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Heat inactivation of viruses in apple cl MM 109 by use of daily temperature cycles

Daglige rytmer i temperaturen under varmebehandling af æblegrundstammen MM 109

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Summary

Tips from apple plants MM 109, with the viruses chlorotic leaf spot (CLSV), platycarpa scaly bark (SBV), Spy epinasty and decline (EDV), 'Virginia Crab' stem pitting (SPV) and the agent which produces graft-union-breakage (GUB) in 'Virginia Crab', were produced free of these diseases by alternating exposures to dry heat in daily cycles and to normal greenhouse temperature (20-25 °C). No 'Virginia Crab' stem grooving virus was found in any of the plants. Seven different daily cycles were investigated. The high temperature of these cycles were: 32 °C for 24 hours, 34 °C for 24 hours, 36 °C for 8 hours, 36 °C for 24 hours, 38 °C for 4 hours, 38 °C for 8 hours, and 38 °C for 24 hours. Tips were taken at about 2 weeks intervals from the start of the treatments to 21 weeks later.

The survial of the plants in the heat-treatments was much better when they were exposed to high temperature for only part of the day compared to the survival achieved after continuous exposure to high temperature.

The CLSV and SBV were easily eliminated in nearly all tips, which were treated for more than 2 weeks. GUB, EDV and SPV were eliminated in this order with increasing difficulty. Some separation of the viruses seems to have taken place.

The results demonstrate, that it is possible greatly to improve the survival of actively growing plants by variation of the temperatures during heat treatment over what is conventional with continuous heath and still get the therapeutic effect.

Resumé

Til varmebehandlingsforsøg med forskellige temperaturer i en fast daglig rytme blev anvendt planter af æblegrundstammen MM 109, som spontant var inficeret med klorotisk bladplet virus (CLSV), platycarpa skællet bark virus (SBV), Spy epinasti virus (EDV), 'Virginia Crab' grubet ved virus (SPV) og det smitstof, som fremkalder podningsbrud i 'Virginia Crab' (GUB). 'Virginia Crab' rillet ved blev ikke påvist i nogen af de i forsøget anvendte eller fremstillede planter.

De anvendte behandlinger var: 32°C vedvarende, 34°C vedvarende, 36°C vedvarende, 38°C vedvarende, 36°C i 8 timer fulgt af 16 timer ved ca. 20°C, 38°C i 4 timer fulgt af ca. 20°C i 20 timer, 38°C i 8 timer fulgt af ca. 20°C i 16 timer.

Planterne tålte varmebehandlingen bedst, hvor den høje temperatur kun anvendtes en del af døgnet. Planter udsat for vedvarende høj temperatur led stærkt under behandlingen.

Skudspidser af de behandlede planter blev udtaget med ca. 2 ugers mellemrum fra behandlingernes begyndelse til deres afslutning efter 21 uger. Skudspidserne podedes på små æblefrøplanter for at fremstille nye planter.

CLSV og SBV (tabel 1 og 2) blev inaktiveret i næsten alle de skudspidser, som var behandlet i mere end 2 uger. GUB, EDV og SPV (tabel 3, 4 og 5) inaktiveredes i nævnte rækkefølge med tiltagende vanskelighed.

Resultaterne viser, at det er muligt at forbedre overlevelsen af planter i vækst under varmebehandling meget stærkt ved i varmebehandlingen at indskyde daglige perioder med ca. 20°C. Den ønskede sygdomseliminerende virkning kunne stadig opnås.

Introduction

Good results of virus inactivation have been achieved by placing the plants in hot air for a certain length of time, generally some weeks (*Hollings* 1965). However such treatment often kills many plants before the virus is inactivated.

It is reasonable to assume that a better survival of plants could be obtained if the plants were kept at a lower temperature for a period during the treatment.

Accordingly some authors state that the survival of plants at the high temperature necessary for virus inactivation can be improved if the temperature is lowered somewhat during a part of the treatment period, (*Mellor* and *Fitzpatrick* 1961, *Brierley* 1964, *Mellor* and *Stace-Smith* 1967) although not in regular cycles.

An experiment in which the therapeutic treatment consisted of regular and pre-determined fluctuations in daily cycles was reported by *Hamid* and *Locke* (1961). One hour at 45°C followed by 23 hrs. at 25-30°C in daily cycles for 2 or 6 weeks gave excellent survival of the eye pieces of resting potato tubers and complete elimination of leafroll. Other combinations were equally effective.

The experiment presented here was set up to try if similar daily temperature cycles could improve the survival of actively growing plants of apple during heat treatment and still inactivate the viruses in the apple plants.

A preliminary report of a part of this work has been presented (*Larsen* 1966).

Material and methods

Young plants of the apple understock MM 109 from a stool bed at the experiment station was used in the experiment. Plants from the stool bed have indexed on a range of indicator plants in a manner previously described (*Larsen* 1970).

No symptoms could be found on indexings with the indicators: *Malus* 'Lord Lambourne' cl M 139, 'Jonathan', 'Gravenstein', 'Guldborg', 'Belle de Boskoop', 'Cox Orange' cl CO 1 Nyon, 'Golden Delicious', 'Spartan', 'Early McIntosh', *M. robusta* cl No. 5, *M. sargentii* and *Cydonia oblonga* cl C 7/1.

The indexings with *M.* 'Virginia Crab' cl K 6 showed the stem pitting and graft-union-breakage symptoms. Similarly *M.* Spy 227 showed leaf pattern, epinasty, bark necrosis and death. *M.* 12740-7A showed chlorotic leaf spot, and *M. platycarpa* cl LA 17T2 showed leaf pattern and scaly bark.

These indexings indicated that the MM 109 plants used were spontaneously infected with the stem pitting virus of 'Virginia Crab', the epinasty and decline virus of Spy 227, the scaly bark virus of *Malus platycarpa*, the chlorotic leaf spot virus of R 12740-7A, and the pathogen causing graft-union-breakage in 'Virginia Crab' as described earlier (*Larsen* 1970). The stem grooving symptom was not observed in any of the indexings.

Plants for the heat-treatments were potted in a light soil-peat mixture placed in 16 cm clay pots and were growing actively before they were used in the experiment.

The heat-chambers used for the experiment were placed in a greenhouse which was kept at 20°C. Natural daylight was the main source of illumination, supplemented with cool-white fluorescent light tubes for 6 hours during the middle of the night. The average wattage was 85 watts per square meter. The heat chambers are 1.5 m × 2.0 m × 0.75 m and equipped with a 1200 watt electric heater controlled by a contact-thermometer. The air in the chamber was kept continuously circulating by a small fan. This gives a reasonably good control of temperature with deviations from the set temperature generally within ± 1°C.

The temperature cycles were achieved by moving the plants in and out of the heat-chambers as necessary. The temperature treatments were:

32°C for 24 hrs. = 32°C constl. for the full period of time
34°C » 24 hrs. = 34°C » » » » » » »
36°C » 24 hrs. = 36°C » » » » » » »
38°C » 24 hrs. = 38°C » » » » » » »
36°C » 8 hrs. = the plants were in the heat chamber at 36° for 8 hrs. and in the greenhouse at 20°C for 16 hrs. a day.
38°C » 4 hrs. = the plants were in the heat-chamber at 38°C for 4 hrs. and in the greenhouse at 20°C for 20 hrs. a day.
38°C » 8 hrs. = the plants were in the heat-chamber at 38°C for 8 hrs. and in the greenhouse at 20°C for 16 hrs. a day.

For practical reasons the 4 and 8 hrs. of high temperature treatment were given during the daytime. The heat treatments were carried out from March to end of August 1962.

About five tips, one half to one cm long, were removed every other week from the plants from each treatment and grafted onto apple seedlings according to the method described by *Cambell* (1962).

The seedling was decapitated and given a longitudinal cut to fit the wedgeshaped lower part of the tip obtained from the plants growing in the heat-chamber. The grafted area was covered with a latex band, and the graftings were covered with small polyethylene bags and placed in a shaded greenhouse in order to facilitate the take of the grafts.

The plants arising from the tip-grafts were indexed with *Malus* cl. 12740-7A, cl. Spy 227, 'Virginia Crab' K6 and *M. platycarpa* cl LA P 17 T2 in order to disclose which tips had been freed of the pathogenic agents which produce symptoms on these indicatorplants.

The indicator plants were inspected for at least 2 years after insertion of infector in case of R 12740-7A, Spy 227 and *M. platycarpa* and 4 years in case of 'Virginia Crab'.

Indexing of the plants made by tip-grafts was normally started within 3 years after the heat-treatment, but many of the indexings were repeated later if the early indexings had been negative.

Results

SURVIVAL OF PLANTS DURING TREATMENT

None of the treatments killed the plants during the 21 weeks. However, the plants of the continuous high temperature were very weakned. The plants grown at 38°C were so weak, that they produced no new growth after 6 weeks and none of the tips taken thereafter were viable enough to establish new plants when grafted.

Plants in treatments with cycling temperatures during the heat inactivation periods, 38°C for 8 hrs., 38°C for 4 hrs. survived very well and produced viable tips throughout the whole experimental period.

THERAPEUTIC EFFECT

The results of the indexings of plants made by tips from the various treatments are given as fractions on the Tables below. The numerator indicates the number of plants which produced symptoms in the indicator plant, and the denominator indicates the number of plants made by tipgrafts and indexed. A dash, -, indicates that plants were made from the treatment but none survived to be indexed. A question mark indicates that this result of the indexing was doubtful.

Effect on chlorotic leaf spot (CLSV)

Symptoms recorded on *M. platycarpa* and on R 12740-7A showed, that CLSV was esaily inactivated from the tips by most of the used treatments

Table 1. Effect of different temperature cycles on inactivation of chlorotic leaf spot virus (CLSV) in MM 109. Indexing on Malus platycarpa and R 12740-7A

Weeks	32°C - 24 h.		34°C - 24 h.		36°C - 24 h.		38°C - 24 h.		36°C - 8 h.		38°C - 4 h.		38°C - 8 h.	
	M.p.*	R.*	M.p.	R.	M.p.	R.	M.p.	R.	M.p.	R.	M.p.	R.	M.p.	R.
2	—	—	2/4	0/4	3/5	0/5	3/3	0/3	1/1	0/1	1/1	0/1	1/1	0/1
4	0/1	0/1	0/1	0/1	0/4	0/4	0/1	0/1	0/2	0/2	—	—	0/2	0/2
6	0/3	0/3	?0/4	0/4	0/3	0/3	—	—	0/5	0/5	1/3	1/3	?1/3	0/3
8	0/1	0/1	0/5	0/5	0/5	0/5	—	—	0/4	0/4	?1/4	2/4	0/5	0/5
10			0/3	0/3	0/4	0/4	—	—	0/5	0/5	0/1	0/2	0/5	0/5
12			0/2	0/2	0/4	0/4	—	—	0/2	0/2	—	—	0/2	0/2
14			0/2	0/2	0/2	0/2	—	—	0/2	0/2	0/1	0/1	0/4	0/4
16			0/1	0/1	0/3	0/3	—	—	0/2	0/2	0/2	0/2	0/2	0/2
19			1/3	0/3	0/3	0/3	—	—	0/3	0/3	1/1	1/1	0/3	0/3
21			0/3	0/3	0/1	0/1			0/2	0/2	—	—	0/3	0/3

* M.p. = *Malus platycarpa* R = R 12740-7A
Numerator = number of tips, which produced symptoms in indicator
Denominator = number of tips indexed
A dash indicates, that plants were made from the treatment, but none survived to be indexed. A question mark indicates, that this result was inconclusive

and even after as short a treatment period as 2 weeks.

Table 1 shows the results with R 12740-7A and *M. platycarpa*. The results were very similar with small differences indicating that *M. platycarpa* more readily produced symptoms than R 12740-7A did.

Table 2. Effect of different temperature cycles on inactivation of platycarpa scaly bark virus (SBV) in MM 109. Indexing on Malus platycarpa

Weeks	32°C -24h.	34°C - 24 h.	36°C -24h.	38°C -24h.	36°C - 8h.	38°C - 4h.	38°C -8 h.
2	—	2/4	4/5	3/3	1/1	?0/1	1/1
4	0/1	0/1	0/4	0/1	0/2	—	0/2
6	0/3	0/4	0/3	—	0/5	0/3	0/2
8	0/1	0/5	?1/5*	—	0/4	0/3	0/5
10		0/3	0/4	—	0/5	0/1	0/5
12		0/2	0/4	—	0/2	—	0/2
14		0/1	0/2	—	0/2	0/1	0/4
16		0/1	0/3	—	0/2	0/2	0/2
19		0/3	0/3	—	0/3	0/1	0/3
21		1/3	0/1	—	0/2	—	0/3

* possible attenuation, see text
Numerator = number of tips, which produced symptoms in indicator
Denominator = number of tips indexed
A dash indicates, that plants were made from the treatment, but none survived to be indexed.
A question mark indicates, that this result was inconclusive.

Effect on scaly bark (SBV)

Two weeks were apparently too short a period for any of the treatments to inactivate this virus from the tips, but after 4 weeks most of the treatments gave a good control (Table 2) with the exception of 1 tip at 34°C for 24 hrs. for 21 weeks and possibly 1 tip at 36°C for 24 hours for 8 weeks.

Effect on Spy epinasty and decline (EDV)

This syndrome was rather difficult to elimimate from the tips of MM 109. At 32°C for 24 hours it took at least 6 weeks, at 34°C for 24 hrs., 10 weeks, at 36°C for 8 hrs. 12 weeks, at 36° for 24 hrs. 6 weeks, and at 38°C for 8 hrs. 16 weeks to obtain inactivation in all tips (Table 3). Shorter periods gave only incomplete control, and 38°C for 4 hrs. had virtually no effect on this syndrome.

Table 3. Effect on different temperature cycles on inactivation of Spy espinasty and decline virus in MM 109. Indexing on Malus cl Spy 227

Weeks	32°C -24h.	34°C -24h.	36°C -24h.	38°C -24h.	36°C -8h.	38°C - 4 h.	38°C -8h.
2	—	4/4	5/5	3/3	1/1	1/1	1/1
4	1/1	1/1	4/4	1/1	2/2	—	0/2
6	0/3	1/4	0/3	—	4/5	3/3	1/3
8	0/1	1/5	0/5	—	1/4	4/4	1/5
10		0/3	0/4	—	1/5	0/1	0/5
12		0/2	0/4	—	0/2	—	0/2
14		0/2	0/2	—	0/2	1/1	1/4
16		0/1	0/3	—	0/2	2/2	0/2
19		0/3	0/3	—	0/3	1/1	0/3
21		0/3	0/1	—	0/2	—	0/3

Numerator = number of tips, which produced symptoms in indicator

Denominator = number of tips indexed

A dash indicates, that plants were made from the treatment, but none survived to be indexed

A question mark indicates, that this result was inconclusive.

Effect on 'Virginia Crab' stem pitting (SPV)

The most effective treatment with SPV appeared to be 34°C for 24 hrs., 36°C for 8 hrs. and 36°C for 24 hrs. All these treatments gave rather good control, when applied for 10 weeks and more (Table 4). The control was not complete, as a few tips still had the SPV even after prolonged time in these treatments. The other treatments produced very few tips free of SPV.

Table 4. Effect of different temperature cycles on inactivation of 'Virginia Crab' stem pitting virus in MM 109. Indexing on Malus 'Virginia Crab' cl K 6

Weeks	32°C -24h.	34°C -24h.	36°C -24h.	38°C -24h.	36°C -8h.	38°C - 4h.	38°C -8h.
2	—	4/4	5/5	3/3	1/1	1/1	1/1
4	0/1	1/1	4/4	1/1	2/2	—	0/2
6	?2/3	1/4	?1/3*	—	4/5*	3/3	0/3
8	0/1	2/5	?1/5*	—	1/4	4/4	1/5
10		0/3	0/4	—	0/5	?1/1	0/5
12		0/2	1/4*	—	?1/2*	—	1/2*
14		0/2	0/2	—	0/2	0/1	1/4
16		0/1	0/3	—	0/2	2/2	0/2
19		1/3	0/3	—	0/3	1/1	?1/3*
21		0/3	0/1	—	0/2	—	0/3

* possible attenuation, see text

Numerator = number of tips, which produced symptoms in indicator

Denominator = number of tips indexed

A dash indicates, that plants were made from the treatment, but none survived to be indexed

A question mark indicates, that this result was inconclusive

Effect on 'Virginia Crab' graft-union-breakage (GUB)

The experiment here showed that the agent which caused graft-union-breakage in 'Virginia Crab' (*Larsen* 1970) can be eliminated from the tips of MM 109 by several of the treatments (Table 5). The treatments 34°C for 24 hrs., 36°C for 8 hrs. and 38°C for 8 hrs. had good effect when applied for 6 weeks and more, although a few tips which might still contain the agent appeared. The other temperature cycles had little or no effect under the conditions of this experiment.

Table 5. Effect of different temperature cycles on inactivation of 'Virginia Crab' graft-union-breakage in MM 109. Indexing on Malus 'Virginia Crab' cl K 6

Weeks	32°C -24h.	34°C -24h.	36°C -24h.	38°C -24h.	36°C -8h.	38°C - 4h.	38°C -8h.
2	—	3/3	4/5	3/3	?0/1	?0/1	1/1
4	?0/1	0/1	3/4*	1/1	2/2	—	0/2
6	?1/3	0/4	0/3	—	0/5	3/3	?0/3
8	0/1	0/5	0/5	—	0/4	3/4	?0/5
10		?1/3	0/4	—	0/5	0/1	0/5
12		0/2	?1/4	—	0/2	—	0/2
14		0/2	0/2	—	0/2	?0/1	0/4
16		0/1	0/3	—	0/2	2/2	0/2
19		0/3	0/3	—	0/3	1/1	0/3
21		0/3	0/1	—	0/2	—	0/3

Numerator = number of tips, which produced symptoms in indicator

Denominator = number of tips indexed

A dash indicates, that plants were made from the treatment, but none survived to be indexed

A question mark indicates, that this result was inconclusive

* = possible attenuation, see text

Separation of viruses

Several treatments produced tips in which separation of viruses seems to have taken place. A

number of tips were free of CLSV and SBV and still had EDV, SPV and GUB. Judging from the available results, tips which contained one or two but not the third of the syndromes EDV, SPV and GUB were also produced (Table 6).

Table 6. Number of plants with various combinations of the syndromes Spy epinasty and decline (EDV), 'Virginia Crab' stem pitting (SPV) and 'Virginia Crab' graft-union-breakage (GUB)

EDV	SPV	GUB	No. of plants
÷	+	+	1
÷	÷	+	1
÷	+	÷	8
+	÷	÷	4
+	+	÷	13

Only a few of these clones are still available for repeated indexing in order to check if the separation still hold true.

All the plants mentioned in Table 6 were free of CLSV and SBV.

Attenuation

There were in a few instances apparent contradiction between results where the indexings done within a few years after the heat-treatment showed negative results, and later indexings showed positive results (Tables 2, 4 and 5). This is an indication that the heat treatments did not in those cases accomplish complete elimination but merely an attenuation of the pathogenic agents in question. The indexings shown in the Tables are the latest available.

Discussion

The experiment was carried out mainly in order to test, if short or long daily interruptions of the heat-treatment would improve the survival of actively growing plants and keep them in a condition, which enabled them to produce many viable tips and at the same time achieved the therapeutic effect.

As could be expected, the survival was greatly improved, when the heat-treatment lasted for 4 or 8 hrs. per day compared with continuous exposure to the same temperature. Apparently 8 hours of high temperature daily (36°C for 8 hrs. and 38°C for 8 hrs.) is sufficient to give as good a therapeutic effect as the same temperature used continuously. A constant 38°C weakned the MM 109 plants so much, that no viable tips could be found after 4 weeks.

With the effective temperature cycles it was generally so, that the longer the time from the start of the treatment the better the chance to find virus-free tips. In a few cases (Table 2, 34°C for 24 hrs. and Table 4, 34°C for 24 hrs.) tips were still virus infected after 21 and 19 weeks, respectively, even if tips taken earlier had proved to be free of the viruses in question. This same phenomenon has been noted by *Welsh & Nyland* (1965).

The various viruses were eliminated from the tips with different ease. The chlorotic leaf spot virus (CLSV) and the scaly bark virus (SBV) were comparatively easily eliminated, whereas Spy epinasty and decline (EDV) and the 'Virginia Crab' stem pitting (SPV) were more difficult to eliminate. The results gave a slight indication to the effect, that EDV was more easily eliminated than SPV (Table 3 and 4).

This is in accordance with the findings of *Welsh & Nyland* (1965) but in contrast to the results of *Cropley* (1968), who found the opposite order of ease of elimination.

The "mildest" treatment, 38°C for 4 hrs. had virtually no effect on EDV and SPV. The pathogenic agent which causes graft-union-breakage in 'Virginia Crab' was also eliminated by many of the treatments used here. It was more easily eliminated than EDV and SPV.

The difference in the indexing results between R 12740-7A and *M platycarpa* (Table 1) is difficult to explain. The leaf pattern symptoms recorded on *M. platycarpa* were those generally ascribed to chlorotic leaf spot virus. Still, some of these tips producing leaf pattern symptoms in *M. platycarpa*, did not produce any symptoms in R 12740-7A.

Whether any of the treatments inactivated one or all of the viruses from the entire plant of any of the treated plants was not disclosed in this experiment as the facilities did not permit indexing of the treated plants themselves.

Some separation of viruses seems to have

taken place as shown by the indexings carried out so far. In view of the fact that in some cases later indexings demonstrated that tips which earlier indexings had rated as virusfree, still had virus, more indexings will be needed in order to prove, that separation of viruses has in fact taken place.

Hamid and *Locke* (1961) demonstrated, that it was possible to get therapeutic effect in dormant plant parts, such as potato tubers and parts hereof with daily temperature treatments in cycles where the high temperature lasted for only part of the day. The present report shows, that the same holds true for actively growing plants, the apple understock MM 109 and at the same time the survival of the plants was greatly improved compared with the survival experienced with the conventional continuous heat-treatment.

The shortest high-temperature period, 4 hours, at the highest temperature used here, 38°C, was apparently not enough to give sufficiently good therapeutic effect. Higher temperatures for short periods might give still more favourable results.

The results of this experiment taken together with the results of *Hamid* and *Locke* (1961) indicate that the principle of using cycles of high and low temperatures in heat-therapy can improve survival of many plants and still give good therapeutic effect.

Also, the use of cycling-temperatures in heat-inactivation opens up possibilities for achieving better results with heat-inactivation in plants which are sensitive to heat and with viruses difficult to inactivate.

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