CATT Assesment Florensis

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Sciara and Scatella in Lisianthus young plants

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Contents

Summary

Florensis sometimes encounters issues with small flying insects (*Sciara* sp. (rouwmug) and *Scatella* sp. (oevervlieg)) being present on young Lisianthus plants, an end product of Florensis. These insects are not affecting plant quality but they are a nuisance and not positive for the image of the product. Florensis is interested in a sustainable and non-chemical way to tackle issues with small flying insects (*Sciara sp.* (rouwmug) and *Scatella sp.* (oevervlieg)) being present on young Lisianthus plants. Wageningen Food & Biobased Research (WFBR) independently tested if Controlled Atmosphere Temperature Treatments (a heat treatment combined with Controlled Atmosphere) could be a viable option to kill these insects without affecting plant quality.

Florensis organized rearing the insects and evaluating plant quality and insect mortality (in a double blind way). Experimental set up and treatments were performed by Wageningen Food & Biobased Research as well as the data evaluation and reporting.

Goal of this project is to evaluate if a CATT treatment can be developed that kills *Sciara* and *Scatella*, but does not damage the young Lisianthus plants. This report is intended for the management of Florensis, to help in deciding if CATT can be a practical solution to prevent *Sciara*/ *Scatella* infestation of end products. After a pretest to check if treatment parameters were in the right ballpark and to check feasibility of procedures etc., a range of CATT treatments have been tested to find a combination with sufficient insect mortality while maintaining sufficient plant product quality.

Main conclusions

Most promising results found in this project:

- Treatment with 42°C in ambient air for 8 hours: No living *Sciara* or *Scatella* were found and the plants (Lisianthus rostia White and Lisianthus rostia pink) did not show any damage or reduced growth, both after treatment and after replanting. In the pretest however some survival was observed.
- Similar mortality but slightly reduced growth which is overcome after replanting was observed at:
    - 36°C for 24 hours at 10% CO_2 and 15% O_2
    - 39°C for 8 hours at 65% CO_2 and 1% O_2
    - 39°C for 8 hours at 65% CO_2 and 15% O_2
    - 42°C for 8 hours at 65% CO_2 and 1% O_2
- For Scatella no hard conclusions regarding mortality of treatments can be made because the number of Scatella per bucket were insufficient and too variable.
- No additional plant quality issues were observed during replanting that were not clear already after 14 days.

Recommendations

- Retest selected promising treatments, with a focus on the 8 hours at 42°C in ambient air to check reproducibility.
- Combine with time series at 42°C in ambient air. If with less time the *Sciara* and *Scatella* are also killed, it could be better for the plants. Also try some longer time periods than 8 hours to get answers on the next questions: How long can the plant handle 42°C before showing damage? 24 hours is too long, but can the treatment also be done overnight? How critical is the 8 hours of treatment?
- Since the *Scatella* numbers were too low to make a conclusion; standardize the breeding of *Scatella* and try to scale up the breeding for more flies in the buckets.
- It seems that *Scatella* is more difficult to kill than *Sciara*. When the breeding of *Scatella* is sufficient, focus on testing with *Scatella*. If the right CATT conditions for *Scatella* are found check if also *Sciara* is killed.
- Check if treatment is effective on naturally infected plant products
- Screen more cultivars/qualities since sensitivity for quality loss might vary between cultivars/qualities.

1 Acknowledgements

We are thankful for the pleasant cooperation with Florensis in this project. Roy van Heesch the client, delegated the actual work to William Barbier and Eric de Groot (insect rearing and mortality assessments) and Anne Vromans (plant quality assessments). They managed to focus on getting the details of these experiments right, despite COVID-19 restrictions and the always present commercial pressure of the normal daily operations of a busy company like Florensis.

2 Introduction

Florensis sometimes encounters issues with small flying insects (*Sciara sp.* (rouwmug) and *Scatella sp.* (oevervlieg)) being present on young Lisianthus plants, an end product of Florensis. These insects are not affecting plant quality but they are a nuisance and not positive for the image of the product. Florensis is interested in a sustainable and non-chemical way to tackle this issue.
Wageningen Food & Biobased Research has been involved in developing Controlled Atmosphere Temperature Treatments, using heat treatments combined with Controlled Atmosphere (adapted oxygen and carbon dioxide levels) for postharvest treatment of various combinations of plant products and insects/mites. These CATT treatments have to be optimized for every plant-pest combination to ensure sufficient insect mortality while maintaining sufficient plant product quality. Florensis asked Wageningen Food & Biobased Research to *independently* evaluate a range of CATT treatment conditions to evaluate the potential of this type of treatment on young Lisianthus plants, financed by Florensis. In order to reduce costs, Florensis organized rearing the insects and evaluating plant quality and insect mortality (in a double blind way). The experimental set up and treatments were performed by Wageningen Food & Biobased Research, as well as the data evaluation and reporting. Temperature, oxygen, carbon dioxide concentration and treatment duration are the main variables in a CATT treatment. By varying these four variables, a range of CATT recipes is screened to evaluate the potential to find a treatment in which the insects are killed without affecting plant quality.

So the goal of this project is to evaluate if a CATT treatment can be developed that kills *Sciara* and *Scatella*, but does not damage the young Lisianthus plants. This report is intended for the management of Florensis, to help in deciding if CATT is a potential practical solution to prevent *Sciara*/ *Scatella* infestation of end products.

3 Materials and methods

3.1 Experimental setup pretest

Before the main experiment started a pretest was performed to see how the plants and insects reacted on the intended temperatures and treatment durations to be used. This was done in order to minimize the chance of chosen conditions for the main experiment being too severe (e.g. killing all the plants) or too mild (not killing flies). Also other practicalities such as fitting objects in the intended treatment containers etc. could be checked to prevent issues in the main experiment.

3.1.1 Lisianthus cuttings and insects

For the pretest, rooted cuttings of four different cultivars of *Lisianthus grandiflorum* (DW 12/20) were provided by Florensis (see ***Table 1***). The trays with the different cultivars were cut into 8 parts, so that every cultivar could be tested in all treatments.

Table 1 Cuttings of Lisianthus cultivars in the pretest

Cultivar	Code in report	Code	Code	Tray
Chroma II Blue Picotee	(GR)	900149110	50166	6001
Alissa 2 Green	(GR)	900149777	50739	6001
Celeb 2 Madonna	(MD)	900149759	50355	6001
Rosita 3 Pure white	(PW)	900149708	31174	6001

Florensis provided the insects in separate buckets with substrate containing *Sciara* or *Scatella*, prepared by Florensis. Rearing and preparation procedures were not shared with WFBR.

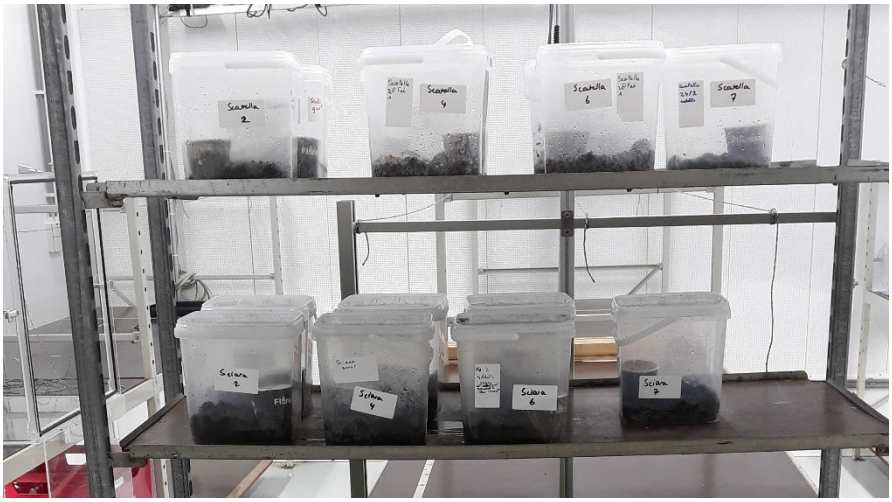

Figure 1 Buckets with Sciara or Scatella

3.1.2 Pretest treatments

To see how the plants and insects reacted on the different intended conditions three temperatures (36, 39 and 42°C) and two durations (8 and 24 hours) were tested (see ***Table 2***).

Table 2 Temperature and time conditions of the pretest. CO_2 and O_2 levels were ambient (0% and 21%)

Object number	Temperature (°C)	Time (h)
5	20	24
8 (*)	20	24
7	36	8
1	36	24
4	39	8
3	39	24
2	42	8
6	42	24

*: (only cuttings, no insects)

Figure 2 Preparing the objects for the pretest

Every object number had one bucket of *Sciara*, one bucket with *Scatella*, and the four Lisianthus cultivar young plants. The objects were placed in the cells with the right temperature. It became clear that in the main experiment maximum two cultivars of cuttings would fit in a conditioned atmosphere container together with the two buckets *Sciara* and *Scatella*.

After treatment the objects were placed in a conditioned room at 20°C 60% RV where they could cool down. After cooling, the young plants were placed in a box to be transported to Florensis, together with the buckets *Sciara* and *Scatella*.
Florensis scored the quality of the Lisianthus and the surviving/dead *Sciara* and *Scatella* after 1 and 5 days.

Figure 3 The objects during the pretest. Top: control at 20°C; Bottom: at treatment temperature

3.2 Experimental setup main experiment

The main experiment has tested a wide variety of temperatures and gas conditions based on the outcome of the pretest and WFBR experience /educated guesswork from previous experiments with white fly (*Bemisia tabaci*) and Western flower trips (*Frankliniella occidentalis*).

3.2.1 Lisianthus cuttings and insects

For the main experiment, rooted cuttings of two different cultivars of *Lisianthus grandiflorum* were provided by Florensis namely Lisiantus rostia White and Lisiantus rostia Pink; six trays of each cultivar.

The trays were cut in 8 parts on arrival and placed at 20°C 60% RV before treatment the next day.

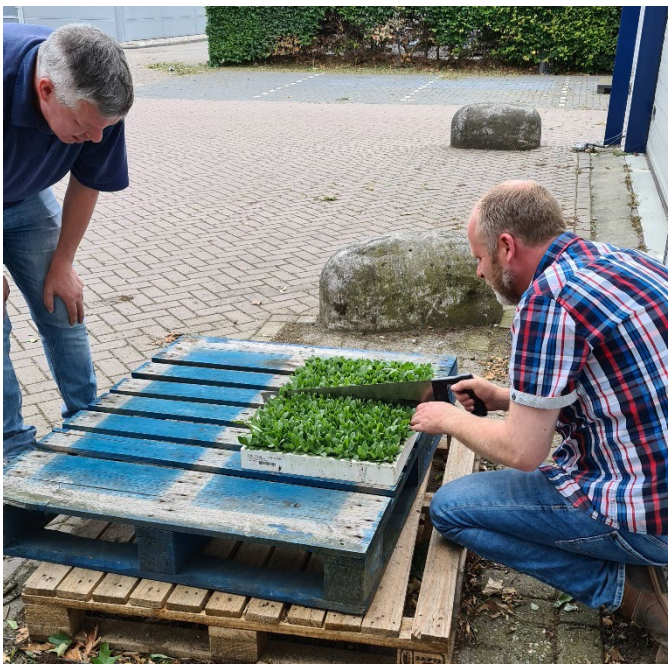

Figure 4 Sawing the trays in 8 pieces

The insects were prepared and provided by Florensis in 40 buckets with *Sciara* and 40 with *Scatella*.

Figure 5 Buckets with Sciara and Scatella for the main experiment

3.2.2 Treatments main experiment

In the pretest, Lisianthus turned out to react reasonably well at the proposed temperatures. Therefore, the same temperatures are used in the main experiment namely 36, 39 and 42°C combined with a variation of CO_2/O_2 levels.

For the CATT treatments, every object (except for untreated controls) was placed in a 67l stainless steel container connected to a high-tech flow system, enabling continuous flushing of all individual containers with preselected mixtures of oxygen, carbon dioxide and nitrogen (inert balance gas). These containers are placed in temperature controlled climate rooms, see Figure 6 bottom panels.

Each container had one bucket of *Scatella*, one bucket with *Sciara*, 1/8 tray with Lisianthus rostia White and 1/8 tray with Lisianthus rostia Pink. Temperature loggers (Logtag, New Zealand), or RH/T loggers (Escort, Switserland) were placed between the bucket and the cuttings. After closing the containers, the gas concentrations were quickly flushed to about the intended conditions and plugged into the flow through system, with a gas flow with concentrations as displayed Table 3 and Table 4.

Oxygen and carbon dioxide levels were checked several times during the treatment using a calibrated portable gas analyser (Checkpoint II, Dansensor, Denmark).

Figure 6 top: preparing objects to put in container. Below: objects in container, closing the containers and connect to flow through system

During the main experiment these parameters were selected:

- Temperature: 36°C, 39°C and 42°C (control at 20°C)
- Time: 8 and 24 hours treatment
- O_2 levels: 1%, 15%, 25% (control ambient 21%)
- CO_2 levels: 10%, 40%, 65% (control ambient 0%)

Because a complete square design would result in a too large experiment, it was decided that 36°C and 42°C included part of the treatments, while 39°C included all treatments (see Table 3 and Table 4). In total 40 treatments were performed.

During the treatment, gasses fed to the containers were humidified using gas wash bottles to create high humidity (~100%) during the treatment.

Table 3 Conditions of the containers with the objects in the main experiment 8 hours treatment.

Object	Temperature (°C)	Time (hours)	CO2 (%)	O2 (%)
1	36	8	0	21
3	36	8	10	15
5	36	8	65	1
7	36	8	65	25
9	39	8	0	21
11	39	8	10	1
13	39	8	10	15
15	39	8	10	25
17	39	8	40	1
19	39	8	40	15
21	39	8	40	25
23	39	8	65	1
25	39	8	65	15
27	39	8	65	25
29	42	8	0	21
31	42	8	10	15
33	42	8	65	1
35	42	8	65	25

Table 4 Conditions of the containers with the objects in the main experiment 24 hours treatment.

Object	Temperature (°C)	Time (hours)	CO2 (%)	O2 (%)
2	36	24	0	21
4	36	24	10	15
6	36	24	65	1
8	36	24	65	25
10	39	24	0	21
12	39	24	10	1
14	39	24	10	15
16	39	24	10	25
18	39	24	40	1
20	39	24	40	15
22	39	24	40	25
24	39	24	65	1
26	39	24	65	15
28	39	24	65	25
30	42	24	0	21
32	42	24	10	15
34	42	24	65	1
36	42	24	65	25
37	20	24	0	21
38	20	24	0	21
39	20	24	0	21
40	20	24	0	21

After treatment the objects were placed in a conditioned room at 20°C 60% RH to cool down. After cooling, the cuttings were placed in a box and transported to Florensis, together with the buckets *Sciara* and *Scatella,* for quality- and mortality assessment by Florensis.

3.2.3 Scoring by Florensis

Florensis scored the quality of the Lisianthus and the surviving/dead *Sciara* and *Scatella* in the following weeks as shown below. Florensis was not informed which treatment code was connected to which treatment to enable blind scoring to prevent unintentional scoring bias.

The quality of Lisianthus young plants were scored after arrival at Florensis, 14 days after treatment and after replanting in week 36 (~8 weeks after treatment). The scoring was done in five categories:

1 = dead
2 = almost dead, plant does not grow anymore
3 = severe damage; plant is splitting
4 = little (leave) damage
5 = plant is good; no damage

The last quality observation after replanting was done differently, using 3 categories: 1=bad, 2= less, 3=good.

Sciara survival was scored upon arrival at Florensis and after 5, 8 and 14 days after treatment. *Sciara* was scored per bucket in categories:
0 = no *Sciara* alive
1 = 1-25 *Sciara* alive
2 = 25-50 *Sciara* alive
3 = 50-100 *Sciara* alive
4 = 100-250 *Sciara* alive

Scatella survival was scored as the counted number of insects alive per bucket.

During counting the *Scatella*, also unidentified moths were found in some buckets. These moths are scored as number of moths found.

3.2.4 Calculating mortality and relative plant quality

To evaluate the effects of treatments, mortality per treatment is calculated. However, *Sciara* is scored in categories, so calculating the percentage insects killed is not possible since the categories are not evenly distributed (category 1 and 2 difference of 25 insects; 3 of 50 insects and 4 of 150 insects). As solution the average of the category is taken to measure the amount of insects resulting in:
0 = 0
1 = (1+25)/2 = 13
2 = (26+50)/2 = 38
3 = (50+100)/2 = 75
4 = (100-250)/2 = 175
These numbers are used to calculate the percentage mortality:

%mortality = 1-(nr of living insects per treatment/nr amount of living insects of untreated control)*100%

Relative plant quality was calculated by averaging the quality score from the untreated controls and setting this on 100% for every moment of observation. The treatments were then expressed as % of the accompanying controls.

4 Results

4.1 Results pretest

All three temperatures showed little quality loss for 8 hours treatments on all four cultivars compared with untreated controls. Only the quality of Celeb 2 Madonna was slightly less at 42°C. After 24 hours of treatment more quality issues were observed. The least at 36°C and the most at 42°C, as could be expected. Celeb 2 Madonna and Chroma II Blue Picotee had the most damage; Alissa 2 Green had the least damage.

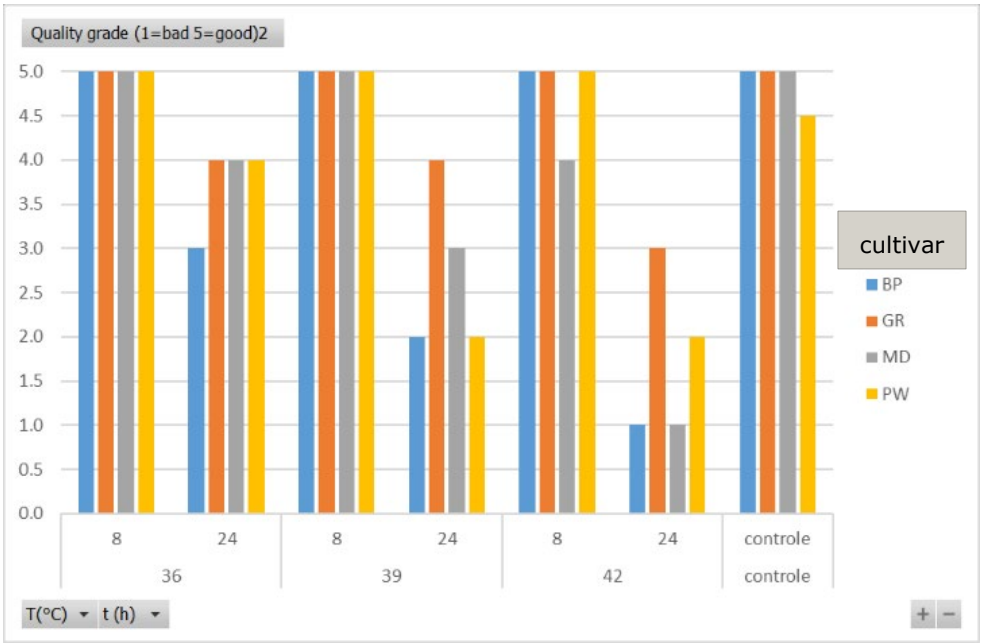

Figure 7 Plant quality of 4 cv's after heat treatments in pretest

The rearing of the insects appeared to be a challenge, especially to get large and even amounts in the buckets.
However, the results give reason to use the chosen treatment temperatures in the main experiment. Insects were killed (without the expected added value of CA conditioning) and the Lisianthus cuttings could handle at least 8 hours treatment.

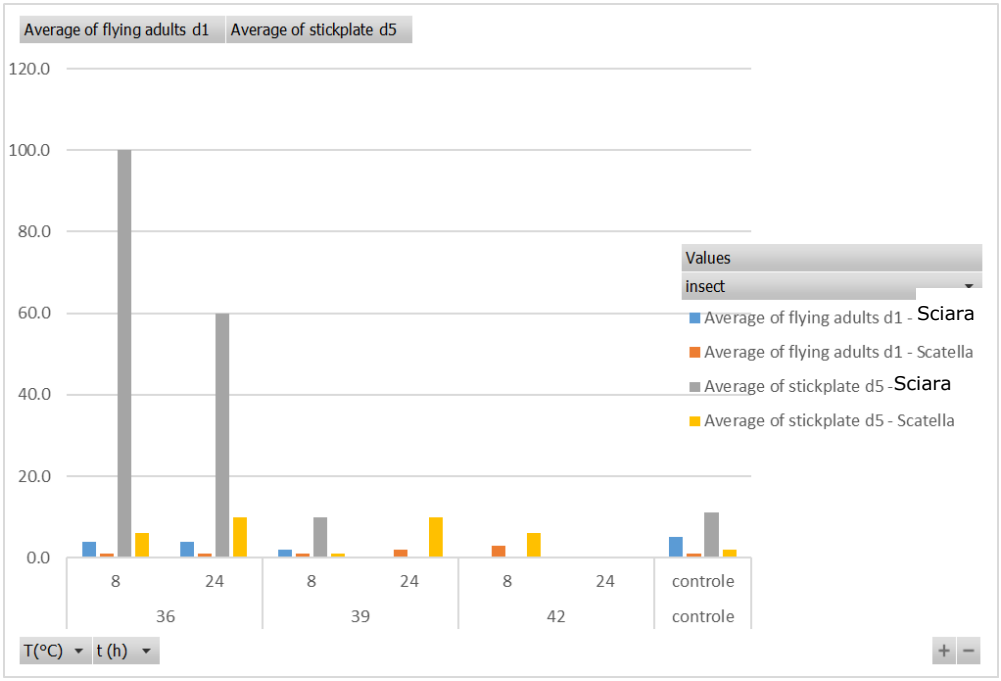

Figure 8 Insect survival after heat treatments in pretest

Relative humidity in this pretest was not controlled, resulting in visibly affected (dry) plugs at the borders of the cut trays, especially at the 24 hours treatments. In the main experiment extra attention was paid to make sure humidity was high during the treatments.

4.2 Results main experiment

4.2.1 Conditions during treatment

During the treatment the CO_2 and O_2 levels were measured several times. In Table 5 and Table 6 the average of the measured levels are given. The measured concentrations were about the intended concentrations. The relative large volume of the insect buckets covered with fine mesh is likely to have equilibrated O_2 and CO_2 levels slowly in the start of the conditioning, making precise conditioning not so easy. The actual concentrations however, were close enough to the intended levels for this experiment to enable evaluation of effects of a wide range of conditioning, as intended.

Table 5 Intended and measured conditions of the containers with the objects in the main experiment 8 hours treatment

Object	Temperature (°C)	Time (h)	Intended CO2 (%)	Intended O2 (%)	Measured CO2 (%)	Measured O2 (%)
1	36	8	0	21	0.4	20.9
3	36	8	10	15	9.0	15.8
5	36	8	65	1	58.6	1.8
7	36	8	65	25	57.9	23.9
9	39	8	0	21	0.4	19.8
11	39	8	10	1	9.0	1.7
13	39	8	10	15	8.6	14.2
15	39	8	10	25	8.6	23.0
17	39	8	40	1	33.8	2.2
19	39	8	40	15	33.5	14.6
21	39	8	40	25	33.8	22.2
23	39	8	65	1	54.5	1.9
25	39	8	65	15	57.1	15.3
27	39	8	65	25	57.2	24.8
29	42	8	0	21	0.5	20.2
31	42	8	10	15	8.9	14.0
33	42	8	65	1	55.6	2.0
35	42	8	65	25	59.9	23.3

Table 6 Intended and measured conditions of the containers with the objects in the main experiment 24 hours treatment

Object	Temperature (°C)	Time (h)	Intended CO2 (%)	Intended O2 (%)	Measured CO2 (%)	Measured O2 (%)
2	36	24	0	21	0.5	20.1
4	36	24	10	15	9.4	15.3
6	36	24	65	1	60.5	1.9
8	36	24	65	25	59.4	24.9
10	39	24	0	21	0.5	19.4
12	39	24	10	1	9.3	1.9
14	39	24	10	15	8.7	14.3
16	39	24	10	25	8.2	22.1
18	39	24	40	1	35.0	2.3
20	39	24	40	15	34.2	17.0
22	39	24	40	25	31.5	23.3
24	39	24	65	1	57.1	2.0
26	39	24	65	15	55.8	20.1
28	39	24	65	25	59.4	24.0
30	42	24	0	21	0.5	19.1
32	42	24	10	15	9.8	14.2
34	42	24	65	1	56.7	2.2
36	42	24	65	25	58.9	24.5

4.2.2 Results of 24 hours treatment

In Figure 9 the mortality of *Sciara* and *Scatella* combined and the Lisianthus young plant quality of 24 hours treatment is given (scored 14 days after treatment). After 24 hours of treatment there may be a good killing of insects, however, the plant quality drops dramatically especially when the temperature is higher, or with higher CO_2 levels. This means that 24 hours treatment is too long for Lisianthus young plants, especially in combination with other gas conditions than ambient.

If you look at the results of *Sciara* and *Scatella* separately, (see Figure 10) it is clear that *Sciara* is killed with all 24 hour treatments (except for 36°C, 21% O_2 and 0% CO_2). For *Scatella* also quite a lot of treatments seems effective. However, the amounts of *Scatella* in the buckets are too low to take any conclusions. In the control buckets (20°C, 21% O_2 and 0% CO_2) the amount of *Scatella* were 7, 1, 1 and 0. Since also in one control bucket no living *Scatella* was present, a bucket of a treatment with no living *Scatella* is hopeful, but no hard conclusions can be taken.

In the Annex 1.1-1.4 also the scores of *Sciara* and *Scatella* as well as plant quality directly after treatment are shown, but results shown here give the best impression of overall effects.

Figure 9: 24 hours treatment scored after 14 days: top: Average mortality of Sciara and Scatella combined. bottom: relative plant quality: 100%= plant good, 80% = little (leave) damage; 60% = severe damage; plant is splitting; 40% = plant almost dead, does not grow

anymore, 20% = plant dead; Green bar is good result, red bar is bad result, orange bar is in between result.

Figure 10: 24 hours treatment scored after 14 days: top: Sciara mortality (%) bottom: Scatella mortality (%). Green bars is good result; red bar is bad result; orange bar is in between result.

4.2.3 Results of 8 hours treatment

An 8 hours treatment is better for the plants as can be seen in Figure 11. The average mortality of *Sciara* and *Scatella* combined however, is much less than after 24h treatment. However, there are large differences in mortality between *Sciara* and *Scatella* as can be seen in Figure 12.

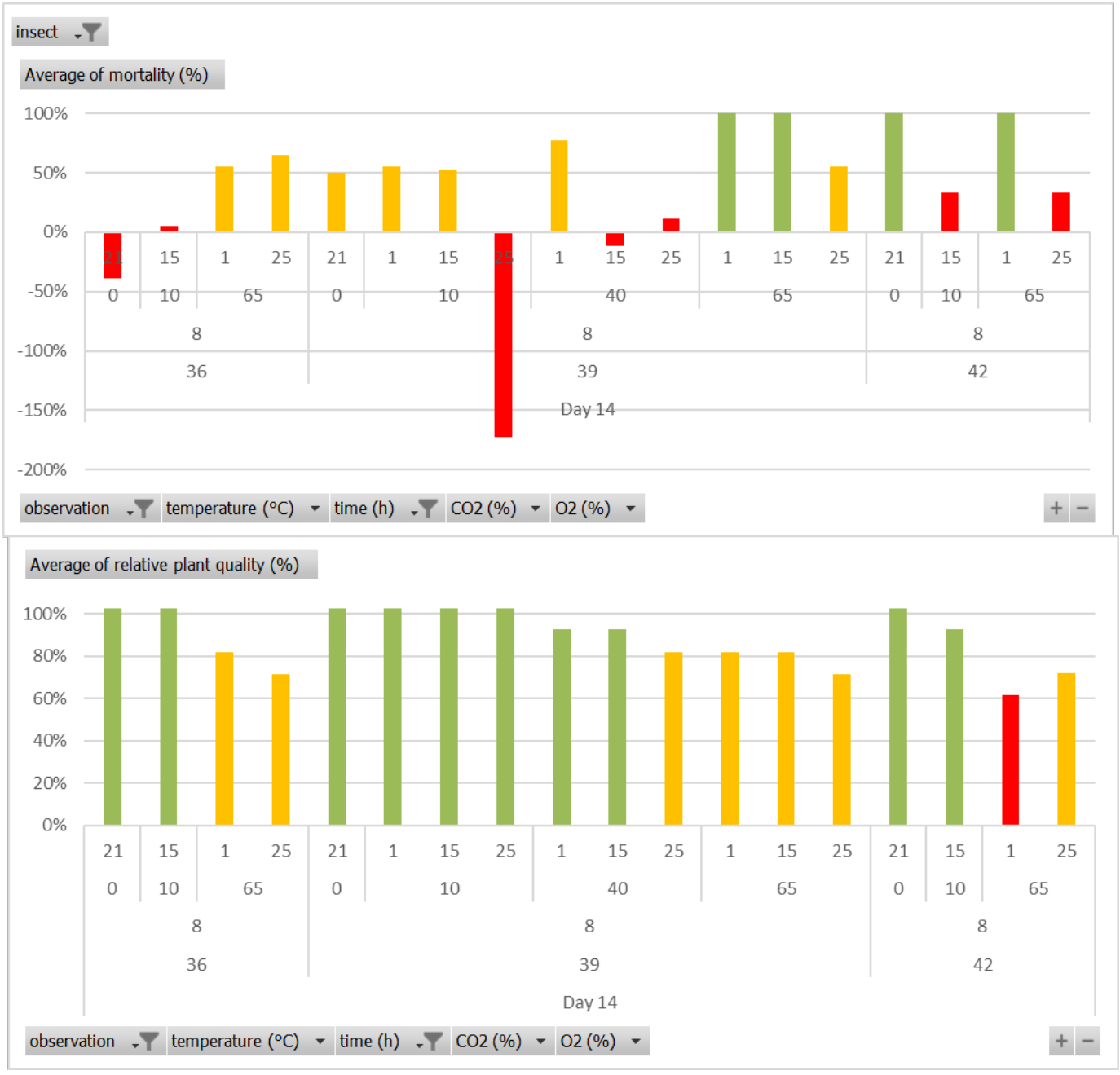

Figure 11: 8 hours treatment scored after 14 days: top: Average mortality of Sciara and Scatella combined. bottom: relative plant quality: 100%= plant good, 80% = little (leave) damage; 60% = severe damage; plant is splitting; 40% = plant almost dead, does not grow anymore, 20% = plant dead; Green bar is good result, red bar is bad result, orange bar in between result.

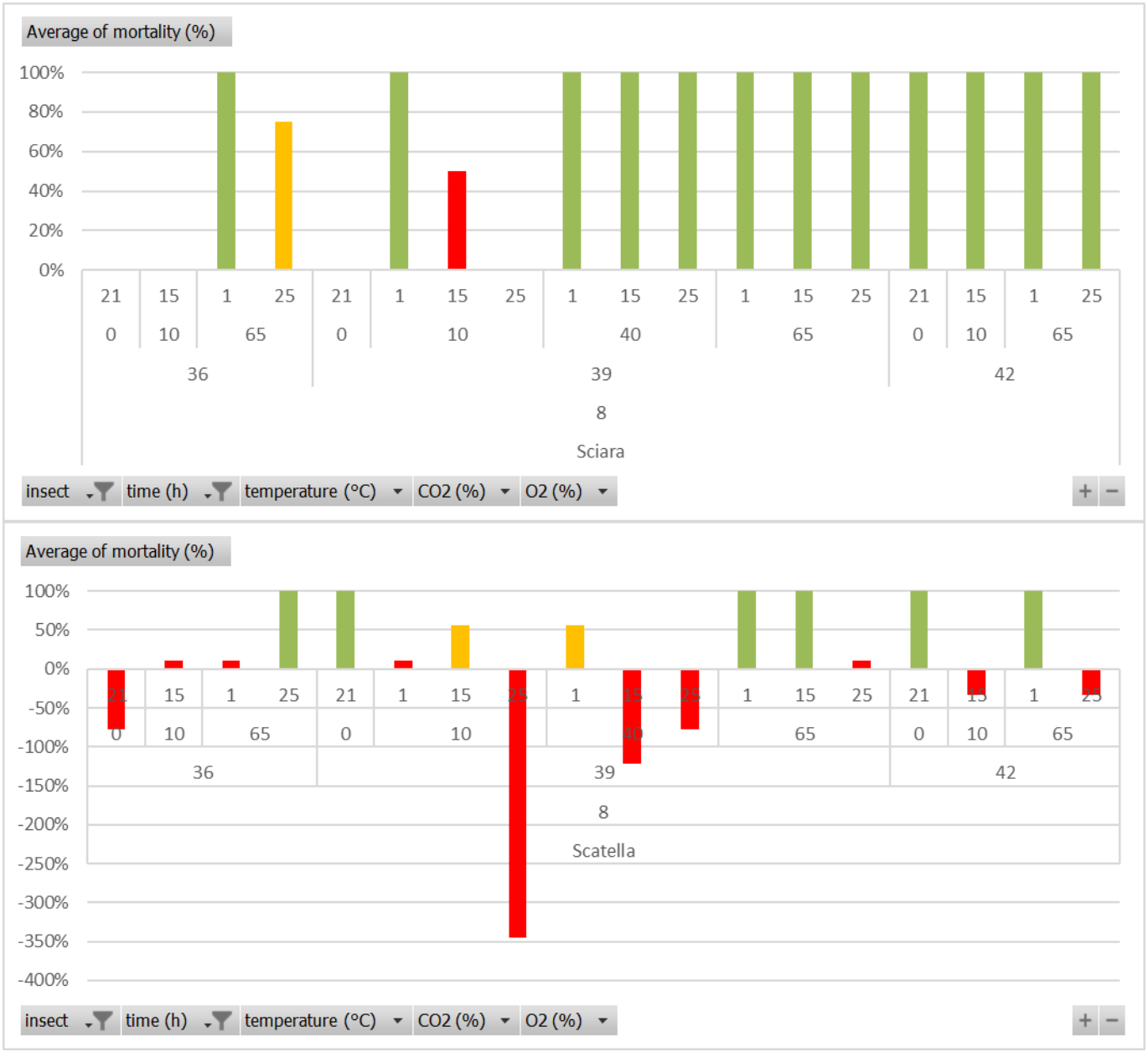

Figure 12: 8 hours treatment scored after 14 days: top: Sciara mortality (%) bottom: Scatella mortality (%). Green bars is good result; red bar is bad result; orange bar is in between result.

Sciara is killed at all conditions at 42°C and at 39°C with CO_2 levels 40% or higher. For 36°C 1% O_2/65% CO_2 seems to be a good way to kill *Sciara*. For *Scatella* the results are less clear. For six buckets a negative mortality is seen, meaning there are more living insects in the bucket than in the control buckets. These are not likely treatment results, but effect of the very low numbers of *Scatella* per bucket resulting in relatively large variation between buckets. There are also some buckets with no living *Scatella*. This is hopeful, especially 42°C with ambient air (21% O_2 and 0% CO_2). This is the only tested condition with 100% mortality for *Sciara* and *Scatella* together with a good plant quality. However, as described above, the conclusions about *Scatella* results have to be taken with precaution because of the low numbers of *Scatella* in all buckets, including the control buckets.

4.2.4 Differences between cultivars of Lisianthus

Different Lisianthus cultivars can react differently on the CATT treatment. In the main experiment cultivars Lisianthus rostia White and Lisianthus rostia pink were used. In Figure 13 the results of both cultivars are shown separately for all treatments. The cultivars showed more or less the same

sensitivities towards damaging conditioning. As seen in previous figures above, for both cultivars 8 hours treatment is better than 24 hours treatment and the CO_2 level should not be too high. Both cultivars show no damage at the promising CATT condition with 100% mortality of *Sciara* and *Scatella* namely 42°C 21% O_2 and 0% CO_2.

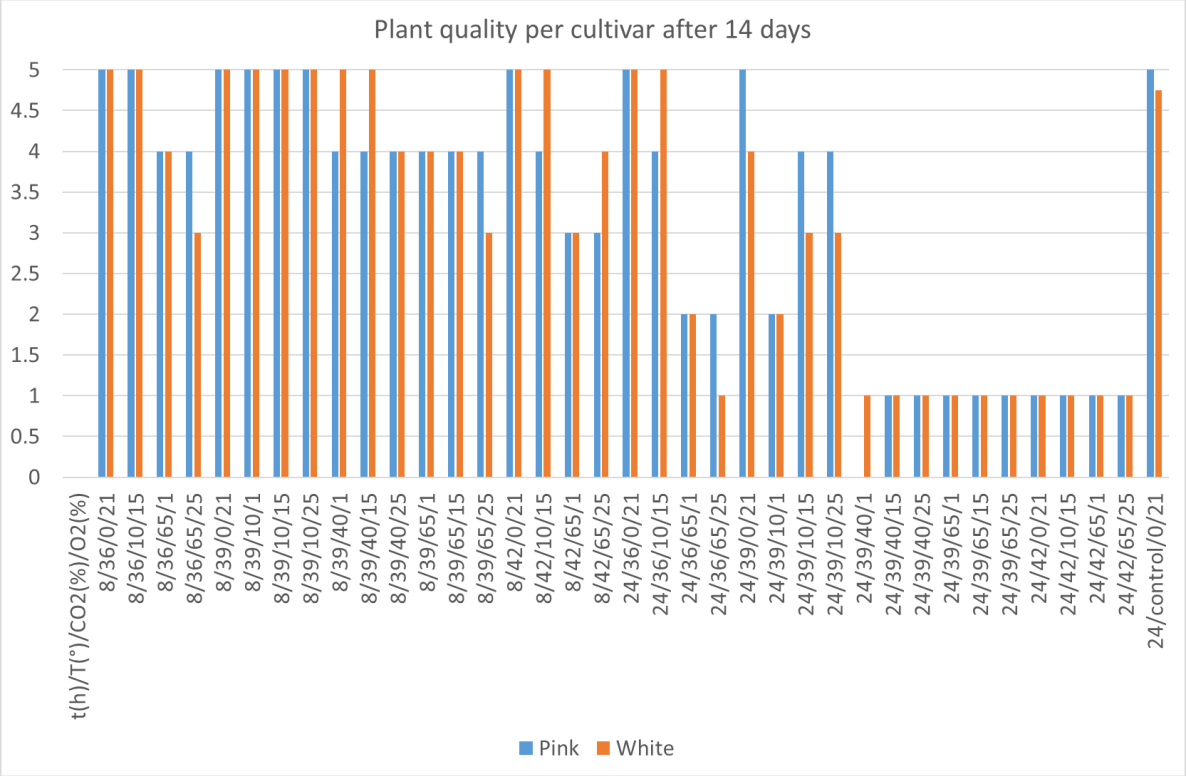

Figure 13 Result CATT treatment on plant quality after 14 days per cultivar. Code shows time (h)/temperature (°C)/%O_2/%CO_2.

The quality of the treated plants was also monitored after replanting. Final quality observation was after 8 weeks, see figure below. Treatments with big quality issues, like 24 hours 42°C, were not replanted. Main observation was that after replanting no new or other damage was observed, compared to the quality loss observed after up to 14 days. For many treatments where limited quality loss was observed at day 1/14, no quality loss was observed after 8 weeks.

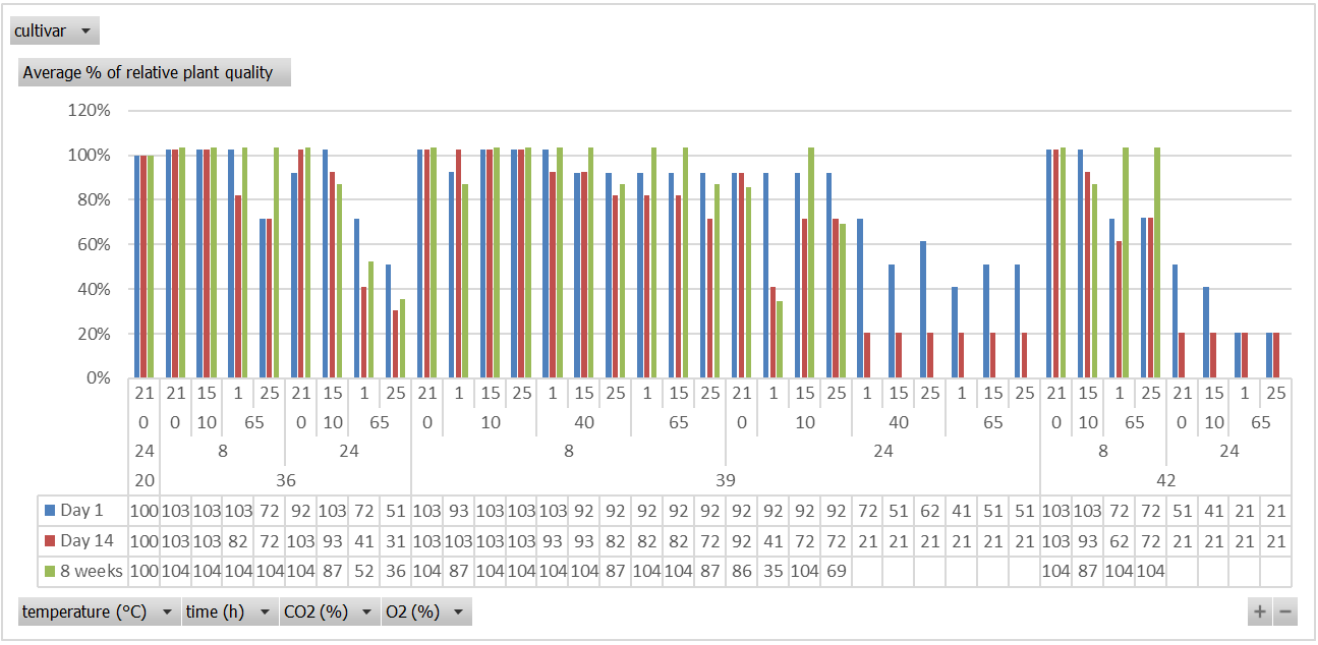

Figure 14: Effect of treatment conditions on relative plant quality directly and after 14 days after treatment. After replanting quality was also determined after 8 weeks. A relative plant quality > 100% means a treatment scored better than the untreated controls

To summarize the effects of the various tested conditions in one table, for each treatment the %mortality (after 14 days) and the %relative plant quality (both after 14 days and 8 weeks) was averaged in Table 7.

Table 7: Overview of the overall treatment effects with averaged mortality of Scatella and Sciara and averaged relative quality of the used cultivars.

				(mortality+quality)/2	
				mortality d14	mortality d14
temperature (°C)	**time (h)**	**CO2 (%)**	**O2 (%)**	quality d14	quality 8 wks
20	**24**	0	21	50%	50%
36	**8**	0	21	32%	32%
36	**8**	10	15	54%	55%
36	**8**	65	1	69%	80%
36	**8**	65	25	61%	77%
36	**24**	0	21	68%	69%
36	**24**	10	15	96%	93%
36	**24**	65	1	71%	76%
36	**24**	65	25	65%	68%
39	**8**	0	21	76%	77%
39	**8**	10	1	79%	71%
39	**8**	10	15	86%	87%
39	**8**	10	25	-35%	-34%
39	**8**	40	1	85%	91%
39	**8**	40	15	41%	46%
39	**8**	40	25	47%	49%
39	**8**	**65**	1	91%	102%
39	**8**	**65**	15	91%	102%
39	**8**	65	25	64%	71%

39	**24**	0	21	40%	37%
39	**24**	10	1	71%	67%
39	**24**	10	15	41%	57%
39	**24**	10	25	75%	73%
39	**24**	40	1	60%	50%
39	**24**	40	15	60%	50%
39	**24**	40	25	60%	50%
39	**24**	65	1	60%	50%
39	**24**	65	15	60%	50%
39	**24**	65	25	60%	50%
42	**8**	0	21	101%	102%
42	**8**	10	15	63%	60%
42	**8**	65	1	81%	102%
42	**8**	65	25	53%	68%
42	**24**	0	21	60%	50%
42	**24**	10	15	60%	50%
42	**24**	65	1	60%	50%
42	**24**	65	25	60%	50%

5 Conclusions

A range of CATT treatments have been tested to find a combination with sufficient insect mortality while maintaining sufficient plant product quality.
Most promising treatments found in this project:

- 42°C in ambient air for 8 hours. No living *Sciara* or *Scatella* were found and the plants (Lisianthus rostia White and Lisianthus rostia pink) did not show any damage or reduced growth, both after treatment and after replanting. In the pretest however, some survival was observed.
- Similar mortality, but slightly reduced growth which is overcome after replanting, was observed at: 36°C for 24 hours at 10% CO_2 and 15% O_2
  39°C for 8 hours at 65% CO_2 and 1% O_2
  39°C for 8 hours at 65% CO_2 and 15% O_2
  42°C for 8 hours at 65% CO_2 and 1% O_2

Other conclusions:

- For *Scatella* no hard conclusions regarding mortality of treatments can be made because the number of *Scatella* per bucket were insufficient and too variable.
- *Sciara* is killed at 42°C with all gas conditions tested, but also at 39°C with CO_2 levels 40% or higher.
- (Almost) every 24 hours treatment at 36, 39 or 42°C gives sufficient mortality, however the plant quality declines dramatically.
- With an 8 hours treatment the plant quality is better and the mortality of the insect can be achieved with certain treatments.
- No additional plant quality issues were observed during replanting that were not clear already after 14 days.

6 Recommendations

This report describes a first test with CATT treatments with the aim to find a combination with sufficient mortality on *Sciara* and *Scatella* while maintaining Lisianthus plant quality. One treatment looks most promising for further development: 42°C in ambient air for 8 hours. Not having to use control of oxygen/carbon dioxide levels during treatment, would greatly simplify the technical setup of a treatment facility. However, there are some things we recommend to look into before using this treatment in practice.

- Retest selected promising treatments, with a focus on the 8h at 42°C in ambient air to check reproducibility.
- Combine with time series at 42°C in ambient air. If with a short treatment the *Sciara* and *Scatella* are also killed, it could be better for the plants. Also try some longer time periods than 8 hours to get answers on the next questions: How long can the plant handle 42°C before showing damage? 24 hours is too long, but can the treatment also be done overnight? How critical is the 8 hours of treatment?
- Since the *Scatella* numbers were too low to make a conclusion; standardize the breeding of *Scatella* and try to scale up the breeding for more flies in the buckets.
- It seems that *Scatella* is more difficult to kill than *Sciara*. When the breeding of *Scatella* is sufficient, focus on testing with *Scatella*. If the right CATT conditions for *Scatella* are found check if also *Sciara* is killed.
- Check if treatment is effective on naturally infected plant products
- Screen more cultivars/qualities since sensitivity for quality loss might vary between cultivars/qualities.

If results of this additional testing in ambient air does not result in sufficient mortality without affecting plant quality, CATT conditions found to have good effects in this experiment, can be further explored and refined.

Annex 1 Raw data provided by Florensis

1.1 White

Plants	Object nr	Temp (°)	Time (h)	CO2 (%)	O2 (%)	Direct after treatment (3 july)	14 day's after treatment
White	1	36	8	0	21	5	5
White	2	36	24	0	21	4	5
White	3	36	8	10	15	5	5
White	4	36	24	10	15	5	5
White	5	36	8	65	1	5	4
White	6	36	24	65	1	3	2
White	7	36	8	65	25	3	3
White	8	36	24	65	25	2	1
White	9	39	8	0	21	5	5
White	10	39	24	0	21	4	4
White	11	39	8	10	1	5	5
White	12	39	24	10	1	4	2
White	13	39	8	10	15	5	5
White	14	39	24	10	15	4	3
White	15	39	8	10	25	5	5
White	16	39	24	10	25	4	3
White	17	39	8	40	1	5	5
White	18	39	24	40	1	3	1
White	19	39	8	40	15	4	5
White	20	39	24	40	15	2	1
White	21	39	8	40	25	4	4
White	22	39	24	40	25	3	1
White	23	39	8	65	1	4	4
White	24	39	24	65	1	2	1
White	25	39	8	65	15	4	4
White	26	39	24	65	15	2	1
White	27	39	8	65	25	4	3
White	28	39	24	65	25	2	1
White	29	42	8	0	21	5	5
White	30	42	24	0	21	2	1
White	31	42	8	10	15	5	5
White	32	42	24	10	15	2	1
White	33	42	8	65	1	3	3
White	34	42	24	65	1	1	1
White	35	42	8	65	25	4	4
White	36	42	24	65	25	1	1
White	37	control	24	0	21	4	4
White	38	control	24	0	21	5	5
White	39	control	24	0	21	5	5
White	40	control	24	0	21	5	5

1 = dead, 2 = almost dead, plant does not grow anymore; 3 = severe damage; plant is splitting; 4 = little (leave) damage; 5 = plant is good; no damage

1.2 Pink

Cultivar	Object nr	Temp (°)	Time (h)	CO2 (%)	O2 (%)	Direct after treatment (3 july)	14 day's after treatment
Pink	1	36	8	0	21	5	5
Pink	2	36	24	0	21	5	5
Pink	3	36	8	10	15	5	5
Pink	4	36	24	10	15	5	4
Pink	5	36	8	65	1	5	4
Pink	6	36	24	65	1	4	2
Pink	7	36	8	65	25	4	4
Pink	8	36	24	65	25	3	2
Pink	9	39	8	0	21	5	5
Pink	10	39	24	0	21	5	5
Pink	11	39	8	10	1	4	5
Pink	12	39	24	10	1	5	2
Pink	13	39	8	10	15	5	5
Pink	14	39	24	10	15	5	4
Pink	15	39	8	10	25	5	5
Pink	16	39	24	10	25	5	4
Pink	17	39	8	40	1	5	4
Pink	18	39	24	40	1	4	wortel eraf, uit proef gehaald.
Pink	19	39	8	40	15	5	4
Pink	20	39	24	40	15	3	1
Pink	21	39	8	40	25	5	4
Pink	22	39	24	40	25	3	1
Pink	23	39	8	65	1	5	4
Pink	24	39	24	65	1	2	1
Pink	25	39	8	65	15	5	4
Pink	26	39	24	65	15	3	1
Pink	27	39	8	65	25	5	4
Pink	28	39	24	65	25	3	1
Pink	29	42	8	0	21	5	5
Pink	30	42	24	0	21	3	1
Pink	31	42	8	10	15	5	4
Pink	32	42	24	10	15	2	1
Pink	33	42	8	65	1	4	3
Pink	34	42	24	65	1	1	1
Pink	35	42	8	65	25	3	3
Pink	36	42	24	65	25	1	1
Pink	37	control	24	0	21	5	5
Pink	38	control	24	0	21	5	5
Pink	039(1)	control	24	0	21	5	5
Pink	039(2)	control	24	0	21	5	5
Pink	40	control	24	0	21	5	5

1 = dead, 2 = almost dead, plant does not grow anymore; 3 = severe damage; plant is splitting; 4 = little (leave) damage; 5 = plant is good; no damage

1.3 *Sciara*

Living *Sciara* were counted and scored as categories. 0 = 0; 1 = 1-25; 2 = 25-50; 3 = 50-100 and 4 = 100-250

	Before shipment	1 jun	HIA after shipmement		6 jun observations		9 jun observations		15 jun observations	
Bucket	**Observations HIA**	**Treatment WUR CATT nr**	**Obervations**	**Remarks**	**5 day's After shipment**	**adults flying**	**8 day's After shipment**	**Remarks**	**after 14 days sticky plate**	**Remarks**
Sci 032	larvae	1		flying adults	3	50-100	4	100-200 no sticky plate	4	levend
Sci 038	larvae	2	moving larvae		0		1	0-25	3	70
Sci 024	larvae	3	moving larvae		3	50-100	4	100-200 no sticky plate	4	levend
Sci 025	larvae	4	Larvae on top not moving		0		0		0	0
Sci 028	larvae	5	Larvae on top not moving	dried out soil	0		0		0	0
Sci 004	larvae	6	Larvae on top not moving		0		0		0	0
Sci 021	larvae	7	Larvae on top not moving		1	0-25	1	0-25	1	9
Sci 030	larvae	8	Larvae on top not moving		0		0		0	0
Sci 036	larvae	9	moving larvae		0		4	100-200 no sticky plate	4	levend
Sci 040	larvae	10	Larvae on top not moving		0		0		0	0
Sci 015	larvae	11	Larvae on top not moving	dried out soil	0		0		0	0
Sci 005	larvae	12	Larvae on top not moving		0		0		0	0
Sci 013	larvae	13	Larvae on top not moving		2	25-50	2	25-50	2	30
Sci 009	larvae	14	not moving larve		0		0		0	0
Sci 018	larvae	15	some moving larvae		1	0-25	2	25-50	4	130
Sci 008	larvae	16	Larvae on top not moving		0		0		0	0
Sci 029	larvae	17	Larvae on top not moving	dried out soil	0		0		0	0
Sci 022	larvae	18	Larvae on top not moving		0		0		0	0
Sci 019	larvae	19	not moving larve		0		0		0	0
Sci 039	larvae	20	Larvae on top not moving		0		0		0	0
Sci 026	larvae	21	not moving larve		0		0		0	0
Sci 006	larvae	22	Larvae on top not moving		0		0		0	0
Sci 014	larvae	23	Larvae on top not moving		0		0		0	0
Sci 012	larvae	24	Larvae on top not moving		0		0		0	0
Sci 033	larvae	25	Larvae on top not moving		0		0		0	0
Sci 037	larvae	26	Larvae on top not moving		0		0		0	0
Sci 002	larvae	27	Larvae on top not moving		1	0-25	0		0	0
Sci 020	larvae	28	Larvae on top not moving		0		0		0	0
Sci 034	larvae	29	Larvae on top not moving		0		0		0	0
Sci 007	larvae	30	not moving larve		0		0		0	0
Sci 031	larvae	31	Larvae on top not moving		0		0		0	0
Sci 011	larvae	32	not moving larve		0		0		0	0
Sci 023	larvae	33	Larvae on top not moving		0		0		0	0
Sci 035	larvae	34	Larvae on top not moving		0		0		0	0
Sci 016	larvae	35	Larvae on top not moving		0		0		0	0

Bucket	Observations HIA	Treatment WUR CATT nr	Obervations	Remarks	5 day's After shipment	adults flying	8 day's After shipment	Remarks	after 14 days sticky plate	Remarks
Sci 001	larvae	36	Larvae on top not moving		0		0		0	0
Sci 003	larvae	37	moving larvae		3	50-100	4	100-250 no stick plate	4	levend
Sci 027	larvae	38	moving larvae	flying adults	3	50-100	4	50-100 no sticky plate	4	levend
Sci 010	larvae	39	moving larvae	flying adults	3	50-100	4	100-200 no sticky plate	4	levend
Sci 017	larvae	40	Adults flying		3	50-100	3	50-100 some escaped	4	+/- 130

1.4 *Scatella*

	Before shipment	1 jun	HIA after shipment							
Bucket	**Obervations** HIA	**Treatment** WUR CATT nr	**Obervations**	**Remarks**	**5 days after shipment**	**8 days after shipment**	**after 14 days sticky plate**	**overall adult survival**	**overall mortality**	**Remarks**
Scat 015	Larvae & Pupae	1	Larvae on top not moving	mothfly alive	mots only	mots & 1adult scat	4	4	20%	10 motjes
Scat 003	Larvae & Pupae	2					2	2	60%	
Scat 034	Larvae & Pupae	3 →3					2	2	60%	4mots
Scat 026	Larvae & Pupae	4					0	0	100%	1 *sciara*
Scat 014	Larvae & Pupae	5					2	3	40%	1(ontsnapt)
Scat 001	Larvae & Pupae	6	Larvae on top not moving				0	0	100%	
Scat 002	Larvae & Pupae	7					0	0	100%	1 *sciara*
Scat 022	Larvae & Pupae	8			mots only		0	0	100%	
Scat 017	Larvae & Pupae	9	Larvae on top not moving				0	0	100%	
Scat 010	Larvae & Pupae	10	Larvae on top not moving			1 adult scat	5	5	0%	
Scat 039	Larvae & Pupae	11					2	2	60%	
Scat 005	Larvae & Pupae	12					0	0	100%	
Scat 020	Larvae & Pupae	13					1	1	80%	
Scat 037	Larvae & Pupae	14				1 adult scat	4	4	20%	
Scat 029	Larvae & Pupae	15				1 adult scat	10	10	-100%	
Scat 040	Larvae & Pupae	16	Larvae on top not moving			1 adult scat	1	1	80%	
Scat 027	Larvae & Pupae	17					1	2	60%	(ontsnapt)
Scat 019	Larvae & Pupae	18					0	0	100%	
Scat 009	Larvae & Pupae	19	Larvae on top not moving				5	5	0%	
Scat 021	Larvae & Pupae	20	Larvae on top not moving				0	0	100%	
Scat 013	Larvae & Pupae	21					4	4	20%	
Scat 031	Larvae & Pupae	22					0	0	100%	
Scat 030	Larvae & Pupae	23			mots only		0	0	100%	
Scat 011	Larvae & Pupae	24					0	0	100%	
Scat 016	Larvae & Pupae	25					0	0	100%	
Scat 035	Larvae & Pupae	26	Larvae on top not moving				0	0	100%	
Scat 028	Larvae & Pupae	27					2	3	40%	(1ontsnapt
Scat 012	Larvae & Pupae	28					0	0	100%	
Scat 024	Larvae & Pupae	29					0	0	100%	
Scat 036	Larvae & Pupae	30					0	0	100%	
Scat 004	Larvae & Pupae	31					3	3	40%	
Scat 006	Larvae & Pupae	32					0	0	100%	
Scat 008	Larvae & Pupae	33	Larvae on top not moving				0	0	100%	
Scat 023	Larvae & Pupae	34					0	0	100%	
Scat 007	Larvae & Pupae	35					3	3	40%	
Scat 038	Larvae & Pupae	36					0	0	100%	
Scat 032	Larvae & Pupae	37					7	7	0%	
Scat 033	Larvae & Pupae	38	mothfly alive			mots	1	1	0%	42mots
Scat 025	Larvae & Pupae	39	mothfly alive			mots & 1adult scat	0	0	0%	6*sciara* 5 mots
Scat 018	Larvae & Pupae	40	mothfly alive			mots	1	12	0%	11*sciara* 8mothes

1.5 Plant quality after 8 weeks

treatment code	cultivar	insect	temperature (°C)	time (h)	CO2 (%)	O2 (%)	code HIA	plant quality
1	White		36	8	0	21	White 001	3
2	White		36	24	0	21	White 002	3
3	White		36	8	10	15	White 003	3
4	White		36	24	10	15	White 004	2
5	White		36	8	65	1	White 005	3
6	White		36	24	65	1	White 006	1
7	White		36	8	65	25	White 007	3
8	White		36	24	65	25	White 008	
9	White		39	8	0	21	White 009	3
10	White		39	24	0	21	White 010	3
11	White		39	8	10	1	White 011	2
12	White		39	24	10	1	White 012	1
13	White		39	8	10	15	White 013	3
14	White		39	24	10	15	White 014	3
15	White		39	8	10	25	White 015	3
16	White		39	24	10	25	White 016	2
17	White		39	8	40	1	White 017	3
18	White		39	24	40	1	White 018	
19	White		39	8	40	15	White 019	3
20	White		39	24	40	15	White 020	
21	White		39	8	40	25	White 021	2
22	White		39	24	40	25	White 022	
23	White		39	8	65	1	White 023	3
24	White		39	24	65	1	White 024	
25	White		39	8	65	15	White 025	3
26	White		39	24	65	15	White 026	
27	White		39	8	65	25	White 027	2
28	White		39	24	65	25	White 028	
29	White		42	8	0	21	White 029	3
30	White		42	24	0	21	White 030	
31	White		42	8	10	15	White 031	2
32	White		42	24	10	15	White 032	
33	White		42	8	65	1	White 033	3
34	White		42	24	65	1	White 034	
35	White		42	8	65	25	White 035	3
36	White		42	24	65	25	White 036	
37	White		20	24	0	21	White 037	3
38	White		20	24	0	21	White 038	
39	White		20	24	0	21	White 039	3
40	White		20	24	0	21	White 040	
1	Pink		36	8	0	21	Pink 001	3
2	Pink		36	24	0	21	Pink 002	3
3	Pink		36	8	10	15	Pink 003	3
4	Pink		36	24	10	15	Pink 004	3
5	Pink		36	8	65	1	Pink 005	3
6	Pink		36	24	65	1	Pink 006	2
7	Pink		36	8	65	25	Pink 007	3
8	Pink		36	24	65	25	Pink 008	1
9	Pink		39	8	0	21	Pink 009	3
10	Pink		39	24	0	21	Pink 010	2
11	Pink		39	8	10	1	Pink 011	3
12	Pink		39	24	10	1	Pink 012	1
13	Pink		39	8	10	15	Pink 013	3
14	Pink		39	24	10	15	Pink 014	3
15	Pink		39	8	10	25	Pink 015	3
16	Pink		39	24	10	25	Pink 016	2
17	Pink		39	8	40	1	Pink 017	3
18	Pink		39	24	40	1	Pink 018	
19	Pink		39	8	40	15	Pink 019	3
20	Pink		39	24	40	15	Pink 020	
21	Pink		39	8	40	25	Pink 021	3
22	Pink		39	24	40	25	Pink 022	
23	Pink		39	8	65	1	Pink 023	3
24	Pink		39	24	65	1	Pink 024	
25	Pink		39	8	65	15	Pink 025	3
26	Pink		39	24	65	15	Pink 026	
27	Pink		39	8	65	25	Pink 027	3
28	Pink		39	24	65	25	Pink 028	
29	Pink		42	8	0	21	Pink 029	3
30	Pink		42	24	0	21	Pink 030	
31	Pink		42	8	10	15	Pink 031	3
32	Pink		42	24	10	15	Pink 032	
33	Pink		42	8	65	1	Pink 033	3
34	Pink		42	24	65	1	Pink 034	
35	Pink		42	8	65	25	Pink 035	3
36	Pink		42	24	65	25	Pink 036	
37	Pink		20	24	0	21	Pink 037	3
38	Pink		20	24	0	21	Pink 038	3
39	Pink		20	24	0	21	Pink 039 (1)	3
39	Pink		20	24	0	21	Pink 039 (1)	2
40	Pink		36	8	0	21	Pink 040	3

To explore the potential of nature to improve the quality of life

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