GREENHOUSE PRODUCTION OF MICROGREENS: GROWTH MEDIA, FERTILIZATION AND SEED TREATMENTS

by

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ABSTRACT

A series of experiments was conducted with the objective of lessening the greenhouse production time and cost for selected microgreen species. Initially, seeds of table beet (*Beta vulgaris* 'Early Wonder Tall Top'; TB), arugula (*Eruca vesicaria* subsp. *sativa*; AR) and amaranth (*Amaranthus tricolor*; AM) were determined to be of high quality (high germination percentage and rate) across a 15 to 25° C range. The seeding rates recommended commercially (Johnny's Select Seeds) for these crops resulted in the greatest shoot fresh weight/m^2 (economic yield), but in the lowest fresh weight per shoot compared to lower seeding rates.

Peat-lite was found to be superior to lower cost wood or paper fiber mulches as growth media for TB, AR or AM microgreens. Insufficient nutrient levels and low cation exchange capacity, as well as excessive liquid retention or inadequate aeration, probably contributed to reduced growth of the microgreens in the mulches. Increasing concentration of solution fertilization (0, 75 or 150 mg N/L daily) increased shoot growth of AR and TB in all media, but especially in peat-lite. Preplant incorporation of nitrogen fertilizers at 2000 mg N/L of peat-lite affected AR shoot fresh wt/m^2 in the order $Ca(NO_3)_2 > NH_4NO_3 > CO(NH_2)_2 > (NH_4)_2SO_4$. Adding the $Ca(NO_3)_2$ as liquid rather than as solid increased shoot fresh wt/m^2 by 6%. The preplant incorporation of $Ca(NO_3)_2$ in the peat-lite, by stimulating microgreen shoot growth, would reduce production time.

Germinating seeds in a medium within an incubator then sowing the germinated seed-medium mixture on the peat-lite in the greenhouse would reduce production time. Germinating AR and TB seeds in the hydrophilic polymer,

Supersorb C, compared to fine, grade 5 vermiculite resulted in greater and earlier germination. Since the human toxicity of Supersorb C is unknown, only vermiculite was used in further experiments. The optimum water concentration (% of vermiculite dry weight) and incubation period at 20° C for TB was 150% for 5 days (50% germination, 6.5 mm long radicles), and for AR was 200% for 1 day (81.5% germination, 2 mm long radicles). Combining these germination treatments with preplant incorporation of $Ca(NO_3)_2$ at 1000 mg N/L of peat-lite plus daily solution fertilization with 75 mg N/L from 21-5-20, gave the greatest shoot fresh weight per m^2 of AR and TB in the university greenhouse when compared to control seeds and other fertilization treatments.

The final experiment compared the standard ARC Greenhouses production practices for TB microgreen production (control seed, no preplant incorporation of nitrogen fertilizer in the peat-lite, and daily solution fertilization with 100 mg N/L from 21-5-20) with selected seed and fertilization treatments that might hasten production in the greenhouse. Sowing TB seedballs germinated in vermiculite (200% water, 4.5 days at 20° C) increased shoot fresh weight/m^2 by 13% at 15 days after planting. A further increase in shoot fresh weight/m^2 to 52% above that achieved with standard production practices resulted from incorporating $Ca(NO_3)_2$ at 1000 mg N/L into the peat-lite before planting (along with the standard daily 100 mg N/L solution fertilization).

CHAPTER 1

LITERATURE REVIEW

1.1 Microgreen production

Microgreens have been defined as salad crop shoots harvested for consumption within 10 to 20 days of seedling emergence (Lee *et al.*, 2004). Microgreens are not to be confused with sprouts, or baby lettuce that we consume in Mesclun mixes. Microgreens are more mature than sprouts but less mature than baby lettuce that provides variety to the popular Mesclun mixes. In addition, sprouts do not need light to grow, while microgreens are most often grown in the dark until they germinate, at which time they are then exposed to light in order to grow and develop properly (Orr, 2003).

The term "microgreen" covers an endless number of salad greens, some more common than others, including table beet and swiss chard (*Beta vulgaris*), spinach (*Spinacia oleracea*) and ever more exotic greens, exotic in the sense that these greens have never been consumed in such a style. These exotic microgreens include the foliage of pea plants (*Pisum sativum*), mint (*Mentha* sp.), red amaranth (*Amaranthus* sp.), fennel (*Foeniculum vulgare*), chrysanthemum (*Dendranthema/Chrysanthemum* sp.), and many more. Their bright colors, interesting textures, delightful taste, and charming nature garnish the plate, and their high antioxidant capacity makes them healthful foods that introduce important vitamins, minerals, and enzymes to your meal (Orr, 2003).

Epidemiological studies have shown consistently that diets high in fruits and vegetables may contribute to maintenance of health and possibly reduce risk of chronic ailments such as coronary heart disease, cataract, cancer, diabetes, and Alzheimer's disease (Tucker, 2003; Willett, W.C., 2002). Fruits and vegetables rich in pigments such as anthocyanins, betalains, or carotenoids are potent sources of antioxidants (Fernandex-Lopez *et al.*, 2001), with table beets being rich in betalain and folic acid (Nutrient Data Laboratory, 2003). It has been shown that supplementation of natural antioxidants, through a balanced diet containing enough fruits and vegetables, could be the most effective in protecting the body against various oxidative stresses (Cao *et al.*, 1996). It was shown in cowpea (*Vigna unguiculata*) that plant population density affected the dietary value (e.g. protein, carbohydrate, fiber) of the harvested foliage (Ohler, *et al.*, 1996). Varying the plant population density (through seeding rate) may affect the dietary value (including antioxidant activity) of microgreen crops.

There are numerous references available as guides to raising salad crops to maturity, but to my knowledge, there is no scientific literature available that addresses how to grow and harvest microgreen crops (e.g. growth media, optimum conditions, and seeding rates). The proposed research in this thesis examines several aspects (seed treatments, growth media, and fertilization) that might decrease the time and cost associated with microgreen production.

1.2 Seed Treatments

At least initially, my research will focus on table beet seed, since this vegetable has slow and asynchronous germination (Lee *et al.*, 2004). Table beet (*Beta vulgaris*), a biennial herbaceous dicot in the Chenopodiaceae family, includes four

botanical groups or types: sugar beet, mangelwurzels or mangels, garden beets, and leaf beets (chard and ornamental beets) (Desai *et al.*, 1997). The beet seed is botanically a fruit (termed a seed ball) that can have one (monogerm) to several (multigerm) highly compressed achenes formed by joining several floral units, each with one seed containing a single embryo (McDonald and Copeland, 1997). There are commonly between 2 and 4 (possibly more) embryos to a single seed ball (Taylor *et al.*, 2003). Each achene has the potential to germinate resulting in 3 to 5 plants (McDonald and Copeland, 1997) that develop closely intertwined so they are difficult to thin (Desai *et al.*, 1997).

Taylor *et al.* (2003) noted that there are three major seed factors that negatively influence beet seed germination: 1) the mucilaginous layer that surrounds the seed ball that can not be washed off, 2) the ovary cap tenacity of the seed ball structure, and 3) the presence of phenolic germination inhibitors.

Cultivars with a greater incidence of mucilage had lower germination potential (Taylor *et al.*, 2003). The mucilage layer defined as "a well hydrated insoluble polysaccharide mixture with internal cross linking that provides strength and physical structure" reduced both the rate and percentage of germination. The ovary cap (operculum) is described as the non-embedded portion of the ovary and remnants of the stigma and style. This structure is a barrier to oxygen diffusion, and it can be very strong and tightly bound, making it difficult for the radicle to protrude. Removal of the ovary cap, or raising it, increased germination percentage (Taylor *et al.*, 2003). Taylor *et al.* (2003) found that phenolic compounds inhibited seed germination of table beet seeds and thus recommended that the seeds be rinsed in water before sowing. Interestingly, seeds with mucilage leaked more phenolic compounds than

those without mucilage, which was attributed to a greater concentration of phenolics in seeds with a mucilaginous layer.

Khan *et al.* (1992) found that matric conditioning of table beet seeds speeded seedling emergence in laboratory studies. Matric conditioning is the seed exposure to low water potentials created by matric forces to a solid carrier (Pill, 1995). Such controlled seed uptake of water permits seeds to advance to the end of phase 2 of imbibition (the lag phase), but prevents the seeds from entering phase 3, the second phase of water uptake which coincides with radicle emergence (Job *et al.*, 2000). The earlier emergence of table beet seeds with matric conditioning was enhanced further by washing the seeds following conditioning, presumably by increasing removal of water soluble germination inhibitors (Khan *et al.*, 1992). Lee *et al.* (2004) examined several seed treatments to advance greenhouse establishment of beet and chard (both *Beta vulgaris*) microgreens. The seed treatments were matric priming (-1.0 MPa at 12° C for 6 days in fine vermiculite) or various soaks (water, 20°C for 48 hours; hydrogen peroxide, 0.3% at 20° C for 48 hours; hydrogen chloride, 0.3M at 20° C for 2 hours; or sodium hypochlorite, 4% at 20° C for 3 hours). While germination percentage was little affected, appreciable germination advancement in both crops was achieved using all seed treatments. The most pronounced seedling emergence advancement, however, was gained by germinating seeds in fine grade vermiculite and sowing the germinated seed and vermiculite mixture.

Katzman *et al.* (2001) examined several seed treatments to enhance germination of spinach (*Spinacia oleracea*, another member of the Chenopodiaceae). Removal of the pericarps hastened germination in all cultivars and increased germination in some cultivars. They found that the greatest germination rate and

percentage in intact seeds resulted from soaking the seeds in 0.5% sodium hypochlorite for 4 hours, followed by rinsing in water for 15 hours, followed by soaking in 0.3% hydrogen peroxide. In fact, the combined treatments had a synergistic, not merely additive, effect on germination. The treatments may have weakened the seed covering tissues, counteracted the effects of inhibitors in the pericarp, and the hydrogen peroxide may have provided supplemental oxygen for respiration and other metabolic activities.

1.3 Growth Media

Greenhouse crops typically grow in peat-based media, the "peat-lites," in which *Sphagnum* peat is amended with horticultural vermiculite and/or perlite to provide suitable air and water retention properties in the media. Such media are quite costly ($3.50 to 5.00 per compressed cubic foot = $1.75 to 2.50 per cubic foot when decompressed (Griffin Product catalog, 2004). Given that microgreen production is short term, part of the proposed research is to determine other, less expensive growth media to effectively substitute for peat-lite. To this end, microgreens will be grown in recycled newspaper fiber or wood fiber, both available commercially as mulch components of hydroseeding systems. There is no literature pertaining to the use of these two media as sole components of growth media in plant production. Some literature has examined the growth of plants in media amended with paper mill sludge (e.g. Chong and Cline, 1993).

1.4 Fertilization

There is no literature associated with fertilization of microgreens, in part because microgreen is a fairly recent term, and partly because the production of microgreens is in its infancy.

It has long been known that either nitrate or ammonium salts will serve as nitrogen sources for plant growth (Barker and Mills, 1980). For most plant species, nitrate nitrogen results in greater vegetative and/or reproductive growth than ammoniacal nitrogen. For instance, total and mean fruit weights and fruit size increased when hydroponically grown cucumbers (*Cucumis melo*) were supplied with increasing nitrate (from 25% to 100%) relative to ammonium (Kotsiras *et al.*, 2005). Since NO_3-N and NH_4-N account for more than 70% of the total cations and anions taken up by plants (van Beusichem *et al.*, 1988), it is not surprising that nitrogen form can have marked effects on crop performance. Ammonium toxicity can be manifested as greatly restricted root growth with root discoloration (Maynard and Barker, 1969). Aerial symptoms of ammonium toxicity include leaf chlorosis and necrosis, epinasty, and stem lesions (Maynard and Barker, 1969). Seed germination and seedling growth can be damaged severely by ammonium ions (Barker *et al.*, 1970; Patnaik *et al.*, 1972). Pill *et al.* (1978) noted that ammonium nutrition, compared to nitrate nutrition of tomatoes in sand culture, resulted in reduced shoot growth, reduced fruit number and weight, reduced leaf xylem pressure potential, and reduced leaf Ca, Mg, and NO_3 concentrations. Blossom-end rot, a disorder associated with low fruit Ca and water stress, was evident only under ammonium nutrition.

Application of soluble fertilizer through the irrigation water (solution fertilization, "fertigation") is a widely practiced method of fertilizing horticultural crops. It may be applied by subsurface irrigation, as a growth medium drench,

through trickle irrigation, or as a foliar spray (liquid fertilization). Compared with 100 mg N/liter, 400 mg N/L as solution fertilization twice daily increased the shoot dry weight of celery, lettuce, broccoli, and tomato transplants by 37%, 38%, 61% and 38% respectively, after one month of growth (Masson *et al.*, 1991). High N fertilization accelerated shoot growth at the expense of root growth, except for tomato where a 16% increase of root dry weight was observed. Soundy *et al.* (2005) evaluated lettuce transplant growth in response to 0, 30, 60, 90 and 120 mg N/liter fertigated every 1, 2, 3, or 4 days. As N concentrations increased, shoot dry weight and leaf N concentration increased, and root:shoot ratios decreased linearly or quadratically. Transplants fertigated every second to third day with 60 to 90 mg N/liter had optimal root systems to achieve the highest pulling success rate from flats. Cornillon (1999) observed that increasing N concentration in a complete fertilizer daily liquid fertilization increased tomato transplant shoot fresh weight but had little effect on the shoot dry weight.

CHAPTER 2

SEEDLOT GERMINATION

2.1 Introduction

Initially, the seed count (weight per seed number) of the species was determined. In order to characterize seed germination of the seed species used in my research, a seed germination assay at different temperatures was conducted.

2.2 Materials and Methods

Seed used in my research were purchased from Johnny's Selected Seed (Winslow, Maine). The species selected were: table beet (*Beta vulgaris* 'Early Wonder Tall Top'), arugula (*Eruca vesicaria* subsp. *sativa*) and amaranth (*Amaranthus tricolor*). Throughout this thesis, the three species will be abbreviated to TB, AR and AM for table beet, arugula and amaranth, respectively.

To establish the seed count, 5 samples of 100 seeds were randomly selected from the seed bags, and then weighed to three decimal places. From this data, the number of seeds per gram was determined (Table 2.1).

For the germination assay, four replications of 100 seeds each were placed on two layers of germination paper (Germination blotter No. 385; Seedburo, Chicago, IL) moistened with 15 ml of deionized water contained within 125 X 80 X 20 mm transparent polystyrene boxes. Germination was assayed at 15, 20 or 25° C incubators in the dark. The replications (boxes) were arranged in completely randomized design.

The numbers of seeds germinated (having a visible radicle) were recorded and removed daily until no further germination occurred. From this data, the angular transformation (arcsine.√%) of the final germination percentage (FGP), days to 50% of FGP (G_{50}, an inverse measure of germination rate), and days between 10 and 90% of FGP (G_{10-90}, an inverse measure of germination synchrony) were calculated and subjected to analysis of variance.

2.3 Results and Discussion

The seed weights of the three species were ranked TB > AR > AM (Table 2.1). The TB seed is actually a seed ball (botanically, a fruit) that can have one to several highly compressed achenes; each achene has the potential to germinate resulting in 3 to 5 plants (McDonald and Copeland, 1997).

Table 2.1 Seed counts for TB, AR, and AM.

Species	Seed count (number/g) (mean ± standard deviation)		
TB	56	±	0.10455
AR	579	±	0.00553
AM	1249	±	0.00242

Seeds of all species were of very high quality with average germination of 97.6 percent (Table 2.2). For all species the G_{50} increased as germination temperature increased, such that G_{50} was more than double at 15° C compared to 25° C. The G_{10-90} was greater for TB than for AR or AM. The lowered germination synchrony of TB may reflect the different rates of germination of each achene within the seed ball, and many depend on the position of an individual ovary within the seed ball relative to the

growth medium surface. Epicotyls from seedlings may take longer to emerge if the ovary is positioned downward in the growth medium. Even within the range of 15 to 25° C, germination of all species was high and fairly rapid. In a greenhouse maintained at energy-saving lower temperatures (e.g. 15° C), crop establishment would be satisfactory, but slightly slower.

Table 2.2 Final germination percentage (FGP) and its angular transformation (Asfgp), days to 50% of FGP (G_{50}) and days between 10 and 90% of FGP (G_{10-90}) of TB, AR, and AM at 15, 20 or 25° C[z].

Species and germination temperature (°C)	FGP (Asfgp)	G_{50} (days)	G_{10-90} (days)
TB			
15	97 (81) a	5.1 a	2.7 a
20	97 (81) a	2.9 b	2.1 a
25	99 (85) a	2.1 c	2.7 a
	LSD (8) ns	LSD 0.3 ***	LSD 1.0 ns
AM			
15	94 (81) a	2.4 a	1.8 a
20	97 (83) a	1.5 b	1.0 b
25	96 (80) a	0.7 c	1.5 ab
	LSD (13) ns	LSD 0.1***	LSD 0.6*
AR			
15	100 (88) a	1.4 a	1.0 b
20	99 (87) a	0.7 b	1.5 a
25	99 (85) a	0.6 c	1.1 b
	$LSD_{0.05}$ (4) ns	$LSD_{0.05}$ 0.1 ***	$LSD_{0.05}$ 0.4 *

[z] ***,**,*,ns Significant at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, or not significant, respectively. Means with the same letter in a column for a given species can not be considered to be different by $LSD_{0.05}$.

2.4 Conclusions

Seeds of all species were of high quality as revealed by high germination percentage and rate (low G_{50}) across a temperature range of 15 to 25° C.

CHAPTER 3

SEED SOWING RATE

3.1 Introduction

There is no published research on the effect of seed sowing rate on the seedling emergence and growth of microgreen vegetables. Consequently, selected seed sowing rates were relative to those used at ARC Greenhouses which uses rates recommended by Johnny's Selected Seed (Winslow, ME).

3.2 Materials and Methods

Plastic 601 inserts, measuring 17 X 12 X 4 cm, were filled to a 2.5 cm depth with proprietary peat-lite growth medium (Pro-mix BX; Premier Brands, New Rochelle, N.Y.). A waxed paper ring (10.8 cm diameter x 2 cm depth; 92 cm^2) cut from a waxed paper tub was pressed into the growth medium at the center of each 601 with about 1 cm above the growth medium surface. This ring provides a subset for seedling harvest so that seedlings within the ring should be free of any border effects. Seed sowing rates selected for the three microgreen species were 0.25, 0.50, 0.75, and 1.00-fold of the commercially recommended rate (Table 3.1). Seeds of each species were broadcast evenly onto the surface of the growth medium, and then covered with a thin (1 to 2 mm) layer of Jiffy Mix Plus. The four replications (601 inserts) of each seed sowing rate for each species were arranged in randomized complete blocks on a greenhouse bench (22° C day, 20° C night) under natural light during April-May. The

inserts were irrigated with water as needed. The TB, AR and AM seeds were planted on 5 April, 27 April, and 6 May, respectively.

Table 3.1 Seed sowing rates for TB, AR and AM.

Species	Seed density at the 1X rate (g/m^2)	($no./m^2$)	Seed sowing rate[z] 0.25	0.50	0.75	1.00 (g seed/204 cm^2)
TB	202	11312	1.02	2.03	3.05	4.06
AR	55	31845	0.28	0.56	0.84	1.12
AM	83	103667	0.42	0.85	1.27	1.69

[z]Proportions of the commercially recommended 1X rate (Johnny Select Seeds); 204 cm^2 is the area of the 601 insert into which the seeds were sown.

When the lamina of the first true leaves were estimated to be *ca* 2 cm long in any of the treatments of a species, seedlings of all treatments were harvested. For TB, AR and AM, the seedling harvest was 14, 10 and 9 days after planting, respectively. On the day of harvest, the shoots of all seedlings within the ring were cut with sharp scissors at the peat-lite surface, and their fresh weights determined within several minutes of harvest. The number of seedling shoots was also counted. The lamina length of the first true leaf also was measured. Seedling shoots then were placed in a labeled paper bag and allowed to dry for one week at 65° C before shoot dry weights were determined. All data were subjected to analysis of variance.

3.3 Results and Discussion

For all species, increasing seeding rate resulted in a linear increase in number of shoots/m^2 (Table 3.2). As seeding rate increased, shoot fresh weight/m^2 increased linearly, but fresh weight/shoot decreased. This decrease in individual shoot weight with increasing seeding rate reflects increasing interplant competition for resources such as water, nutrients, and light. The increasing interplant competition with increasing population density resulted in decreased length of the first true leaf at harvest of all three species (Table 3.3).

Table 3.2 Shoot fresh and dry weights of TB, AR and AM at 14, 9, and 9 days after planting, respectively, in response to seed sowing rate[z].

Species and recommended rate (g seed/m^2)		Shoot density (no/m^2)	Fresh weight (mg/shoot)	Fresh weight (g/m^2)	Dry weight (mg/shoot)	Dry weight (g/m^2)
TB						
0.25	50	5478 c	271 a	1473.7 c	16.3 a	881.0 c
0.50	101	12360 b	160 b	1947.3 b	11.3 b	1383.8 b
0.75	151	14958 b	130 bc	1887.6 b	8.8 b	1283.7 b
1.00	201	21945 a	121 c	2450.1 a	9.4 b	1837.1 a
Linear (F)		***	***	***	***	***
Quadratic (F)		ns	***	ns	*	ns
AR						
0.25	14	6882 d	176 a	1201.5 c	10.9 a	774.7c
0.50	28	13518 c	134 b	1752.5 b	8.0 b	1053.7 b
0.75	41	19031 b	111 c	2094.5 b	6.7 c	1276.3 b
1.00	55	29073 a	91 d	2624.6 a	5.5 d	1589.2 a
Linear (F)		***	***	***	***	***
Quadratic (F)		ns	Ns	ns	*	ns
AM						
0.25	21	20880 d	73 a	1518.3 d	4.0 a	843.2 d
0.50	42	31426 c	57 b	1747.9 c	3.0 b	931.5 c
0.75	62	49789 ab	44 c	2186.1 b	2.6 c	1272.5 b
1.00	83	51966 a	45 c	2368.4 a	2.7 c	1400.3 a
Linear (F)		***	***	**	***	**
Quadratic (F)		ns	ns	ns	*	ns

[z] ***,**,*,ns Significant at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, or not significant, respectively. Means within a column for a species can not be considered different by $LSD_{0.05}$.

Table 3.3 Approximate length of first true leaf lamina at time of harvest of TB, AR and AM.

Length of first true leaf lamina (mm)

Seeding rate	TB	AR	AM
0.25	13 – 19	13 – 16	13 – 19
0.50	6 – 13	10 – 13	6 – 13
0.75	3 – 6	5 - 10	3 – 6
1.00	0 – 3	0 – 5	0 – 3

3.4 Conclusions

The commercially recommended seeding rate (1X in this study) resulted in the greatest shoot fresh weight/m^2 but the lightest individual shoots at the time of harvest in all three crops. Seeding rates lower than this recommended rate resulted in lower yield/unit bench area and thus, lower economic yield.

CHAPTER 4

GROWTH MEDIA

4.1 Introduction

The peat-lite media used at ARC Greenhouses is expensive. In an attempt to reduce growth media cost in the production of microgreens, growth of TB, AR and AM in two mulch materials was compared to that in peat-lite.

4.2 Materials and Methods

One bale each of two fibrous mulch materials was acquired from Central Fiber Corporation (Canton, OH; www.centralfiber.com): Second Nature Wood Fiber Mulch and Second Nature Paper Fiber Mulch. Both products are used primarily as hydroseeding mulches to reduce soil erosion. Each bale weighed 22.7 kg (50 lb) and measured 66 X 43 X 23 cm (26 X 17 X 9 in.). Five samples of each of these fiber mulch media and of the peat-lites used at ARC Greenhouses (Jiffy Mix 921 (80/20) and Jiffy Mix Plus) were sent to the University of Delaware Soil Testing Laboratory. The results of these tests are shown in Table 4.1.

Table 4.1 Chemical properties of four growth media, as determined by the University of Delaware soil testing laboratory (mean ± s.d., n = 5).

Variable[z]	Wood fiber mulch	Paper fiber Mulch	Jiffy mix 921 (80/20)	Jiffy mix plus
CEC (meq/L)	26.7 ± 2.1	29.0 ± 1.3	74.5 ± 6.3	90.7 ± 4.1
Organic matter (%)	99.7 ± 0.7	96.2 ± 0.7	36.9 ± 1.7	63.0 ± 3.3
Total carbon (%)	50.1 ± 0.5	44.7 ± 0.5	20.7 ± 1.2	37.2 ± 1.7
Total nitrogen (%)	0.53 ± 0.1	0.47 ± 0.1	0.54 ± 0.1	0.80 ± 0.02
C:N	97.3 ± 16.2	102.4 ± 34.2	38.6 ± 2.4	46.2 ± 2.3
pH	4.7 ± 0.1	7.7 ± 0.1	5.7 ± 0.1	4.50 ± 0.1
Ions (mg/L)				
P	3.6 ± 0.8	3.0 ± 0.7	19.4 ± 5.1	46.6 ± 9.2
K	102.3 ± 0.1	9.4 ± 0.7	126.7 ± 3.7	185.4 ± 19.2
Ca	121.0 ± 7.2	888.9 ± 125.0	427.7 ± 20.1	686.9 ± 5.5
Mg	18.2 ± 0.9	15.9 ± 1.2	327.6 ± 18.2	345.6 ± 32.1
S	8.5 ± 0.7	21.3 ± 1.9	80.4 ± 7.8	137.2 ± 10.1
Mn	5.5 ± 0.5	2.2 ± 0.1	6.1 ± 0.3	4.9 ± 0.4
Zn	0.4 ± 0.1	0.9 ± 0.1	48.5 ± 5.9	30.4 ± 2.8
Fe	6.0 ± 0.7	3.6 ± 0.3	80.4 ± 7.8	137.2 ± 10.1

[z]pH by 1:1 (volume), soil: distilled water.
Organic matter by weight loss by ignition.
CEC (cation exchange capacity) by summing cations at pH 7.0.
The extractant used for determining ion concentrations was Mehlich 3. Mehlich 3 extractant = 0.2N CH_3COOH + 0.25N NH_4NO_3 + 0.15N NH_4F + 0.013N HNO_3 +0.001 M EDTA.
Recommended Soil Testing Procedures for the Northeastern United States, 2nd edition, Agricultural Experiment Station, University of Delaware, Newark, DE.

Into each of two, 76-liter (20 gallon) plastic garbage cans was added 37.9L (10 gallons) of municipal water. To the water was added 303 ml each of stock solution A and stock solution B, the two stock solutions that formulate the hydroponic nutrient solution used at ARC Greenhouses. The composition of the stock solutions and the concentration of nutrient elements in the final diluted solution are shown in

Table 4.2. The diluted solution had a pH of 6.37 and an electrical conductivity (EC) of 1.95 dS/m (mmho/cm), respectively.

Fiber mulch was broken manually into smaller, manageable "chunks" and then was added to the nutrient solution within the garbage can and allowed to become fully saturated by soaking overnight.

Table 4.2 The composition of the stock solutions and of the final, diluted solution used to hydrate the wood and paper fiber mulches.

Stock solution	Salt	Concentration (lb/100 gal. stock) (g/L)		Concentration of diluted solution (1 stock: 125 water) (mg/L)
A	KNO_3	28.7	34	270
	$MgSO_4$	29.2	35	278
	KH_2PO_4	22.8	27	214
	Micronutrients	[2 gallons]	[7.6 L]	60
B	KNO_3	28.7	34	270
	$Ca(NO_3)_2$	86.4	104	825
	Sprint 330	3.1	3.7	29

[z]Concentrations (mg/L) of micronutrients in the diluted solution: Mn 0.5, Zn 0.15, Cu 0.15 and Mo 0.10.

Each mulch, and the peat-lite (Jiffy Mix 921; PL), was spread about *ca* 2.5 cm deep in 52 X 25 X 5 cm (20.5 X 10 X 2 in.) standard flats. To provide a subsample for harvesting and weighing, a plastic 17 x 12 cm 601 insert whose bottom had been cut off leaving only a 2.5 cm tall wall was pressed into the center of the medium, with its long axis parallel to that of the flat.

Onto the growth medium, seeds were evenly broadcast at 24.96, 6.80, and 10.24 g/flat for TB, AM and AR, respectively. The growth medium in all seeded flats then was covered with a thin layer (*ca* 2 mm deep) of Jiffy Mix Plus, since it proved to be impossible to cover seeds with the non-friable mulches. These flats then were

placed on a bench in a greenhouse (25° C day, 20° C night) under natural light (16-27 June). The four replications (flats) of each growth medium were arranged in randomized complete blocks by plant species (TB, AR and AM). The flats received irrigation as needed, and no further fertilization.

All three species of microgreens (TB, AR and AM) were harvested at 11 days after planting, when the first true leaves were 1.3 to 2.5 cm long. The seedlings were cut with a pair of sharp scissors at the growth medium surface within the 601 insert. These seedling shoots were counted and their fresh weights determined. Fresh weight per shoot and per unit of growth medium area were subjected to analysis of variance.

4.3 Results and Discussion

The shoot fresh weight/m^2 of all three crops was greatest in peat-lite (Table 4.3). For TB and AM, wood mulch resulted in greater shoot fresh weight/m^2 than paper mulch; but for AR, paper mulch gave greater shoot fresh weight/m^2 than wood mulch. The fresh weight/shoot of TB and AR followed the same trends as did shoot fresh weight/m^2, however fresh weight/shoot of AM was unaffected by growth medium. The shoot population density for TB was greatest in peat-lite, least in paper mulch, and intermediate in wood mulch. The population densities of AM and AR were unaffected by the growth medium.

Table 4.3 Shoot fresh weight and shoot population density of TB, AR and AM microgreens at 11 days after planting in response to growth in three growth media.

Species	Growth Medium	Fresh wt per shoot (mg)	Shoot fresh wt per m^2 ($g\ m^{-2}$)	Shoot population (number/m^2)
AM	Paper mulch	11	480 c	45220
	Wood mulch	11	628 b	55330
	Peat-lite	23	1032 a	45189
	$LSD_{0.05}$[z]	Ns	110*	ns
AR	Paper mulch	51 b	1986 b	38880
	Wood mulch	44 c	1504 c	33939
	Peat-lite	71 a	2544 a	35943
	$LSD_{0.05}$	4***	313***	ns
TB	Paper mulch	39 c	712 c	18432 b
	Wood mulch	47 b	1050 b	22406 ab
	Peat-lite	101 a	2537 a	25165 a
	$LSD_{0.05}$	7***	324***	4697*

[z] ***,**,*,ns Significant at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, or not significant, respectively. Means within a column for a species with the same letter are not significantly different by $LSD_{0.05}$.

The decreased growth in the mulches compared to peat-lite may be the result of lower cation exchange capacity (CEC), and lower concentrations of phosphorous, magnesium, sulfur, zinc, and iron (Table 4.1). The nutrient solution used to hydrate the mulches (Table 4.2) contained insufficient levels of the above listed nutrients to sustain vigorous microgreen growth. If such mulches were to be used for microgreen production, they would need to be solution fertilized frequently to support adequate crop growth. The higher C:N ratio of the mulches (97.3 for wood mulch and 102.4 for the paper mulch) compared to 38.6 for the Jiffy Mix 921 (Table 4.1), may have resulted in greater microbial immobilization of N in the mulches. The

tissues of microorganisms have a C:N of about 30:1, and if they are to decompose organic materials, they quickly remove any soluble N present around them (Handreck and Black, 1984).

The mulches appeared to retain more moisture than the peat-lite. Before the mulches were spread into the flats, any excess moisture was removed by manual squeezing. For the paper mulch, this resulted in "balling," making it very difficult to spread evenly throughout the flat, and less receptive to even-seeding because the seeds had a tendency to roll into the cracks and crevices of the medium. Throughout the entire growth period, the mulches retained so much liquid that root penetration appeared to be impeded, possibly as a consequence of insufficient aeration.

4.4 Conclusions

Peat-lite was superior to the two mulches in supporting microgreen growth. Insufficient nutrient levels and CEC, as well as excessive liquid retention and inadequate aeration probably contributed to reduced growth of the microgreens in the mulches.

CHAPTER 5

POST-EMERGENCE FERTILIZATION

5.1 Introduction

The results of the previous experiment revealed reduced seedling growth in the fiber mulches than in the peat-lite. In all media, seedlings appeared slightly chlorotic. In an attempt to increase growth in all media, but especially in the fiber mulches, the present experiment was undertaken. In this study, the growth of TB and AR in peat-lite or wood or paper fiber mulches was examined in response to post-emergence daily application of solution fertilization at three concentrations. During the planning of this research, I learned that the microgreens at ARC Greenhouses are solution fertilized daily with 100 mg N/L from 21-5-20 fertilizer.

5.2 Materials and Methods

The materials and methods were identical to those of Experiment 4 except that AM was removed from the study, and three concentrations of solution fertilization, 0, 75, and 150 mg N/L from 21-5-20 (Peters Excel All Purpose; Scotts-Sierra Horticultural Products Co., Marysville, OH) were added to the experiment. The solutions were prepared within 75.7L (20-gallon) garbage cans by adding 0, 14, or 27g of the 21-5-20 to 37.8L (10 gallons of water) for the 0, 75, or 150 mg N/L, respectively. The flats were irrigated using a watering can to beyond saturation every morning with the appropriate fertilizer solution, beginning at 2 days after planting for

the AR, and 3 days after planting for the TB (when seedling emergence was first evident). Flats (replications) were arranged on the greenhouse bench in randomized complete blocks with four replications. The greenhouse temperatures were set at 27° C day and 20° C night under natural light from 11-25 July.

The shoots of both species were harvested at 15 days after planting. Shoots were excised with sharp scissors at the growth medium surface within the 601 insert, counted, and the total shoot fresh weight determined. Also, shoot length and first true leaf lamina length were measured. On the following day, photographs of representative freshly harvested shoots external to the 601 insert and of root systems were taken. During this same day, the pH and EC of the media were determined on samples of growth medium: reverse osmosis water (1:1, volume). All data were subjected to analysis of variance.

Bruce Cobb, proprietor of ARC Greenhouses, was concerned that residual fertilizer salt on the shoot surfaces may increase the salty taste of the microgreens, when ingested. In response, we instituted a taste test of the harvested TB microgreens. It was thought that the pungency of the AR would "mask" any salt taste. Three stations were established in the Fischer Greenhouse head house. At each station were three paper plates labeled A, B, and C containing freshly harvested, tap water-rinsed TB shoots. The randomly assigned locations of the shoots from the solution fertilization treatments are shown in Table 5.1. Water bottles and instruction/data sheets were placed by each station (Table 5.2). Seven people tested samples at the three stations, to provide a total of 21 stations sampled.

Table 5.1 Taste test for shoot saltiness: random assignment to the A, B, or C plates at three stations of TB shoot samples that had received 0, 75, or 150 mg N/L daily from solution fertilization with 21-5-20.

Shoot samples from three solution fertilization concentrations (mg N/L)									
Station:	1			2			3		
Plate:	A	B	C	A	B	C	A	B	C
	0	75	150	75	150	0	150	0	75

Table 5.2 The instruction/data sheet for the saltiness taste test to be completed by each taster at a station.

TASTE TEST

Please taste the shoots in the 3 dishes labeled A, B, and C and rank them as to salty taste. In between tasting samples, rinse your mouth with the water provided. Taste each sample as many times as you feel necessary to make a determination of relative salty taste. Then complete the following:

Station 1
Your name _____ (for statistical purposes only)

Write MOST, LEAST, or MEDIUM with respect to salty taste of the 3 samples in the blank. If you feel there is no difference between samples then state which ones are the "same".

A _____ B _____ C _____

Please now repeat the process at the other two stations…..either now or sometime during the rest of the day.

5.3 Results and Discussion

5.3.1 Shoot Growth

Shoot fresh weight/m^2 of AR was greater in peat-lite than in either mulch, and generally was increased with increasing concentration of daily solution fertilization (Table 5.3). Fresh weight/shoot of AR growing in peat-lite decreased as solution fertilization concentration increased from 0 to 150 mg N/L. This decrease in individual shoot fresh weight was associated with a great increase in shoot population density from 41,557 shoots/m^2 with 0 mg N/L to 126,262 shoots/m^2 with 150 mg N/L. Thus, increasing emergence percentage or seedling survival occurred in the peat-lite as a consequence of increasing concentration of solution fertilization. At the high population densities with 75 or 150 mg N/L in peat-lite, individual shoot growth was doubtless suppressed by increasing competition for resources such as light, water and nutrients, although reduced light available to seedlings due to mutual shading is the most likely factor limiting seedling growth. It was noted that at 150 mg N/L, there was considerable rot of the AR seedlings, presumably a consequence of excessive crowding and restricted air movement favoring pathogen attack. Such crops could have been harvested earlier.

Both shoot fresh weight/m^2 and fresh weight/shoot of seedlings growing in the mulches increased as daily solution fertilization increased from 0 to 150 mg N/L (Table 5.3). Masson *et al.* (1991) found that 1-month old seedlings of several vegetable transplants were heavier following 400 mg N/L rather than 100 mg N/L twice daily solution fertilization. Soundy *et al.* (2005) found that increasing N up to 120 mg N/L in solution fertilization increased lettuce transplant shoot growth. Cornillon (1999) observed that increasing N concentration in daily solution

fertilization increased tomato transplant shoot fresh weight. The increase in individual shoot fresh weight with increasing concentration of solution fertilization (Table 5.3) means that, in contrast to seedlings growing in peat-lite, individual plants were not subjected to levels of competition that would restrict growth. Indeed, shoot population density in the mulches were much lower than in peat-lite suggesting that the mulches were incapable of supporting high percentages of crop establishment with daily applications of 75 or 150 mg N/L. Fresh weight/shoot were similar in all three media, but because of the lower population density in the mulches than in the peat-lite, shoot fresh weight/m^2 was lower in the mulches. It remains unknown whether the mulches could support similar shoot fresh weight/m^2 as the peat-lite if a shoot population density in the mulches had been similar to those in the peat-lite. As observed in the previous experiment, the mulches remained very wet. Such hypoxic conditions in the mulches presumably reduced seedling emergence.

Fresh weight/shoot and shoot fresh weight/m^2 for TB were greater for seedlings growing in peat-lite than in the mulches (Table 5.3). In all three media, shoot fresh weight/m^2 and per plant increased with increasing solution fertilization concentration, while shoot population density generally decreased. The increasing salinity from increasing concentration of solution fertilization appeared to cause decreased seedling emergence, such that interplant competition decreased, resulting in heavier seedling shoots and increased shoot weights/m^2. It is surprising that shoot population density was generally lower in the peat-lite than in the mulches. The greater seedling growth in peat-lite, however, compensated for the lower population density. Similarly, the greater population density in the mulches compensated for reduced shoot fresh weight/m^2 to such an extent that daily solution fertilization with

75 or 150 mg N/L resulted in shoot fresh weight/m^2 similar to that achieved in peat-lite that received only water (0 mg N/L).

Final pH of the peat-lite was lower than of the wood and paper mulches for both crops (Table 5.3), with nitrogen concentration having little or no effect.

Even though growth medium significantly affected final EC, values were too low (approximately 0.1 to 0.3 mmho/cm) to influence plant growth.

Table 5.3 Shoot growth of AR and TB in three growth media at 15 days after planting, and final pH and electrical conductivity (EC) of the growth medium, in response to daily solution fertilization with different N concentrations from 21-5-20[z].

Species and growth medium	N concn (mg/L)	Fresh wt. (mg/shoot)	Fresh wt. (g shoots/m^2)	Population (shoots/m^2)	Final pH	Final EC (dS/m)
AR Peat-lite	0	147 a	5865 b	41557 c	6.48 c	0.14 b
	75	95 b	8410 a	94752 b	6.24 c	0.11 b
	150	70 bc	8080 a	126262 a	6.19 c	0.20 a
Paper mulch	0	51 c	2012 ef	41179 c	7.55 a	0.20 a
	75	69 bc	2650 d	38750 c	7.56 a	0.19 a
	150	85 b	3148 c	37005 c	7.50 a	0.20 a
Wood mulch	0	43 c	1628 df	38196 c	7.15 b	0.15 a
	75	73 bc	2163 e	29599 d	7.04 b	0.13 b
	150	92 b	2419 de	26392 d	7.03 b	0.11 b
$LSD_{0.05}$ (2-way)		32	426	6427	0.27	0.05
Growth medium (G)		***	***	***	***	***
N concn (N)		ns	***	***	ns	ns
G X N		***	ns	***	ns	ns
TB Peat-lite	0	137 b	2858 b	21686 cde	5.73 d	0.11 d
	75	245 a	4316 a	17983 e	5.87 d	0.09 d
	150	257 a	4608 a	18491 de	5.89 d	0.14 cd
Paper mulch	0	63 c	1908 c	30307 a	6.84 b	0.34 a
	75	102 bc	2832 b	27795 ab	7.11 a	0.24 b
	150	145 b	3219 b	22960 bcd	7.25 a	0.19 bc
Wood mulch	0	49 c	1237 d	25212 bc	6.48 c	0.23 b
	75	94 bc	2148 c	22877 cde	6.53 c	0.13 cd
	150	131 b	2842 b	21816 cde	6.43 c	0.14 cd
$LSD_{0.05}$		56	528	4907	0.23	0.06
Growth medium (G)		***	***	***	***	***
N concn (N)		***	***	***	*	***
G X N		ns	ns	ns	ns	**

[z] ***,**,*,ns Significant at $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, or not significant, respectively.

5.3.2 Saltiness Taste Test

The assumption was that the increasing concentration of solution fertilization would increase the salty taste of the TB greens. Thus, 0 ppm N would be the least salty, 75 ppm N would be medium salty, and 150 ppm N would be the most salty. Out of 21 sample stations, only one station resulted in a "correct" response, 8 were completely "wrong," and 12 stations had two of the responses reversed (Table 5.4).

Table 5.4 Results of the saltiness taste test on TB after receiving daily solution fertilization at 0, 75 or 150 mg N/L.

Sample Frequency	Response
1	correct
8	completely wrong
8	"medium" correct, but "least" and "most" reversed
3	"most" correct, but "medium" and "least" reversed
1	'least" correct, but "most" and "medium" reversed

5.4 Conclusions

Growth of TB and AR shoots were lower in the wood and paper mulches than in the peat-lite, although AR shoot growth was suppressed more than that of TB. Increased concentration of solution fertilization increased shoot growth of both crops in all media, but resulted in decreased shoot population density of both crops in all media, except for AR in peat-lite which showed a marked increase in population density.

Concentrations of daily solution fertilization did not affect the level of salty taste of TB microgreens.

CHAPTER 6

PRE-PLANT NITROGEN FERTILIZATION OF GROWTH MEDIUM

6.1 Introduction

Bruce Cobb suggested that increasing the preplant level of nitrogen in the peat-lite (Jiffy Mix 921) might increase microgreen seedling growth. Ideally, a preplant fertilizer addition at a particular concentration would increase seedling shoot growth without adversely affecting seed germination and seedling growth through an osmotic effect. It was decided that several nitrogen-bearing granular fertilizers should be incorporated into the peat-lite before planting. Of concern with such fertilizers would be the nitrogen form (nitrate, ammonium, or urea), their solubility in water, and the concentration to use.

Slow release N-bearing fertilizers would not be an option for two reasons. Firstly, their rate of release would be too low to provide nutrients to the microgreens which are harvested within a few weeks of planting. Secondly, Bruce hopes to recycle the peat-lite he uses, which would require the medium to be pasteurized. The heat and moisture from such a treatment would cause a sharp increase in nutrient release from the slow-release fertilizers and result in an excessive level of soluble salts in the medium.

If dry, granular fertilizer were to be incorporated uniformly throughout the growth medium, the dissolution of these fertilizers would result in localized high soluble salt concentrations. Consequently, in this experiment, the same quantities of

each fertilizer, in both the dry and dissolved form, were incorporated to determine whether distribution (or dissolution rate) of the salts within the growth medium had an effect on microgreen growth.

6.2 Materials and Methods

Four nitrogen-bearing fertilizers at 500, 1000, 2000 or 4000 mg N/L were selected (Table 6.1). These fertilizers provided nitrogen as the ammoniacal or nitrate form, with ammonium nitrate providing both. The amines of urea reduced to ammonium.

Table 6.1 Nitrogen-bearing fertilizers and their concentrations used as preplant additions to peat-lite.

Fertilizer	Formula	Analysis (N-P_2O_5-K_2O by weight)	Salt index[z]	N concn (mg/L) 500	1000	2000	4000 (g fertilizer / L water)
Urea	$CO(NH_2)_2$	45-0-0	75	1.11	2.22	4.44	8.89
Ammonium nitrate	NH_4NO_3	34-0-0	105	1.47	2.94	5.88	11.76
Calcium nitrate	$Ca(NO_3)_2$	15.5-0-0	53	3.23	6.45	12.90	25.81
Ammonium sulphate	$(NH_4)_2SO_4$	21-0-0	69	2.38	4.76	9.52	19.05

[z]Rate of dissolution relative to $NaNO_3$ with an arbitrary value of 100.

These fertilizers were incorporated into the peat-lite (Jiffy Mix 921) as both solid and solution. The treatment unit was a 601 insert containing 0.5 L (2.5 cm depth) of peat-lite. A total of 2 L of peat-lite was required for 4 replications of the fertilizer treatment. It was determined that the peat-lite could receive 150 ml of water per liter without drainage, and that by adding the water the peat-lite was sufficiently friable for its dispensation into the 601 inserts. For 4 replications, 2 L of peat-lite and

300 ml of the fertilizer solution were required. The addition of the 300 ml of fertilizer solution per 2 L of peat-lite is termed the liquid (L) treatment. Table 6.2 shows the weight of fertilizer per liter of water; however only 300 ml of the solution was added to the 2 L of peat-lite. For the solid (S) treatment, 300 ml of reverse osmosis water was added to 2 L of the peat-lite and then the required weight of dry fertilizer was uniformly incorporated into the soil (Table 6.2). The water was added so that the initial moisture content of the peat-lite would not confound treatment effects.

Table 6.2 The amount of fertilizer for each nitrogen concentration required per 2L of peat-lite, either dry or dissolved in 300 ml of reverse osmosis water.

Fertilizer	N concn (mg/L)			
	500	1000	2000	4000
	(g fertilizer / 2 L peat-lite)			
Urea	0.33	0.67	1.33	2.67
Ammonium nitrate	0.44	0.88	1.76	3.53
Calcium nitrate	0.97	1.94	3.87	7.74
Ammonium sulphate	0.71	1.43	2.86	5.72

When the required weights of fertilizers were added to the reverse osmosis water (Table 6.2), they all dissolved readily except for the ammonium sulphate; so, the liquid application of ammonium sulphate was deleted from the study. The study thus consisted of a 3 (N-bearing fertilizer) X 4 (N concentration) X 2 (dry vs. liquid) factorial, and the dry application of ammonium sulphate at 4 concentrations. The control treatment was peat-lite that received no preplant fertilizer addition to the peat-lite. The pH and EC of the dissolved fertilizers were determined (Table 6.3).

Table 6.3 The pH and EC of the fertilizer solutions before they were mixed in the peat-lite. No statistical procedures could be performed on these single value readings.

Fertilizer[z]	N concn (mg/L)	pH	EC (mmho/cm)
Urea	500	6.49	0.37
	1000	5.58	0.37
	2000	6.61	0.37
	4000	6.64	0.37
Ammonium nitrate	500	6.54	2.77
	1000	6.48	4.79
	2000	6.41	8.83
	4000	6.29	17.66
Calcium nitrate	500	6.46	3.82
	1000	6.42	6.62
	2000	6.31	11.71
	4000	6.14	20.90

[z] Ammonium sulfate was excluded because it failed to dissolve.

The media were placed into the 601 inserts and leveled at 2.5 cm depth. AR seeds were then broadcast over the surface (at the 1X rate of 1.12g seeds over the 204 cm^2 of the surface) and then covered with *ca* 2 mm of Jiffy Mix Plus. Treatments (601 inserts) were arranged in randomized complete blocks in a greenhouse set at 27° C day and 20° C night, and seedlings were grown under natural light from 27 June - 14 June. Flats were irrigated with water as needed, however the media were irrigated as little as possible to prevent leaching of the fertilizer.

At 14 days after planting, all shoots were cut with sharp scissors at the growth medium surface, and their fresh weight determined. All media were sampled on the same day as the harvest, and the final pH and EC determined (1:1 volume; growth medium: reverse osmosis water). Shoot fresh wt/m^2 and final pH and EC of the media were subjected to analysis of variance.

6.3 Results and Discussion

Fertilizer type, nitrogen concentration, and fertilizer state (solid vs. liquid application) all affected AR shoot fresh weight/m^2 (Tables 6.4.1 and 6.4.2), but none of the interactions was significant (Table 6.4.3). Shoot fresh weight/m^2 was greatest with calcium nitrate, followed by ammonium nitrate, then urea, then ammonium sulfate (Table 6.4.2). The control media which received no preplant nitrogen fertilizer other than that already present in it, supported similar shoot fresh weight/m^2 as media to which ammonium sulfate had been incorporated. These results are in agreement with others who found reduced vegetative and/or reproductive growth in crops receiving ammonium nitrogen rather than nitrate nitrogen (Barker and Mills, 1980; Kotsiras *et al.*, 2005). Although no symptoms of ammonium toxicity such as leaf chlorosis and necrosis, epinasty, or stem lesions (Maynard and Barker, 1969) were observed, fertilizers that provided nitrogen either partly or wholly (NH_4NO_3, $(NH_4)_2SO_4$, $CO(NH_2)_2$) resulted in less seedling growth than $Ca(NO_3)_2$. Barker *et al.* (1970) and Patniak *et al.* (1972) observed that seed germination and seedling growth can be damaged severely by ammonium ions. Reasons for reduced growth with ammonium than with nitrate nitrogen include reduced uptake of other cations such as Ca and Mg, and increased plant water stress (Pill *et al.*, 1978).

Table 6.4.1 Shoot fresh weight of AR at 14 days after planting, and final pH and EC of the growth medium, in response to the type, state and concentration of preplant nitrogen fertilization of the peat-lite (all data)[z].

Fertilizer	Form	N concn (mg/L)	Shoot fresh weight/m^2	Final pH	Final EC (dS/m)
Urea	Liquid	500	2188	6.16	0.09
		1000	2314	6.26	0.08
		2000	2532	6.37	0.09
		4000	2224	6.67	0.09
	Solid	500	2195	6.42	0.09
		1000	2192	6.38	0.08
		2000	2268	6.53	0.09
		4000	2151	6.61	0.10
Ammonium nitrate	Liquid	500	2207	6.31	0.09
		1000	2467	6.38	0.08
		2000	2569	6.26	0.09
		4000	2476	6.35	0.09
	Solid	500	2180	6.08	0.09
		1000	2447	6.09	0.08
		2000	2479	6.23	0.09
		4000	2133	6.30	0.10
Calcium nitrate	Liquid	500	2486	6.23	0.09
		1000	2758	6.36	0.09
		2000	2889	6.25	0.11
		4000	2454	6.29	0.09
	Solid	500	2348	6.15	0.14
		1000	2522	6.16	0.09
		2000	2533	6.28	0.09
		4000	2513	6.18	0.09
Ammonium sulfate	Solid	500	1983	6.18	0.09
		1000	2073	6.08	0.10
		2000	1996	6.06	0.09
		4000	1509	6.07	0.20
Control (no fertilizer)	-	-	1828	6.18	0.10

[z] Statistical analysis appears in Table 6.4.3.

Table 6.4.2 Shoot fresh weight of AR at 14 days after planting, and final pH and EC of the growth medium, in response to the type, state and concentration of preplant nitrogen fertilization of the peat-lite (statistically significant means, excluding ammonium sulfate and control)[z].

Treatment	Shoot fresh weight/m^2	Treatment	Final pH
Liquid	2462 a	Ammonium nitrate-liquid	6.3 c
Solid	2320 b	Ammonium nitrate-solid	6.1 d
		Calcium nitrate-liquid	6.3 c
Ammonium nitrate	2372 b	Calcium nitrate-solid	6.2 d
Calcium nitrate	2568 a	Urea-liquid	6.4 b
Urea	2258 c	Urea-solid	6.5 a
N concn (mg/L)		N concn (mg/L)	
500	2258	500	6.2
1000	2436	1000	6.2
2000	2540	2000	6.3
4000	2322	4000	6.4
	L*		L**
	Q**		Q^{ns}
Ammonium sulfate (solid only)[y]	1890		
Control (no fertilizer)	1828		6.2

[z] ***,**,* Significant at $P \leq 0.001$, $P \leq 0.01$, or $P \leq 0.05$; L= linear; Q= quadratic; means with the same letters within a column for a treatment are not significant, different by $LSD_{0.05}$.

[y] See Table 6.4.3 for analysis of variance.

Table 6.4.3 Statistics associated with Tables 6.3.1 and 6.3.2.

Source of variation	Level of significance[z] Shoot fresh wt/m^2	Final pH	Final EC
1. Factorial (excluding ammonium sulfate and the control)			
Fertilizer (F)	***	***	ns
N concn (N)	***	**	ns
N concn lin (NL)	*		
N concn quad (NQ)	**		
F X N	ns	ns	ns
S vs. L (SL)	***	ns	ns
F X SL	ns	**	ns
N X SL	ns	ns	ns
F X N X SL	ns	ns	ns
2. One-way, all solid fertilizers and the control (excluding liquid fertilizers)			
Treatment	***	***	**
3. One-way, all liquid fertilizers and the control (excluding liquid fertilizers)			
Treatment	***	**	***

[z] ***,**,ns Significant at $P \leq 0.001$, $P \leq 0.01$, or not significant, respectively.

Increasing nitrogen concentration (from 500 to 2000 mg N/L) increased shoot fresh weight/m^2, but the quadratic response (Table 6.4.2) revealed that shoot fresh weight/m^2 was decreased when nitrogen increased from 2000 mg N/L to 4000 mg N/L. This is a typical response of plants to factors necessary for growth. Growth responds positively to increasing nutrient levels, and then reaches a maximum, beyond which growth may stabilize during luxury consumption, and may decline when toxic levels are reached (Handreck and Black, 1984). Increasing concentration of

ammonium nitrate and calcium nitrate resulted in linear increases in electrical conductivity of the fertilizer solutions added to the growth medium (Table 6.4). Another reason for decreased growth with 4000 mg N/L from NH_4NO_3 or $Ca(NO_3)_2$ may be water stress induced osmotically by the high concentration of dissolved salts at the time of media preparation and during early seedling growth (Table 6.3). By 14 days after planting, however, growth medium EC was very low (Table 6.4.1) and little affected by treatment (Table 6.4.3). An EC value of 0.15 to 0.50 dS/m (1 medium: 2 water) is recommended as being "suitable for seedlings and media high in organic matter," such as the peat-lites (Bunt, 1988).

Dissolving the fertilizers in reverse osmosis water before adding them to the growth medium gave 6% greater shoot fresh weight/m^2 than incorporation of dry fertilizer (Table 6.4.2). This increased growth may reflect a more even distribution of the fertilizer so that roots of all seedlings benefited from this immediately available (soluble) nutrition. Fertilizer granules as they dissolve would result in regions of high salt concentration interspersed by regions devoid of the preplant nitrogen fertilizer addition. The time needed for granule dissolution would cause a delay in availability of nutrients from this form of application compared to incorporation of dry granules in the growth medium. Even though preplant incorporation of the nitrogen-bearing fertilizers as liquids rather than solids increased yield by 6%, the dissolution of the fertilizer may represent an extra labor input that the grower may not want to endure.

Use of ammonium bearing fertilizers contributes to a decrease in growth medium pH while use of nitrate-bearing fertilizers contributes to increased growth medium pH (Handreck and Black, 1984). In this study however, the final pH of the growth medium was greater for urea than for ammonium nitrate and calcium nitrate,

and was least for ammonium sulfate (Table 6.4.2). It is possible that insufficient microbial urease was present in the growth medium to cause the conversion of urea to ammonium, so that less oxidation of ammonium to nitrate by nitrification led to reduced accumulation of protons and therefore to increased growth medium pH, and to less shoot growth than occurred with nitrate bearing fertilizers. While increasing concentrations of ammonium nitrate and calcium nitrate which led to increasing EC at the time of media preparation, increasing urea concentration failed to increase EC (Table 6.3). This response is likely due to the absence of microbial urease to break down the urea. Elliott (1988) noted that while urea hydrolysis has been studied extensively for many years in field soils, only recently has this process received attention in soilless growth media. He found that ureolytic activity was not consistently affected by length of cropping (poinsettia or anemone) in peat-lite or bark-modified peat-lite, and that interactions of media type, fertilization system, and length of cropping indicated the complexity of the development of ureolytic activity in soilless growth media.

6.4 Conclusions

Preplant incorporation of $Ca(NO_3)_2$ resulted in the greatest shoot fresh weight/m^2 for AR, followed by ammonium nitrate, urea, and then ammonium sulfate. Liquid and solid $Ca(NO_3)_2$ at 2000 ppm gave 58% and 39% increase, respectively, in shoot fresh weight/m^2 than the control (no preplant or post emergence fertilization). Fertilizer type, form and concentration have very little effect on final pH, and no effect on final EC.

CHAPTER 7

SEED TREATMENTS

7.1 Introduction

To speed microgreen production, Bruce Cobb would like to use the pregermination technique described in Lee *et al.* (2004). This technique involves mixing seeds in very fine (grade 5) moist exfoliated vermiculite, allowing a large percentage of the seeds to germinate before broadcasting the vermiculite-germinated seed mixture onto the growth medium. To be of value to commercial microgreen production, the system of pregermination must be fairly simple and must apply (with minor adjustments) to all species. In this experiment, the germination "medium" will be the grade 5 vermiculite and a coarse grade hydrophilic polymer, Supersorb C. Both of these materials are highly visible, so that the individual sowing the germinated seed-medium mixture will be able to sow the seed evenly over the peat-lite growth medium.

7.2 Materials and Methods

The moisture concentration of two potential media in which the microgreen seeds are germinated will be varied to determine the optimal moisture concentration for germination and radicle growth. Lee *et al.* (2004) used 150% water (percentage of the vermiculite dry weight) in the fine (number 5 grade) exfoliated vermiculite to germinate seeds of TB and swiss chard. In the present study,

concentrations of 100, 150, 200 or 250% water in the vermiculite (% of dry weight) were selected to determine their effects on the germination and radicle growth of TB and AR seeds. As another seed germination medium, a coarse grade of a cross-linked polyacrylamide hydrophilic copolymer (Supersorb C, Aquatrols, Cherry Hill, NJ) was selected. In a preliminary study, radish seeds were mixed in 2.5 % Supersorb C (Supersorb C weight in water (W/V)). Within one day, the seeds had germinated throughout the hydrated Supersorb C, even at the bottom of the beaker. Thus, the coarse nature of the Supersorb C with hydrated particle up to 0.5 cm diameter permitted adequate aeration of the seeds at the bottom of the beaker. Supersorb C concentrations of 2.0, 2.5, 3.0 or 3.5% were selected for the present study. The control treatment was the germination of TB or AR seeds on germination paper moistened with deionized water.

The volume of vermiculite and Supersorb C and the weight of seeds used in this study were based on the area of a 601 insert and 1X seeding rates. The 601 is 0.01318 the area of an ARC Greenhouses trough. It was assumed that 5 L of vermiculite or Supersorb C would permit easy distribution of the seed-medium mixture, so that a medium volume of 66 ml would be needed for the 601 insert. At the 1X seeding rate in a 601 insert, 4.06 g of TB seeds and 1.12 g of AR seeds would be needed.

To determine the hydrated volume, 2.0, 2.5, 3.0 or 3.5 g of Supersorb C was added to 100 ml of deionized water. After 30 minutes of frequent stirring, the hydrated Supersorb C was transferred to a graduated cylinder, and the volumes estimated as 110, 120, 125, or 130 ml, respectively.

In an earlier study (see Table 2.2), the G_{50} of TB and AR at 20° C was established as 2.9 and 0.7 days, respectively. AM was deleted from this study owing to its infestation with *Rhizoctonia solanii*. Since 20° C is a common average room temperature, it was decided to use this as the seed germination temperature. To bracket the G_{50} times, separate vermiculite and Supersorb C samples were prepared for 1, 2, or 3 days for AR, and 1, 2, 3, 4, or 5 days for TB. Thus, the experiment consisted of two species (TB and AR) in two media (vermiculite or Supersorb C) at 4 "concentrations," replicated 4 times for each of the sampling days (5 for TB, 3 for AR). Control seeds were sown on moistened double blotters as described in earlier studies. The vermiculite and Supersorb C were placed in opaque 100 ml cups, capped with clear lids that had been punctured slightly to provide aeration to the seeds.

At each day of the germination count, the percentage germination and the mean radicle length of approximately 100-seed subsamples (are all 100 seeds of the control treatment) were determined, and the experimental unit discarded. With TB, when the first seed within a seed ball germinated, it was classified as "germinated".

7.3 Results and Discussion

Supersorb C as a germinating medium for TB seeds gave earlier germination than vermiculite as a germinating medium at 20° C (Table 7.1). By day 2, Supersorb C resulted in 10 to 20% germination and radicle lengths averaging 2.4mm, whereas no seeds had germinated in vermiculite. Increasing the Supersorb C concentration from 2 to 3.5% resulted in decreased germination percentage and radicle length on day 2, but in subsequent days Supersorb C concentration had little to no effect on germination or radicle length. The optimal Supersorb C concentration was

2.5%, which on day 3 resulted in 69.3% germination and 8.5mm long radicles. On day 3, the control seeds had only 20.2% germination and radicles 4.3mm long.

Increasing moisture content in vermiculite at 20° C increased germination percentage of TB on days 3, 4, 5, and 6, and increased radicle length on days 3, 4, and 5 (Table 7.1). Such increases can be explained by the increase in matric potential as moisture content increased. The moisture characteristic curve for this material revealed matric potential (ψ_m) values of -0.4, -0.3, -0.2, and -0.1 MPa at 100, 150, 250, and 300% (w/w) water (Khan *et al.*, 1992). In fact, the reduced water availability at -0.4 MPa (100% water) severely restricted germination and radicle growth throughout the course of the experiment. By day 6 and 7, radicles were too long in both Supersorb C and vermiculite to be measured. The optimal vermiculite treatment was 150% water on day 5, which gave 50% germination and radicles 6.5mm long. On day 5, the control seeds had 82.3% germination with radicles 10.5mm long. The lower germination in the vermiculite than in the control seeds may reflect reduced water and/or aeration. Lee *et al.* (2004) found that germinating TB seeds in vermiculite was a satisfactory way to advance establishment of this crop as a microgreen. They further found that various seed treatments prior to germination such as priming and soaking in hydrogen peroxide failed to improve TB establishment.

It remains unknown whether the long (*ca* 1 cm) radicles of TB seeds germinated in Supersorb C or vermiculite remain intact during sowing. Mechanical injury to the root system of the seedlings could hinder subsequent growth and development.

For AR, one day of germination in either Supersorb C or vermiculite at 20° C resulted in satisfactory germination (Table 7.2). The optimal Supersorb C

treatment was 2% solid on day 1 which gave 90% germination and radicles 1mm long, compared to 62.5% germination in the control treatment which had radicles 1.5 mm long. There was little or no effect of Supersorb C concentration on germination percentage or radicle length.

Increasing moisture content in vermiculite increased AR germination percentage and radicle length at 20° C (Table 7.2). The treatment deemed optimal was 200% moisture in the vermiculite for 1 day, which gave 81.5% germination and radicles 2 mm long, compared to 62.5% germination and 1.5mm long radicles for the control treatments. It is possible that further advancement in establishment in AR could be achieved by priming the seeds in vermiculite at 50% moisture for 3 days at 12° C (this achieves -1.0 MPa) before increasing the moisture content to 150% for 1 day at 27° C, since Lee and Pill (2005) found this two-stage, priming-germinating system to be advantageous for radish, kale, and amaranth microgreens, with similar germination rates to AR.

Table 7.1 Daily germination percentage (and its angular transformation) and radicle length of TB in response to Supersorb C and vermiculite germination media at varying concentrations of solid to water.

	Day 2			Day 3			Day 4		
Treatment[z]	Germ. %	Radicle length (deg.)	(mm)	Germ. %	Radicle length (deg.)	(mm)	Germ. %	Radicle length (deg.)	(mm)
Control	0.8	(2.5) c	0.3c	20.2	(26.5) c	4.3 c	65.0	(53.9) a	10.0 bc
Supersorb 2.0	18.8	(25.6) a	3.3 a	54.5	(47.6) b	9.8 a	50.3	(45.2) b	1.8 abc
2.5	19.3	(26.0) c	2.5 ab	69.3	(56.4) a	8.5 ab	68.0	(55.6) a	14.3 a
3.0	10.0	(18.2) b	2.0 ab	58.5	(49.9) b	9.3 a	74.3	(59.5) a	13.0 ab
3.5	10.0	(18.2) b	1.8 b	58.3	(49.8) b	8.5 ab	64.3	(53.6) a	11.3 abc
Vermiculite 100	0	(0) c	0 c	0	(0) f	0 d	1.0	(3.9) a	0.5 e
150	0	(0) c	0 c	2.5	(7.9) e	1.8 d	32.0	(34.2) c	5.8 d
200	0	(0) c	0 c	9.3	(17.6) d	4.8 c	28.3	(32.1) c	8.8 cd
250	0	(0) c	0 c	20.3	(26.4) c	7.3 b	65.0	(54.0) a	11.8 abc
1-way ($LSD_{0.05}$)		(3.7) ***[y]	1.3 ***		(5.2) ***	1.8***		(7.9) ***	3.1***
Factorial									
Medium (M)		(***)	***		(***)	***		(***)	***
Concn (C)		(***)	***		(***)	***		(***)	***
M X C		(***)	***		(***)	***		(***)	***
	Day 5			Day 6			Day 7		
Treatment[z]	Germ. %	Radicle length (deg.)	(mm)	Germ. %	(deg.)		Germ. %	(deg.)	
Control	82.3	(65.8) b	10.5 c	93.5	(75.9) b	--[x]	96.5	(79.5) b	--[x]
Supersorb 2.0	100.0	(90.0) a	15.0 b	100.0	(90.0) a		100.0	(90.0) a	
2.5	100.0	(90.0) a	21.3 a	100.0	(90.0) a		100.0	(90.0) a	
3.0	100.0	(90.0) a	10.0 c	100.0	(90.0) a		100.0	(90.0) a	
3.5	100.0	(90.0) a	9.5 cd	100.0	(90.0) a		100.0	(90.0) a	
Vermiculite 100	3.0	(7.1) d	1.3 e	13.5	(21.5) e		23.0	(28.4) c	
150	50.0	(45.0) c	6.5 d	73.8	(59.3) d		100.0	(90.0) a	
200	62.5	(52.5) c	10.0 c	82.8	(66.0) c		100.0	(90.0) a	
250	100.0	(90.0) a	16.3 b	100.0	(90.0) a		100.0	(90.0) a	
1-way ($LSD_{0.05}$)		(8.3) ***	3.2 ***		(5.6) ***			(2.8) ***	
Factorial									
Medium (M)		(***)	(***)		(***)			(***)	
Concn (C)		(***)	(***)		(***)			(***)	
M X C		(***)	(***)		(***)			(***)	

[z] 2.0 to 3.5% Supersorb C in water (wt./vol.) and 100 to 250% water in grade 5 vermiculite (wt./dry wt.).

[y] *** Significant at $P \leq 0.001$. Means followed by the same letter within a column for a germination day are not significantly different by $LSD_{0.05}$.

[x] Radicles were too long and tangled to be measured.

Table 7.2 Daily germination percentage (and its angular transformation) and radicle length of AR in response to Supersorb C and vermiculite germination media at varying concentrations of solid to water.

	Day 1			Day 2			Day 3		
	Germ.	Radicle length		Germ.	Radicle length		Germ.	Radicle length	
Treatment[z]	%	(deg.)	(mm)	%	(deg.)	(mm)	%	(deg.)	(mm)
Control	62.5	(52.5) d	1.5 b	100	(90)	13.5 c	100	(90)	32.0 ab
Supersorb 2.0	90.0	(74.6) a	1.0 b	100	(90)	7.8 d	100	(90)	17.0 c
2.5	84.8	(68.7) bc	1.3 b	100	(90)	8.3 d	100	(90)	17.0 c
3.0	88.3	(18.2) f	2.0 ab	100	(90)	9.5 d	100	(90)	14.5 cd
3.5	86.0	(18.2) f	1.8 b	100	(90)	8.8 d	100	(90)	13.0 d
Vermiculite 100	47.0	(43.2) e	1.1 b	100	(90)	12.3 c	100	(90)	31.3 ab
150	66.3	(54.9) d	1.6 b	100	(90)	17.0 b	100	(90)	30.5 b
200	81.5	(66.0) c	2.0 ab	100	(90)	17.0 b	100	(90)	30.8 b
250	87.0	(69.2) b	3.0 a	100	(90)	218 a	100	(90)	33.8 a
1-way ($LSD_{0.05}$)		(2.7) ***[y]	1.1 ***		(NA)	2.5***		(NA)	2.8 ***
Factorial									
Medium (M)		(***)	***		(NA)	***		(NA)	***
Concn (C)		(ns)	***		(NA)	***		(NA)	ns
M X C		(*)	***		(NA)	***		(NA)	ns

[z] 2.0 to 3.5% Supersorb C in water (wt./vol.) and 100 to 250% water in grade 5 vermiculite (wt./dry wt.).
[y] *** Significant at $P \leq 0.001$. Means followed by the same letter within a column for a germination day are not significantly different by $LSD_{0.05}$. NA= not applicable.

7.4 Conclusions

Germinating seeds in the hydrophilic polymer, Supersorb C, compared to vermiculite resulted in greater and earlier germination of both TB and AR seeds.

The germination media at 20° C deemed optimal for TB were 2.5% Supersorb C for 3 days (69.3% germination, 6.5 mm radicles) and vermiculite with 150% water for 5 days (50% germination, 6.5 mm radicles).

The germination media at 20° C deemed optimal for AR were 2% Supersorb C for 1 day (90% germination, 1 mm radicles) and vermiculite with 200% water for 1 day (81.5% germination, 2 mm radicles).

CHAPTER 8

COMBINING SEED AND FERTILIZER TREATMENTS

8.1 Introduction

In this experiment, the treatments that gave the optimal results in Experiments 5, 6 and 7 are combined. In Experiment 5, while TB growth in peat-lite was greatest with 150 mg N/L applied daily as solution fertilization, AR growth was decreased as daily fertilization increased from 75 to 150 mg N/L. However, the 150 mg N/L treatment hastened AR growth to such an extent that excessive shoot crowding by the time of harvest resulted in considerable shoot rot. Plants from this treatment could have been harvested earlier, reducing production time. Thus, 150 mg N/L was selected as the optimal concentration of solution fertilization.

From the results of Experiment 6, the preplant fertilizer addition that gave the greatest shoot weight of TB and AR was $Ca(NO_3)_2$ at 2000 mg N/L. Although the liquid application gave greater shoot weight than the solid, the solid application was selected because it may be the more convenient incorporation method in a commercial microgreen production operation.

The effect of combining preplant N addition with solution fertilization has not been determined. Thus, the fertilization treatments in this experiment included preplant incorporation of solid $Ca(NO_3)_2$ at 2000 mg N/L alone; daily solution fertilization at 150 mg N/L from 21-5-20; these two treatments combined ($Ca(NO_3)_2$ at 2000 mg N/L plus 150 mg N/L solution fertilization); and, these two treatments at

one-half concentration each (preplant $Ca(NO_3)_2$ at 1000 mg N/L plus 75 mg N/L solution fertilization). This last treatment was included because of concern that by combining the preplant and solution fertilization treatments that separately maximized growth, excessive soluble salts may restrict growth.

From Experiment 7, TB germination was 50% and radicle length was 6.5 mm when the seeds were incubated in vermiculite at 150% water concentration for 5 days. Increasing the water concentration to 200% increased germination to 62.5% and resulted in radicles that might be too long, so long (*ca* 10 mm) that mechanical injury might occur when broadcasting the seed. For AR, 1 day in incubation in vermiculite with 200% water resulted in 81.5% germination and 2 mm long radicles. Since a single water concentration in the vermiculite across all microgreen species would be more convenient commercially, 200% water was selected, with an incubation period at 20° C of 4.5 days for TB and 1 day for AR.

As for seed incubation in Supersorb C, the optimal polymer concentration in water for TB was 2.5% for 3 days, yielding 69.3% germination and 8.5 mm long radicles. For AR, the optimal Supersorb C concentration was 2% for 1 day, which resulted in 90% germination and 1 mm long radicles. As with vermiculite, a single Supersorb C concentration would be more convenient in commercial microgreen production, thus 2% was selected, with 1 and 3 days incubation at 20° C for AR and TB, respectively.

8.2 Materials and Methods

The methods used in this experiment were identical to those described in Experiments 5, 6, and 7, respectively, for solution fertilization, preplant fertilizer

incorporation into the peat-lite, and seed germination. The three seed treatments and 5 fertilizer treatments combined factorially are listed in Table 8.1.

Table 8.1 The 3 seed treatments and 5 fertilizer treatments factorially combined in Experiment 8.

Treatment code	Seed treatment[z]	Fertilization treatment[y]
1	C	CK
2	C	CN
3	C	SF
4	C	CN + SF
5	C	0.5 CN+ 0.5SF
6	V	CK
7	V	CN
8	V	SF
9	V	CN+SF
10	V	0.5CN+ 0.5SF
11	S	CK
12	S	CN
13	S	SF
14	S	CN+SF
15	S	0.5CN+0.5SF

[z] Seed treatment: C = control, V = germination at 20° C in vermiculite (200% water; 1 day for AR and 4.5 days for TB), S = germination in Supersorb C (2%; 1 day for AR and 3 days for TB).
[y] Fertilization treatment: CK = check, CN = preplant $Ca(NO_3)_2$ at 2000 mg N/L, SF = solution fertilization at 150 mg N/L daily; 0.5 CN = one-half CN; 0.5 SF = one-half SF.

Each flat comprised a fertilization treatment. Three 601 inserts, with their bottoms removed, were inserted into each standard flat containing the Jiffy Mix 921. These inserts were randomly assigned to the three seed treatments. Seeds (TB at 4.06g and AR at 1.12g) were broadcast within the boundaries of the inserts. Between the inserts and the flat walls, nontreated seeds of the appropriate species were

broadcast at a rate estimated to be similar to that within the inserts. The broadcasted seeds were covered with a 2 mm layer of Jiffy Mix Plus. The flats were arranged in a randomized complete block design with 4 replications and the experiment was conducted in mid-August under natural light. When seedlings of a treatment within a species first reached harvestable stage, shoots from all treatments were harvested.

On the day of harvest (15 and 13 days after sowing for TB and AR, respectively), all shoots within a 601 insert were cut with sharp scissors at the growth medium surface and immediately weighed to provide shoot fresh weight per unit area of growth medium. Then, a subsample of 100 randomly selected shoots was weighed, from which an estimate of fresh weight per shoot was calculated. The pH and EC of a growth medium sample from within each 601 insert were measured on the same day as shoot harvest.

Owing to the large size of this experiment, it was divided. First, control versus Supersorb C was tested, then control versus vermiculite.

8.3 Results

The interaction of seed treatment (control vs. germination in Supersorb C) and fertilization treatment affected shoot fresh weight/m^2 and population density of AR (Table 8.2.1). Shoot fresh weight/m^2 at 13 days after planting was generally greater from AR seeds that were germinated in Supersorb C before sowing than from control seeds. For control seeds, shoot fresh weight/m^2 in media containing $Ca(NO_3)_2$ (1000 mg/L or 2000 mg/L) were greater than those that received only 150 mg N/L solution fertilization or no additional fertilizer (the check treatment). Shoot fresh weight/m^2 from seeds germinated in Supersorb C were greatest with the combination of $Ca(NO_3)_2$ preplant fertilization with solution fertilization (at both half-or full-rates

of each), followed by $Ca(NO_3)_2$ or solution fertilization, and were least in the check medium. Fresh weight/shoot was 9.7% greater with Supersorb C than from the control seeds. Fresh weight/shoot generally followed shoot fresh weight/m^2, in that values were greatest when preplant $Ca(NO_3)_2$ incorporation was combined with solution fertilization (half- or full-rates of each), followed by $Ca(NO_3)_2$ and solution fertilization separately, and values were least in the check medium. Population density was similar in all treatments except for lower values for control seeds that received half rates of both $Ca(NO_3)_2$ and solution fertilization. Final growth medium pH and EC were unaffected by seed treatment but were affected by fertilization (Table 8.2.1). The combination of preplant incorporated $Ca(NO_3)_2$ at 2000 mg N/L and 150 mg N/L solution fertilization depressed final pH to 5.29 relative to other treatments which ranged from pH 5.73 to 5.99. Final growth medium EC was greatest with 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization, lowest with the check, and intermediate with all other treatments.

Table 8.2.1 Shoot fresh weight per plant and per unit area and population density of AR at 13 days after planting, and final growth medium pH and electrical conductivity (EC), in response to seed treatment (control and Supersorb C) and fertilizer treatment.

Seed treatment (ST)[z]	Fertilization treatment (FT)[y]	Shoot fresh weight (mg/shoot)	Shoot fresh weight m^{-2} (g)	Population density (no. m^{-2})	Final pH	Final EC (dS m^{-1})
Control	Check	70	1745 g	25083 ab	6.18	0.062
	CN	86	2411 de	28620 a	5.92	0.206
	SF	101	1774 fg	17735 c	5.81	0.214
	0.5 CN+ 0.5 SF	110	2347 de	21342 bc	5.78	0.180
	CN+SF	104	2638 cd	25537 ab	5.29	0.388
Supersorb C	Check	79	2151 ef	27293 a	5.81	0.128
	CN	102	2824 bc	27708 a	6.04	0.142
	SF	105	2854 bc	27518 a	5.98	0.156
	0.5 CN+ 0.5 SF	115	3243 a	28244 a	5.67	0.236
	CN+SF	119	3059 ab	25808 ab	5.29	0.276
$LSD_{0.05}$			391	4976		
Significances[x]	ST	**	***	**	ns	ns
	FT	***	***	*	***	***
	ST X FT	ns	*	*	ns	ns
Main effect means						
Control		94 b	2183 b	23664 b	5.80 a	0.210 a
Supersorb C		104 a	2826 a	27314 a	5.76 a	0.188 a
Check		75 c	1948 c	26188 a	5.99 a	0.095 c
CN		94 b	2618 a	28164 a	5.98 a	0.174 b
SF		103 ab	2314 b	22627 b	5.89 ab	0.185 b
0.5 CN+ 0.5 SF		113 a	2795 a	24793 ab	5.73 b	0.208 b
CN+SF		112 a	2848 a	25672 ab	5.29 c	0.332 a
$LSD_{0.05}$		11	276	3518	0.20	0.078

[z] Control= raw seed and Supersorb= seed germination in 2% Supersorb C for 1 day at 20° C.
[y] Check= no additional fertilizer added to the peat-lite; CN= preplant addition of solid $Ca(NO_3)_2$ at 2000 mg N/L of peat-lite; SF= daily application of 150 mg N/L solution fertilizer from 21-5-20; .05= one-half rate.
[x] ***, **, *, ns Significant at $P \leq 0.001$, $P \leq 0.01$, or $P \leq 0.05$, or not significant, respectively. Means within a column and grouping followed by the same letter are not significantly different by $LSD_{0.05}$.

Table 8.2.2 Shoot fresh weight per plant and per unit area and population density of AR at 13 days after planting, and final growth medium pH and electrical conductivity (EC), in response to seed treatment (control and vermiculite) and fertilizer treatment.

Seed treatment (ST)[z]	Fertilization treatment (FT)[y]	Shoot fresh weight (mg/shoot)	Shoot fresh weight m^{-2} (g)	Population density (no. m^{-2})	Final pH	Final EC (dS m^{-1})
Control	Check	68 f	2623	38317 a	6.93	0.122 fg
	CN	117 e	3194	27573 b	6.68	0.108 g
	SF	174 bc	4161	24133 bc	6.63	0.268 bcd
	0.5 CN+ 0.5 SF	142 d	3969	27935 b	6.73	0.202 defg
	CN+SF	173 bc	3764	22175 c	6.57	0.324 bc
Vermiculite	Check	80 f	2876	36011 a	6.92	0.132 efg
	CN	156 cd	4178	26841 b	6.73	0.222 cdefg
	SF	187 ab	4755	25531 bc	6.55	0.242 bcde
	0.5 CN+ 0.5 SF	204 a	4868	23982 bc	6.88	0.346 b
	CN+SF	178 b	4822	27188 b	6.57	0.528 a
$LSD_{0.05}$		18		4182		0.115
Significances[x]	ST	***	***	ns	ns	***
	FT	***	***	***	***	***
	ST X FT	***	ns	*	ns	*
Main effect means						
Control		135 b	3542 b	28026 a	6.71 a	0.205 b
Vermiculite		161 a	4300 a	27911 a	6.73 a	0.294 a
Control		74 c	2749c	37164 a	6.92 a	0.127 c
CN		136 b	3686b	27207 b	6.71 bc	0.165 c
SF		181 a	4458a	24832 b	6.59 cd	0.255 b
0.5 CN+ 0.5SF		173 a	4419a	25959 b	6.80 b	0.274 b
CN+SF		175 a	4293a	24681 b	6.57 d	0.426 a
$LSD_{0.05}$		13	311	2957	0.12	0.082

[z] Control= raw seed and Vermiculite= seed germination in fine grade (5) vermiculite with 200% d. water for 1 day at 20° C.

[y] Check= no additional fertilizer added to the peat-lite; CN= preplant addition of solid $Ca(NO_3)_2$ at 2000 mg N/L of peat-lite; SF= daily application of 150 mg N/L solution fertilizer from 21-5-20; .05= one-half rate.

[x] ***, **, *, ns Significant at $P \leq 0.001$, $P \leq 0.01$, or $P \leq 0.05$, or not significant, respectively. Means within a column and grouping followed by the same letter are not significantly different by $LSD_{0.05}$.

In comparing control seeds of AR with those germinated in vermiculite, shoot fresh weight/m^2 was increased 21.4% by germinating the seeds in vermiculite before planting (Table 8.2.2). Shoot fresh weight/m^2 was greatest with 150 mg N/L solution fertilization (SF), 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF), and 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF), followed by 2000 mg N/L $Ca(NO_3)_2$ (CN), and was least with the check medium (CK). Thus solution fertilization alone or in combination with preplant $Ca(NO_3)_2$ incorporation increased shoot fresh weight/m^2. Fresh weight/shoot generally followed the same patterns as shoot fresh weight/m^2 in response to seed and fertilizer treatments. Population density was little affected by seed treatment and was generally suppressed by all fertilization treatments compared to the check medium.

Final growth medium pH was unaffected by seed treatment, but as with the previous experiment, the lowest pH was found in growth media receiving 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF). Final growth medium EC was influenced by the interaction of seed and fertilization treatments. Other than elevated EC with 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN + SF) in media supporting seedlings germinated in vermiculite, the 0.238 dS/m range of EC in all other treatments was small.

Shoot fresh weight/m^2 and fresh weight/shoot of TB from seeds germinated in Supersorb C were 25.9% and 21.4% greater, respectively, than those from control seeds (Table 8.3.1). Shoot fresh weight/m^2 and fresh weight/shoot followed similar trends in response to fertilization treatment in that the greatest values were with 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF) and the lowest values were achieved in the check media, other treatments yielding

intermediate values. Plant population density was affected only by fertilization treatment with all fertilization treatments giving greater values than the control.

Final pH was affected only by fertilization treatment with 150 mg N/L solution fertilization (SF) and 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF) giving lower values than the other treatments. Although final EC was affected by the interaction of seed treatment and fertilization treatment, the 0.552 dS/m range was small.

Table 8.3.1 Shoot fresh weight per plant and per unit area and population density of TB at 15 days after planting, and final pH and electrical conductivity (EC) in response to seed treatment (control and Supersorb C) and fertilizer treatment.

Seed treatment (ST)[z]	Fertilization treatment (FT)[y]	Shoot fresh weight (mg/shoot)	Shoot fresh weight m^{-2} (g)	Population density (no. m^{-2})	Final pH	Final EC (dS m^{-1})
Control	Check	182	1926	10592	5.92	0.238 c
	CN	214	3722	17317	5.82	0.780 a
	SF	242	3485	14589	5.52	0.686 ab
	0.5 CN+ 0.5 SF	237	3616	15214	5.72	0.462 abc
	CN+SF	263	4125	15704	55.43	0.532 abc
Supersorb	Check	229	2861	12502	5.81	0.348 bc
	CN	275	4174	15171	5.84	0.292 c
	SF	299	4581	15430	5.38	0.564 abc
	0.5 CN+ 0.5 SF	292	4697	16276	5.80	0.464 abc
	CN+SF	285	4939	17376	5.37	0.790 a
$LSD_{0.05}$						0.353
Significances[x]	ST	***	***	ns	ns	ns
	FT	***	***	***	***	*
	ST X FT	ns	ns	ns	ns	*
Main effect means						
Control		227 b	3375 b	14683 a	5.68 a	0.540 a
Supersorb		276 a	4250 a	15351 a	5.64 a	0.492 a
Control		205 c	2393 c	11547 b	5.87 a	0.293 b
CN		244 b	3948 b	16244 a	5.83 a	0.536 ab
SF		270 a	4033 b	15010 a	5.45 b	0.625 a
0.5 CN+ 0.5 SF		264 ab	4156 ab	15745 a	5.76 a	0.463 ab
CN+SF		274 a	4532 a	16540 a	5.40 b	0.661 a
$LSD_{0.05}$		22	413	1729	0.22	0.250

[z] Control= raw seed and Supersorb= seed germination in 2% Supersorb C for 3 days at 20° C.
[y] Check= no additional fertilizer added to the peat-lite; CN= preplant addition of solid $Ca(NO_3)_2$ at 2000 mg N/L of peat-lite; SF= daily application of 150 mg N/L solution fertilizer from 21-5-20; .05= one-half rate.
[x] ***, **, *, ns Significant at $P \leq 0.001$, $P \leq 0.01$, or $P \leq 0.05$, or not significant, respectively. Means within a column and grouping followed by the same letter are not significantly different by $LSD_{0.05}$.

Table 8.3.2 Shoot fresh weight per plant and per unit area and population density of TB at 15 days after planting, and final pH and electrical conductivity (EC) in response to seed treatment (control and vermiculite) and fertilizer treatment.

Seed treatment (ST)[z]	Fertilization treatment (FT)[y]	Shoot fresh weight (mg/shoot)	Shoot fresh weight m^{-2} (g)	Population density (no. m^{-2})	Final pH	Final EC (dS m^{-1})
Control	Check	166 e	2705 h	16333 abc	6.33	0.316
	CN	268 d	4207 f	15795 bc	6.06	0.582
	SF	268 d	4676 e	16631 abc	6.09	0.682
	0.5 CN+ 0.5 SF	284 cd	4676 e	16592 abc	6.34	0.294
	CN+SF	299 cd	5469 cd	19343 a	5.87	0.818
Vermiculite	Check	362 b	3391 g	15230 c	6.31	0.474
	CN	298 cd	5087 de	17565 abc	6.13	0.642
	SF	306 c	5649 c	18469 abc	6.09	0.570
	0.5 CN+ 0.5 SF	362 b	6294 b	17465 abc	6.67	0.528
	CN+SF	415 a	6937 a	16723 abc	5.92	1.510
$LSD_{0.05}$		36	444	3358		
Significances[x]	ST	***	***	ns	ns	*
	FT	***	***	*	***	***
	ST X FT	**	*	**	ns	ns
Main effect means						
Control		260 b	4347 b	16739 a	6.14 a	0.538 b
Vermiculite		321 a	5471 a	17090 a	6.22 a	0.745 a
Control		194 d	3048 e	15781 b	6.32 b	0.395 b
CN		283 c	4647 d	16680 ab	6.09 c	0.612 b
SF		294 c	5163 c	17550 a	6.09 c	0.626 b
0.5 CN+ 0.5 SF		323 b	5485 b	17029 a	6.51 a	0.411 b
CN+SF		357 a	6203 a	17533 a	5.90 d	1.164 a
$LSD_{0.05}$		25	314	1187	0.18	0.279

[z] Control= raw seed and Vermiculite= seed germination in fine (grade 5) vermiculite with 200% d. water for 4.5 days at 20° C.

[y] Check= no additional fertilizer added to the peat-lite; CN= preplant addition of solid $Ca(NO_3)_2$ at 2000 mg N/L of peat-lite; SF= daily application of 150 mg N/L solution fertilizer from 21-5-20; .05= one-half rate.

[x] ***, **, *, ns Significant at $P \leq 0.001$, $P \leq 0.01$, or $P \leq 0.05$, or not significant, respectively. Means within a column and grouping followed by the same letter are not significantly different by $LSD_{0.05}$.

For TB seeds germinated in vermiculite (as in Supersorb C), the greatest shoot fresh weight/m^2 and fresh weight/shoot resulted from 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF), and the lowest values were achieved in the check media, other treatments yielding intermediate values (Table 8.3.2). For the control seeds, fresh weight/shoot was similar with all fertilizer treatments, all of which gave greater response than the check treatment. Although significantly affected by the interaction of seed and fertility treatments, the 4013 seedlings/m^2 range across all treatments was small.

Final growth medium pH and EC were affected by fertilization treatments and not by the seed treatments. The greatest growth medium pH occurred with 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF) and the least with 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF), but the 0.61 range between these treatments was small. Final EC was greater with 2000 mg N/L $Ca(NO_3)_2$ plus 150 mg N/L solution fertilization (CN+SF) than with all other fertilization treatments.

8.4 Discussion

The single treatment that provided the greatest gain in economic yield (shoot fresh weight/m^2) was germination of seeds before sowing. For AR, germinating the seeds in vermiculite or Supersorb C before sowing resulted in 21.4% (Table 8.2.1) and 29.5% (Table 8.2.2) increases in shoot fresh weight/m^2, respectively, than was achieved by sowing raw seed. For TB, the corresponding increase was 25.9% when seeds were germinated in either vermiculite or Supersorb C (Tables 8.3.1 and 8.3.2). The sowing of germinated seeds in order to gain earlier and greater yields

was the logic behind a sowing technique termed "fluid drilling" in which germinated seeds are suspended and transferred to the seedbed in a gel carrier (Pill, 1991). Fluid drilling planters extrude the gel-contaminated seed mixture as thin streams or as "blobs." If a planter could be developed that would extrude gel over a 25 cm (10 in.) wide swath, the width of the commercial trays, the fluid drilling technique could be adapted to microgreen production. Seeds could be germinated in the gel (Pill and Watts, 1983; Pill and Mucha, 1984) and even stored in the gel under refrigeration for up to 12 days (Pill and Fieldhouse, 1982).

For pregerminated AR seeds, combining the preplant $Ca(NO_3)_2$ incorporation (at 1000 or 2000 mg N/L) and the daily solution fertilization (at 75 or 150 mg N/L) resulted in the greatest shoot fresh weight/m^2 (Tables 8.2.1 and 8.2.2). Problematic was the fact that when AR seeds were germinated in Supersorb C, both the calcium nitrate and solution fertilization treatments gave the same shoot fresh weights/m^2 statistically as the calcium nitrate plus solution fertilization treatments (Table 8.2.1). Similarly, when AR seeds were germinated in vermiculite, the solution fertilization treatment (150 mg N/L) gave similar shoot fresh weights/m^2 as the calcium nitrate and solution fertilization treatments. Had the preplant $Ca(NO_3)_2$ been dissolved before incorporation, yields may have been increased as revealed in Experiment 6 (Tables 6.4.1 and 6.4.2). If a single fertilization treatment were to be selected for AR, it would be preplant incorporation of 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF), since it provided the greatest shoot fresh weight/m^2 numerically (not necessarily statistically) for plants established from seeds germinated in Supersorb C or vermiculite. Since doubling both the preplant $Ca(NO_3)_2$ to 2000 mg N/L and the solution fertilization to 150 mg N/L did not increase yield,

use of this treatment would increase production cost and increase the risk of environmental contamination with fertilizer salts.

For TB seeds germinated in vermiculite or Supersorb C, the greatest amount of fertilizer supplied (CN + SF) gave the greatest shoot fresh weight/m^2 and fresh weight/shoot numerically or statistically (Tables 8.3.1 and 8.3.2). The increased shoot fresh weights with this treatment compared to the preplant incorporation of 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF) treatment, in contrast to the response for AR in which yields were decreased or unaffected (Tables 8.2.1 and 8.2.2), may reflect the high salt tolerance of TB. Maas (1986) reported that the EC of saturated soil extracts can reach 4-6 dS/m without yield reduction of table beet.

In a commercial operation in which many microgreen species are grown, the fertilizer treatment that provides maximum economic yield at least economic and environmental cost ideally should be determined for each species and used. From a practical standpoint, however, it would be difficult to establish different fertilization regimes for different crop species. Based on the results of this experiment, the single most "suitable" fertilizer treatment for divergent crops such as AR and TB would be preplant incorporation of 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF).

Although Supersorb C was at least as effective as vermiculite as a germination medium, this cross-linked polyacrylamide gel may pose (unknown) health risks if the harvested microgreens become contaminated with it. Hence, vermiculite is the germination medium of choice.

The selected optimal fertilizer treatment (0.5CN+0.5SF), compared to the check fertilizer treatment for AR seeds germinated in vermiculite, decreased shoot population density, but increased both shoot fresh weight/m^2 and fresh weight/shoot (Table 8.2.2). This response revealed that the preplant incorporation of 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF) treatment stimulated fewer shoots to grow more rapidly. Presumably, the earliest emerging AR shoots gained a competitive advantage, especially for light, suppressing or even causing death of later emerging seedlings so that population density decreased, leaving more resources for the remaining shoots. For TB germinated in vermiculite, the check and preplant incorporation of 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF) treatments had no effect on fresh weight/shoot and yet shoot fresh weight/m^2 increased greatly (Table 8.3.2) with the added fertilizer. This increase in shoot fresh weight/m^2 was associated with a numerically greater shoot population (although not significantly different), which would suggest that interplant competition even at high shoot population density was insufficient to suppress growth of individual shoots.

Although seed treatment had no effect on growth medium pH at the end of the experiment, the vermiculite carrier of the germinated seeds resulted in increased growth medium EC at the end of the experiment (Tables 8.2.2 and 8.3.2). Presumably, ions held on the exchange sites of the vermiculite (*ca* 200 meq/L; Handreck and Black, 1984) were displaced into the growth medium solution to cause this increase. The increase in growth medium EC at the end of the experiment with the 1000 mg N/L $Ca(NO_3)_2$ plus 75 mg N/L solution fertilization (0.5CN+0.5SF) treatment compared to the check media that supported AR growth (Table 8.2.2) may

be attributed to the fertilizer addition to the media. However, this fertilizer treatment did not result in a greater final EC in growth media supporting TB growth (Table 8.3.2). In comparing the final growth medium pH with these two fertilization treatments (check vs. 0.5CN + 0.5 SF), the reversed magnitude for AR and TB defies explanation.

8.5 Conclusions

For both crops, sowing germinated seeds in a vermiculite or Supersorb C carrier greatly increased shoot fresh weight/m^2 and fresh weight/shoot. The single fertilization treatment that gave the greatest shoot fresh weight/m^2 of both crops with the least cost and least likelihood of environmental pollution was preplant incorporation of $Ca(NO_3)_2$ at 1000 mg N/L of growth medium plus 75 mg N/L solution fertilization from 21-5-20 (0.5CN+0.5SF).

CHAPTER 9

A COMMERCIAL TRIAL OF SELECTED TREATMENTS

9.1 Introduction

The treatments from Experiment 8 that resulted in the greatest shoot fresh weight per m^2 were used at ARC Greenhouses during January, 2006. It was decided that using Supersorb C as a germination medium should be deleted from this study since it is not known whether inadvertent ingestion of this cross-linked polyacrylamide from harvested shoots could have a toxic effect on consumers of the microgreens. Owing to space constrictions, only TB was included in this experiment.

9.2 Materials and Methods

The ten treatments of the 2 (seed treatment) X 5 (fertilizer treatment) are listed in Table 9.1. Treatment 1 represents the normal production practice at ARC Greenhouses.

Table 9.1 Seed and fertilization treatments selected for use in a commercial production trial of TB microgreens.

Treatment code	Seed treatment	Preplant $Ca(NO_3)_2$ (mg N/L peat-lite)	Solution fertilization (mg N/L, daily)
1	Control	None	100
2	Control	None	150
3	Control	1000	0
4	Control	1000	100
5	Control	1000	150
6	Vermiculite[z]	None	100
7	Vermiculite	None	150
8	Vermiculite	1000	0
9	Vermiculite	1000	100
10	Vermiculite	1000	150

[z] Vermiculite = germination of seedballs at 20° C for 4.5 days in vermiculite moistened with 200% water.

The experiment was conducted in 671 cm long x 254 cm wide x 2.5 cm deep (22 ft x 10 in. x 1 in.) troughs. Each trough contained a fertilizer treatment, but one-half of the length contained the control seed treatment and the other half contained the vermiculite treatment. Thus five troughs comprised a replication, and there were three replications. The experiment was arranged in randomized complete blocks as shown in Figure 9.1, with each horizontal rectangle representing half a trough.

Figure 9.1 The randomized complete block design of the commercial trial experiment. Treatment code numbers are explained in Table 9.1.

REP I

1	6
8	3
10	5
2	7
9	4

REP II

7	2
4	9
6	1
3	8
5	10

REP III

8	3
10	5
4	9
1	6
2	7

The 38 L of growth medium required to fill a trough was spread on plastic and the 40 g of $Ca(NO_3)_2$ was incorporated manually (treatments 3, 4, 5, 8, 9, and 10), before placing the peat-lite in the trough. Troughs were color-coded for the appropriate concentration of solution fertilization. Fertilizer solutions (0, 100, and 150 mg N/L) were prepared in color-coded 20-gallon plastic garbage cans by dissolving weighed amounts of 21-5-20 in warm water (Table 9.2).

Table 9.2 Composition of the solution fertilization treatments.

Color code	Solution N concn (mg/L)	g 21-5-20/L	g 21-5-20/20 gallon
YELLOW	0	0	0
BLUE	100	0.48	36
RED	150	0.71	54

At the standard (1X) seeding rate of TB, 154g of seed were required for each half-trough. Since 5 L of vermiculite permitted distribution of seeds over the peat-lite within a trough, 2.5 L of vermiculite would be required for a half-trough. Fifteen cylindrical white plastic containers (143 cm wide by 12.7 cm deep; 7.5 in. by 5in.) were filled with 2.5 L of fine (grade 5) vermiculite. Reverse osmosis water was added at 200% of the vermiculite dry weight to each container, and the vermiculite and water were mixed thoroughly until the vermiculite was uniformly moist. In each of these containers, 154g of 'Early Wonder Tall Top' TB seeds were thoroughly mixed in the moist vermiculite. A tight-fitting lid with a single 1 cm long cut was secured on each container. The 15 containers were placed in darkness in an incubator (at the University of Delaware) at 20° C for 4.5 days so that the seedballs could germinate.

On the planting day (12 January, 2006) the plastic containers with the germinated seedballs and vermiculite were transported to ARC Greenhouses. All troughs were irrigated with the appropriate solution fertilization before planting. Control (non-treated) seeds and the vermiculite-germinated seed mixtures were broadcast over each half trough, and then Jiffy Mix Plus was used to cover the sown seeds to a depth of *ca* 0.5 cm. The greenhouse was set at constant 21° C with natural light. Wire coat hangers, with an enclosed area of 435 cm^2, were pressed into the growth medium at three randomly selected locations within a half trough. At 5, 10, and 15 days after planting (17, 22, and 27 January), seedlings were cut at the growth

medium surface from the randomly located subsample areas, and the shoots placed in zip-lock bags. The bags were placed in an ice chest and brought back to the University of Delaware. Shoot fresh weights of the entire bag contents and of 25 randomly selected seedlings from each bag were determined. Shoot fresh weight/m^2 was determined by multiplying the shoot fresh weight per 435 cm^2 by 23. Fresh weight/shoot was calculated by dividing the fresh weight of the 25 seedlings by 25. Seedling population/m^2 was estimated by dividing the shoot fresh weight/m^2 by the fresh weight per shoot. All these data were subjected to analysis of variance.

9.3 Results and Discussion

Shoot fresh weight/m^2 was affected by seed treatment at each harvest and by fertilizer treatment at the third (15 DAP) harvest (Table 9.5). Fresh weight/shoot was affected only by seed treatment at each harvest and by the interaction of seed treatment with harvest. Shoot population density was affected only by seed treatment at the first (5 DAP) harvest, leading to the significant harvest by seed treatment interaction.

Sowing seedballs germinated in vermiculite compared to sowing non-treated seedballs increased shoot fresh weight/m^2 by 232%, 50%, and 18% at the 5, 10, and 15 DAP harvests, respectively (Tables 9.4 and 9.5). Thus, the economic yield benefit diminished at each harvest, but was still substantial (18%) at the 15 DAP harvest. Shoot population density at the 5 DAP harvest was increased 150% by using germinated seed (17,029/m^2) rather than control seed (6823/m^2), but at subsequent harvests seed treatment had no effect on shoot population density (Table 9.5). Fresh weight/shoot from seeds germinated in vermiculite was greater than that of control seeds at each harvest (Table 9.5). The fresh weight/shoot from control seeds at 5 DAP

(26.2 mg) was artificially high because it was impossible to harvest the very small shoots without including particles of growth medium which were too numerous to remove. Thus, it is likely that the 35% greater fresh weight/shoot from germinated seeds relative to control seeds at 5 DAP is greatly underestimated. The benefit in fresh weight/shoot from germinated seed was 61% and 24% at 10 and 15 DAP, respectively, similar to the benefit in shoot fresh weight/m^2 at these two harvests. Consequently, the actual increase in fresh weight/shoot from germinated seed compared to control seed at 5 DAP is likely to be closer to the 232% increase observed in shoot fresh weight/m^2 at the first harvest.

Only between 10 and 15 DAP did fertilizer treatment affect shoot fresh weight/m^2 (Tables 9.3 and 9.4). Preplant incorporation in the peat-lite of 1000 mg N/L from $Ca(NO_3)_2$ combined with daily solution fertilization with 100 mg N/L from 21-5-20 gave a 28% increase in shoot fresh weight/m^2 over the average of other fertilizer treatments, irrespective of seed treatment (Table 9.4).

Table 9.3 Analysis of variance for shoot fresh weight/m^2 and per plant and population density of 'Early Wonder Tall Top' TB grown under commercial conditions at 5, 10 and 15 days after planting (DAP) in response to seed and fertilizer treatments.

Source of variation	Shoot fresh weight (g/m^2)	Shoot fresh weight (mg/shoot)	Shoot population density (number/m^2)
5 DAP			
Seed treatment (ST)	***	**	***
Fertilizer treatment (FT)	ns	ns	ns
ST X FT	ns	ns	ns
10 DAP			
Seed treatment (ST)	***	***	ns
Fertilizer treatment (FT)	ns	ns	ns
ST X FT	ns	ns	ns
15 DAP			
Seed treatment (ST)	*	**	ns
Fertilizer treatment (FT)	0.05	ns	ns
ST X FT	ns	ns	ns
Harvest (H)	***	***	***
ST	***	***	*
H X ST	ns	*	***
FT	ns	ns	ns
H X FT	ns	ns	ns
ST X FT	ns	ns	ns
H X ST X FT	ns	ns	ns

[z] ***,**,ns Significant at $P \leq 0.001$, $P \leq 0.01$, or not significant, respectively.

Table 9.4 Shoot fresh weight per area of 'Early Wonder Tall Top' TB as influenced by seed and fertilizer treatments at 5, 10 and 15 DAP.

Fertilizer[z]	Shoot fresh weight (g/m^2) Control[y]	Vermiculite[y]	Mean
5 DAP			
0 + 100	188	619	403 a
0 + 150	203	540	372 a
1000 + 0	120	654	387 a
1000 + 100	228	595	411 a
1000 + 150	163	579	371 a
	mean 180 b[x]	597 a	
10 DAP			
0 + 100	932	1515	1223 a
0 + 150	1018	1164	1091 a
1000 + 0	830	1651	1240 a
1000 + 100	948	1359	1154 a
1000 + 150	830	1117	974 a
	mean 911 b	1361 a	
15 DAP			
0 + 100	1574	1780	1677 b
0 + 150	1646	1710	1678 b
1000 + 0	1349	1773	1561 b
1000 + 100	1782	2398	2090 a
1000 + 150	1476	1738	1607 b
	mean1560 b	1880 a	

[z]First number = $Ca(NO_3)_2$ preplant incorporation (mg N/ L growth medium) and second number- mg N/L applied daily as solution fertilization from 21-5-20.
[y] Control= non-treated seedballs and vermiculite = incubation of seeds in vermiculite moistened with 200% water for 4.5 days at 20° C.
[x] Means in a row or column within a harvest followed by the same letter are not significantly different by $LSD_{0.05}$.

Table 9.5 Shoot fresh weight/m^2 and fresh weight/shoot and population density of 'Early Wonder Tall Top' TB as influenced by seed treatment at 5, 10 and 15 DAP.

Harvest (DAP)	Seed treatment	
	Control	Vermiculite[y]
	Shoot fresh weight (g/m^2)	
5	180 f	597 e
10	911 d	1361 c
15	1565 b	1880 a
$LSD_{0.05}$= 185		
	Shoot fresh weight (mg/shoot)	
5	26 e[z]	35 d
10	43 d	69 c
15	83 b	100 a
$LSD_{0.05}$ = 9.1		
	Shoot population density (number/m^2)	
5	6823 c	17029 b
10	22588 a	20583 ab
15	19263 ab	19236 ab
$LSD_{0.05}$ = 4352		

[z] Means within a variable followed by the same letter are not significantly different.
[y] Vermiculite = incubation of seeds at 20° C in vermiculite moistened with 200% water, 4.5 days for TB.

The use of germinated seeds and preplant $Ca(NO_3)_2$ addition plus 100 mg N/L daily solution fertilization (1000 + 100), compared to the standard production procedure (control seeds and daily 100 mg N/L solution fertilization) increased shoot fresh weight/m^2 from 1574 g/m^2 to 2398 g/m^2, an increase of 52% (Table 9.4). Compared to this treatment (1000 + 100), increasing the solution fertilization to 150 mg N/L daily when combined with preplant $Ca(NO_3)_2$ incorporation in the peat-lite (1000 + 150) decreased shoot fresh weight/m^2 to levels achieved with other treatments (0 +

100, 0 + 150, or 1000 + 0), suggesting a toxic effect from excessive soluble salts. Since fertilizer treatments had no effect on shoot fresh weight/m^2 until >10 DAP would suggest that all fertilizer treatments provided sufficient nutrition to crops up to 10 DAP. Between 10 and 15 DAP for media with 1000 mg N/L from $Ca(NO_3)_2$, however, the additional 100 mg N/L daily solution fertilization increased growth, while 0 or 150 mg N/L solution fertilization failed to increase growth, perhaps from N deficiency and toxicity, respectively.

A more definitive response to fertilizer treatments probably would have been possible if more than 3 replicate blocks had been used, and if the plant environment had been more desirable. The troughs sloped downwards slightly (towards the left in Figure 9.1), and it was noted that occasionally the media in the upper end of the trough would dry to such an extent between daily solution fertilization that plants wilted. Conversely, seedlings in the lower ends of the troughs never wilted. Opaque walls near each end of the troughs caused shading to the upper end of the trough in the morning, and to the lower end of the trough in the afternoon. Even though treatments were randomly assigned within a block, treatment position affected the plant environment (at least growth medium moisture and incident light) and confounded treatment effects so that with only 3 replications, high variability diminished treatment effects. Had more space and labor been available more replications should have been used.

Sowing germinated seeds rather than non-treated seeds, by increasing shoot fresh weight/m^2, could reduce microgreen production time in the greenhouse. Ideally seeds of all microgreen crop species could be germinated in vermiculite in a non-greenhouse incubator area with temperature control. The use of greenhouse space

(expensive-to-operate space) during the period from seed sowing to seed germination would be avoided. If 1000 mg N/L $Ca(NO_3)_2$ were preplant incorporated in the peat-lite, in combination with the standard ARC Greenhouses practice of daily solution fertilization with 100 mg N/L 21-5-20, economic yields would be increased further.

9.4 Conclusions

Compared to the standard ARC Greenhouses production practices (control seed and daily 100 mg N/L solution fertilization), the sowing of TB seedballs germinated in vermiculite (200% water in the vermiculite, 4.5 days at 20° C) increased shoot fresh weight/m^2 at 15 DAP, by 13%. A further increase to 52% was achieved by incorporating $Ca(NO_3)_2$ at 1000 mg N/L into the peat-lite before planting.

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