T. Pl.ziekten 4 (1960): 264–267

SOME EXPERIENCES IN MAINTAINING YELLOWS-TYPE VIRUSES[1]

Met een samenvatting: Enige ervaringen met het in stand houden van virussen behorende tot het „yellows"-type

BY

V. VALENTA

Institute of Virology, Czechoslovak Academy of Sciences, Bratislava

The leafhopper-borne yellows-type viruses are defined almost exclusively according to the symptoms they cause in selected differential hosts. These symptoms are, however, rather variable and their character depends on many factors, among which the intrinsic properties of individual strains play an important part. The keeping of type culture collections is, therefore, of primary importance for obtaining reproducible results. As yet, however, very little information is available as to how such a collection can be maintained without the expenditure of much time and space in view of the appreciable losses of virus material which regularly can occur in the winter months.

The experience obtained in the course of several years' work with the yellows-type viruses in our laboratory might be, therefore, of some interest. Although we have been working mainly with European viruses, the results obtained would undoubtedly also hold good for similar viruses occurring elsewhere in the world.

The viruses under consideration can only be maintained by continuous passaging in susceptible living substrates, either plant or insect.

Continuous maintenance of yellows-type viruses in viruliferous lines of the appropriate leafhopper vector will almost certainly remain restricted to those viruses which cannot be maintained more advantageously in plants. Apart from the fact that it is more difficult to maintain leafhoppers than plants there would always be a risk that some would escape and spread or introduce undesired infections.

Before proceeding further, the question deserves attention, whether the growing of whole infected plants could be replaced by culturing their tissues in vitro. Unfortunately this is as yet an almost unexplored problem, but there are some indications that such a method might be possible (MARAMOROSCH, 1958). If efficiently worked out, a tissue-culture method would carry many advantages.

So far then, plants remain the most convenient substrate for the maintenance of yellows-type viruses. These plants should:

(a) be sufficiently susceptible to the given virus, but not so sensitive that the infection would appreciably reduce their length of life;

(b) offer adequate facilities for doing virus transmissions by any method desired (grafting, dodder, vectors);

(c) be perennial and/or vegetatively propagable;

(d) be easy to keep under usual greenhouse conditions; and

(e) resist infection by mechanically transmitted viruses (e.g. tobacco mosaic virus, etc.).

[1] Accepted for publication 27 June, 1960.

Unfortunately these requirements are not always compatible and, depending on the aims pursued, the use of more than one plant species would usually be neccessary.

The present differential diagnosis of European yellows-type viruses is based mainly on the behaviour of some solanaceous hosts, plants belonging to other families offering only supplementary, although also important criteria. It would seem, therefore, that the plants needed for our purposes would be Solanaceae and this has proved to be largely true. Several were examined in fairly extensive trials with a number of strains of the stolbur and Czechoslovak potato witches' broom viruses differing in virulence. Subsequently other viruses were included, but these were examined in a restricted range of hosts.

Of the annuals, Samsun tobacco *(Nicotiana tabacum* L. var. Samsun) gave very good results, especially with viruses not affecting its length of life. For instance one strain of the Czechoslovak type II of potato witches' broom virus has been kept without passaging for over five years in cuttings repeatedly taken from plants, which all originated from a single plant in which this strain was first isolated. The great disadvantage of Samsun tobacco is its high susceptibility to infection with Tobacco mosaic virus and, therefore, the variety Xanthi necroticum probably would prove to be more suitable. Preliminary tests have shown that it is about as susceptible to yellows-type viruses as Samsun. *N. tabacum* is, however, seemingly either immune or at least highly resistant to infection with some of the yellows-type viruses and in these cases the use of other plant species is imposed.

Of the perennials, *Cyphomandra betacea* SENDT., *Physalis alkekengi* L. and *P. franchettii* MAST. were found unsuitable. *Atropa belladonna* L. and *Solanum tuberosum* L. gave good results with the viruses of the potato witches' broom group only, but transmissions to fresh hosts were necessary after a time. With *Solanum pseudocapsicum* L. (a florists' variety) irregular results were obtained in graft transmissions to new hosts. *Nicotiana glauca* GRAH. gave the best results and is now currently used for maintaining virus strains in our collection. These viruses are the following:

Stolbur virus
Potato witches' broom virus –
Canadian types 1 and 2
Czechoslovak types I, II and III
German type (a strain obtained from Dr. BODE, Braunschweig, differing somewhat from the virus described by SPRAU (1954))
Crimean yellows virus

The German type of potato witches' broom virus causes in *N. glauca* leaf symptoms consisting of vein clearing, but no other adverse effects. The remaining viruses mentioned are either wholly symptomless in *N. glauca*, or cause only some transient flower symptoms.

Experiments are still in progress with other European yellows-type viruses (parastolbur, metastolbur, clover phyllody and clover dwarf) and preliminary results (MIŠIGA, unpublished) indicate that *N. glauca* is susceptible to at least some of them.

Several of the *N. glauca* plants infected in the first experiments have been kept for over three years. They were cut back from time to time in order to produce

new growth and did not show any signs of being adversely affected by the presence of virus, nor was there any evidence of changes affecting the virus itself. On several occasions cuttings were rooted and the new plants thus obtained behaved in the same manner as those from which they originated.

In this way it has been possible to reduce the previously rather high labour and space requirements to a minimum and wholly to eliminate losses of virus material in the winter months.

The solanaceous species are, however, neither good food plants for the leafhopper vectors nor plants from or to which the viruses can be easily transmitted by means of these vectors. If, therefore, research has to be done on problems involving leafhopper transmissions, other plant species must be used. Of these, clovers seem to be the most promising. They are susceptible to the majority, if not all of the known European yellows-type viruses. White clover plants *(Trifolium repens* L.) can easily be propagated by cuttings even after being infected with stolbur or clover phyllody viruses. We have been keeping strains of these viruses in this way for several years without transmissions to fresh plants. In winter care must be taken that the glasshouse temperatures do not go beyond 15–20°C, higher temperatures leading to considerable losses of plants. Sufficient experience has not yet been gained with the parastolbur and Crimean yellows viruses, which are more pathogenic for clovers than are the former two viruses. Furthermore it is impossible to maintain the clover dwarf virus by vegetative propagation of infected clover plants, because this virus is lethal to clover. It has been maintained in our collection either in continuous culture of the leafhopper *Euscelis plebejus* (FALL.) on white clover, or in *Vinca rosea* plants. The latter plant species was found suitable for maintaining also other yellows viruses, but it was found not to be adaptable as a virus source for further transfers to unrelated hosts.

Members of Compositae and other families (e.g. *Calendula officinalis* L., *Senecio vulgaris* L., *Taraxacum officinale* WEB., *Reseda lutea* L. etc.), some of which are susceptible to infection by means of leafhoppers, proved unsuitable for long-term maintenance of the viruses so far examined.

SUMMARY

The problem of maintaining yellows-type viruses in culture collections is discussed.

Nicotiana glauca GRAH. was found to be the most suitable plant host for general purpose cultures of the following viruses: stolbur virus, Canadian types 1 and 2, Czechoslovak types I, II and III and a German type of the potato witches' broom virus and the Crimean yellows virus.

Other hosts are, however, necessary for work involving leaf-hopper transmissions. For this purpose white clover *(Trifolium repens* L.) gave the most promising results.

SAMENVATTING

Het is van veel betekenis verschillende typen van „yellows"-virussen, die door cicaden worden overgebracht, voor vergelijkend onderzoek in stand te houden. Het bleek dat *Nicotiana glauca* GRAH. hiertoe de geschiktste plantesoort is voor de volgende virussen: het stolbur virus, het heksenbezemvirus van de aardappel

(te weten de Canadese typen 1 en 2, de Tsjechoslowaakse typen I, II en III en het Duitse type) en het „yellows"-virus van de Krim. Daar *N. glauca* zich echter minder leent tot het verrichten van onderzoek over de overdracht van deze virussen door cicaden, is het noodzakelijk voor dit doel andere plantesoorten te gebruiken. Witte klaver *(Trifolium repens* L.) heeft in dit opzicht de meestbelovende resultaten opgeleverd.

ACKNOWLEDGEMENT

The author wishes to thank Mr. J. M. TODD for revision of the English text.

REFERENCES

MARAMOROSCH, K., – 1958. Viruses that multiply in both plants and insects. Trans. N.Y. Acad. Sci. II, 20: 383–393.

SPRAU, F., – 1954. Untersuchtungen über das Zwergstrauch-Virus an Kartoffeln. Mitt. biol. Bundesanst. Berlin-Dahlem 80: 146–151.