

Phytoprotection



Effects of controlled atmosphere temperature treatment (CATT) for *Phytonemus pallidus* management on plant survival, yield and growth of strawberry transplants

Effets du traitement de température en atmosphère contrôlée (CATT) pour gérer *Phytonemus pallidus* sur la survie, le rendement et la croissance des plants de fraises transplantés

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[See table of contents](#)

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Article abstract

Cyclamen mite (*Phytonemus pallidus* Banks) can be eliminated from strawberry (*Fragaria × ananassa* Duchesne) planting material when transplants are subjected to a controlled atmosphere temperature treatment (CATT) at 35 °C, 50% CO₂ and 10% O₂ under high relative humidity for 48 h. In our previous research, CATT reduced cyclamen mite numbers by 99.9%, but its effects on plant vigour were not assessed. The objective of this study was to assess the survival, yield and growth of different strawberry cultivars ('Albion', 'Murano', 'Seascape') and plant types (bareroot plants, trayplants) that received CATT before planting compared to untreated control plants. CATT slightly increased the risks of plant mortality, and plant losses were greater for trayplants than for bareroot plants. CATT resulted in increased runner production for 'Albion' transplants but caused no effects on other cultivars. Fruit yields were either unaffected or decreased by CATT, suggesting stimulation of vegetative rather than reproductive growth. Based on these results, disinfection of strawberry transplants using CATT would be more suitable for plants destined for propagation rather than for fruit production. Our research highlights the need to develop a universal and easily adoptable treatment effective against multiple pests and pathogens to produce clean strawberry transplants.

Effects of controlled atmosphere temperature treatment (CATT) for *Phytonemus pallidus* management on plant survival, yield and growth of strawberry transplants

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Cyclamen mite (*Phytonemus pallidus* Banks) can be eliminated from strawberry (*Fragaria* × *ananassa* Duchesne) planting material when transplants are subjected to a controlled atmosphere temperature treatment (CATT) at 35 °C, 50% CO₂ and 10% O₂ under high relative humidity for 48 h. In our previous research, CATT reduced cyclamen mite numbers by 99.9%, but its effects on plant vigour were not assessed. The objective of this study was to assess the survival, yield and growth of different strawberry cultivars ('Albion', 'Murano', 'Seascape') and plant types (bareroot plants, trayplants) that received CATT before planting compared to untreated control plants. CATT slightly increased the risks of plant mortality, and plant losses were greater for trayplants than for bareroot plants. CATT resulted in increased runner production for 'Albion' transplants but caused no effects on other cultivars. Fruit yields were either unaffected or decreased by CATT, suggesting stimulation of vegetative rather than reproductive growth. Based on these results, disinfection of strawberry transplants using CATT would be more suitable for plants destined for propagation rather than for fruit production. Our research highlights the need to develop a universal and easily adoptable treatment effective against multiple pests and pathogens to produce clean strawberry transplants.

Keywords: *Fragaria* × *ananassa*, pest management, *Phytonemus pallidus*, runner production.

[Effets du traitement de température en atmosphère contrôlée (CATT) pour gérer *Phytonemus pallidus* sur la survie, le rendement et la croissance des plants de fraises transplantés]

Le tarsonème du fraisier (*Phytonemus pallidus* Banks) peut être éliminé des transplants de fraisiers (*Fragaria* × *ananassa* Duchesne) soumis à un traitement de température en atmosphère contrôlée (CATT) à 35 °C, 50 % de CO₂ et 10 % d'O₂ sous une humidité relative élevée pendant 48 h. Dans notre recherche précédente, le traitement CATT a réduit le nombre de tarsonèmes du fraisier de 99,9 %, mais ses effets sur les plants n'ont pas été évalués. L'objectif de cette étude était d'évaluer l'effet du traitement CATT sur la survie, le rendement et la croissance de différents cultivars ('Albion', 'Murano', 'Seascape') et types de plants (plants à racines nues, trayplants). La mortalité après la plantation était plus grande pour les plants traités. Le traitement CATT a entraîné une augmentation de la production de stolons pour les transplants du cultivar 'Albion', mais n'a eu aucun effet sur les autres cultivars. Les rendements en fruits n'ont pas été affectés ou ont été diminués par le traitement CATT, ce qui suggère qu'il pourrait stimuler la croissance végétative aux dépens de la croissance reproductive. La désinfection des transplants de fraisiers à l'aide du traitement CATT conviendrait donc mieux aux plantes destinées à la multiplication plutôt qu'à la production de fruits.

Mots-clés : *Fragaria* × *ananassa*, lutte antiparasitaire, *Phytonemus pallidus*, production de stolons.

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INTRODUCTION

Cyclamen mite (*Phytonemus pallidus* Banks; Acari: Tarsonemidae) is a significant pest of strawberry (*Fragaria × ananassa* Duchesne; Rosales: Rosaceae) that is difficult to control once it becomes established in fields. In temperate climates, there are several overlapping generations of cyclamen mite from May to November after which adult females overwinter in the crown of strawberry plants (Jeppson *et al.* 1975; Patenaude *et al.* 2020). *Phytonemus pallidus* feeds on the upper surface of young leaves and flower buds, causing abnormal plant growth (Zhang 2003). Damage such as wrinkled leaves, leathery fruits and stunted appearance occurs on severely infested plants (Fountain and Cross 2018). Stenseth and Nordby (1976) estimated that 35 cyclamen mites per leaflet reduced yields by 22% on 'Senga Sengana'. Passive dispersal by wind, farm equipment and field workers is possible, but cyclamen mite mainly spreads to nearby plants on runner tips growing from infested crowns (Alford 2007). Therefore, the occasional presence of cyclamen mite on strawberry transplants may increase the risk of population outbreaks in fruit-producing fields. As cyclamen mite inhabits the folds of young leaves, it is unreachable by most natural predators (Easterbrook *et al.* 2001; Fitzgerald *et al.* 2008; Tuovinen and Lindqvist 2010) and unaffected by miticides with a contact mode-of-action (Fountain *et al.* 2010). Establishing new strawberry fields with clean planting material is the most effective way to prevent cyclamen mite infestations (Gobin and Bangels 2008; Tuovinen and Lindqvist 2010).

Applying heat treatments to strawberry transplants is an efficient approach to eliminate cyclamen mite before planting. One method to produce mite-free plants is to submerge strawberry transplants in hot water (Davis and Blair 1951; Smith and Goldsmith 1936). To eliminate foliar nematodes (*Aphelenchoides fragariae* Ritzema-Bos), stem nematodes (*Ditylenchus dipsaci* Kühn) and, concurrently, cyclamen mites, it has been advised to treat strawberry runners in hot water at 46.1 °C for 10 min (MacLachlan and Duggan 1979; Staniland 1953). More recently, Hellqvist (2002) found that all adult cyclamen mites on infested runners were killed 6.5 min after being immersed in water at 46 °C. However, hot-water treatments can delay plant growth by two to three weeks (Buchner 1991) and may cause cross-contamination of pathogens such as *Xanthomonas fragariae* (Turechek and Peres 2009; Turechek *et al.* 2013). Cyclamen mite can also be eliminated from strawberry mother-plants (cultivars 'Darselect', 'Elsanta', 'Figaro', 'Korona', 'Sonata', 'Symphony', 'Honeoye', 'Lambada' and 'Evi II') with a 99.8% efficacy by applying a controlled atmosphere temperature treatment (CATT) during which transplants are subjected to 35 °C, 50% CO₂, 10% O₂ and high relative humidity for 48 h (van Kruistum *et al.* 2012, 2014; Verschoor *et al.* 2015).

Plant disinfection protocols are developed to eliminate pests and pathogens, but plant susceptibility to abiotic stressors often limits the success of CATT and other heat treatments (Mitcham *et al.* 2006). Resistance to heat, low O₂ or high CO₂ levels can differ considerably between plant species and even between cultivars (Neven and Johnson 2017). Severe damage occurred on cucumber, eggplant, mustard and green pepper plants at CO₂ concentrations above 50%, but only slight leaf discolouration occurred on strawberries at a CO₂ concentration of 67% (Gong *et al.* 2018). More significant yield reductions were reported for the cultivar 'Sonata' than for 'Honeoye' when strawberry plants were submerged in hot water at > 46 °C for > 20 min

(Daugaard and Lindhard 2007). Flowering and bud break were reduced for strawberry cultivars 'Ventana', 'Camino Real', 'Diamante', 'Oso Grande' and 'Camarosa' after plants were bagged dry or wet and submerged in hot water at 44 °C or 48 °C for 60 to 240 minutes, but 'Strawberry Festival' was remarkably less affected (Turechek and Peres 2009). Strawberry plants established and grew normally in the field after CATT, but CATT was only tested on mother-plants and not fruit-producing plants (van Kruistum *et al.* 2012, 2014; Verschoor *et al.* 2015).

In previous work, we tested the CATT method developed by Dutch research teams (van Kruistum *et al.* 2012, 2014; Verschoor *et al.* 2015) on a smaller scale using the same parameters effective against cyclamen mite (35 °C, 50% CO₂, 10% O₂ and high relative humidity for 48 h) (Bernier *et al.* 2023). After four weeks, infested strawberry transplants that received CATT before planting were destructively sampled for cyclamen mites. CATT reduced adults and larvae numbers by 99.9% in two greenhouse experiments compared to untreated control plants (Bernier *et al.* 2023). These results suggested that CATT could be replicated with the same efficacy, but the treatment's effects on strawberry plants were not assessed.

The objective of this study was to determine the effects of CATT on the survival, yield and growth of bareroot and trayplant strawberry transplants of cultivars commonly grown in Quebec. CATT was tested on strawberry mother-plants and fruiting-plants of day-neutral cultivars to determine whether it can be successfully applied to transplants intended for propagation and those intended for fruit production. We anticipate that the results of this study will complement those obtained with CATT against cyclamen mite in our previous studies and provide the necessary information on the suitability of using CATT as part of an integrated management strategy for cyclamen mite.

MATERIALS AND METHODS

Treatment description

From May to October 2021, the growth of strawberry mother-plants (propagation) and fruiting-plants (fruit production) was measured on two commercial farms in southern Quebec (Trois-Rivières [46.3, -72.6] and Île d'Orléans [46.9, -70.9]). Transplants that received CATT (35 °C, RH > 90%, 50% CO₂ and 10% O₂ for 48 h) before planting were compared to control plants that were not treated. All transplants were transferred from a freezer to a cooler (4 °C) for thawing for three days before CATT or planting (control plants). Two identical CATT units built for a previous research work at Université Laval were used simultaneously to treat transplants (Bernier *et al.* 2023). These units were made from airtight plastic-pallet boxes with all the required instruments to attain and control CATT parameters (35 °C, RH > 90%, 50% CO₂, 10% O₂). Before starting a CATT treatment, strawberry plants were taken out of the cooler and spread out on five stacked plastic bakery trays that would fit inside one CATT unit. Experimental units (plots) were planted on five consecutive days, with each planting date corresponding to a treatment replicate. Plot size varied according to the site and the cropping system (Table 1).

Table 1. Summary of experiments with mother-plants and fruiting-plants carried out in Trois-Rivières and Île d'Orléans (QC, Canada). Each experiment compared plants that were untreated or subjected to a controlled atmosphere temperature treatment (RH > 90%, 50% CO₂, 10% O₂, and 35°C for 48 h).

Systems	Locations	Cultivars	Plant types	Pre-plant treatment	No. of plots	No. plants per plot
Mother-plants	Trois-Rivières	Clery, Seascape	Bareroot	CATT	10	30
				Control	10	30
	Île d'Orléans	Albion	Trayplant	CATT	5	84
				Control	5	84
		Yambu	Trayplant	CATT	5	84
				Control	5	84
Fruiting-plants	Trois-Rivières	Albion, Murano, Seascape	Bareroot, trayplant	CATT	30	24
				Control	30	24
	Île d'Orléans	Albion	Bareroot	CATT	5	150
				Control	5	150
		Seascape	Bareroot	CATT	5	150
				Control	5	150

Strawberry mother-plants

At the Trois-Rivières site, dormant bareroot transplants were planted May 10-14 2021 in two staggered rows on raised beds (height: 18 cm) covered with black polyethylene mulch (Figure 1A). In-row plant spacing was 25 cm and bed width was 76 cm. Straw mulch was placed in the alleys (width: 62 cm) and plants were fertigated through drip tape according to standard management practices. Plots were arranged in a complete randomized factorial design with two pre-planting treatments (CATT and control) and two cultivars ('Clery' and 'Seascape'). All plots were in one raised bed (total length of 95 m) with a buffer of 1 m between plots. Flowers were removed approximately once a week to promote runner production and prevent fruit production for the duration of the growing season.

At the Île d'Orléans site, dormant trayplants grown from runner tips collected on the same farm the previous year were planted from May 3-7 2021 in a tabletop growing system under high tunnel (35 × 4.6 × 3.5 m) at a density of 12 plants per linear meter (Figure 1B). There were three rows of rectangular pots (100 × 17 × 16.3 cm; Model 6MEBK; Bato Plastics BV, Zevenbergen, NL) filled with coconut fiber (Dutch Plantin BV, Boekel, NL) under one high tunnel, with 2 m between rows. Plants were watered and fertilized with drip irrigation several times a day, with a schedule and rates decided by the grower. Mother-plants of two cultivars ('Albion' and 'Yambu') were grown separately in two high tunnels. For each cultivar, experimental plots of 84 trayplants were randomly assigned on the tabletops, and there was an empty buffer zone of 1 m between plots. Each week, flowers were cut and runners crawling on the pots were repositioned to face the alleys (Figure 1B).

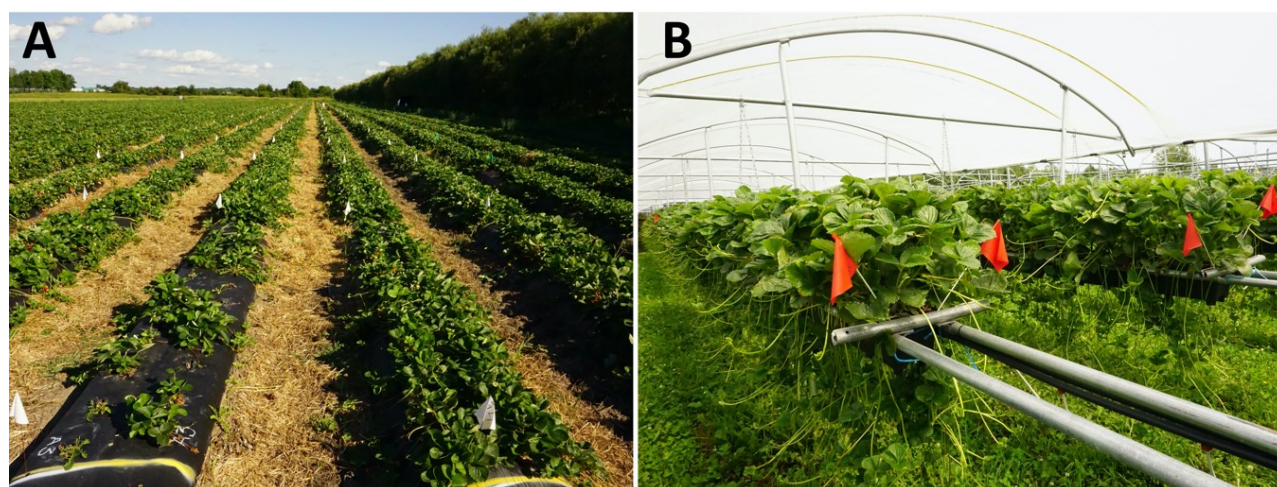


Figure 1. (A): The experimental plots with strawberries fruiting-plants grown on raised beds at the Trois-Rivières site (July 28). **(B):** The tabletop system used to grow strawberry mother-plants at the Île d'Orléans site (July 13). Photographs by Virginie Bernier.

At both sites, the numbers of dead mother-plants were recorded in late June and the numbers of daughter plants produced per experimental plot were counted in late July - early August. In Trois-Rivières, runners were harvested on July 29, and runner tips with sufficient visible peg roots were counted as viable daughter plants, as described by Durner *et al.* (2002). At Île d'Orléans, runners from 'Yambu' mother-plants were collected on July 19 and 27 and August 13, and data was combined to determine the average production of daughter plants per mother-plant. On July 20, runner tips cut the previous day were placed in a mix of coconut fiber (Dutch Plantin BV, Boekel, NL) and blond sphagnum peat moss (Pro-Moss Fine, Premier Tech, Rivière-du-Loup, QC, Canada) to grow plug plants (50 mL of substrate per cell), with plants from each plot in separate plug trays. Plug plants were grown under a shade house where they were watered with an overhead sprinkler irrigation system several times a day. Management of the nursery area (irrigation, fertilization, pest management, etc.) followed a schedule and rates decided by the grower. The numbers of leaves were counted three weeks after planting from 10 randomly sampled plug plants per experimental plot. Crown size was estimated by measuring the long and short axes across the crown surface with a digital caliper, using the values to calculate the ellipse perimeter. Similar to the methods for 'Yambu' plants, runners from 'Albion' mother-plants were collected on July 22 and August 6, and trayplants (250 mL of substrate per cell) were produced from the daughter plants collected on July 22. Growth was measured on October 14 before trayplants were placed in a freezer for winter storage. The numbers of leaves and branch crowns were counted, and the crown size was calculated from the measurements of 15 randomly selected trayplants per experimental plot.

Fruiting-plants

At the Trois-Rivières site, dormant bareroot transplants (obtained by the grower from a commercial nursery) and dormant trayplants (produced on the same farm the previous year from runner tips) were planted from May 10-14 2021 in the same field described for the mother-plant experiment. The plots were arranged in a randomized block factorial design, with two pre-planting treatments (CATT and control), two plant types (bareroot and trayplant), three cultivars ('Albion', 'Murano' and 'Seascape') and five replicates. Each block was a raised bed planted with all factor combinations on the same day. Flowers located on plot edges were cut so that the yield data was averaged for 20 instead of 24 plants.

At the Île d'Orléans site, the experiment was similar to that at the Trois-Rivières site. Dormant bareroot transplants ('Albion' and 'Seascape', obtained by the grower from a commercial nursery) were planted separately May 3-7 2021 in two sections of the same field. In each field section, a randomized block design was used in which the CATT and control replicates planted on the same date were randomly assigned within one of the five blocks.

The same yield and growth parameters were evaluated at both sites throughout the growing season. At both sites, the numbers of dead fruiting-plants were recorded in late

June. At the Trois-Rivières site, strawberries were harvested every two or three days from mid-June to early September. Total yield and average fruit weight were determined for each plot. At the end of the season (September 7), crown size, number of branch crowns and aboveground dry biomass were measured on three randomly selected plants per experimental plot. Fruit and runners were removed before plants were dried individually in paper bags at 65 °C for 84 h. Strawberries were harvested at the Île d'Orléans site from late June to late September. Crown size, number of branch crowns and aboveground dry biomass were measured on September 24-25.

Data analyses

The effect of CATT on the growth of strawberry mother-plants and fruiting-plants at both sites was determined using analyses of variance (ANOVA) with the lme function (nlme package [Pinheiro *et al.* 2023], R Software [R Core Team 2021]). Separate models were used for each plant system (mother-plants, fruiting-plants), and for each site (Trois-Rivières, Île d'Orléans). The planting date was considered as a random effect in all models. For experiments at the Trois-Rivières site, CATT, cultivar and plant type for the fruiting-plants experiment were the fixed effects. For experiments at the Île d'Orléans site, separate ANOVAs were used for each cultivar, and CATT was the only fixed effect.

Plant mortality rates (number of dead plants out of the total number of plants per plot) were arcsine transformed prior to analysis, as recommended for percentage values (Sokal and Rohlf 1994). For the mother-plants experiment at the Trois-Rivières site, an ANOVA for mortality rate was not possible due to the high proportions of zeros in the dataset. The same issue occurred for the fruiting-plants experiment at the Trois-Rivières site, but the analysis was performed with a binomial model using the function glmer (package lme4). For all the other parameters, analyses using the normal distribution and untransformed data were prioritized unless a square root or a log transformation of the response variables was required to meet the normality assumptions as suggested by the Box-Cox method (Box and Cox 1964). For the number of branch crowns of 'Albion' daughter plants and the number of leaves per 'Yambu' daughter plants at the Île d'Orléans site, a Poisson distribution regression model (function glmer, package lme4) was used since data were limited to a few discrete values. Following ANOVA, Tukey's HSD (Honestly Significant Differences) tests (function emmeans, package emmeans [Lenth 2023]) were performed to identify differences among factor means. All data analyses were performed in R 4.1.2 (R Core Team 2021) at the 0.05 level of significance.

RESULTS

Strawberry mother-plants

At the Trois-Rivières site, all strawberry bareroot plants survived in 17 of the 20 experimental plots and mortality was negligible (<10%) in the three remaining plots. 'Clery' mother-plants produced significantly more daughter plants than 'Seascape' mother-plants ($F = 221.2$; $df = 1, 12$; $P < 0.001$)

(Figure 2A). CATT did not affect the number of daughter plants ($F = 0.3$; $df = 1, 12$; $P = 0.579$), but the interaction between CATT and cultivar was significant ($F = 4.8$; $df = 1, 12$; $P = 0.049$). However, no difference between CATT and control mother-plants was detected by the Tukey's multiple comparison method (Figure 2A).

At the Île d'Orléans site, CATT had a significant effect on mortality of 'Yambu' ($F = 17.8$; $df = 1, 4$; $P = 0.014$) but not 'Albion' ($F = 0.2$; $df = 1, 3$; $P < 0.670$) mother-plants (Table 2). One CATT replicate was removed from the ANOVA for

'Albion' mother-plants due to a high mortality rate following pre-plant treatment (77.4%). The numbers of leaves ('Albion': $F = 0.0$; $df = 1, 4$; $P < 0.853$ / 'Yambu': $F = 0.4$; $df = 1, 4$; $P = 0.577$), crown size ('Albion': $F = 1.1$; $df = 1, 4$; $P = 0.363$ / 'Yambu': $F = 0.5$; $df = 1, 4$; $P = 0.513$) and branch crowns ('Albion': $F = 0.0$; $df = 1, 4$; $P = 0.981$) of daughter plants were not affected by CATT for both cultivars (Table 2). CATT caused an increase in the number of daughter plants for 'Albion' ($F = 85.2$; $df = 1, 4$; $P < 0.001$), but not for 'Yambu' ($F = 5.6$; $df = 1, 4$; $P = 0.078$) (Figures 2B and 2C).

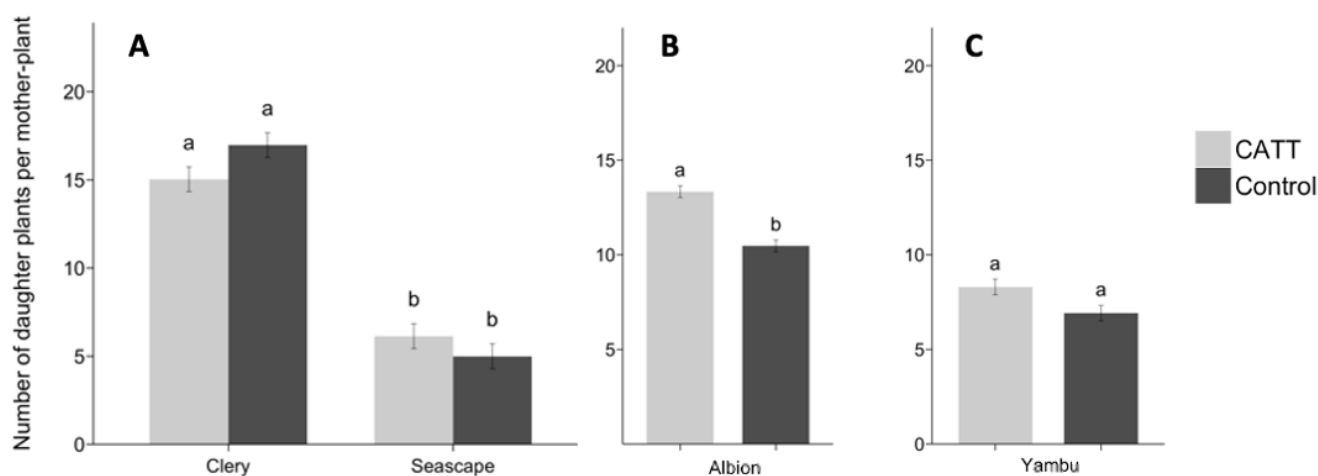


Figure 2. Mean (± SE) number of daughter plants produced by mother-plants that were subjected to controlled atmosphere temperature treatment (CATT) before planting compared to untreated controls. (A): Production of daughter plants at the Trois-Rivières site by bareroot mother-plants of 'Clery' and 'Seascape' cultivars grown in raised beds. Production of daughter plants at the Île d'Orléans site by trayplants of the cultivars 'Albion' (B) and 'Yambu' (C) grown in a table-top system. Means with different letters within each graph are significantly different according to Tukey's HSD at $P < 0.05$.

Table 2. Mortality rate of strawberry mother-plants grown on tabletops at the Île d'Orléans site and quality of their daughter plants

Cultivar	Pre-plant treatment	Mortality rate of mother-plants (%)	Quality of daughter plants		
			Crown size (cm)	Number of leaves	Number of branch crowns
Albion	CATT	0.57 (± 0.56)	4.9 (± 0.2)	8.1 (± 0.3)	1.7 (± 0.1)
	Control	0.94 (± 0.64)	5.1 (± 0.2)	8.1 (± 0.3)	1.7 (± 0.1)
Yambu	CATT	3.60 (± 0.52) b	3.2 (± 0.1)	3.4 (± 0.1)	Not measured
	Control	0.52 (± 0.52) a	3.1 (± 0.1)	3.5 (± 0.1)	

Note. Means (± SE) followed by different letters within a cultivar set are significantly different according to Tukey's HSD (Honestly Significant Differences) at $P < 0.05$.

Statistical models used:

- Mortality rates: mixed model ANOVA with a normal distribution (arcsine transformation)
- Crown size: mixed model ANOVA with a normal distribution (no data transformation)
- Number of leaves (Albion): mixed model ANOVA with a normal distribution
- Number of leaves (Yambu): mixed model ANOVA with a Poisson distribution
- Number of branch crowns (Albion): mixed model ANOVA with a Poisson distribution

Fruiting-plants

At the Trois-Rivières site, CATT had a significant effect ($\chi^2 = 7.7$; $df = 1$; $P = 0.006$) on mortality rates of fruiting strawberry plants (Table 3). There was a $60.0 \pm 16.2\%$ chance that at least one strawberry plant in a plot would not survive after CATT while there was a $13.9 \pm 9.80\%$ chance of mortality in control plots. There was a significant interaction between CATT and cultivars on yield ($F = 5.9$; $df = 2, 44$; $P = 0.006$) (Table 3). CATT reduced strawberry yields for

'Albion' ($P < 0.001$) and 'Seascape' ($P = 0.005$), but not for 'Murano' ($P = 0.819$) (Figure 3). There was also a significant interaction between CATT and plant type on yield ($F = 11.7$; $df = 1, 44$; $P = 0.001$), crown size ($F = 4.8$; $df = 1, 164$; $P = 0.030$) and dry biomass ($F = 4.3$; $df = 1, 164$; $P = 0.039$) (Table 3). CATT reduced yields of trayplants ($P < 0.001$) but not those of bareroot plants ($P = 0.341$) (Figure 4). No significant differences between CATT and control fruiting-plants were detected by the Tukey's multiple comparison method for crown size and dry biomass (Table 4).

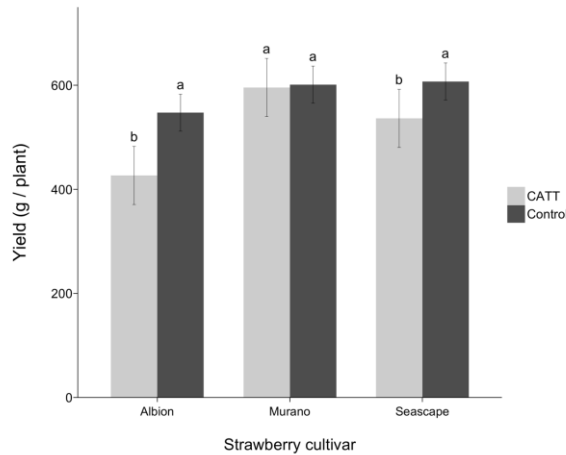


Figure 3. Average (\pm SE) fruit yields of three day-neutral strawberry cultivars ('Albion', 'Murano', 'Seascape') grown at the Trois-Rivières site that were subjected to controlled atmosphere temperature treatment (CATT) before planting compared to controls without CATT. Bars that do not share the same letter within a cultivar set are significantly different at $P < 0.05$ according to Tukey's HSD.

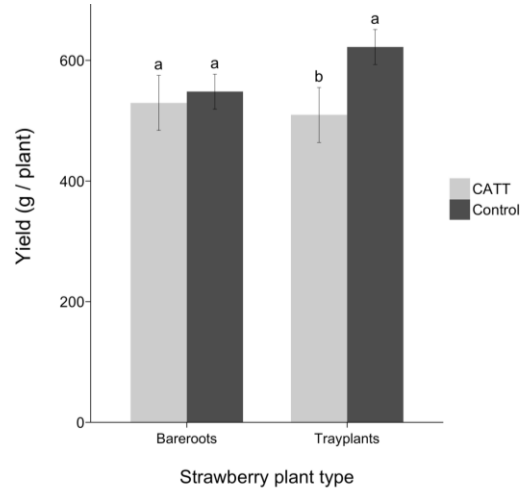


Figure 4. Average (\pm SE) fruit yields of day-neutral strawberry cultivars per plant types grown at the Trois-Rivières site that were subjected to controlled atmosphere temperature treatment (CATT) before planting compared to controls without CATT. Bars that do not share the same letter within a cultivar set are significantly different at $P < 0.05$ according to Tukey's HSD.

Table 3. Analysis of variance results for the effects of pre-plant treatment (CATT, control), cultivar ('Albion', 'Murano', 'Seascape') and plant type (trayplants, bareroots) and their interactions on the growth and yields of fruiting-plants at the Trois-Rivières site

Source of Variation	df	P values					
		Mortality rate	Yields	Average fruit size	Crown size	Branch crowns	Dry biomass
Pre-plant treatment	1	0.006	< 0.001	0.526	0.596	0.568	0.470
Cultivar	2	0.041	< 0.001	< 0.001	0.005	0.005	0.015
Plant type	1	0.273	0.056	0.001	0.875	0.310	0.109
Pre-plant treatment \times Cultivar	2	0.541	0.006	0.192	0.173	0.425	0.067
Pre-plant treatment \times Plant type	1	0.656	0.001	0.597	0.030	0.707	0.039
Cultivar \times Plant type	2	0.645	< 0.001	< 0.001	0.052	0.34	0.028
Pre-plant treatment \times Cultivar \times Plant type	2	NA	0.619	0.709	0.391	0.144	0.878

Note. Statistical models used:

- Mortality rate: binomial model (only possible for simple or double interactions)
- Yields: mixed model ANOVA with a normal distribution
- Average fruit size: mixed model ANOVA with a normal distribution
- Crown size: mixed model ANOVA with a normal distribution (square root transformation)
- Branch crowns: mixed model ANOVA with a normal distribution (square root transformation)
- Dry biomass: mixed model ANOVA with a normal distribution (log transformation)

At the Île d'Orléans site, CATT did not significantly affect mortality rates ('Albion': $F = 0.1$; $df = 1, 4$; $P = 0.814$ / 'Seascape': $F = 0.7$; $df = 1, 4$; $P = 0.436$), yields ('Albion': $F = 1.3$; $df = 1, 4$; $P = 0.320$ / 'Seascape': $F = 4.6$; $df = 1, 3$; $P = 0.122$), average fruit weight ('Albion': $F = 0.4$; $df = 1, 4$; $P = 0.570$ / 'Seascape': $F = 0.8$; $df = 1, 4$; $P = 0.433$), crown size ('Albion': $F = 1.3$; $df = 1, 4$; $P = 0.322$ / 'Seascape': $F = 3.2$; $df = 1, 4$; $P = 0.147$), number of branch crowns ('Albion': $F = 0.5$; $df = 1, 4$; $P = 0.534$ / 'Seascape': $F = 1.1$; $df = 1, 4$; $P = 0.357$) nor dry biomass ('Albion': $F = 0.8$; $df = 1, 4$; $P = 0.428$ / 'Seascape': $F = 0.6$; $df = 1, 4$; $P = 0.472$) of both cultivars (Table 5).

Table 4. Effect of pre-planting treatment's interaction with plant type on fruiting-plants strawberries' crown size and dry biomass at the Trois-Rivières site

Plant type / Pre-plant treatment	Crown size (cm)	Dry biomass (g)
Bareroot / CATT	9.3 (± 0.2)	43.7 (± 2.5)
Bareroot / Control	8.8 (± 0.2)	41.4 (± 1.6)
Trayplants / CATT	8.9 (± 0.2)	37.6 (± 2.0)
Trayplants / Control	9.2 (± 0.2)	42.1 (± 2.0)

Note. Means (\pm SE) followed by different letters within a cultivar set are significantly different according to Tukey's HSD at $P < 0.05$.

Statistical models used:

- Crown size: mixed model ANOVA with a normal distribution (square root transformation)
- Dry biomass: mixed model ANOVA with a normal distribution (log transformation)

Table 5. Mortality rate, yields, average fruit weight, crown size, number of branch crowns and dry biomass of 'Albion' and 'Seascape' cultivars grown at the Île d'Orléans site

Cultivar / Pre-plant treatment	Mortality rate (%)	Yields (g/plant)	Average fruit weight (g/fruit)	Crown size (cm)	Branch crowns	Dry biomass (g)
Albion / CATT	0.9 (± 0.5)	346 (± 21)	15.9 (± 0.2)	6.9 (± 0.3)	2.9 (± 0.2)	29 (± 2)
Albion / Control	0.8 (± 0.5)	362 (± 21)	15.8 (± 0.2)	6.5 (± 0.3)	3.0 (± 0.2)	27 (± 2)
Seascape / CATT	3.2 (± 1.7)	272 (± 5)	12.2 (± 0.1)	8.0 (± 0.3)	4.4 (± 0.4)	24 (± 3)
Seascape / Control	1.5 (± 1.2)	284 (± 5)	12.1 (± 0.1)	7.2 (± 0.3)	3.8 (± 0.4)	27 (± 3)

Note. Means (\pm SE) followed by different letters within a cultivar set are significantly different according to Tukey's HSD at $P < 0.05$.

Statistical models used:

- Mortality rate: mixed model ANOVA with a normal distribution (arcsine transformation)
- Yields: mixed model ANOVA with a normal distribution
- Average fruit weight: mixed model ANOVA with a normal distribution
- Crown size (Albion): mixed model ANOVA with a normal distribution (log transformation)
- Crown size (Seascape): mixed model ANOVA with a normal distribution (square root transformation)
- Branch crowns (Albion): mixed model ANOVA with a normal distribution (log transformation)
- Branch crowns (Seascape): mixed model ANOVA with a normal distribution (square root transformation)
- Dry biomass (Albion): mixed model ANOVA with a normal distribution (log transformation)
- Dry biomass (Seascape): mixed model ANOVA with a normal distribution (square root transformation)

DISCUSSION

At both sites, CATT increased the risk of strawberry mortality after planting. For mother-plants at the Île d'Orléans site, a mortality rate of 3.6% for the cultivar 'Yambu' subjected to CATT was not excessive ($< 10\%$) but still undesirable on a commercial farm given the higher production costs of trayplants compared to bareroot plants (Gambardella *et al.* 2016). Moreover, nearly 100% survival after transplanting process is expected for trayplants since they maintain more root hairs than bareroot plants that rapidly absorb water and nutrients (Durner *et al.* 2002). All trayplants were from the

same origin, but uneven conditions during cold storage reduced the initial vigour of trayplants in some boxes. Nevertheless, our findings highlight the fact that CATT could have detrimental effects when applied to weaker plants. The quality of plant material has been reported as an important factor affecting plant survival after hot-water submersion treatment (Hellqvist 2002; MacLachlan and Duggan 1979). Similarly, mortality rates were higher for CATT plants compared with control plants in the experiment with fruiting-plants at the Trois-Rivières site. However, expected mortality rates cannot be quantified due to the binomial model used. Application of CATT at 50% CO₂, 10% O₂ and 35 °C under high relative humidity for 48 h provided nearly

complete control of cyclamen mite without causing harmful, irreversible consequences on plant vitality (van Kruistum *et al.* 2012). Although the difference from control plants was not significant, van Kruistum *et al.* (2014) reported between 4.3 to 10.0% plant loss after treating mother-plants of different cultivars, origins and plant types with CATT. Mortality rates of strawberry plants recorded in this study can thus be considered as acceptable since they were less than the plant loss rate observed in the Netherlands.

Results obtained on both farms suggested that the type of strawberry transplants exposed to CATT influenced survival and growth during the season. Mortality was significantly higher for trayplants of the cultivar 'Yambu' after CATT than for the control trayplants at the Île d'Orléans site. At the Trois-Rivières site, yields were reduced by 18% for fruiting trayplants treated with CATT compared to control trayplants. On the other hand, plant survival and yields of bareroot plants were similar between CATT and control plants. As strawberry trayplants are usually cold-stored with their new roots in peat substrate and with their younger leaves (Gambardella *et al.* 2016), their lower tolerance than bareroot plants to CATT might be explained by damage caused to their roots and foliage during the treatment. Trayplants are used because they grow faster than bareroot plants after planting, provided that their root system remains healthy during cold storage and transplanting (Giménez *et al.* 2009). In the days after transplanting, leaves on some CATT trayplants at both sites became brown and died before new shoots emerged from the crown. If the plant is unable to restore its leaf surface area rapidly enough, defoliation of the strawberry transplant may lead to reduced plant growth and fewer crowns (Albregts *et al.* 1992; Kerkhoff *et al.* 1988). Given the greater susceptibility of trayplants than bareroot plants to negative effects of CATT and the larger space requirement for trayplants during treatment, future research with CATT should focus on bareroot plants.

Runner production was the same for CATT and control mother-plants at the Trois-Rivières site and for the cultivar 'Yambu' at the Île d'Orléans site, but CATT mother-plants of the cultivar 'Albion' at the Île d'Orléans site produced significantly more daughter plants. The regulation of vegetative and reproductive growth in strawberries is influenced by photoperiod and temperature (Serçe and Hancock 2005), and runner production occurs during days with extended daylight and with higher temperatures (Durner *et al.* 1984). CATT and control 'Albion' mother-plants were exposed to the same photoperiod and temperature from planting in May until runner harvest in July-August, suggesting that the stress induced by the 48 h of CATT treatment may have stimulated runner production for this cultivar. Heat tolerance of strawberry plants can be improved by gradual exposure to higher temperatures (Gulen *et al.* 2016). There are positive effects of heat stress including accumulation of heat-stable proteins (Gulen and Eris 2004), increased chlorophyll content (Kesici *et al.* 2013) and greater abundance of heat-shock proteins (Brown *et al.* 2016). As strawberry trayplants were exposed to 35 °C for 48 h, a possibility is that CATT treatment enhanced their thermotolerance which promoted better performance than control plants during summer heat events. The objective of CATT is to control cyclamen mite by

producing mite-free plants, and increased runner production would be a positive secondary effect. Fortunately, production of more runners for 'Albion' using CATT did not affect the quality of daughter plants in the nursery at the Île d'Orléans site. Since CATT and control daughter plants were grown under the same conditions (substrate, irrigation, nutrient supply, cell size, etc.), any major differences would likely have been due to the treatment, and no such differences were observed.

Yield reductions of 21.9% and 11.6% for 'Albion' and 'Seascape' fruiting-plants at the Trois-Rivières site suggested that CATT treatment negatively impacted flowering. Adverse effects on reproductive growth after the application of heat treatments have been reported in other studies. During initial experiments in the Netherlands, applying CATT at a higher temperature (38 °C) and a lower O₂ concentration (1%) inhibited flowering of some strawberry cultivars (van Kruistum *et al.* 2012). Qiu *et al.* (1993) mentioned that flowering was more likely to be affected than plant survival after hot-water treatments, implying that shorter exposure times and lower temperatures should be applied to treat strawberry plants intended for fruit production. Flowering and bud break were reduced for most cultivars after strawberry plants were bagged dry or wet and immersed in hot water at 44 °C or 48 °C for 60, 120, 180 or 240 minutes (Turechek and Peres 2009). Daugaard and Lindhard (2007) reported that, for the cultivars 'Sonata' and 'Honeoye', marketable yields were significantly reduced when plants were treated in hot water at temperatures above 44 °C for 10 minutes or longer. Some cultivars are less sensitive to heat treatments (Qiu *et al.* 1993; Turechek and Peres 2009), as was the case for 'Murano' at the Trois-Rivières site. Yield did not decline for bareroot fruiting-plants at the Île d'Orléans site. At the Trois-Rivières site, yield declines were observed when data were combined per cultivar for bareroot plants and trayplants (Figure 3). As discussed above, trayplants were more sensitive to CATT so they may have influenced downward yield calculations. Due to the inconsistent results obtained during fruiting-plant experiments at both sites, CATT effect on flowering and fruit production in fruiting-plants warrants further investigation.

Our experiments with strawberry mother-plants and fruiting-plants indicated that disinfection of planting material using CATT can affect growth to varying degrees depending on plant type and cultivars. Overall, CATT stimulated vegetative growth but not reproductive growth. Since the axillary meristems of strawberry plants can differentiate into either a branch crown capable of bearing inflorescences or a runner, an increase in flowering often corresponds to a reduction of runner production and vice versa (Hytönen and Kurokura 2020). While runner production was 25% higher for 'Albion' mother-plants treated with CATT at the Île d'Orléans site, fruit production was reduced for two cultivars out of three for fruiting-plants grown at the Trois-Rivières site. Similarly, Turechek and Peres (2009) observed a net increase in runner production ('Camarosa' and 'Diamante') in their first greenhouse trial after strawberry plants were treated at 44 °C or 48 °C in hot water, and flowering was adversely affected. Application of CATT may be more appropriate for plants used for propagation than fruit production, as is the current practice in the Netherlands.

The potential of CATT to reduce the incidence of cyclamen mite infestations in the field would only be substantial if a large number of transplants is treated. CATT application at a larger scale would certainly be challenging in terms of technology, investment costs and logistics. For instance, the duration of disinfection treatment (48 h) could be a major limitation knowing that thousands of transplants need to be treated at once in a commercial setting, thus requiring large CATT facilities. However, there is a need for alternative control methods for cyclamen mite, and producing mite-free plants is a promising approach since the pest can be easily spread on nursery stock. Disinfection treatments using heat have proven efficacy against arthropods and pathogens transported on planting material, but they usually increase the risks of adverse effects on plant growth and yield. Therefore, research should focus on developing a universal treatment to produce clean strawberry transplants. Growers will be more likely to adopt such a treatment if several pest and disease management benefits can be expected.

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