PRAKTIJKONDERZOEK
PLANT & OMGEVING

Alternatieven voor methylbromide voor desinfestering van uitgangsmateriaal en bloemisterijproducten

Mogelijkheden van heet water behandelingen, controlled atmosphere, alternatieve fumiganten en combinaties hiervan

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Sector Glastuinbouw
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PPO Glastuinbouw

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FAIR CT98 4259

"New Quarantine Treatments for Horticultural and Timber Products as Alternatives to Methyl Bromide Fumigation "

Individual Progress Report for the period

from **01-04-1999** to **01-04-2002**

Type of contract	Shared-cost research project
Total cost	1.378.000 ECU
EC contribution:	689.000 ECU (50 % of the total cost)
Participant N° 02	
Total cost:	575.510 ECU
EC contribution:	287.755 ECU (50 % of the total cost)
Commencement date:	01-04-99 Duration: 3 years
Completion date:	31-03-02
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Participant 02:	Research Station for Floriculture and Glasshouse Vegetables (PBG) - *Contractor* Department of Plant and Product Quality Group Product Quality and Group Crop Protection **Since October 2001 the name of the institute has changed to:** Applied Plant Research Division Glasshouse Horticulture In dutch: Praktijkonderzoek Plant en Omgeving (PPO) Sector Glastuinbouw

Contents:

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From 01 04 2000 till 15 02 2003

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Objectives:

The aim of the project is to develop a range of post-harvest plant quarantine treatments for timber and horticultural products to prevent the spread of non-indigenous pests and diseases into and around the European Union.
The project will investigate the following techniques which previous work has shown have potential as quarantine disinfestation treatments.

- Heat treatment of timber
- Composting of bark and wood chips
- Hot water dipping of ornamental plants and cuttings
- Extreme controlled atmospheres treatments of ornamental plants and cuttings
- Alternative fumigant treatments of ornamental plants and cuttings (phosphine and plant volatiles)
- Combination treatments

Much of the work relies on physical treatments that are not subject to pesticide regulations. Of the chemical treatments involved, phosphine already has widespread registration and its use should cause no problems. No plant volatiles are as yet registered for use although there is intense interest in this area and the registration of some compounds is foreseen in the medium term. The use of carbon dioxide in extreme controlled atmospheres may require an extension of existing registration.

With the exception of the heat treatment of timber all of the techniques are novel in their application to the commodities concerned. It will therefore be necessary initially to establish the viability of the techniques for the effective quarantine treatment of selected commodities. The proposal also combines investigation of the temperature indicator system with refinement of the heat penetration equations for timber, providing an integrated system that will be applicable to both quarantine procedures and to kiln quality control for general use.
Techniques will be developed to produce effective quarantine treatments for a range of commodities against selected pests and to define the limits of their applicability. Where effective treatments are developed, these will be submitted to appropriate international bodies, such as the European and Mediterranean Plant Protection Organisation (EPPO), for adoption.
The work will concentrate on insect pests. However in all cases the possibility of control of pathogens will be considered and where the techniques are considered suitable (primarily heat treatment of timber and composting), the effect of treatments on relevant pathogens will also be investigated.

Actions in the project (over the full project):

Task 4: Hot Water Dipping of Ornamental Plants and Cuttings

- *Sub Task 4.1:* Equipment Development for Hot Water Treatments
- *Sub Task 4.2:* Testing of Hot Water Treatments on Plants
- *Sub Task 4.3* Testing of Hot Water Treatments on Pests
- *Sub Task 4.4* Development of Hot Water Treatment Schedules

Task 5: Controlled Atmosphere Treatments

- *Sub Task 5.1:* Development of Experimental Equipment for Controlled Atmosphere Treatments
- *Sub Task 5.2:* Development of Commercial Equipment for Controlled Atmosphere Treatments
- *Sub Task 5.3:* Testing of Controlled Atmosphere Treatments on Plants
- *Sub Task 5.4* Testing of Controlled Atmosphere Treatments on Pests
- *Sub Task 5.5* Development of Controlled Atmosphere Treatment Schedules

Task 6: Alternative Fumigants

- *Sub Task 6.1* Testing Alternative fumigants on plants
- *Sub Task 6.2* Testing Alternative fumigants on pests

Task 7: Investigation of Combination Treatments

- *Sub Task 7.1* Investigation of Combination Treatments against pests
- *Sub Task 7.2* Development of Combination Treatment Schedules and Equipment

Task 8: Publication of Quarantine Treatment Schedules

Work scheme

The project team has evaluated the proposed combinations of insects and plants and treatments. We have suggested the combinations that are relevant for the floriculture industry. This proposal has been discussed in the project meeting in December 1999 at Aalsmeer. Here we show the combinations to be tested by PBG and CSL only. The scheme can be complated by other participants.

Workscheme.
Division of tasks as agreed on by project partners PBG and CSL, December 1999
HW: Hot water treatment
CA: Controlled atmosphere treatment
AF: Alternative fumigants treatment
**:* T.palmi *experiments on flowers will not be carried out (see text)*

	Pest species	Yucca sp.		Rose		Chrysanthemum			Poinsettia
		plant	stem	flower	cutting	flower	rooted cutting	cutting	cutting
HW	*Opogona sacchari*	-	PBG	-	-	-	-	-	-
	Bemisia tabaci	-	-	-	-	-	-	-	CSL
	Thrips palmi	-	-	-	CSL	-	CSL	CSL	-
	F. occidentalis	-	-	-	PBG	-	PBG	PBG	-
	Liriomyza spp.	-	-	-	-	-	CSL	CSL	-
	Nematoda	-	-	-	PBG	-	-	-	-
CA	*Bemisia tabaci*	-	-	-	-	-	-	-	PBG
	Thrips palmi	-	-	CSL	CSL	*	CSL	CSL	-
	F. occidentalis	PBG	-	PBG	PBG	PBG	PBG	PBG	-
	Liriomyza spp.	-	-	-	-	PBG	CSL	CSL	-
AF	*Bemisia tabaci*	-	-	-	-	-	-	-	PBG
	Thrips palmi	-	-	CSL	CSL	*	CSL	CSL	-
	F. occidentalis	PBG	-	PBG	PBG	PBG	PBG	PBG	-
	Liriomyza spp.	-	-	-	-	PBG	CSL	CSL	-
CA + AF	*Bemisia tabaci*	-	-	-	-	-	-	-	PBG
	Thrips palmi	-	-	CSL	CSL	*	CSL	CSL	-
	F. occidentalis	PBG	-	PBG	PBG	PBG	PBG	PBG	-
	Liriomyza spp.	-	-	-	-	PBG	CSL	CSL	-

In the workscheme above all relevant insect – plant combinations are shown that should be tested by PBG and CSL in Hot water (HW), Controlled atmosphere (CA), Alternative fumigants (AF), and Combination (CA + AF) treatments. By mutual agreement with CSL, PBG should test in hot water (HW) treatments *Opogona sacchari* on yucca, *Frankliniella occidentalis* on cuttings of rose and Chrysanthemum, and plant-pathogenic nematodes on rose. We opted for not treating flowers (rose and Chrysanthemum) with hot water because serious *Botrytis* problems can be expected in wetted flowers.

In CA, AF, and Combination treatments PBG should test *F. occidentalis* on flowers and cuttings of rose and chrysanthemum and on yucca plants, *Lyriomyza* spp. on Chrysanthemum flowers and *Bemisia tabaci* on poinsettia cuttings.

PBG is not allowed to work with *Thrips palmi* because of its quarantine status in the Netherlands. All experiments with *T. palmi* will be carried out by CSL. As an alternative, PBG will carry out HW and CA treatments on the Western Flower Thrips (WFT) *Frankliniella occidentalis*, next to AF and combination treatments as was originally agreed on in the contract. WFT is a serious world-wide pest in a range of horticultural products and a reason for extensive use of Methyl Bromide. Since testing

T. palmi on flowers or potted plants would be extremely difficult because of its quarantine status and the resulting research limitations, PBG and CSL agreed on choosing the non-quarantine WFT as a model for these on-plant studies.

Research activities during the whole project:

Task 4: Hot water dipping of ornamental plants and cuttings

Sub Task 4.1: Equipment development for hot water treatments

This sub-task has been completed and a full report of the findings was given in the progress report for the period from 01-04-99 to 31-09-99.

Sub Task 4.2 : Testing of hot water treatments on plants

4.2.1. Cut flowers

4.2.1.1. Cut roses

Introduction

Although application of a hot water treatment for cut roses is not an obvious way of disinfecting them because of the danger for *Botrytis* blight after treatment, these treatments were used to test the reaction of cut flower development on hot water treatments. In these two experiments the main goal was to investigate if high-temperature damage on bud opening occurred, and if yes, at what temperature.

Materials and methods

Roses were harvested at the research station. Flowers were hydrated for 24 h at 5 °C in water. Hot water treatment was carried out in a waterbath, with circulation. Exposure time started at the moment the flowers were immersed. After treatment, the flowers were cooled down in a bath at 20 °C, for 10 minutes. After treatment, stems were recut and placed in the interior room (20 °C, 60 % RH, 12 h light per day at 14 $\mu mol.m^{-2}.s^{-1}$), 10 stems per treatment. All stems were examined daily until the end of the vase life.

Results and discussion

For roses the development of the flower bud during vase life is considered to give insight in damage of developmental processes in plants. Flower bud opening was inhibited by some hot water treatments. In the first experiment (Fig 4.2.1.1.1.) the 10 minutes 50 °C treatment caused a complete inhibition of the bud opening. The other treatments, like a much shorter 50 °C treatment did not cause changes in bud opening.

In the next experiment (Fig 4.2.1.1.2) the 10' 50 °C again caused damage. A longer 45 °C treatment (30 minutes) was also harmful. There is a clear temperature-time interaction on the inhibition of bud opening. Other harmful effects of the hot water treatments were browning of the calyx. This was always seen in the treatments that caused inhibition of bud opening, but also after the 10 minutes 45 °C treatment in the second experiment (Table 4.2.1.1.1).

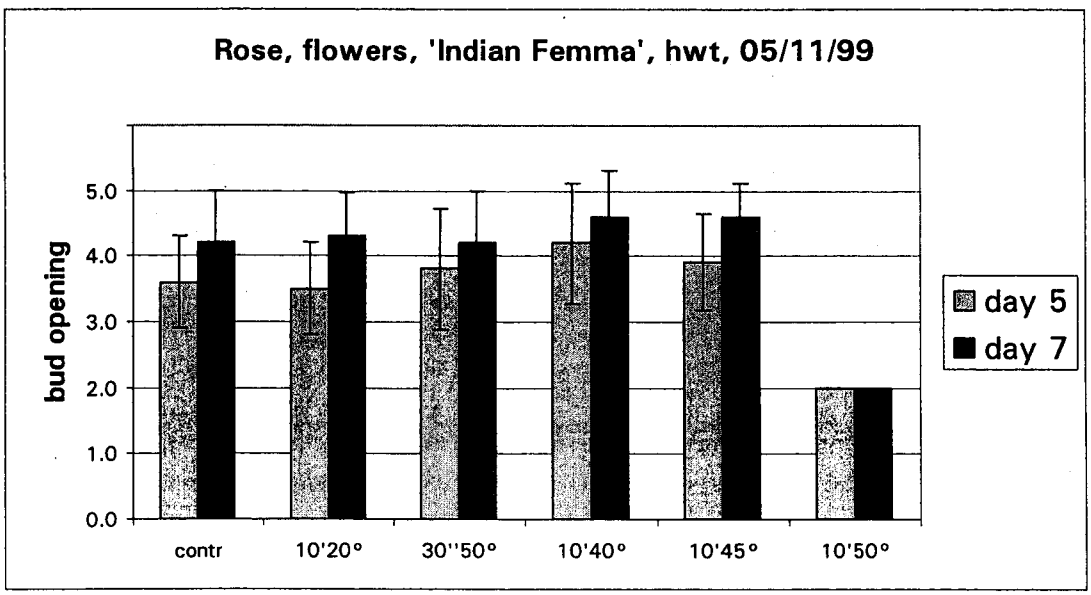


Fig 4.2.1.1.1. Flower bud opening of rose variety 'Indian Femma' , measured after 5 and 7 days after the hot water treatments. Bud opening classes: 1 = closed pointed bud, 2 = cylindrical bud, 3 = half open flower, 4 = open flower, 5 = open flower and anthers visible, end of bud opening process

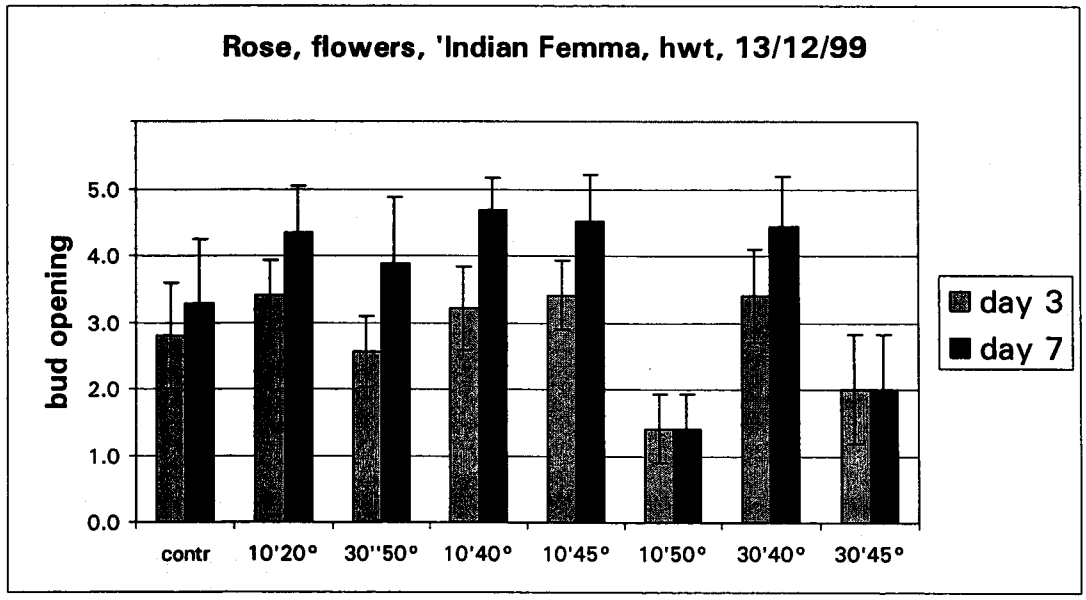


Fig 4.2.1.1.2. Flower bud opening of rose variety 'Indian Femma' , measured after 3 and 7 days after the hot water treatments. Bud opening classes, see Fig 4.2.1.1.1.

Table 4.2.1.1.1. Visual damage caused by hot water treatments of cut roses 'Indian Femma' in Nov 1999 and Dec 1999

HWT, cut roses 'Indian Femma', 11/99

treatm		temp	
1	control		No visual damage
2	10 min	20	No visual damage
3	30 sec	50	No visual damage
4	10min	40	No visual damage
5	10 min	45	No visual damage
6	10 min	50	leaves and flower dead

HWT, cut roses 'Indian Femma', 12/99

treatm		temp	
1	control		No visual damage
2	10 min	20	No visual damage
3	30 sec	50	No visual damage
4	10min	40	No visual damage
5	10 min	45	browning of calyx
6	10 min	50	leaves and flower dead
7	30 min	40	No visual damage
8	30 min	45	browning of calyx

4.2.1.2. Anthurium flowers

Introduction

For Anthurium flowers a hot water treatment appears to be a possible way of disinfestation. Anthurium is a cut flower of tropical origin, so was expected to have a tolerance against high temperatures, and *Botrytis* infection is not known to be of major problems in this commodity.

Materials and methods

Anthurium flowers were harvested at the research station. Flowers were hydrated for 24 h at 20 °C in water for 24 h.

Hot water treatment was carried out in a waterbath, with circulation. Exposure time started at the moment the flowers were immersed. After treatment, the flowers were cooled down in a bath at 20 °C, for 10 minutes.

After treatment, stems were recut and placed in the interior room (20 °C, 60 % RH, 12 h light per day at 14 $\mu mol.m^{-2}.s^{-1}$), 10 stems per treatment. All stems were examined daily until the end of the vase life.

Results and discussion

Treating the Anthurium flowers in the same way as the roses in Fig 4.2.1.1.1 and 4.2.1.1.2 showed that the 50 °C treatment caused severe damage. The spathe turned brown within a few days. The number of brown spathes was almost 100% in both experiments (Fig 4.1.1.2.1 and 4.1.1.2.2). Also, the 30' 45 °C treatment caused damage: 40 % showed browning of the spathes. The undamaged stems had the same vase life as the control.

Obviously, the fact that Anthurium is a crop of tropical origin, did not coincide with a higher high-temperature tolerance.

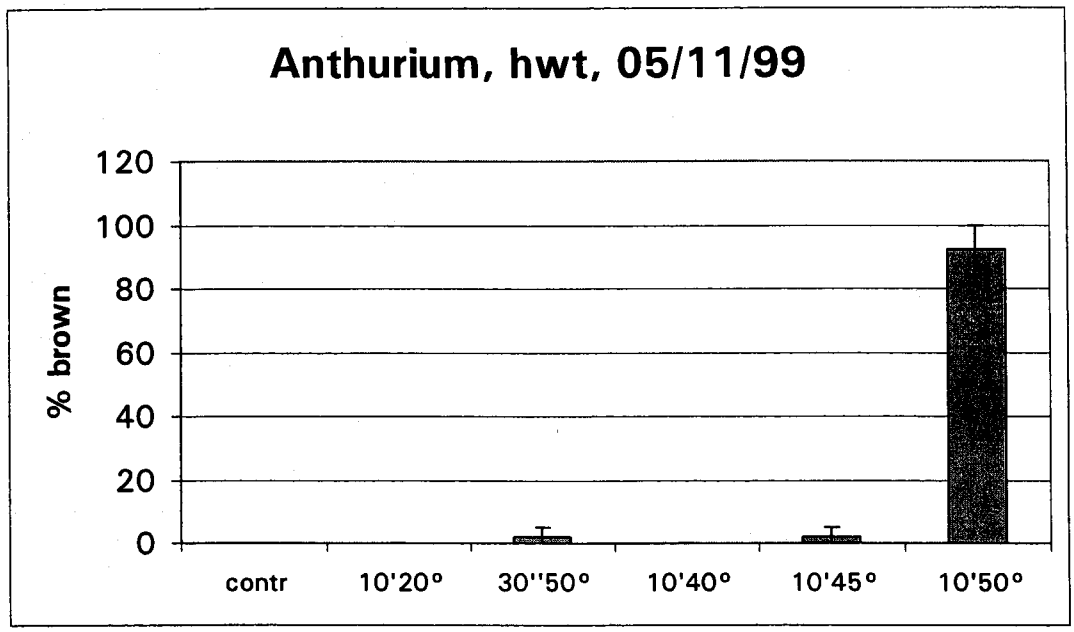


Fig 4.1.1.2.1. Percentage of brown spathes of Anthurium 5 days after the hot water treatments. First experiment.

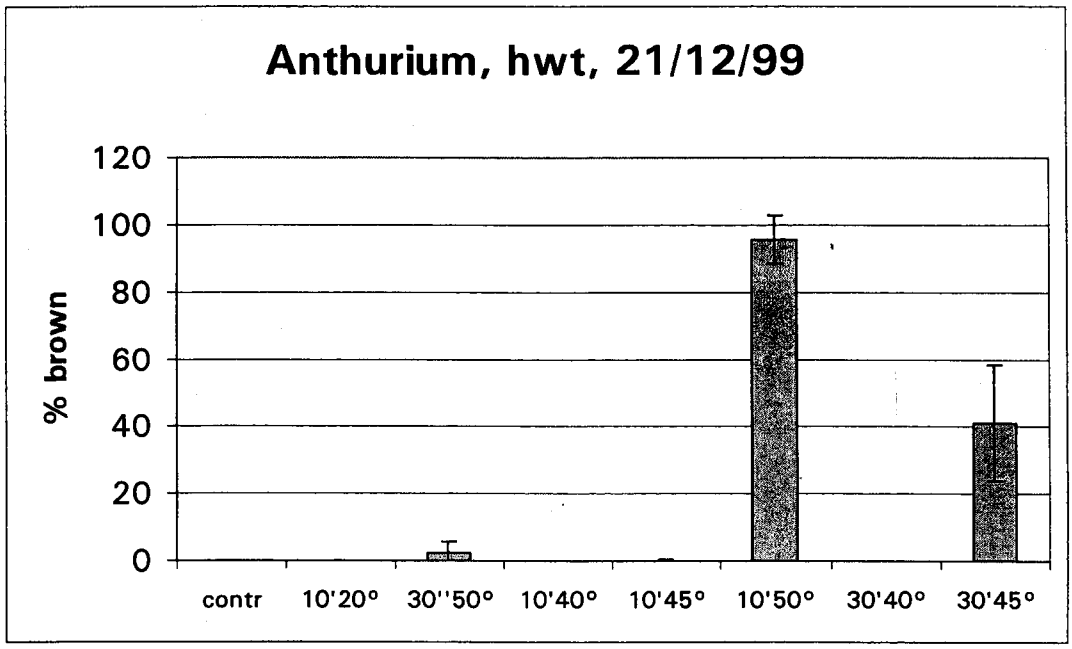


Fig 4.1.1.2.2. Percentage of brown spathes of Anthurium 5 days after the hot water treatments. Second experiment.

4.2.2. Cuttings and planting material

A possible field for application of hot water treatments is in plant cuttings and other plant parts like Yucca stems. These plant materials are transported all over the world.

4.2.2.1. Yucca stems

Introduction

Yucca stems are imported from Middle-American countries. Here, several pests can be present in the material, which often are not visible in the material at the moment of exporting/importing. Yucca stems can be stored for longer periods, thus enabling disinfestation during storage. At the time this project started Yucca stems were mainly transported in unrooted stage. During the last two years however, an increase is seen in import of rooted stems; the rooting process is conducted in the producing countries. Besides development of roots, sometimes also undifferentiated callus is formed along the rim of the stem. From this callus roots can develop in a later stage.
After testing hot water treatments on unrooted stems in the first project year, rooted stems were tested in the second year.

Material and methods

Unrooted or rooted stems were purchased at a local company. Hot water treatment was carried out in a waterbath, with circulation. Exposure time started at the moment the stems were immersed. After the hot water treatment, the stems were cooled down in a bath at 20 °C, for 10 minutes. Control plants were immersed in 20 °C for 10 minutes. After the treatment, stems were planted in 14cm pots in commercial potting soil and placed in a greenhouse at a temperature of 20 °C, 7 stems per treatment.

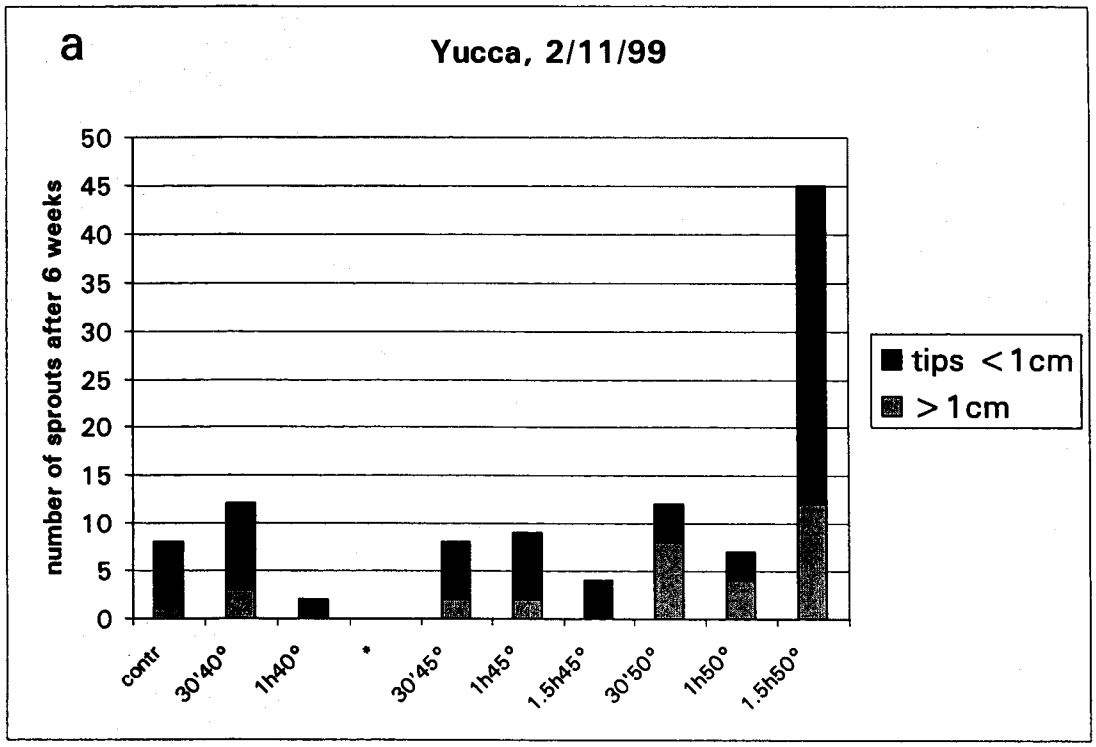
Effects of the hot water treatment on Yucca stems was investigated by counting the number of developing sprouts during a 5 month period after planting. The sprouts were divided in small green tips < 1 cm, or larger ones < 10 cm and > 10 cm.
The first experiment was carried out in November 1999.
A second experiment with unrooted stems has been carried out in March 2000, with 40, 42, 43, 44, 45 and 50 °C for 30, 60 and 120 minutes. After the treatment, stems were planted in the same way as described above.

A third experiment was carried out with rooted stems or stems with callus in September 2000. The 1 hour 44 and 47 °C treatments were chosen. Root- and shoot development were observed.

In order to measure actual temperatures in the centre of the yucca stems a 40, 45 and 50 °C treatment was given to three stems. Small thermometers were inserted in the stems, boreholes sealed, and temperatures monitored during the treatment.

Results and discussion

In the first experiment the untreated control stems developed about 20 shoots. The hot water treatments caused a slight decrease or increase of sprout development, with the 1.5 hours 50 °C treatment showing a remarkable high sprout development (Fig. 4.2.2.1.1a and 4.2.2.1.1b). However, these shoots developed only in the middle part of the stem, not in the top part, where they should develop.



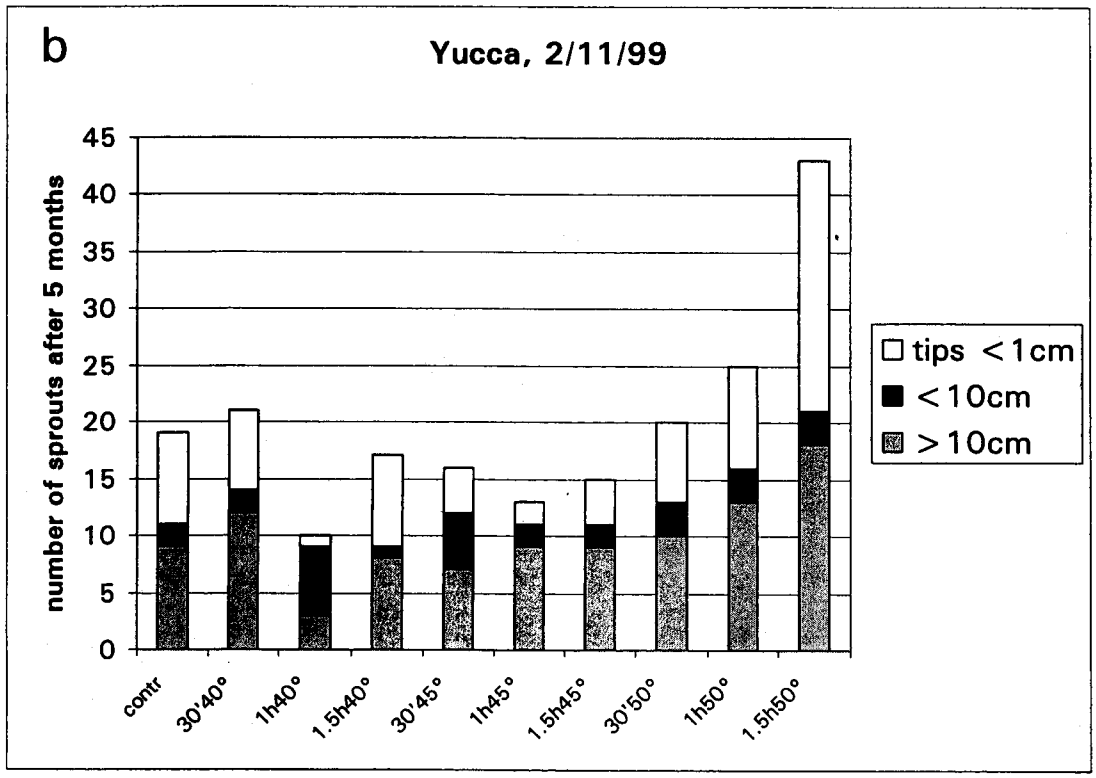


*Fig 4.2.2.1.1a and b. Number of green sprouts developed on Yucca stems after hot water treatments on Nov 2, 1999. Numbers are a total of 7 stems. After 6 weeks (4.2.2.1a) sprouts were divided in two classes: < 1 cm and > 1 cm. For the results of the second measurement after 5 months (4.2.2.1b, same plants) a division in three classes was made: < 1 cm, >1 but < 10 cm, and > 10 cm. * = not determined*

Although the root development was not quantified, it was observed that the hot water treatments did not cause differences in root development. Also the shoot morphology was not influenced by the treatments.

The second experiment showed that even after long treatments with high temperatures the shoot development was satisfactory (Fig 4.2.2.1.2 a). There was considerable variation between and within treatments (Fig 4.2.2.1.2 b and c), but it was clear that even the treatments with 50 °C were not harmful for the plants.

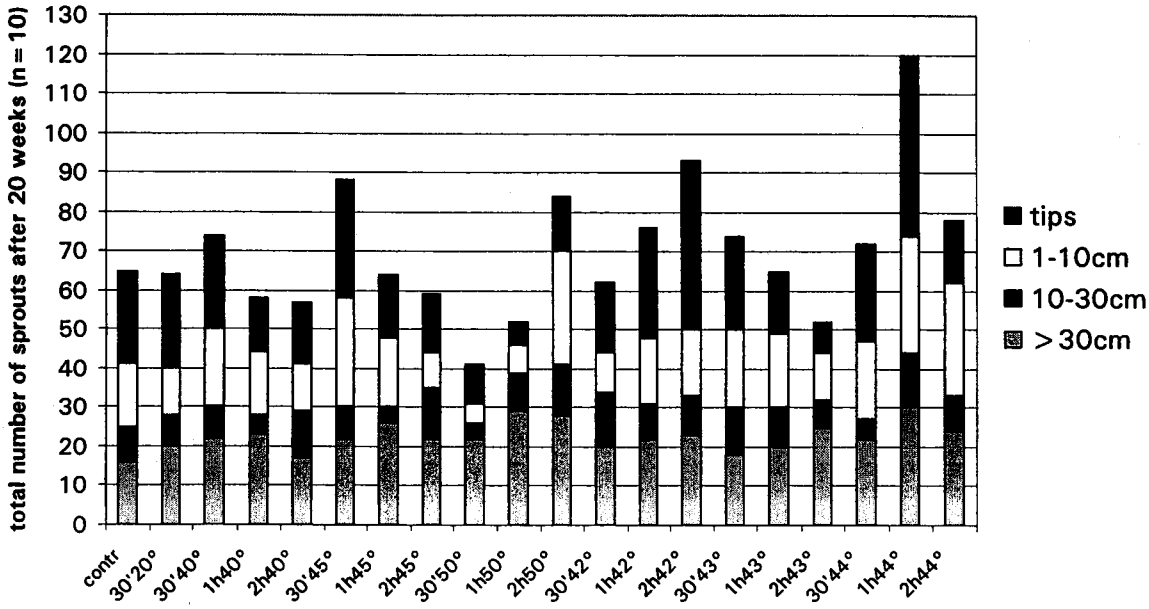


Fig. 4.2.2.1.2 a Yucca, hot water treatment of bare stems. Total number of sprouts of 10 stems per treatment after a growing period of 20 weeks in the greenhouse.

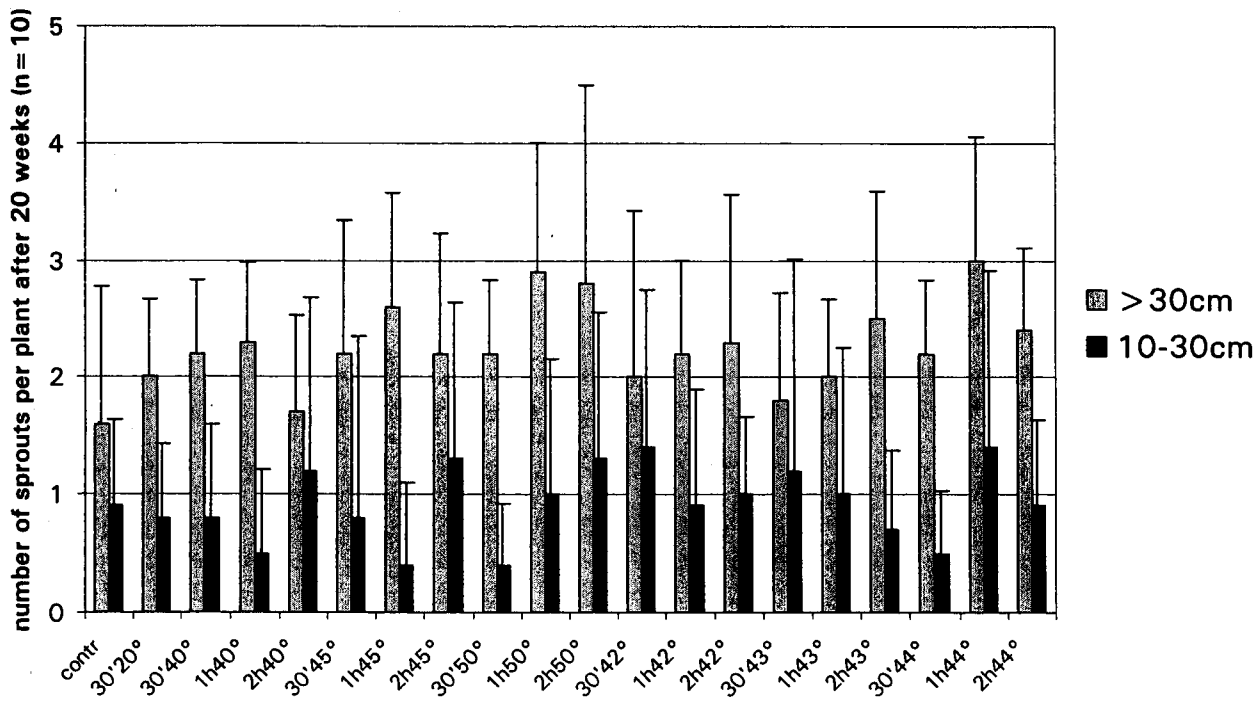


Fig 4.2.2.1.2.b. Yucca, hot water treatment of bare stems. Number of sprouts per stem after a growing period of 20 weeks in the greenhouse.

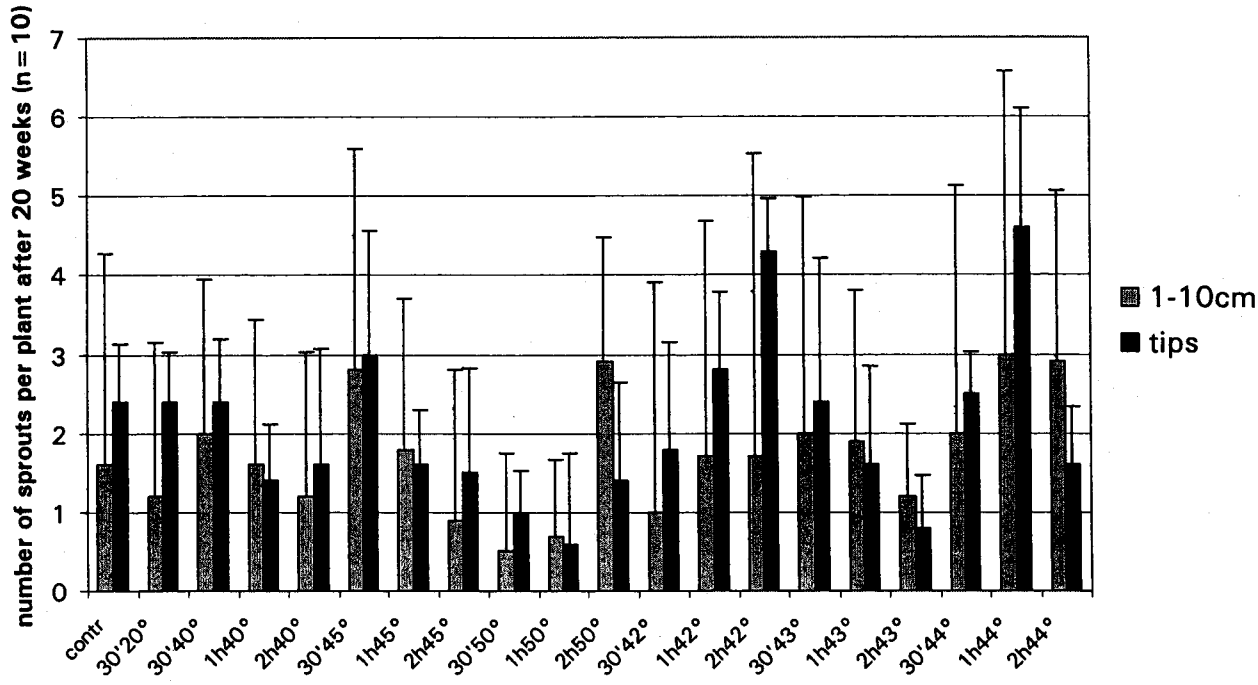


Fig. 4.2.2.1.2 c. Yucca, hot water treatment of bare stems. Number of sprouts per stem after a growing period of 20 weeks in the greenhouse.

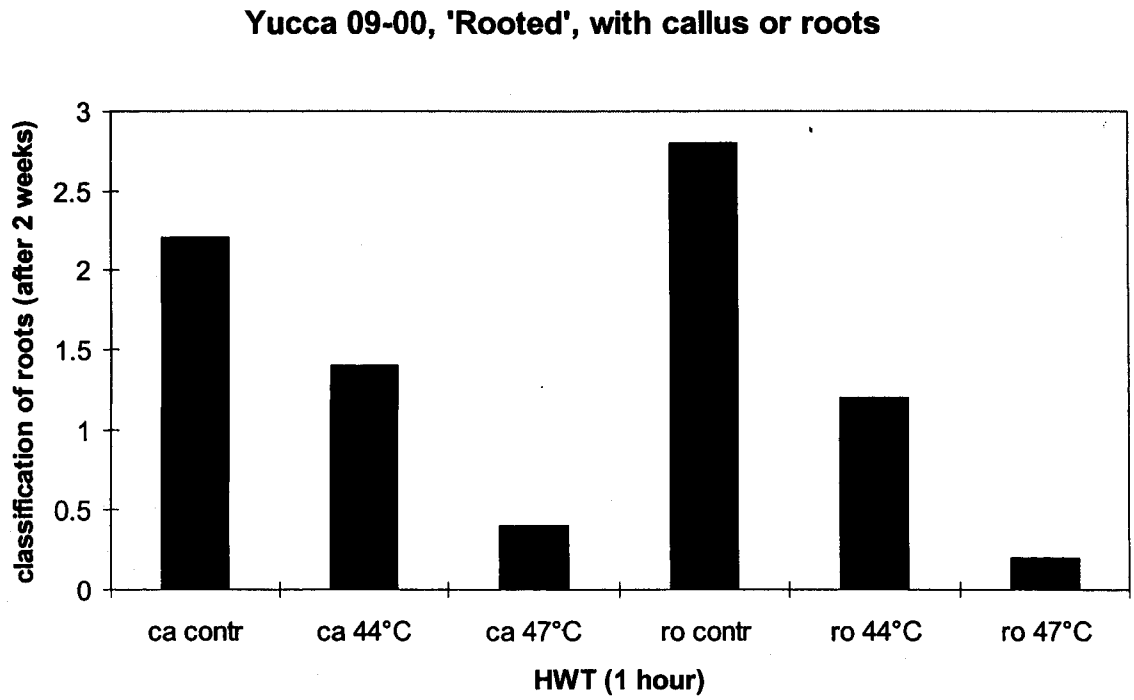


Fig 4.2.2.1.3 a. Root development two weeks after hot water treatment of Yucca stems with roots (ro) or callus (ca) present before hot water treatment. Classification: 0 = no roots, 1 = some healthy roots, 2 = intermediate number of healthy roots, 3 = many healthy roots

Treating rooted yucca stems or stems with callus for one hour in water of 44 °C or 47 °C caused a decrease of root development after two weeks (Fig 4.2.2.1.3 a). In order not to destroy the plants for quantifying root development a visual classification of the number of roots was used. Control plants developed a normal root system, which was classified between 2 and 3. Lower amounts of roots were classified as a 1 or 0 when no roots were seen. Although root development was hampered in the beginning, after 3 months the shoot development was not negatively influenced by the hot water treatments (Fig 4.2.2.1.3 b). Probably the retardation in root development was compensated during the growing period of three months.

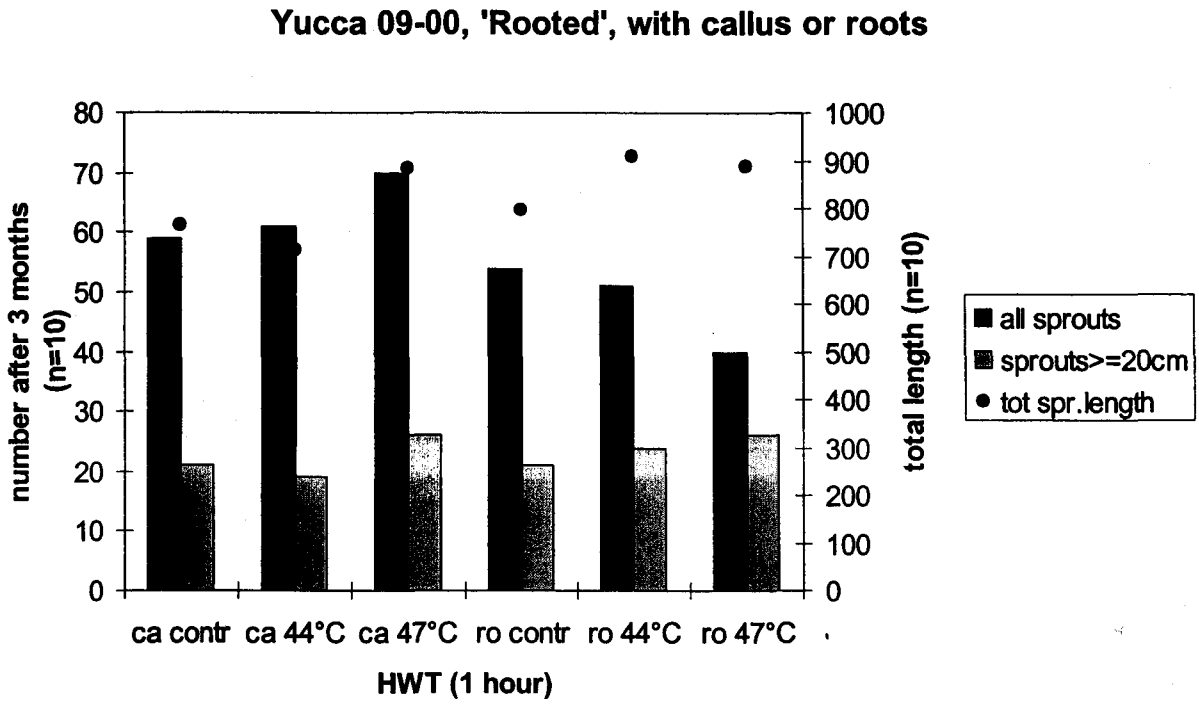


Fig 4.2.2.1.3 b. Number of sprouts developed after 3 months after hot water treatments of yucca stems with callus (ca) or roots (ro) present before the hot water treatments.

Since it is mainly the number of sprouts larger than 20 cm that count for the quality judgement, there is no negative effect on the quality of the plants at the moment of sale.

The actual temperature in the centre of the stems probably lags behind. Actual temperatures were measured in order to know if a shorter duration of the treatment would be possible. In Fig 4.2.2.1.4 the temperature curves show that it lasts about 40 minutes before the core of the stem has reached the same temperature as the water in the bath.

This means that a treatment duration of 30 minutes or less is not suitable for killing insects that are located in the centre of the

stem.

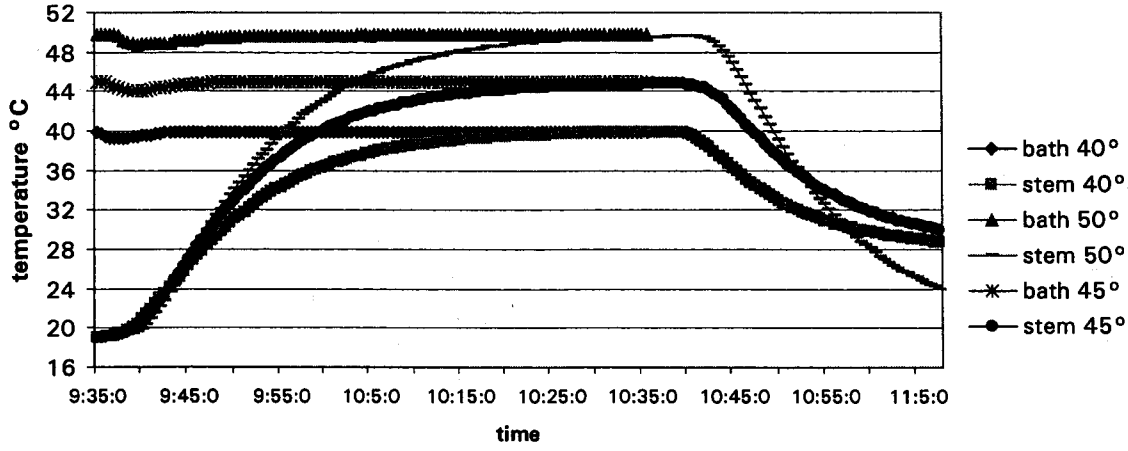


Fig 4.2.2.1.4. Yucca: measured temperatures in the water baths and in the centre of the stems, during a one-hour treatment at different temperatures, followed by a cooling period of 30 min. in a bath of approx. 20°C.

4.2.2.1b. Dracaena stems

Introduction

A meeting with a Yucca grower revealed that besides Opogona in Yucca, they can have problems with bark beetles in *Dracaena spp* stems. These bark beetles (*Xyleborus ferrugineus*) can cause damage in the plants during culture in the greenhouse. We decided to test whether the same hot water treatment as applied to Yucca was possible for Dracaena too.

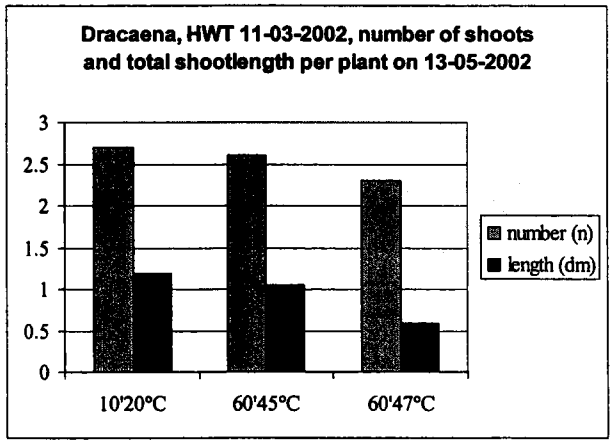
Material and methods

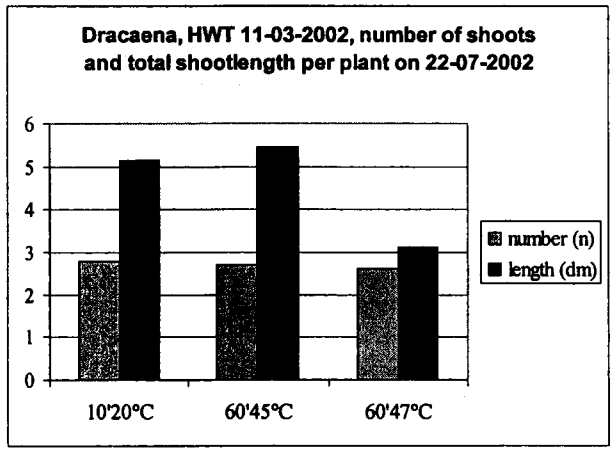
Tests were carried out with rooted stems. They were provided by a grower. Since the aim of the test was to investigate the temperature-resistance of the stems, non-infected material was used, so no special precautions had to be taken during cultivation of the plants. Ten stems per treatment were used. The control treatment consisted of 10 minutes in 20 °C, the other two treatments consisted of 1 hour 45 °C, followed by 10 minutes 20 °C, and 1 hour 47 °C, followed by 10 minutes 20 °C. Date of treatment was March 11, 2002. After treatment the stems were potted in commercial potting soil, and grown in a greenhouse, following the normal growth circumstances for *Dracaena*. After 2 months of cultivation the number of sprouts was counted and their length was measured, and another two months later they were counted and measured again. Root formation was checked visually.

Results and discussion

In Fig 4.2.2.1b.1 the shoot length and numbers are shown. It is clear that a treatment of 1 hour 45 °C is not harmful. At both counting dates the stems that had been treated for 1 hour at 47 °C showed shorter and fewer shoots than the controls (see Appendix 4, Fig 1). In this treatment no new roots had developed in the second period of growth (between the first and second counting). Possibly the roots that were present have been

damaged by the 1 hour 47 °C treatment. Since rooted stems were used, it cannot be said whether root development of unrooted stems would have been inhibited too. Also, in this treatment (1 h 47 °C) the shoots did not develop at the upper end of the stem, but on a lower position, as can be seen on the photo in appendix 4. The shoots of the plants of the 1 h 45 °C treatment also had a somewhat lower position as the control, but this was not considered to be a real quality loss.





4.2.2.2. Chrysanthemum cuttings

Introduction

Also, non-woody plant material is transported over the world. Production of cuttings often takes place in the (sub-) tropics, while the end product is produced in greenhouses in temperate zones. Chrysanthemum cuttings are produced in large quantities, can be stored during several days, thus allowing disinfecting treatments.

When testing the hot-water treatments for insects on plant cuttings, it seemed that the inundation was not complete. Some experiments were carried out in order to improve the wetting of the plant material during the hot water treatment.

Material and methods

Unrooted cuttings and rooted cuttings in soil blocks were purchased at a local company.

Hot water treatment was carried out in a waterbath, with circulation. Exposure time started at the moment the plants were immersed. After the hot water treatment, the plants were cooled down in a bath at 20°C, for 10 minutes.

After the treatment, unrooted cuttings were treated with Rhizopon (a rooting hormone product), planted in soil blocks, rooted during one week, hardened during one week, in some experiments the cuttings were planted in 11cm pots with commercial potting soil and put in a greenhouse at 20°C, 10 cuttings per treatment. In other experiments cuttings were measured after two weeks.

For rooted cuttings, after the treatment, the plants were planted in 11cm pots with commercial potting soil and put in a greenhouse at 20°C, 10 plants per treatment.

For the stress treatments, rooted cuttings in soil blocks were purchased at a local company. Part of the plants were stressed by keeping them dry for a few days, until they started wilting. Subsequently the hot water treatments were applied. A range of preconditioning treatments has been applied prior to several hot water treatments.

Results and discussion

In Fig 4.2.2.2.1 and Fig 4.2.2.2.2 experiments with respectively unrooted and rooted Chrysanthemum cuttings are shown. The growth after a 3 week period showed no significant effects on length development, with an exception for the 2 minutes 50 °C treatment, which caused some growth inhibition. For rooted cuttings several treatments caused growth inhibition, especially the 45 and 50 °C treatments.

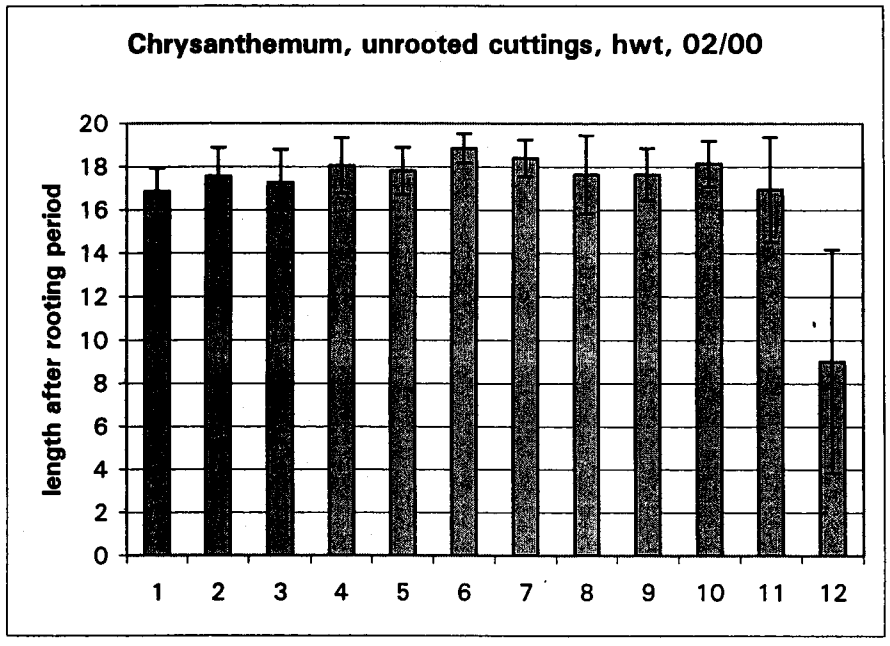


Fig 4.2.2.2.1. Length of Chrysanthemum plants after a 3 week period after the water treatment of the unrooted cuttings.

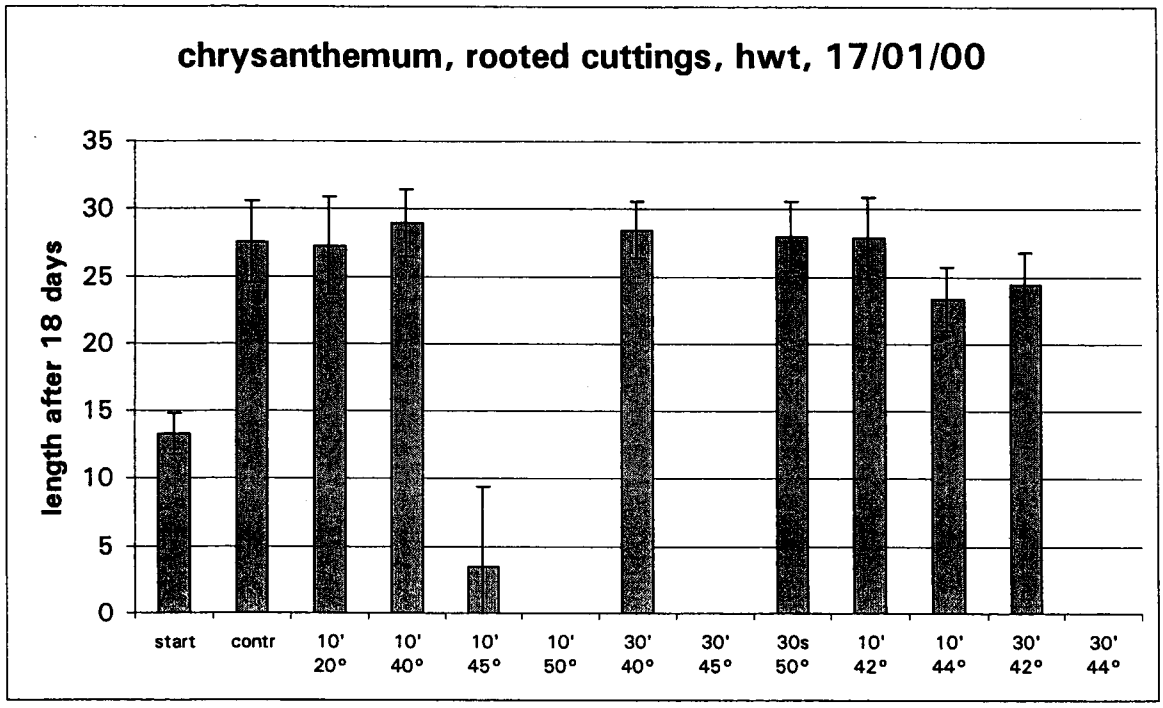


Fig 4.2.2.2.2. Length of Chrysanthemum plants after a 18 days growth period after hot water treatment of rooted cuttings.

Besides some effects on growth there were significant effects on the leaves during further development. Of the unrooted cuttings only the 2 minutes 50 °C treatment caused the older leaves to turn brown. But in the rooted cuttings there were white spots in the leaves, with sometimes entirely white leaves (Table 4.2.2.2.1). Although, for instance, the 10 minutes 42 °C treatment did not cause inhibition in growth, the spotting in the leaves probably will cause a significant decrease in appreciation by growers.

Table 4.2.2.2.1. Visual damage of Chrysanthemum plants, 'Reagan white', after hot water treatment of the rooted cuttings. Observed on the same plants as shown in Fig 4.2.2.2.2.

treatm		temp	
1	control		
2	10 min	20	
3	10 min	40	
4	10 min	45	7 plants dead, 3 plants leaves dead
5	10 min	50	all plants dead
6	30 min	40	3 plants with small white spots
7	30 min	45	all plants dead
8	30 sec	50	
9	10 min	42	2 plants with small white spots
10	10 min	44	older leaves with white spots, or completely white
11	30 min	42	older leaves with white spots, or completely white
12	30 min	44	all plants dead

Because drought stress before the hot water treatments could increase the stress tolerance (Hara et al, 1997), and thus the amount of damage, several tests on this item have been performed. Measuring length after 5 weeks showed that the stressed

cuttings had less damage than the non-stressed ones. In the first experiment (Fig 4.2.2.2.3) the positive effects of pre-stress were stronger for Vyking. In the second experiment (Fig 4.2.2.2.4) both varieties reacted in the same way.

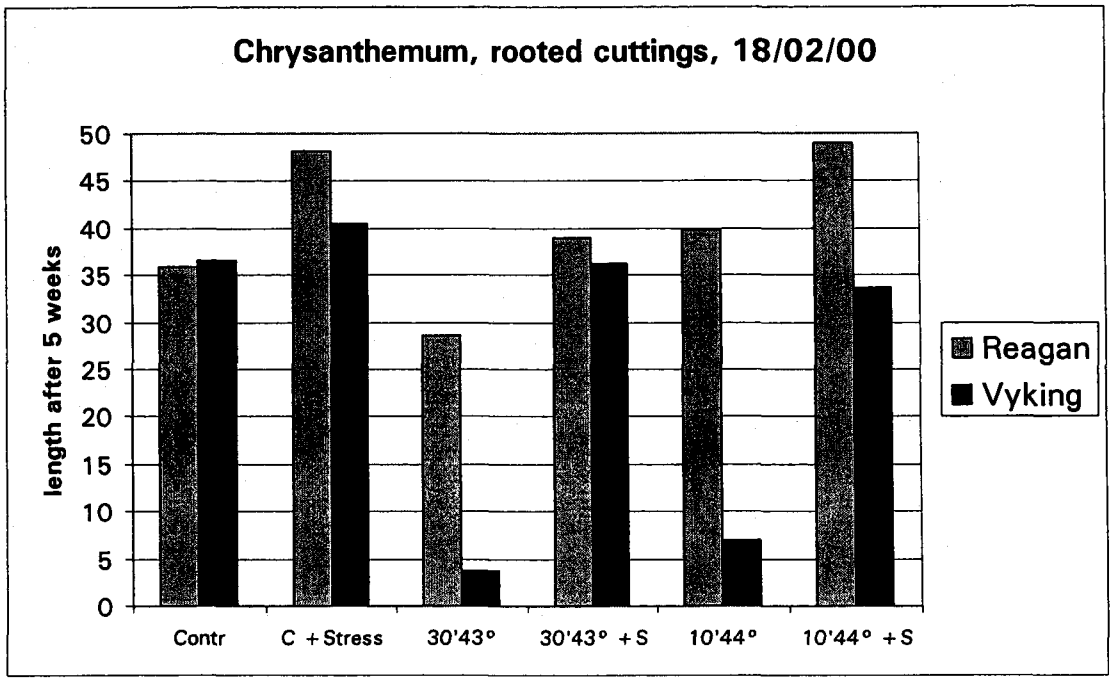


Fig 4.2.2.2.3. Length of Chrysanthemum plants (varieties 'Reagan' and 'Vyking') after a 5 weeks growth period after the hot water treatment of rooted cuttings with or without a drought stress treatments prior to the hot water treatment.

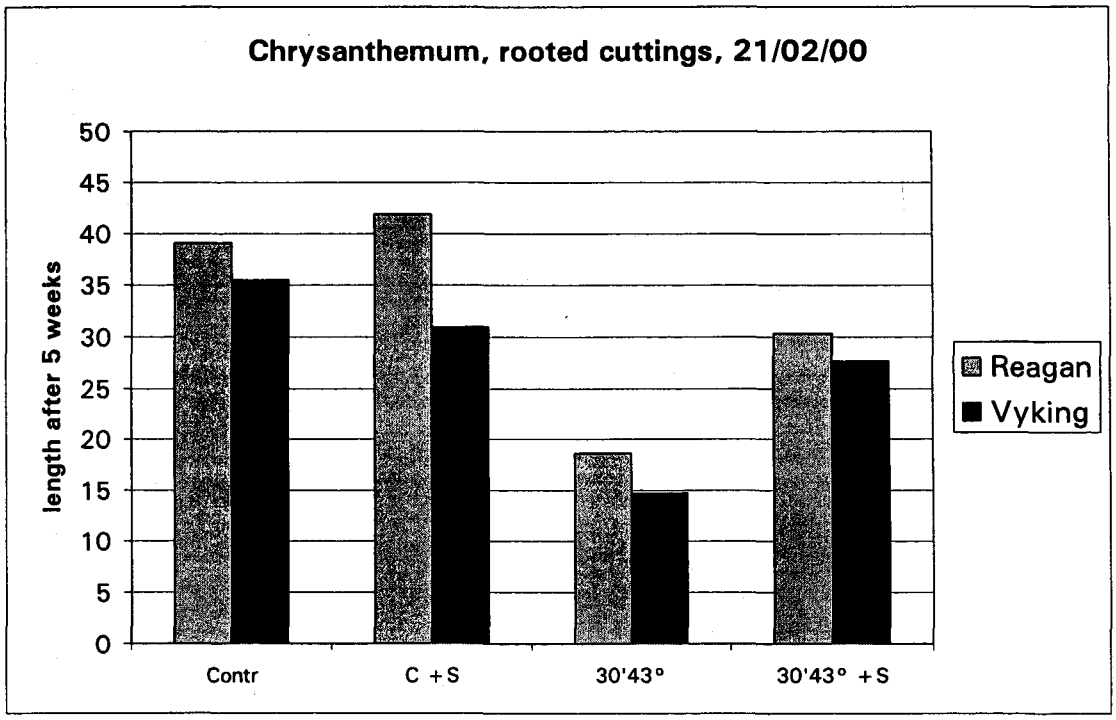


Fig 4.2.2.2.4. Length of Chrysanthemum plants (varieties 'Reagan' and 'Vyking') after a 5 weeks growth period after the hot water treatment of rooted cuttings with or without a drought stress treatment prior to the hot water treatment.

The development of visual damage during growth after the treatments is shown in Table 4.2.2.2.2, see also Appendix 4, Figs 3 and 4. There is less development of white spots when the plants have been stressed before the hot water treatment. These observations on stressed plants indicate that tolerance for hot water treatments may be dependent on the pre-treatment of the material. This can be regarded as an advantage for disinfesting methods: the plants can be 'hardened' before the treatment. However, it can also be a disadvantage: it is always difficult to trace the 'stress history' of material, so the actual tolerance for hot water treatments often will be a guess.

Table 4.2.2.2.2. Visual damage of Chrysanthemum plants which are stressed or non-stressed before the hot water treatment. Observed on the same plants as shown in Fig 4.2.2.2.3 and 4.2.2.2.4.

treatm		temp	Reagan	Vyking
18/02				
1	control			
1 stress	control			
2	30 min	43 °C	young leaf: white spots	upper leaves: white / dead
2 stress	30 min	43 °C	upper leaves: white spots	some young leaf: white spots
3	10 min	44 °C	young leaf: white spots	young leaf: white / dead
3 stress	10 min	44 °C	upper leaves: white spots	some leaves: white spots
21/02				
1	control			
1 stress	control			
2	30 min	43 °C	upper leaves: white/brown	upper leaves: white/brown
2 stress	30 min	43 °C	upper leaves: white spots	upper leaves: white spots

Two further experiments were done to precondition (harden) the cuttings, to make them more resistant to high temperatures. Two temperatures were applied (Table 4.2.2.2.3)

Table 4.2.2.2.3 Damage on 'Reagan White' Chrysanthemum cuttings after hot water treatments after prior stress treatments. Desiccation was expressed as loss of fresh weight (FW). Damage was classified as 0=no damage, 5= dead

Hot water treatment	Control (10' 20°C)	30' 43 °C	30' 45°C
Stress pre-treatment			
Exp 1, 16 Aug 2000			
Control	0	5	5
24 h 35 °C in plastic bag	1	3	5
2h 35 °C in water	0	3	5
2 h 40 °C in plastic bag	1	2	4
1 h 35 °C + 1 h 40 °C in water	2	4	5
Exp 2, 29 Aug 2000			
Control	0	5	5
5 % desiccation	0	4	5
10 % desiccation	0	3	5
15 % desiccation	1	3	5

It is clear that even with a pre-stress treatment a hot water treatment of 30' 45 °C still gives unacceptable damage. Desiccation is not improving the tolerance of the plant for hot water treatments.

Two experiments were performed to achieve a better wetting of the cuttings, in order to get possible present insects in better contact with the hot water. The use of the wetting agent "Zipper" (Asepta, The Netherlands) and vacuum was chosen. In Table 4.2.2.2.4 the damage caused by the treatments is shown.

In the first experiment both the vacuum treatment and the wetting agent caused unacceptable damage. In the second experiment only the wetting agent caused extra damage when compared with the 30' 45 °C treatment only.

Table 4.2.2.2.4. Damage caused by measures to improve wetting of the plant material. Damage was classified as 0=no damage, 5= dead. 'Reagan White' cuttings were used.

Treatment	Damage class
Exp 1, 27 July 2000	
Control 10' 20°C	0
30' 43°C	0
5' vacuum treatment (in water) + 30' 43°C	4
30' 43°C in water with 0.005% wetting agent	4
Exp 2, 2 Aug 2000	
Control 10' 20°C	0
30' 43°C	2
5' vacuum treatment (in water) 500 mbar + 30' 43°C	2
5' vacuum treatment (in water) 100 mbar + 30' 43°C	2
30' 43°C in water with 0.0005% wetting agent	3
30' 43°C in water with 0.005% wetting agent	3

In Table 4.2.2.2.5 the growth and damage levels of unrooted chrysanthemum cuttings after several hot water treatments are given. These experiments included thrips larvae and adults, as described in Tables 4.3.2.8.and 4.3.2.9. For the growth numbers, means of all four experiments are given.

Table 4.2.2.2.5. Length and damage level of chrysanthemum cuttings 'Reagan White' three weeks after a hot water treatment of unrooted cuttings. Means of four experiments.

pre-treatment	treatment	length	% damaged leaves
none	10 ' 20 °C	19.4	0
none	30 ' 43 °C	8.4	11
none	30 ' 45 °C	2.2	33
2 h 35 °C	10 ' 20 °C	20.3	1
2 h 35 °C	30 ' 43 °C	19.1	10
2 h 35 °C	30 ' 45 °C	12.0	37
1 h 35 °C + 1 h 40 °C	10 ' 20 °C	20.3	5
1 h 35 °C + 1 h 40 °C	30 ' 43 °C	20.0	1
1 h 35 °C + 1 h 40 °C	30 ' 45 °C	27.0	13

A pre-treatment of 2 hours 35 °C gave no reduction of the percentage of damaged leaves, while a pre-treatment of 1 hour 35 °C + 1 hour 40 °C gave a good 'protection' against the subsequent hot water treatments of 30'43 and 30'45 °C. Yet, a damage

level of 13 % of the leaves scorched, yellow or brown still is too high for commercial practice. The total treatment consisting of 1 hour 35 °C + 1 hour 40 °C, followed by 30 minutes 43 °C appeared to be the most promising.

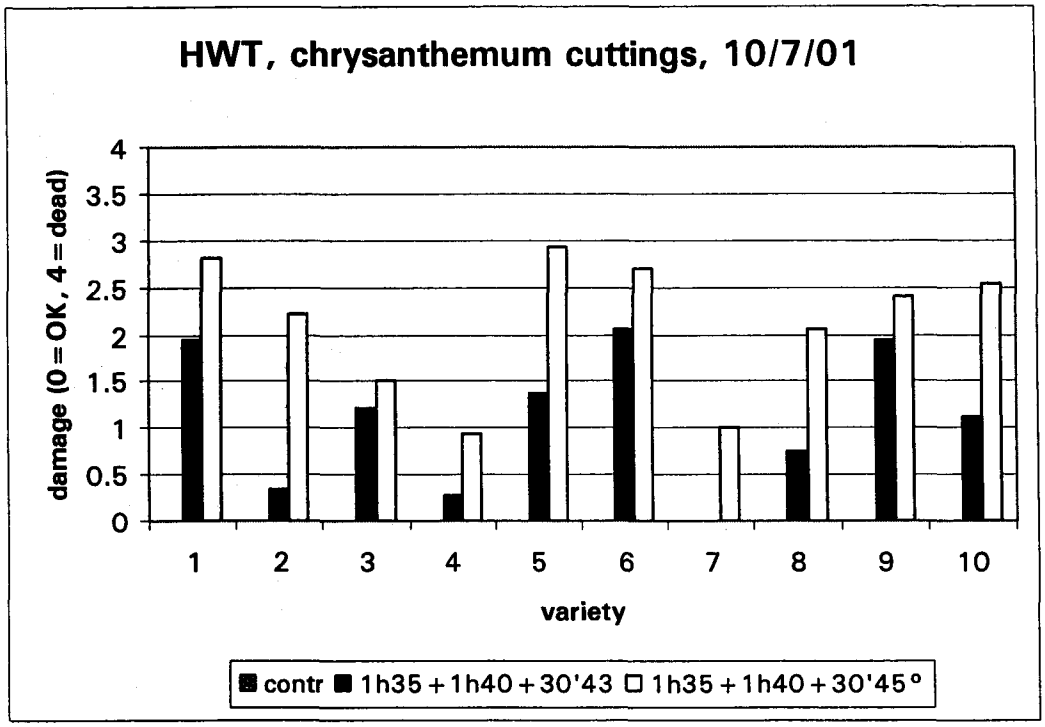
In order to test whether there are big differences in hot water treatment tolerance between different varieties, two variety experiments were carried out. Unrooted cuttings of different varieties were treated with the pre-treatment (1 hour 35 °C + 1 hour 40 °C) followed by 30 minutes 43 °C or 30minutes 45 °C, both followed by a cooling down of 10 minutes 20 °C. Fifteen cuttings per treatment were used. In Table 4.2.2.2.6 the names of the varieties are given.

Table 4.2.2.2.6. Chrysanthemum varieties used in variety experiments 1 and 2

Nr	Experiment 1	Experiment 2
1	Harlekijn	Harlekijn
2	Merced	Reagan Cream Elite Arie
3	Miramar	Reagan White
4	Rage	Rocky
5	Reagan Cream Elite Arie	Stallion
6	Reagan White	Tigerrag
7	Rocky	Weldon Dark
8	Stallion	Eugene Ivory
9	Tigerrag	Miramar
10	Weldon Dark	Rage

In Fig 4.2.2.2.5 and 4.2.2.2.6 the results of experiment 1 are shown. Leaf damage was scored between 0 (no damage) and 4 (dead). There was more damage at 45 °C, as expected, but there was also considerable damage for some varieties at 43 °C . The damage levels were very different between the varieties. 'Rage' and 'Rocky' showed almost no damage, while 'Harlekijn' and 'Reagan White' showed to be susceptible to heat damage.

The second experiment was designed as a repetition of the first. 'Merced' was replaced by 'Eugene Ivory' because the former was not available at that moment. In Figure 4.2.2.2.6 the damage and length of the cuttings after three weeks of growth are shown. In this experiment damage levels were higher, especially in the 30'45 °C treatment. Pictures of some of these plants can be found in appendix 5.



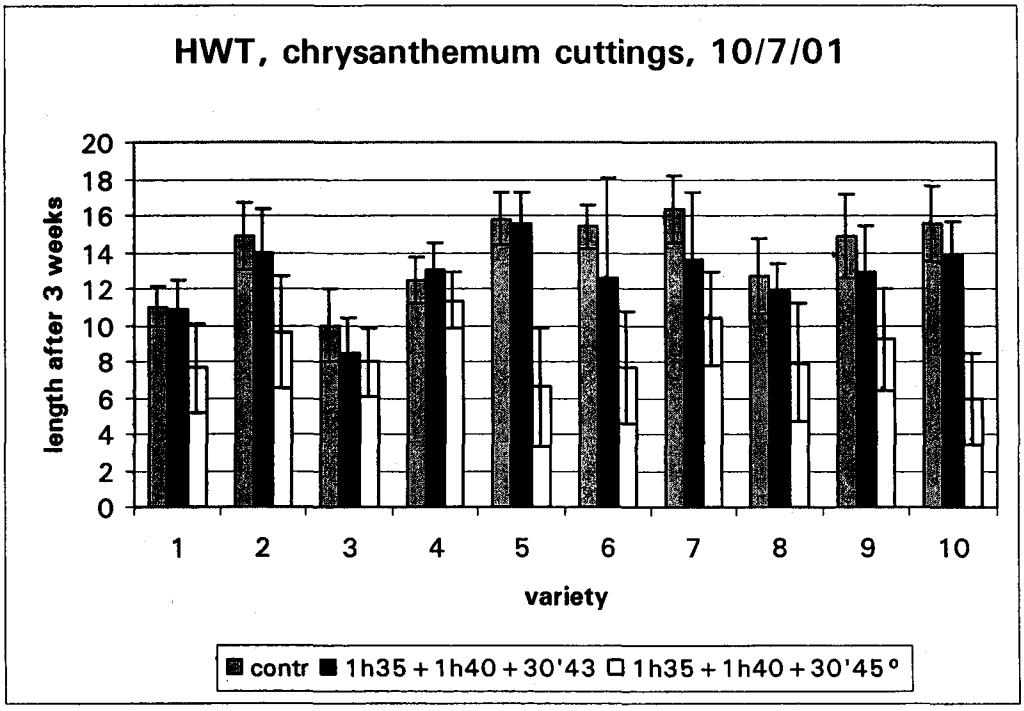


Fig 4.2.2.2.5. Leaf damage (upper graph) and plant length (lower graph) of chrysanthemum cuttings 3 weeks after hot water treatment. Control plants: no damage. Variety numbers refer to the names in Table 4.2.2.2.5, first experiment.

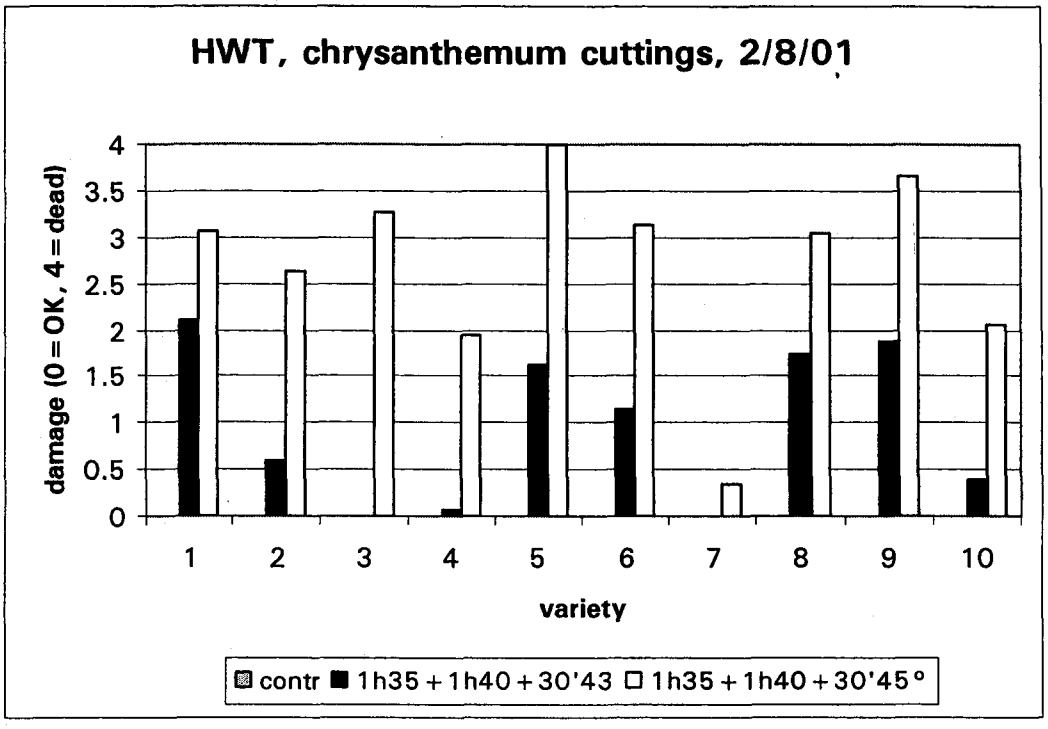
The length of the plants after 3 weeks culture in the greenhouse showed considerable difference within the treatments, especially in the 45 °C treatments (See Appendix 4, Fig 2). Because different varieties have a different growth rate, the decrease in length between the control plants and the hot-water-treated plants must be studied. In Table 4.2.2.2.7 the length of the hot-water-treated cuttings as percentage of the length of the

controls is shown for both experiments. There are differences between the first and the second experiment, meaning that a variety which performed well in the first experiment ('Rage') did not so in the second, and vice versa ('Reagan White').

Table 4.2.2.2.7. Length of cuttings after 3 weeks after hot water treatments of 1 hour 35 °C + 1 hour 40 °C followed by 30 minutes 43 °C or 30 minutes 45 °C. Length is expressed as percentage of the length of the control plants.

Variety	Experiment 1		Experiment2	
	30'43 °C	30'45°C	30'43°C	30'45°C
Harlekijn	98.5	69.3	82.6	45.1
Merced	93.3	64.4		
Miramar	84.9	80.2	92.7	14.8
Rage	105.1	91.3	92.1	73.8
Reagan Cream Elite Arie	98.2	41.7	100.5	59.0
Reagan White	81.9	49.5	109.0	33.9
Rocky	83.5	63.4	102.9	72.7
Stallion	93.8	62.7	77.3	0.9
Tigerrag	86.7	62.2	83.4	33.8
Weldon Dark	89.2	38.1	78.7	75.4
Eugene Ivory			88.0	52.9

The difference in heat tolerance between the varieties means that if a hot water treatment has to be used to disinfect cuttings, it should be checked first if the current variety is resistant to the hot water treatment.



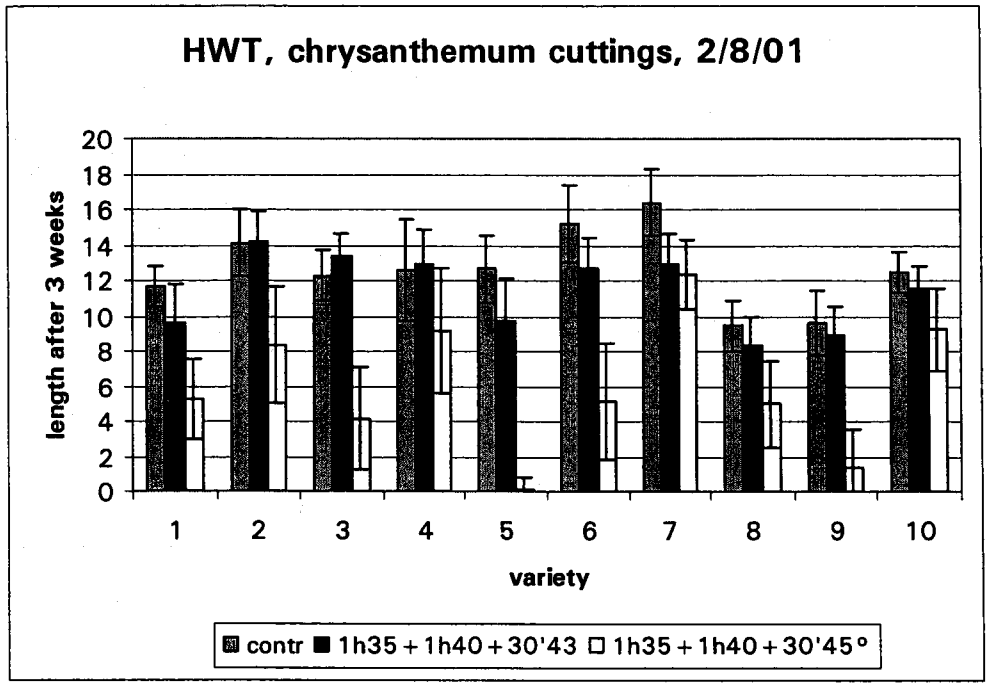


Fig 4.2.2.2.6. Leaf damage (upper graph) and plant length (lower graph) of chrysanthemum cuttings 3 weeks after hot water treatment. Control plants: no damage. Variety numbers refer to the names in Table 4.2.2.2.5, second experiment.

4.2.2.3. Rose cuttings

Introduction

Production of clean planting material for rose culture is crucial when aimed for a biological cultivation method. Like in Chrysanthemum cuttings chemical residue is a disadvantage, so heavily spraying of the mother plants is no option. Hot water dipping of the rooted or unrooted cuttings could be an alternative for disinfestation with Methyl Bromide.

Materials and methods

Unrooted cuttings were made from rose stems purchased from a local grower. The upper five-leave of each stem was used. After the hot-water treatments the cuttings were dipped in a commercial rooting powder, containing auxine, and placed in perlite (grain size 2 mm) in a tent in the greenhouse. Relative humidity in the tent was 100%. Perlite temperature was kept at 23 °C. After three weeks root weight and shoot length were measured. Per treatment 20 cuttings were used.

Rooted cuttings were purchased from a local company specialised in propagation of roses. They were rooted in rockwool blocks of approximately 8x8x8 cm. After hot water treatment they were planted on potting soil in a greenhouse. Growth was measured after 4 weeks in the greenhouse.

Results and discussion

In Fig 4.2.2.3.1 and 4.2.2.3.2 the results of one experiment are shown.

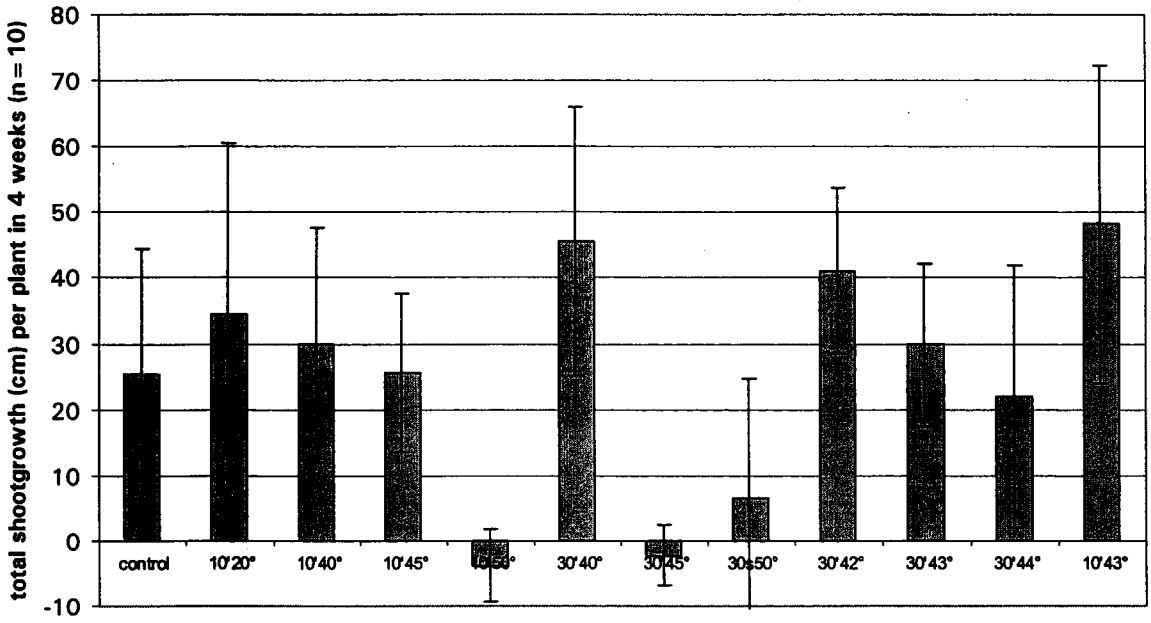


Fig.4.2.2.3.1. Rose 'Valentino', rooted cuttings in rockwool blocks. Hot water treatments. Total shoot growth per plant in 4 weeks in the greenhouse after the treatment.

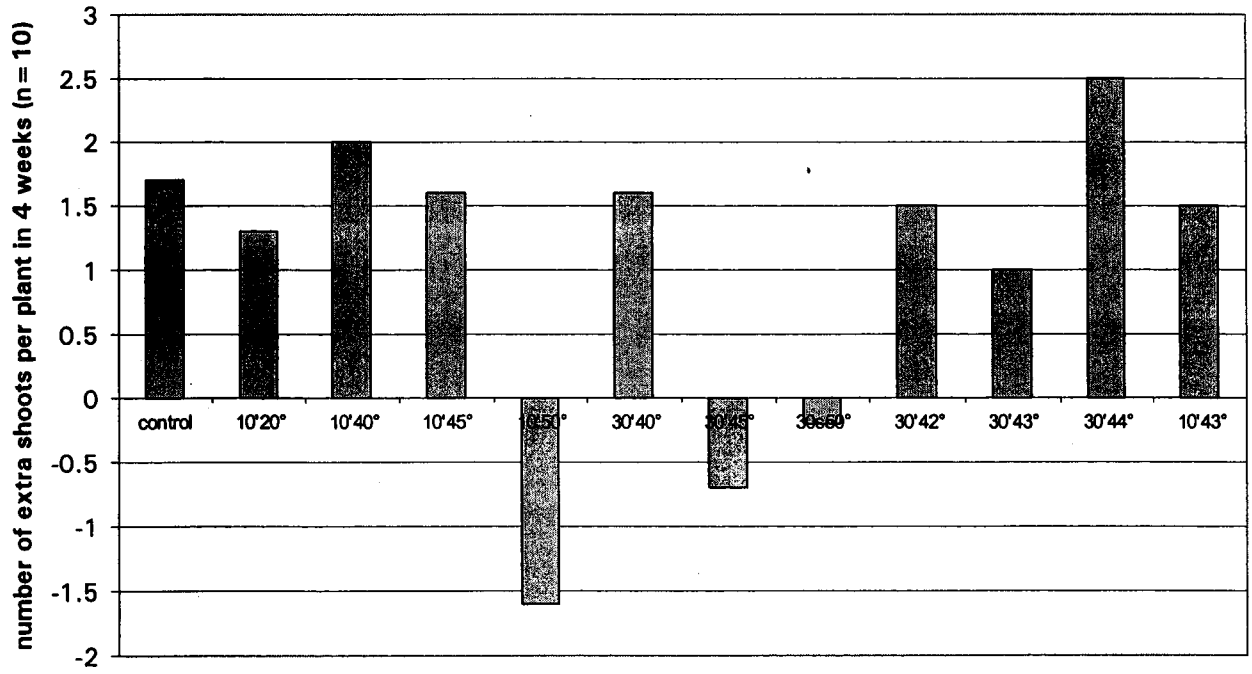


Fig.4.2.2.3.2. Rose 'Valentino', rooted cuttings in rockwool blocks. Hot water treatments. Number of extra shoots, grown per plant in 4 weeks in the greenhouse after the treatment.

It is clear that there is a lot of variability in shoot growth within the treatments. This is a normal feature in young rose plants, making it difficult to see significant differences between treatments. However, it can be concluded that temperatures higher than 45 °C for longer than 10 minutes are detrimental for further growth of the cuttings.

For unrooted rose cuttings we investigated if a pre-treatment was possible, in order to enhance the heat-tolerance of the cuttings. Comparable with the chrysanthemum cuttings we chose for a pre-treatment of 1 hour 35 °C + 1 hour 40 °C treatment. We applied this pre-treatment in the water bath, as well as in a plastic bag in a climate room. The hot water treatment following the pre-treatment was 30 '43 °C or 30 '45 °C. Three different varieties were tested: 'First Red', 'Indian Femma' and 'Red Berlin'.

Table 4.2.2.3.1. Shoot length (cm) and root weight (g) plus standard deviations (st dev) of 'First Red' rose cuttings three weeks after a hot water treatment with a pre-treatment.

Pre-treatment	Treatment	Shoot		Root	
		length	st dev	weight	st dev
none	10' 20 °C	0.6	0.94	0.77	0.44
1 h 35 °C + 1 h 40 °C in plastic bag	30 '43 °C	5.7	5.52	0.72	0.37
1 h 35 °C + 1 h 40 °C in plastic bag	30 '45 °C	3.8	5.57	0.68	0.40
1 h 35 °C + 1 h 40 °C in water	30 '43 °C	3.3	4.82	0.47	0.45
1 h 35 °C + 1 h 40 °C in water	30 '45 °C	2.5	4.41	0.39	0.29

Table 4.2.2.3.2. Shoot length (cm) and root weight (g) plus standard deviations (st dev) of 'Indian Femma' rose cuttings three weeks after a hot water treatment with a pre-treatment.

Pre-treatment	treatment	Shoot		Root	
		length	st dev	weight	st dev
none	10' 20 °C	10.2	3.13	0.92	0.29
1 h 35 °C + 1 h 40 °C in plastic bag	30 '43 °C	11.7	3.29	0.74	0.38
1 h 35 °C + 1 h 40 °C in plastic bag	30 '45 °C	8.5	2.98	0.86	0.32
1 h 35 °C + 1 h 40 °C in water	30 '43 °C	12.5	3.07	0.96	0.19
1 h 35 °C + 1 h 40 °C in water	30 '45 °C	11.7	3.14	0.95	0.39

Table 4.2.2.3.3. Shoot length (cm) and root weight (g) plus standard deviations (st dev) of 'Red Berlin' rose cuttings three weeks after a hot water treatment with a pre-treatment.

Pre-treatment	treatment	Shoot		Root	
		length	st dev	weight	st dev
none	10' 20 °C	0.4	1.12	0.93	0.19
1 h 35 °C + 1 h 40 °C in plastic bag	30 '43 °C	0.9	1.58	0.72	0.18
1 h 35 °C + 1 h 40 °C in plastic bag	30 '45 °C	0.6	1.33	0.77	0.32
1 h 35 °C + 1 h 40 °C in water	30 '43 °C	1.3	2.52	0.90	0.23
1 h 35 °C + 1 h 40 °C in water	30 '45 °C	2.1	2.76	0.88	0.21

The very low values for the length of the control cuttings of 'First Red' and 'Red Berlin' could be caused by the relatively short (10 minutes) treatment in the water bath. It is possible that the dormant buds in the leaf axils will develop easier when previously soaked in water for a few hours. Probably a proper control treatment would have been 2.5 hours in water of 20 °C. These low control values prevent a good comparison of the effect of the treatments on the growth and root development of the cuttings. Variability within the treatments was high. Yet, we see that the 30'45 °C treated cuttings tended to have shorter shoots than the 30 '43 °C treated cuttings. There was not much difference between a pre-treatment in a plastic bag or in a water bath. In 'Indian Femma' the control cuttings showed normal shoot development, with only the 30'45 °C treatment after pre-treatment in a bag being slightly shorter.
In general, these hot water treatments for unrooted rose cuttings gave positive results. However, like with Chrysanthemum cuttings, there are differences between varieties, which is a considerable disadvantage for use in practice.

4.2.2.4. Rose bushes

Introduction

When roses are grown in soil, a new crop is often started with rooted bushes. These are roots (often from the *Rosa canina*-type), grafted with a cut rose variety. The roots are produced by outdoor planting of *R. canina* cuttings in soil. These rooted plants are harvested, shoots removed, and new shoots of the desired cut rose variety are grafted upon them. The roots can be infected by nematodes during the outdoor cultivation. Several nematode species, like *Meloidogyne hapla, Pratylenchus vulnus* and *P. penetrans* can cause damage in rose culture, of which *M. hapla* can cause serious growth retardation in glasshouse rose culture. Thus, an alternative for disinfestation with Methyl Bromide is desirable. In bulb culture and also in miniature tree culture hot water treatments are used to kill nematodes. However, rather long treatments times at relatively high temperatures are necessary to reach complete disinfestation.

Materials and methods

Rose bushes of half a year old, consisting of *R. canina* 'inermis' roots, grafted with cv 'Circus' were purchased from a local company. 10 bushes per treatment were used. In the first experiment bushes were hot-water treated without any pre-treatment, or with a pre-treatment by keeping them in a climatised room at 40 °C for 2 or 24 hours, wrapped in plastic. Treatment date was in March 2001. In the second experiment (July 2001), the pre-treatment of 24 hours was replaced by a treatment of 1 hour 35 °C + 1 hour 40 °C in a water bath.
Bushes were planted in coconut fibre in a greenhouse and development was measured after 6 (first experiment) and 11 (second experiment) weeks by counting the number of new sprouts, weight of new sprouts and examining root development by classification: 0 = no roots, 1 = several roots, 2 = a normal healthy root system.

Results and discussion

In Fig 4.2.2.4.1 the weight of newly developed sprouts in the first experiment is shown. The treatments without pre-treatment show that 43 °C for 30 and 60 minutes does not give any damage. Higher temperatures lead to fewer and smaller shoots. A pre-treatment of 2 hours at 40 °C did improve the tolerance for hot water dipping to 45 °C (30 minutes). A 24 hour 40 °C pre-treatment was detrimental for all plants. The effect on the number of sprouts was equal to the effect on the sprout weight (data not shown).
In Fig 4.2.2.4.2. the root growth is shown. It is clear that root development is less susceptible for damage by hot water treatment than shoot growth. At 47 °C a decrease of the number of roots was seen. The 24 hour pre-treatment at 40 °C was also very harmful for root growth.
In Towson and Lear (1982) a treatment of 24 hours at 37.8 °C, wrapped in plastic, followed by 35 minutes hot water treatment at 48.3 °C was lethal to all *P. vulnus* without thermal injury to the plants. In our first experiment the plants were more susceptible for hot water treatments.

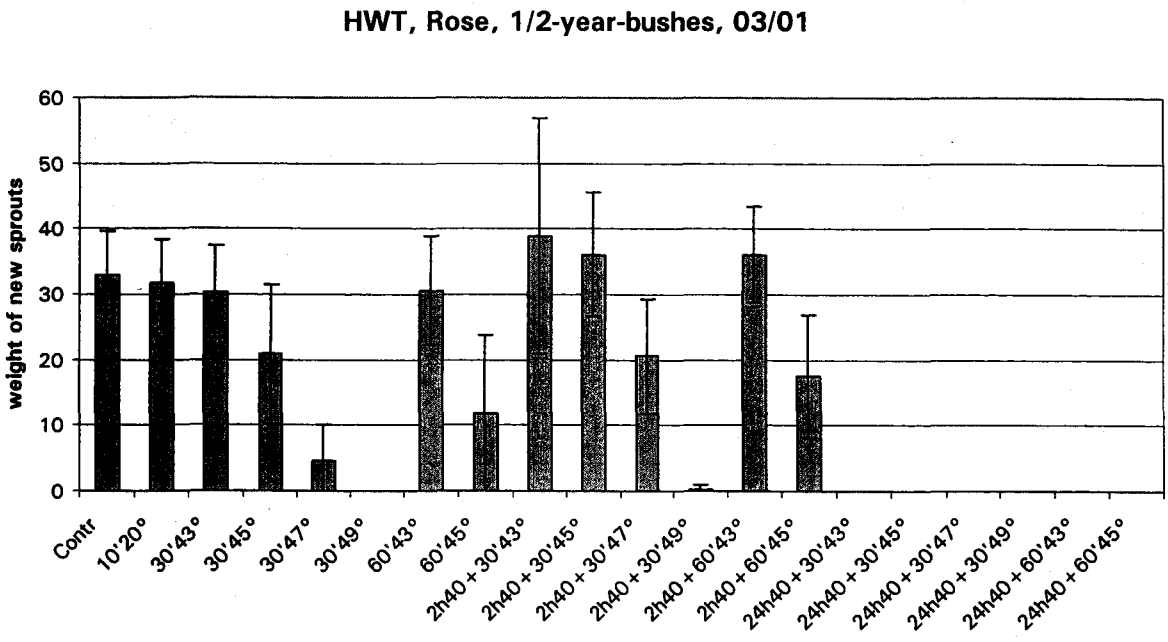


Fig 4.2.2.4.1. Weight of new sprouts (grams) formed 6 weeks after a hot water treatment of rose bushes. First experiment.

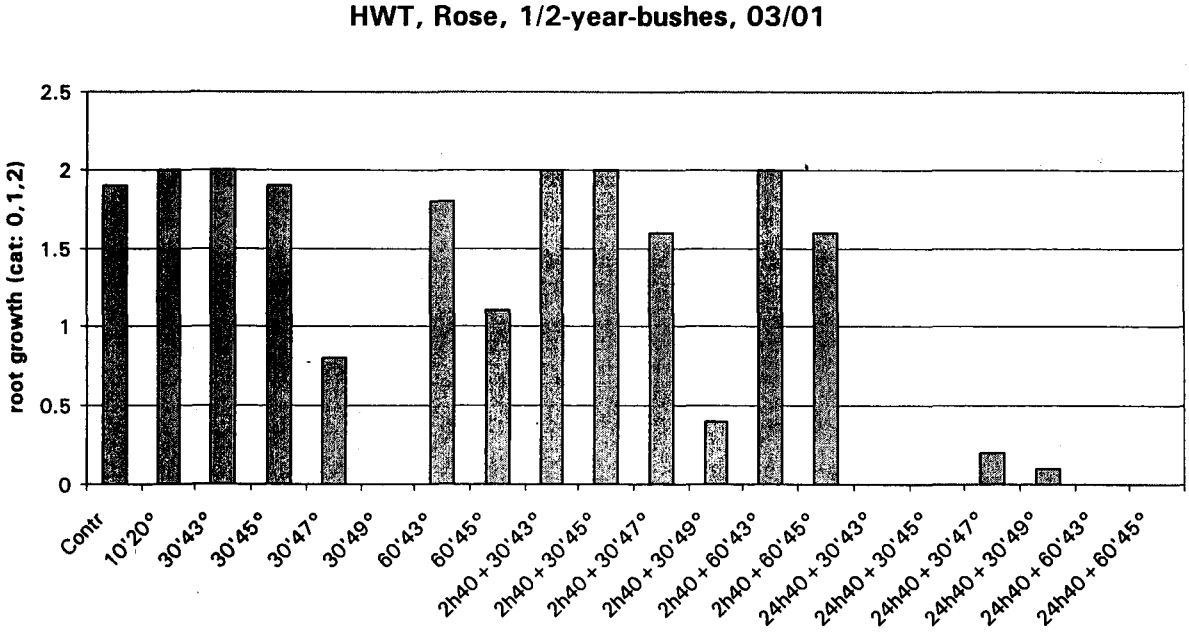
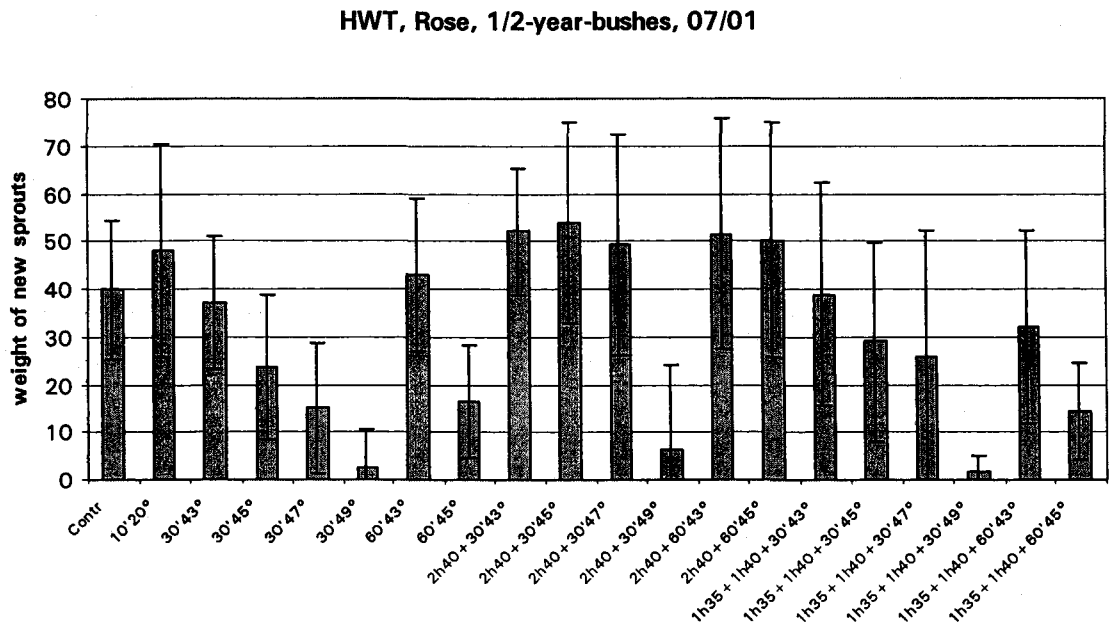


Fig 4.2.2.4.2. Root growth after a hot water treatment of rose bushes. 0 = no roots, 1 = several roots, 2 = normal root development. First experiment.

In the second experiment we shortened the 24 hour pre-treatment to 2 hours. Besides, we added a pre-treatment in a water bath.
In Fig 4.2.2.2.3 the weight of the new sprouts is shown. Here again, like in the first experiment, the treatments with temperatures above 43 °C caused lower shoot weights, when the plants were not pre-treated. In this experiment the 30 '47 °C and the 60 ' 45

°C treatments after a 2 h 40 °C pre-treatment were better than in the first experiment. However, the 30'49 °C treatment again was harmful for the plants. The 1 hour 35 °C + 1 hour 40 °C pre-treatment was not as good as the 2 hours 40 °C pre-treatment, because all plants developed less sprout weight. The effect on the number of sprouts again was very similar to the effect on sprout weight (data not shown)



Since the temperature tolerance of these rose bushes does not exceed 45 °C or 47 °C, a hot water treatment for disinfestation for nematodes was not possible.

Sub task 4.3: Testing of hot water treatments on pests

4.3.1. Hot water treatments on *Opogona sacchari*

Introduction

In 1999 a rearing with the Banana moth *Opogona sacchari* was started. Because of its long generation time (ca. 3 months) it is very time consuming to expand the rearing such that high numbers of larvae can be obtained for the HW treatments. This means that we mainly depend on infested material from growers. In the beginning of 2000 a large amount of infested Yucca stems was obtained. Larvae from these stems were used in the experiments.

In earlier experiments the Banana moth was treated with Hot water (HW) dips separately from the stems. Regardless of the exposure time (15, 30 or 45 min.) the mortality of the larvae at 44°C was 100%. In the next experiments larvae in the Yucca stems were treated with HW dips.

Material and method

In order to test HWT larvae mortality in vitro, three Banana moth larvae of the same size (as a reflection of age) were put in a 60 ml glass vial. Vials were sealed with a paper plug and tape before HW dipping. Immediately after a HW treatment vials were cooled in water of 20 °C for 10 minutes.
Percentages mortality were determined directly after each treatment.

In order to test HWT larvae mortality in situ, five stems with larvae were used for each treatment. (In the first experiment we used six stems per treatment). Hot water treatment was carried out in a waterbath, with circulation. Exposure time started at the moment the water temperature had reached the treatment temperature again. Immediately after a HW treatment stems were cooled down in water of 20 °C for 10 minutes.

The number of alive and dead larvae were counted directly after each treatment. Therefore the bark of the stem had to be removed.

For the experiments with Opogona eggs, adults, reared in a separate insect cage, were placed in an insect cage together with clean Yucca stems. After one week the stems were divided in two groups, one for control, one for the hot water treatment. After the hot water treatment the stems were put back in an insect cage, both groups separately, in order to enable the surviving eggs to develop. Three weeks later, the stems were checked under the bark for living larvae, eggs and dead larvae. After this observation the stems were immersed in water, and a few days later the water was checked for any floating larvae that had been missed during the first observation or originated from the core of the stem.

Results and discussion

Both the untreated control treatment and the control treatment in which the vials were put in 20 °C water for 10 min. resulted in no Banana moth mortality. At this stage only one temperature was tested. Regardless of the exposure time (15, 30 or 45 min.) the mortality of the larvae at 44 °C was 100%. See table 4.3.1.1.

Table 4.3.1.1. Hot-water treatment of Opogona sacchari larvae

treatment	replicates	mean size larvae (± stdev.)	mean % mortality (± stdev.)
control: untreated	5	1.4 (± 0.5) cm	0 (± 0.0)
control: 10 min. 20°C	5	1.5 (± 0.5) cm	0 (± 0.0)
15 min. 44°C	5	1.2 (± 0.3) cm	100 (± 0.0)
30 min. 44°C	5	1.3 (± 0.3) cm	100 (± 0.0)
45 min. 44°C	5	1.3 (± 0.3) cm	100 (± 0.0)

When whole stems were dipped, the untreated control treatment and the control treatment in which the stems were put in 20 °C water for 10 min. resulted in no Banana moth mortality. The number of larvae in each stem varied. In the last experiment only one stem was used in the untreated control treatment, in order to know if there were alive larvae in the batch of stems used for HW dips.
The mortality of the larvae was 100% at 44°C and 47°C with an exposure time of at least 30 minutes and at 50°C with an exposure time of at least 15 minutes. See table 4.3.1.2.

Table 4.3.1.2. Hot-water treatment of Opogona sacchari larvae in Yucca stems

Experiment no.	treatment	# stems	# larvae alive	# larvae dead
1	control: untreated	6	47	0
	control: 10 min 20°C	6	29	0
	30 min. 40°C	6	10	19
	30 min. 44°C	6	0	13
2	control: untreated	5	24	0
	15 min. 44°C	5	10	64
	15 min. 45°C	5	6	25
	5 min. 50°C	5	2	11
3	control: untreated	5	171	0
	30 min. 44°C	5	0	19
	5 min. 50°C	5	3	25
	15 min. 50°C	5	0	134
4	30 min. 44°C	5	0	32
	60 min. 44°C	5	0	64
	30 min. 47°C	5	0	49
	60 min. 47°C	5	0	12

In order to test the effectiveness of the 60 min 47 °C treatment on Opogona eggs, we used stems that had been infected shortly before the hot water treatment. In Table 4.3.1.3. the results of the first experiment are shown. The number of larvae found in the control stems was rather high, indicating a good reproduction capacity of the adult Opogona moths we used. In the hot water treated stems no larvae were found, indicating a 100% mortality of Opogona eggs. Two surviving *Sciara* larvae were found, indicating that not all insects were susceptible for this treatment.
This experiment was repeated twice, with the same results. The only difference being the low number (15) of larvae found in the control treatment in the second experiment. In the third treatment a few larvae were seen during the hot water treatment. This could

indicate that the yucca stems were not completely free from Opogona larvae from the start, or that the eggs had hatched already at the time of the treatment. No dead larvae were found in all three experiments.

Table 4.3.1.3. Total number of larvae developed after a hot water treatment of Yucca *stems infected with* Opogona *eggs. Larvae under the bark were counted after a 2 or 3 weeks incubation period after the treatment. Larvae in the core of the stem were counted after three day immersion (larvae in core). Three separate experiments are shown.*

		Alive larvae	Larvae in core
Exp 1	Control (untreated)	47	6
	60 '47 °C + 10' 20 °C	0	0
Exp 2	Control (untreated)	15	0
	60 '47 °C + 10' 20 °C	0	0
Exp 3	Control (untreated)	40	29
	60 '47 °C + 10' 20 °C	0	0

4.3.1b. Hot water treatments on *Xyleborus ferrugeneus*

Introduction

In chapter 4.2.2.1b has been mentioned the treatment of *Dracaena* stems with a hot water treatment in order to kill bark beetles *Xyleborus ferrugeneus.* The growth test in that chapter was carried out wit clean stems of *Dracaena*. In this chapter we describe one experiment wit stems, infected with *Xyleborus ferrugeneus* in various stages.

Material and method

Infected stems were obtained from a local grower. For the first experiment only 10 *Dracaena* stems were found. Two stems were kept for control. Eight stems were hot water treated. The control treatment was not put in water, the other treatment consisted of 1 hour 47 °C, followed by 10 minutes 20 °C. Alive and dead larvae and adults were counted immediately after the treatments. Bark and stem cores of both groups were placed separately in insect cages in order to check whether eggs still developed.

In the second experiment *Dracaena* as well as *Yucca* stems that were infected with *Xyleborus ferrugeneus* were treated as described for the first experiment.

Results and discussion

In Table 4.3.1b.1. the results of the first experiment are given. In the two untreated control stems all stages were found, wit many adults. In the hot water treated stems only dead larvae and dead adult were found. The material that was hot water treated did not yield any larvae after two weeks storage in an insect cage, indicating that the eggs were killed too. This shows that the hot water treatment is suitable for killing *Xyleborus ferrugeneus* bark beetles.

In the next experiment three Dracaena as well as three Yucca stems were used. Results are shown in Table 4.3.1b.2. In the untreated stems many (alive) adults were found. In the treated stems also mainly (dead) adults were found, only a few larvae. The material stored in insect cages did not yield any surviving larvae after two weeks.

Table 4.3.1b.1. Total number of larvae developed after a hot water treatment of Dracaena *stems infected with* Xyleborus ferrugeneus. *Eggs, larvae, pupae and adults were counted after a 2 or 3 weeks incubation period after the treatment.*

treatment	alive				dead			
	eggs	larvae	pupae	adults	eggs	larvae	pupae	adults
control	15	3	1	>10	0	0	0	0
60 '47 °C + 10' 20 °C	0	0	0	0	0	8	0	3

Table 4.3.1b.2. Total number of larvae developed after a hot water treatment of Dracaena *and* Yucca *stems infected with* Xyleborus ferrugeneus. *Eggs, larvae, pupae and adults were counted after a 2 or 3 weeks incubation period after the treatment.*

treatment	Plant	alive				dead			
		eggs	larvae	pupae	adults	eggs	larvae	pupae	adults
control	*Yucca*	0	0	0	20	0	0	0	0
	Dracaena	0	0	0	15	0	0	0	0
60 '47 °C + 10' 20 °C	*Yucca*	0	0	0	0	0	2	0	> 34
	Dracaena	0	0	0	0	0	0	0	11

Conclusive remarks on disinfestation of *Yucca* and *Dracaena* stems infected with *Opogona sacchari* or *Xylebores ferrugeneus*.

The results above show that a disinfestation of Yucca and Dracaena stems is possible with a hot water treatment of 60 '47 °C + 10' 20 °C. However, the results described in Chapter 4.2.2.1 and 4.2.2.1b show that relatively small growth retardation is seen in both *Yucca* and *Dracaena* plants after the treatment. Also, the sprouts develop slightly lower on the stem than in the controls.
Consulting a Yucca/Dracaena grower about the importance of these growth deviation we learned that there is hardly any margin for quality loss acceptable. We concluded that further fine-tuning of the treatment would be needed before a relatively expensive large-scale experiment could be carried out. Due to lack of time in this project these fine-tuning and large-scale experiments have been omitted.

4.3.2. Hot water treatments on the Western Flower Thrips *Frankliniella occidentalis*

Introduction

Larval and adult Western Flower Thrips (WFT) were treated with Hot water (HW) dips separately from their host plants as a first step, for a rough determination of a dose response curve. These results combined with the phytotoxicity study of the plants (sub-task 4.2), will narrow down the possibilities to be tested in the more laborious on-plant experiments. Since WFT lays her eggs in leaf tissue, the HW experiments of eggs are carried out in this *in vivo* situation.

Material and method

WFT eggs

Four adult female WFT were placed on a Chrysanthemum leaf in a small container (11 x 7 x 7 cm). The leaves were placed in a vial with the stem in 6 ml water. The containers were sealed with thrips-proof gauze and incubated at 22 °C. After 48 hours the thrips were removed and the leaves were checked for oviposition spots before obtaining an HW treatment. Immediately after a HW treatment leaves were cooled in water of 20 °C for 10 minutes. The leaves were placed in the containers again, and after 3 and 5 days emerged larvae were counted and removed.

Five experiments were carried out in which different HW treatments were tested (Table 4.3.2.1); experiments 2 and 3, and experiments 4 and 5, are replicates.

WFT larvae and adults

For first familiarisation with the material, five adult or five larval (L1 and L2) WFT were collected in 6 ml vials with help of an aspirator, after which the vials were sealed with a rubber plug. Thrips received a HW treatment (10 vials per treatment) and immediately thereafter were cooled in water of 20 °C for 10 minutes. After cooling down and again after 20 hours, numbers of alive and dead (immobile) thrips were determined.

The experiment was carried out two times according to this method (exp. 1 and 2 in Table 4.3.2.2). A third experiment was carried out with important adjustments in the experimental set-up. In this case larvae were placed into the vials with a small paint brush in stead of the aspirator, which resulted in lower larval control mortality. In both adult and larval trials a Chrysanthemum rayflower was put in the vials to prevent dehydration of the thrips. This also resulted in a lower control mortality (result of this pilot trial is not shown).

WFT on cuttings

WFT larvae or WFT adults were put in a plastic bag with 10 Chrysanthemum cuttings (variety Reagan White). The larvae were placed directly into a bag with a small paint brush. The adults were collected in a vial with help of an aspirator; the vial without a plug was placed in the plastic bag. The plastic bags were sealed and incubated at 22 °C for 24 hours to enable WFT to crawl into the growing tips of the cuttings.

In later experiments the method of collecting WFT was changed back to using an aspirator, because this was less time-consuming. A control treatment was always involved in the experiments, in order to compare the mortality caused only by handling.

After the 24 hour period cuttings were treated in hot water in the plastic bags, or they were taken out prior to the hot-water treatment.

When testing the tolerance of Chrysanthemum cuttings for a hot-water dip, it appeared that a pre-treatment with moderate high temperatures was beneficial for the tolerance (see 4.2.2.2). The effect of this pre-treatments of WFT larvae and adult mortality was not known. This is tested in several experiments.

Results and discussion

WFT eggs

Preliminary results (Table 4.3.2.1) show that a 30 minutes HW treatment of 41 °C or higher results in (almost) no emerging WFT larvae from Chrysanthemum leaves. With higher temperatures, also shorter treatment times can be applied for the same effect.

Table 4.3.2.1. Numbers of emerging WFT larvae 3 and 5 days after HW treatment of eggs in Chrysanthemum leaves

experiment no.	treatment	# leaves	# larvae after 3 d from 10 leaves	# larvae after 5 d from 10 leaves	total # larvae from 10 leaves
1	untreated	10	46	12	58
1	20°C, 10 min.	10	78	17	95
1	43°C, 20 min.	10	0	1	1
2	untreated	10	2	86	88
2	20°C, 10 min.	10	6	112	118
2	41°C, 10 min.	10	0	98	98
2	41°C, 30 min.	10	0	0	0
3	untreated	10	4	0	4
3	20°C, 10 min.	10	6	0	6
3	41°C, 10 min.	10	9	0	9
3	41°C, 30 min.	10	7	0	7
4	untreated	10	34	7	41
4	42°C, 10 min.	10	3	9	12
4	42°C, 20 min.	10	1	0	1
4	42°C, 30 min.	10	0	0	0
5	untreated	10	8	0	8
5	42°C, 10 min.	10	2	1	3
5	42°C, 20 min.	10	0	0	0
5	42°C, 30 min.	10	0	0	0

WFT larvae and adults

In experiment 1 and 2 the mortality in the untreated larvae and adults was high, especially 20 hours after the HW treatments (Tables 4.3.2.2 and 4.3.2.3). After changes in the experimental set-up (see material en method) this control mortality was much lower (exp. 3, Tables 4.3.2.2 and 4.3.2.3). The mortality in the control groups that received only a cold water dip (10 min., 20 °C), was in experiment 1 and 2 lower than the mortality in the untreated groups. Because we suspected this had to do with the humidity within the vials and its effect on thrips, in experiment 3 a rayflower of Chrysanthemum was added to prevent dehydration of the insects (see also material and method).

It is important that the numbers of immobile larvae and adults are determined not only directly after the dipping, but also 20 hours later. Due to the HW treatment thrips may become immobile but are not necessarily killed. Immobile WFT are regarded as dead 20 hours after HW treatment (Table 4.3.2.2 and 4.3.2.3).

Table 4.3.2.2 shows the effects of several HW treatments on WFT larvae. Only a treatment of 30 minutes 43 °C and 5 minutes 50 °C killed all larvae. More than 90% of the larvae were killed by HW treatments of 20 minutes of 43 °C.

Table 4.3.2.3 shows the effects of several HW treatments on WFT adults. A treatment of 30 minutes 42 °C, 20 minutes 43 °C and 2.5 or 5 minutes of 50 °C, killed all adults. In experiment 3 a 30 minutes treatment of 43 °C left a few thrips adults alive, but those where all dead after 20 hours. More than 90% of the adults were killed by HW treatment of 2 minutes of 50 °C.
Although there is some deviation in results between the experiments described here, it can be concluded that mortality rates are increasing with temperature and duration of the HW treatments. In general, 43 °C is lethal to WFT when treatment duration is in the 20 – 30 minutes range, but temperatures of more than 45 °C are needed for shorter treatment duration. WFT eggs are found to be more vulnerable to HW treatment, which means that future HW experiments should concentrate mainly on larval and adult WFT.

Table 4.3.2.2. Means of percentage immobile WFT larvae (± standard deviation) directly after HW treatment and dead larvae (± standard deviation) 20 hours after HW treatment

experiment no.	treatment	# replicates	% immobile larvae after treatment (± stdev.)		% larval mortality after 20 hr (± stdev.)	
1	untreated	10	9	*13*	70	*20*
1	20°C, 10 min.	10	2	*7*	17	*15*
1	40°C, 10 min.	10	13	*24*	46	*23*
1	40°C, 30 min.	10	46	*26*	82	*17*
1	42°C, 10 min.	10	36	*18*	67	*9*
1	42°C, 30 min.	10	94	*12*	100	*0*
1	50°C, 0.5 min.	10	2	*6*	11	*11*
1	50°C, 1 min.	10	53	*17*	80	*19*
2	untreated	10	60	*37*	39	*27*
2	20°C, 10 min.	10	17	*21*	21	*22*
2	40°C, 30 min.	10	12	*16*	19	*24*
2	40°C, 60 min.	10	20	*20*	32	*38*
2	42°C, 20 min.	10	100	*0*	49	*29*
2	42°C, 30 min.	10	100	*0*	78	*29*
2	50°C, 2 min.	10	100	*0*	98	*6*
2	50°C, 5 min.	10	100	*0*	100	*0*
3	untreated	10	3	*8*	10	*15*
3	20°C, 10 min.	10	18	*22*	21	*15*
3	43°C, 20 min.	10	100	*0*	90	*15*
3	43°C, 30 min.	10	100	*0*	100	*0*
3	45°C, 2 min.	10	3	*6*	12	*14*
3	45°C, 3 min.	10	11	*13*	17	*15*
3	45°C, 5 min.	10	97	*7*	82	*17*
3	50°C, 2.5 min.	10	95	*9*	86	*15*

Table 4.3.2.3. Means of percentage immobile adult WFT (± standard deviation) directly after, and dead adults (± standard deviation) 20 hours after HW treatment

experiment no.	treatment	# replicates	% immobile adults after treatment (± stdev.)		% adult mortality after 20 hr (± stdev.)	
1	untreated	10	7	*13*	74	*15*
1	20°C, 10 min.	10	0	*0*	48	*22*
1	40°C, 10 min.	10	0	*0*	76	*14*
1	40°C, 30 min.	10	36	*35*	76	*27*
1	42°C, 10 min.	10	12	*19*	81	*17*
1	42°C, 30 min.	10	100	*0*	100	*0*
1	50°C, 0.5 min.	10	0	*0*	35	*18*
1	50°C, 1 min.	10	65	*28*	78	*18*
2	untreated	10	2	*6*	6	*12*
2	20°C, 10 min.	10	9	*9*	8	*13*
2	40°C, 30 min.	10	16	*14*	16	*14*
2	40°C, 60 min.	10	29	*34*	54	*31*
2	42°C, 20 min.	10	100	*0*	83	*17*
2	42°C, 30 min.	10	100	*0*	100	*0*
2	50°C, 2 min.	10	100	*0*	98	*6*
2	50°C, 5 min.	10	100	*0*	100	*0*
3	untreated	10	2	*6*	4	*8*
3	20°C, 10 min.	10	7	*12*	13	*13*
3	43°C, 20 min.	10	100	*0*	100	*0*
3	43°C, 30 min.	10	98	*7*	100	*0*
3	45°C, 2 min.	10	11	*12*	16	*11*
3	45°C, 3 min.	10	2	*6*	10	*11*
3	45°C, 5 min.	10	67	*22*	79	*17*
3	50°C, 2.5 min.	10	100	*0*	100	*0*

WFT on cuttings

Results show that after HW treatments we never found 100 % of the larvae back. Some of the thrips were washed off the cuttings; others sat on the plastic.

There was no HW treatment which killed all the larvae. In a HW dip of 43°C for 30 minutes we found 1 or 2 larvae alive (Table 4.3.2.4.). There were no differences in results of the treatments inside or outside the plastic bag. A HW dip of 43°C for 45 minutes and a HW dip of 45°C for 30 minutes gave brown spots on the youngest leaves, immediately after treatment.

A HW treatment of 30 minutes 43°C killed almost all larvae. The larvae, which were alive, were mostly found in the growing tips or in folded leaves. In these places the leaves were dry. Maybe all larvae will be killed when the cuttings are completely wetted. This could be achieved by a vacuum treatment of the soaked cuttings or by adding a wetting agent to the water (see 4.2.2.2.4.)

The experiment is replicated once. In the first experiment the quality of the cuttings was very poor.

Table 4.3.2.4. HW treatment of WFT larvae on unrooted Chrysanthemum cuttings. Treatments were started with 100 larvae.

experiment no.	treatment	in/out plastic	# larvae alive	# larvae dead
1	control: untreated	in	62	6
	control: 10 min 20°C	in	51	8
	30 min. 43°C	in	1	41
	30 min. 43°C	out	0	15
2	control: untreated	in	69	0
	control: 10 min 20°C	in	32	6
	30 min. 43°C	in	1	23
	30 min. 43°C	out	1	51
3	control: untreated	in	55	6
	control: 10 min 20°C	out	20	36
	30 min. 43°C	out	2	22
	30 min. 45°C	out	1	17
4	control: untreated	in	38	14
	control: 10 min 20°C	out	19	12
	30 min. 43°C	out	1	18
	45 min. 43°C	out	5	33

The results show that also in the vacuum or wetting agent treatments a few larvae were not killed. (Table 4.3.2.5.) These methods don't open up new possibilities, because of phytotoxic effects on the cuttings (see 4.2.2.2.4.).

Table 4.3.2.5. HW treatment of WFT larvae on unrooted Chrysanthemum cuttings

Experiment no.	Treatment	in/out plastic	# larvae alive	# larvae dead
1	Control: untreated	in	47	9
	Control: 10 min 20°C	out	51	16
	30 min. 43°C	out	5	33
	Vacuum +30 min. 43°C	out	1	12
	Wetting agent +30 min. 43°C	out	1	7
2	Control: untreated	in	36	15
	Control: 10 min 20°C	out	12	32
	30 min. 43°C	out	5	35
	Vacuum +30 min. 43°C	out	0	20
	Wetting agent +30 min. 43°C	out	2	15

In Table 4.3.2.6. a comparison between the mortality of adults and larvae of WFT on Chrysanthemum cuttings is shown. Although the cuttings were left in the plastic bag during the treatment, many insects were lost. There was no clear difference

in mortality between adults or larvae, so the following experiments were carried out with adults, enabling the quicker collection method with an aspirator.

Table 4.3.2.6. Mortality of WFT adults and larvae on Chrysanthemum cuttings. Numbers are a mean of three similar experiments, carried out with 50 larvae or adults per treatment.

Treatment	Adult / Larvae	% alive	% dead	% lost
10 ' 20 °C	larvae	13	6	81
30 ' 43 °C	larvae	1	18	81
30 ' 45 °C	larvae	1	21	78
10 ' 20 °C	adult	22	9	69
30 ' 43 °C	adult	2	21	77
30 ' 45 °C	adult	1	15	84

In Table 4.3.2.7. the results of several experiments with WFT adults on Chrysanthemum cuttings with a pre-treatment prior to the hot-water treatment are shown. The pre-treatments were applied without the plastic bag, so the % lost insects is very high. Some of the insects remained in the bags so did not get the HWT, as shown in the table. The mortality of a hot-water treatment without pre-treatment was not 100%, a single living adult was found after the 30 ' 45 °C treatment. After a pre-treatment, followed by 30 ' 43 °C or 30 ' 45 °C all insects were dead. The reason of the relatively high mortality in the 10' 20 °C (control) treatment is unknown. Normally this does not cause high mortality rates.

Table 4.3.2.7. Mortality of WFT adults in Chrysanthemum cuttings after a hot-water treatment with or without a pre-treatment. Numbers are a mean of three similar experiments, carried out with respectively 100, 50 and 50 WFT.

pre-treatment	treatment	% alive	% dead	% in bag	% lost
None	10 ' 20 °C	17	10	12	61
None	30 ' 43 °C	3	21	7	69
None	30 ' 45 °C	1	20	8	71
2 h 35 °C	10 ' 20 °C	6	11	5	78
2 h 35 °C	30 ' 43 °C	0	8	5	87
2 h 35 °C	30 ' 45 °C	0	14	5	81
1 h 35 °C + 1 h 40 °C	10 ' 20 °C	0	9	4	87
1 h 35 °C + 1 h 40 °C	30 ' 43 °C	0	13	7	80
1 h 35 °C + 1 h 40 °C	30 ' 45 °C	0	8	5	87

In the next series of experiments extra cuttings were treated in order to investigate the hot water treatment resistance of the cuttings, together with the mortality of mortality of thrips adults and larvae in the same experiment.
Mortality rates of WFT adults are shown in Table 4.3.2.8, and mortality rates of larvae in Table 4.3.2.9. Both tables show means of two similar experiments.
It is clear that the treatments which end with a 30' 45°C treatment cause 100% mortality in both adults and larvae. In 30'43°C one surviving adult was found. Of course the percentage of thrips that was not found back was very high, due to the treatment method in which the cuttings were soaked in the waterbath without a plastic bag. The growth of the cuttings after a three week period is described in chapter 4.2.2.2.

Table 4.3.2.8. Mortality of WFT adults in Chrysanthemum cuttings after a hot-water treatment with or without a pre-treatment. Numbers are a mean of two similar experiments, carried out with 100 thrips at the beginning..

pre-treatment	treatment	% alive	% dead	% in bag	% lost
None	10 ' 20 °C	17	2	4	77
None	30 ' 43 °C	0	17	0	83
None	30 ' 45 °C	0	22	1	77
2 h 35 °C	10 ' 20 °C	0	4	0	96
2 h 35 °C	30 ' 43 °C	1	2	0	97
2 h 35 °C	30 ' 45 °C	0	8	0	92
1 h 35 °C + 1 h 40 °C	10 ' 20 °C	0	3	0	97
1 h 35 °C + 1 h 40 °C	30 ' 43 °C	0	5	0	95
1 h 35 °C + 1 h 40 °C	30 ' 45 °C	0	6	1	93

Table 4.3.2.9. Mortality of WFT larvae in Chrysanthemum cuttings after a hot-water treatment with or without a pre-treatment. Numbers are a mean of two similar experiments, carried out with 100 thrips at the beginning..

pre-treatment	treatment	% alive	% dead	% in bag	% lost
none	10 ' 20 °C	20	18	1	61
none	30 ' 43 °C	0	19	1	80
none	30 ' 45 °C	0	26	0	74
2 h 35 °C	10 ' 20 °C	5	6	1	88
2 h 35 °C	30 ' 43 °C	0	10	1	89
2 h 35 °C	30 ' 45 °C	0	7	1	92
1 h 35 °C + 1 h 40 °C	10 ' 20 °C	2	9	1	88
1 h 35 °C + 1 h 40 °C	30 ' 43 °C	0	12	1	13
1 h 35 °C + 1 h 40 °C	30 ' 45 °C	0	17	1	18

4.3.3. Hot water treatments on plant-pathogenic nematodes

Root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) are common species that can be found in rose trading material. Previous research (van der Meij, 1998) indicates that to kill *Meloidogyne hapla*, HW treatments of 2 hours at 43.5 °C or 1 hour 45 °C is needed, while for *Pratylenchus penetrans* even higher temperatures or longer treatment duration are needed for the same effect. Since the HW treatment of rose bushes (see
4.2.2.4) causes severe damage at these temperatures a possible way to decrease the damage is to give the bushes a hardening pre-treatment.
In two experiments with rose bushes has been shown that even after a hardening pre-treatment there is still too much plant damage for a commercial application.

Sub Task 4.4 Development of hot water treatment schedules

Although the hot water treatment of *Yucca* and *Dracaena* stems infected with *Opogona sacchari* or *Xyleborus ferrugineus* still was not perfect, here a schedule of the hot water treatment is given.

Treatment schedule:

The water bath should be much bigger than the volume of the plant material. This over-capacity avoids the temperature to drop too much after immersing the ambient-temperature *Yucca*- or *Dracaena* stems. An estimated of a 3- to 4-fold over-capacity is necessary.

The water should be pre-heated to 47 °C before immersion. Stems should be completely immersed during treatment, so due to the buoyancy of the material, it should be made heavier by placing a grid on top of the stems and putting weights on it. The duration of the immersion must be 1 hour. After 1 hour the stems must be immersed in a waterbath of 20 °C, to cool down.

If stems must be packed after the hot water treatment, they should be left to dry for a few hours (depending on the circumstances) before packing.

Task 5: Controlled atmosphere treatments

Sub Task 5.1: Development of experimental equipment for controlled atmosphere treatments

For application of the CA treatments stainless steel vessels are designed and manufactured. Six vessels are available for experiments. They have been tested for leakage, also when exposed to temperature changes. No leakage was observed.

To create controlled atmosphere conditions a gas-mixing equipment has been installed in Nov/Dec 1999, and could be used from January 2000. The performance of the equipment has been tested by comparing the mixed gasses with calibrated gas mixtures. Deviation between mixed and calibrated gasses was less than 1%.

Sub Task 5.2: Development of commercial equipment for controlled atmosphere treatments

In January and February 2000 there have been meetings with the director and the technical advisor of the company (RUVOMA G.A. Verhart BV, The Netherlands) that carries out the disinfestation of ornamentals in the flower auction in Aalsmeer. Besides ornamentals the company is also involved in disinfestation of other commodities. At the moment possibilities for application of CA (removal of oxygen, no enhanced CO_2 levels) for commodities that allow long treatment duration (a.o. cacao-beans, but also wooden furniture) is under their investigation. Preliminary results show that very long treatment duration (days to weeks) are needed to kill the range of insects they tested.

Sub Task 5.3: Controlled atmosphere (CA) phytotoxicity studies

5.3.1. Cuttings

5.3.1.1. Chrysanthemum cuttings

Introduction
For cuttings of Chrysanthemum it is possible to treat them with Controlled Atmosphere (CA) during storage or transport. Cuttings can be treated when still unrooted, but it is also possible to treat them after a rooting period on pressed soil blocks.

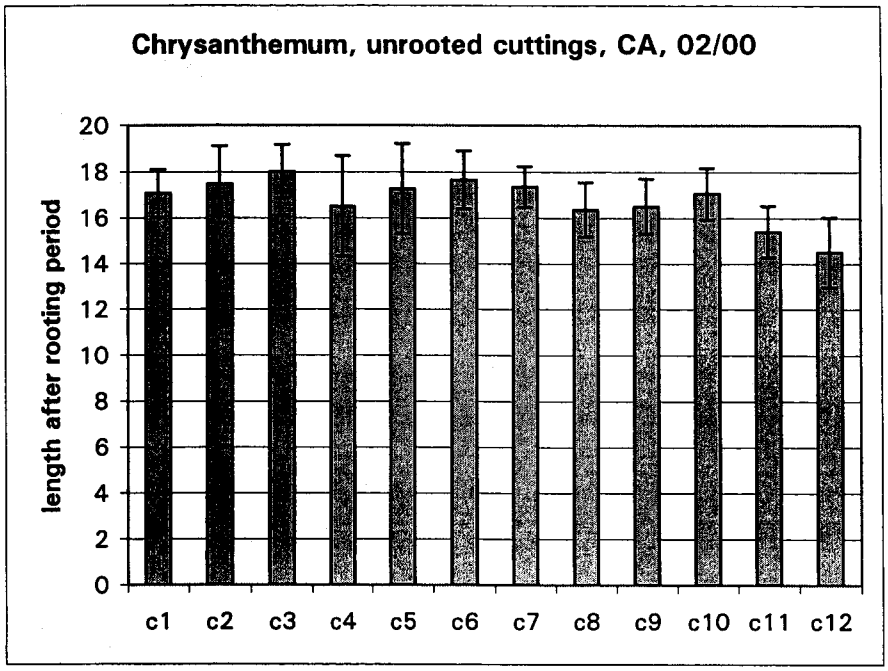
Material and methods
Unrooted cuttings were purchased at a local company.

After the CA treatment, cuttings were treated with Rhizopon, planted in soil blocks, rooted during one week, hardened during one week, planted in 11cm pots with commercial potting soil and put in a greenhouse at 20°C, 10 cuttings per treatment. Rooted cuttings in soil blocks were purchased at a local company.

CA treatment was carried out in 56 litre vessels, at 20°C. Unrooted cuttings were treated dry, except for one treatment, where the lower part of the cuttings were put in water (Fig 5.3.1.1.1). Vessels were flushed during 30 minutes at a flow of 10 litre/min, with circulation with a ventilator in the vessel, after which concentrations were measured, ventilation was shut off and exposure time started. Control cuttings were untreated.

Plant length and visual damage were measured.

Results and discussion
Plant length after the 3-week growth period was only slightly influenced by the long treatments (Fig 5.3.1.1.1). Only in treatment 12 a small decrease in length was seen. Rooted cuttings reacted in a similar way as unrooted cuttings (Fig 5.3.1.1.2.): the 24 hour treatments caused a decrease in length after 17 days. Effects of CA on rooted cuttings are shown in Fig 5.3.1.1.2. Only the 24 hours 0.1 % oxygen, 60 % CO_2 treatment had effect on the length.



c1 = control
c2 = 4h 0.1,60
c3 = 4h 1,60
c4 = 4h 5,60
c5 = 4h 0.1,77
c6 = 4h 1,77
c7 = 4h 5,73
c8 = 8h 0.1,60
c9 = 8h 1,60
c10 = 8h 5,60
c11 = 24h 1,40 dry
c12 = 24h 1,40 water

Fig 5.3.1.1.1. Length of Chrysanthemum plants 3 weeks after the CA treatments of the unrooted cuttings. Treatment codes e.g. c8 = 8h 0.1,60 to be read as: treatment 8, 8 hours CA application with 0.1 % oxygen, 60 % CO_2 (N_2 supplemented to 100%). For treatment c12, cuttings were put in water.

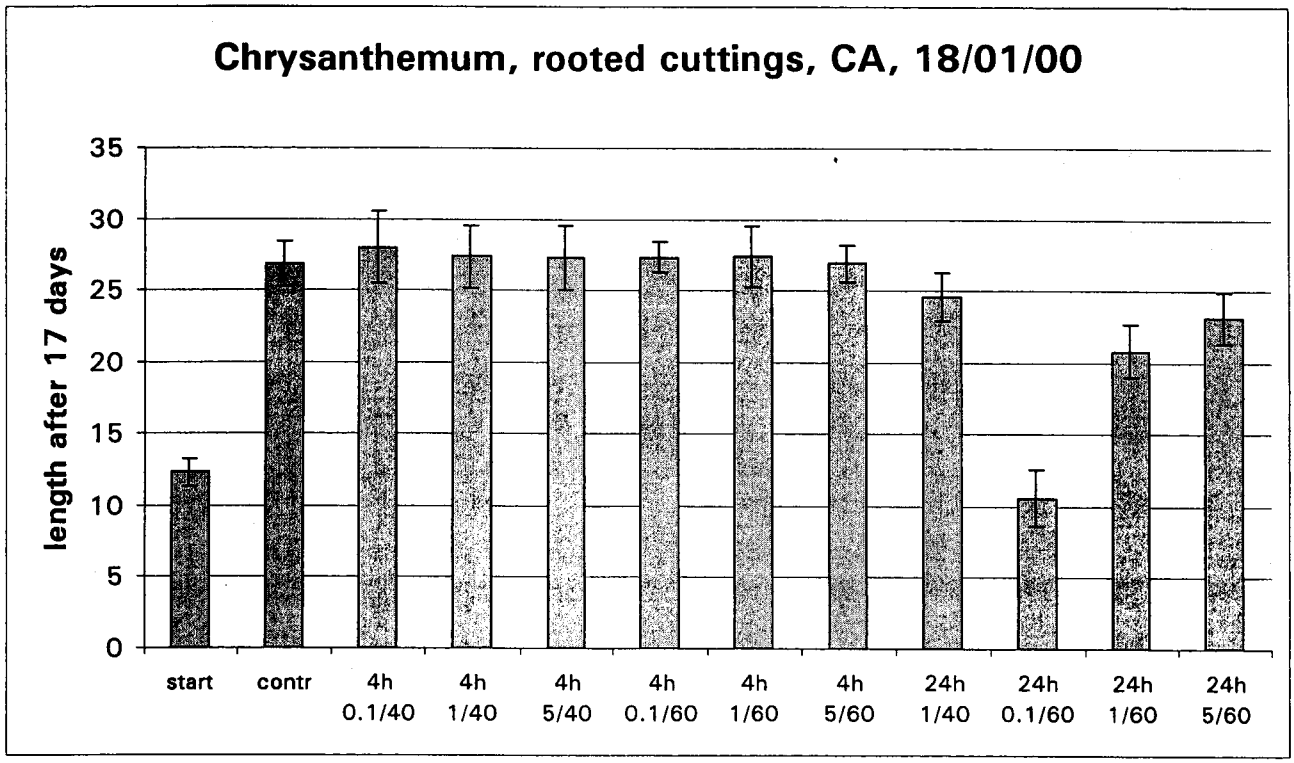


Fig 5.3.1.1.2. Length of Chrysanthemum plants after a 17 days growth period after CA treatment of rooted cuttings. Labels on x-axis e.g. 4h1/60 to be read as: 4 hours CA application with 1% oxygen, 60% CO_2 (N2 supplemented to 100%). 'Start' = the initial length of the cuttings.

During the growth of the cuttings damage developed in the plants from rooted cuttings (Table 5.3.1.1.1). In the plants from unrooted cuttings no damage was seen.

High CO_2 levels combined with low oxygen levels caused damage. More damage is seen with longer treatments duration.

Table 5.3.1.1.1. Visual damage of Chrysanthemum plants 'Reagan White' from 10 rooted cuttings treated with CA.

treatm		02	CO2	
1	control			
2	4h	0.1	40	
3	4h	1	40	
4	4h	5	40	all plants: little brown spots
5	4h	0.1	60	8 plants: little brown spots
6	4h	1	60	4 plants: little brown spots
7	4h	5	60	8 plants: little brown spots
8	24h	1	40	white spots, some dead leaves
9	24h	0.1	60	all young leaves dead
10	24h	1	60	all leaves brown or white spots or dead
11	24h	5	60	all leaves brown or white spots or dead

5.3.1.2. Rose cuttings

Introduction

For rose culture, cuttings without an infection with insects is important especially for an organic rose culture. Normally the plants from which the cuttings are produced are sprayed with pesticides to prevent infection. Also, disinfection with Methyl Bromide is used. When pesticide-free cuttings are required, disinfection with CA is one of the possibilities.

Material and methods

Rooted rose cuttings cv 'Valentino' were purchased from a local company. The rooting medium was rockwool in blocks of 8x8x8cm.
The treatment of the cuttings with CA has been carried in 56-litre vessels (see 5.3.1.1). After treatment, the blocks were placed on potting soil in 14-cm pots, and placed in the greenhouse. After 4 weeks growth period the total shoot growth was measured, and the number of shoots developed during this period was counted. 10 plants per treatment were used.

Results and discussion

In fig 5.3.1.2.1. the total length of the shoots per plant is shown. Although there is a large deviation, caused by the inherent inhomogeneity of the plant material, it can be concluded that there are no big differences between the treatments. Not only the length of the shoots determines the quality of growth after treatments, but also the number of shoots; too high numbers lead to a too bushy plant, too low numbers lead to growth retardation. In fig 5.3.1.2.2. the mean number of shoots per plant is shown. It can be concluded that there is no negative effect of the treatments.

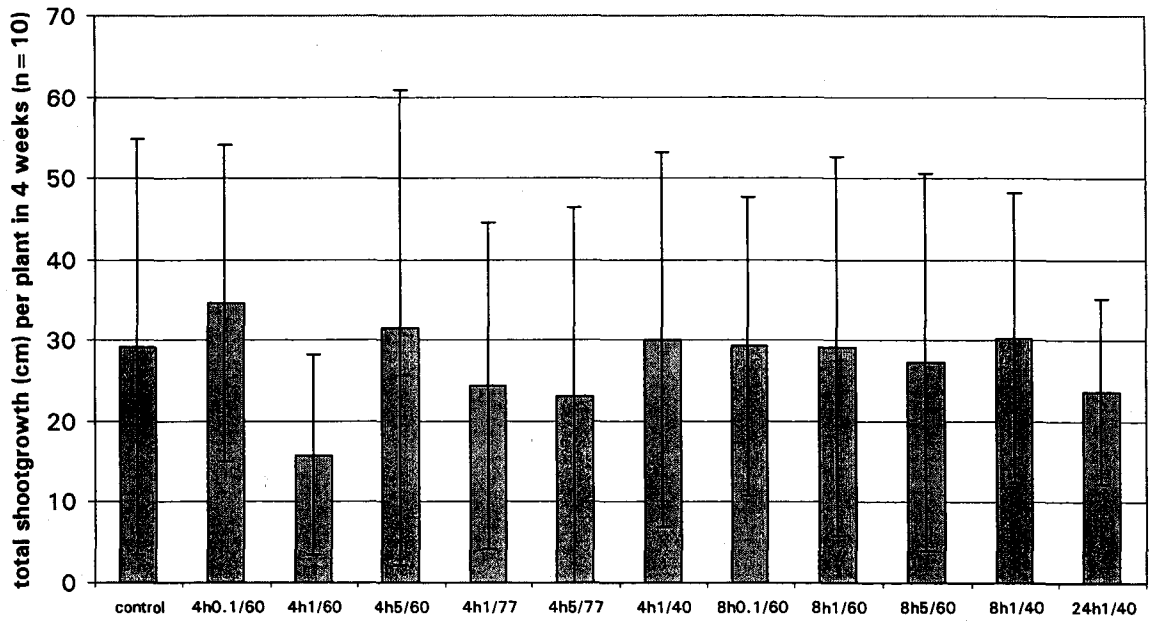


Fig 5.3.1.2.1. Mean total shoot length per rose plant grown from rooted cuttings after a CA treatment. Treatments are indicated with: duration (4h, 6h or 24h), % oxygen (0.1,1 or 5) and % CO2 (40, 60 or 77), the remaining % is supplemented by nitrogen (not indicated).

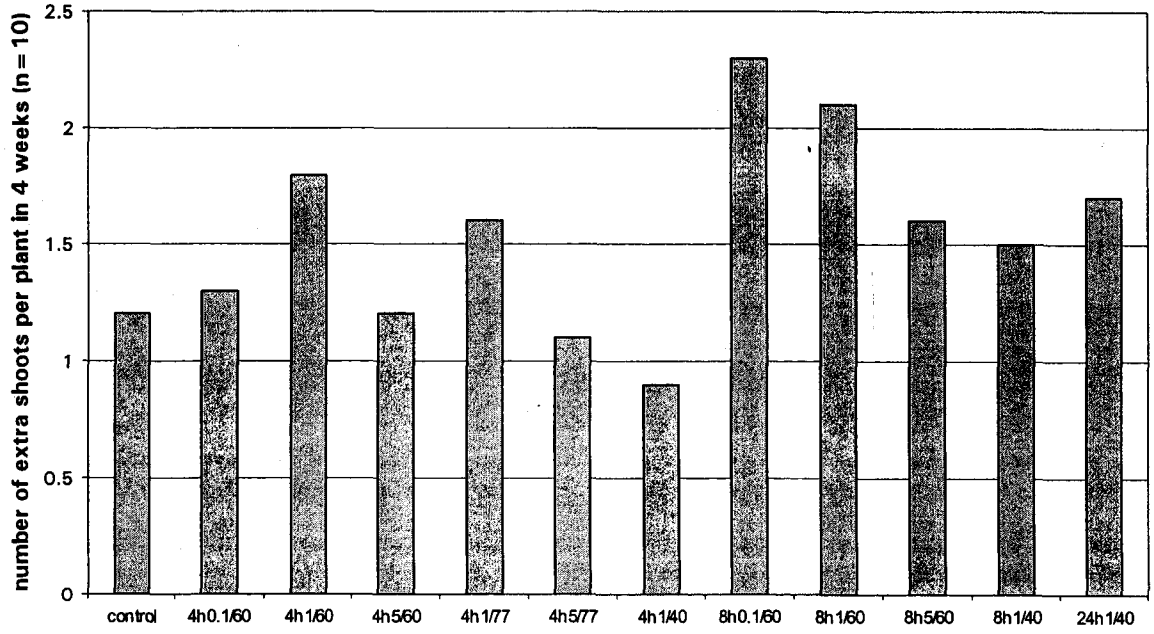


Fig 5.2.1.2.2. Mean number of shoots formed on a rose plant grown from rooted cuttings after a CA treatment. Treatments are indicated with: duration (4h, 6h or 24h), % oxygen (0.1,1 or 5) and % CO2 (40, 60 or 77), the remaining % is supplemented by nitrogen.

5.3.2. Flowers

During the transport chain of flowers it is possible to treat flowers with CA as disinfestation. Chrysanthemum and rose flowers have been used to test the phytotoxicity of several CA treatments.

5.3.2.1. Chrysanthemum flowers

Introduction

For this test an orange-brown Chrysanthemum variety was chosen, in order to see possible colour changes caused by the treatments.

Material and methods

Flowers variety 'Tiger' were purchased with roots at a local grower. Stems were cut off under water, at 20cm above the roots. Stems were hydrated for 24 hours in water at 5°C.

CA treatment was carried out in 56 litre vessels, with stems in water, at 20°C. Vessels were flushed during 30 minutes at a flow of 10 litre/min, with circulation with a ventilator in the vessel, after which concentrations were measured, ventilation was shut off and exposure time started.

After treatment, stems were recut and placed in the interior room (20°C, 60%RH, 12h light per day at 14μmol.m^{-2}.s^{-1}), 10 stems per treatment. All stems were examined daily until day 14.

Results and discussion

CA treatments on Chrysanthemum flowers, variety 'Tiger', did not cause any short-term effects on vase life. Since a 14 days vase life was expected to be sufficient to see effects, the ultimate day of vase life was not recorded; all flowers were discarded after 14 days (Table 5.3.2.1.1). Damage was only seen in treatment 9, plants showing damaged leaves and brown flower centres. In treatment 11 the first day a bleaching of the flowers was observed, but this had disappeared the next day.

Table 5.3.2.1.1. Visual damage on Chrysanthemum flowers treated with CA.

CA, Chrysanthemum flowers, 'Tiger', 02/00

treatm		02	CO_2	
1	control			
2	4h	0.1	40	
3	4h	1	40	very little damage at day 14
4	4h	5	40	
5	4h	0.1	60	
6	4h	5	60	
7	4h	5	77	
8	8h	1	40	
9	8h	0.1	60	leaf damage, brown flowers
10	8h	5	60	
11	24h	1	40	pale flowers on day 1, gone on day 2
12	4h	21	0.06	
13	24h	21	0.06	

5.3.2.2. Rose flowers

Introduction

For these tests two common rose varieties were chosen: one ('First Red') with dark red flowers and dark leaves, the other ('Frisco') with yellow flowers, and lighter leaves. In this way it was possible to see possible damage in both types of material.

Material and method

Flowers were purchased directly after cutting at a local grower. Stems were hydrated for 24 hours in Chrysal RVB at 5°C.

CA treatment was carried out in 56 litre vessels, with stems in water at 20°C. Vessels were flushed during 30 minutes at a flow of 10 litre/min, with circulation with a ventilator in the vessel, after which concentrations were measured, ventilation was shut off and exposure time started. Control flowers were not treated.

After treatment, stems were recut and placed in the interior room (20°C, 60%RH, 12h light per day at 14μmol.m^{-2}.s^{-1}), 10 stems per treatment. All stems were examined daily until the end of the vase life.

Results and discussion

Vase life of rose 'First Red' was shorter after a 4 hours CA treatment, especially when the oxygen levels were very low (treatment 2 to 4, Fig 5.3.2.2.1). Combined with higher CO_2 levels there was even more effect of low oxygen levels (treatment 5 to 7). Also a longer application of the CA caused a shorter vase life. Bud opening during vase life was poor over the whole lot, but was even poorer in the treatments with low oxygen and longer application times (Fig 5.3.2.2.2)

Vase life of 'Frisco' roses was much less influenced by these CA treatments (Fig 5.3.2.2.3). All flowers opened during vase life, irrespective of the treatment (Fig 5.3.2.2.4).

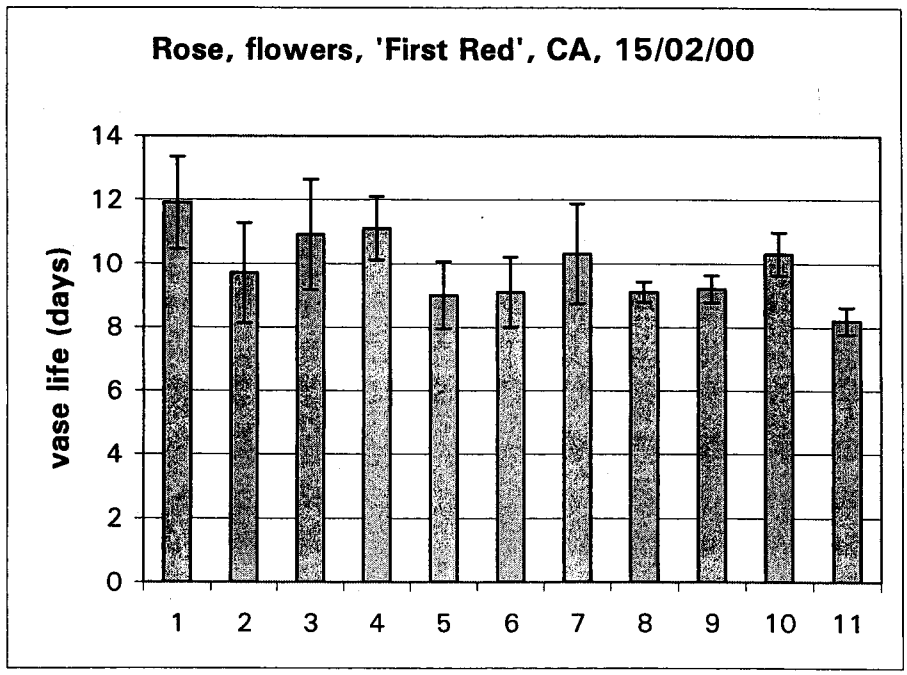


Fig 5.3.2.2.1. Vase life of rose 'First Red' after CA treatment. Treatment codes as in Fig 5.3.1.2.1.

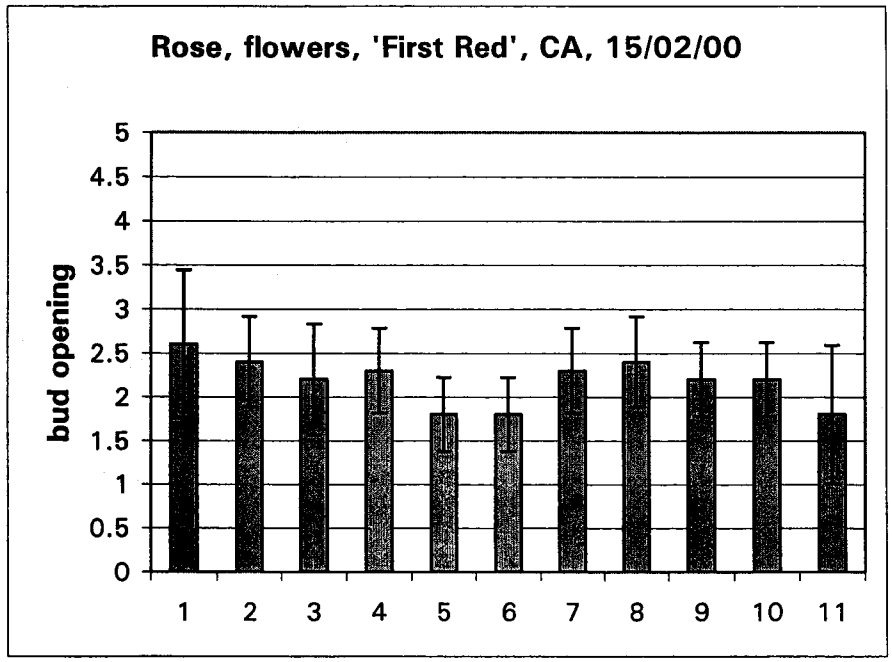


Fig 5.3.2.2.2. Bud opening of rose 'First Red' after CA treatment. Bud opening classification as in Fig 4.2.1.1.1.

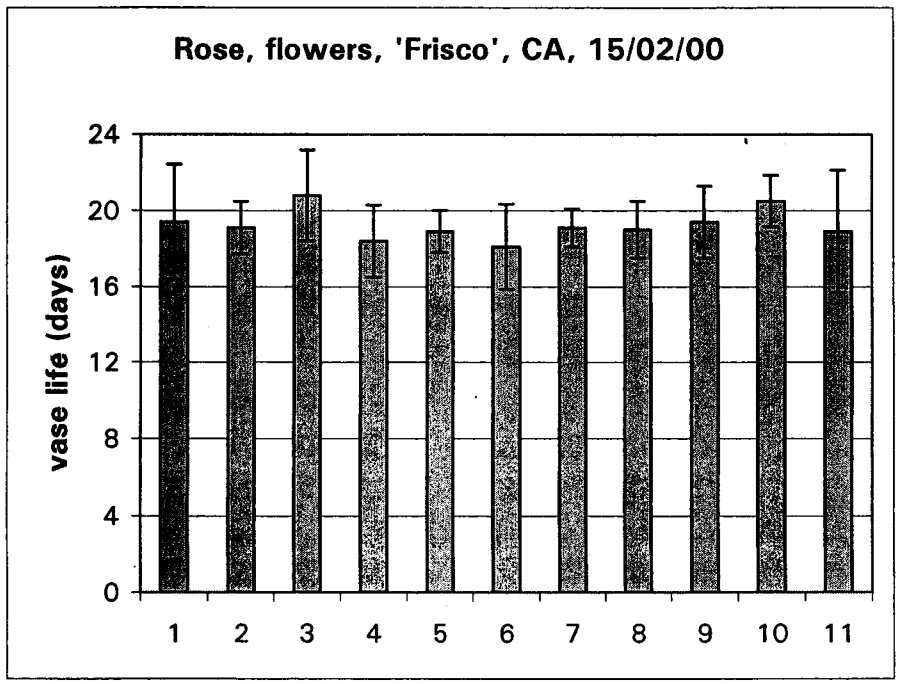


Fig 5.3.2.2.3. Vase life of rose 'Frisco' after CA treatment. Treatment codes as in Fig 5.3.1.2.1.

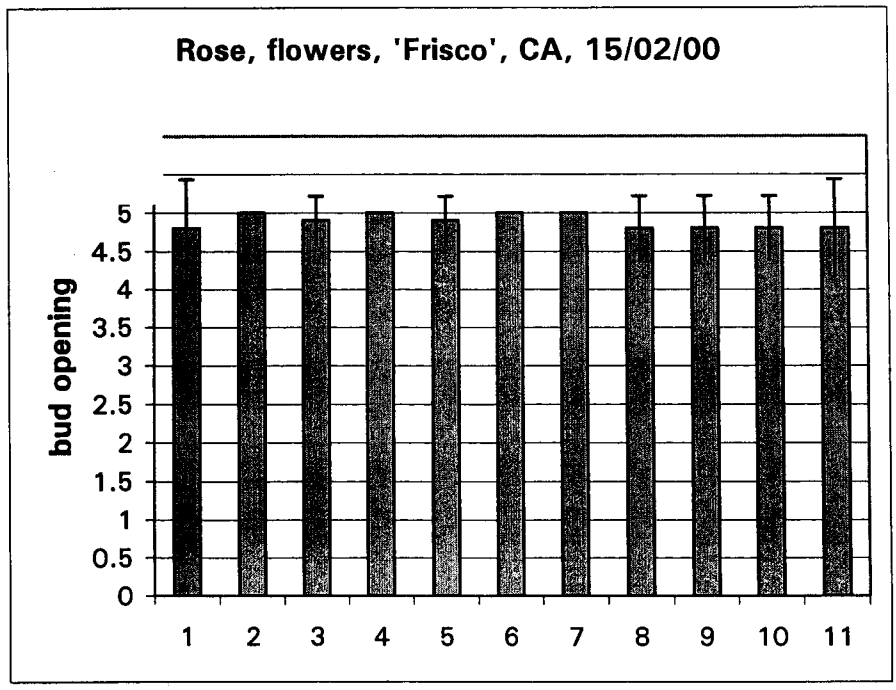


Fig 5.3.2.2.4 . Bud opening of rose 'Frisco' after CA treatment. Bud opening classification as in Fig 4.2.1.1.1.

Visual damage occurred in both varieties: the leaves of 'First Red' showed blackish spots within the first week (Table 5.3.2.2.1) and also showed leaf drying in the first week. This phenomenon was also seen in the control plants, but was accelerated by the CA treatment. In 'Frisco' only dark spots in the leaves were seen (Table 5.3.2.2.2).

Table 5.3.2.2.1. Visual damage during vase life in rose 'First Red' .

treatm		02	CO2	
1	control			Leaf drying on day 13
2	4h	0.1	60	Dark leaves and leaf drying on day 7
3	4h	1	60	Dark leaves and leaf drying on day 7
4	4h	5	60	Dark leaves and leaf drying on day 7
5	4h	0.1	77	Dark leaves and leaf drying on day 5
6	4h	1	77	Dark leaves and leaf drying on day 5
7	4h	5	73	Dark leaves and leaf drying on day 5
8	8h	0.1	60	Dark leaves and leaf drying on day 6
9	8h	1	60	Dark leaves and leaf drying on day 6
10	8h	5	60	Dark leaves and leaf drying on day 8
11	24h	1	40	Dark leaves, -drying, -abscission day 6

Table 5.3.2.2.2. Visual damage during vase life in rose 'Frisco' .

treatm		02	CO2	
1	control			
2	4h	0.1	60	Dark spots on the leaves on day 6
3	4h	1	60	Dark spots on the leaves on day 5
4	4h	5	60	Dark spots on the leaves on day 5
5	4h	0.1	77	Dark spots on the leaves on day 5
6	4h	1	77	Dark spots on the leaves on day 5
7	4h	5	73	Dark spots on the leaves on day 5
8	8h	0.1	60	Dark spots on the leaves on day 6
9	8h	1	60	Dark spots on the leaves on day 6
10	8h	5	60	Dark spots on the leaves on day 6
11	24h	1	40	Dark spots on the leaves on day 5

Sub Task 5.4: Testing of controlled atmosphere treatments on pests

5.4.1. CA-treatments on *Frankliniella occidentalis*

Introduction
Larval and adult Western Flower Thrips (WFT), *Frankliniella occidentalis*, were treated with Controlled Atmosphere (CA) separately from their host plants for a rough determination of a dose response curve. These results will be combined with the phytotoxicity studies (Sub Task 5.3), in order to narrow down the possibilities to test in on-plant experiments.

Material and method
The research on the effects of CA on pests was started with *Frankliniella occidentalis*. Larvae and adults were reared on Chrysanthemum flowers. Adults were moved from the flowers into small 30 ml vessels by means of an aspirator. Larvae were picked up with a moisturised paintbrush. Each vessel contained 5 larvae (L1 and L2) or 5 adults. The vessels were capped with a septum and the gas mixture was applied through a syringe, with another syringe as gas outlet. The vessels were flushed during 10 minutes with humidified air of the desired gas composition. Then the 4-hour treatment started, without flushing. After this 4-hour period the vessels were flushed with air for 10 minutes. The number of living (mobile) and 'dead' (immobile) adults or larvae was counted one and 24 hours after the end of the treatment. Each treatment consisted of 5 vessels. The results are expressed as percentages, because sometimes not all individuals could be traced back after the treatment, and sometimes more than 5 adults were present in the vessels.

Results and discussion
The CA conditions used in these experiments did not cause high mortality rates (Table 5.4.1). When the counting was done 24 hours after the treatment relatively many insects were alive. Observations after one hour can give an overestimation of the lethal effect of the treatments; the CO_2 in the gas mixture causes immobility, but the insects may recover after some time in normal atmospheric conditions (e.g. 30/3, adult). However, there can also be increase in mortality, as seen with larvae on 4/4. This may be caused by a natural mortality, which would have occurred also without a CA treatment. It is not possible to draw many conclusions from these differences, due to a lacking control treatment.
Further experiments, with higher CO_2 levels or with longer duration were carried out in order to enhance the mortality rate of CA treatments for *Frankliniella occidentalis.*
In Fig 5.4.1. is shown that there is no treatment that yields a 100% mortality rate, although CO2 percentages of 77 % were used.
The exposure time is probably too short, so in the next experiment the treatments which gave the best results were applied for 24 hours. In Fig 5.4.2. the results show that even with a 24 hour duration there was not a 100% mortality rate. Extending the duration of the treatments was not appropriate: in chapter 5.3.2.2. is described that these treatments cause phytotoxic reactions in roses and Chrysanthemum.

Table 5.4.1. Percentage of living and immobile larvae or adults of Frankliniella occidentalis after a treatment with Controlled Atmosphere. Means of 5 vessels are presented.

		CA conditions			% inactive	% mortality (after
date	stage	% O_2	% CO_2	% N_2	(after one hour)	24 hours)
23/3	adult	1	60	39	42	18
		5	60	35	57	39
		1	77	22	81	29
		5	73	22	82	24
30/3	adult	0	0	100	8	12
		0	20	80	12	19
		0	50	50	76	46
		0	77	23	40	40
4/4	larvae	0	0	100	8	23
		0	20	80	4	32
		0	50	50	24	28
		0	77	23	36	44
11/4	adult	0	0	100	11	17
		0	35	65	35	34
		2	0	98	8	16
		2	35	63	66	66

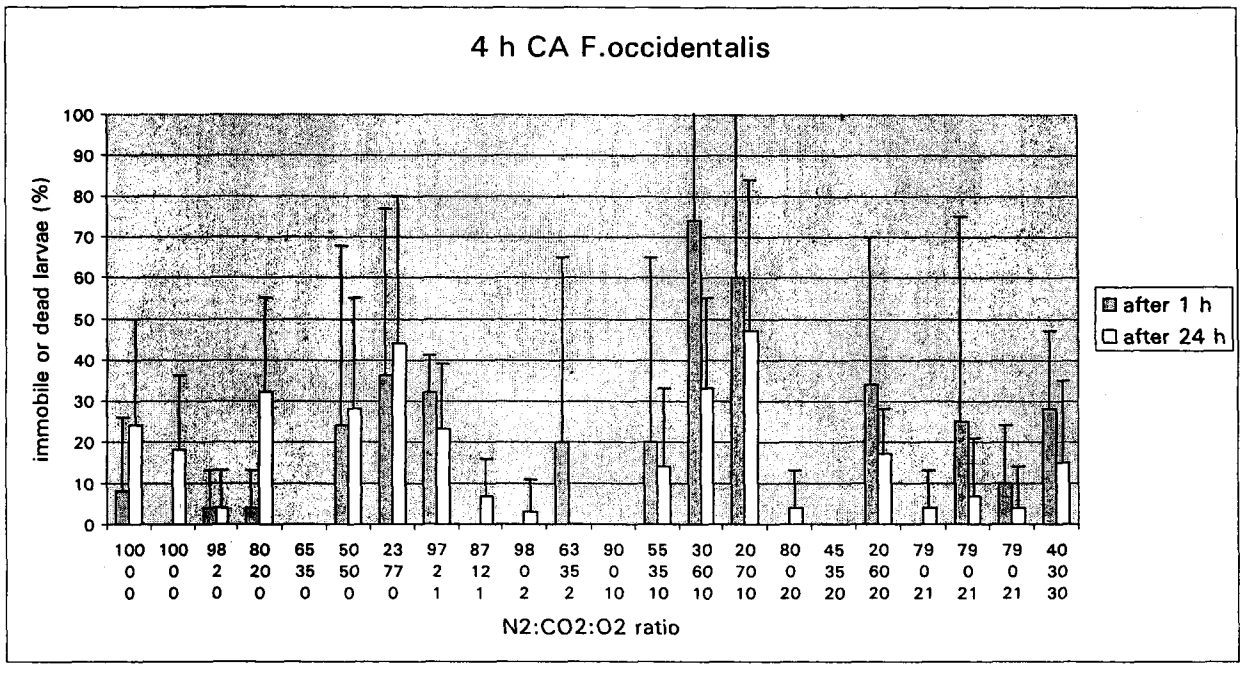


Fig 5.4.1.Percentage of immobile or dead larvae of Frankliniella occidentalis after a treatment with Controlled Atmosphere. Means of 5 vessels are presented.

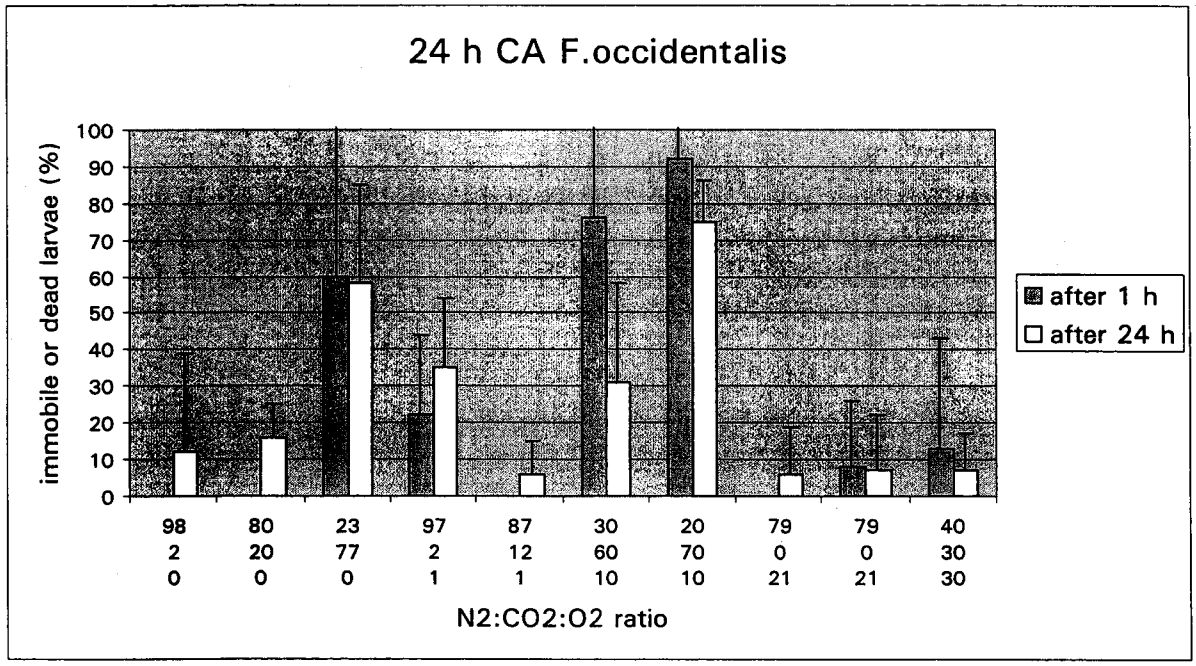


Fig 5.4.2. Percentage of immobile or dead larvae of Frankliniella occidentalis after a treatment with Controlled Atmosphere. Means of 5 vessels are presented.

5.4.2. CA-treatments on *Bemisia tabaci*

Introduction

Bemisia tabaci can be a problem in many crops. In *Poinsettia* cuttings it often leads to problems. Cuttings can be sprayed or gassed with MeBr to disinfect. Treatment with CA during transport could be an alternative.

Materials and methods

Larval *Bemisia tabaci* were treated with Controlled Atmosphere (CA) in on-plant experiments.

Larvae were reared on the Poinsettia plants. CA treatment was carried out in 56 litre vessels. Vessels were flushed during 30 minutes at a flow of 10 litre/min, with circulation with a ventilator in the vessel. After 30 minutes the ventilation was shut off and exposure time of 24 hour started. After treatment the plants were placed in the greenhouse. We used 4 plants per treatment. The CA conditions that were used were based on the results with *Frankliniella occidentalis* (Fig 5.4.2). In Table 5.4.2. the treatments are shown.

Table 5.4.2. CA conditions in Bemisia tabaci on Poinsettia plants experiments.

Treatment	% O2	% CO2	% N2
1	21	0	79
2	0	77	23
3	10	60	30
4	10	70	20

Results and discussion

After 2 days we wanted to count the percentage of dead larvae for the first time. All plants of treatment 2, 3, and 4 were dead. Since the larvae of *Bemisia* are positioned inside the leaf tissue, and need living tissue to survive, all larvae had died too. Apparently *Poinsettia* is much more susceptible for damage by CA treatments than the crops tested before.

5.4.3. General remark on CA effects on pests

Due to the non-promising mortality rates in the before described experiments we have decided not to investigate the possibilities of CA treatments alone any further.

Task 6: Alternative fumigants

Essential oils are potential alternative fumigants to be used against pests. However, a literature survey has shown that the most promising fumigants do not yield a 100% mortality rate for the insects. Mortality rates are always improved by combining the fumigant treatment with Controlled Atmosphere (CA). Former research in our institute has shown the same. Thus, we have concluded that we should focus on Task 7: the combination treatments.

Sub Task 6.1 Testing alternative fumigants on plants

Since these experiments were planned to be performed with plants and insects together in one vessel, and control treatments often include a fumigant treatment without CA, the type of experiments to be done in Task 6 are carried out anyhow.

Phytotoxicity of Methyl Bromide on cut flowers.

In April 2001 2 bunches of roses (cv 'Orange Unique') and three bunches of Chrysanthemums (cv 'Europa') were brought to RUVOMA, the company which disinfects ornamentals with Methyl Bromide before export to countries like Japan.

Phytotoxicity was determined after 24 hours on the vase in indoor test rooms.

In Chrysanthemum some of the small buds turned brown after 24 hours. In roses the sepals showed a slight discoloration/yellowing.

This means that a Methyl Bromide treatment is not completely unharmful for the plants. However, the companies that import flowers that have been treated with Methyl Bromide never have rejected flowers because of phytotoxic damage, indicating that the damage is small, up to practice criteria.

Sub Task 6.2 Testing alternative fumigants against pests

See remark under Task 6

Task 7: Combination treatments

Sub Task 7.1 Testing combination treatments on plants

In the experimental set-up for Task 7 a large part of the experiments were performed with plants and insects simultaneously. Results of these experiments show both phytotoxic effects as well as insect mortality.

To begin with, insect mortality was investigated. Then, the most promising fumigants were tested in combination with cut flowers.

For sake of time and space, the effects of combination treatments on plants will be described together with the effects on pests in Sub Task 7.2.

Sub Task 7.2 Testing combination treatments against pests

Introduction

The effects of alternative fumigants, like essential oils, on insect mortality always improve when combined with controlled atmosphere (CA) during fumigation.

In our experiments we have used several different CA conditions, the choice was based on previous research as described in this report, or on CA conditions mentioned in literature about combination treatments (De Gelder et al, 1997 and 1998).

Materials and methods

The insects used for combination treatments were Western Flower Thrips (WFT, *Frankliniella occidentalis*) adults and larvae and the aphid *Macrosiphum euphorbiae*, all wingless stages. In later experiments also the aphid *Aulacorthum solani* was used. Insects were caught and held during fumigation in little plastic cages with a piece of Chrysanthemum leaf inside. Thrips adults were caught with an aspirator, thrips larvae and aphids were placed inside the little cages with a brush. There was one insect species per cage. The used numbers per cage were 25 for thrips adults and larvae and 20 for the aphids.

The cages had thrips gauze on two sides to enable air circulation for penetration of the fumigants and establishment of the CA condition.

For the fumigation iron 56 litre vessels were used. The cages with the insects were placed inside the vessel, on a rack. From experiment 8 we started adding flowers to the vessel. Ten rose or five Chrysanthemum stems were placed in a vase in the vessel. After closing the vessel, the ventilator inside the vessel was switched on. The vessel was flushed with the desired CA during 20 minutes at a flow of 10 litre/min. The gas mixture from the CA-installation was moisturised by leading it through water. The used CA conditions varied between the experiments (see also the results). As a control, little cages with insects were placed on a table in the same room. After flushing, the valves of the vessel were closed and the fumigants were injected through the septum on a filter paper. The ventilator was placed just below the filter paper, to stimulate evaporation. The fan was functioning during the whole treatment period. When two fumigants were combined in one vessel, the moments of application could differ. Lavender oil and hop oil, for instance were applied in order to protect the plant against the phytotoxic effects of insect-toxic fumigants. These 'protecting oils' were injected one hour before the insecticide fumigant was injected (Table 7.2.8). In other combinations of fumigants, which were chosen to increase the insecticide effect, the

fumigants were injected immediately after each other, like piperonylbutoxide and propanal (Table 7.2.23).
Depending on the experiment the vessel was closed after flushing for 2 hours, 2 hours and 40 minutes or 3 hours and 40 minutes (see also the results). This leads to a total treatment time of 2 hours and 20 minutes, 3 hours or 4 hours respectively. One hour after opening the vessel, the number of immobile insects was counted. Immobile insects were placed in a clean cage on a moist paper. They were observed again the next day. Insects which did not move after touching gently were recorded as dead.
After the treatment the flowers were placed in the postharvest rooms in vases with tap water and observations for phytotoxic damage were made on the next week. In some experiments, as a control, flowers were treated with only CA, without the essential oil. Always, control flowers were placed in the vicinity of the vessels, together with insects in cages (as described earlier), as a control to handling.

Results and discussion
In Table 1 the results of the first combination treatment are given. Linalole was used as fumigant, because it has been described as a potential alternative for MeBr (De Gelder et al, 1997 and 1998). None of the treatments gave a 100% mortality for the three insect groups. Thrips adults are more susceptible than larvae. In vessel 2 and 4 drops of linalole were found on the insect cages, probably because the filters were too thin, causing the oil dripping through them.

Table 7.2.1. Insect mortality of combination treatments with 2 ml linalole as fumigant and different CA conditions. Treatment duration was 2 hours and 20 minutes. Fan switched on for a total of 40 min. For thrips 25 larvae and 25 adults were used, for aphids 20 individuals were used.

vessel	CA conditions			Essential oil	% mortality (after 24 hours)		
	%CO2	%O2	%N2	linalole	thrips adult	thrips larvae	*M. euphorbiae*
1	0	10	90	-	0	0	0
2	0	10	90	+	100	72	5
3	10	10	80	-	22	0	0
4	10	10	80	+	79	24	0
5	40	10	50	-	0	4	15
6	40	10	50	+	72	12	25
	Control,air			-	0	0	0

In Table 7.2.2. the results of a replication are shown. Mortality rates were lower, especially for the aphids (*M. euphorbiae*). Although the ventilators were functioning during the whole treatment, the filter papers were still moist by oil. Probably the mortality rate could be increased by a longer treatment, both because more oil can evaporate and the insects are exposed to the oil for a longer period.

Table 7.2.2. Insect mortality of combination treatments with 2 ml linalole as fumigant and different CA conditions. Treatment duration was 2 hours and 20 minutes. For thrips 25 larvae and 25 adults were used, for aphids 20 individuals were used.

vessel	CA conditions			Essential oil	% mortality (after 24 hours)		
	%CO2	%O2	%N2	linalole	thrips adult	thrips larvae	*M. euphorbiae*
1	0	10	90	-	4	20	0
2	0	10	90	+	20	24	0
3	10	10	80	-	0	0	0
4	10	10	80	+	36	16	0
5	40	10	50	-	4	20	0
6	40	10	50	+	16	24	0
	control, air			-	0	0	0

In Table 7.2.3. the results of a longer treatment are shown. Mortality rates improved, but still were not 100% . There was still some residual oil in the filter papers. The time length of the treatment was 4 hours, which is the logistic maximum time a disinfestation of ornamentals (at 20 °C) may last.

Table 7.2.3. Insect mortality of combination treatments with 2 ml linalole as fumigant. Treatment duration was 4 hours. For thrips 25 larvae and 25 adults were used, for the aphid Macrosiphum euphorbiae 20 individuals were used.

vessel	CA conditions			Essential oil	% mortality (after 24 hours)		
	%CO2	%O2	%N2	linalole	thrips adult	thrips larvae	*M. euphorbiae*
1	0	10	90	-	0	0	0
2	0	10	90	+	44	20	10
3	10	10	80	-	0	8	0
4	10	10	80	+	96	40	25
5	40	10	50	-	20	0	20
6	40	10	50	+	88	28	80
	control, air			-	0	0	0

Since linalole did not give satisfying mortality rates the essential oil p-cymene was used in the next experiment (De Gelder et al, 1997 and 1998). The same CA conditions were applied, and we chose to treat the insects during 4 hours. Results are shown in Table 7.2.4. Mortality rates were much higher than with linalole, also for the aphids. Now also, not all oil had evaporated from the filter paper.

Table 7.2.4. Insect mortality of combination treatments with 2 ml p-cymene as fumigant. Treatment duration was 4 hours. For thrips 25 larvae and 25 adults were used, for aphids 20 individuals were used.

vessel	CA conditions			Essential oil	% mortality (after 24 hours)		
	%CO2	%O2	%N2	p-cymene	thrips adult	thrips larvae	*M. euphorbiae*
1	0	10	90	-	0	0	0
2	0	10	90	+	96	76	40
3	10	10	80	-	0	0	0
4	10	10	80	+	96	76	55
5	40	10	50	-	28	8	0
6	40	10	50	+	100	84	80
	control, air			-	4	0	0

In the next experiment another aphid was included: *Aulacorthum solani*. Because the rearing of this species was not successful it was only used in three out of the six treatments, as shown in Table 7.2.5. Treatment number 6 gave inexplicable low mortality rates, so this experiment was repeated. Results are shown in Table 7.2.6.

Table 7.2.5. Insect mortality of combination treatments with 2 ml p-cymene as fumigant. Treatment duration was 4 hours. For thrips 25 larvae and 25 adults were used, for both aphid species 20 individuals were used. A. solani was included in treatment 4,5 and 6 and control only.

vessel	CA conditions			Essential oil	% mortality (after 24 hours)			
	%CO2	%O2	%N2	p-cymene	thrips adult	thrips larvae	*M. euphorbiae*	*A. solani*
1	0	10	90	-	0	0	0	
2	0	10	90	+	92	100	55	
3	10	10	80	-	0	4	5	
4	10	10	80	+	92	80	45	40
5	40	10	50	-	8	4	0	0
6	40	10	50	+	16	8	0	0
	control, air			-	0	0	0	0

Table 7.2.6. Insect mortality of combination treatments with 2 ml p-cymene as fumigant. Treatment duration was 4 hours. For thrips 25 larvae and 25 adults were used, for both aphid species 20 individuals were used. A. solani was included in treatment 5 and 6 and control only.

vessel	CA conditions			Essential oil	% mortality (after 24 hours)			
	%CO2	%O2	%N2	p-cymene	thrips adult	thrips larvae	*M. euphorbiae*	*A. solani*
1	0	10	90	-	8	0	0	
2	0	10	90	+	100	40	65	
3	10	10	80	-	0	4	0	
4	10	10	80	+	100	88	50	
5	40	10	50	-	44	16	10	0
6	40	10	50	+	92	84	80	90
	control, air			-	0	0	0	0

Although mortality rates were not 100% we decided to include cut flowers in the next experiment. Both essential oils were used, in order to check possible phytotoxic reactions on both oils. In Table 7.2.7. the data of insect mortality are shown.
In each vessel ten rose stems (commercial bud stage) or ten Chrysanthemum stems, both commercial stage, were used.
Phytotoxicity was checked immediately after finishing the treatment. In vessel 2 and 3 the leaves of the Chrysanthemums were shiny (see Appendix 4, Fig 5), and the petals of the flowers were partly discoloured (Appendix 4, Fig 6). In vessel 5 and 6 we saw no effect on the leaves of the roses, but there was a brownish discoloration of the outer rose petals (see Appendix 4, Fig 7 and 8)

Table 7.2.7. Insect mortality of combination treatments with linalole and p-cymene as fumigants. Treatment duration was 2 hours and 40 min. Total 3 hours. For thrips 25 larvae and 25 adults were used, for both aphid species 20 individuals were used. A. solani was included in treatment 1,2,3, and control only.

vessel	flower	CA conditions			Essential oil (ml)		% mortality (after 24 hours)			
		%CO2	%O2	%N2	linalole	p-cymene	Thrips adult	Thrips larvae	*M. euphorb*	*A. solani*
1	Chrys	25	15	60	0	0	12	8	0	0
2	Chrys	25	15	60	1.6	0.4	76	16	10	0
3	Chrys	25	15	60	2	0	28	24	30	35
4	Rose	25	15	60	0	0	0	0	20	
5	Rose	25	15	60	1.6	0.4	80	4	45	
6	Rose	25	15	60	2	0	0	16	0	
		Control, air					4	0	0	0

In the next experiment (Table 7.2.8) lavender oil was used prior to the essential oil p-cymene or the fumigant propanal, which was described as effective against insects, but non-phytotoxic on lettuce (Hammond et al, 2000). A 1 hour pre-treatment with lavender oil, a supposedly non-phytotoxic (but also supposedly non-insect-toxic) essential oil was thought to prevent phytotoxic effects of the insect-toxic oils, by saturating the plant with a non-toxic oil. Treatment duration was 4 hours in total: 20 min flushing to reach CA conditions, 1 hour pre-treatment with lavender oil, 2 h 40 ' for p-cymene or propanal. This experiment was carried out with Chrysanthemums ('variety 'Euro') only. In all next experiments *Aphis fabae* was used as aphid species because rearing of this species was more successful than the other aphids. None of the treatments with p-cymene caused high mortality rates in trips larvae and aphids. Propanal showed very high mortality rates, but also was very phytotoxic. Phytotoxicity was not clearly decreased by pre-treatment with lavender oil. One of the disadvantages of this pre-treatment is that the whole treatment will last longer, which can cause logistical and quality problems.

*Table 7.2.8. Insect mortality of combination treatments with lavender oil, p-cymene and propanal as fumigants. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 4 hours in total. For thrips 25 larvae and 25 adults were used, for Aphis fabae 20 wingless stage individuals were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. * = damage due to droplets of oil on leaves. F= flower, L= leaves, S= sepals*

vessel		Fumigant (ml applied)			% mortality (after 24 hours)			Phytotoxicity Chrysanthemum		
		lavender	p-cymene	propanal	Thrips adult	Thrips larvae	*Aphis fabae*	F	L	S
1	CA				0	0	0	0	0	0
2	CA	2			56	8	0	-*	-*	0
3	CA	2	1		44	24	0	0	-	0
4	CA	2	2		100	24	0	-	--	--
5	CA	4	2		20	20	10	0	--	--
6	CA			5	100	92	100	--	--	--
contr	air				0	0	0	0	0	0

In the following experiment only *Aphis fabae* was used, because they proved to have low mortality rates. Some of the previous treatments were repeated, and a lower concentration of propanal, with or without lavender oil, was used. For this experiment roses (cv 'Orange unique') as well as Chrysanthemums (cv 'Euro') were used. In Table 7.2.9 the mortality and phytotoxicity is shown. Only in vessel 6 a 100% mortality is seen. There had been some difficulties in injecting propanal in vessel 5, due to plugging of the injection needles, so this can be the cause of no mortality and no phytotoxicity in that treatment.

*Table 7.2.9. Insect mortality of combination treatments with lavender oil, p-cymene and propanal as fumigants. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 3 hours in total. For Aphis fabae a mix of 25 wingless and winged stage individuals were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. * = damage due to droplets of oil on leaves. F= flower, L= leaves, S= sepals*

vessel		Fumigant (ml applied)			% mortality	Phytotoxicity					
		Lavender	p-cymene	propanal	*Aphis fabae*	Chrys			Rose OU		
						F	L	S	F	L	S
1	CA	2			0	--*	0	0	--	0	0
2	CA	2	2		0	0	-*	0	--	--	--
3	CA	4	2		0	--*	-	0	--	--	0
4	CA		2		0	-*	-*	0	0	--	0
5	CA			2.5	0	0	0	0	0	0	0
6	CA	2		2.5	100	--*	-*	-	--	--	--
contr	air										

Here again damage by oil droplets was seen. But in roses lavender oil vapour caused damage in the leaves. Comparing the phytotoxicity in vessel 4 and 2 in roses an increase of damage by lavender oil is seen, rather than the expected decrease. For Chrysanthemums this is less clear.
The following experiment was concentrated on propanal. Because some phytotoxic effects were seen in the previous experiment, a pre-treatment with lavender oil was included, see Table 7.2.10. Treatment duration was 3 hours in total: 20 minutes flushing,1 hour for pre-treatment with lavender oil (if applied) and 1 h 40' for propanal.

Lower application rates of propanal were used because 2.5 ml was phytotoxic and caused 100% mortality of aphids. Again rose 'Orange Unique' and Chrysanthemum 'Euro' were used. From the former experiments it is obvious that thrips larvae are harder to kill than thrips adults, for that reason in the following experiments only thrips larvae were used.

*Table 7.2.10. Insect mortality of combination treatments with lavender oil and propanal as fumigants. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 3 hours in total. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. * = damage due to droplets of oil on leaves. F= flower, L= leaves, S= sepals*

vessel		Fumigant (ml)		% mortality		Phytotoxicity					
		lavender	propanal	Thrips larvae	A. fabae	Chrys			Rose		
						F	L	S	F	L	S
1	CA		0.1	12	61	0	-	0	0	0	0
2	CA	1	0.1	100	80	0	--	0	-	0	0
3	CA		0.25	100	83	0	--	0	0	0	0
4	CA		1.0	100	60	0	--	0	0	--*	0
5	CA	1	1.0	100	77	0	--	0	--	--*	--
6	CA		2.5	100	71	0	--	0	--	--*	--
contr	air			0	0	0	0	0	0	0	0

An application of 0.25 ml propanal gave the highest mortality. Although the aphid mortality was not yet 100%, an increase of the amount of propanal did not cause higher mortality rates. Probably there is considerable variation in the toxic effect of propanal on aphids. Some severe phytotoxic effects of propanal on rose as well as chrysanthemum were seen. An application of 2.5 ml propanal caused severe damage. Instead of decreasing the phytotoxicity of propanal, lavender oil increased the occurrence of phytotoxic damage in the plants.

In order to investigate whether a longer treatment period would cause higher mortality rates an experiment with different treatment duration was carried out (Table 7.2.11). The 0.1 and 0.5 ml concentrations were applied for 2 and 4 hours.

*Table 7.2.11. Insect mortality of combination treatments with propanal as fumigant. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 2 or 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. * = damage caused by handling. F= flower, L= leaves, S= sepals*

vessel	Time (h)		ml propanal applied	% mortality		Phytotoxicity					
						Chrys			Rose		
				Thrips larvae	A. fabae	F	L	S	F	L	S
1	2	CA	0.1	10	0	0	0	0	0	0	0
2	2	CA	0.5	0	16	0	0	0	0	0	0
3	4	CA	0.1	5	16	0	-*	0	0	-	0
4	4	CA	0.5	5	20	0	0	0	0	0	0
contr		air		0	0	0	-*	0	0	0	0

An unexplainable low mortality was seen. No technical reason could be found. Yet, because also a low phytotoxic reaction is seen it could be that something went wrong with the application of propanal. As far as it is possible to draw conclusions from this experiment, no clear increase of mortality was seen by a longer application.

In another experiment the effect of CA on the mortality of propanal was tested. Because of the low mortality rate in the former experiment a higher dose was applied (0.75 ml), see Table 7.2.12.

Table 7.2.12. Insect mortality of combination treatments with propanal as fumigant. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 2 hours in total. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals

vessel		ml propanal applied	% mortality		Phytotoxicity					
					Chrys			Rose		
			Thrips larvae	A. fabae	F	L	S	F	L	S
1	CA	0.75	69	0	0	-	0	0	--	-
2	air	0.75	88	23	0	-	0	0	--	-
contr	air	0	12	0	0	0	0	0	0	0

Whether propanal was combined with CA or not, did not make much difference for the mortality. Although we expected that a treatment in CA would give a higher mortality, the opposite was seen. For phytotoxicity no difference was seen between the two treatments.

Another possible fumigant of which insect toxicity is known, is acetaldehyde. In the next experiment the effect on insect mortality of several concentrations of acetaldehyde is tested, see Table 7.2.13. CA conditions were altered slightly (35% CO_2, 15% O_2, 50% N_2) because Hartsell et al (1979) have described this CA as the best combination with acetaldehyde to kill aphids on lettuce.

*Table 7.2.13. Insect mortality of combination treatments with acetaldehyde as fumigant. CA means: 35% CO_2, 15% O_2 and 50% N_2. Treatment duration was 2 or 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel	Time (h)		ml acetaldehyde applied	% mortality		Phytotoxicity					
				Thrips larvae	A. fabae	Chrys			Rose		
						F	L	S	F	L	S
1	2	CA	2	100	96	0	--	--	0	--*	0
2	2	CA	3	100	100	0	--	--	0	-*	0
3	4	CA	2	100	100	0	--	--	--	--*	-
4	4	CA	3	100	100	0	--	--	--	-*	-
5	4	air	3	94	100	0	--	--	--	-*	0
6	4	CA	0	0	0	0	--	0	0	-*	0
		air	0	0	0	0	0	0	0	-*	0

A very high insect mortality was seen in all treatments with acetaldehyde involved. Phytotoxicity, however, was very high too. The leaf damage in Chrysanthemum in vessel 6 appears to be caused by CA alone. When acetaldehyde was applied for 4

hours, roses also suffered from phytotoxic damage in flowers and sepals. The leaf damage in roses was mainly caused by a seasonal postharvest problem often occurring in roses in winter, and maybe only increased in vessel 1 and 3 by the fumigant treatment. Considering the results, lower concentrations can be tested.
In Table 7.2.14 results of the next experiment are shown. A lower concentration of acetaldehyde was tested, and also the effects of lavender oil and propanal with or without CA were tested. The 'usual' CA conditions were used again (25% CO_2, 15% O_2, 60% N_2). Total duration of the treatment was 4 hours.

*Table 7.2.14. Insect mortality of combination treatments with lavender oil, propanal or acetaldehyde as fumigants. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml applied)			% mortality		Phytotoxicity					
		lavender oil	Propanal	acetaldehyde	Thrips larvae	A. fabae	Chrys			Rose		
							F	L	S	F	L	S
1	CA	1			23	3	0	0	0	--	--	-
2	air	1			100	23	0	0	0	--	-*	0
3	CA		1		100	100	0	--	0	0	-*	0
4	air		1		100	94	0	-	0	0	--	-
5	CA				20	6	0	0	0	0	-*	0
6	CA			0.2	43	4	0	--	0	0	-*	0
	air				50	0	0	0	0	0	-*	0

A surprisingly high thrips mortality was seen with lavender oil in air, which was even higher than in CA. Propanal in CA gave a 100% mortality for both insect species and was about equal to propanal in air. A low concentration of acetaldehyde gave low mortality rates. Lavender oil showed to be phytotoxic for roses, especially when applied in CA. Propanal caused phytotoxic reactions in leaves of both crops, with only a slight difference between CA and air. Even the low concentration of acetaldehyde caused severe phytotoxic reactions in Chrysanthemum leaves. CA alone did not cause phytotoxic reactions. The results for lavender oil are confusing; it is not known as toxic for insects, and the high mortality in thrips larvae without CA is difficult to explain.

Further investigation of propanal and p-cymene was carried out in the next experiment (Table 7.2.15).Two rose varieties were used, 'Orange Unique' (=Rose OU) and 'Vendela' (=Rose V), together with Chrysanthemum 'Euro'.

*Table 7.2.15. Insect mortality of combination treatments with propanal or p-cymene as fumigants. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 3 or 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Two rose varieties were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel	time (h)		Fumigant (ml applied)		% mortality		Phytotoxicity								
			propanal	p-cymene	Thrips larvae	A. fabae	Chrys			Rose OU			Rose V		
							F	L	S	F	L	S	F	L	S
1	4	air	1		100	97	0	-	0	0	-*	-	0	--	-
2	3	air	1.25		100	88	0	-	0	0	-*	-	0	--	-
3	4	air		1	100	20	0	--	0	--	--	-	--	--	--
4	3	CA	1		100	69	0	--	0	0	-*	0	-	-	-
5	3	CA			0	0	0	-	0	0	-*	--	0	0	0
6	3	CA	1		100	94	0	--	0						
					23	0	0	0	0	0	-*	0	0	0	0

The treatment in vessel 1 was a repetition of vessel 4 in Table 7.2.14, and in vessel 2 the dose was increased slightly, because a 100% mortality was not reached in the former experiment. Both treatments still did not give a 100% mortality, and rather severe plant damage. CA in combination with 1 ml propanal decreased mortality. P-Cymene again was not toxic enough for aphids. Here also, CA alone caused leaf damage in Chrysanthemum. Vessel 4 and 6, same conditions, showed a large difference in mortality.

Hop oil (from *Humulus lupulus*) is also mentioned as a protector against phytotoxic effects of other insect-toxic fumigants. In Table 7.2.16 an experiment with hop oil and propanal is described. Treatment duration was 4 hours in total for the combination of oils, hop oil or lavender oil were applied 1 hour before injecting propanal. Treatments with only propanal lasted 3 hours.

*Table 7.2.16. Insect mortality of combination treatments with lavender oil, hop oil or propanal as fumigants. CA means: 25% CO_2, 15% O_2 and 60% N_2. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml applied)			% mortality		Phytotoxicity					
		Hop oil	Lavender oil	propanal	Thrips larvae	A. fabae	Chrys			Rose OU		
							F	L	S	F	L	S
	CA	1			12	61	0	-	0	0	0	0
	CA			1.25	100	80	0	--	0	0	--	-
	CA	1		1.25	100	83	0	--	0	0	--	-
	CA		1	1.25	100	60	0	--	0	--	--	--
	CA			1	100	77	0	--	0	0	--	-
	CA			1	100	71	0	--	0			
	air				0	0	0	0	0	0	0	0

In the results of this experiments can be seen that still not all aphids were killed by propanal, there was even a lower mortality than in the previous experiment. Hop oil did not prevent phytotoxicity caused by propanal.
In the next experiment yet another fumigant was tested: REP 4. The precise formula of this agent is unknown, as it was kindly provided for testing by Plant Research International, Wageningen.
Three different concentrations with or without CA were tested, see Table 7.2.17.
Overall insect mortality was low. Phytotoxic effects were considerable, especially on rose flowers. The leaf damage in chrysanthemums in vessel 1 t/m 3 might be caused by CA (compare Table 7.2.15).

*Table 7.2.17. Insect mortality by treatment with REP4 as fumigant. CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 3 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Two rose varieties were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		ml REP4 applied	% mortality		Phytotoxicity								
			Thrips larvae	A. fabae	Chrys			Rose OU			Rose V		
					F	L	S	F	L	S	F	L	S
1	CA	0.5	24	18	0	-	0	-	-*	0	-	0	0
2	CA	1	3	32	0	-	0	-	-*	0	-	-	0
3	CA	2	45	6	0	--	0	--	0	-	-	0	-
4	air	0.5	36	9	0	0	0	--	0	0	--	0	0
5	air	1	33	14	0	0	0	--	0	0	--	0	0
6	air	2	36	22	0	0	0	--	0	0	--	0	0
contr	air		0	20	0	0	0	0	-*	0	0	-*	0

A higher quantity of REP4 was tested and also the fumigants S-Limonene and trans-cinnamaldehyde, see Table 7.2.18. The fumigant trans-cinnamaldehyde was combined with a different CA because this agent readily oxidises to the non-volatile cinnamic acid (Smid et al, 1996).

*Table 7.2.18. Insect mortality by treatment with REP4, S-Limonene (S-Lim) or trans-cinnamaldehyde (t-cin) as fumigants. CA1 means: 25% CO_2, 15% O_2 and 60% N_2, CA2 means: 25% CO_2, 2% O_2 and 73% N_2. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Two rose varieties were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel	time (h)		Fumigant (ml applied)	% mortality		Phytotoxicity								
				Thrips larvae	A. fabae	Chrys			Rose OU			Rose V		
						F	L	S	F	L	S	F	L	S
1	4	CA1	2 REP4	68	0	0	-	0	-	0	-	--	-*	-
2	4	CA1	4 REP4	45	23	-	--	0	--	--	-	-	--	--
3	3	CA1	2 S-Lim	100	40	0	--	0	0	--	--	0	--	--
4	3	CA1	4 S-Lim	100	92	0	--	0	--	--	--	--	--	--
5	3	CA2	5 t-cin	70	8	0	-	0	-	-*	-	-	-*	-
6	3	CA2		14	0	0	-	0	0	-	0	0	0	0
contr		air		26	0	0	0	0	0	-*	0	0	-*	0

Still, REP4 was not 100% toxic for both insects. S-Limonene gave 100% thrips mortality but not to aphids. Trans-cinnamaldehyde did not cause high mortality rates. Phytotoxic damage was severe in most treatments.

In the next experiment we tested propanal again, and we tested whether the phytotoxic effect of p-cymene (compare Table 7.2.15) could be counteracted by lavender oil.

*Table 7.2.19. Insect mortality by treatment with propanal (p), lavender oil(lo) and p-cymene(p-c). CA means: 25% CO_2, 15% O_2 and 60% N_2. When two fumigants were combined, the first was applied for 1 hour, followed by the other for the remaining treatment time. Treatment duration was 3 or 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Two rose varieties were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

Vessel	time (h)		Fumigant (ml applied)	% mortality		Phytotoxicity								
				Thrips larvae	A. fabae	Chrys			Rose OU			Rose V		
						F	L	S	F	L	S	F	L	S
1	4	CA		33	10	0	-	0	-	0	-	--	-*	-
2	3	CA	1 p	100	84	-	--	0	--	--	-	-	--	--
3	4	air	1 p	100	53	0	--	0	0	--	--	0	--	--
4	3	CA	1 lo	0	13	0	--	0	--	--	--	--	--	--
5	3	CA	2 p-c	100	67	0	-	0	-	-*	-	-	-*	-
6	3	CA	1 lo + 2 p-c	100	75	0	-	0	0	-	0	0	0	0
Contr		air		0	6	0	0	0	0	-*	0	0	-*	0

Results in Table 7.2.19 show that a 3 or 4 hour treatment with 1 ml propanal was phytotoxic and still did not give 100% mortality for aphids. Propanal in CA gave a somewhat higher mortality than when applied in air. Phytotoxicity was high in most treatments.

Because *Aphis fabae* still was the toughest to kill, this experiment was carried out with A. fabae only (Table 7.2.20). A probable cause for low mortality rates could be that one of the two flower crops we used, could absorb high amounts of propanal. Thus, in vessel 1-3 only Chrysanthemums were used, and in vessel 4-6 only roses. The amount of flowers per vessel was equal to previous experiments, so two bunches of Chrysanthemums or two bunches of roses (two varieties) in each container.

*Table 7.2.20. Insect mortality by treatment with propanal (p) and/or piperonylbutoxide (pip). CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Two rose varieties were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Propanal (ml applied)	% mortality	Phytotoxicity								
			A. fabae	Chrys			Rose OU			Rose V		
				F	L	S	F	L	S	F	L	S
1	CA		13	0	-*	0						
2	CA	1	46	0	--	0						
3	air	1	30	0	--	0						
4	CA		13				0	--	0	0	--	0
5	CA	1	50				--	--*	-	0	--	-
6	air	1	78				0	0	-	0	--	--
contr	air		17	0	0	0	0	0	0	-*	0	0

No obvious differences in mortality were seen when chrysanthemums or roses were present in the vessels, so probably there is not a big difference in the interaction between the crop and the amount of propanal absorbed. Here, we see that an application of propanal in air gave a higher mortality rates than when applied in CA. This is contradictory to the results in Table 7.2.19. Probably these inconsistent results are caused by high a variability in effect between the same treatments.

*Table 7.2.21. Insect mortality by treatment with propanal (prop) and/or piperonylbutoxide (pip). CA means: 25% CO_2, 15% O_2 and 60% N_2. Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml applied)		% mortality		Phytotoxicity					
		pip	prop	Thrips larvae	A. fabae	Chrys			Rose V		
						F	L	S	F	L	S
1	CA		1	100	100	0	--	0	0	--	-
2	CA	2.5		0	3	0	0	0	0	0	0
3	CA	2.5	1	100	95	0	--	0	--	--	--
4	CA	1		0	5	0	0	0	0	0	0
5	CA	1	1	94	98	0	--	0	0	--	--
6	CA			0	27	0	-	0	0	0	0
contr	air			0	0	0	0	0	0	0	0

In the next experiment (Table 7.2.21) piperonylbutoxide was combined with propanal. Piperonylbutoxide often is combined with other insecticides, because it increases the effect of the latter. Now we see a 100% mortality in *A. fabae* with 1 ml propanal for 4 hours (compare Tables 7.2.19 and 7.2.20). Piperonylbutoxide alone was not toxic for plants or insects. A positive effect on mortality by propanal could not be detected, because propanal alone did cause 100% mortality.

In the next experiment a much lower concentration of propanal was used, in order to see if piperonylbutoxide could increase the mortality. Also the effect of piperonylbutoxide on the REP4 effect was tested, see Table 7.2.22.

*Table 7.2.22. Insect mortality by treatment with propanal (prop), piperonylbutoxide (pip) and/or REP4. CA means: 25% CO_2, 15% O_2 and 60% N_2 . 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel	time (h)		Fumigant (ml applied)	% mortality		Phytotoxicity					
				Thrips larvae	A. fabae	Chrys			Rose V		
						F	L	S	F	L	S
1	4	CA	0.25 prop	0	0	0	-	0	0	0	0
2	4	CA	1 pip	0	0	0	0	0	0	0	0
3	4	CA	1 pip + 0.25 prop	16	4	0	0	0	0	0	0
4	3	CA		17	13	0	-	0	0	0	0
5	3	CA	1 pip + 0.5 REP4	40	30	0	0	0	0	-*	0
6	3	CA	0.5 REP4	12	0	0	0	0	0	0	0
contr		air		100	4	0	0	0	0	0	0

Comparing vessel 1 and 3, an increase if the effect of propanal can be seen. Also the effect of REP4 was increased by piperonylbutoxide (compare vessel 5 and 6). With these low concentrations of propanal and REP4 no phytotoxic damage occurred, but mortality rates for the insects were low too.

An intermediate concentration of propanal was used in the next experiment, see Table 7.2.23. A 0.5 ml propanal treatment did not kill all aphids, as expected, but in this case piperonylbutoxide did not increase the mortality, when included in the treatment. The amount of piperonylbutoxide did not matter.

*Table 7.2.23. Insect mortality by treatment with propanal (prop), piperonylbutoxide (pip). CA means: 25% CO_2, 15% O_2 and 60% N_2 . Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml applied)	% mortality		Phytotoxicity					
			Thrips larvae	A. fabae	Chrys			Rose V		
					F	L	S	F	L	S
1	CA	0.5 prop	100	75	0	--	0	0	--	--
2	CA	0.25 pip	0	5	0	-	0	0	0	-
3	CA	0.25 pip + 0.5 prop	100	13	0	--	0	0	--	--
4	CA	1 pip	0	25	0	-	0	0	0	0
5	CA	1 pip + 0.5 prop	100	13	0	-	0	0	--	-
6	CA		0	5	0	--	0	0	0	0
contr	air		0	0	0	0	0	0	0	0

In Table 7.2.24 the effect of piperonylbutoxide on 1 ml REP4 action is shown. Also 0.5 ml propanal plus a high quantity of piperonylbutoxide was tested .
The low mortality rates of REP4 were not greatly improved by piperonylbutoxide. For propanal, aphid mortality still was too low, even with 2.5 ml piperonylbutoxide.

*Table 7.2.24. Insect mortality by treatment with propanal (prop), piperonylbutoxide (pip) and/or REP4. CA means: 25% CO_2, 15% O_2 and 60% N_2 . Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml applied)	% mortality		Phytotoxicity					
			Thrips larvae	A. fabae	Chrys			Rose V		
					F	L	S	F	L	S
1	CA	1 REP4	21	33	0	-	0	--	--	0
2	CA	2.5 pip	0	35	0	--	0	0	0	-
3	CA	2.5 pip + 1 REP4	5	49	0	--	0	0	--	--
4	CA	2.5 pip + 0.5 prop	100	42	0	--	0	0	--	-
5	CA	0.5 prop	95	41	0	--	0	0	--	-
6	CA		0	30	0	0	0	0	0	0
contr	air		0	36	0	0	0	0	0	0

Because the former used fumigants all gave more or less disappointing results, a final set of experiments was done with conventional insecticides used during culture of horticultural crops.

In Table 7.2.25 the results are shown of fumigation with three different insecticides. One of them, Agent A, does not have a permit on the Dutch market in any form, so the active ingredient is not mentioned. The other insecticides, however, do not have a permit to be applied for this kind of treatments, but we have included them for experimental purposes. Orthene (acefaat) and Methomex (methomyl) are well-known commercial insecticides, which are known to have (some) effect as a vapour.

*Table 7.2.25. Insect mortality by treatment with Orthene (Ort), Methomex (Met) or 'Agent A' (A) in air. Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml or gram applied)	% mortality		Phytotoxicity					
			Thrips larvae	A. fabae	Chrys			Rose V		
					F	L	S	F	L	S
1	air	0.5 A	100	100	0	0	0	0	--	0
2	air	0.1 A	57	100	0	0	0	0	0	0
3	air	0.1 Ort	9	0	0	0	0	0	0	0
4	air	0.5 Ort	5	0	0	0	0	0	0	0
5	air	0.5 Met	4	15	0	0	0	0	0	0
6	air	0.5 Met	23	49	0	0	0	0	0	0
contr	air		0	33	0	0	0	0	0	0

Agent A was 100% effective for thrips as well as aphids in a quantity of 0.5 ml. Orthene and Methomex were not very effective. No phytotoxicity was seen, except for 2 out of 5 rose stems, which had leaf damage.

Agent A was included in the next experiment (Table 7.2.26) in lower quantities, and combined with piperonylbutoxide. Also, Pirimor (pirimicarb) and Decis (deltamethrin) were included. Pirimor is known to have mainly effect on aphids, and Decis is a broad-spectrum insecticide which is known for good effects on thrips.

*Table 7.2.26. Insect mortality by treatment with 'Agent A' (A), piperonylbutoxide (pip), Pirimor (Pir) or Decis (Dec) in air. Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

Vessel		Fumigant (ml or gram applied)	% mortality		Phytotoxicity					
			Thrips larvae	A. fabae	Chrys			Rose V		
					F	L	S	F	L	S
1	air	0.1 A	35	75	0	0	0	0	0	0
2	air	0.2 A	96	100	0	0	0	0	--	-
3	air	0.4 A	100	100	0	0	0	-	--	-
4	air	0.1 A + 2 pip	26	54	0	0	0	0	0	0
5	air	0.5 Pir	32	14	0	0	0	0	0	0
6	air	0.5 Dec	29	17	0	0	0	0	0	0
Contr	air		16	0	0	0	0	0	0	0

Apparently at least a quantity of 0.4 ml of Agent A is necessary to reach 100% mortality in both insects. However, at this concentration a clear phytotoxic effect is seen in roses, in contradiction with vessel 1 in the former experiment. Adding piperonylbutoxide to the low quantity of Agent A did not increase the mortality, compare vessel 1 and vessel 4. Comparing vessel 1 in this experiment with vessel 2 in the former shows again that there is considerable variability in the outcome of the same treatments.
Pirimor and Decis did not cause a high mortality.

In the last experiment higher quantities of Decis (deltamethrin) were used, and the insecticide Curater (carbofuran). Also, the effect of application of Agent A in CA was tested.

*Table 7.2.27. Insect mortality by treatment with 'Agent A' (A), Decis (Dec) or Curater (Cur) in air or CA (10% CO_2, 2% O_2 and 88% N_2). Treatment duration was 4 hours. 25 thrips larvae and a mix of 25 wingless and winged stage individuals of Aphis fabae were used. Phytotoxicity was scored as 0 = no damage, - = damage, -- = severe damage. F= flower, L= leaves, S= sepals, * = postharvest damage not caused by treatment*

vessel		Fumigant (ml applied)	% mortality			Phytotoxicity					
			White fly	Thrips larvae	A. fabae	Chrys			Rose V		
						F	L	S	F	L	S
1	air	1 Dec	100	48	82	0	0	0	0	--	-
2	air	3 Dec	100	100	54	--	--	-	--	--	--
3	air	5 Dec	100	100	56	--	--	--	--	--	--
4	air	1 Cur	100	0	13	0	0	0	0	0	0
5	air	5 Cur	100	6	0	0	-*	0	0	0	0
6	CA	0.1 A	100	45	89	0	0	0	0	0	0
contr	air		100	0	0	0	-*	0	0	0	0

An application of 3 and 5 ml Decis proved to be 100% effective for white fly and thrips larvae, but not for aphids. Besides, there was considerable phytotoxicity. Curater only killed white fly, and 0.1 ml Agent A in CA did not give higher mortality than the means of the previous experiments with the same quantity of Agent A in air (respectively 46 and 88% mortality for thrips larvae and aphids).

General conclusion for the combination treatments.

From the previous experiments it has become clear that we have not found any fumigant or insecticide that was 100% effective in killing thrips an aphids in 3 or 4 hours. Although we sometimes observed a 100% mortality by some treatments, the results showed to be inconsistent. Besides this inconsistency we have shown that there are considerable phytotoxic effects by almost all fumigants we have used. Whether the toxic effect on insects acts via the same mechanism as the toxic effect on plants cannot be concluded from this research. It is not likely though.
The general conclusion after this extensive testing of many fumigants has to be that the possibility is small to find a fumigant that causes a 100% mortality in insects, but causes no phytotoxic damage.

Significant delays or difficulties experienced during the last year reporting period

Due to illness of the project leader the final report has been delayed till february 2003.

Deliverables

- Organisation of the 6 month meeting at PBG, 14 12 1999, Aalsmeer.
- Production of Poinsettia cuttings for experiments carried out by CSL.
- Presentation of a poster for the 'Open Doors Day' (celebrating the 100 years anniversary of the institute). Title: Insectenvrije export, Alternatieven voor Methylbromide, E.A.M. Beerling, J.J. Fransen, A. de Gelder, G. Slootweg & J. Tolsma.
- Publication in a Growers Bulletin of LTO-Nederland with a request to provide plant material infected with *Opogona* (see Appendix 1)
- Organisation of the 5th participants meeting at PPO-Glasshouse Horticulture, 12 11 2001, Aalsmeer.
- Presentation of a poster at the 'Gewasbeschermingsmanifestatie KNPV' ('Crop Protection Manifestation') February 7, 2002. Title: Nieuwe methoden voor het insectenvrij maken van uitgangsmateriaal en bloemisterijproducten, Bertin Boertjes, Anita Hazendonk, Laxmi Kok, Nollie Marissen en Casper Slootweg. Abstract published in 'Gewasbescherming 33 (2002) p 22, (see Appendix 2 for abstact)
- Presentation of a poster at the Technical Conference, Plant Health and Seeds Inspectorate of the Department of Food, Environment and Rural Affairs, in Southampton, UK, 7 – 9 January 2002. Title: Hot water treatments on the Sugar Cane Borer, *Opogona sacchari*, in Yucca stems, Anita Hazèndonk, Casper Slootweg, Nollie Marissen (see Appendix 3)
- Publication in 'Vakblad voor de Bloemisterij' in preparation, due to be submitted in March 2003

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Appendix 1

Bananenvlinders gezocht!

Op het PBG Aalsmeer is deze zomer een project gestart waarbij alternatieven voor methylbromide begassingen van bloemisterijproducten worden onderzocht. Per 2002 wordt namelijk het gebruik van methylbromide in de EU en dus ook in Nederland verboden. Dit project wordt deels door de Europese Unie en deels door het Ministerie van LNV gefinancierd. Een mogelijk alternatief is het toepassen van een warmwater-behandeling. We dompelen bijvoorbeeld chrysantenstek met daarop trips in een warmwaterbad en kijken daarna of alle insecten dood zijn, maar natuurlijk ook of er geen schade aan het product optreedt. Er worden verschillende temperaturen uitgeprobeerd en ook de duur van de behandeling wordt gevariëerd. We willen deze proeven nu ook gaan doen met de Bananenvlinder *(Opogona sacchari)*. Dit quarantaine insect kan voor grote schade zorgen in o.a. Yucca, Dracena en Cycas palmen. Voor het onderzoek hebben we de komende maanden veel besmet materiaal nodig. We doen daarvoor een beroep op u! **Graag ontvangen we zoveel mogelijk met Bananenvlinders besmet plantmateriaal** zodat we dit onderzoek kunnen uitvoeren.
Voor het sturen van materiaal of informatie kunt u terecht bij:

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Appendix 2

Abstract of poster, published in 'Gewasbescherming' 33 (2002) p 22

Nieuwe methoden voor insectenvrij maken van uitgangsmateriaal en bloemisterijproducten

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Bloemisterijproducten die geëxporteerd worden naar een land met een nultolerantie worden momenteel begast met methylbromide, indien ze niet insectvrij zijn. Ook bij desinfectering van uitgangsmateriaal wordt methylbromide toegepast. Het gebruik van methylbromide is over enkele jaren niet meer toegestaan in Nederland e.a. westerse landen.

Voor uitgangsmateriaal is onderzoek gedaan naar mogelijkheden met warmwaterbehandelingen.
Bananenboorder (*Opogona sacchari*) larven in Yuccastammen worden door een warmwaterbehandeling gedood zonder dat fytotoxiciteit optreedt. Door een voorbehandeling kunnen halfjaarstruikjes van roos een warmwaterbehandeling verdragen die dodelijk is voor nematoden.
Voor stekmateriaal van o.a. chrysant is de warmwaterbehandeling die 100% doding van *Frankliniella occidentalis* (californische trips) veroorzaakt ook schadelijk voor het plantmateriaal. Dit geldt ook wanneer er methoden gebruikt zijn om stekmateriaal warmte-stress bestendiger te maken.

Behandeling van snijbloemen gedurende 4 uur met een luchtsamenstelling afwijkend van normale atmosfeer (CA) veroorzaakte geen 100% sterfte van californische trips en bladluizen. Verscheidene etherische oliën zijn onder verschillende CA-condities getest. Het combineren van CA met etherische olie leidt tot een hogere mortaliteit, maar is vaak fytotoxisch. Er zijn geen behandelingen gevonden die 100% doding geven van de gebruikte insecten en die geen of acceptabele productschade veroorzaken.

Voor andere toepassingen dan export naar nultolerantie landen is 100% doding niet altijd noodzakelijk.

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APPLIED PLANT RESEARCH

Nieuwe methoden voor het insectenvrij maken van uitgangsmateriaal en bloemisterijproducten

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Probleemstelling

Bloemisterijproducten die geëxporteerd worden naar een land met een nultolerantie worden momenteel begast met methylbromide, indien ze niet insectvrij zijn. Ook bij desinfectering van uitgangsmateriaal wordt methylbromide toegepast. Het gebruik van methylbromide is binnenkort niet meer toegestaan in Nederland e.a. westerse landen.
In dit project wordt gezocht naar alternatieven voor methylbromide. Het streven is dat eventuele alternatieven volledige sterfte geven van de insecten en geen schade aan het plantmateriaal veroorzaken. Daarnaast moet de methode voldoende kort duren om inpasbaar te zijn in de handelsketen.

Methode

Warmwaterbehandelingen :
Yuccastammetjes deels geïnfecteerd met larven van de bananenboorder (*Opogona sacchari*), halfjaarstruikjes van roos en chrysantenstek met of zonder trips ondergingen een warmwaterbehandeling met verschillende temperatuur en behandelingsduur. De niet geïnfecteerde planten zijn daarna opgekweekt onder omstandigheden conform de praktijk om eventuele fytotoxiciteit waar te nemen. In de geïnfecteerde yuccastammetjes werd de mortaliteit van opogona larven direct na de behandeling gemeten na het verwijderen van de bast. Trips sterfte in chrysantenstek werd gemeten door het aantal levende en dode trips op de bladeren te tellen.

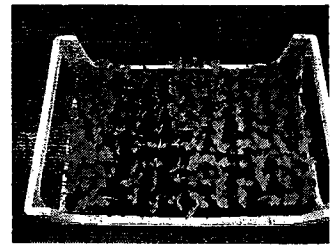
Begassingen :
Snijbloemen (roos en chrysant) ondergingen in begassingscontainers een behandeling met Controlled Atmosphere (CA), een behandeling met een chemische stof of een behandeling met een combinatie van CA en chemische stof. Bij de gebruikte chemische stoffen lag de nadruk op etherische oliën. De behandelingsduur was 3 of 4 uur. De gebruikte stoffen werden via het septum toegediend. Mortaliteit van californische trips (*Frankliniella occidentalis*), larven en adulten, en bladluis (*Aphis fabae*) werd 24 uur na behandeling beoordeeld. Na een week werden de bloemen op schade beoordeeld.

Temperatuur (°C)	Tijd (min.)	# larven	larvale sterfte (%)
controle	controle	281	0
20 °C	10'	29	0
40 °C	30'	29	66
44 °C	15'	74	87
44 °C	30'	64	100
44 °C	60'	181	100
45 °C	15'	31	81
47 °C	30'	49	100
47 °C	60'	199	100
50 °C	5'	41	73
50 °C	15'	134	100

Tabel 1: Warmwaterbehandelingen larven van Opogona in yucca.

Resultaten

Warmwaterbehandelingen :
Opogona larven in yuccastammen kunnen door een warmwaterbehandeling gedood worden (Tabel 1), geen van de behandelingen was fytotoxisch. Na een voorbehandeling kunnen halfjaarstruikjes van roos een warmwaterbehandeling verdragen die volgens de literatuur dodelijk is voor nematoden.
Voor chrysanten stekken is de temperatuur/tijdsduur combinatie die 100% doding van californische trips veroorzaakt schadelijk voor de stekken.
Een methode om het stekmateriaal warmtestress bestendiger te maken is uitgewerkt. De maximale temperatuur/tijdsduur combinatie die niet fytotoxisch is veroorzaakt geen totale doding van trips. Tussen de chrysantencultivars zijn er verschillen in gevoeligheid voor warmwaterbehandeling (Foto).



Begassingen
Behandeling van snijbloemen gedurende 4 uur met CA veroorzaakt geen 100% sterfte van californische trips en bladluizen. Verscheidene etherische oliën zijn onder verschillende CA-condities getest. Het combineren van CA met etherische olie leidt tot een hogere mortaliteit, maar is vaak fytotoxisch. Er zijn geen behandelingen gevonden die 100% doding geven van de gebruikte insecten zonder productschade te veroorzaken.
In tabel 2 staat een voorbeeld van één van de uitgevoerde experimenten.

Chemische stof: p-cymeen / propanol
Totale behandeltijd: 3 of 4 uur
Bloemen: Chrysant cv. Euro / roos cv. 1: Orange unique / roos cv. 2: Versilia
Insecten: Larven californische trips (*Frankliniella occidentalis*) , zwarte bonenluis (*Aphis fabae*)

Container	Behandeltijd (uur)	CA condities %CO2	%O2	%N2	Stof a) (ml)	% Sterfte na 24 uur trips	luis	Effect op bloemen b) chrys. A	B	C	roos1 A	B	C	roos2 A	B	C
1	4	lucht			1.0 prop.	100	97	0	-	0	0	-*	-	0	-	-
2	3	lucht			1.25 prop.	100	88	0	-	0	0	-*	-	0	-	-
3	4	lucht			1.0 cym.	100	20	0	-	0	-	-	-	-	-	-
4	3	25	15	60	1.0 prop.	100	69	0	-	0	0	-*	0	-	-	-
5	3	25	15	60		0	0	0	-	0	0	-*	-	0	0	0
6	3	25	15	60	1.0 prop.	100	94	0	-	0						
Controle		lucht				23	0	0	0	0	0	-*	0	0	0	0

a) cym. = p-cymeen, prop. = propanol
b) * = na-oogst schade die niet veroorzaakt is door de begassing of de gebruikte stof
A = bloem, B = blad, C = kelkblad

Tabel 2: Resultaten begassingsexperiment.

Onderdeel van LNV-programma 338 "Signalering en Beheersing van Plaaginsecten, Mijten en Slakken". Dit project wordt mede gefinancieerd door de Europese Unie.

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WAGENINGEN UR

Appendix 3

Hot water treatments on the Sugar Cane Borer, *Opogona sacchari*, in Yucca stems.

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Introduction

- A range of quarantine treatments for timber and horticultural products as alternatives to methyl bromide fumigation are being developed under an EU funded project lead by CSL with partners from Applied Plant Research Division Glasshouse Horticulture (NL), Forestry Commission Research Agency (UK) and Enterprise Ireland (Eire).
- Treatments must reliably achieve high levels of insect mortality, have minimal effects on plant material and subsequent growth, and be of sufficiently short duration.
- Applied Plant Research (NL) has investigated hot water treatments on *Opogona sacchari* larvae and Yucca stems.

Methods

Experiments 1 & 2

Opogona sacchari larvae contained in glass vials or concealed within Yucca stems were dipped in hot water at 44°C for 15, 30 or 45 minutes. Post treatment the vials were cooled in water at 20°C for 10 minutes before larval survival was recorded.

Experiment 3

South American produced 'rooted' Yucca stems without leaves were treated soon after arrival in the Netherlands. Two treatment groups, (i) stems with callus only (no roots), and (ii) stems with roots. Hot water treatments were conducted as above before stems were potted up and grown on in a glasshouse. After 3 months growth was recorded.

Results

Experiments 1 & 2

Total mortality of larvae in vials was obtained at 44°C after a treatment of only 15 minutes. To kill larvae concealed within Yucca stems treatments of 30 minutes at either 44°C or 47°C, or 15 minutes at 50°C (Table 1). No larval mortality was recorded in the control treatments.

Experiment 3

In all stems, a hot water treatment of 1 hour at 44°C and at 47°C had no effect on total sprout length, nor on the number of sprouts bigger than 20cm (a parameter of plant quality) (Figure 1). Only the total number of sprouts was slightly negatively affected on the stems with roots.

Conclusions

- A hot water treatment of at least 30 minutes at 44°C or at 47°C achieved total mortality of *O. sacchari* larvae. The effect of hot water treatment on eggs will also be determined.
- Yucca stems can tolerate hot water treatments of 1 hour at 44°C or 47°C without reducing plant quality.
- Treatment schedules will be developed for plant material moving in trade.

Table 1 Hot water treatments of *Opogona sacchari* larvae in Yucca stems

Experiment	Treatment	No. of stems	No. of larvae	Larval mortality (%)
A	control: untreated	6	47	0
	control: 10 min 20°C	6	29	0
	30 min. 40°C	6	29	66
	30 min. 44°C	6	13	**100**
B	control: untreated	5	24	0
	15 min. 44°C	5	74	87
	15 min. 45°C	5	31	81
	5 min. 50°C	5	13	85
C	control: untreated	5	171	0
	30 min. 44°C	5	19	**100**
	5 min. 50°C	5	28	68
	15 min. 50°C	5	134	**100**
D	control: untreated	1	9	0
	30 min. 44°C	5	32	**100**
	60 min. 44°C	5	64	**100**
	30 min. 47°C	5	49	**100**
	60 min. 47°C	5	12	**100**
E	control: untreated	1	30	0
	60 min 44°C	5	117	**100**
	60 min 47°C	5	187	**100**

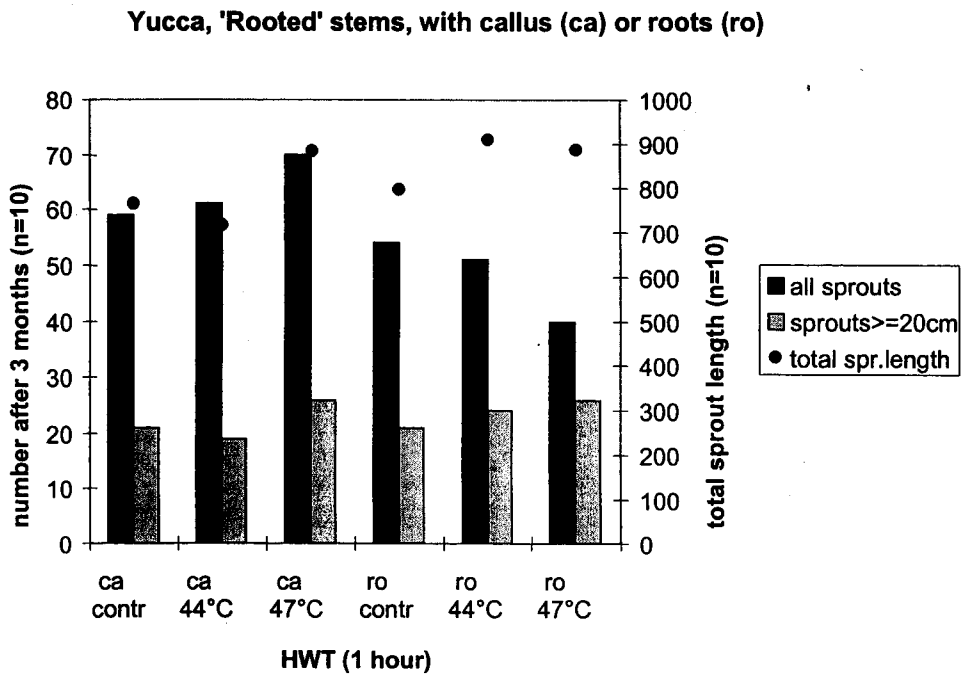

Figure 1. Post treatment new sprout growth shown as: all sprouts, sprouts bigger than 20cm and the total sprout length, of 10 plants, 3 months after a hot water treatment (HWT) of 1 hour at different temperatures of 'rooted' Yucca stems which had roots or only callus when treated.

Appendix 4 Effect of water treatments on plants

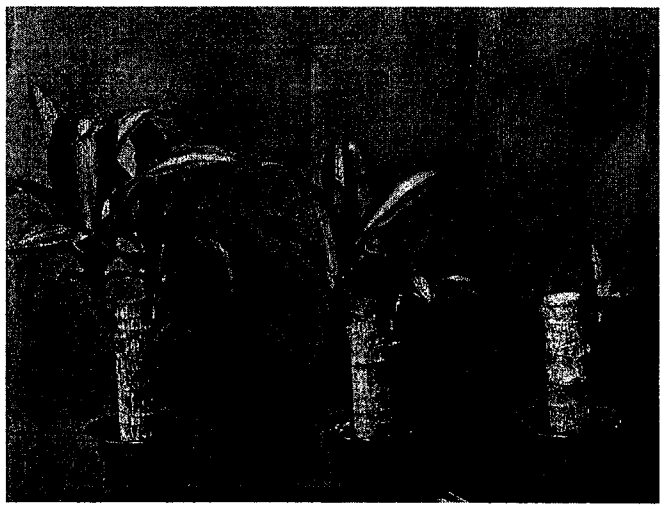

Fig 1. Dracaena stems after 4 months culture after a hot water treatment. From left to right: control, 1 hour 45 °C, 1 hour 47 °C .

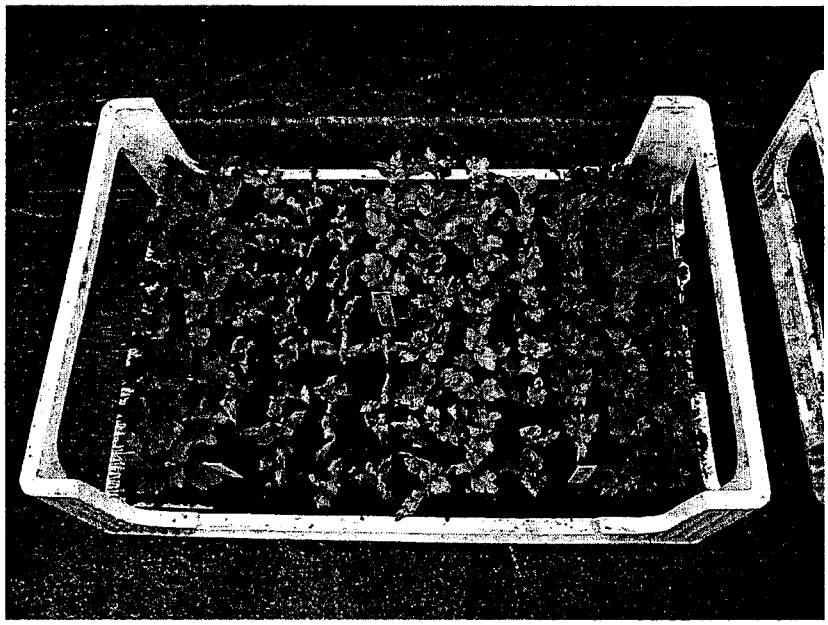

Fig 2. Four different varieties of Chrysanthemum cuttings after three weeks of growth after a hot water treatment of 1 hour 35 °C + 1 hour 40 °C (pre-treatment), followed by 30 minutes 45 °C plus 10 minutes 20 °C. Plants originate from experiment 2.

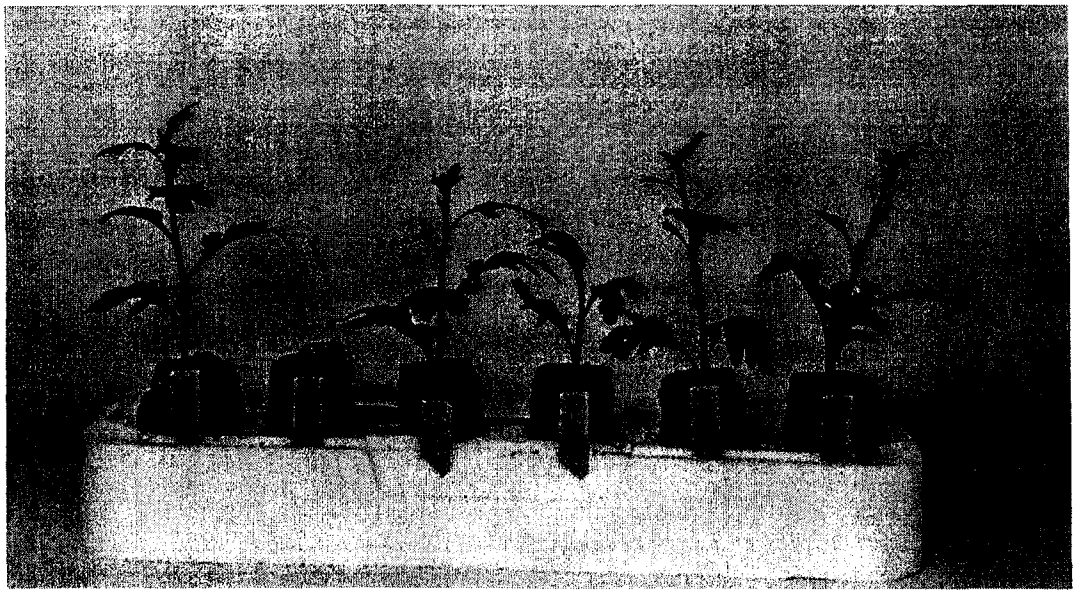

Fig 3. Chrysanthemum cuttings grown after a hot water treatment of 30 minutes 43 °C with several pre-treatments. Pre-treatments from left to right: 1: Control, 2:10% dehydration, 3: 24 hours 35 °C dry, in plastic, 4: 2 hours 35 °C in water, 5: 2 hours 40 °C dry, in plastic, 6: 1 hour 35 °C + 1 hour 40 °C in water.

Fig 4. Detail of pre-treatment 6 in picture above.

Fig 5. Leaf damage in Chrysanthemum 'Euro' after treatment with p-cymene (right vase). Picture taken 24 hours after end of treatment.

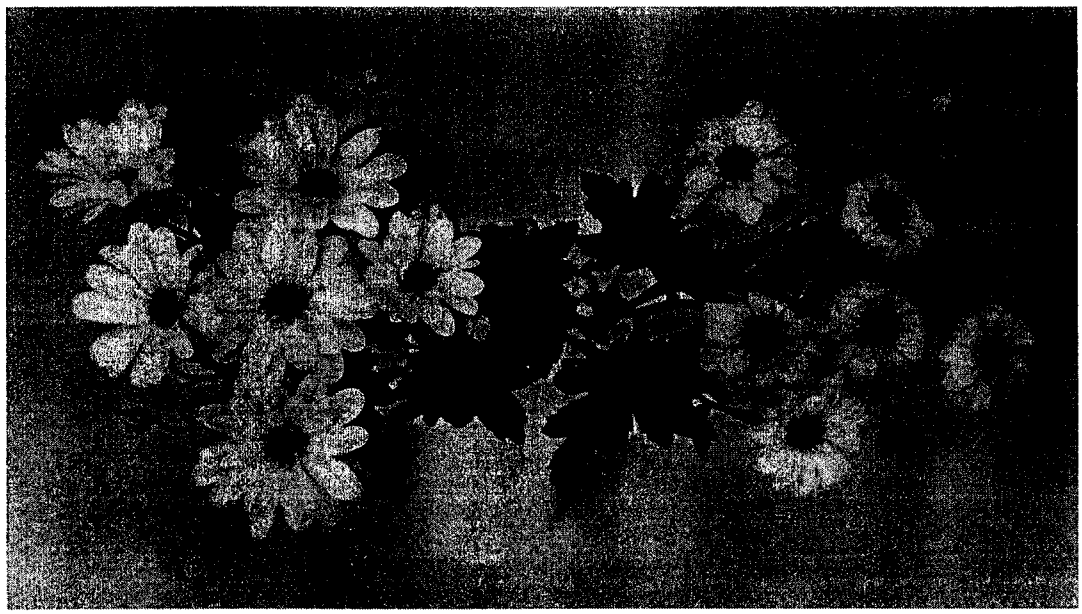

Fig 6. Flower damage in Chrysanthemum 'Euro' after treatment with linalole (right flower). Picture taken 24 hours after end of treatment.

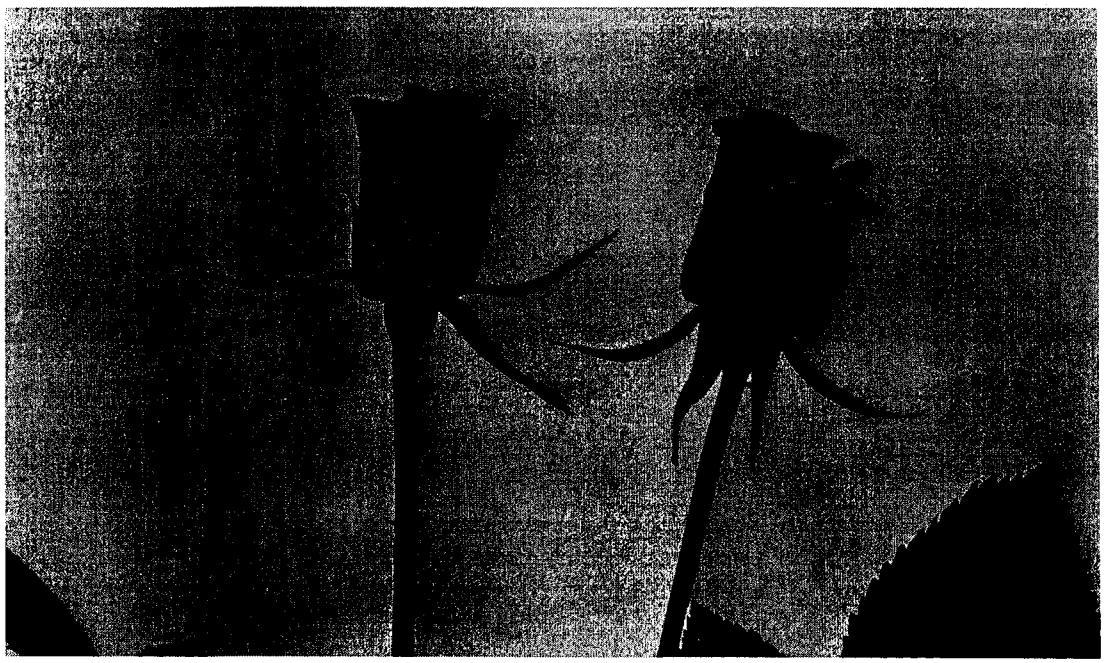

Fig 7. Flower bud damage in rose 'Orange Unique' after treatment with p-cymene (right). Picture taken 24 hours after end of treatment.

Fig 8. Flower bud damage in rose 'Orange Unique' after treatment with linalole (right). Picture taken 24 hours after end of treatment.