Factors Affecting Petri Dish Condensation in Tissue Culture (CU) Chambers

By

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Foreword

Hello Reader,

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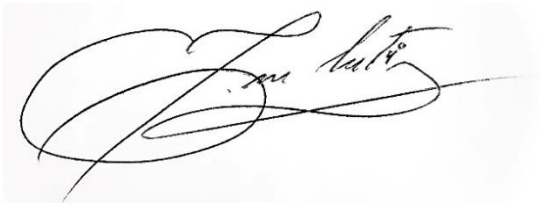
Henry Imberti, Sr VP of Engineering



Introduction

In this report, we will investigate the effects of infrared lighting, light intensity, stacking, as well as constant temperature and varied temperature between the day and night cycle. We will also investigate how the amount of condensation changes throughout the course of the day and how petri dish placement inside the chamber affects condensation. The goal of this report is to provide a comprehensive analysis of the best ways to reduce condensation in tissue culture chambers.

Materials and Methods

This experiment was conducted in a Percival Scientific CU-36L5 chamber, specifically SO #IO3FI (Figure 1A). The chamber was fitted with a fluorescent light (FL) (Philips F17T8/TL841 Alto, 367938) on the top tier. The second, fourth, and fifth tiers contained two standard SciWhite (SW) tiles with a color temperature of 4000K and a CRI of 90. The third tier contained a PetriClear (SW+IR) tile with a color temperature of 5500K and a CRI of 90 which aimed to match the fluorescent light composition and had alternating SciWhite and Infrared LED chips (Figure 1B). The infrared lighting tiles used in this experiment had an equal number of infrared and white LEDs. This chamber is ideal for our testing purposes as it matches the style of chamber that previously had condensation issues.

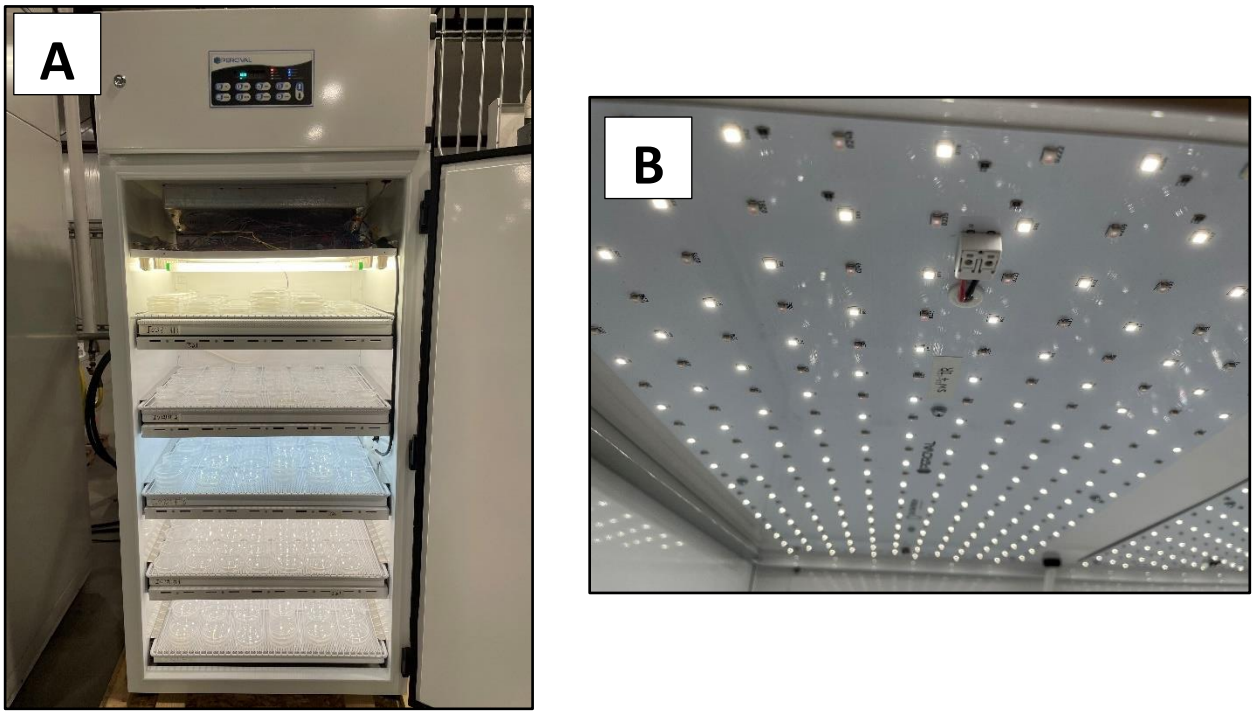


Figure 1: Percival Scientific CU-36L5 Chamber with the door open (A) and Tile Design of the SW+IR tile (B)
A: A CU-36L5 chamber was fitted with Fluorescent tubes on Shelf 1, SciWhite tiles on Shelves 2, 4 & 5, and a SW+IR tile on Shelf 3. You can see that the SciWhite LEDs on the SW+IR shelf are slightly cooler in temperature
B: Image of the SW+IR tile showing the alternating SciWhite and Infrared LED chips. As a note, you can tell that the infrared is on by the slightly pink color on them, this can be captured by most cameras but cannot be seen by the naked eye.

All experiments were conducted using a photoperiod of 14/10, specifically lights on from 6 am to 8 pm, and all lights, except for the infrared LEDs, were turned off from 8 pm to 6 am. The infrareds were on for the entirety of the day/night cycle. For the first and third experiments, the temperature was held constant throughout the day/night cycle at 28°C. The second and fourth experiments were conducted at a day temperature of 28°C and a night temperature of 20°C to test the effect of temperature differential between the day and night cycles. All experiments were conducted over the course of at least 2 days, except for the amount of condensation occurring throughout the day which monitored the amount of condensation over the course of a single day. Unless otherwise noted, all measurements were taken between 8:00 am and 8:30 am. As shown in Experiments 5, 6, & 7, the amount of condensation decreased throughout the day, so this gave us a better estimate of the worst-case scenario.

Experiments were conducted at light intensities ranging from 50±3 µmol m^{-2} s^{-1} to 140±5 µmol m^{-2} s^{-1}. Light intensities were measured weekly using a LI-COR LI-180 Spectrometer and measured at the center of the chamber. Shelves were filled with petri dishes at 70% max capacity, 36 petri dishes in a 6x6 grid (Figure 2A), and at 100% capacity (Fully Loaded), 49 petri dishes in a 7x7 grid (Figure 2B). Single indicates a single petri dish (Figure 3B). For the tier that contained stacked dishes, triple stacked indicates three petri dishes stacked on top of each other (Figure 3C), double stacked indicates two petri dishes stacked on top of each other (Figure 3C), half empty dish indicates conditions where the top or bottom of an empty petri dish was placed on top of a petri dish (Figure 3E), empty dish indicates where an empty petri dish was placed on top of the petri dish (Figure 3F). The term “underneath” indicates where an empty petri dish was placed underneath a stack of petri dishes (Figure 3D).

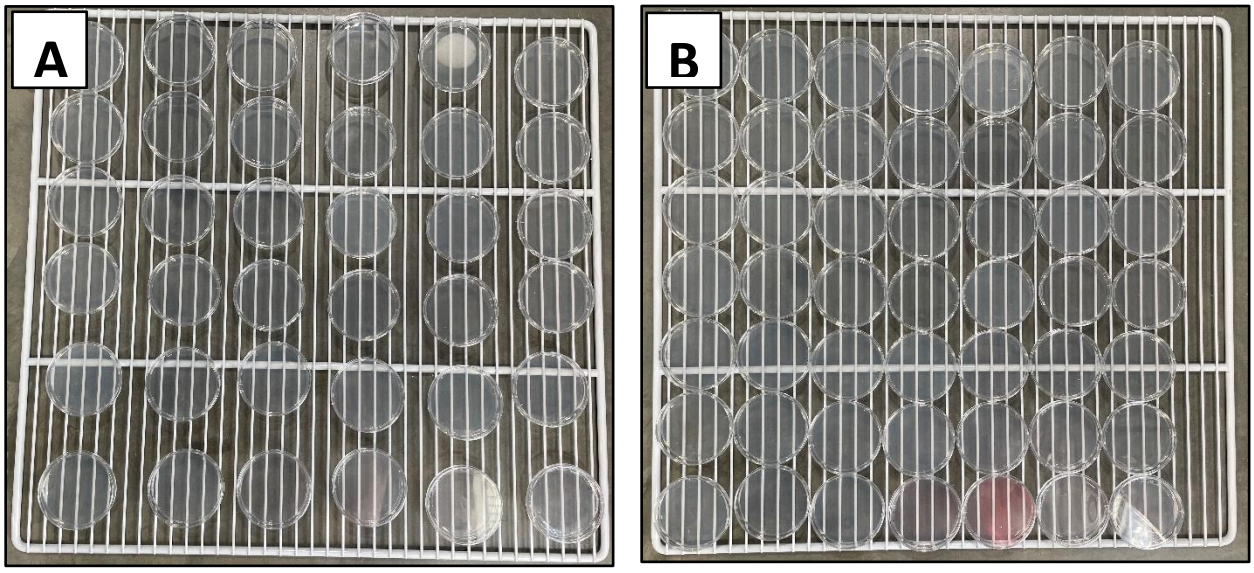


Figure 2: Difference between the 70% - Control (A) which had 36 petri dishes and the 100% - Fully Loaded (B) condition which had 49 plates on a shelf

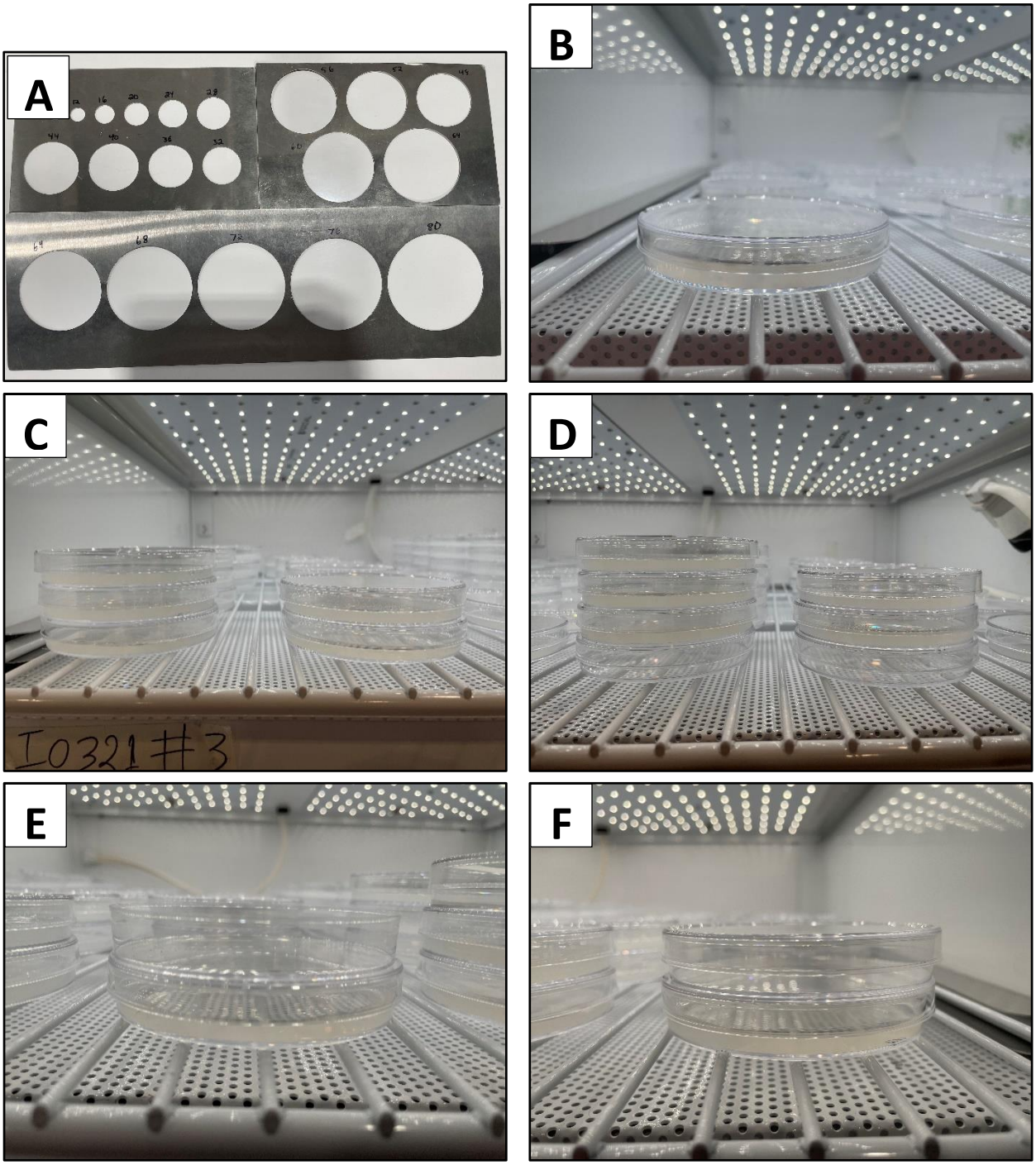


Figure 3: Condensation gauges that were used which ranged from 4-80 mm in diameter in 4 mm increments (A) Differences in stacked petri dish conditions: Single Stacked (B), Triple stacked (C, Left), Double stacked (C, Right), Triple stacked w/ Underneath (D, Left), Double stacked w/ Underneath (D, Right), ½ empty petri dish placed on top (E), and whole empty petri dish placed on top (F)

The area of condensation was measured using a series of gauges (Figure 3A) that ranged from 4 to 80 mm in diameter in increments of 4 mm, the area of condensation was then calculated using the following equation, where D is the diameter.

$$Area = \frac{(D^2\pi)}{4} \ (Equation\ 1)$$

Percent condensation was calculated by dividing this area by the total area of the petri dish lid, which was 5542 mm^2 or 8.6 in^2. As a note, the condensation was not always circular in shape as the gauges were, and in these instances our best judgement was used to estimate the amount of condensation was present as if it were a circular shape. This was done by matching the amount of empty space inside of the circular gauge to the amount of condensation that was outside of the gauge.

The petri dishes in the experiment contained 0.8% agar (Research Products International, Phyto Agar, CAS 9002-18-0). The procedure for making the petri dishes was as follows.

1. Add RO water to a glass bottle or mason jar with a plastic cap no more than ½ full.
2. Add Phyto Agar at a concentration of 8 g/L to the glass bottle, do not stir/shake.
3. Microwave for 6-8 minutes, until boiling occurs, and all the agar has fully dissolved, the solution should be clear with a yellowish tint and no particles floating in it. If needed, gently swirl the bottle to fully dissolve the agar near the end of microwaving. When microwaving, it is critical to loosen the lid ¼ to ½ turn to allow for steam to escape while keeping the lid secure.
4. Allow to cool to room temperature until you can touch the jar comfortably, do not allow the liquid agar to sit at room temperature for too long or the agar will solidify in the bottle. (If it does, add more RO water and microwave again, it should return to liquid phase)
5. Sterilize the area where you will be pouring the plates with 10% bleach or another cleaning solution. Ideally, this would all be done under a laminar flow hood to minimize contamination.
6. Wearing gloves, briefly remove the lid and pour the agar into a petri dish between 70% and 90% full, this will be 30-40 mL for standard 100mm petri dishes. Partially cover the petri dish with the lid until the agar has cooled, the agar should solidify within 10-15 minutes.
7. Place the lid fully back onto the bottom petri dish, store the petri dishes for at least 24 hours at the experimental conditions before taking any measurements to prevent condensation that was formed during the pouring process to impact the results.

Petri dishes in this experiment were used until medium sized bacterial colonies had formed and were replaced with new petri dishes as needed. On Fridays, any small colonies of bacteria were sprayed with 10% bleach in order to extend the life of the plates.

Results & Discussion

Test #1: Effect of Petri dish placement inside the chamber

The first test was conducted to see how much of an effect petri dish placement on the shelf had on the amount of condensation present. As shown in Tables 1A & 1B, petri dishes at the front of the chamber had more condensation than those at the back of the chamber. This is likely caused by the plenum design and the way that air is circulated in the chamber. Another factor in this is that the petri dishes at the front of the chamber had more exposure to ambient conditions than those at the back of the chamber while measurements were taken since the door was open for 10-to-30-minute intervals while measurements were taken.

Environmental Conditions

Light Intensity	50 µmol m^{-2} s^{-1}	**Photoperiod**	14/10	**Day Temp.**	28°C
Light Type	SciWhite	**Loading**	70% and 100%	**Night Temp.**	28°C

Control – Percent Condensation (A)

Distance from Left Hand Corner (in)		3.5	7.5	11.5	16	21	25.5		
		A	B	C	D	E	F	Average	S.D.
3	1	0%	0%	0%	0%	0%	0%	0%	0%
7.5	2	0%	0%	0%	0%	0%	1%	0%	0%
12	3	0%	0%	0%	0%	0%	2%	0%	1%
16.5	4	0%	13%	11%	36%	29%	3%	15%	14%
20.5	5	28%	30%	16%	27%	14%	11%	21%	8%
25	6	19%	11%	16%	14%	0%	0%	10%	8%
	Average	8%	9%	7%	13%	7%	3%	8%	
	S.D.	12%	12%	8%	16%	12%	4%		11%

Fully Loaded, Percent Condensation (B)

Distance from Left Hand Corner (in)		3	6.5	10	14	17.5	21.5	25		
		A	B	C	D	E	F	G	Average	S.D.
2	1	0%	0%	0%	0%	0%	0%	0%	0%	0%
6.5	2	0%	0%	0%	0%	0%	0%	0%	0%	0%
10.5	3	0%	0%	0%	0%	0%	0%	0%	0%	0%
14	4	0%	11%	0%	2%	0%	0%	0%	1%	3%
17.5	5	0%	11%	19%	14%	0%	0%	9%	12%	7%
21	6	8%	23%	30%	23%	16%	20%	21%	24%	9%
24.5	7	19%	16%	42%	35%	14%	14%	41%	19%	14%
	Average	9%	8%	12%	9%	5%	7%	6%	8%	
	S.D.	13%	12%	16%	15%	6%	9%	11%		10%

Table 1: Percent Condensation under various loading conditions, Control at 70% Capacity, 36 petri dishes (A), and Fully Loaded at 100% Capacity, 49 petri dishes (B). No Temperature Differential between day and night temperature. Under 50 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod. All measurements were taken at 8 am. Data are means of 2 replicates, averages and standard deviation by row, column, and overall is shown
Light green indicates the least amount of condensation, yellow is the average, and red is the maximum.

Test #2: Effect of Shelf Tier Placement on Percent Condensation

The second test was conducted to see how much shelf-to-shelf variation there was in the chamber, after noticing inconsistencies between data collected on Shelf 2 and Shelves 4 and 5 (Figure 4). This shelf-to-shelf variation was likely caused by the Fluorescent lighting since it gave off more excess heat than the LED tiles on the other tiers did. It is important to note that Fluorescent lights were installed on Shelf 1, and SW+IR lighting was installed on Tier 3. This excess heat from the top shelf led to slight temperature differentials between the shelves caused by the way air flows throughout the chamber when the door is closed. As a result of this test, all the experimental results shown in the figures below, except for Fluorescent and SW+IR, were conducted on either Shelf 2 or Shelf 4 and 5. This causes some issues when trying to compare one graph versus another, but overall, this shelf-to-shelf was likely between 5% and 10% and had a negligible effect on the conclusions drawn in this report.

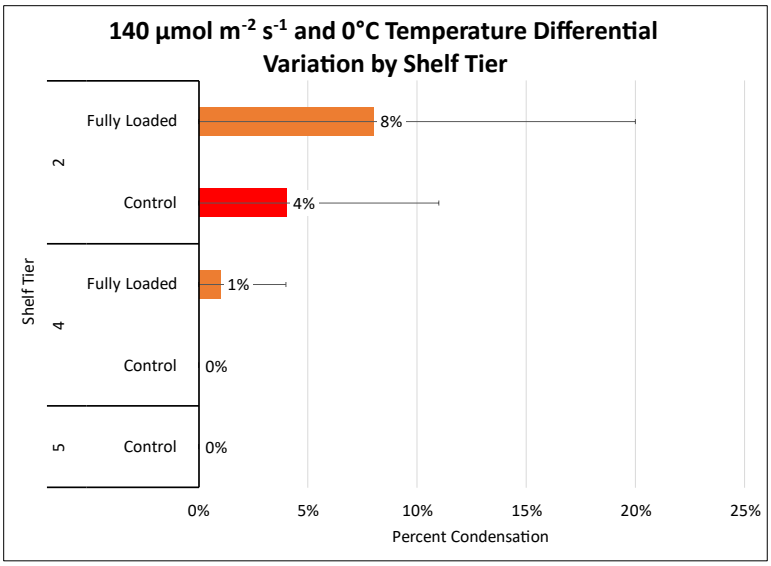


Figure 4: Percent Condensation variation by tier, under both Fully Loaded (Orange) and Control (Red) loading conditions. Under 140 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod, and no temperature differential.
All measurements were taken at 8 am.
Data are means of 72 (Control) and 98 (Fully Loaded) replicates ± S.D.

Experiments 1 & 2: 50 µmol m^{-2} s^{-1} Light Intensity, 0°C & 8°C Temp. Differential

Under 50 µmol m^{-2} s^{-1} light intensity, petri dishes under SW+IR lighting had less condensation than all other experimental conditions (Figure 5A & 5B). While there does not appear to be a drastic change between SW+IR, Fully Loaded, or Control conditions this is largely due to the petri dish placement inside the chamber as mentioned above (Figure 4), and the difference in the front two rows of the chamber was more significant (Tables 1A & 1B). At an 8°C Temperature differential, condensation was increased in the Double and Triple stacked conditions on the bottom petri dish (Figure 5A & 5B) compared to no temperature differential. It is important to note that the top and middle petri dishes under these conditions did not show significant levels of condensation and are not shown in the figures. Condensation also increased in both the Fully Loaded and Control conditions under an 8°C Temperature differential as compared to 0°C temperature differential. Condensation is formed when cool air from the plenum flows over the petri dish during the night cycle, since the temperature inside of the petri dish to is slightly above the temperature of the air outside, causing condensation to form on the inside lid of the petri dish. When there is a temperature differential between day and night conditions the effect is more extreme because the outside of the petri dish cools down much faster than the inside of a petri dish can. The humid, warm air on the inside of the petri dish then condenses on the inside lid of the petri dish, which is cooler due to the temperature differential on the outside of the petri dish. Petri dishes under SW+IR lighting did not form condensation under an 8°C temperature differential, which means that the addition of infrared lighting was able to prevent condensation from forming by heating up the inside of the petri dish lid. Previous studies have shown that temperature differentials as low as 0.5°C can cause condensation to form on the lid of petri dishes (Finer).

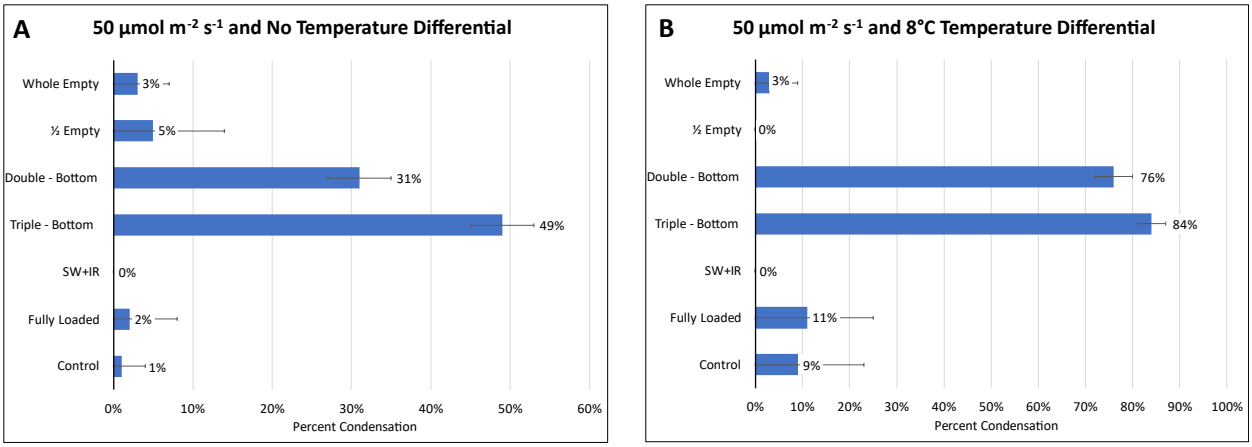


Figure 5: Percent Condensation under varying Day/Night temperature differentials, No Temperature Differential (A) and 8°C Temperature Differential (B). Under 50 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod. All measurements were taken at 8 am.
Data are means of at 12-98 replicates ± S.D.

Experiments 3 & 4: 140 µmol $m^{-2}s^{-1}$ Light Intensity, 0°C & 8°C Temp. Differential

Experiments conducted at 140 µmol m^{-2} s^{-1} had similar results to those conducted at 50 µmol m^{-2} s^{-1} as more condensation was observed at an 8°C temperature differential than at no temperature differential (Figure 6A & 6B). This difference was most notable under Triple stacked conditions as the bottom dish went from 36% average condensation to 87% when a temperature differential was introduced. The middle petri dish under this condition increased from 0% to 12%. SW+IR was identical to Fluorescent lighting under this scenario, as both produce extra heat compared to the control condition that warms up the inside of the petri dish lid and prevents condensation from forming on the petri dish lid.

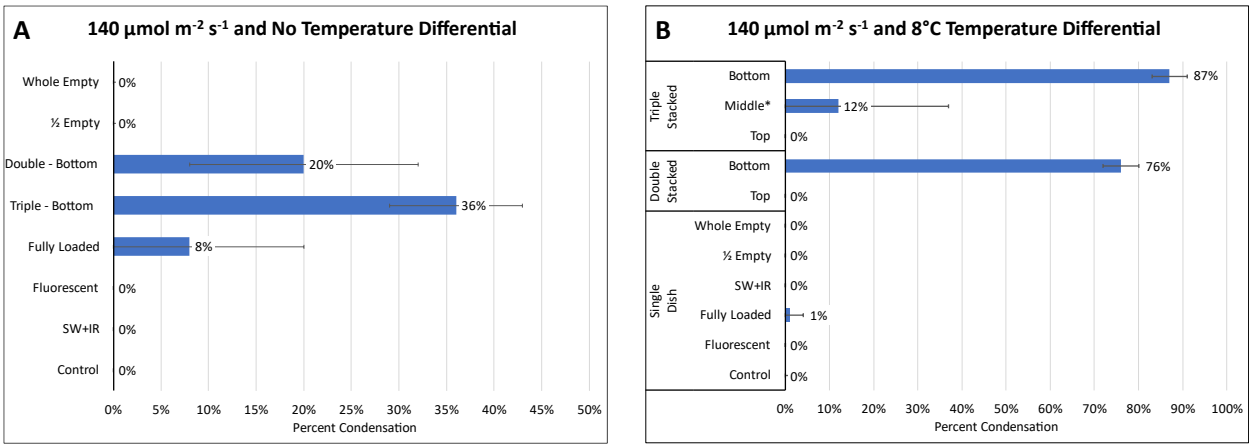


Figure 6: Percent Condensation under varying Day/Night temperature differentials, No Temperature Differential (A) and 8°C Temperature Differential (B). Under 140 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod. All measurements were taken at 8 am.
Data are means of at 12-144 replicates ± S.D.
*large S.D. was noticed under this condition, as the front few rows of the chamber had 50+% condensation on the middle petri dish, while the back rows had no condensation on the middle petri dish, similar to Table 1A & 1B

Experiment 5: 140 µmol $m^{-2}s^{-1}$ Light Intensity, 8°C Temp. Differential, Throughout the Course of One Day

Experiment 5 was conducted in order to assess how the time measurements were taken affected the amount of condensation formed. This experiment was conducted at 140 µmol m^{-2} s^{-1} and an 8°C temperature differential. In general, the amount of condensation decreased as the day went on. This is because the excess heat generated from the lights warmed up the lid of the petri dish throughout the course of the day, eliminating condensation that had formed during the night cycle. There were a few cases in which the condensation increased from the 0.5 to the 2-hour mark. This is likely due to the amount of time the door was open for measurements to be taken, since the ambient environment was around 24°C compared to the desired temperature of 28°C. It took roughly 30 minutes to measure all the stacked petri dishes on the various shelves, leaving only one hour for conditions to settle. This led to increased condensation on a few data points. Data taken at hours 6 and 10 had more than 3 hours to reach steady state before the door was opened, which is why this trend was not observed at either of those time periods. Triple and double stacking petri dishes caused there to be much more condensation than when there is only

a single petri dish (Figure 7A), with triple stacking having a more extreme effect. The top dish in the triple stacked conditions had more condensation under fluorescent lighting than under the control or SW+IR (0% throughout) conditions (Figure 7B). This is likely because the dish was so close to the lights that there was a significant difference in temperature between the lid of the petri dish and the cool air being supplied by the chamber, causing a temperature imbalance in the petri dish. This difference was less extreme in the control conditions since the SciWhite LED tiles put off less heat than the inefficient fluorescent bulbs. On the middle petri dish in triple stacked conditions, there was less condensation on petri dishes under SW+IR lighting than under control or fluorescent lighting (Figure 7C), which means the infrared kept the surface of both lids warm enough to prevent condensation from forming. This means that the infrared light can penetrate through a petri dish with agar, which could potentially cause issues if the agar gets too much heat and warms up, causing heat stress in plants. This was briefly tested in Experiment 9, and there did not appear to be any issues caused by the infrared lighting (Figure 11). More tests would need to be conducted to see if the infrared lighting is an issue for plant growth. On the bottom dish under both triple and double stacked conditions, the most condensation occurred (Figures 7D & 7F). This is because the dishes are the closest to the cool air being circulated by the plenum, and the furthest from the light source which supplies heat that reduces the condensation. Under both situations, SW+IR did the best job at reducing the amount of condensation in a short time and had less condensation on average. Placing an empty petri dish underneath the stacked petri dishes caused less condensation to form under both the control (SciWhite) and SW+IR lighting conditions (Figure 7B-7F). This is likely because the air being supplied by the chamber through the plenum had more time to warm up before contacting the petri dishes, leading to a smaller temperature differential and less condensation. Interestingly, the opposite was noticed under Fluorescent lighting (Figure 7B-7F). This is because the empty petri dish underneath put the other petri dishes closer to the fluorescent light fixture that radiates much more heat than the LED tiles and caused a temperature differential that led to the formation of condensation.

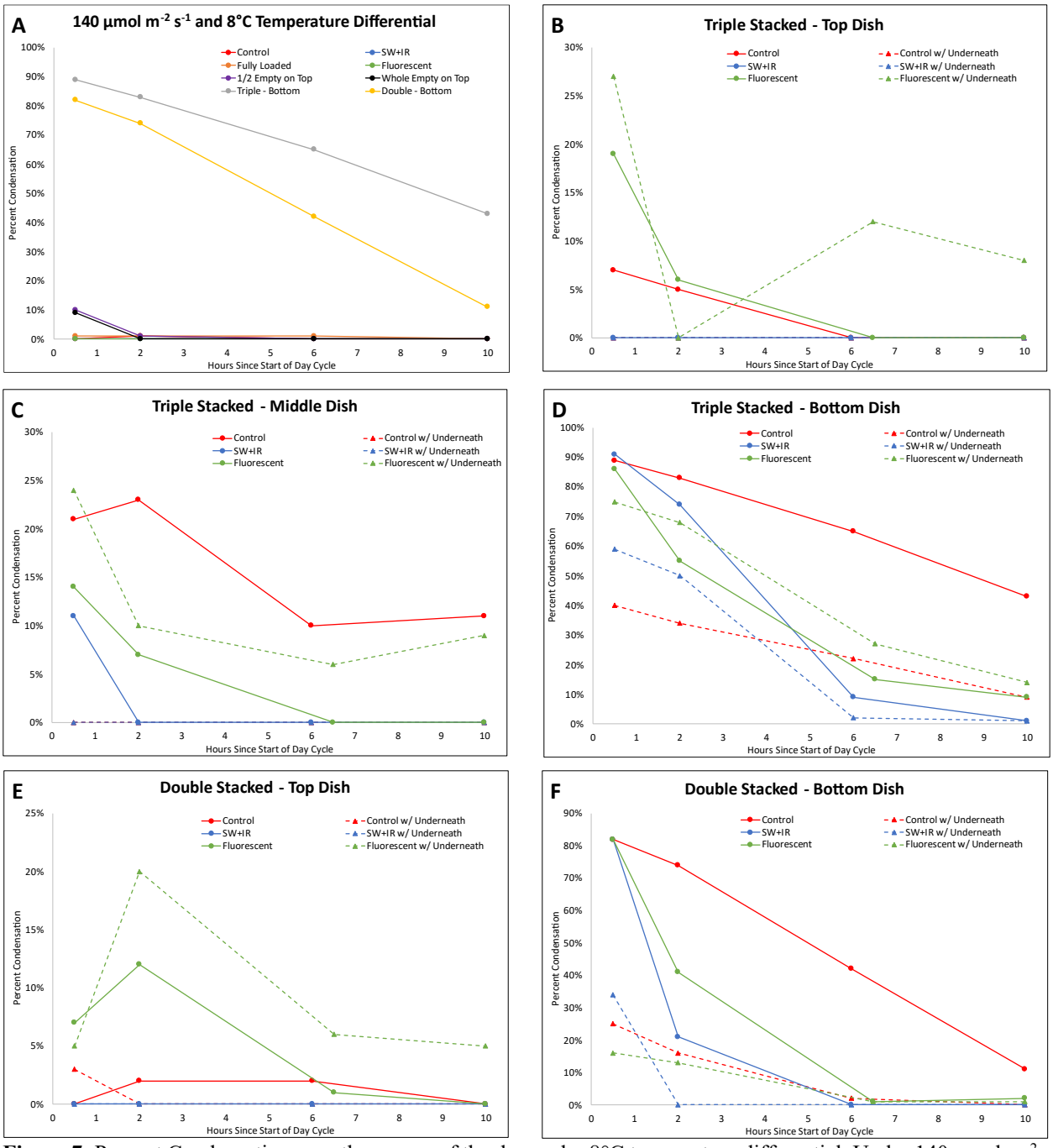


Figure 7: Percent Condensation over the course of the day under 8°C temperature differential. Under 140 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod. Comparisons were between all previously tested conditions (A), Triple stacked petri dishes under SciWhite (control), SW+IR, and Fluorescent lighting with and without an empty petri dish underneath the stack, the condensation on the top (B), middle (C), and bottom (D) petri dishes were observed. Double stacked petri dishes under SciWhite (control), SW+IR, and Fluorescent lighting with and without an empty petri dish underneath the stack, the condensation on the top (E) and bottom (F) petri dishes were observed.
Hour '0' indicates the start of the Day cycle, at 6:00 am.
Data are means of at 6-49 replicates ± S.D, conducted over the course of one day.

Experiment 6: 50 µmol $m^{-2}s^{-1}$ Light Intensity, 8°C Temp. Differential, Throughout the Course of One Day

Experiment 6 was conducted to observe how the amount of condensation varied over the course of the day under 50 µmol m^{-2} s^{-1} and the same temperature differential of 8°C as in Experiment 5, which was conducted at 140 µmol m^{-2} s^{-1}. This experiment only compares the Control (SciWhite), SW+IR, and SW+IR with a petri dish underneath since the fluorescent light bulbs could not change light intensity. The results of this experiment were consistent with Experiment 5 in that condensation was reduced throughout the day, the bottom petri dish had the most condensation, and that an empty petri dish underneath stacked petri dishes helped to reduce the amount of condensation formed under LED lighting (Figure 8A & 8B). Additionally, the SW+IR light fixture did a better job than the control at reducing condensation in a shorter amount of time. With both the stacked and stacked with an empty petri dish underneath condition having virtually no condensation at the 6-hour mark for double stacked and 10-hour mark for triple stacked. It is best to plan experiments and data collection at times where condensation has been shown to be the lowest, near the end of the day cycle. Additionally, thanks to the functionality of the Intellus system on Percival chambers, the starting point of the day cycle can be manipulated so that measurements are taken at the end of the day cycle. It is important to note that no condensation was observed on the top petri dish in either condition (not shown).

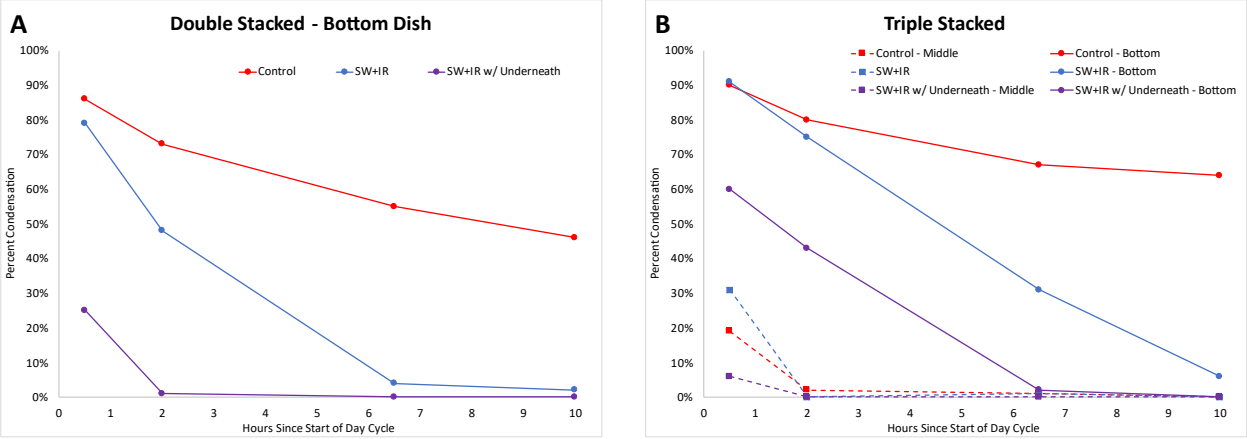


Figure 8: Percent Condensation over the course of the day under 8°C temperature differential. Under 50 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod. Comparisons of Double stacked (A) and Triple stacked (B) petri dishes under SciWhite (control) and SW+IR with and without an empty petri dish underneath the stack.
Hour '0' indicates the start of the Day cycle, at 6:00 am.
Data are means of at 6-36 replicates ± S.D, conducted over the course of one day.

Experiment 7: 50 µmol $m^{-2}s^{-1}$ Light Intensity, 0°C Temp. Differential, Throughout the Course of One Day

Experiment 7 was conducted to observe how the amount condensation changed throughout the day when there was no change in temperature between the day and night cycles. Compared to Experiment 5 (Figure 7) and 6 (Figure 8), there was a much smaller change in condensation throughout the course of the day (Figure 9). Only the bottom dish in the double stacked condition experienced a significant change in condensation throughout the course of the day. Any condensation that had formed was not significantly reduced under the control (SciWhite lighting), and the SW+IR lighting prevented condensation from being formed at all. This also helps to validate that any light source helps to reduce condensation by heating up the lid of the petri dish, since all the experimental conditions had slightly less condensation as time went on. As a note, the control was on Tier 2 for this experiment, while fully loaded was on Tier 4 and the shelf-to-shelf variation (Figure 4) is likely what caused the fully loaded to have less condensation than the control in this experiment.

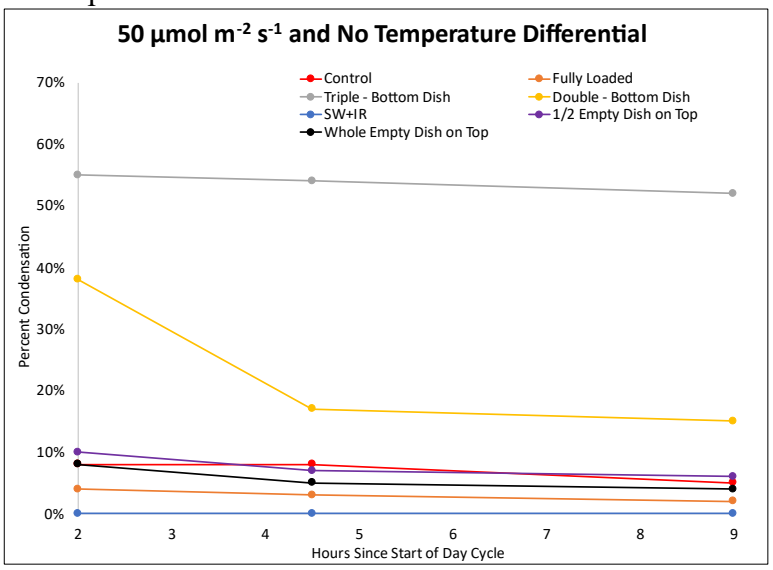


Figure 9: Percent Condensation over the course of the day under no temperature differential.
Under 50 µmol m^{-2} s^{-1} light intensity, 14/10 hour photoperiod.
Hour '0' indicates the start of the Day cycle, at 6:00 am.
Data are means of at 6-49 replicates ± S.D, conducted over the course of one day.

Experiment 8: 50 & 140 µmol $m^{-2}s^{-1}$ Light Intensity, 8°C Temp. Differential, With and Without Ramped Temperature Change

Experiment 8 was conducted to understand if ramping the 8°C temperature change reduced condensation compared to an immediate temperature change. In these experiments, the "Ramped" condition experienced the 8°C temperature change over the course of 2 hours in 1°C increments every 15 minutes at both the beginning and end of the day cycle. The "Immediate" condition was programmed to achieve the temperature change as quickly as possible. The chamber was able to achieve this temperature change in less than 15 minutes while warming up and while cooling down. The effect of ramping was tested at both 50 µmol m^{-2} s^{-1} and 140 µmol m^{-2} s^{-1}. Ramping was shown to reduce the amount of condensation compared to the immediate temperature change in all stacking conditions at 140 µmol m^{-2} s^{-1} (Figure 10A & 10B). At 50 µmol m^{-2} s^{-1} condensation in stacked petri dishes was slightly higher under ramped conditions than under the immediate temperature change, this is likely due to a combination of natural variation, since the differences are minor, and that measurements were taken 15 minutes after the final temperature change in ramped conditions, and 2 hours after the temperature change in immediate conditions.

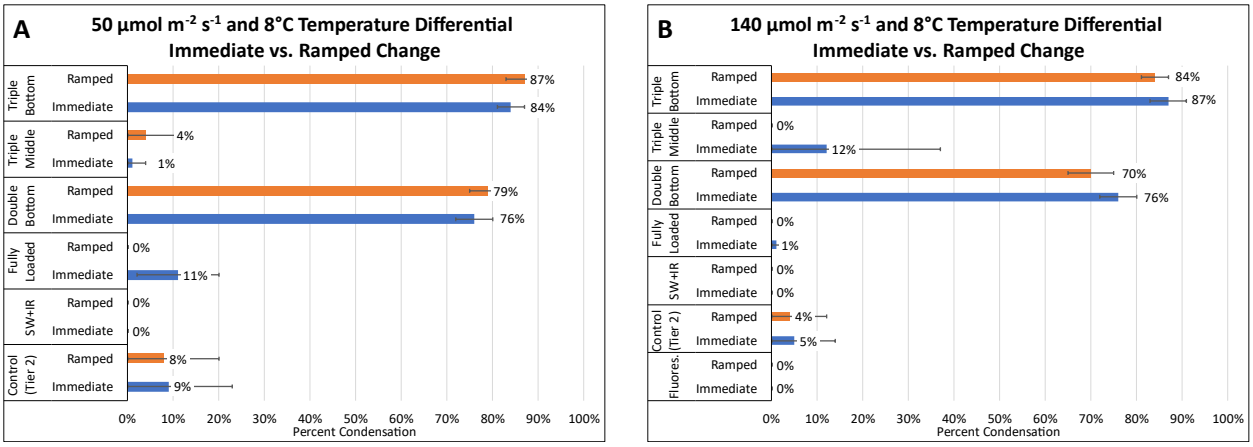


Figure 10: Percent condensation under an 8°C temperature differential comparing the effects of Immediate and Ramped temperature change. Conducted at 50 µmol m^{-2} s^{-1} (A) and 140 µmol m^{-2} s^{-1} (B). 14/10 hour photoperiod All measurements were taken at 8am.
Data are means of 12-98 replicates ± S.D.

Experiment 9: Effect of Infrared Lighting on Plant Growth

Experiment 9 was conducted to test whether the infrared lighting used by the SW+IR tiles would have any effect on plant growth in a tissue culture environment. Only two plates were used in this study and were donated by the Walley Lab in the Plant Pathology, Entomology, and Microbiology Department at Iowa State University. The plates contained *Arabidopsis thaliana* Wild Type and Mutant seeds that had been used for a separate root assay and were placed into the chamber at 3.5 weeks old. Our main concern was that the added infrared lighting could cause the agar to increase in temperature and cause heat stress in the plants, since it was shown to reduce condensation in the bottom petri dishes in stacked conditions. Based on the results of this experiment (Figure 11), there does not appear to be any negative effects caused by the infrared lighting and plants grown under SW+IR and SciWhite lighting appeared identical. There did not appear to be any morphological or color differences between the two conditions. The chamber was set to maintain a constant temperature which should account for any additional heat produced by the infrared LEDs. Any temperature difference created by the infrared LEDs on the agar is likely too small to cause any noticeable differences between the plants. Previous studies have shown variation in hypocotyl and cotyledon growth under large (9°C) temperature differentials in *Arabidopsis thaliana* (Afshar), although no experiments were found that studied the effects of minor temperature changes like what the infrared may cause. Further experiments with much larger sample sizes would need to be conducted over the life cycle of the plants in order to verify that the infrared lighting does not influence plants grown on petri dishes or in in vitro culture.

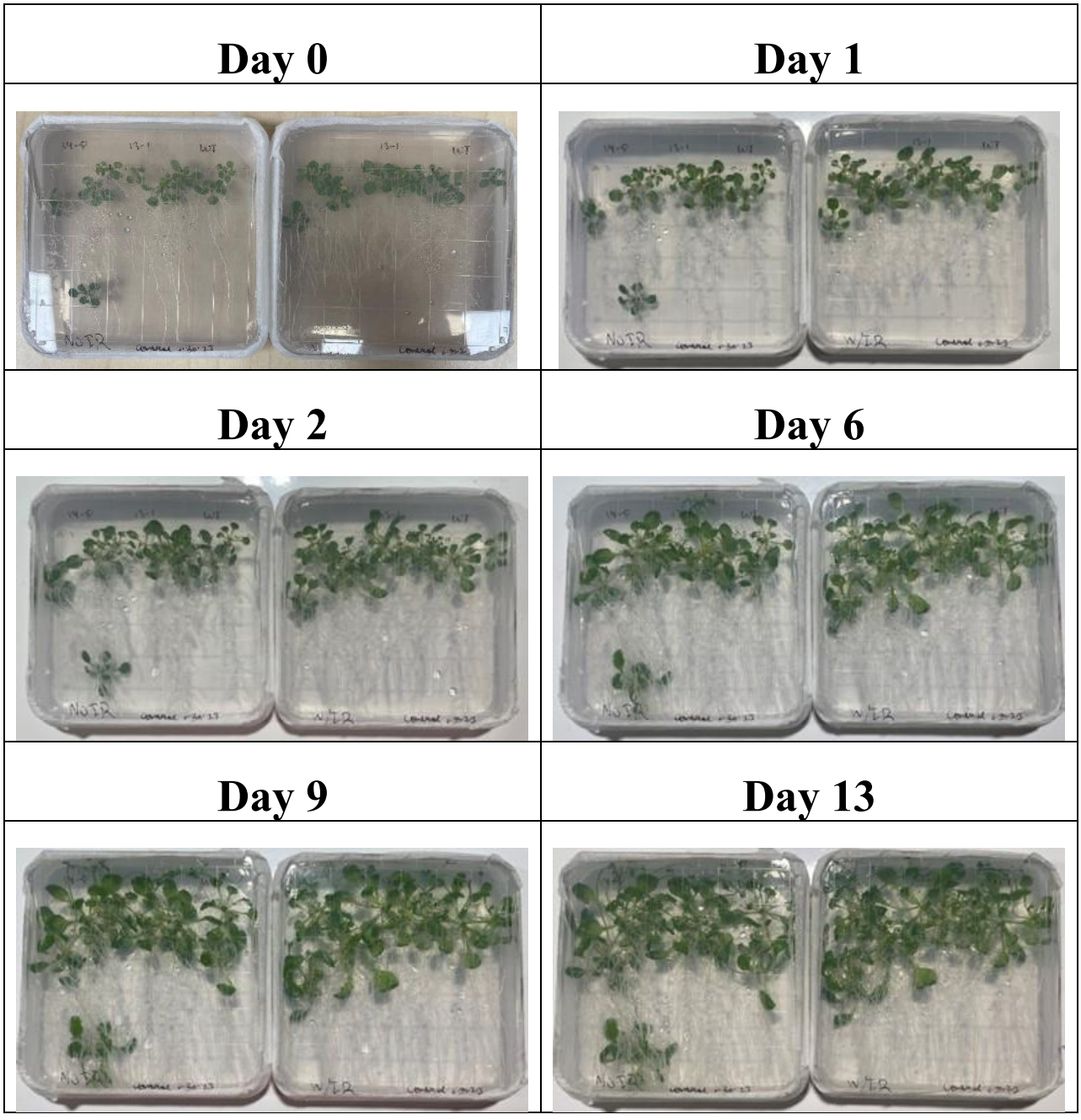


Figure 11: Comparison of *Arabidopis thaliana* seedlings grown under SciWhite (Left, labeled No IR) and SW+IR (Right, labeled W/ IR) conditions. Plants were brought to conditions at 3.5 weeks and left for 10 days.

Conclusion

Overall, light intensity, temperature differential, stacking, shelf loading, time since the start of the day/night cycle, placement inside of the chamber, and ramping all impacted amount of condensation formed on the petri dish lid, although at varying degrees.

SW+IR lighting was found to be one of the best ways to reduce the amount of condensation formed. No condensation was formed on single stacked petri dishes in the temperature range of 20-28°C, light intensity of 50-140 µmol m^{-2} s^{-1} under normal and fully loaded conditions. Condensation on the bottom petri dishes in double and triple stacked conditions was observed, but this condensation was less than under SciWhite and Fluorescent lighting and went away in a shorter amount of time.

As light intensity increased, the amount of condensation formed decreased in all trials. This is because when the light intensity increases there is more heat being produced by the LEDs, since they are running at higher voltages, which keeps the surface temperature of the lid slightly warmer which reduces the amount of condensation that is formed. While increasing the light intensity does reduce condensation, it was not a huge difference in most cases. Additionally, a light intensity of 140 µmol m^{-2} s^{-1} is above what most tissue culture is conducted at and could cause other issues if set this high. This is an important variable to check when trying to troubleshoot condensation issues but is not the most important factor. We would recommend increasing light intensity in 10-20 µmol m^{-2} s^{-1} increments, if possible, if experiencing condensation issues if other solutions have been attempted without solving the problem.

Temperature differentials between the day and night cycle had a significant impact on the amount of condensation formed on the petri dish lid. The temperature differential slightly increased the condensation formed in both the control and ½ empty petri dish conditions. The greatest difference was comparing the bottom and middle dishes in stacked-dish conditions. Under 140 µmol m^{-2} s^{-1} when triple stacked, the middle dish increased from 0% condensation to 12% condensation, and the bottom dish increased from 36% to 87%. The double stacked conditions also increased from 0% to 12% on the top dish and 20% to 76% on the bottom dish (Figure 6A & 6B). The amount of condensation under control and fully loaded conditions was also increased. As shown in Experiments 5 & 6 this condensation does decrease throughout the day (Figure 7 & 8), however the shear amount of condensation present throughout the day is a problem. As a result, we highly recommend eliminating or reducing the temperature differential as much as possible, if condensation is causing issues with experiments. Minor fluctuations in temperature as small as 0.5°C have been shown to cause condensation (Finer).

If a temperature differential is needed, it would be best to ramp up and down the temperature over a 1-to-2-hour period, or longer, at the beginning and end of the day. This will reduce the difference in temperature between the inside of the petri dish and the outside environment and, in turn, reduce condensation formed. In most cases, this change was minor and ramping will likely not completely prevent condensation from forming.

Stacking petri dishes had by far the largest impact on the amount of condensation present on petri dish lids. When double stacked, the bottom condensation ranged from 20% (140 µmol m^{-2}

s^{-1}, no temperature differential) to 76% (140 µmol m^{-2} s^{-1}, 8°C temperature differential). When triple stacked the bottom condensation ranged from 36% to 87% and the middle dish ranged from 0% to 21%, under the respective conditions (Figure 6). This is significantly more condensation than any other condition. For comparison, the control and fully loaded conditions each ranged from 0% to 9% and 0% to 11% average condensation, respectively, over the course of the experiments. When petri dishes are stacked on top of each other, the top dishes insulate the petri dishes below them. This prevents light and heat from reaching the lid of the petri dish, which prevents the removal of any condensation that has formed, while also keeping the dish itself warmer for longer, leading to a larger temperature differential between the inside of the petri dish and the ambient environment in the chamber. The bottom petri dishes are constantly being fed cool air from the plenum which leads to a significant temperature difference between the inside and the outside of the petri dish leading to large amounts of condensation forming on the lid of the petri dish. Not stacking petri dishes seems to be one of the easiest fixes to significantly reduce the amount of condensation occurring. This would be one of the first troubleshooting steps to take if experiencing condensation issues. If stacking petri dishes is necessary, double stacking the dishes had significantly less condensation than triple stacking and placing an empty petri dish underneath stacked petri dishes in chambers with LED lighting was shown to reduce condensation (Figures 7 & 8). As a note, the opposite was true for Fluorescent lighting, and placing an empty petri dish underneath stacked petri dishes is not advised for chambers with Fluorescent lighting.

Shelf loading appeared to have minor effects on the amount of condensation formed. When comparing the control and fully loaded conditions. The fully loaded condition typically had 1% to 4% more condensation when all other conditions were equal. This difference is minor compared to a lot of other variables studied in this report. This could be a remedy if the chamber is severely overloaded and shows proof that operating above 70% capacity in the chamber does make a difference, although minor.

Three experiments were conducted to analyze the amount of condensation throughout the day, and in all cases, condensation decreased or stayed the same throughout the day. This had a significant impact on all the conditions that experienced condensation. The change over the course of the day was even more drastic when a temperature differential of 8°C was used. The chamber can very quickly achieve an 8°C drop in temperature whereas it takes a much longer time for the petri dish to cool down by 8°C. This causes condensation to form at the beginning of the night cycle as warm, humid air is trapped in the petri dish causing it to condense on the cooler surface of the petri dish lid. Throughout the day, the inside petri dish warms back up slowly compared to the ambient environment in the chamber and reduces the condensation on the lid of the petri dish. Additionally, heat and infrared light (SW+IR) radiating from the light fixtures helps to reduce this condensation by warming the lid of the petri dish. Knowing this, it is advised to take data as late in the Day cycle as possible in order to minimize the amount of condensation. Thanks to the Intellus system on Percival Scientific chambers, the starting times of the Day/Night cycle can easily be adjusted to fit around time constraints of data collection.

As noted in Test 1, the front of the chamber experiences significantly more condensation than the rear of the chamber. This is caused by two factors. The first is that the front petri dishes were closer to the door than rear petri dishes, as the door was open for 15–30-minute periods when taking measurements which exposed the front petri dishes to ambient conditions more than the rear petri dishes which introduced unintentional temperature swings. The other factor is the way air circulates within the chamber, warm air is drawn forward and up the chamber as cool air is supplied from the rear of the chamber. This exposes the front few rows of petri dishes to additional temperature swings that lead to the formation of condensation on the lid of petri dishes. This could be a potential fix if experiencing condensation issues and if there is unused space near the rear of the shelf. Most labs and researchers are likely utilizing the majority of if not all the shelf space and this will not be a great solution in those situations.

Overall, for people experiencing condensation issues, SW+IR lighting and not stacking petri dishes had the greatest impact on reducing condensation issues. The petri dishes used in this experiment did not contain live plants, and only contained agar, which may cause results to vary slightly compared to in vitro culture. Further experiments conducted on petri dishes with plants being grown inside of them would clarify any differences caused by this. In short, to prevent condensation we recommend using SW+IR lighting at desired light intensities, reducing or eliminating temperature differentials between the Day and Night cycle, and not overloading the chamber or stacking petri dishes. Based on the findings of this report, following these steps should prevent, or significantly reduce, any condensation from forming on petri dishes.

References

Afshar, Tina, et al. "The effect of temperature on the germination of Arabidopsis thaliana seeds." *The Expedition* 6 (2016).

Finer, J.E., Finer, J.J. A simple method for reducing moisture condensation on Petri dish lids. *Plant Cell Tiss Organ Cult* 91, 299–304 (2007). https://doi.org/10.1007/s11240-007-9292-6

Imberti, D On the Effect of Infrared Radiation on Petri Dish Condensation in CU Series Environmental Growth Chambers, Percival Internal Report (2023)

Imberti, H Petri Dish CU Condensation Lab Report, Percival Internal Report (2020)