

A QUICK AND EFFICIENT INOCULATION METHOD FOR THE SAP-TRANSMISSION OF VIRUSES FROM WOODY TO HERBACEOUS HOSTS¹

Met een samenvatting: Een snelle en efficiënte methode voor de overdracht van virussen van houtige naar kruidachtige waardplanten

BY

TH. M. BERG

Laboratorium voor Bloembollenonderzoek, Lisse

In recent investigations on a virus disease of poplars (BERG, 1962) attempts to transmit the causal virus to herbaceous hosts by means of the "fresh leaf disc method" (YARWOOD, 1953) were successful. Additional experiments were conducted to see whether it was possible to demonstrate the virus in the inner bark of the diseased poplars by a direct inoculation method.

In this trial different cultivars of *Populus canadensis* MOENCH (Section *Aigeiros* Duby) were used. Inoculation pieces, about 20 cm long, were cut out of branches bearing leaves with symptoms. A few drops of a 0.01 M phosphate buffer pH 8 were pipetted onto the upper surface of leaves of the test plants, which had been dusted previously with carborundum. In this case the primary leaves of cowpea (*Vigna sinensis* Endl. var. 'Black') served as the local lesion host.

After the leaves had been removed from the inoculation piece the outer cortex was peeled off with a sterilized knife (fig. 1A). By so doing the inner green bark was exposed. The inoculation was then performed by wiping this fresh green surface over the test leaves (fig. 1B). Immediately following inoculation the leaves were thoroughly washed with water.

Upper parts of branches from which the outer cortex could be easily peeled off, provided the best inoculation material. Using this method of inoculation the virus could be detected in diseased mother trees not only during the growing season but also during the winter rest period.

Investigations were made to determine whether this inoculation method could be applied to other tree viruses. Therefore inoculation pieces were cut out of branches of 14 different woody hosts, which were known to be virus-diseased. This inoculation material was cut in February at the end of the dormancy period. The phosphate buffer used had a pH of 7.5. In this preliminary experiment transmission could be obtained in 50 percent of the tested virus-diseased trees (table 1). The inoculation with four *Prunus* species and one each of *Rosa* X 'Circus', *Syringa vulgaris* L. and *Ribes sanguineum* Pursh proved unsuccessful. In the case of the *Prunus* species difficulty was encountered in peeling off the outer cortex, which might account for this negative result.

It seems possible to obtain virus transmission from trees to herbaceous plants by the above described method where conventional methods are unsuccessful.

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TABLE 1. Results of virus transmission experiments from woody to herbaceous hosts.
Resultaten van virusoverbrengingsproeven van houtige naar kruidachtige planten.

Herbaceous hosts <i>Kruidachtige waard-planten</i>	Symptoms on herbaceous hosts caused by inoculation with: <i>Symptomen op kruidachtige waardplanten, veroorzaakt door inoculatie met:</i>		
	<i>Hydrangea macrophylla</i> D. C.	<i>Forsythia intermedia</i> Zab. var. 'Spectabilis'	<i>Ligustrum obtusifolium</i> Sieb. et Zucc. var. 'Regulianum'
<i>Nicotiana tabacum</i> L. var. 'White Burley'	— ¹	local and systemic ring symptoms after 6–10 days <i>lokale en systemische ringsymptomen na 6–10 dagen</i>	—
<i>N. tabacum</i> L. var. 'Samsun'	—	local and systemic ring symptoms after 6–20 days <i>lokale en systemische ringsymptomen na 6–20 dagen</i>	—
<i>N. Debneyi</i> Domin.	—	local ring symptoms after 6 days <i>lokale ringsymptomen na 6 dagen</i>	—
<i>Chenopodium amaranticolor</i> Coste & Reyn	local lesions after 10 days <i>lokale lesies na 10 dagen</i>	local lesions; systemic after 15 days <i>lokale lesies; systemisch na 15 dagen</i>	no local symptoms; systemic after 14 days <i>geen lokale symptomen; systemisch na 14 dagen</i>
<i>Gomphrena globosa</i> L.	local lesions after 7 days <i>lokale lesies na 7 dagen</i>	—	—
<i>Cucumis sativus</i> L.	—	local light spots; systemic after 14 days <i>lokale lichte vlekken; systemisch na 14 dagen</i>	local light spots; systemic after 14 days <i>lokale lichte vlekken; systemisch na 14 dagen</i>
<i>Vigna sinensis</i> Endl. var. 'Black'	—	red local lesions; systemic after 14 days <i>rode lokale lesies; systemisch na 14 dagen</i>	—
<i>Petunia hybrida</i> Vilm.	—	severe local necrotic and ring symptoms; systemic after 8 days <i>ernstige lokale necrotische en ringsymptomen; systemisch na 8 dagen</i>	—
Conclusions <i>Conclusies</i>	transmitted virus probably hydrangea ring-spot virus (BRIERLEY & LORENTZ, 1957) <i>overgebracht virus waarschijnlijk het hydrangea-ringspot-virus</i>	probably more than one virus transmitted <i>mogelijk meer dan een virus overgebracht</i>	—

¹ No reaction was observed on this test plant.
Geen reactie gezien op deze toetsplant.

<i>Daphne mezereum</i> L. var. 'Grandiflora'	<i>Sambucus rasemosa</i> L.	<i>Laburnum anagyroides</i> Med.	<i>Ribes rubrum</i> L.
—	—	—	ringspot symptoms; systemic after 10 days <i>ringvlekken-symptomen;</i> <i>systemisch na 10 dagen</i>
local lesions after 10 days <i>lokale lesies na 10 dagen</i>	chlorotic spots; syste- mic after 14 days <i>chlorotische vlekken;</i> <i>systemisch na 14 dagen</i>	no local symptoms; systemic after 14 days <i>geen lokale symptomen;</i> <i>systemisch na 14 dagen</i>	local ring symptoms <i>lokale ringsymptomen</i>
—	—	—	local lesions; systemic after 14 days <i>lokale lesies; systemisch</i> <i>na 14 dagen</i>
—	—	—	local light spots; syste- mic after 14 days <i>lokale lichte vlekken;</i> <i>systemisch na 14 dagen</i>
—	—	—	local ring symptoms; systemic after 14 days <i>lokale ringsymptomen;</i> <i>systemisch na 14 dagen</i>
—	—	—	transmitted virus pro- bably "lepelblad virus" (VAN DER MEER, 1960) <i>overgebracht virus</i> <i>waarschijnlijk het "le-</i> <i>pelblad-virus"</i>

SAMENVATTING

Bij onderzoeken over een virus van populier (BERG, 1962) bleek het mogelijk het virus in de bast van een zieke tak door directe inoculatie aan te tonen. Hiertoe werd van een stuk tak de schors dun afgeschild (fig. 1A) en de toetsplant, *Vigna sinensis*, met het verse, groene snijvlak direct geïnoculeerd (fig. 1B). Op deze wijze kon behalve in ontbladerde takken in de zomer het virus ook worden aangetoond in virusziek winterstek.

Deze inoculatie-methode werd toegepast op andere viruszieke bomen. Van getoetste gedeelten van takken, afkomstig van 14 verschillende soorten houtige waardplanten, werd in zeven gevallen virusoverdracht verkregen op één of meer kruidachtige plantesoorten (tabel 1).

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FIG. 1A. For explanation see text.
Voor de verklaring zie tekst.



FIG. 1B. For explanation see text.
Voor de verklaring zie tekst.