Effects of seeding density and cultivar on productivity of baby spinach grown hydroponically in deep water culture systems

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Abstract: Three spinach cultivars, Carmel, Space, and Seaside (F1), were evaluated in regards to their suitability for Deep Water Culture (DWC) hydroponic production according to the procedure presented in Cornell Controlled Environment Agriculture (CEA) Baby Spinach Handbook. Carmel consistently had the highest sprout count and produced the highest fresh weight (FW) among the three cultivars. Space performed moderately well; more data is needed for statistical robustness and verification under more typical higher-light conditions. With respect to Carmel, seeding fewer cells, but at a higher reduced sprouting rate and yield, but not severely. For all three cultivars, pericarps (seed coats getting stuck on cotyledons time of harvest) found in manually harvested baby spinach of marketable size were rare.

1. Introduction:

DWC hydroponics is the soilless culture of plants whose roots are immersed in a circulating, aerated nutrient solution that contains all necessary ions for plant growth, typically practiced in ponds. Cornell CEA has worked extensively to develop a production protocol for all-year DWC baby spinach[1].

Spinach has historically been a difficult crop to grow hydroponically, especially compared to lettuce, for several reasons including:

(1) Susceptibility to *Pythium* infection, especially *P. aphanidermatum* and *P. dissotocum*[3]
(2) Presence of pericarps in harvested product
(3) Costly labor inputs (seeding & harvest)

Cornell CEA, Biological & Environmental Engineering (BEE), and Element Farms, Inc. collaborated to evaluate three different cultivars for germination and grow out potential in DWC systems. Additionally, reliable data was gathered on medium usage per flat (when filled by hand).

Experiments were performed on the Ithaca campus at the Dimock Lab and Kenneth Post Lab House where the flats were stored in a germination chamber and grew out in a bench-scale DWC system, respectively.

This work builds on the foundation laid by Cornell CEA, especially Drs. D. de Villiers, T. Shelford, N. Mattson, R. Langhans, and L. Albright, whose previous work on DWC hydroponics and spinach production made these experiments possible.

Summary of Recommended Sowing Protocol: The Cornell CEA Hydroponic Baby Spinach Production Handbook suggests best practices for sowing spinach for DWC applications. Expanded polystyrene flats are used with common cell configurations of 13 cells by 26 cells on 1” centers. Speedling and Beaver Plastic flats are the most widely-used. Flats are seeded according to a three-step protocol.

Figure 1: Speedling 338 Flat

In the first step, termed the “first pass”, the flat is loosely filled with pre-wet medium - ideally a sterile, soilless mix - and scraped such that each cell is evenly filled to the top. Then the flat is dibbled using the tools described in the following sections. Next, the tray is seeded with 1-3 seeds per cell. Seeds should be dropped directly in the center of cells. Finally, the flat is filled with more medium, scraped, and dibbled again - this time with a shallower dibbler. This is the “second pass”. After seeding, flats are stored in a high humidity environment with no light, after which they are floated in production ponds until harvest.

2. Materials and Methods:

Two trials were conducted comparing the seeding densities and cultivars. Experiments were performed in a dark climate controlled growth chamber, where germination occurred, and a conventional glass greenhouse where the flats grew out in a bench-scale DWC system with typical hydroponic nutrient solution as suggested by previous Cornell CEA experiments.

2.1 *Germination Chamber and Greenhouse Description:*

Experiments were conducted in Ithaca, NY (42◦26056.2” N 76◦28008.3” W). Germination occurred in a climate controlled chamber (10 ft^2) with no light and constant temperature of 24C.

Grow out occurred in a conventional glass greenhouse. The DWC system was located on a bench in the NE corner of the room. Other experiments were simultaneously conducted on other benches in the same room. As such, no supplemental light was provided.

2.2 DWC System Description:

Grow out took place in two steel tubs (24” by 48” by 12”) which fit five cut flats (described below) when completely full. Nutrient solution flowed freely between the two channels through a ¾” hose. Tubs were insulated with ¾ in. styrofoam boards on their exterior to minimize heat transfer and reduce cooling load.

A 1/6HP submersible pump located in the corner of one tub drew nutrient solution in from both tubs. The solution was pumped through a venturi air injector, followed by a ¼ HP inline chiller that kept the water temperature at 19C. Water was returned to the tubs through 1” PVC.

In addition to the venturi air injector, dissolved oxygen (DO) was supplemented with an air pump (General Hydroponics 110V, 8W) attached to cylindrical 1” air stones. Two air stones were placed on the bottom of each channel to maintain DO levels close to saturation. DO was saturated as checked with a YSIPro20 DO meter (with galvanic membrane) when the system was at half-capacity.

Sections of the tubs that did not have flats covering them were covered with styrofoam to prevent growth of algae and contaminants entering the water.

In between trials, the tubs were emptied, cleaned, and sanitized with Greenshield to minimize risk of root infection and ensure consistent nutrient solution conditions between trials.

Figure 2: Bench-Scale DWC System at half- capacity

2.3 Nutrient Solution Conditions:

The stock solutions recommended in the Cornell CEA Baby Spinach Handbook were prepared. The day before the flats were removed from the germination chamber for floating, stock solutions were added to RO water in the tubs in a 1:1 ratio (A:B) to achieve an electrical conductivity (EC) of 1200-1400uS/cm. This naturally maintained pH between 5.7 and 6.3 without the addition of any additional acid or base.

Over the course of each trial growout, RO water and stock solutions were added to maintain the EC/pH within the desired range as checked by handheld meters, calibrated at the beginning of every trial, every two days. Water level in the tubs was maintained so that growing flats were not shaded by the tub walls.

Tables 1 & 2: Stock Solution Recipes [1]

STOCK A	
These chemicals are added to 300 L of RO water	
Calcium Nitrate	29160.0 g
Potassium Nitrate	6132.0 g
Ammonium Nitrate	840.0 g
Sprint 330 Iron - DTPA (10% Iron)	562.0 g

STOCK B	
These chemicals are added to 300L of RO water	
Potassium Nitrate	20378.0 g
Monopotassium Phosphate	8160.0 g
Potassium Sulfate	655.0 g
Magnesium Sulfate	7380.0 g
Manganese Sulfate*H_2O (25% Mn)	25.6 g
Zinc Sulfate*H_2O (35% Zn)	34.4 g
Boric Acid (17.5% B)	55.8 g
Copper Sulfate*$5H_2O$ (25% Cu)	5.6 g
Sodium Molybdate*$2H_2O$ (39% Mo)	3.6 g

2.4 Greenhouse Abiotic Conditions:

Greenhouse ambient temperature was maintained at 24C from 8am-8pm and 19C at night by an automated Argus system.

The greenhouse, which is a relatively new, clean structure has a transmissivity of about 85%. The DLI average in the greenhouse for each trial is estimated from outside light records, these transmissivity values, and shade curtain position. The results are reported in the appendix. Given light was not regulated between trials, it is improper to compare directly across trials.

2.5 Flats:

Speedling 338 flats were used for all trials. The 26 row flats were cut to 22 rows in order to fit tightly in the channels. Halves of these cut flats were considered for different treatments and are comprised of 10 rows by 13 cells per row, or 130 cells total, with two rows separating the respective halves which were filled with medium, but not seeded.

Cells are rectangular on the top, spaced on one-inch centers and tapered to a circular hole on the bottom. The cut flats had dimensions of 13.5” by 22.5” by 1.75”.

2.6 Dibbling Tools:

A combination of conventionally built and 3D-printed dibbling tools were used for these experiments. Conventionally built dibblers constructed are described in the CEA Spinach Production System Report:

> “To control depth of seeding precisely and firm the medium above the seed uniformly, two dibbling tools were made for each type of flat used. The first compressed the medium in the cell by 5/16 inches. After seeding and covering with additional medium, the medium in the cell was again compressed, this time by ¼ inch. The purpose of covering seed and firming the medium above it is to encourage roots to penetrate downwards rather than popping out of the soil. Compression of the medium above and below the seed was quite light, and depth of seeds not great, in both cases just enough to eliminate “pop-ups” as a serious problem, while not impeding root penetration or shoot emergence. Use of a standardized procedure and special tools

ensured uniformity of conditions seed to seed, and repeatability across experiments. Questions as to ideal degree of soil compression above and below the seed were not systematically investigated experimentally, but were determined by trial and error and recourse to experience.[2]"

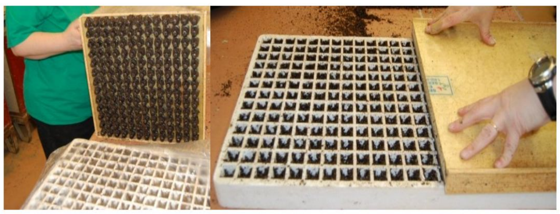
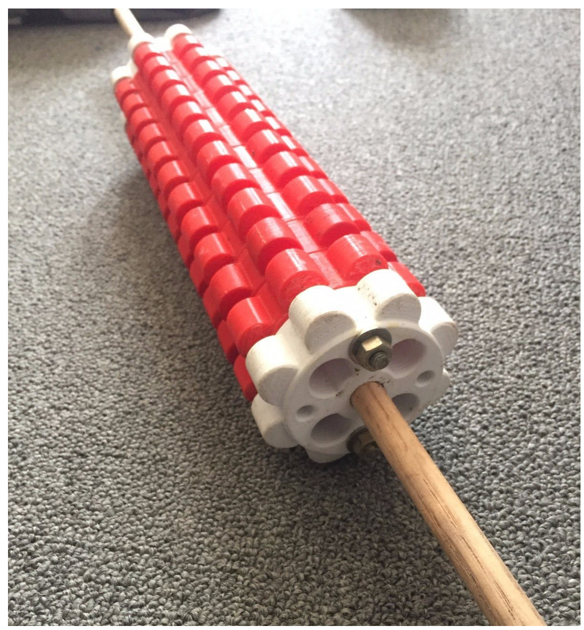


Figure 3: First pass plate dibbler

Olav Imsdahl (BS/M.Eng Mechanical Eng., Cornell 2017), designed and 3D-printed rolling dibblers from ABS polymer at the Rapid Prototyping Lab on Cornell's Ithaca campus. The individually printed pieces were bolted together, as shown. The 3D-printed dibbler worked well for the second pass, as the protruding points are shallower, which allowed it to roll easily across the flat surface, resulting in consistent compression and time savings when compared to use of wooden single-row dibblers.

Given the similar performance of the cells that had been dibbled with the wooden and 3D-printed rolling dibblers in a prior test run, the 3D-printed rolling dibbler was used in these experiments.

At the radius considered, deeper first pass printed dibblers did not roll easily across the flat surface, and instead the plate dibbler pictured in **Figure 3** was used in all experiments for the first pass.



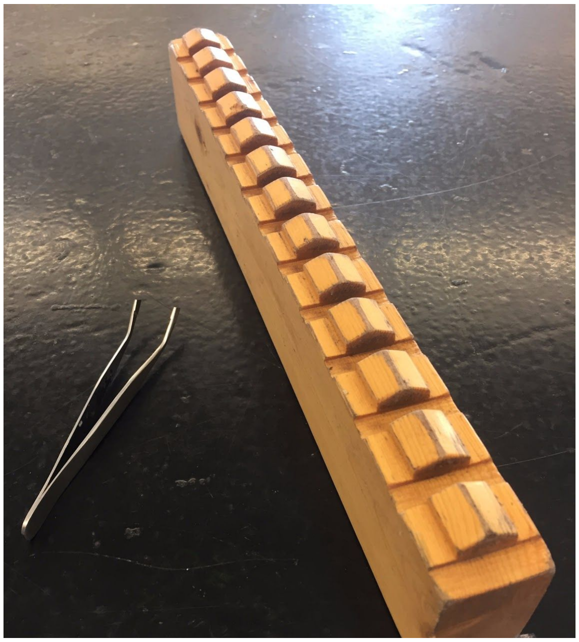


Figure 4: Single 3D printed roller (left); whole flat roller with dowel for a handle (top); wooden dibbler & forceps for handling seeds (bottom)

2.8 Seeds

All seeds tested were procured from Jonny's Seeds Inc. and were ordered in September 2017. When not in use, they were stored in a cool, dry environment in the headhouse. The reported germination rates are: Carmel - 84%, Seaside - 97%, Space - 99%.

Figure 5: Seed packets with reported rate

2.9 Sowing, Germination, and Floating Procedure:

The recommended procedure from CEA was followed closely. Before seeding, flats were cleaned of soil/debris, disinfected with a Greenshield solution, thoroughly rinsed to remove any residue, and allowed to dry.

Sungro Propagation mix, composed of sphagnum peat moss, horticultural perlite, horticultural vermiculite, wetting agent, and starter nutrients was used. RO was added to the dry medium at a ratio of 0.6kg RO water per 1 kg dry medium and mixed in five-gallon buckets. This moisture content was chosen given the trial run results and the fact that the dry medium was easier to work with and stuck to the dibbler less. The medium and water were well-mixed, and the lid was kept on the buckets except when mixing or filling flats to mitigate moisture loss.

When filling the flats for the first pass, a ¼" metal mesh screen was used as a sieve to remove any clumps from the medium and ensure homogenous, loosely-packed filling of cells. Excess medium was scraped off the top of the flat with a straight edge. The plate dibbler was used to create consistent dibbles in each cell. Subsequently, flats were seeded at the appropriate density.

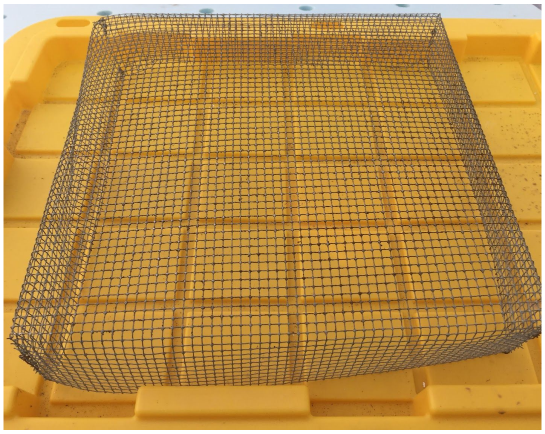


Figure 6: Metal mesh sieve

Post-seeding, another layer of medium was added on top of the seeds, following the same sieving procedure described for the first pass. Finally, the rolling dibbler was used to compress the medium on top of the seeds in the second pass by rolling the dibbler across the flat surface back and forth several times.

Once all flats were seeded, filled, and dibbled they were stacked randomly. Non-seeded guard flats were placed on the top and bottom of the stack. The entire stack of six-seven flats was wrapped in PVC plastic wrap to prevent moisture loss. The stack was moved into a larger, rigid plastic bin for transportation to the germination chamber, where the flats were stored for 60 hours in the dark at 24C before removal for inspection and floating.

Figure 7: Flats just after floating

Upon removal from the germination chamber, flats were inspected for pests and abnormalities. Throughout the experiment, no pests were found in seedlings. All germinations appeared successful upon removal from the chamber - meaning there were some visible sprouts protruding from the medium and many more young roots protruding out of the bottoms of flats. After this step, the flats were carefully floated in the tubs, where they remained until removal for harvest.

2.10 Seedling Sprout Count:

On the sixth day after flotation in the tubs, the total visible seedling sprouts were counted for each treatment. This count included “popups” (sprouts that withered because their roots did not reach the water), which were uncommon. Earlier sprout counts were performed in the first trial, and found to be a poor indication of the final number of seedlings present at time of harvest.

2.11 Harvesting Protocol:

Flats were harvested when the fastest growing treatment's leaves reached marketable size, which occurred between 14 and 16 days after flotation in the tubs. This designation was subjective between trials, but for each case determined by the larger leaves of the Carmel treatments. Leaves were not allowed to get longer than 3" in length before harvest, which was the ceiling for leaf size and provides a point of reference.

Figure 8: Close Up of Harvested Flat

Harvesting was performed by hand with scissors. A handful of leaves would be carefully grasped from the top. The stems would then be cut about halfway down the stems of the longest leaves. This meant the smaller, shorter leaves from each flat were also harvested with shorter stems, since they were lower in the canopy. A large variance in leaf size was found for all treatments, which was due both to the non-simultaneous germination observed, and the presence of young, second true leaves in addition to the older, larger, first true leaves. Harvest weight (as well as medium usage) was measured with a 20lb digital scale accurate to one gram directly after leaves were harvested. As can be seen in Figure 8, many small leaves go unharvested.

2.11 Trial Timeline

Seeding Density Trial
Seed: 10/11
Float: 10/13
Harvest: 10/28

Cultivar Comparison Trial
Seed: 10/28
Float: 10/30
Harvest: 11/17

2.12 Statistical Analysis

The statistical analysis was the same for each trial. The average and standard deviation of each treatment was calculated for both final sprouts and fresh weight - except Space, for which only one flat was seeded.

The results from one well-germinating flat chosen as a representative sample are also reported to show the intra-flat variation to be expected, even when germination occurs at a high-percentage of the reported rate.

Results:

Figure 9: Carmel (left) and Seaside (right) just before harvest

The observed data, averages, and standard deviations are reported. Despite having the lowest reported germination, Carmel had the highest sprout count of the three cultivars tested. Carmel averaged about 97.5% of its reported germination rate. Final sprouting was stable for Carmel across the trials. Both Space and Seaside germinated and produced seedlings at a relatively high rate, as well, but at significantly lower rates than reported.

Carmel yielded better than the other cultivars on a per flat basis. Space yielded nearly as well as Carmel in the single flat observed. More data is needed to substantiate the expected differences in germination and yield between these two cultivars, if any. More data on Space yield in a more tightly controlled environment is available [4]. Seaside, at least grown in the low-light conditions of November in Ithaca, produced a short, bushy canopy compared to the Carmel and Space. The latter two cultivars produced longer stemmed leaves that sat higher above the cotyledons and were easier to harvest.

Pericarps at time of harvest were infrequent and hard to spot as cotyledons become tangled in the lower leaves. However, it was rare to spot a pericarp on cotyledons after a week in the ponds as they were pushed off as the cotyledons grew or by growing true leaves, and they were easily spotted at this time in the crop cycle.

In the Space and Carmel treatments, fewer cotyledons ended up in the harvested spinach, in general, due to the longer stems of the largest leaves and the nature of the cotyledons to fold over under the true leaves compared to Seaside. Overall, this resulted in almost no pericarps ending up in harvested product - on the scale of 0-1 per half-flat. Thus, data on methods to prevent and reduce pericarps was unattainable at this scale.

The results from the density trial suggest seeding three seeds per cell negatively affects both germination and yield, but this test should be repeated to ensure reliable results and to establish the magnitude of difference, or possible methods to mitigate the decrease in yield.

Figure 10: One-week old flat, few pericarps

Table 3: Cells seeded according to [1, 2, 1, 2] … or [3, 0, 3, 0…] pattern giving average density of 1.5 seeds per cell for each trial

Carmel, Varying Seeding Density							
Trial Seed Date	10/11						
Density	*Variable*						
Possible	195	Seeds					
GH Daytime Temp	24	C					
	Sprout Percentage	Std. Dev	Mean Fresh Weight (g)	Std Dev (g)	Flat	Final Sprouts	Fresh Weight
N. Carmel - 83% reported - 1.5 Seeds per Cell	80.5%	4.8%	151.3	6.3	1	150	144
					2	159	157
					3	158	148
					4	161	156
N. Carmel - 83% reported - 3 Seeds per Cell, alternating	75.9%	5.2%	144.3	7.5	1	144	134
					2	155	152
					3	144	146
					4	149	145

Table 4: Cells seeded according to [1, 2, 1, 2] pattern with different cultivars

Carmel, Seaside, Space Comparison							
Trial Seed Date	10/28						
Density	1.5	seed per cell					
Possible	195	Seeds					
GH Daytime Temp	24	C					
	Sprout Percentage	Std. Dev	Mean Fresh Weight (g)	Std Dev (g)	Flat	Final Sprouts	Fresh Weight (g)
N. Carmel - 83% reported	80.6%	5.1%	150.3	4.3	1	162	145
					2	150	155
					3	158	149
					4	159	152
Seaside - 97% reported	73.8%	4.7%	94.5	6.8	1	145	102
					2	150	97
					3	139	86
					4	142	93
N. Space - 99% Reported, whole flat	75.6%	-	145.5	-	L	144	142
					R	151	149

Table 5: Intra-flat Variation in a cut Carmel (F1) flat

Row Number	Final Sprout Count	Rate
1	16	82%
2	17	87%
3	19	97%
4	19	97%
5	15	77%
6	18	92%
7	17	87%
8	13	67%
9	16	82%
10	16	82%
11	17	87%
12	11	56%
13	18	92%
14	14	72%
15	18	92%
16	14	72%
17	14	72%
18	17	87%
19	14	72%
20	14	72%
21	17	87%
22	11	56%

Total	345
Total Possible	429
Avg. Rate	80.4%
Row Avg.	15.7
Row Std.	2.32

Discussion:

These experiments correspond to first steps in a comprehensive comparison of the cultivars represented in these experiments. Ideally, the study would have been conducted in a growth chamber with consistent DLI, temperature, and relative humidity. Future work will pursue a more stringently controlled growing environment to gather more reliable yield data. However, the germination results are consistent and largely independent of fluctuations in greenhouse light conditions. It is peculiar that the cultivar with lowest reported germination, Carmel, had the highest overall sprout percentage (nearly 100% of the reported rate) and also the most vigorous growth during the short 14-18 day cycle considered. Space also performed relatively well when compared to Seaside - which was found to be the least suitable for DWC hydroponic production given a lower sprout rate, short stems, and the slow growth observed.

In future tests, it would be helpful to dig up cells where no sprouts appear and observe the seed condition. If it could be deduced whether or not the seed germinated in the cell, impactful germination procedure alterations could be narrowed down more easily. For instance, if the seed germinated but the seedling withered and died before it could breach the medium surface or its roots reached the nutrient solution, different cell dimensions, seed positions in cells, or compaction/dibbling schemes could be analyzed. On the other hand, if non-sprouting cells were found to have non-germinated seeds, different germination temperatures, a wider range of medium moisture contents, or duration spent in the germination chamber could be investigated, among other factors. In any case, it seems the current procedure is sub-optimal for Space and Seaside, which sprouted far less frequently than their reported rate.

Trial Three's results suggest that Carmel does not suffer an extreme reduction in sprouts or final yield when fewer cells are seeded at a higher density. A custom-designed flat with half as many cells as the speedling 338, but spaced twice as far apart, would cut medium costs roughly in half. The relative costs of medium and seeds are such that making up for slightly reduced sprouting rate with more seeds and using half the medium would result in large proportional decrease of input costs per flat. These preliminary tests corroborate similar results found for Eagle cultivars and highlight the possibility and potential for a flat design custom for baby spinach production [2].

As mentioned previously, the seedling count even four days after floating, was not necessarily indicative of the final sprout count. This suggests methods to imbibe seeds may be necessary in an attempt to synchronize germination and sprout timing and speed up the germination of the slowest seeds. If the last seedlings are emerging a full five days after the first, there is a clear loss of productivity and maximum potential of the seeds is not being realized. Cornell CEA has done extensive work on imbibing procedures. It is yet to be tested if the methods are feasible at a large scale or if imbibed seeds would be compatible with industrial seeding machines, which may have a problem with wet seeds.

This study employed 3D-printed dibbling tools which were found to be a viable alternative to milled, wooden tools. For Spinach production, the dibbling tools are essential. Without the dibblers, germinating seeds would not root properly; they become stringy and pop out of the cells. However, the customized tools conventionally used in Cornell greenhouses are not easy to make for the average grower and require specialized equipment. Nor are these tools widely available.

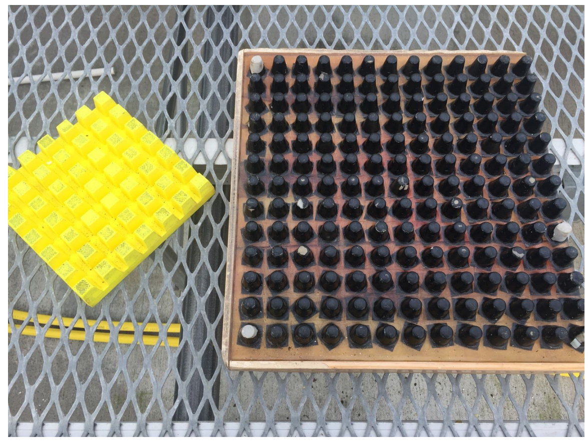


Figure 11: 3D-Printed alternative to plate dibbler

Dibbler CAD designs can be shared, modified, and printed on many campuses, labs, or at a local machine shops/incubators, which are widely-available to growers. Proliferation of custom-designed, 3D-printable horticultural tools that are compatible with commonly used flats, trays, and pots could help standardize sowing and grow out procedures for experimenters and give growers access to the tools they need to optimize production or save time on labor.

The time for printing and amount of material required depend on the size of the dibbler, density (amount of fill), and the printer itself, among other things. It is helpful to be creative with cutting out unnecessary fill; the rolling dibblers printed have circles cut out in addition to the holes for bolts. This can significantly cut down time for printing and reduce material costs.

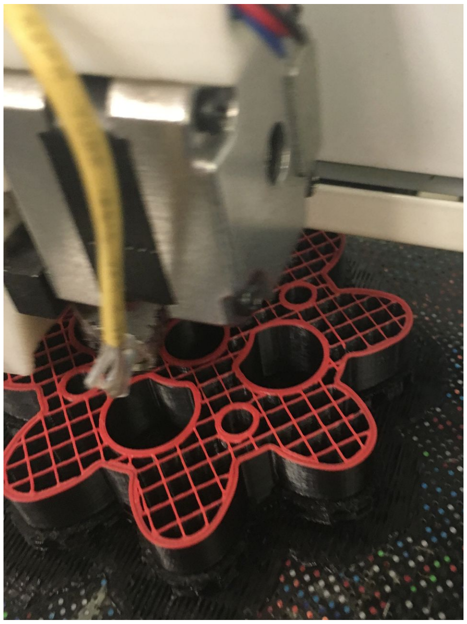


Figure 9: Rolling dibbler mid print

References

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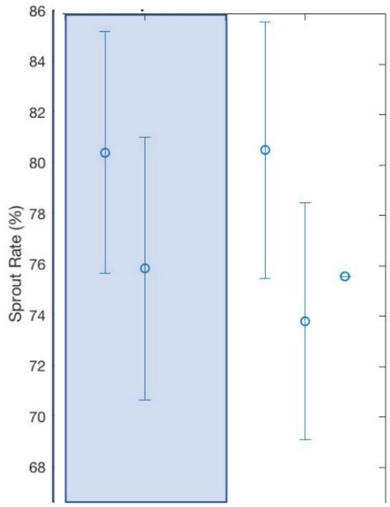
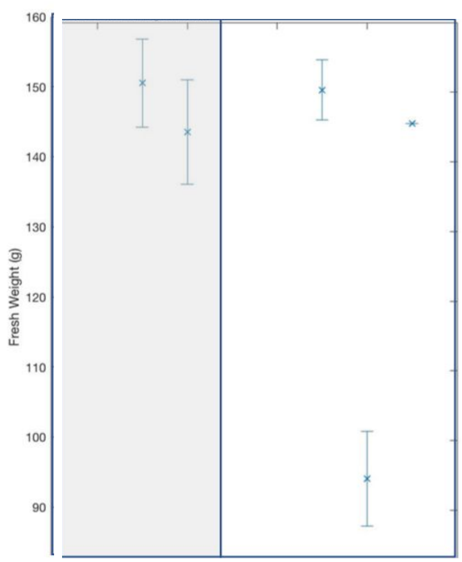
Appendix:



Density Cultivar



Density Cultivar

Medium Use & Fixed Input Cost Estimates:

Sungro Propagation Mix (dry) used to fill cut flats: 592g ± 7g

When scaled to a full sized: 700g

At $17 per bag (2017 price), this comes out to $0.68/flat.

Of the medium used, 80% was for the first pass, and the remaining 20% came from refilling the seeded, dibbled cells to the top in the second pass.

Seed Use and Cost Estimates

$480 for 1 million seeds, or $0.16 to seed a 338 flat with one seed per cell, which obviously scales linearly to the 1.5 seeds per cell used for calculations.

Speedling Tray and Cost estimate:

$10 per flat, 30 uses per flat = $0.33/flat