

# Steam thermotherapy for strawberry disease management: long-term effects, planting timing, and heat-tolerance mechanisms

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## Summary

Several of the strawberry pathogens that affect production in Florida are introduced from nursery transplants. Our research has demonstrated that *Colletotrichum acutatum*, *Botrytis cinerea*, *Xanthomonas fragariae*, *Phytophthora* spp., and the emerging pathogen, *Neopestalotiopsis* spp., are often harbored on quiescently infected transplants. In this project, we determined the long-term effect of thermotherapy on strawberry transplants under nursery production conditions. Results from five nursery/cultivar combinations showed a cultivar/nursery-dependent effect, which seemed to be related to the initial transplant quality. Transplants treated at the nursery and planted at the UF-GCREC field had less than 5% mortality across various nursery sources and cultivars. We further tested the optimum planting time after thermotherapy and found that, regardless of the cultivar, plant mortality remained low and indistinguishable from the non-treated control, suggesting storage for up to 7 days in a cooler (4°C) after treatment does not affect the quality of transplants. Aerial images were obtained from commercial fields that received transplants that were treated and non-treated in nurseries, but analysis to calculate plant mortality is still being processed. The experiment to optimize the pre-heat/cool-down step is still ongoing due to COVID-19 closures.

## Thermotherapy

Our standard thermotherapy protocol includes a pre-heat treatment at 98.6°F (37°C) for 1 hour, a cool-down step at room temperature for 1 hour, and a

thermotherapy step at 111.2°F (44°C) for 4 hours. The plant sauna devices used for thermotherapy at the UF-GCREC in Florida and nurseries in North Carolina and Canada are shown in Fig. 1.



**Figure 1.** Plant sauna devices used for thermotherapy at the UF-GCREC (upper panels) and in nurseries in North Carolina and Canada (lower panels).

## Methods

### Long-term effects of thermotherapy on strawberry transplants under nursery production conditions

Planting stock of several strawberry cultivars was heat-treated on-site at two nurseries in North Carolina and one nursery in Canada using our standard thermotherapy protocol. Treated plants were stored in a cooler overnight and then planted, or were planted soon after treatment. Irrigation and

fertilization were implemented according to growers' standard practices. Plant mortality was evaluated approximately one month after planting and immediately before the harvest of strawberry transplants. Thereafter, treated and control plants were delivered and planted at the UF-GCREC. Plant mortality was evaluated weekly until mid-January. Some transplants were planted in commercial fruit production fields in Florida. Plant mortality in commercial fields was evaluated by analyzing drone images.

### Optimum planting time after thermotherapy

Transplants of 'Florida Radiance', 'Florida127', 'Florida Beauty', and 'Florida Brilliance' were heat-treated at the UF-GCREC with the standard thermotherapy protocol and stored in a cooler at 4°C. Treated and control plants were planted at 2-day intervals, beginning from the overnight storage for up to 7 days after storage. Plant mortality was evaluated weekly until mid-January (early season).

### Optimization of the pre-heat/cool-down step of the current protocol for the treatment of strawberry transplants

To determine whether the time for the pre-heat step at 37°C for 1 hour plus the cool-down step for 1 hour could be shortened, whole transcriptome shotgun sequencing, also called RNA sequencing (RNA-Seq), was performed to reveal changes in gene expression of heat shock proteins over time. Two strawberry cultivars, Strawberry Festival and Florida127, were selected for the experiment. Transplants grown in 1-liter pots and kept in the greenhouse for evaluation of plant survival after treatment (bioassay). Total RNA was extracted using commercial kits and sent to Novogene for library preparation and sequencing. The raw sequencing data were analyzed using bioinformatic tools to identify genes associated with heat tolerance and differences in gene expression among samples.

## Results

### Long-term effects of thermotherapy on strawberry transplants under nursery production conditions

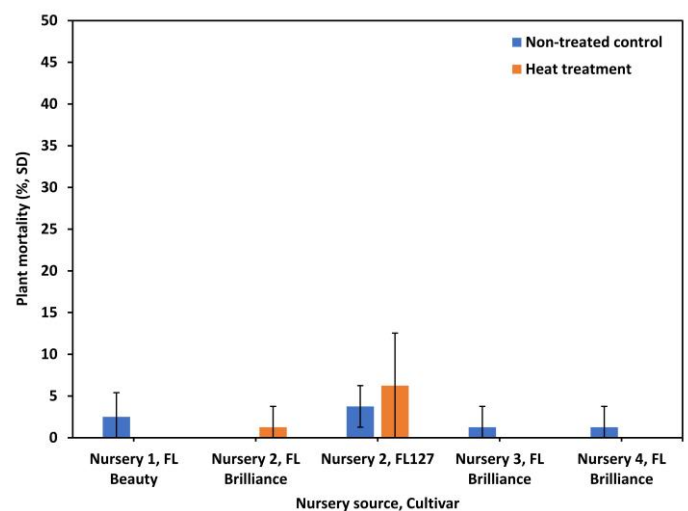
The results of field evaluation before harvest in strawberry nurseries in North Carolina showed a cultivar/location-dependent effect on plant mortality

(Table 1). While plant mortality did not differ between treated and non-treated groups in Nursery 1-Location 2 ('Florida Brilliance') and Nursery 2-Location 1 ('Florida127'), higher plant mortality in treated plots was observed in Nursery 1-Location 1 ('Florida Beauty') and particularly Nursery 2-Location 3 ('Florida Radiance'). In contrast, control plants in Nursery 2-Location 2 ('Florida Beauty') had a higher mortality rate than the treated ones (Table 1).

Transplants delivered to and planted at the UF-GCREC stayed vigorous throughout the period of evaluation with less than 5% mortality across various nursery sources and cultivars (Fig. 2). Currently, the data on plant mortality in commercial fields for transplants from treated and non-treated plots in nurseries are still being processed.

**Table 1.** Long-term effect of thermotherapy on strawberry transplants before the harvest in strawberry nurseries

Nursery-Location	Cultivar	Treatment	Total plant #	Mortality (%)
Nursery 1 - #1	FL Beauty	Control	2324	2.71
...	...	Heat treatment	3538	12.27
Nursery 1 - #2	FL Brilliance	Control	5599	0.61
...	...	Heat treatment	5888	0.51
Nursery 2 - #1	FL127	Control	2793	33.05
...	...	Heat treatment	1987	33.37
Nursery 2 - #2	FL Beauty	Control	2922	7.73
...	...	Heat treatment	3561	2.19
Nursery 2 - #3	FL Radiance	Control	2252	3.37
...	...	Heat treatment	3795	47.38



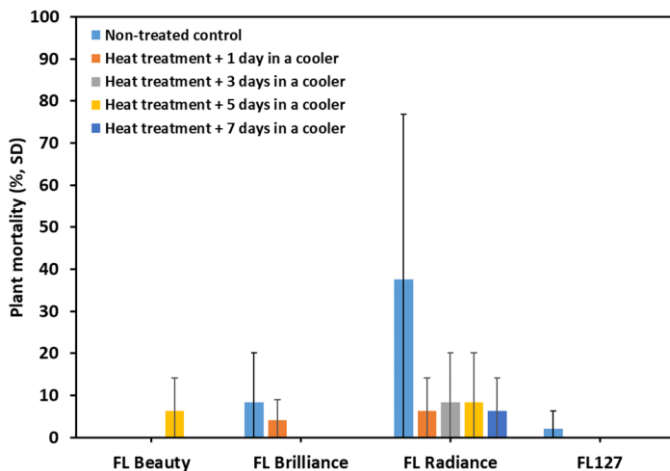
**Figure 2.** Long-term effects of thermotherapy on strawberry transplants after 13 weeks of planting at the UF-GCREC.

### Optimum planting time after thermotherapy

During the period of evaluation, most of treated and control plants remained vigorous and healthy with less than 10% mortality across treatments and cultivars (Fig. 3). The only exception was the non-treated Florida Radiance showing a higher mortality rate of approximately 40%. This high mortality was later found to be attributed to the quality of planting stock as pathogen isolations showed the presence of *Phytophthora* species and other secondary pathogens (data not shown). Our results indicate the storage of strawberry transplants in a cooler (4°C) for up to 7 days after thermotherapy does not affect the establishment of strawberry transplants.

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**Figure 3.** Effect of storage at 4°C after heat treatment on strawberry transplant establishment. The data presented here are from 13 weeks after planting at the UF-GCREC.

### Optimization of the pre-heat/cool down step of the current protocol for the treatment of strawberry transplants

Eight weeks after treatment, all plants grown in the greenhouse remained healthy without showing any adverse effects from the treatment (data not shown). Before performing whole transcriptome shotgun sequencing, high-quality RNA must be obtained. However, we experienced setbacks due to COVID-19 closures.