laitue. Mededelingen Faculteit voor Landbouwwetenschappen Rijksuniversiteit Gent 50/3b: 1295–1300

Vetten HJ, Lesemann D-E and Dalchow J (1987) Electron microscopical and serological detection of virus-like particles associated with lettuce big-vein disease. Journal of Phytopathology 120: 53–59