

TRANSMISSION OF RATTLE VIRUS AND *ATROPA*
BELLADONNA MOSAIC VIRUS BY NEMATODES¹

*Met een samenvatting: Over de overbrenging van ratelvirus en van mozaïekvirus
van Atropa belladonna door aaltjes*

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Among the many characters rattle virus and *Atropa belladonna* mosaic virus (*Belladonna* mosaic virus, SMITH, 1946, 1957) have in common, is that of being soil-borne and attacking the roots of tobacco grown in infected soil. They differ in that rattle virus may or may not pass into the stems and leaves after such an infection, whereas *Atropa belladonna* mosaic virus always remains confined to the roots.

Because these two viruses are soil-borne and have other features in common, similar experiments were done to test possible transmission by nematodes. In these experiments nematodes from infected soil were transferred to uninfected soil and to previously infected soil in which the nematodes had been killed. Elimination of nematodes was attempted by heating at 60°C or 120°C or by rubbing thin layers of soil with a block of wood on a flat concrete tile, to avoid drying out the soil. By the latter method only nematodes and organisms of equal or larger size are destroyed. However, not all nematodes were dead in the soil treated in this way for use in inoculation experiments.

Sources of nematodes were a light sandy soil from Lisse infected with rattle virus and a sandy soil from Baarn infected with *Atropa belladonna* mosaic virus. Both soils contained several species of tylenchids and dorylaims but no *Xiphinema*.

Nematodes were collected from these soils by elutriation and sieving (SEINHORST, 1956). The nematode suspensions obtained were practically free from other animals. Neither insects nor spiders were seen but in a few cases some rotatorians and tardigrades were present. The number of soil particles in the suspensions used for inoculation was very small.

After the different treatments, the soil to be inoculated was put in small flowerpots or plastic bags (the contents of each was 150 to 250 g). Suspensions of nematodes collected from 500 g of soil were added to each of the pots or bags to be inoculated. Usually not more than eight hours elapsed between the beginning of the extraction of nematodes from the soil and their placement in the pots or bags.

After inoculation of the soil with nematodes, one tobacco plant was planted in each pot or bag. In the experiments with *Atropa belladonna* mosaic virus the roots of these plants were pressed out ten days after planting. The sap was rubbed with carborundum on leaves of healthy tobacco plants to determine the

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presence or absence of virus. In experiments with rattle virus this was done only with plants which still did not show symptoms in the leaves thirty days after planting.

TABLE 1. Transmission of *Atropa belladonna* mosaic virus by nematodes from infected soil.
Overdracht van mozaïekvirus van Atropa belladonna door nematoden uit geïnfecteerde grond.

Soil used in experiment <i>Grond gebruikt in proef</i>	Treatment of soil <i>Behandeling van de grond</i>	Inoculated with <i>Geïnoculeerd met</i>	Results Number of tobacco plants <i>Aantal tabaksplanten</i>	
			healthy <i>gezond</i>	with virus <i>met virus</i>
infected soil <i>geïnfecteerde grond</i>	—	—	1	24
” ”	rubbed <i>gewreven</i>	—	3	2
” ”	”	nem. from uninfected soil <i>nem. uit gezonde grond</i>	4	1
” ”	”	nem. from infected soil <i>nem. uit geïnfecteerde grond</i>	none <i>geen</i>	5
uninfected soil <i>gezonde grond</i>	”	—	15	none <i>geen</i>
” ”	”	nem. from uninfected soil <i>nem. van gezonde grond</i>	10	none <i>geen</i>
” ”	”	nem. from infected soil <i>nem. van geïnfecteerde grond</i>	2	3
potting compost <i>potgrond</i>	sterilized <i>gestoomd</i>	—	10	none <i>geen</i>
”	”	nem. from infected soil <i>nem. van geïnfecteerde grond</i>	none <i>geen</i>	10

The results of experiments with nematode suspensions from soil infected with *Atropa belladonna* mosaic virus are given in table 1; those of experiments with nematode suspensions from soil with rattle virus in table 2. In none of the experiments was virus found in tobacco plants grown in soil free of nematodes. On the other hand when nematodes from infected soil were transferred to soil made nematode free or nematode and virus free, or to uninfected natural soil or to sterilized potting compost the tobacco grown in these soils often became heavily infected with virus. It is very improbable that, in these experiments, the virus was transmitted with soil particles, bacteria or fungi. Otherwise rubbing of the infected soil could not have caused such a marked reduction in the incidence of virus infection as compared to untreated soil. Therefore it must be concluded that nematodes were the vectors of *Atropa belladonna* mosaic virus and rattle virus.

Inoculation of virus- and nematode free soil with *Hoplolaimus uniformis* and *Hemicycliophora* sp. collected from nematode suspensions, which had been extracted from soil infected with rattle virus, did not result in infection of tobacco plants by this virus. The remainder of these suspensions was infective, however. Therefore it is improbable, that *Hoplolaimus uniformis* or *Hemicy-*

TABLE 2. Transmission of rattle virus by nematodes from infected soil.
Overdracht van ratelvirus door nematoden uit geïnfecteerde grond

Soil used in experiment <i>Grond gebruikt in proef</i>	Treatment of soil <i>Behandeling van de grond</i>	Inoculated with <i>Geïnoculeerd met</i>	Results Number of tobacco plants <i>Aantal tabaksplanten</i>	
			healthy <i>gezond</i>	with virus <i>met virus</i>
infected soil <i>geïnfecteerde grond</i>	— rubbed <i>gewreven</i>	—	2 14	13 1
" "	heated at 60°C for 10 min. <i>gedurende 10 min.</i> " " " " <i>op 60°C verhit</i>	—	2	none <i>geen</i>
" "	" "	nem. from infected soil <i>nem. uit geïnfecteerde grond</i>	none <i>geen</i>	2
" "	sterilized <i>gestoomd</i>	—	2	none <i>geen</i>
" "	" "	nem. from infected soil <i>nem. uit geïnfecteerde grond</i>	none <i>geen</i>	8
" "	" "	" "	3	3
mixture of infected soil and potting compost (1:1) <i>mengsel geïnfecteerde grond en</i> <i>potgrond (1:1)</i>	" "	—	2	none <i>geen</i>
" "	" "	nem. from infected soil <i>nem. uit geïnfecteerde grond</i>	none <i>geen</i>	6
infected soil <i>geïnfecteerde grond</i>	" "	<i>Hoplolaimus uniformis</i> from infected soil, 16–42 nem. per bag	5	none <i>geen</i>
" "	" "	<i>Hoplolaimus uniformis</i> uit <i>geïnfecteerde</i> <i>grond</i> , 16–42 <i>nem. per zakje</i>	5	none <i>geen</i>
" "	" "	<i>Hemicycliophora</i> sp. from infected soil, 20–70 nem. per bag	1	4
" "	" "	<i>Hemicycliophora</i> sp. uit <i>geïnfecteerde</i> <i>grond</i> , 20–70 <i>nem. per zakje</i>		
" "	" "	nem. from infected soil minus <i>H. unifor-</i> <i>mis</i> and <i>Hemicycliophora</i> sp. <i>nem. uit geïnfecteerde grond zonder H. uni-</i> <i>formis en Hemicycliophora</i> sp.		

cliophora sp. are vectors of the rattle virus. Experiments with other nematode species are in progress.¹

SAMENVATTING

Met ratelvirus en mozaïekvirus van *Atropa belladonna*, twee op elkaar gelijkende grondvirussen, werden proeven gedaan over een mogelijke overbrenging door aaltjes.

Nematoden uit met virus besmette grond werden toegevoegd aan natuurlijke, onbesmette grond, aan tot 60°C of 120°C verhitte en aan gewreven grond. Door wrijven van de grond worden de meeste aaltjes en andere organismen van dezelfde afmetingen gedood. In de aldus behandelde en geïnoculeerde grond werden tabaksplanten geteeld. Bij de proeven met mozaïekvirus van *Atropa belladonna*

¹ Recent experiments by H. H. SOL and J. W. SEINHORST indicate, that *Trichodorus pachydermus* SEINHORST is a vector of rattle virus.

werden tien dagen na het planten de wortels uitgeperst en het sap met carborundum uitgewreven op bladeren van gezonde tabaksplanten. Bij de proeven met ratelvirus werd dit alleen gedaan met planten, die dertig dagen na het planten geen symptomen vertoonden.

Virusinfectie trad niet op in de proeven, waar aaltjes niet de overbrengers konden zijn. Aaltjes uit besmette grond brachten in zeer veel gevallen virus naar onbesmette grond over (tabellen 1 en 2).

Hoplolaimus uniformis en *Hemicycliophora* sp. brengen het ratelvirus waarschijnlijk niet over. De proeven worden voortgezet met andere soorten aaltjes.

REFERENCES

- SEINHORST, J. W., - 1956. The quantitative extraction of nematodes from soil. *Nematologica* 1: 249-268.
SMITH, K. M., - 1946. Virus diseases of farm and garden crops. Worcester, (England), p. 92.
SMITH, K. M., - 1957. A textbook of virus diseases. 2nd ed. London, p. 105-106.