CATT: a New and Non-Chemical Pest and Nematode Control Method in Strawberry Planting Stock

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Keywords: *Fragaria x ananassa,* nurseries, frigo plants, disinfection, *Phytonemus pallidus, Meloidogyne hapla, Xanthomonas fragariae*

Abstract

As an alternative to MeBr fumigation a 48h Controlled Atmosphere Temperature Treatment (CATT) was developed and scaled up by Wageningen UR in cooperation with the Dutch plant propagating association Plantum. This results in an excellent de-infestation and 99.8 % mortality of the strawberry tarsonemid mite (*Phytonemus pallidus)*. This non-chemical and sustainable method provides a healthy production of highly qualified strawberry runners in the field. From 2009 CATT is up scaled to a commercial level and widely applied by Dutch nurseries. In 2011 this CATT method was successfully modified to eradicate also the root knot nematode *Meloidogyne hapla* (>99.7% mortality), which was not effectively controlled by MeBr fumigation. For an effective killing of the root knot nematodes, temperature must be raised to 40 ºC. In several experiments the optimum conditions for a high mortality of both tarsonemids and nematodes was studied. This leads into an adapted CATT of 20 hours at a temperature of 35 ºC and 50 % CO2 followed by 20 hours at a temperature of 40 ºC. In 2012 this adapted CATT was successfully upgraded and tested under field conditions. Additional research in 2013 leads to the conclusion that cross infection of plants by the bacterial Q-disease *Xanthomonas fragariae* during CATT treatment is unlikely.

INTRODUCTION

Until 2008 methyl bromide (MeBr) fumigation was applied in The Netherlands to de-infest strawberry mother planting stock from strawberry tarsonemid mites (*Phytonemus pallidus*). After cold storage in early spring the strawberry runners were treated in a specially equipped fumigation chamber. This treatment was highly effective and killed the tarsonemid mites for at least 99.8 %. Because of the unfavourable side effects of methyl bromide on the ozone layer, it was internationally agreed by the Montreal Protocol in 1987 to phase out the use of this fumigant. In the Netherlands this fumigation method for strawberry runners (mother plants) for raising of planting stock

was banned from 2008 onwards. As an alternative of MeBr fumigation a Controlled Atmosphere Temperature Treatment (CATT) was developed and scaled up by Wageningen UR in cooperation with the Dutch plant propagating association Plantum (Van Kruistum et al., 2012). Two commercial companies provide the CATT protocol as a phytosanitary treatment of strawberry runners.

Strawberry frigo plants are treated after cold storage and before planting during 48 hours at a temperature of 35 ºC and 50 % CO_2 in very humid conditions. This results in an excellent disinfestation and a mortality of 99.8 % of the strawberry tarsonemid mite. CATT is now a proven and durable method, widely applied by Dutch producers of mother planting stock. This method provides a healthy production of highly qualified strawberry runners in the field and is part of the Elite certification of the Netherlands Inspection Service for Horticulture (Naktuinbouw).

This physical CATT method is not only effective in killing strawberry tarsonemids but is also effective in killing other insects on different products (Michael and Whiting, 1996; Held et al., 2001; Liu, 2003).

Recently it was also found that the plant parasitic nematodes *Meloidogyne hapla* and *Pratylenchus penetrans* can be considerably reduced by application of CATT. Clean source material on nematode-free soil prevents the increase of the nematode population resulting in a reduction of the use of chemical soil disinfectants. In 2011 the standard 48h CATT method was successfully modified to eradicate also root knot nematodes *Meloidogyne hapla*, which was not effectively controlled by MeBr fumigation. However for an effective killing of the root knot nematodes, temperature must be raised to 40 ºC. In several experiments the optimum for a high mortality of both tarsonemids and nematodes was studied. This leads into an adapted CATT of 20 hours at a temperature of 35 ºC and 50 % CO_2 followed by 20 hours at a temperature of 40 ºC (Van Kruistum et al., 2014).

This paper summarizes CATT experiments focused on optimizing of eliminating the root knot nematode *M. hapla* in mother planting stock at a same high level of killing tarsonemids. In 2012 further field research was carried out with this adapted CATT. Because of possible risks for cross infection of the bacterial Q-disease *Xanthomonas fragariae* during CATT, in 2013 additional experiments were carried out to study the risks of cross contamination under high disease pressure.

MATERIALS AND METHODS

CATT equipment

The experiments concerning the Controlled Atmosphere and temperature conditions were realized by using a flow through system at Food & Biobased Research (FBR) Wageningen UR. In this system mass-flow controllers are used to flush gas mixtures at steady and well-defined rates through containers with strawberry plants. The stainless steel containers with a content of 65 liters were placed in special temperature controlled rooms (Fig. 1). During 2011 and 2012 in these containers experiments were conducted at a range of temperatures: 35-40 ºC and CA-conditions during 40-48 hours.

Plant material

One bundle of 15-20 healthy runners of different varieties and origins together with 5 infected plants formed one experimental unit. After treatment 2-5 healthy runners of each variety and origin and 5 runners with tarsonemids or with nematodes infected roots were potted separately and placed under favorable growing conditions at 20 ºC in

the greenhouse after treatment. Plants were examined on aberrations and vigor 1 and 4 weeks after potting up. All treatments were compared with untreated controls stored at 4 ºC during CATT.

Mortality tarsonemids and nematodes

Young, still unexpanded leaflets from 5 infected plants were checked on population development of tarsonemid mites by Berlese extraction directly after CATT and 5 weeks after potting up according to the described method of Fried, 2000. Mortality of nematodes after CATT was assessed in the laboratory by root incubation in mist chambers according Van Galen-Van Beers et al. (2002). The final counting of survived nematodes per gram root was performed 6 weeks after incubation. Analysis of variance was applied with Genstat windows version 10.

Field experiment

In 2012 the optimized adapted CATT method for killing tarsonemids and the root knot nematode *M. hapla* was tested under field conditions with a wide range of mother planting stock, originating from different nurseries. 16 samples of 5 to 10 thousand strawberry plants each of different varieties, origin and plant types (SE2, A, A + and light waiting bed plants), were treated according to the standard method: 48h at 35 °C and the new method: shorter treatment for 40 hours and increased temperature in the second phase to 40 °C. CATT was carried out at the two commercial companies: Ruvoma, Montfoort NL and Van Acht Cooling, Sint-Oedenrode NL (Fig. 2). At a smaller scale the standard and new CATT method was also applied in the experimental equipment of Food & Biobased Research (FBR). During CATT temperatures were recorded at several places. After treatment from 24 to 26 April and a short storage period, small samples of 20 plants each including untreated were transplanted in the field at Vredepeel NL on May 8, 2012. From 4 weeks after planting the field plots were assessed on plant establishment, plant loss and initial formation of runners in a 2 weekly interval until the end of July 2012.

Xanthomonas fragariae

In May and October 2013 experiments on possible cross infection by the Q-organism *Xanthomonas fragariae (Xf)* were carried out in the experimental CATT system of FBR Wageningen. A+ plants 'Elsanta' were briefly grown in the greenhouse. One and two weeks before CATT, the plants were infected with *Xf*. Just before the onset of CATT, rhizomes were dipped in a suspension of *Xf*. In this way, high (symptomatic), moderate (latent) and low (rhizome) infection levels were created. To test the risk of dissemination of *Xf* in the CATT room, inoculated rhizomes were placed next to the not inoculated target rhizomes. Figure 3 shows the experimental lay-out in one of the CATT containers.

In addition to plant material also *Xf* suspensions were put into the CATT containers. Catch plates were placed to monitor splash dispersal and bacterial dispersal through water leakage. The air was sampled to identify spread through the air. To test whether there was survival and spread of the pathogen during CATT, treated materials were collected and a bio-PCR was performed. To this end plant extracts were plated on R2A medium. As a control *Xf* suspensions were plated and samples were spiked with *Xf*. Plates were incubated for 2 weeks at 25°C, after which *Xf*-like colonies were counted. Bacterial suspensions were washed from the plates, DNA was extracted from suspensions and tested with a TaqMan assay. In addition, individual *Xf*-like colonies were tested with the TaqMan assay. By using a bio-PCR only living *Xf* is demonstrated.

RESULTS AND DISCUSSION

Optimisation adapted CATT

In a final experiment in 2011 it was decided to work with a combination of a temperature of 35 °C, the standard temperature for CATT, with an increase of temperature to 40 °C during different intervals from 12 until 28 hours in the second part of CATT but with shortening of the total treatment period to 40 hours. In this trial strawberry cultivars are used infected with *M. hapla* (cv. Lambada) or with *P. penetrans* (cv. Sonata). In addition also healthy plants of cv. Lambada, Elsanta and the everbearer Evi II were treated, including plants contaminated with strawberry tarsonemid mites.

At 12 and 16 hours 40 °C (preceded by 28 and 24 hours at 35 °C) mortality of *M. hapla* was over 97 % compared to the untreated control (Table 1). At 20 and 24 hours 40 °C (preceded by respectively 20 and 16 hours at 35 °C), mortality was over 99 % and at 28 hours 40 °C even 100 %. Plants infected with *P. penetrans* were less available, so a limited number of objects was studied. At 12 hours 40 °C mortality of *P. penetrans* nematodes was nearly 68 %, after 20 hours at 40 °C nearly 80 % and up to 28 hours at 40 °C 90 %. All the treatments have led to an excellent control of strawberry tarsonemid mites > 99.8 % (Table 2). Plant establishment and development 4 weeks after CATT in almost all cases was satisfactory to good. However, it was evident that an increase in the number of hours at 40 °C and a decrease in the number of hours at 35 °C, plant development is slightly less compared to the untreated control. Only healthy plants of cv. Elsanta and the everbearer Evi II gave a moderate to bad plant development after treatment during 28 hours at 40 °C.

Field experiment

In 2012 the adapted CATT proved to be equally successful as the standard method, except for object F: new CATT method, carried out in the experimental system at FBR (Table 3, Fig. 4). Because of the relatively large amount of plant material in the containers, in this object an increase in temperature has occurred from about 1.2 °C above set point as a result of respiration. At the other treatment places (Van Acht and Ruvoma), temperatures during new CATT treatment were not above set point.

It became clear that the upper limit of 40 °C is critical during treatment; only 1 °C over this limit results in clear damage. Uniform temperature distribution during treatment is required, so the plants shall not to be packed too tight in the box during treatment. Also sufficient humidification and control of the proper oxygen concentration is important.

Cross infection Xf

After CATT Xf in water was killed for 100 % while outside the CATT area at room temperature the bacteria survived in the water. It did not matter whether the standard CATT was used or the adapted CATT where temperature in the second phase reaches 40 °C. This clearly shows that CATT conditions are finally fatal for "unprotected" bacterial cells.

In plant material the case is different. Xf infected strawberry plants were still found to carry viable cells of the pathogen after CATT (Table 4). The bacterial cells are protected by the plant and are not killed by the standard CATT. Whether Xf will be killed during the adapted CATT has not been studied.

Knowing that the standard CATT does not kill Xf in infected plant material, the question remains whether cross-contamination can occur during the treatment. In theory this is possible by leaking water, movement through the air of *Xf* in aerosols and through water splashes. In air no living Xf bacteria cells were found. In condensation or dripping water only a small amount of Xf was detected. In this experiment only at a high inoculum density in one of the six samples of healthy target plants *Xf* was detected (Table 4).

CONCLUSIONS

The experiments of 2011 proved that control of the nematodes *M. hapla* and *P. penetrans* is successful by application of an adapted CATT treatment with a duration of 40 hours at 50 % CO_2 and in the second phase warming-up plant temperature from 35 °C to a maximum of 40 °C. As the number of hours at 40 °C is increasing, control of *M. hapla* is better and plant vitality is slightly less. Treatment during 28 hours at 40 °C can result in plants with a poorer vitality. Treatments during 20 or 24 hours at 40 °C results in plants with a sufficient to good quality. To reduce the risk to the plants even more, at best can be chosen for the duration of treatment of 20 hours at 35 °C, followed by 20 hours at 40 °C. Mortality of *M. hapla* is over 99 % and *P. penetrans* is controlled for 80%. Plant vitality and development is good and strawberry tarsonemid mites is controlled for at least 99.8 %.

In a comprehensive field experiment during 2012 with different varieties, origins and plant types these results for the adapted CATT on plant quality were confirmed with the restriction that a temperature of 40 °C is upper critical level. Application of this optimized CATT will prevent further dispersion of both tarsonemids and *Meloidogyne hapla* nematodes in the strawberry chain on propagation fields and later also on production fields.

The experiments from 2013 showed that the CATT method gives an acceptable risk of cross-contamination of the Q-bacteria *Xanthomonas fragariae* (*Xf*). It is not known whether the *Xf* population surviving CATT can infect strawberry plants. In the trials only at a high level of infection, provided by inoculation of leaves, small scale spread of *Xf* was found. Our experiments simulated that heavily infected plants from the field were put through CATT immediately after harvest. In practice, plants with visible symptoms will be degraded and taken from the market. Furthermore most plants are treated in spring after a period in the cold (frigo) store. To effect of cold storage of strawberry plants on the vigour of the *Xf* population is not known. The effects of the adapted CATT on dispersion risk of *Xf* are not yet determined.

In pilot experiments in 2013 it was confirmed that CATT also can be used for sustainable treatment of insect, mite and nematode (Q)-pests in the chain of the international trade of plant materials and fresh products. Optimize the gas composition and temperature combination of a CATT treatment on tomato miner fly (*Tuta absoluta*), thrips (*Frankliniella occidentalis*), codling moth (*Cydia pomonella*) and nematodes as *Meloidogyne chitwoodii* are part of this research. The effects of CATT on the products (tomatoes, chrysanthemum, apples and potatoes) are also studied.

ACKNOWLEDGEMENTS

This research was funded by the Dutch Ministry of Economic Affairs, The Propagating Association Plantum, Product Board for Horticulture (Productschap Tuinbouw) and Netherlands Inspection Service for Horticulture (Naktuinbouw).

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Tables

Table 1. Mortality of *M. hapla* and *P. penetrans* in bare rooted strawberry plants after adapted CATT during 40 hours in different intervals of temperatures, starting with 35 °C (Phase 1) and finishing with 40 °C (Phase 2). Wageningen/Lelystad NL, April 2011.

		M. hapla			*P. penetrans*		
Object	# hours 40 °C	# per gram root		% mortality	# per gram root		% mortality
1	0 (control)	232.6	b	-	255.3	a	-
2	12	6.2	a	97.3	84.6	b	66.9
3	16	5.0	a	97.9			
4	20	0.6	a	99.7	51.2	b	79.9
5	24	0.9	a	99.6			
6	28	0.0	a	100.0	25.4	b	90.1
F prob.		0.04			0.005		

Table 2. Mortality tarsonemids after Berlese extraction, directly after adapted CATT and 5 weeks after potting up infected plantmaterial in the glasshouse. Lelystad NL, April-May 2011.

	April 4, 2011		May 9, 2011	
# hours 40 °C	# tarsonemids per plant	% mortality	# tarsonemids per plant	% mortality
0 (control)	9.5	0	104.8	0
12	0	100	0.2	99.8
16	0	100	0.1	99.9
20	0	100	0	100
24	0	100	0	100
28	0	100	0.1	99.9

Table 3. Effect of standard and adapted CATT on plant loss and plant establishment (1 = plants died; 6 = sufficient vigor; 10 = excellent growth and development) in up-scaling field trial. Vredepeel NL, 2012.

Object code	Description	% Plant loss on June 27	Plant establishment on July 18
A	Control	0 a	7,1 bc
B	CATT standard Van Acht	4,3 a	7,3 c
C	CATT adapted Van Acht	5,1 a	7,4 c
D	CATT adapted Ruvoma	7,5 a	7,1 c
E	CATT standard FBR	10,0 a	6,9 b
F	CATT adapted FBR	32,2 b	6,3 a

Table 4. Survival and spread of *Xanthomonas fragariae* during CATT. Wageningen NL, May & October 2013.

Inoculation level plants	*Xf* before CATT	*Xf* after CATT	Bio-PCR after CATT
Rhizome infection	$2 * 10^6$	$16 * 10^6$	*Xf* proved
Xf latent	$54 * 10^6$	$26 * 10^6$	*Xf* proved
Xf symptomatic	$67 * 10^6$	$26 * 10^6$	*Xf* proved
Infection level healthy target plants:			
Low	0	0	None *Xf*
Moderate	0	0	None *Xf*
High	0	$0.027 * 10^6$	*Xf* proved in 1 of 6

Figures

Fig. 1. Experimental CATT system used for eliminating tarsonemids (*Phytonemus pallidus*) or the plant parasitic nematodes *P. penetrans* and *M. hapla* in strawberry runners. Food & Biobased Research, Wageningen UR.

Fig. 2. Commercial CATT of strawberry mother planting stock in equipped rooms. December 2011.

Fig. 3. Experimental lay-out trial dispersion *Xanthomonas fragariae* during CATT. Wageningen NL, October 2013.

Fig. 4. CATT field trial 2012, planting date May 8. Row in middle foreground according adapted CATT, far left untreated, 2nd row from left according standard CATT. Vredepeel NL, July 31 2012.