

EFFECT OF HEAT TREATMENT OF YOUNG PLANTS
ON FREEING CHRYSANTHEMUMS FROM VIRUS B
BY MEANS OF MERISTEM CULTURE¹

*Het effect van warmtebehandeling van jonge planten op het elimineren van
virus B van chrysanten door middel van meristeme-cultures*

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Meristem culture provides a means of obtaining virus-free plants from cultivars of chrysanthemum which are wholly infected with virus B. In an effort to improve the efficiency of the method plants were subjected to a temperature of 37°C for periods of 0, 10, 20, 30, 40, 50 and 60 days, respectively, prior to isolation of the meristems. The three chrysanthemum cultivars used, 'Blanche Poitevine Suprême', 'Migoli' and 'Chatsworth' reacted differently both to heat treatment and to meristem culture. However, their reactions had two points in common. First, heat proved to be non-essential: even without heat plants could be produced in which virus B was no longer present. Secondly, with increasing duration of the heat treatment the relative number of plants free from virus B increased; but since prolonged heat treatment had an unfavourable effect on the cultivation of the meristems, the absolute number of plants free from the virus eventually decreased.

INTRODUCTION

In the Netherlands the testing of chrysanthemum mother plants for the presence of viruses is undertaken to improve the state of health of the commonly grown cultivars. In the course of this work it was found that some cultivars are wholly infected with chrysanthemum virus B. We therefore focused our attention on methods for freeing these cultivars from this virus. BRIERLEY & LORENTZ (1960) obtained healthy tip cuttings from mosaic-infected chrysanthemum after having kept the plants for 2-8 months at 35°C. QUAK (1960) succeeded in freeing chrysanthemum plants from virus B by means of meristem culture. Meristem culture of chrysanthemums has also been used in Great Britain with a view to removing other viruses. Attempts by HOLLINGS, STONE & NORRISH (1962) to eliminate the chrysanthemum stunt virus by meristem culture were unsuccessful. However, tips from heat-treated plants of 'Balcombe Perfection' infected with green flower virus produced plants which flowered normally, whereas the original heat-treated stools still bore green flowers.

Since, in the Netherlands, the results of meristem culture to eliminate virus B were encouraging, it seemed worth while to make a systematic study of the effect that pretreatment of young plants with heat might have on the effectiveness of this method of obtaining virus-free plants.

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MATERIALS AND METHODS

From each of the cultivars 'Blanche Poitevine Suprême', 'Migoli' and 'Chatsworth', supposed to be wholly infected with virus B, 245 rooted cuttings were transplanted into wooden boxes, 35 plants per box. For a check on the presence of virus B, ten leaf samples of each cultivar were taken and tested by sap inoculation on *Petunia hybrida* Vilm. 'Celestial'. All of the 30 plants proved to be infected with virus B, and it was taken for granted that the remaining plants of the same lots were also infected with this virus.

The experiment was started about two weeks after topping with 'Blanche Poitevine Suprême' on March 18, with 'Migoli' on April 17, and with 'Chatsworth' on May 27, 1963. The boxes were placed in a heat chamber maintained at $37^{\circ}\text{C} \pm 2^{\circ}$, illuminated by fluorescent tubes eight per m^2 for 18 hours per day. In order to obtain plants subjected to heat treatments of 0, 10, 20, 30, 40, 50 and 60 days respectively, boxes were removed from the heat chamber at ten-day intervals. Some hours after removal from the heat chamber the meristems were excised under aseptic conditions and placed on the surface of a nutrient agar in small Pyrex tubes: 96 meristems of each cultivar per treatment. The medium used was that developed by MOREL (personal communication), supplemented with kinetin at 0.1 ppm and α -NAA at 1 ppm.

When the plantlets had two leaves of about 1 cm diameter and roots, they were transferred to small pots filled with a light soil mixture and grown until big enough to be tested by sap inoculation on *P. hybrida* 'Celestial'. All plants were tested a second time, about six months later. Only when the test plants showed no reaction in both tests, were the chrysanthemums considered to be free from virus B.

RESULTS AND DISCUSSION

The total numbers of plants obtained from meristems and the numbers and percentages of plants free from virus B among them, are given in Table 1.

Although from each cultivar plants free from this virus were obtained, the response of the cultivars to the treatments differed in some respects. The plants of the cultivar 'Blanche Poitevine Suprême' survived up to 60 days of high temperature. However, with the cultivars 'Migoli' and 'Chatsworth', the heat

TABLE 1. Number of plants obtained by meristem culture after heat treatment.

Duration of heat treatment	'Blanche Poitevine Suprême'			'Migoli'			'Chatsworth'		
	Number of plants obtained	Plants free from virus B		Number of plants obtained	Plants free from virus B		Number of plants obtained	Plants free from virus B	
		Number	%		Number	%		Number	%
0 days	34	3	8.8	56	11	19.6	9	1	11.1
10 "	34	18	52.9	25	16	64.0	41	19	46.3
20 "	40	30	75.0	6	6	100.0	33	12	36.4
30 "	64	58	90.6	10	7	70.0	19	6	31.6
40 "	49	43	87.7	—	—	—	20	18	90.0
50 "	12	11	91.7	—	—	—	20	12	60.0
60 "	32	30	93.7	—	—	—	—	—	—

treatment had to be terminated after 30 and 50 days respectively, because of the death of the plants.

The fact that the 'Migoli' plants did not survive for a period longer than 30 days and that the 'Chatsworth' plants did not survive for longer than 50 days does not necessarily mean that this survival period is a varietal characteristic, for this may have been connected with the condition of the plants.

A short heat treatment had a favourable effect on the growth of the meristems of both 'Blanche Poitevine Suprême' and 'Chatsworth'. The number of meristems of the cultivar 'Migoli' that developed into plantlets was smaller when excised from heat-treated plants than from the untreated control plants.

Although the three cultivars behaved differently, there were some points in common. First, in no case did heat treatment prove to be essential for the elimination of virus B. With all three cultivars, plants free from this virus were obtained without any heat treatment at all. Secondly, an interesting point is that all three of the percentage lines have an upward tendency (Fig. 1). This means that with increasing duration of the heat treatment the relative number of plants free

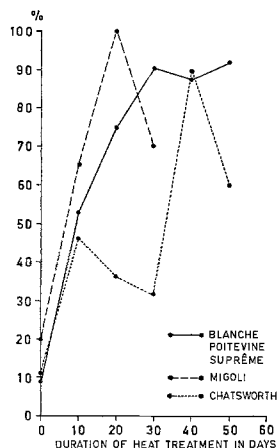


FIG. 1. Percentages of healthy plants of some cultivars amongst the total number of plants obtained by meristem culture.

from virus B also increased. However, because prolonged heat treatment had an unfavourable effect on the cultivation of the meristems the absolute number of plants free from virus B decreased eventually.

Because of the differences in behaviour of the three cultivars used it may be concluded that no general procedure for freeing chrysanthemum cultivars from virus B by meristem culture in conjunction with heat treatment can be prescribed.

SAMENVATTING

Door middel van meristeemcultuur kan het virus B uit volledig besmette chrysantherassen worden geëlimineerd. Teneinde het rendement van deze methode te verhogen, werd het effect bestudeerd van een voorbehandeling van het materiaal met warmte. De planten werden gedurende respectievelijk 0, 10, 20, 30, 40, 50 en 60 dagen bij een temperatuur van 37°C gehouden, alvorens de vegetatiepunten ervan werden geïsoleerd.

De reactie der drie gebruikte cultivars, 'Blanche Poitevine Suprême', 'Migoli' en 'Chatsworth' op de behandelingen liep weliswaar in enkele opzichten uiteen, doch er waren punten van overeenkomst. Vooreerst was warmtebehandeling niet noodzakelijk om planten vrij van virus B te verkrijgen. Verder nam met de duur van de warmtebehandeling het relatieve aantal gezonde planten eveneens toe. Daar langdurige warmtebehandeling het opkweken der meristemen ongunstig beïnvloedde, nam het absolute aantal gezonde planten evenwel af.

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REFERENCES

- BRIERLEY, P. & P. LORENTZ, - 1960. Healthy tip cuttings from some mosaic-diseased Asiatic chrysanthemums: some benefits and other effects of heat treatment. *Phytopathology* 50: 404-408.
- HOLLINGS, M., O. M. STONE & M. E. NORRISH, - 1962. *Ann. Rep. Glassh. Crops Res. Inst., Littlehampton*: 90.
- QUAK, F., - 1960. Meristeemcultuur van anjers en andere bloemisterijgewassen. *Jaarversl. Inst. Plantenziektenk. Onderz.* : 84-85.