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EARLY BROWNING OF PEA, A DISEASE CAUSED BY A SOIL- AND SEED-BORNE VIRUS[1]

Met een samenvatting:
Vroege verbruining van erwt, een ziekte veroorzaakt door een via de grond en met het zaad overgaand virus

BY

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INTRODUCTION

A peculiar disease of pea (*Pisum sativum* L.) has been known to the Agricultural Advisory Service in the north-western part of the Netherlands since 1949 (ZIJDEWIND, personal communication). The disease starts early in the growing season, often in the first days of May, and is characterized by brown patches in the crop due to partial necrosis of plants or even the death of entire plants. For this reason it has been named "early browning of pea".

In 1953 the second author succeeded in transmitting from diseased plants a virus which induced typical symptoms in pea plants following mechanical inoculation. During surveys since 1957 of legume viruses occurring in this country (BOS & VAN DER WANT, 1958), the disease has increasingly attracted our attention. We now know that it is present in the north- and south-western parts of the Netherlands, although it generally occurs only sporadically. At present the disease is also known to occur in eastern England (GANE, manuscript).

Since the disease seemed to be of potential practical and also scientific importance, more detailed investigations were started in 1957.

DESCRIPTION OF THE DISEASE

The description to be given here is based on observations made on the pea variety 'Rondo', which is grown predominantly in this country. In other varieties the symptoms are essentially the same.

The first symptoms appear early in the season, often during the first part of May, i.e. about two months after sowing. The stems, petioles and leaves of diseased plants show purplish-brown necrotic discolorations, irregularly distributed over the plant (fig. 1). The discoloration starts as a vascular necrosis which extends into some surrounding tissue. In the stipules and leaflets the veins often become necrotic, causing localized wilting and the eventual death of adjacent interveinal tissue. In many cases this symptom is restricted to a few leaves or even to sectors of leaflets or stipules, the rest of the plant remaining normal. Frequently, however, the tops of infected plants are stunted and somewhat distorted, showing slight overall yellowing or a faint mottling. Often the

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[2] These studies were started by the second author and continued after 1958, by the first author. Present address of the second author: Laboratory of Virology, Agricultural University, Wageningen.

tops of the plants become necrotic, in which case new sprouts may arise from the base.

Sometimes plants recover to a certain extent in their new growth, but later the newly formed parts may also develop severe necrosis. These plants and also plants becoming infected at a late stage of development often show a typical fleck- and ring-like purplish-brown necrotic pattern of the pods. If, however, the pod is invaded when young, it remains smaller than normal and may be distorted. The seeds in infected pods may show faint chlorotic and somewhat finely wrinkled spots (fig. 2).

Affected plants may be found scattered throughout a field, but more commonly occur in patches which may measure as much as several meters in diameter (fig. 3). In extreme cases, large areas of a field are affected. When infection occurs early in the season, necrosis may lead to a brownish coloration of the crop. After some time the retarded growth or death of the plants allows a heavy development of weeds in the affected parts of the field. Early browning occurs especially on light or sandy clay soils.

Investigations on the inciting virus

In 1953 a virus was transmitted mechanically from diseased pea plants to a number of plant species. It induced typical systemic symptoms in pea plants, and local lesions in tobacco and French bean leaves. The isolate unfortunately was lost before it had been accurately identified. The appearance in the field and the early time of infection suggested that the virus might be soil-borne and might have many features in common with tobacco rattle virus. This suspicion was strengthened by the fact that the symptoms incited in tobacco closely resembled those of tobacco rattle.

When in 1957 the virus was reisolated, a detailed investigation was undertaken to identify the cause of the disease and to study its epidemiology in order to be able to devise methods of control. In view of its suspected relationship with tobacco rattle virus the early-browning virus was compared with that virus in many of the experiments to be described below. In the following text the names of both viruses will be abbreviated to EBV and RV.

a. *Materials and methods*

Since no differences were observed between various isolates of EBV, several isolates were used, without especially mentioning their origin. Most experiments, however, were made with an isolate present in a batch of seed. The RV isolate was obtained from Dr. Sol who isolated it from an infested tulip field by planting tobacco in a soil sample.

Plant experiments were carried out in an insect-proof greenhouse. Temperatures varied from 18–23 °C. During winter additional fluorescent lighting was provided. The plants were grown in sterilized compost soil contained in 4- or or 6-inch clay pots.

The techniques used for virus transmission, etc. were those described by Bos et al. (1960). Additional special techniques will be described later with the presentation of the results.

b. *Host-plant reaction*

1. Host range

Several plant species were tested by mechanical inoculation with EBV and RV respectively in order to determine the host range of EBV, and to compare reactions to the two viruses. Because of lack of greenhouse space only those plant species showing questionable or no symptoms were checked for virus by back-inoculation to cucumber seedlings (var. 'Gele tros'). A summary of the results is given in table 1. Details of the symptoms produced by both viruses in important host and/or test plants are presented in the section on symptomatology together with a discussion of the relationships between the two viruses.

TABLE 1. Summary of results of host-range experiments with early-browning and rattle viruses.
Samenvatting van de resultaten der waardplantenproeven met vroege-verbruinings-virus en ratelvirus.

Plant species *Plantesoort*	Early-browning virus *Vroege-verbruiningsvirus*		Rattle virus *Ratelvirus*	
	Symptoms *Symptomen*	Results of back inoculation[1] *Resultaten van terug-inoculatie*[1]	Symptoms *Symptomen*	Results of back inoculation[1] *Resultaten van terug-inoculatie*[1]
Amaranthus caudatus L.	–[2]	–[2]	(S)[4]	S[3]
	–	–	S	S
Antirrhinum majus L.	(S)	S+[5]	(S)	S
	S	S+		
Arachis hypogaea L.	L[3]	–	L	–
Beta vulgaris L.	L	–	L	–
	–	–	L	–
Brassica napus L.	–	–	–	–
	–	–		
Brassica oleracea L. var. *botrytis*	–	–	–	–
Brassica rapa L.	–	–	–	–
Callistephus chinensis Nees	L	S	LS	S
	–	L		

[1] In most cases only the non-inoculated plant parts were tested for the presence of virus by means of back inoculation.
In de meeste gevallen werden slechts de niet geïnoculeerde plantedelen getoetst op aanwezigheid van virus door middel van terug-inoculatie.

[2] – = No symptoms observed, or no virus present as revealed by means of back inoculation.
Geen symptomen waargenomen, of geen virus aangetoond bij terug-inoculatie.

[3] S = Systemic symptoms oberserved, or virus present in systemically invaded tissue.
Systemische symptomen waargenomen, of virus aanwezig in systemisch besmet weefsel.

[3] L = Local symptoms observed, or virus present in locally infected tissue.
Locale symptomen waargenomen, of virus aanwezig in lokaal besmet weefsel.

[4] Parentheses point to the fact that the symptoms mentioned or results of back inoculation were not clear.
Haakjes wijzen op het feit dat de genoemde symptomen of de resultaten van terug-inoculatie niet duidelijk waren.

[5] The + signs indicate the presence of large quantities of virus as revealed by a large number of local lesions on cucumber cotyledons used for back inoculation.
De + tekens wijzen op de aanwezigheid van grote hoeveelheden virus zoals bleek uit een groot aantal lokale lesies op de komkommerzaadlobben, gebruikt voor terug-inoculatie.

TABLE 1, continued (see notes table 1, page 370)

Plant species *Plantesoort*	Early-browning virus *Vroege-verbruiningsvirus*		Rattle virus *Ratelvirus*	
	Symptoms *Symptomen*	Results of back inoculation *Resultaten van terug-inoculatie*	Symptoms *Symptomen*	Results of back inoculation *Resultaten van terug-inoculatie*
Capsicum annuum L.	LS		LS	
	–		–	
	L	LS		
Carum carvi L.	–		–	
Chenopodium amaranticolor Coste & Reyn	L		L	
	L	–	S	–
	L	(L)		
Chenopodium quinoa Willd.	L(S)	–	L(S)	–
	L	–	(S)	–
Chrysanthemum carinatum Schb.	L	L		
Chrysanthemum maximum Ramond	L	–		
Cichorium endivia L.	–	–	–	–
	–	L		
Crotalaria spectabilis Roth.	–		–	
Cucumis sativus L. var. 'Gele tros'	L	L	L	L(S)
Cucurbita pepo L.	L		L	
	L	–		
Dahlia variabilis Cav.	–	–		
Datura stramonium L.	L	(S)	L	–
Dianthus caryophyllus L.	–	–	–	–
Dolichos lablab L.	–		–	
Eschscholtzia californica Cham.	(L)	–		
Gaillardia sp.	–	S	–	S
	–	S^+		
Glycine soja S. et Z.	L	–	L	–
	–		–	
Gomphrena globosa L.	LS	S	LS	
	L	S	L	–
	L	L		
Lathyrus odoratus L.	–	S^+	–	
Lycopersicum esculentum Mill. var. 'Ailsa Craig'	–	–	(L)	(S)
	–	(L)		
– – var. 'Bonny Best'	–	L	LS	S
	–	–	LS	S
Medicago lupulina L.	–	–	–	–
Medicago sativa L. var. 'Du Puits'	–	–	–	–
	–	–	–	–
Nicotiana glauca Grah.	LS	–	LS	
Nicotiana glutinosa L.	S		LS	S
Nicotiana rustica L.	–	S	LS	
Nicotiana tabacum L. var. 'White Burley'	L	S	LS	S
	L	S^+		
Petunia hybrida Vilm.	LS	S^+	LS	S
	L		S	S
	LS	S^+		

TABLE 1, continued (see notes table 1, page 370)

Plant species *Plantesoort*	Early-browning virus *Vroege-verbruiningsvirus*		Rattle virus *Ratelvirus*	
	Symptoms *Symptomen*	Results of back inoculation *Resultaten van terug-inoculatie*	Symptoms *Symptomen*	Results of back inoculation *Resultaten van terug-inoculatie*
Phaseolus aureus Roxb.	(L)	–	(L)	–
Phaseolus lunatus L.	–		(L)	
Phaseolus vulgaris L. var. 'Beka'	LS		L	
	L		–	
	L	L		
– – var. 'Bountiful'	L	–	L	–
– – var. 'Dubbele zonder draad'	L		L	
– – var. 'Furore'	L		L	
– – var. 'Pinto'	LS		L	
– – var. 'Saxa'	LS		–	
– – var. 'Topcrop'	LS		–	
Phlox drummondii Hook.	LS	LS^+		
Physalis floridana Rydb.	–	–	(S)	S
	–	–		
Pisum sativum L.	LS	S	L	–
Solanum tuberosum L. var. 'Bintje'	–	–	–	–
– – var. 'Eersteling'	–	–	(L)	–
Spinacia oleracea L.	–	–	–	–
Stellaria media Vill.	–	–	–	–
Tagetes erectus L.	–		–	
Tetragonia expansa Murr.	L	–	L(S)	–
Trifolium incarnatum L.	–		–	
Trifolium pratense L.	–		–	
	–	–	–	–
Trifolium repens L.	–		–	
	–	–	–	–
Tropaeolum majus L.	S	S	S	S
	–	LS		
Vicia faba L. var. 'Driemaal wit'	–	S	–	–
	–	S	–	–
	–	LS^+		
Vicia sativa L.	–		–	
Vigna sinensis (L.) Savi 4 different unidentified varieties	–	–	L	–
– – var. 'Black eye'	–	–	L	–
Viola tricolor L.	–	LS		
Zinnia elegans Jacq.	(S)	S	LS	S
	–	S^+		

To obtain information concerning the epidemiology of the early-browning disease, important agricultural and horticultural plant species were tested in field experiments for susceptibility to natural infection. As will be discussed in detail in the section on virus transmission, the EBV is soil-borne and is furthermore very persistent in the soil.

The field tests, carried out in 1958, 1959, 1960 and 1961, were located each year on a different portion of a field which in 1957 had produced a pea crop showing serious and very homogeneous infection. Each year the presence of virus in the soil was checked by surrounding the trial field by a strip of highly

sensitive 'Eroica' or 'Koroza' peas. In 1960 and 1961 especially, these peas showed a high percentage of infection indicating that all plant species included in the field were exposed to infection from the soil. Each plant species was sown in duplicate, most of them in fields of 12 m^2.

Except for peas, no naturally infected plant species ever showed symptoms caused by EBV. In 1960 and 1961 the various plant species were systemically checked for virus by taking random samples of both aboveground and underground parts at specific time intervals throughout the vegetative period. These samples were tested in the greenhouse by means of sap inoculation to cucumber seedlings (var. 'Gele tros'). Before testing, all root samples were thoroughly cleaned of adhering soil. The data obtained are assembled in table 2.

The results demonstrate that in addition to peas, other plant species may play a role in the epidemiology of the disease, presumably as symptomless carriers. Very striking is the fact that most samples of broad bean (*Vicia faba*) contained large amounts of virus, especially in the aboveground parts. A high percentage of samples of all clover species tested (including lucerne) showed natural infection with EBV, even though it was not possible to infect them by mechanical inoculation.

The important agricultural crops, such as cereals, sugar beet and potato, never contained virus. Among the artificial hosts, *Eschscholtzia californica* and *Tropaeolum majus* proved highly susceptible to natural infection. Of the weed species tested, only *Solanum nigrum* showed infection in a number of samples.

2. Pea varietal reaction

All 21 pea varieties tested in the greenhouse appeared to be susceptible to the virus. These included the round blue peas: 'Fertilas', 'Mansholt's Pluk', 'Pauli', 'Rondo', 'Rovar', 'Servo', 'Unica', 'Vares' and 'Virtus'; the marrowfats: 'Big Ben', 'Emigrant' and 'Zelka'; the maple peas: 'Aureool' and 'Dolfijn'; the grey peas: 'Erioica', 'Ivora', 'Koroza' and 'Vinco', and the horticultural wrinkled green peas: 'Celsior', 'Juwel' and 'Wyola'. The latter three varieties showed a less severe reaction indicating some resistance to the virus.

Since these initial tests, a screening for resistance among pea material has been made by HUBBELING (manuscript) of the Division for Plant Disease Resistance of the Institute of Phytopathological Research.

3. Symptomatology

A survey of the plant species tested and a summary of their reactions to EBV and to RV has already been given in table 1. A short description of symptoms in some important host and/or test plants will be presented here in an abbreviated form.

Beta vulgaris L., sugarbeet, var. 'Kuhn P.' Both viruses usually produce faint chlorotic local lesions in some plants. EBV: lesions about 4 mm diameter, sometimes ring-shaped. RV: lesions about 2 mm diameter, sometimes surrounded by a narrow necrotic zone.

Callistephus chinensis Nees, China aster. EBV: in one out of four plants some brown, desiccated local lesions. RV: in all inoculated plants, similar but enlarging local lesions and a systemic, irregularly distributed necrosis along the veins, leading to curling and deformation of the leaves.

Chenopodium amaranticolor Coste & Reyn. Both viruses: yellow local lesions, which were largest with EBV.

Cucumis sativus L., cucumber, var. 'Gele tros' (fig. 4). Both viruses: chlorotic local lesions

TABLE 2. Summary of results of natural-infection tests with plant species grown in a contaminated field. Presence of virus was tested bij back inoculation to cucumber seedlings. *Samenvatting van de resultaten der natuurlijke-besmettingsproeven met plantesoorten, gegroeid op besmette grond. Aanwezigheid van virus werd getoetst door terug-inoculatie op komkommerzaailingen.*

	Aboveground parts *Bovengrondse delen*	Underground parts *Ondergrondse delen*
Cultivated plants/*Cultuurplanten*		
Avena sativa L.	0/9[1]	0/11
Beta vulgaris L.	1/18?[2]	0/19
Carum carvi L.	0/7	0/7
Cichoreum endivia L.	0/18	0/18
Eschscholtzia californica Cham.	0/12	9/12
Helianthus annuus L.	0/15	0/15
Hordeum vulgaris L.	0/9	0/10
Linum usitatissimum L.	1/4?	1/4
Lupinus luteus L.	0/17	0/16
Medicago lupulina L.	5/18+[3]	6/18
Medicago sativa L.	8/21+	8/21
Papaver somniferum L.	0/13	2/12
Phaseolus vulgaris L.	3/14?	5/14
Secale cereale L.	0/3	0/3
Solanum tuberosum L. (three varieties/*drie rassen*)	0/23	0/23
Spinacia oleracea L.	0/13	1/13?
Trifolium pratense L.	6/20	9/20
Trifolium repens L.	0/18	4/17
Triticum spp.	0/9	0/11
Tropaeolum majus L.	3/19	6/11
Vicia faba L.	11/14++	6/14
Zinnia elegans Jacq.	1/10	1/10
Weeds/*Onkruiden*		
Capsella bursa pastoris Med.	1/4	0/4
Chenopodium album L.	0/10	0/9
Matricaria inodora L.	0/1	0/1
Poa annua L.	0/5	0/5
Polygonum aviculare L.	0/7	0/7
Polygonum persicaria L.	0/7	0/7
Senecio vulgaris L.	0/1	0/1
Solanum nigrum L.	1/4	3/4
Sonchus oleraceus L.	0/4	0/4
Stellaria media L.	0/8	0/12
Taraxacum officinale Web.	0/1	0/2
Tussilago farfara L.	0/3	1/3

[1] Numerator: number of samples containing virus
Denominator: number of samples tested
Teller: aantal virusbevattende monsters
Noemer: aantal getoetste monsters

[2] ? = Reaction questionable
Reactie niet duidelijk

[3] + sign indicates high concentration of virus as revealed by the number of local lesions obtained on cucumber cotyledons
+ duidt op hoge virusconcentratie, zoals bleek uit het aantal lokale lesies, dat werd verkregen op de zaadlobben van komkommer

in cotyledons of 2–3 mm diameter, sometimes showing faint concentric rings; these lesions appear 3–4 days after inoculation. RV seems to have a tendency to produce diffusely bordered somewhat enlarging lesions. EBV alone was mechanically inoculated to young foliage leaves and produced in them yellowing local lesions, similar to those in cotyledons.

Datura stramonium L., jimson weed. EBV: green rings appeared in old yellowing leaves that had been inoculated. RV: numerous tiny necrotic local lesions.

Eschscholtzia californica Cham., California poppy. Only EBV tested: local lesions, no systemic infection could be demonstrated by back-inoculation. After sowing in contaminated soil (cf. table 2) most plants contained the virus in their roots.

Gomphrena globosa L., globe amaranth. Both viruses: irregularly shaped desiccated local lesions, some systemic lesions of similar colour and shape and a slight systemic distortion. EBV: recovered from systemically invaded leaves.

Lathyrus odoratus L., sweet pea. Although no distinct symptoms were produced, back-inoculation demonstrated the presence of a high concentration of EBV.

Lycopersicum esculentum Mill., tomato, var. 'Bonny Best'. EBV: no symptoms although virus concentration in systemically invaded tissues was high. RV: no local reaction, systemic stunting was accompanied by some leaf narrowing and a faint mosaic.

Nicotiana glauca Grah., tree tobacco. Both viruses: ring-shaped, etch-like local lesions and systemic necrosis along the veins, sometimes distributed in an oak-leaf pattern. EBV: the number of local lesions was smaller, their size larger and the systemic reaction less severe.

Nicotiana glutinosa L. RV only: a rather large quantity of desiccated, irregularly shaped local lesions, ½–2 mm diameter. Both viruses: a diffuse mosaic tending towards mottling, associated with some leaf curling.

Nicotiana rustica L., wild tobacco. EBV: no symptoms, but virus could be recovered. RV: many irregularly shaped, superficially desiccated local lesions, usually smaller than 1 mm, partly enlarging and coalescing, leading to wilting and withering of inoculated leaves; systemic chlorosis and necrosis and final collapse of the plants.

Nicotiana tabacum L., tobacco, var. 'White Burley'. EBV: ring- and arc-like, desiccated local lesions; no systemic symptoms although virus could be recovered from systemically invaded leaves. RV: desiccated ring-like local lesions, smaller in size and in larger number than with EBV, systemic necrotic streaks in stems and necrotic rings and arcs in leaves, resulting in curling and distortion of leaves.

Petunia hybrida Hort., garden petunia. EBV: desiccated local lesions surrounded by a dark grey border, later enlarging to brown rings, 3 mm diameter; systemic mottling and stunting. RV: no local reaction; systemic reaction as described for EBV.

Phaseolus vulgaris L., French bean, var. 'Beka' (fig. 5, below). EBV: ring-shaped local lesions 1 mm in diameter appearing three days after inoculation, gradually enlarging to chocolate brown, necrotic rings of 2–3 mm diameter; necrosis sometimes extending for some distance along the veins; only during winter was a systemic reaction sometimes observed. This reaction started with elongated, internal necrotic areas in the petioles of primary leaves and here and there in the stems; later, on trifoliate leaves, necrotic specks surrounded by a chlorotic ring. Occasionally these leaves showed some veinal necrosis leading to leaf distortion. Systemic reaction in younger leaves consisted of chlorotic flecking and sometimes chlorotic arcs and rings. (The varieties 'Pinto', 'Saxa' and 'Topcrop' also showed this systemic reaction during summer). RV: only numerous tiny necrotic local lesions are formed.

Pisum sativum L., pea, varieties 'Eroica', 'Mansholt's Pluk' (fig. 5, above), 'Rondo' and 'Unica'. EBV: some necrotic local lesions enlarging rapidly, followed quickly by systemic necrosis as described above in the section "Description of the disease". RV: a varying amount of tiny local lesions, without any systemic spread.

Vicia faba L., broad bean, var. 'Driemaal wit'. EBV: symptomless systemic infection. RV: no symptoms, nor could virus be recovered.

Vigna sinensis (L.) Savi, cowpea, four unidentified varieties and 'Black eye'. EBV: no infection. RV: numerous chocolate-brown, small local lesions on all inoculated primary leaves[1].

Zinnia elegans Jacq., zinnia. EBV: only inconspicuous systemic mottling, although large quantities of virus could be demonstrated. RV: irregular brown local lesions and a faint systemic mottling, sometimes serious necrosis leading to death of the plants.

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[1] After finishing the manuscript, we performed an experiment with 'Monarch's Black eye' cowpeas. In contrast to varieties tested previously, this variety reacted to EBV with some chocolate-brown local lesions and a clear systemic mottling. Only EBV could be reisolated from non-inoculated leaves.

We realize that the data summerized in table 1 have only relative value since susceptibility and reaction of some plant species and varieties mentioned turned out to be variable. With this in mind, the following conclusions may be drawn. A close resemblance exists between EBV and RV. There are several plant species in which both viruses are almost or completely indistinguishable, such as cucumber, *Gomphrena*, sugar beet and *Tropaeolum*. In others certain differences were observed which could, however, be quantitative rather than qualitative. This group comprises *Callistephus chinensis*, *Chenopodium amaranticolor*, *Nicotiana glauca*, *N. tabacum*, *Petunia hybrida* and *Zinnia elegans*.

On the other hand, the two viruses can be clearly differentiated with the help of *Datura stramonium*, *Phaseolus vulgaris*, *Vicia faba*, *Pisum sativum* and *Vigna sinensis*, and presumably also on *Lycopersicum esculentum* and *Nicotiana rustica*. The reactions of *Phaseolus vulgaris* and *Pisum sativum* are of special diagnostic value.

Another characteristic difference between the two viruses is that in our field experiments (table 2) EBV could never be isolated from potato varieties known to be highly susceptible to potato stem mottle (= rattle virus). Nor could EBV be detected in roots of cereals. If present in a soil, RV can be easily demonstrated in roots of rye (*Secale cereale*) and *Stellaria media* (NOORDAM, 1956). EIBNER (1959) reported that his so-called *Stellaria*-test demonstrated without fail the presence of RV in the soil. Our experience with EBV is that it could never be recovered from roots of this species, taken either from highly contaminated field soils or from greenhouse soil-transmission tests. In the latter experiments a high percentage of pea plants became infected (cf. also p. 377).

c. *Virus transmission*

As was mentioned before, early browning occurs especially on sandy or light clay soils, and often only in patches. Thus in this respect also the disease resembles the soil-borne tobacco rattle and potato stem-mottle diseases. These facts suggested the likelihood of soil transmission of the virus, which was then studied in detail. During these experiments it was soon discovered that seed transmission plays another important role in the epidemiology of the disease.

Since no insect transmission of soil-borne viruses is known, no detailed insect-transmission tests were done. This was further justified by the fact that the distribution of the virus in the field suggested an absence of air-borne vectors. A few very preliminary tests were made, all of which yielded negative results, and therefore will not be described here. Some experiments were performed to check the possible role of sap transmission in the epidemiology of the disease.

1. Soil-transmission tests

In August, 1957, soil samples were taken in the field from a site where earlier that season peas had shown severe infection. Some of the samples were kept almost undisturbed by placing them immediately into Mitscherlich pots (series A). Another batch of soil was taken to the laboratory in a plastic bag and then put into 10-inch clay pots (series B). After 3 days, all pots were sown with the highly sensitive pea variety 'Rondo', 20 seeds to a pot. The first symptoms of early browning were observed 16 days after sowing. At different dates the numbers of newly attacked plants were recorded on the basis of typical symp-

toms. The results are summarized in table 3. All plants showing symptoms on the first day of recording were indexed for virus by means of back-inoculation to 'Eroica' peas. In all but two cases these tests were positive.

TABLE 3. Summary of results of soil-transmission tests performed in pots in the greenhouse with soil from a contaminated field. Twenty healthy pea seeds (var. 'Rondo') were sown in each pot. At different dates newly infected plants were recorded on the basis of characteristic symptoms observed.
Samenvatting van de resultaten van een in de kas verrichte potproef met grond van een besmet perceel ter vaststelling van de overdracht van het vroege-verbruiningsvirus via de grond. In iedere pot werden 20 gezonde erwtezaden (ras 'Rondo') gezaaid. Op verschillende data werden de nieuw geïnfecteerde planten genoteerd op grond van waargenomen karakteristieke symptomen.

Series *Serie*	Date of observation, indicated as number of days after sowing *Datum van waarneming, aangeduid als aantal dagen na zaaien*						
	18	21	28	31	43	73	Totals *Totalen*
A. Undisturbed soil in Mitscherlich pots (11 pots in total) *Ongestoorde grond in Mitscherlich-potten (11 potten in totaal)*	7	6	12	4	16	66	110 (out of 220 seeds)
B. Disturbed soil in 10 inch clay pots (12 pots in total) *Gestoorde grond in bloempotten met een diameter van 25 cm (12 potten in totaal)*	6	3	15	7	12	120	163 (out of 240 seeds)

In all our experiments with other viruses where pea plants were used, the planting of pea seeds in sterilized soil has never given rise to plants showing early-browning symptoms. The results obtained in the above experiment therefore clearly confirmed the soil transmissibility of the virus. Table 3 shows that there was very early infection of some plants, after which more and more were diseased. There was no difference between undisturbed and disturbed soil.

To test the persistence of the virus in soil, the soil samples mentioned above were kept in the glasshouse, being watered only now and then to prevent the soil from drying out completely. Four months after sampling (on December 24), 'Eroica' peas were again sown in these pots. The development of the plants was normal, indicating that the virus was no longer present.

In a third experiment performed in 1958, the same soil samples were used together with new samples (taken May 12) of undisturbed soil (series C) and of disturbed soil (series D). All pots were sown with 'Rondo' and 'Eroica' peas. Some of the pots were placed in the glasshouse and others outside. Only peas growing in series C and D, both inside and outside, developed symptoms of the disease (recorded June 26, results have not been read in detail).

Further evidence of soil transmission of the virus was obtained from the field experiments described in the section on host range. The occurrence of the virus in the roots of a number of plant species which rarely or never show infection

of the aboveground parts (cf. table 2), points further to infection from the soil.

The field trials described in the section on host range were also intended to study the influence of different crops on the persistence and concentration of the virus in contaminated soil. The following year therefore, each plot was sown entirely with sensitive 'Rondo' peas. Careful observations and countings of diseased and healthy plants repeated during three years did not reveal any influence attributable to the crop of the previous year. Data fluctuated heavily and no correlation existed between replicates.

2. Seed-transmission tests

In 1958, Mr. A. C. M. MULDERS of the Dutch Agricultural Advisory Service in Western Noord-Brabant sowed seeds of diseased pea plants, var. 'Rondo', harvested in 1957, in his garden on sandy soil. Some of the plants which emerged showed symptoms of the disease. This pointed to the possible seed transmission of the virus. To study this in more detail, a series of experiments was performed using seeds of the variety 'Rondo'. Results of the most important trials will be reported here.

In the early experiments seed transmission was checked on the basis of symptoms in plants grown from seed obtained from a diseased patch in a field, followed by back-inoculation from these plants. The seeds were sown in sterilized compost soil in clay pots in the greenhouse. In the first trial (October 1958) no characteristic symptoms developed, although many plants showed wilting of basal leaves and some stunting. Back-inoculation, however, revealed the presence of virus in many of these slightly abnormal plants. From the data obtained it could be concluded that normal-looking seeds produced virus-free plants, whereas a high percentage of seeds showing some wrinkling and greenish-grey discoloration of the seed-coat gave rise to virus-infected plants. For later experiments seeds showing the above symptoms were selected. They always produced a high percentage of virus-containing plants. In the greenhouse typical early-browning symptoms rarely developed. To check their ability to produce characteristic symptoms, abnormal seeds were sown outside (August 1959) in pots containing a sandy soil or a very heavy clay soil, both taken from fields which had never shown any sign of the disease. On both soil types some plants showed typical symptoms, indicating that environmental conditions play an important role in the production of symptoms after infection from the seed.

Subsequently seeds were tested directly for the presence of virus. In these trials seeds were soaked in water for one night. They were then ground in the presence of a little water and the mash was used as inoculum. The results of only one representative experiment will be mentioned here.

From an average sample of pea seeds, var. 'Rondo', obtained from an infected patch in November 1959, 10 seeds were taken at random and tested separately for virus by means of inoculation to three cucumber seedlings. The numbers of lesions obtained were as follows:

Seed no 1:	numerous	6:	numerous
2:	numerous	7:	3
3:	0	8:	0
4:	3	9:	1
5:	0	10:	0

These high numbers of local lesions obtained with single seeds indicate the presence of rather large quantities of virus in infected seeds. In our experience, seeds of diseased pea plants provide generally the best source of virus for greenhouse experiments. The water in which infected seeds had been soaking, was also tested for infectivity but did not contain virus.

To study the location of the virus in the infected seeds, 10 seeds showing typical symptoms were soaked for one night and separate inoculations were made with seed coats, germs, and cotyledons to 8 cucumber seedlings. The numbers of local lesions obtained were 3, 127 and 156 respectively. This indicates substantial presence of virus in the embryos.

In connection with a search for a practical method of seed testing, it was considered important to check whether the presence of virus in the seed is always associated with visible symptoms in these seeds.

In July, 1959, from an average sample of 100 'Rondo' seeds obtained from a diseased crop, 25 suspected seeds were selected. These seeds were tested in groups of 10, 10 and 5 seeds respectively, on three cucumber seedlings each, all with very clear and positive results. The remaining healthy looking seeds were tested in groups of 10, 20 and 45 seeds. The first group produced only one questionable local lesion, and the second only two, but the last group gave a clearly positive reaction. In November of the same year, 60 healthy looking seeds were selected from an average sample taken from a diseased field. They were tested in groups of 5 seeds. Only one group out of twelve produced numerous local lesions. Another 10 normal-looking seeds were tested separately, without producing any local lesions. These results indicate that generally normal-looking seeds do not contain virus.

The experiments performed so far clearly show an association of virus infection with wrinkling and greyish-green discoloration of the seed coat. Further studies are in progress.

Another aspect studied was the spread of the infection into pea plants from the seeds, the so-called secondary infection, and especially the relation between infection and the production of symptoms. To investigate this problem the following trials were made.

Seeds were used that showed characteristic symptoms of early browning. As a check, 10 of these seeds were tested individually on cucumber seedlings. Nine seeds contained virus. On March 2, 1961, 150 clay pots were sown with these seeds, using two seeds per pot. After germination on March 8, the pots were divided into three groups and placed (a) in a greenhouse at 22 °C, (b) in a greenhouse at 15 °C, and (c) in the open. An average sample of freshly germinated seedlings, tested this same day by inoculation to 'Beka' beans, was found to contain virus. To test the plants for virus, each week 10 plants, taken at random from each series, were back-inoculated separately to four cucumber seedlings. The results obtained are given in table 4. These show that from the time of germination an average of 37 % of the plants was infected. No increase of infection occurred with time, and no differences in infection percentages could be observed between the different series. Thus no influence of conditions was noticed. The first symptoms were observed only a long time after germination: in series (a) 27 days after germination, in series (b) 68 days after germination, and in series (c) 47 days after germination. Summarizing it may be con-

TABLE 4. Results of back-inoculation tests with pea plants grown from infected seeds and performed on a series of dates after emergence. Date of sowing, March 2, 1961; date of emergence, March 8, 1961. Numerator: number of plants containing virus; denominator: number of plants tested.
Resultaten van de terug-inoculaties van erwteplanten, opgekweekt uit besmet zaad. Deze toetsingen werden verricht op een serie opeenvolgende data na opkomst. Zaaidatum 2 maart 1961, datum van opkomst 8 maart 1961. Teller: aantal virusbevattende planten; noemer: aantal getoetste planten.

Dates of testing *Toetsdata* / Series *Serie*	March *maart*		April *april*				May *mei*		Totals *Totalen*
	16	23	6	13	20	27	4	11	
a. Greenhouse 22 °C *Kas 22 °C*	7/10	3/10	6/10	5/10	0/10	4/10	6/10	1/10	32/80
b. Greenhouse 15 °C *Kas 15 °C*	4/10	1/10	4/10	3/10	5/10	3/10	6/10	2/10	28/80
c. Outdoors *Buiten*	3/10	1/10	5/10	4/10	1/10	5/10	4/10	6/10	29/80
Totals/*Totalen*	14/30	5/30	15/30	12/30	6/30	12/30	16/30	9/30	89/240
Percentages	47	17	50	40	20	40	53	30	37

cluded that not all infected seeds produce infected plants, that infection of plants from the seeds does not always lead to the production of symptoms, and that external conditions may play a role in the production of symptoms.

To check the possible role of other plant species in spreading the virus in their seeds, a few pilot experiments were made.

French bean plants, *Phaseolus vulgaris*, var. 'Beka', infected artificially, show under certain conditions systemic symptoms including distortion of the pods. Moreover, in our field trials, virus could sometimes be isolated from naturally infected plants (cf. table 2). Seeds harvested from artificially infected plants showing systemic symptoms, were therefore tested for virus by back-inoculating five groups of five seeds each, after soaking, to cucumber seedlings and 'Beka' plants. All results were negative.

Broad bean plants, *Vicia faba*, often show a high concentration of virus upon exposure to natural infection (cf. table 2), however, without producing symptoms. Seed transmission was therefore tested in this plant species. In the field 46 broad bean plants were mechanically inoculated on emergence. After five weeks three plants taken at random were tested individually for virus with positive results. Sixteen days after harvesting the mature pods, a sample of 10 soaked seeds was ground and inoculated to 10 cucumber seedlings. Seven local lesions were obtained, indicating the presence of some virus. A similar test with seeds obtained from our trial field on infested soil, gave negative results. One week later these tests were repeated with germs of seeds only. The results were again negative. After a further eleven days from the first-mentioned batch of seeds, 10 whole seeds were ground and from another 10 seeds the germs only were used. Inoculations with this material did not reveal any virus. To test this further, 9 seeds were sown in pots. Leaf samples of the resulting plants were tested twice, but without any indication of virus. The results of these experi-

ments suggest that broad bean plants, although infected systemically, rarely produce infected seeds.

To study the possibility of contaminating previously virus-free soil by sowing infected seeds, the following experiments were carried out; the first in the glasshouse, the second in the open.

In the first experiment in each of 10 clay pots containing sterilized soil, four virus-infected seeds (var 'Rondo') were sown (October 29, 1959). All seeds produced diseased plants. Back-inoculations to cucumber seedlings revealed the presence of the virus in all but two plants. Later (December 4, 1959) the same soil was sown with healthy pea seeds. None of the resulting plants showed any sign of infection. A second and similar test was performed in the open, in pots containing sterilized soil (diseased seeds sown May 18, 1961, healthy seeds September 13, 1961). No diseased plants were obtained from the healthy seeds nor could virus be demonstrated in these plants by means of back-inoculation. Thus the growing of diseased plants in sterilized soil did not lead to contamination of the soil.

In addition a number of field trials were carried out. Two experiments, in which samples containing infected seeds were sown on clay soils, resulted in some diseased plants in the one crop and no symptoms in the other. Crops derived from healthy seeds sown on these plots the following years showed no infection. In contrast, a plot of sandy soil sown with infected seeds yielded plants with distinct symptoms of the disease (36 plants per 25 m^2; 1961). Moreover, the following year, a crop grown from healthy seed on the same plot showed a high incidence of infection, whereas an adjacent plot sown with healthy pea seeds for the first time, did not produce any diseased plants. This test clearly proved the possibility of contaminating soil from infected seeds.

3. Sap-transmission tests

In the field early browning shows no evident spread during the season. Nevertheless tests were performed to check the possibility of virus transmission by contact between plants. From other work on the virus it was already known that it is easily sap-transmissible. In the case of cucumber cotyledons, sap transmission is possible even without using carborundum as an abrasive.

In one test leaves of a pea plant showing distinct disease symptoms were gently rubbed over the surface of cotyledons of cucumber seedlings, young leaves of pea plants and primary leaves of French bean seedlings without the use of an abrasive. Out of 10 cucumber seedlings 7 showed 30 characteristic local lesions. The pea and bean plants, however, did not produce any indication of virus infection. In another experiment, diseased pea plants were swept thoroughly through three rows of healthy pea plants, each row comprising 5 pots containing 4 pea plants per pot. None of the 60 pea plants became infected.

d. *Properties of the virus in plant sap*

Tolerance to dilution: Dilutions were made of sap from virus-containing cucumber cotyledons. Testing these dilutions by means of inoculation to cucumber seedlings, the dilution end-point of EBV was found to lie between 10^{-4} and 10^{-5} and of RV to be higher than 10^{-5}.

Thermal inactivation: Using sap from cucumber cotyledons the thermal inactivation point was estimated to be between 65 and 70 °C for EBV and between 70 and 75 °C for RV.

Ageing in vitro: Both EBV and RV were still infective in expressed tobacco sap after 147 days. In a later experiment with EBV only, the virus was still active in sap of pea plants after six months, but not after 8 months.

The data reported here suggest a high degree of similarity between the two viruses. Since virus properties in plant sap are rather variable, as was discussed by Bos et al. (1960), the slight difference in thermal inactivation point should not be given too much weight. Moreover the higher thermal inactivation point of RV may be connected with its higher concentration in cucumber cotyledons, suggested by the dilution end-points of the two viruses. The relativity of the importance of virus properties in plant sap for virus characterization is further demonstrated by the differences between the properties of RV mentioned above and those reported in the literature. According to CADMAN & HARRISON (1959) the dilution end-point of RV is 10^{-5} – 10^{-6}. These authors found the thermal inactivation point to be between 80 and 85 °C, whereas VAN DER WANT (1952) reported 75–80 °C. With an aster isolate of this virus, ZSCHAU (1958) found it to be between 70 and 80 °C. According to VAN DER WANT (1952), RV has an ageing *in vitro* of less than one month, whereas SCHMELZER (1957) found the virus to be still active after 260 days.

e. *Electron microscopy*

Preparations were made according to the dip-method. A check on the magnification of the electron microscope (Philips EM. 100) was obtained by adding polystyrene balls. Measurements were made following the technique by Bos et al. (1960).

The number of virus particles observed was generally very low. Moreover, there was usually considerable end-to-end aggregation of virus particles, which hindered measuring. In only a few preparations there was a sufficient number of discrete particles present (fig. 6). All the preparations showed a characteristic bimodal distribution. In one preparation, the mean lengths of the short and long components were estimated, after correction for the magnification of the microscope on the basis of the measured size of the polystyrene balls, as 112 and 225 mμ respectively. Measurements of other preparations led to the following averages: 104 and 217, 106 and 212, 112 and 225, 107 and 220, 88 and 200, 113 and 225 mμ[1]. On this basis the normal lengths of the particles were estimated to be about 105 and 210 mμ. In view of their variation these data may need further exact confirmation.

Morphologically the EBV and the RV closely resemble each other in several respects. In both cases the rods are short, rigid and thick and have bimodal distribution curves. The viruses differ, however, both in absolute lengths and in the ratio average length of short particles to average length of long particles. For RV the lengths are 70 and 180 mμ (PAUL & BODE, 1955) or 75 and 185 mμ

[1] Dr. J. BRANDES, Institut für landwirtschaftliche Virusforschung der B.B.A., Braunschweig, Germany, was so kind as to check our measurements. Measurement of the last but one preparation led to his estimation of normal lengths 98 mμ and 209 mμ. Measurements of 85 particles in a preparation made by himself gave normal lengths of 102 and 205 mμ respectively.

(HARRISON & NIXON, 1959) and the ratio about 1:2.6. For EBV the particles are longer and the ratio is very close to 1:2.

f. *Serology*

Serological experiments were carried out in the Institute of Phytopathological Research by Mr. D. Z. MAAT with material provided by us. No serological relationship could be demonstrated between the two viruses with low-titre antisera available so far. Detailed results of these investigations by MAAT will be published shortly.

g. *Discussion of results*

The early-browning disease of peas has many features in common with diseases in other crops caused by the tobacco rattle virus, such as in potato (potato stem mottle, ROZENDAAL (1947), and ROZENDAAL & VAN DER WANT (1948)) and in bulbous ornamentals (VAN SLOGTEREN, 1958).

From the host-range tests it was concluded that the pea early-browning virus and tobacco rattle virus have many hosts in common. In many cases these hosts even react similarly. In other cases there are characteristic differences, however, and it is possible to distinguish the viruses easily on the basis of reactions on *Phaseolus vulgaris* and *Pisum sativum*.

In their transmission the EBV and RV again closely resemble each other. Both viruses are soil-borne. SOL et al. (1960) and SOL & SEINHORST (1961) could demonstrate the transmission of RV by the nematode *Trichodorus pachydermus* Seinhorst. The short persistence of virus in soil contained in pots in our greenhouse experiments and the fact that no contamination of sterilized compost soil could be demonstrated, points to the existence of a soil-borne vector of the EBV. Recently VAN HOOF (1962) has shown that *Trichodorus pachydermus* and *T. teres* Hooper both act as vectors of this virus. In peas the EBV is seed-transmitted. Since making this observation in 1958, seed transmission to a low percentage of the progeny of infected plants of shepherd's purse has been reported for RV (CADMAN & LISTER, 1961).

Tests regarding dilution end-point, thermal inactivation point and ageing *in vitro* did not reveal any characteristic differences between the two viruses.

The results discussed so far suggest that the EBV might be a strain of RV, differing slightly from the type-virus in host range and symptomatology and being especially adapted to infect pea plants. Critical evidence of the existence of basic differences between the two viruses, however, was provided by the electron microscope studies. The viruses resemble each other in gross morphology, and in having a bimodal distribution curve, but differ in absolute lengths and in their respective ratio length of short particles to length of long particles.Serology later confirmed the difference between the viruses (MAAT, to be published).

From these data it can be concluded that there is a group of at least two morphologically and serologically different, although morphologically and biologically closely related, soil-borne viruses of the rattle-type. In this respect the situation resembles that of the ringspot group of soil-borne viruses, which are biologically related but differ in intrinsic properties. Future detailed serological tests with high-titre antisera may reveal distant serological relationships

between EBV and RV as was demonstrated for other groups of morphologically related viruses by WETTER & QUANTZ (1958) and later also by BERCKS (1960). The American and Japanese soil-borne wheat mosaic viruses, having lengths of 130 mμ (GOLD et al., 1957) and 150–160 and 170 mμ (SAITO et al., 1961) respectively, and the seed-borne barley stripe mosaic virus (130 mμ) (GOLD et al., 1954), might also belong to the rattle virus group.

Symptomatologically the early-browning disease of peas has several features in common with the many so-called "streak virus diseases" of pea described in the literature, all of which are characterized by more or less severe necrosis occurring in purplish-brown or black "streaks" on stems, petioles and veins. Sometimes parts of leaves or entire leaves die, leading to the death of the whole plant. The name "streak" for these diseases is incomplete since the term as such does not indicate whether the streaking or striping is necrotic or chlorotic.

In the U.S.A. in particular, several "streak" diseases of pea have been described. The following viruses were reported as incitants of "streak" in peas: alfalfa mosaic virus (ZAUMEYER, 1938), cucumber mosaic virus (WHIPPLE & WALKER, 1941), "Wisconsin pea streak" virus (HAGEDORN & WALKER, 1949), red clover vein-mosaic virus and beet mosaic virus (QUANTZ, 1958), "Minnesota pea streak" virus and "P.O. streak" virus (KIM & HAGEDORN, 1959), "a new virus related to *the* pea streak virus" (ZAUMEYER & PATINO, 1959), red clover vein-mosaic virus in complex with bean yellow mosaic virus (SCHROEDER et al., 1959) and a virus related to tobacco ringspot virus or to alfalfa mosaic virus (SCHROEDER et al., 1960). Although in many cases the causative viruses have not been identified in detail, the information so far available indicates that all these diseases differ etiologically and, in particular, epidemiologically from the early-browning disease. None of them has been shown to be seed- and soil-borne.

The epidemiology of early browning is not yet fully understood. The results of the investigations show that soil-transmission is one important factor. In the Netherlands six to eight years elapse at a minimum between two successive pea crops. Nevertheless new pea crops often show severe infection. On the other hand contaminated soil kept in pots for only a few months appeared to lose its infectivity. The long persistence of virus in the soil in the field is thus evidence of the role played by other cultivated and wild hosts. Whether or not the virus may persist in the nematode vectors is not yet known.

LISTER (1960) presumed that the spread of virus through seeds may have a wide application among soil-borne viruses, and CADMAN & LISTER (1961) further suggested that seed-transmission might be a characteristic feature of some kinds of soil-borne viruses. Early browning of pea seems to be a typical example of a seed-transmitted, soil-borne virus. Thus far, pea is the only host in which seed-transmission of the virus is known to occur to any appreciable extent. However, seed-transmission in other hosts also seems likely.

Infection of healthy plants by contact with plants infected from seed or from the soil, if it occurs, presumably does not play a major role in spreading the disease. In the field the disease occurs in patches and is always associated with pockets of light soil, the infection never extending noticeably beyond these pockets. We think therefore, that aboveground transmission of the virus, e.g. by insects, is not important. Moreover, insect-transmission has never yet been demonstrated for any of the other soil-borne viruses. Thus a low percentage

of infected seeds seems unlikely to result in serious damage to a crop. Seed infection is only a menace in soils "receptive" to the virus. The only explanation available so far for the wide distribution of the EBV in light-type soils appears to be that of dissemination in the seeds.

CONTROL OF THE DISEASE

The most important, and in most cases the only means of control of virus diseases is their prevention. One of the first preventive methods is the use of highly resistant or, if possible, immune varieties. All varieties of pea at present grown in the Netherlands appear to be susceptible (cf. pea varietal reaction). The presence of some resistance to the virus in a number of garden-pea varieties suggested the possibility of improving resistance by breeding. Since 1959 a breeding programme has been in progress in the Netherlands, involving a number of private breeders and also the Institutes of Plant Breeding and Horticultural Plant Breeding, in co-operation with the Plant Disease Resistance Department of the Institute of Phytopathological Research. The results obtained so far will shortly be published by HUBBELING and KOOISTRA.

Another way of prevention is that of avoiding sources of infection. Susceptible, or even partially resistant varieties should not be grown on fields contaminated with the virus, since they may help to maintain or increase the level of virus in that soil. For the same reason, no other susceptible crops, even those showing no symptoms, such as broad bean, alfalfa, black medick, red clover and presumably white clover and French bean, should be grown on contaminated fields. Moreover weeds, which often thrive in those patches where the pea plants suffer from the disease, should be removed, since some of them are also susceptible to the virus (table 2). Although as yet no proof is available, these susceptible cultivated plants and weeds might be important in spreading the virus through their seeds.

As the virus inciting stem mottle in potato and the early-browning virus are both known to occur in the same type of sandy clay soils, it might happen that the two are present together in the same field. The presence of rattle virus alone is not a menace to peas since this virus has not been found to be capable of attacking peas. Neither is there reason to believe that the presence of early-browning virus alone is dangerous to potatoes susceptible to stem mottle.

Until more information is available concerning the virus vector and its biology, no cultural methods to control the vector or to reduce soil-transmission can be advised (cf. also VAN HOOF, 1962).

At present the only means of avoiding sources of infection is by the use of virus-free pea seeds. For this reason the General Netherlands Inspection Service for Seeds of Field Crops and for Seed Potatoes (N.A.K.) started as early as 1959 seriously to inspect for the presence of early-browning virus in pea crops presented by farmers for certification. If more than one diseased plants occurs per are (100 m^2) the entire field is rejected. A few diseased plants occurring sporadically lead to a lowering of the grade of health. Possibilities for checking seed lots for the presence of virus are at present under investigation. Since infected seeds may not only give rise to diseased plants, but may also bring about soil contamination (if the soil is "receptive"), the use of certified seed with a high grade for health is recommended.

Summary

A description is given of a virus disease of pea called "early browning", which is characterized by irregularly distributed necrosis of stems, petioles, leaf veins, interveinal tissue and pods.

Because of certain similarities with tobacco rattle virus the early-browning virus was compared with the former. Like rattle virus it has a wide experimental and natural host range. In a number of hosts the symptoms produced by the two viruses are either identical or only quantitatively distinguishable. Some species, however, show characteristic differences, in which connection *Phaseolus vulgaris* and *Pisum sativum* have proved of particular value as differential hosts.

It was demonstrated that the virus may be soil-borne in areas of sandy clay and that it is seed-borne in peas. The virus could be recovered from a number of plant species, especially from legumes and from some weeds growing on contaminated soil. Sometimes the presence of the virus could be demonstrated only in the underground parts of these plants. Except for peas, no other susceptible species ever showed symptoms following natural infection. No virus could be recovered from cereals, *Stellaria media* or potato varieties known to be susceptible to rattle virus.

The dilution end-point of the early-browning virus in sap of infected pea and tobacco plants was found to be 10^{-4} to 10^{-5}. Its thermal inactivation point is 65–70 °C and longevity *in vitro* 6 to 8 months. These data closely resemble those for rattle virus.

Morphologically the viruses resemble each other in that both are relatively short, thick rods with a bimodal length distribution curve. The early-browning virus differs from rattle virus, however, in that its particles are longer (about 105 and 210 mμ). The ratio of length of short particles to length of long particles (1 : 2) also differs from that of rattle virus.

In order to control early-browning disease it is suggested that no susceptible pea varieties or other susceptible crops should be grown on contaminated soil. Efficient weed control is recommended.

The use of virus-free pea seed is advocated. As early as 1959 the General Netherlands Inspection Service for Seeds of Field Crops and for Seed Potatoes (N.A.K.) extended its inspection of peas grown for seed to include the early-browning disease.

Samenvatting

Sinds 1949 is in het noord- en zuidwesten van Nederland een incidenteel voorkomende, doch potentieel belangrijke erwteziekte bekend die, naar uit het hier beschreven onderzoek blijkt, door een virus wordt teweeggebracht. Bij dit onderzoek is voornamelijk aandacht besteed aan het in Nederland meest geteelde ras 'Rondo'. De ziekte werd onlangs ook in het oosten van Engeland waargenomen.

De verschijnselen (fig. 1) bestaan uit paarsbruine, onregelmatig over de plant verdeelde verkleuringen. Ter plaatse is het weefsel afgestorven. Deze necrose begint in de vaatbundels van de stengel en zet zich van hieruit onregelmatig voort in de bladstelen en de nervatuur. Hierdoor kan het tussennervige blad-

weefsel afsterven en kan zelfs topsterfte optreden. Soms herstellen de planten zich min of meer. Laat in het seizoen vertonen de peulen vaak paarsbruine, necrotische vlekken en kringen. De zaden zijn dan dikwijls vlekkerig en licht gekreukt (fig. 2).

De zieke planten kunnen verspreid over een perceel erwten voorkomen, maar meestal bevinden zij zich pleksgewijs bijeen (fig. 3). Als gevolg hiervan doet de aantasting zich als een plaatselijke verbruining van het gewas voor. Aangezien de verschijnselen betrekkelijk vroeg in het seizoen optreden, werd de ziekte „vroege verbruining van erwt" genoemd.

Daar de ziekte in haar voorkomen enige overeenkomst vertoont met de door ratelvirus veroorzaakte ziekten bij tabak en aardappel, werd een uitvoerig onderzoek verricht ter vergelijking van het vroege-verbruiningsvirus en het ratelvirus. Ook was dit van belang omdat het erwtevirus met sap onder meer op tabak kan worden overgebracht en het op deze plant symptomen veroorzaakt die sterk aan de verschijnselen van de ratelziekte doen denken.

Evenals ratelvirus heeft het vroege-verbruiningsvirus een uitgebreide experimentele en natuurlijke waardplantenreeks (tabellen 1 en 2). Bij enige waardplanten, zoals komkommer (fig. 4) is er geen duidelijk verschil in reactie tussen beide virussen. Bij andere planten is het verschil in symptomen slechts van kwantitatieve aard. Een beperkt aantal soorten gaven echter karakteristieke verschillen te zien. Het erwtevirus kon slechts nu en dan met moeite op tomaat worden overgebracht, hoewel het ratelvirus daarop gemakkelijk duidelijke systemische symptomen geeft. Boon (*Phaseolus vulgaris*) en erwt reageren zeer verschillend op de twee virussen (fig. 5).

Met pot- en veldproeven werd aangetoond dat infectie via de grond plaatsheeft (tabel 3). Gedurende de jaren 1958–1960 werd de vatbaarheid voor natuurlijke infectie van een aantal plantesoorten op met vroege-verbruiningsvirus besmette grond getoetst. Behalve bij erwten werden nooit ziekteverschijnselen waargenomen. De resultaten van het onderzoek op de aanwezigheid van het virus in afzonderlijke boven- en ondergrondse delen der betrokken planten zijn in tabel 2 weergegeven. In vlinderbloemigen, vooral in tuinboon (*Vicia faba*) en luzerne (*Medicago sativa*) werd het vroege-verbruiningsvirus herhaaldelijk aangetroffen. Soms bleken alleen de wortels geïnfecteerd te zijn, zoals bij witte klaver (*Trifolium repens*). Het virus kon nooit worden aangetoond in wortels van granen of van muur (*Stellaria media*) en van een drietal aardappelrassen, die bekend staan als vatbaar voor ratelvirus. Aldus werd aangetoond dat er geen verband bestaat tussen vroege verbruining bij erwt en stengelbont bij aardappel.

Het virus bleek met het zaad van zieke erwten te kunnen overgaan. In kiemplanten die uit besmet zaad opgroeiden, kon in verscheidene gevallen het virus direct na opkomst worden aangetoond (tabel 4), doch de symptomen traden pas later op. Het gelukte de aanwezigheid van het virus in de zaden aan te tonen door ze een nacht in water te weken, ze daarna fijn te wrijven en met de aldus verkregen brij jonge zaadlobben van komkommerplantjes te inoculeren. Besmette zaden bleken de reeds genoemde afwijkingen te vertonen.

Het uitzaaien van virusbevattend zaad bleek alleen op zandpercelen tot een besmetting van de grond te leiden. Op kleigrond of op door middel van warmte gesteriliseerde grond lukte dit niet. Wees dit reeds op de mogelijkheid van het

bestaan van een ondergrondse vector van het virus, door VAN HOOF (1962) is inmiddels aangetoond, dat bepaalde nematode-soorten als zodanig fungeren.

Er werden geen aanwijzingen verkregen voor het bestaan van bovengrondse virusoverbrengers (insekten) of voor de verspreiding van het virus in het gewas als gevolg van contact van zieke en gezonde planten.

De verdunningsgrens van het vroege-verbruiningsvirus in sap van besmette erwten bleek te liggen bij 10^{-4} tot 10^{-5}. De inactiveringstemperatuur bleek 65 tot 70 °C te bedragen en het virus kon bij kamertemperatuur 6 tot 8 maanden in sap worden bewaard. Deze gegevens vertonen veel overeenkomst met die, welke in de literatuur voor ratelvirus zijn vermeld.

Morfologisch vertonen het vroege-verbruiningsvirus en het ratelvirus veel gelijkenis (zie fig. 6 voor het eerste virus). Beide bestaan uit korte, dikke, rechte deeltjes; de frequentiedistributiekromme van de lengte van deze deeltjes is voor beide virussen tweetoppig. De deeltjes van het vroege-verbruiningsvirus zijn echter langer (gemiddeld ongeveer 105 en 210 mμ) dan die van ratelvirus (volgens recente literatuurgegevens 70 en 180 mμ). Uit deze cijfers blijkt dat de verhouding van de korte tot de lange deeltjes bij het erwtevirus 1:2 is en bij het ratelvirus 1:2,6.

MAAT (publikatie in voorbereiding) kon met door hem verkregen antisera van lage titer tegen ratelvirus en vroege-verbruiningsvirus geen serologische verwantschap tussen deze virussen aantonen.

Op grond van de verkregen gegevens moet worden geconcludeerd, dat het vroege-verbruiningsvirus en het ratelvirus twee verschillende virussen zijn, ofschoon zij epidemiologisch en naar hun intrinsieke eigenschappen verwantschap vertonen.

Ter bestrijding van de ziekte is het gewenst geen erwten en andere vatbare gewassen te verbouwen op besmet bevonden grond. Het is belangrijk op besmette percelen bijzondere aandacht te besteden aan de onkruidbestrijding. Het gebruik van virusvrij erwtezaad is noodzakelijk. Daarom wordt door de Nederlandse Algemene Keuringsdienst voor Landbouwzaden en Aardappelpootgoed (N.A.K.) reeds sinds 1959 gekeurd op het voorkomen der ziekte in erwten, geteeld voor zaaizaad.

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FIG. 1. Symptoms of early browning in pea (*Pisum sativum* L.) var. 'Rondo' after natural infection. (Drawing by M. P. van der Schelde).

Symptomen van vroege verbruining in erwt (Pisum sativum *L.*) ras '*Rondo*' *na natuurlijke infectie. (Tekening van M. P. van der Schelde).*

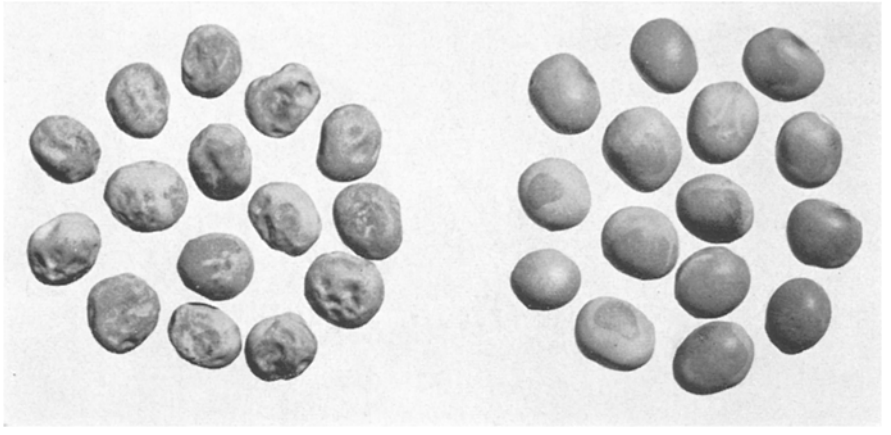

FIG. 2. Symptoms of early browning in pea seed, var. 'Rondo'. Healthy seeds at right.
Symptomen van vroege verbruining in erwtezaad, ras 'Rondo'. Rechts gezonde zaden.

FIG. 3. Early browning, occurring in spots in a pea field.
Vroege verbruining, pleksgewijs voorkomend in het veld.

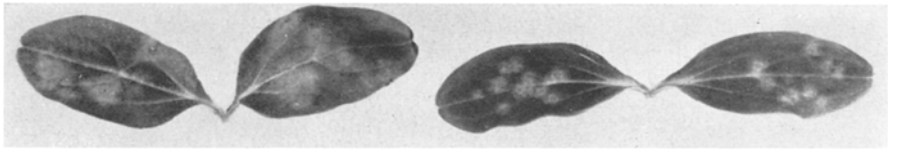

FIG. 4. Local reaction of cucumber cotyledons, var. 'Gele tros', to mechanical inoculation with early-browning virus (right) and rattle virus (left).
Lokale reactie van komkommercotylen, ras 'Gele tros', op mechanische inoculatie met vroege-verbruiningsvirus (rechts) en ratelvirus (links).

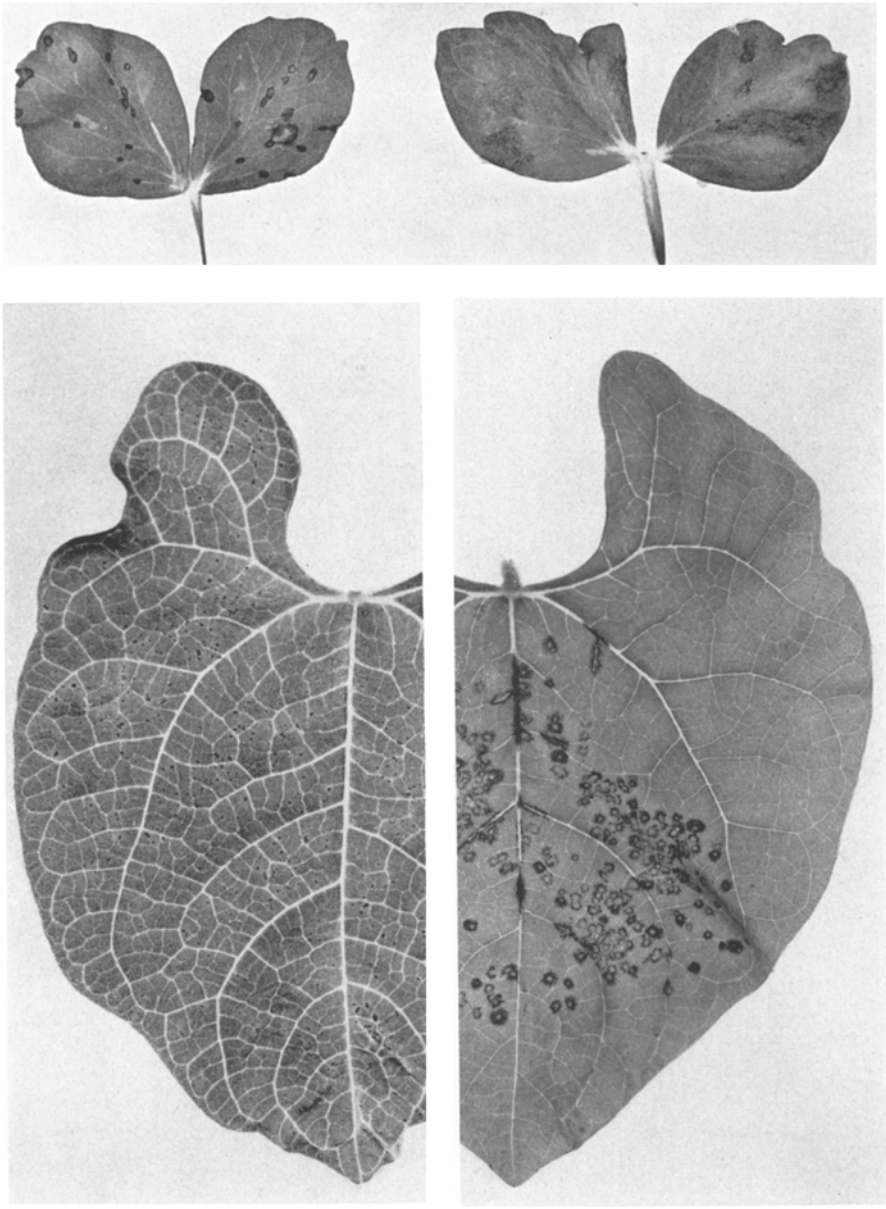

FIG. 5. Local reaction of pea leaflets, var. 'Mansholt's Pluk' (above), and primary leaves of bean, var. 'Beka' (below), to mechanical inoculation with early-browning virus (right) and rattle virus (left).

Lokale reactie van erwteblaadjes, ras 'Mansholt's Pluk' (boven), en enkelvoudige bladeren van boon, ras 'Beka' (onder), op mechanische inoculatie met vroege-verbruiningsvirus (rechts) en ratelvirus (links).

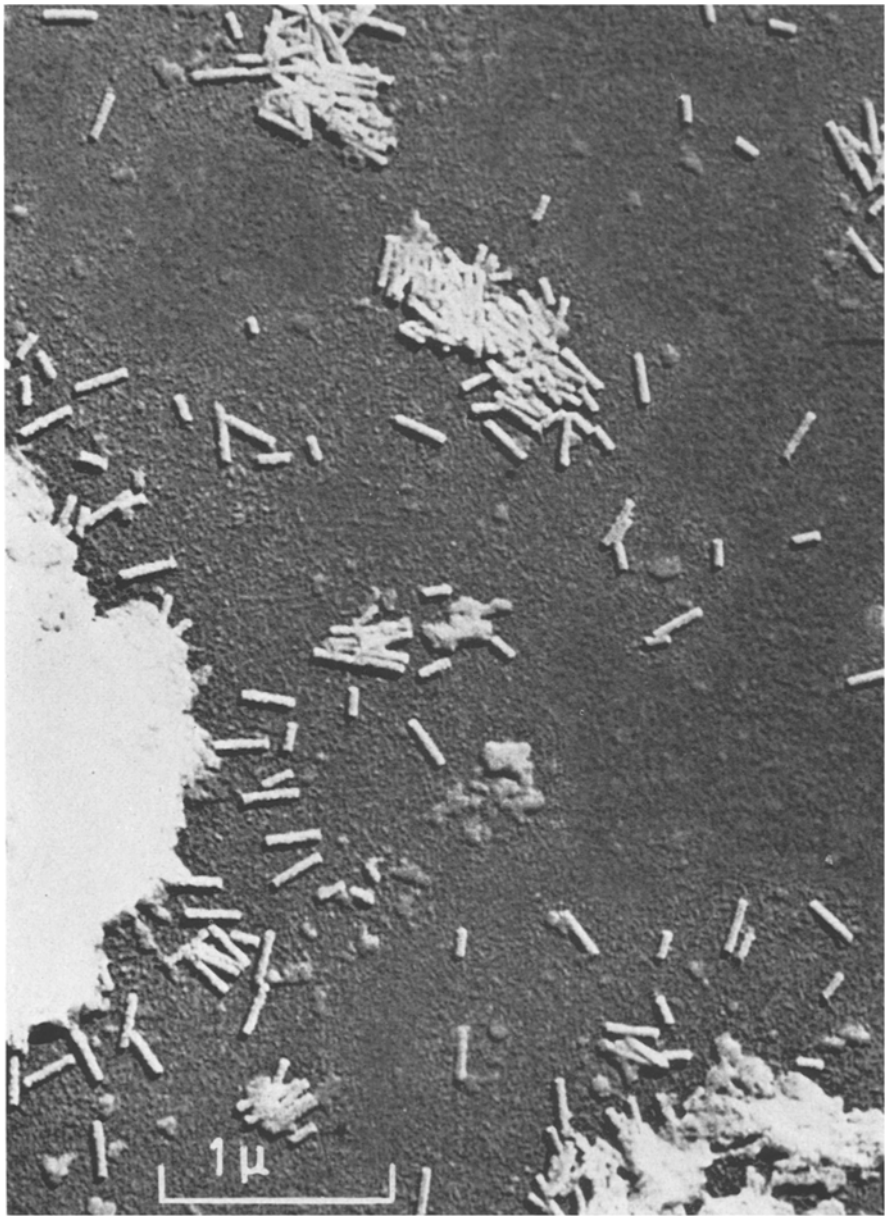

FIG. 6. Electron micrograph of a preparation of the early-browning virus obtained from infected pea by the dip method. Magnification × 30,000. (Photograph made by the Physico-Technical Service for Agriculture, Wageningen).

Elektronenfoto van een preparaat van het vroege-verbruiningsvirus, verkregen van een geïnfecteerde erwteplant door middel van de indoopmethode. Vergroting 30.000 ×. (Foto gemaakt door de Landbouw Fysisch-Technische Dienst, Wageningen).