Neth. J. Pl. Path. 83 (1977) 217–220

Persistence of infectivity of three viruses in plant material dried over $CaCl_2$ and stored under different conditions

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Accepted 29 April 1977

Abstract

The infectivity of three viruses in leaf material dried over $CaCl_2$ and stored under different conditions was tested over a period of seven years. Alfalfa mosaic virus and cucumber mosaic virus kept very well for the entire period both at 4°C and at room temperature, provided they were stored over $CaCl_2$ in well-closed tubes. The infectivity of bean yellow mosaic virus, however, gradually declined, especially at room temperature. In open or loosely closed containers without $CaCl_2$ none of the viruses survived more than three months of storage and the infectivity of bean yellow mosaic virus declined after only two weeks.

Introduction

Preservation of several plant viruses in leaf material dried and stored over $CaCl_2$ at 4°C has proved a simple and efficient technique for maintaining collections (Barradas and Silberschmidt, 1973; Bos, 1969, 1973; McKinney, 1947, 1953). Storage is generally at 4°C, but during mailing of samples temperatures are usually higher, and often no desiccant is present. Therefore, I have tested the effect of some different conditions on the persistence of infectivity of three viruses in desiccated leaf material over a period of seven years.

Materials and methods

The viruses used were alfalfa mosaic virus (AMV) (isolate 425, Wisconsin, dried October 10, 1967), bean yellow mosaic virus (BYMV) (isolate B25, dried February 14, 1969), and cucumber mosaic virus (CMV) (isolate KMV3, dried February 11, 1969). The experiment was started on February 11, 1970, at which time the preparations had already been stored over $CaCl_2$ for ca. $2\frac{1}{2}$, 1 and 1 years, respectively.

Virus-containing leaf material was first ground with a mortar and pestle to a homogeneous powder to allow division into uniform batches for the different treatments and the later testing of uniform samples. Treatments were (A) conventional storage over $CaCl_2$ in a well-closed test tube at 4°C (Bos, 1969), (B) as in A, but at room temperature, (C) in a loosely closed plastic bag at room temperature, (D) in a closed petri-dish at room temperature and (E) in an open Erlenmeyer flask at room temperature.

For each assay of infectivity 5 mg-samples of leaf powder were further ground in a mortar with 5 ml (for AMV and CMV) or 0.5 ml (for BYMV) of 0.01 M phosphate

buffer, pH 7, or water. Based on 5% of dry matter in succulent leaves, this corresponds to final sap dilutions of 1:50 and 1:5, respectively.

Assay was on intact plants in the glasshouse and plants were dusted with 500 mesh carborundum, prior to inoculation. BYMV was tested on primary leaves of four plants of *Phaseolus vulgaris* 'Bataaf', AMV on most leaves of two plants of *Chenopodium amaranticolor*, and CMV likewise on *C. quinoa*. In presence of the viruses these species readily reacted with local lesions.

Results and discussion

Since assay conditions and test plants were not uniform throughout the test and numbers of test plants were too low for reliable quantitative determination of virus infectivity, the results represented in Table 1 are semi-quantitative only.

The results show that CMV (Table 1 C) kept remarkably well for the entire period of nearly seven years, both at 4°C and at room temperature, provided it was stored in a well-closed tube over $CaCl_2$. After storage at room temperature without desiccant and in more or less open connection with the air, infectivity gradually declined to zero in less than four months. However, under such conditions the virus could still be readily recovered after three months of storage.

The same held for the AMV preparation (Table 1A), although nearly $2\frac{1}{2}$ years old at the start of the experiment. Here, however, even under standard conditions infectivity declined towards the end of the experiment. This observation might partly be ascribed to the use of water instead of buffer from 36 months onwards.

BYMV (Table 1B) could hardly be recovered from material stored at room temperature in open containers for two months. It could, however, be recovered after two weeks of storage and thus survive airmail transportation under such conditions to any part of the world. In contrast to AMV and CMV, this virus kept less over $CaCl_2$ in a closed tube at room temperature than at 4°C. Infectivity also gradually deteriorated during long-term storage at 4°C. Similarly, Bhargava (1976) found that watermelon mosaic virus, another potyvirus, kept well over $CaCl_2$ at – 1°C for six months, but infectivity gradually declined to zero at room temperature. Such viruses may have to be reactivated at certain intervals, then multiplied and dried again. However, I found that certain other strains of BYMV keep very well over $CaCl_2$.

Good air-tight closing of tubes, e.g. with rubber stoppers or with screw tops, and the presence of a desiccant to insure complete absence of moisture seem a prerequisite for long-term preservation (see also McKinney, 1953).

Samenvatting

Behoud van het infectievermogen van drie virussen in boven $CaCl_2$ gedroogd plantemateriaal na bewaring onder verschillende omstandigheden

Gedurende een periode van zeven jaar werd op verschillende tijdstippen het infectievermogen getoetst van drie plantevirussen in homogene porties vermalen droog bladmateriaal van geïnfecteerde planten, na bewaring onder verschillende omstandigheden.

Table 1. Persistence of infectivity of three viruses in desiccated leaf material.

Virus and type of storage	Period of storage in months													
	½	2	3	4	7	10[1]	13	16	22	36[2]	42	60	76	84
A. Alfalfa mosaic virus														
at 4 °C: closed tube over $CaCl_2$		+++[3]	+++	+++	++	+++	+++	+++	+++	++	+	++		0[4]
at room temperature:														
closed tube over $CaCl_2$		+++	+++	+++	++	+++	+++	+++	+++	+++	++	++		++
loosely closed plastic bag		++	+	−	−	−	−	−						
closed petri dish		++	±	−	−	−	−	−						
open Erlenmeyer flask		++	+	−	−	−	−	−						
B. Bean yellow mosaic virus														
at 4 °C: closed tube over $CaCl_2$	++	++	++	++	++	++	+	+		+		−	±	0
at room temperature:														
closed tube over $CaCl_2$	++	++	++	+	+	+	+	+		−		−	−	−
loosely closed plastic bag	++	±	−											
closed petri dish	+	−	−											
open Erlenmeyer flask	+	±	−											
C. Cucumber mosaic virus														
at 4 °C: closed tube over $CaCl_2$		+++	+++	++	+++	++	+++		+++	+++		+++	++	0
at room temperature:														
closed tube over $CaCl_2$		++	+++	+++	++	++	+++		+++	+++		+++	++	++
loosely closed plastic bag		++	+	−	−		−							
closed petri dish		+	+	−	−		−							
open Erlenmeyer flask		++	+	−	−		−							

[1] From 10 months onwards for AMV inoculum concentration was 10 fold.
[2] From 36 months onwards water was used instead of buffer and for CMV inoculum concentration was 10 fold.
[3] +++ more than 100 local lesions per primary bean leaf for AMV, or per plant of *C. quinoa* for CMV;
++ 11–100 local lesions per bean leaf for AMV, per plant of *C. quinoa* for CMV or per plant of *C. amaranticolor* for BYMV;
+ 1–10 local lesions;
± less than 1 local lesion.
[4] Not tested because material was no more available.

Tabel 1. Behoud van het infectievermogen van drie virussen in gedroogd bladmateriaal.

Het komkommermozaïekvirus (CMV, Tabel 1C) bleek zeer goed houdbaar bij bewaring boven $CaCl_2$ in gesloten reageerbuisjes. Zowel bij 4°C als bij kamertemperatuur bleek het infectievermogen na beproeving nog erg hoog.

Het luzernemozaïekvirus (AMV, Tabel 1A) was eveneens bij beide temperaturen boven $CaCl_2$ uitstekend houdbaar, ondanks het feit dat het materiaal bij het begin van de proef al bijna $2\frac{1}{2}$ jaar oud was.

Het bonescherpmozaïekvirus (BYMV, Tabel 1B) liep tijdens de bewaring, ook bij lage temperatuur maar vooral bij kamertemperatuur, geleidelijk achteruit in infectievermogen.

Alle drie virussen zijn in gedroogd materiaal in open vaten zonder droogmiddel niet langer dan drie maanden houdbaar. Het bonescherpmozaïekvirus is dan al na enkele weken moeilijk isoleerbaar. Toch lijkt het virus onder die omstandigheden gemakkelijk luchtpostverzending over grote afstanden te kunnen doorstaan.

Acknowledgment

Careful technical assistance by Miss M. P. Schor is gratefully acknowledged.

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