Controlled Atmosphere Temperature Treatment as Sustainable Alternative to Control Strawberry Tarsonemid Mites and Plant Parasitic Nematodes in Strawberry Plants

G. van Kruistum[1,a], H. Hoek[1], J. Verschoor[2] and L. Molendijk[1]
[1] Applied Plant Research, Wageningen University and Research Centre, Lelystad, The Netherlands
[2] Food and Biobased Research, Wageningen University and Research Centre, Wageningen, The Netherlands

Keywords: *Phytonemus pallidus*, plant parasitic nematodes, *Fragaria* × *ananassa*, runner production, controlled atmosphere

Abstract

High qualified strawberry (*Fragaria* × *ananassa*) planting material, mainly for export, is produced by Dutch growers on circa 1100 ha field area in The Netherlands. Planting stock must be destroyed if strawberry tarsonemid mite (*Phytonemus pallidus*) or infection by the plant parasitic nematode *Meloidogyne hapla* is found by the Dutch Quality Board. Also the nematode *Pratylenchus penetrans* can attack strawberry plants severely. The presence of tarsonemid mites in plant material can result in a considerable loss of production. Until 2007 in The Netherlands mother planting stock for runner production was treated with methyl bromide (MeBr) to eliminate tarsonemids. This chemical disinfestation method is banned since 2008. From 2007 the choice was made to optimize the Controlled Atmosphere Temperature Treatment (CATT) to provide a non-chemical and sustainable method for future disinfestation. By application of CATT during 48 h at a temperature of 35°C and 50% CO_2 mortality of the tarsonemid mites is over 99.8%. No harmful irreversible results of CATT on vitality of mother plants in the field and runner production are found. From 2009 CATT is up scaled to a commercial level and widely applied by Dutch producers of planting stock. As a spin-off also plant parasitic nematodes are reduced by application of CATT. Understanding of the influence of different factors during CATT creates also possibilities for optimization this treatment to control *P. penetrans* and *M. hapla* in strawberry plants or in the soil particles attached to the roots. Application of an optimized CATT can prevent further dispersion of these plant parasitic nematodes in the strawberry chain.

INTRODUCTION

Dutch strawberry runner production represents a value of approximately € 70 million. This amount of money is the direct value of the higher qualified material, mainly for export, produced by Dutch growers on circa 1100 ha in The Netherlands. The planting material must be destroyed, if strawberry tarsonemid mite (*Phytonemus pallidus*) is found by the Dutch Quality Board. Until 2007 methyl bromide (MeBr) was used to produce strawberry runners, intended as planting stock, fully free of infestation by strawberry mites. After cold storage in early spring the strawberry runners were treated in a specially equipped fumigation chamber, because there is no soil treatment. In The Netherlands 120 kg of active methyl bromide was used each year for this application. This treatment is highly effective and kills the tarsonemid mites for at least 99.8%. Because of the unfavorable 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 is banned from 2008. Since several years effort is directed towards a non-chemical and durable solution instead of regulatory approval of alternative pesticides.

[a] gijs.vankruistum@wur.nl

Follett and Neven (2006) and Mitcham (2003) reviewed alternative methods and the mode of action for eliminating arthropod pests from a wide range of commodities. During the years 2005 and 2006 several alternative methods were screened for eliminating tarsonemids in strawberry runners (Van Kruistum et al., 2007). Healthy bare rooted runners together with tarsonemid mites infested plants were treated with ozone, water saturated hot air, hot water, at high atmospheric pressure, in water by electrical power or under a wide range of controlled atmosphere (CA) conditions. High temperature CA-treatments were most promising and were optimized to a larger scale during the last years.

Recently it was found that the plant parasitic nematodes *Meloidogyne hapla* and *Pratylenchus penetrans* also can be reduced in mother planting stock by CATT. Understanding of the influence of different factors during CATT creates also possibilities for optimization this treatment to control *P. penetrans* and *M. hapla* in strawberry plants or in the soil particles attached to the roots. This paper summarizes these CATT experiments for eliminating strawberry tarsonemid mites and plant parasitic nematodes in mother planting stock.

MATERIAL AND METHODS

With tarsonemid mites or with plant parasitic nematodes *P. penetrans* or *M. hapla* infested plants and non-infested bare rooted runners ('Elsanta' and 'Sonata') from different growers were potted and placed under favorable growing conditions at 20°C in the greenhouse after treatment. The experiments concerning the controlled atmosphere and temperature conditions were realized by using a flow through system. In this system the gas flows at a steady and well-defined rate through a container with strawberry plants. The stainless steel containers with a content of 70 L were placed in special temperature controlled rooms (Fig. 1). During March 2007 in these containers an experiment was conducted at a range of temperatures: 32-35-38°C at normal air and CA-conditions during 24 or 48 h. In April 2007 the influence of different combinations of CO_2 (50, 70 and 90%) and O_2 (2, 5 and 10%) levels were examined.

One bundle of 15-20 healthy runners together with 5-10 infested plants formed 1 experimental unit. After treatment 2 healthy runners of different origin and 5 infested plants were potted separately. Plants were examined on aberrations and vigor 1 and 3 weeks after potting. Young, still unexpanded leaflets from 5 infested plants are checked on population development by Berlese extraction 3 weeks after potting 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.

In a field experiment runners from 6 origins were planted on 7 June 2007 after treatment by the most optimal CA-conditions at 35°C during 48 h. MeBr fumigation of 50 g m^{-3} during 2 h was used as standard treatment. Non treated runners were used as control plants. Separately natural infested and treated plants were planted. During the season plant establishment, formation of ranks, development of mildew and the population of tarsonemids were assessed.

The field experiments were laid out as randomized block designs in four replications. Analysis of variance was applied with Genstat windows version 10. For some of the responses a logarithm transformation was used and the back transformed means (medians) are presented.

During the season 2008-2009 the CATT method for disinfestation of strawberry plants from tarsonemids was up scaled to a commercial level by different treatments of bigger amounts of plants from different producers of planting stock in a large CA-cell at the company Ruvoma BV in Montfoort-NL (Fig. 2). After treatment the plants were returned to the growers and planted out in the field. The growers followed the establishment of the plants critically during 2009.

RESULTS AND DISCUSSION

CA-treatment (1% O_2 and 50% CO_2) during 48 h at 35 or 38°C resulted in >99% mortality of the tarsonemids (Fig. 3). Most of the runners from the CA-treatment at 38°C died after potting, only at 35°C some plant origins survived with a grand negative effect on plant establishment. Heat treatment under normal atmospheric conditions during 48 h at 38°C gave a lower mortality rate of 88.1% with only a slight negative effect on plant establishment. A low level of O_2 during CA-treatment can enhance plant stress. It is possible that high temperatures during treatment enhances the respiration rate of the runners and have led to a lack of oxygen, followed by anaerobic conditions. In a next experiment the effects of higher O_2 levels were studied. At an O_2 level of 10%, this negative effect on plant establishment was diminished at the same mortality level of >99% (Fig. 4). Remarkable was that at higher temperatures and lower levels of O_2, blossoming was prevented in some plant origins. Runners from some origins were more resistant against the different treatments (Fig. 5). Higher levels of CO_2 reduced also plant establishment.

Only 10% O_2 at 50% CO_2 during 48 h and 35°C gave satisfying results in killing of the tarsonemids >99% at a sufficient plant establishment. This CA-treatment was tested under field conditions and compared with the standard MeBr fumigation. During the field period plant establishment of the MeBr treated runners was slightly lower than CA-treatment; also formation of ranks was significant lower (Table 1). Mildew attack in November was moderate and only minor differences between treatments existed. Plant development in the field was different between the 6 origins (Fig. 6) but no interaction existed between origin and treatment. Monitoring by Berlese extraction showed that during the field period the population of tarsonemids did not develop in the infested plants after CA-treatment or MeBr fumigation. By cross-infestation at the end of the growing season also in the CA- and MeBr treatments a slight increase of tarsonemids was observed.

After several up scaling experiments during 2008-2009 and transplanting the treated mother planting stock in the field by the producers it became clear that this new method can replace the old treatment with MeBr gas fumigation. In some cases when very light plants were treated some more dropouts were reported, possibly due to dehydration of the plants during CATT.

After an exploratory experiment it became clear that CATT also can reduce the number of the plant parasitic nematodes *M. hapla* and *P. penetrans* in infested plants. Further experiments during 2010 learned that for improved effectiveness of CATT on nematode mortality, temperature during CA-treatment must raise to 40°C. In an experiment with *M. hapla* infested plants, different combinations of the standard CATT at 35°C and a higher temperature of 40°C in the 2nd phase were tested on mortality of *M. hapla.* The results show a high mortality of >94% after 12 h treatment at 40°C in phase 2 (Table 2). Although only significance between the control and the treatments was found it is clear that there are possibilities for a combined treatment against tarsonemids and nematodes at acceptable plant vigor after treatment. For nematode mortality a higher temperature seems to be more important than maintenance of CA-conditions. In further experiments also mortality of tarsonemids must be evaluated.

CONCLUSIONS

Compared to the standard MeBr gas fumigation, CATT is at the same level in killing tarsonemids in strawberry runners used for planting stock. During the field experiment was exposed that also use of MeBr can lead to some negative effects on plant establishment and formation of ranks. CATT is in development of the runners under field conditions at least even compared to MeBr fumigation. CATT of mother planting stock is without further use of an alternative chemical suitable for application on a commercial scale and is already replacing MeBr fumigation from 2008. In further experiments also the influence of CATT combined with a higher temperature of 40°C on mortality of the plant parasitic nematodes *P. penetrans* and *M. hapla* is demonstrated. Application of an optimized CATT can prevent further dispersion of both tarsonemids and plant parasitic nematodes in the strawberry chain.

ACKNOWLEDGEMENTS

This research was funded by Plantum NL, 'Productschap Tuinbouw', Naktuinbouw, Senter Novem, Foundation 'Proef & Selectie' and the Dutch Ministry of Agriculture, Nature and Food Quality.

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Tables

Table 1. Plant establishment and mortality of tarsonemids during field conditions of strawberry runners from 6 origins after different treatments. Planting date: 7 June 2007.

Object	Plant establishment[1] 19 July	# ranks/ plant 21 Aug.	Mildew attack[2] 12 Nov.	Mortality (%) tarsonemids		
				20 Aug.	7 Sept.	11 Oct.
A. Control	5.6 [b]	5.5 [b]	2.5 [abc]	0.0 [b]	0.0 [c]	0.0 [c]
B. CATT	5.5 [b]	5.5 [b]	2.7 [bc]	99.1 [a]	99.9 [a]	98.8 [a]
C. MeBr	4.9 [a]	4.8 [a]	2.4 [a]	99.1 [a]	99.6 [a]	98.0 [ab]

[1] Scale 1 tot 10: 1= plants died 6 = sufficient 10 = excellent.
[2] Scale 1 tot 4: 1= no mildew attack; 4 = 100 % mildew.

Table 2. Mortality of *M. hapla* and plant establishment after CATT in phase 1 followed by treatment at 40°C during different time (h) in phase 2 at CA-conditions or in normal air. CATT in the period 18-20 May 2010. Assessment of plant establishment after potting up in greenhouse on 10 June 2010.

Object	Phase 1	Phase 2			Total	%	Plant establishment[1]
	# h 35°C	# h 40°C	% O_2	% CO_2	# h treatment	mortality *M. hapla*	
1. Control	*	*	*	*	0	0	6.8
2. CATT	48	0	*	*	48	64	6.5
3. CATT	36	12	21	0	48	98	4.0
4. CATT	36	12	15	50	48	94	4.6
5. CATT	40	8	21	0	48	73	5.8
6. CATT	40	8	15	50	48	91	5.1
7. CATT	44	4	21	0	48	70	4.9
8. CATT	44	4	15	50	48	56	6.2
9. CATT	28	12	21	0	40	99	5.1
10. CATT	28	12	15	50	40	97	6.2
F prob						0.01	0.27
LSD5%						53	2.3

[1] Scale 1 tot 10: 1= plants died 6 = sufficient 10 = excellent.

Figures

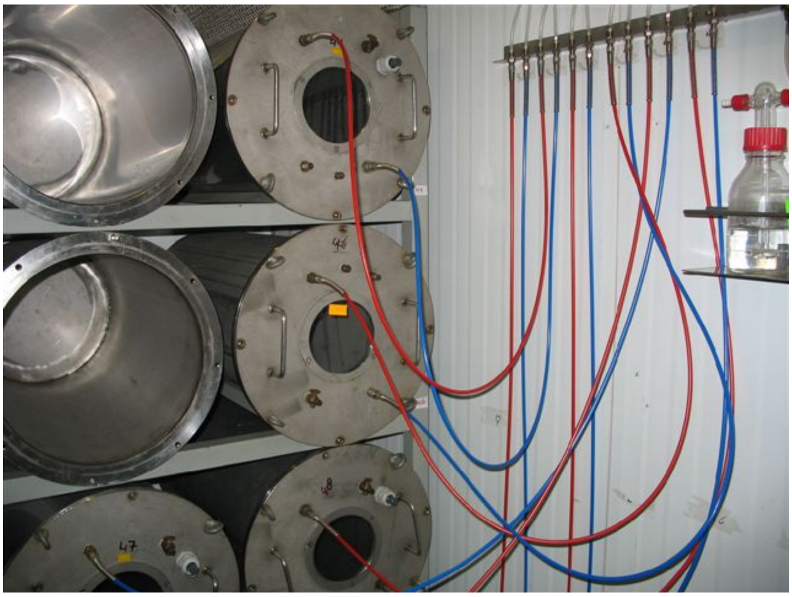

Fig. 1. Experimental CA-equipment used for eliminating tarsonemids (*Phytonemus pallidus*) or the plant parasitic nematodes *P. penetrans* and *M. hapla* in strawberry runners.

Fig. 2. CATT of strawberry mother planting stock on a commercial scale at Ruvoma BV. CA-cell directly after treatment, March 2009.

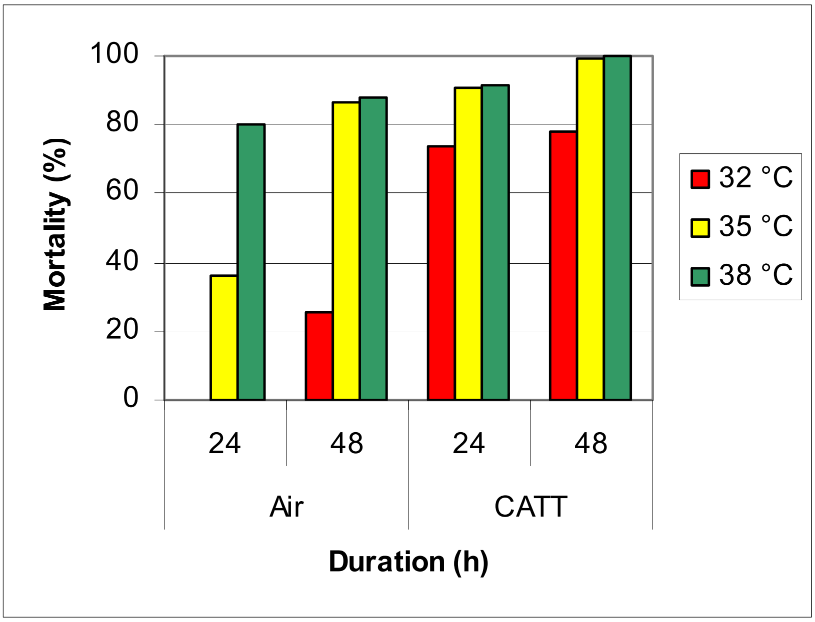

Fig. 3. Mortality (%) of tarsonemids in strawberry runners after treatment in air or by CATT (1% O_2 and 50% CO_2) during 24 or 48 h at 32, 35 or 38°C in March 2007. Replacing 0.0 by 0.1 and 100 by 99.9 analysis of variance on logit (mortality %) including only main effects, showed Air to have lower mortality than CA ($P<0.05$) and T 38 has a higher mortality (%) than T 32 ($P<0.05$).

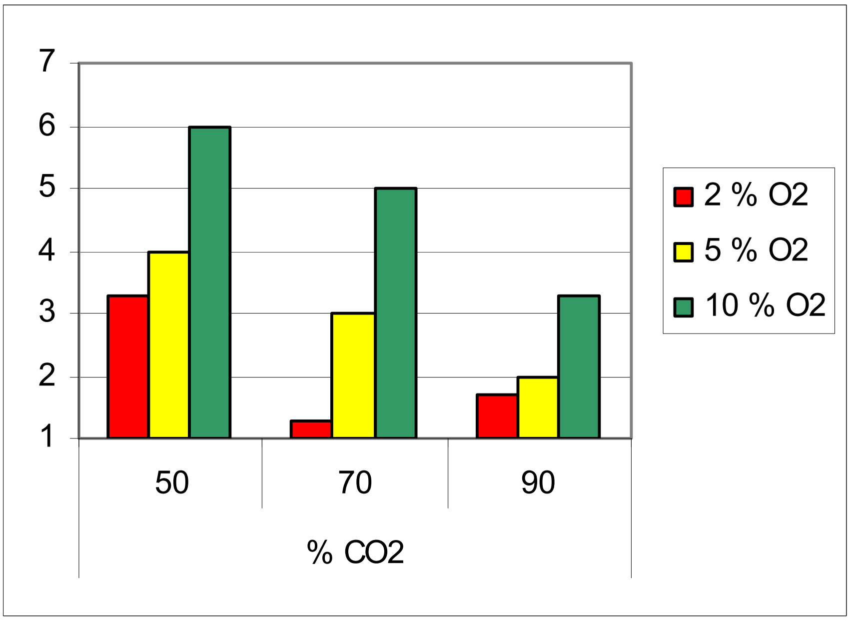

Fig. 4. Plant establishment (1 = plants died; 6 = sufficient vigor; 10 = excellent growth and development) after CA-treatment of strawberry runners at different O_2 and CO_2 combinations during 48 h at 35°C in April 2007. Mortality (%) of tarsonemids in all treatments >99%. Plant establishment in non-treated control: 7.7. Analysis of variance including only main effects, showed plant establishment to be higher for 10% O_2 than for 2 and 5% O_2 ($P<0.05$) and 50% CO_2 to have a higher plant establishment than 70 and 90% CO_2 ($P<0.05$).

Fig. 5. Plant establishment 1 week after potting of 6 origins (colour codes) after CA-treatment of strawberry runners at different O_2 and CO_2 combinations during 48 h at 35°C. Lelystad, April 2007.

Fig. 6. Plant establishment during the field period of runners of 6 origins after different treatments, August 2007.