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Short communication

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Crop growth and viability of seeds on Mars and Moon soil simulants

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Abstract: If humans are going to establish a base on the Moon or on Mars they will have to grow their own crops. An option is to use Lunar and Martian regolith. These regoliths are not available for plant growth experiments, therefore NASA has developed regolith simulants. The major goal of this project was to cultivate and harvest crops on these Mars and Moon simulants. The simulants were mixed with organic matter to mimic the addition of residues from earlier harvests. Ten different crops, garden cress, rocket, tomato, radish, rye, quinoa, spinach, chives, pea and leek were sown in random lines in trays. Nine of the ten species grew well with the exception of spinach. It was possible to harvest edible parts for nine out of ten crops. The total biomass production per tray was highest for the Earth control and Mars soil simulant and differed significantly from Moon soil simulant. The seeds produced by three species were tested for germination (radish, rye and cress). The germination on Moon soil simulant was significantly lower in radish than for the Earth control soil.

Keywords: Extra-terrestrial; Food production; Growth experiment; Regolith; Exobiology

1 Introduction

A (permanent) human settlement on Mars or the Moon is becoming more realistic. Several countries and private companies are preparing for this journey. One of the major issues will be ensuring food availability and safety (Cousins and Cockell 2016). Food can and will be brought along, but for a permanent stay, production of crops on Mars or the Moon to supplement or even supply the total food demand could be a necessity (Graham and Bamsey 2016). There are several reasons to grow fresh food on Mars or the Moon: it is healthy, it contains more flavours and is thus more tasteful than space food, and it saves costly cargo volume in a spacecraft. There are various options to grow food on Mars or the Moon. The first possibility is hydroponics. There is a vast amount of experience in cultivating plants on inert substances, such as rock wool, with a nutrient solution as the medium. Since there is enough water, as ice, on Mars and even on the Moon this is a feasible option (Hui et al. 2013; Möhlmann 2004). However, the rock wool has to be flown in and replaced after one or more crop cycles. Moreover, not all crops thrive very well on rock wool. It is also possible to grow crops directly in water, avoiding the need to bring rock wool. A second option is aeroponics (Maggi and Pallud 2010). The advantage of using aeroponics is that it only requires a nutrient solution and no extra soil-like material. A third option is to use the regolith on Mars or the Moon for crop growth. Only the seeds of the crops would have to be brought to Mars or the Moon. This is, of course, besides the general equipment that is needed for all three options, i.e. lamps, racks, solar panels etc., and a habitat to live in.

Our research focuses on the growth of plant species using the regolith present on Mars and the Moon. Mars regolith is not yet available on Earth. Instead we used NASA's Mars regolith simulant JSC 1A (Carlton et al. 1998). The composition of the simulant is mainly based on information gathered by the Viking landers and the Mars Pathfinder rover. This simulant resembles the actual Mars regolith closely and originates from the Pu'u Nene cinder cone located between Mauna Loa and Mauna Kea on Hawaii (Rickman et al. 2007).

Moon regolith has been brought to Earth. During the Apollo program an experiment with plant growth on actual Moon regolith was conducted (Baur et al. 1974; Ferl and Paul 2010). However, Moon regolith is not available for growing plants at sufficient quantities at the moment. Therefore, instead of actual Moon regolith we used the JSC 1A Moon simulant provided by NASA (Rickman et al. 2007). This regolith simulant originates from an Arizonian

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desert near Flagstaff. It resembles the actual Moon regolith closely; no important deviations from the original regolith are reported as far as we know.

After the successful growth of 14 different plant species on Mars soil simulant and to a lesser extent on Moon soil simulant (Wamelink et al. 2014) we focussed in this experiment on the growth of ten different crop species on the regolith simulants. The main goals were the production of edible crops and the production of seeds of selected species for the next generation. For this purpose, we altered the regolith simulants by adding organic matter, as if we reused the first harvest. We assume that plants will be grown indoors and below ground on Mars or the Moon to avoid the unfavourable conditions outside. This paper describes a first experiment with the focus on fruit setting and seed production, more experiments on e.g. optimal organic matter content or water use efficiency will follow. This short communication reports the most important results of this first experiment to investigate if crop growth and harvest on Mars and Moon regolith could be feasible.

2 Materials and Methods

The experiment was carried out under Earth atmosphere, gravity, and light conditions in a normal greenhouse in Wageningen (51.9692° N, 5.6654° E). During the experimental period, average temperature was 21.1 ± 3.0 °C and air humidity was 65.0 ± 15.5%, based on 24 hour recording at 5 minute intervals (day and night). Daylight lasted 16 hours. Lamps yielding 80 µmol m-2s-1 (HS2000 from Hortilux Schréder) were used when the sunlight intensity was below 150 watt/m². Ambient air was used and no extra CO_2 was added (400 ppm in 2015 in De Bilt, The Netherlands).

2.1 Regoliths

Since the Mars and Moon regoliths are comparable to Earth soils, at least in mineral composition, they can be mimicked by using Earth soils, as has been done by NASA (Carlton et al. not dated; Chevrier and Mathe 2007; Clark 1993; Clark and Van Hart 1981; Gibson 1977; Rickman et al. 2007). The simulants were purchased from ORBITEC (now Sierra Nevada Corporation). As Mars simulant we used JSC-1A Mars-1A regolith simulant. For the Moon we used the JSC1-1A lunar regolith simulant (Carlton et al. 1998; Rickman et al. 2007). Both simulants were manufactured under contract to NASA. As a control we used organic soil (Table 1).

Wamelink et al. (2014) analysed the nutrient availability of the simulants. The analysis revealed that the Moon regolith simulant is nutrient poor, though it contained small amounts of nitrate and ammonium. The Mars regolith also contained traces of nitrates and ammonium, but also a significant amount of carbon. It is still unclear whether or not reactive nitrogen is present in actual Mars regolith (Foley 2003; Mancinelli and Banin 2003). We found that there is only a small amount of reactive nitrogen present in the simulants and that the pH of the simulants is high. The pH of the Moon regolith simulant is so high (9.6, Wamelink et al. 2014) that it may be problematic for many plant species (Wamelink et al. 2005). Water holding capacity of the soils was estimated and was around 30% for the Mars and Moon simulant and 100% for Earth organic soil, all in weight percent.

2.2 Species selection

For this experiment we selected ten different crop species. Three crop species from our earlier experiment (Wamelink et al. 2014) were used again, namely tomato, *Solanum lycopersicum* L. ('Super Sweet 100 F1', Horti Tops), rye, *Secale cereale* L. ('Summer rye', Cruydthoeck) and garden cress, *Lepidium sativum* L. ('broad leaved', Horti Tops). Crops new for this experiment were leek, *Allium ampeloprasum* L. ('Farinto', Pilstar), quinoa, *Chenopodium quinoa* Willd. (AH), pea, *Pisum sativum* L. ('Prince Albert', Tuinservice), radish, *Raphanus raphanistrum subsp. Sativus* (L.) Domin ('Bel image', Horti Tops), spinach, ('Prickly seeded' Tuinservice), rocket, *Diplotaxis tenuifolia* (L.) DC. ('rucola',

Table 1: Content of organic soil used as Earth control (Lentsepotgrond, Horticoop) and the total Nitrogen (Ntot), Phosphorous (Ptot) and Potassium (Ktot) content

Content	Amount
Horticlay	50 kg/ m^3
Swedish peat	20%
Baltic peat	40%
Garden peat	40%
Dolokal	4,7 kg/m^3
Pg mix	1 kg/ m^3
Ntot	12.4 (± 6.3) g/kg
Ptot	2.2 (± 1.7) g/kg
Ktot	18.8 (± 12.1) g/kg

'Wild Rocket', Horti Tops) and chives, *Allium tuberosum* Rottler ex Spreng. 1825 not Roxb. 1832 ('Garlic taste', Horti Tops). Species were selected to provide a representative selection of crops with different edible parts, except for below ground parts (roots, tuber etc.). Leek, radish, rocket and chives were also selected because of their spicy taste. Astronauts on the ISS often complain about the taste of their food and spicy crops may therefore be a welcome addition to their diet (Cooper et al. 2011). Several of our selected crops are also included by Perchonok and Bourland (2002) as part of a diet for a long stay in space.

2.3 Organic matter and bacteria

Organic matter was used to improve and enrich the regolith simulants. Both the Moon and Mars simulant were mixed with organic matter to mimic the addition of organic matter from a previous harvest (Wamelink et al. 2014). The harvest from this earlier experiment was not used since that biomass was used for analyses. Instead, fresh mown grass of *Lolium perennne* L., commonly used in meadows in the Netherlands and as cattle fodder, was used. This is an arbitrary choice; however, *L. perenne* is, as rye, part of the family Gramineae with common properties. The grass was cut into small pieces of approximately 1-4 cm length and then mixed with the regolith, 267g (fresh weight) of grass was mixed with 7,5 L Mars soil simulant and Moon soil simulant per tray. During the experiment a nutrient solution (Table 2) was added each last Friday of the month (except the first month April) to mimic the addition of human faeces and urine. The nutrient solution given is a balanced solution. We assumed that based on the faeces and urine a balanced solution will be made, as was done in the many experiments applying hydroponics for bases on Mars and the Moon (see e.g. Dueck et al. 2016; Meinen et al. 2018).

Table 2: Nutrient content of the solution applied to the growing trays. The EC was 1.45 mS/cm and the pH 5.7. The solution was made by adding 25.2 l Zwakal, 44.2 l BFK, 14.4 l Baskal, 13.8 l Amnitra, 10.4 l Magnitra and 64 l Calsal to 100,000 l of water (standard solution for pot plants at Wageningen UR)

Element/molecule	Concentration (mmol/l)
NH_4	1.10
NO_3	7.79
P	1.50
K	5.11
Ca	3.00
Mg	0.87
SO_4	1.00

Even though no bacteria are present on Mars or the Moon, we did not sterilize the test media or the seeds. We assume that bacteria will be brought to Mars and the Moon to support plant growth and organic matter break down. Which bacteria will be selected will be the topic of further research.

2.4 Experimental design

There were three replicates in the experiment (n=3), i.e. three trays (with punctures in the bottom) with Mars soil simulant, three trays with Moon soil simulant and three trays with the control, Earth potting soil (Figure 1 for the design, Table 1 for potting soil), This results in a randomized complete block design for the total biomass harvest. The plastic trays, 40 * 60 cm, were filled with simulant or organic soil. Underneath the punctured tray a second tray (closed bottom) was placed to keep the soils moist. The ten crops were sown in a line, the order of the species was randomized as well in a split plot design, resulting in a different order of the species for each tray. Between the lines with seeds 5 cm open space was kept. The number of seeds varied per species, 15 for pea, 25 for leek, tomato and rye, 50 for radish, chives, spinach and rocket and 100 for garden cress and quinoa. Seeds were sown on the ninth of April and the experiment lasted till the fifteenth of September 2015, so harvest took place 159 days after the start. Trays were watered once a day by spraying with tap water, except when they received nutrient solution, keeping them moist and the plants turgescent. The amount of water given was not recorded.

2.5 Harvest and measurements

During the experiment and at the end of the experiment the above ground biomass was harvested and, after cleaning, dried in an oven for 48 hours at 70°C. After cooling down the biomass was weighed. During the experiment seeds of three species, rye, cress and radish were harvested and dried for 48 hours at 25°C. The seeds were weighted to calculate the 1000 seeds weight. After storage the seeds were sown on April 5, 2016, after which germination percentages were calculated. The germination experiment was kept until April 25, 2016. We duplicated the same random

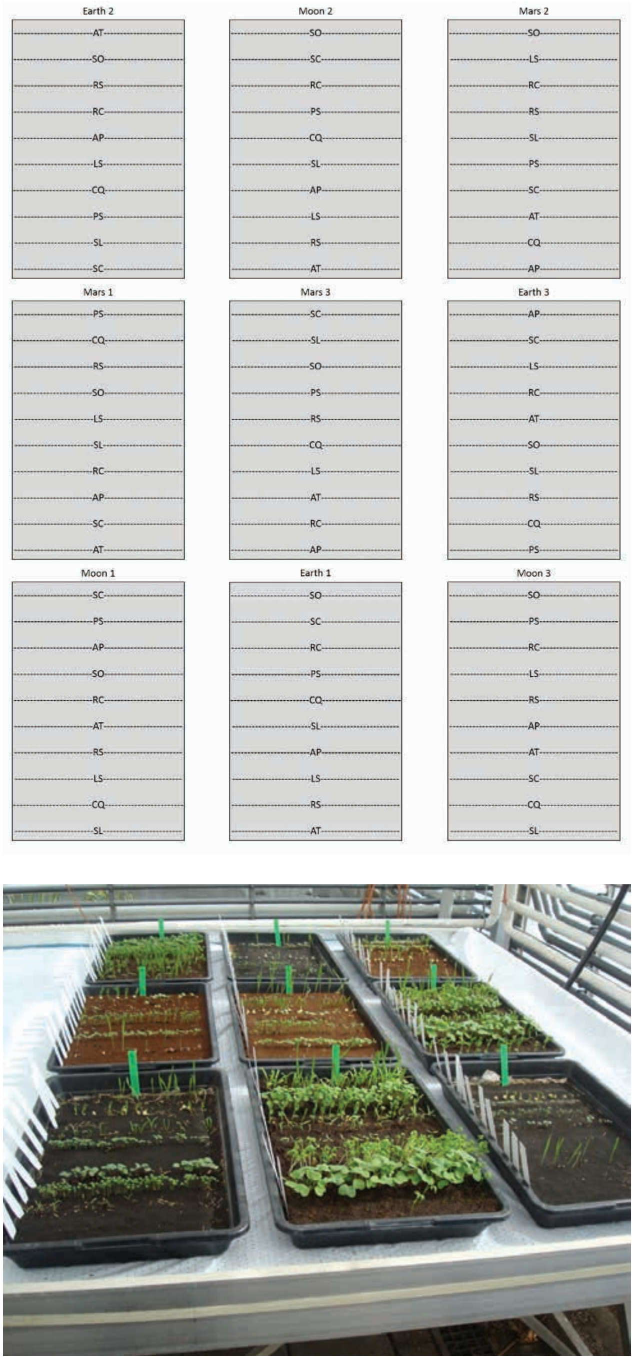

Figure 1: Top panel: experimental design, with SO: Spinach (*Spinacia oleracea*), PS: Peas (*Pisum sativum*), SC: Rye (*Secale cereale*), RC: Rocket or Arugula (*Rucola coltivata*), SL: Cherry tomato (*Solanum lycopersicum*), AP: Leek (*Allium porrum*), LS: Cress (*Lepidium sativum*), RS: Radish (*Rhaphanus satives*), AT: Carlic chives (*Allium tuberosum*) and CQ: Quinoa (*Chenopodium quinoa*). The Hyphens besides the abbreviations for the species indicate the line the seeds of the species were sown (the number of hyphens do not represent the number of seeds). Bottom panel: experiment overview on April 16, 2015, seven days after sowing

design for the trays and the sequence of species per tray as in 2015, with exclusion of the other six species.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Fruit setting and biomass

We were able to harvest radishes and radish seeds, cress and cress seeds, rye seeds, rocket, tomatoes and peas on Mars and Moon soil simulants and Earth organic soil. Chives and leek did grow steadily, but at a low growth rate on all three growing medias (see Appendix 1 for biomass). Quinoa was growing well and formed flowers, but did not form any seeds. The only species that did not grow properly on any of the soils was spinach; the plants started to flower after only a few small leaves were formed. The total aboveground biomass was highest for the Earth control and lowest for the Moon soil simulant (Figure 2). However, Mars soil simulant was not significantly different from the Earth or Moon media soils, also due to the high variation between the three replicas. One of the Mars trays, on the upper right corner of 3*3 lay out of the trays, had a much lower biomass. The harvested dry biomass on Moon soil simulant was lower than the Earth control ($p=0.0318$).

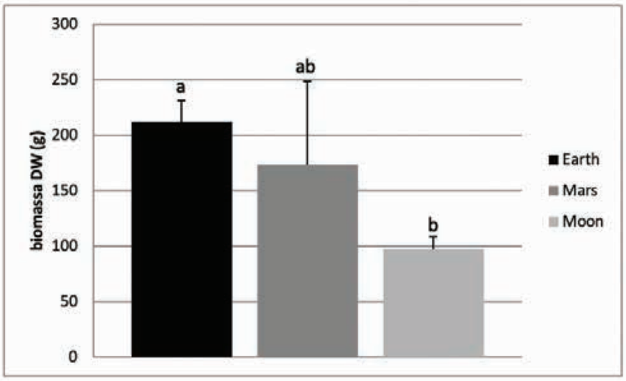

Figure 2: Total aboveground combined dry biomass production for ten different crops, garden cress, rocket, tomato, radish, rye, quinoa, spinach, chives, pea and leek on Mars and Moon soil simulant and Earth organic soil (control). Different letters indicate significant differences ($n=3$, $p< 0.05$)

3.2 Seed weight and germination

For three species, radish, cress and rye it was possible to harvest enough seeds for a germination experiment. The seed weight of radish and rye did not deviate between the three growing media (Figure 3, for background data see Appendix 2). For cress this looks different, the seed weight for the Earth control is higher than for both simulants. However, none of the differences are statistically significant (at p=0.05). For rye all the seeds germinated on all three growing mediums (Figure 4). The germination percentage was lowest for radish. There is a significant difference in germination for the seeds on Earth soil and Moon soil simulant for radish, with a higher percentage for seeds grown on Earth soil. The germination percentage of radish on Mars soil simulant was not significantly different from either the Earth control or Moon soil simulant (at p=0.05). None of the germinated plants died during the 20 days the germination experiment lasted.

4 Discussion

In this limited preliminary experiment, we show that crop growth on Mars and moon soil simulants is possible. The addition of organic matter to both the Mars and Moon regolith simulants resulted in the germination and growth of all ten crop species. Several crops produced fruits and seeds on both Mars and Moon simulants. Compared to our earlier research (Wamelink et al. 2014), the biomass production was significantly higher, and crops such as tomato, rye, pea and radish formed fruit and viable seeds. The biomass production on the Mars soil simulant was comparable to the Earth soil. The biomass production on the Moon soil simulant was significantly lower than for the Earth soil. Earlier research on *Arabidopsis thaliana* (L.) Heynh. and *Tagetes patula* L. (Ferl et al. 2010; Kozyrovska et al. 2006; Zaets et al. 2011) on regolith and Moon rock-simulant also produced flowering plants. However, fruits had never been harvested before on the Mars soil simulant or the Moon soil simulant, as far as we know. Now that we have shown that adding organic matter to the simulants improves plant growth one of the next steps will be to find the optimal amount of organic matter to add. Several ratios should be tested. However, the more organic matter that is needed, the more difficult the first phase of building a ‘soil’ will be and the longer it will take.

Seed production is a necessity to prevent new seeds to be flown in for every generation. The formed seeds have to be viable. The results show that for rye and cress the seeds are viable with almost no seeds that did not germinate. For radish the viability of the seeds grown on Moon soil simulants was lower than on Earth soil. For radish there are reasons for concern, even though germination percentage is still around 50%. A quality indicator is the seed weight and that is reasonable for all of the four tested species, since there are no significant differences between the soils.

We give the biomass as a total per tray, since the ten different species were grown in one tray. For individual analyses of biomass species should be grown individually, which was not the goal of this experiment (individual biomass per species is given in Appendix 2). This should be further investigated, both per species and in combination with each other. The latter to investigate if a mixed crop growth could yield a higher harvest.

Unfortunately, the peas were harvested as a total biomass per treatment and not per tray. Therefore, it was

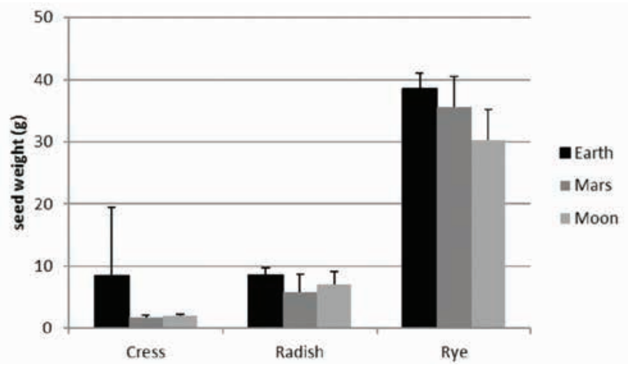

Figure 3: Seed weight of cress, radish and rye harvested from plants grown on Mars and Moon soil simulants and Earth potting soil. None of the differences are statistically significant (n=3, p=0.05, for cress due to the large uncertainty in the Earth control)

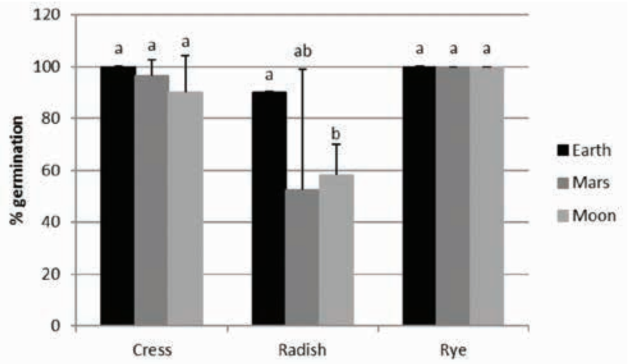

Figure 4: Percent germination for seeds harvested from plants grown on Mars and Moon soil simulants and Earth control potting soil. Different letters indicate significant differences (n=3, p< 0.05)

not possible to evaluate the variance between the three replicas. This had consequences for the evaluation of the total biomass as well. To overcome the problem of missing replicates, we estimated the values for the peas based on the variance of the other species.

This short communication describes a small step towards the final goal, a sustainable agricultural ecosystem for a Moon and Mars colony. More research is necessary to find the optimal organic matter content of the simulant regoliths and the water use efficiency. Also, more information is needed on the physical characteristics of the simulants (and the actual regoliths on Mars and the Moon), especially those that may affect plant growth. Once reliable production in these simulants can be established, further research will include the recycling of organic matter by worms and bacteria, nitrogen fixation by bacteria to overcome to shortage of reactive nitrogen in the regoliths, the recycling of nutrients from human faeces and the application of fungi in symbiosis with crops to acquire nutrients from the soil especially in the early stages of building a proper soil.

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Conflict of interest: Author declares no conflict of interest.

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Appendix 1: Dry biomass (dw) per species. For pea only total biomass for the three trays was recorded. The 'rest' contains dry biomass from all species that could not be identified, presumably mostly cress and further radish, quinoa, pea and rocket. It does not contain leek, chives and tomato

		biomass (g dw)					
simulant	**species**	**tray 1**	**tray 2**	**tray 3**	**total**	**average**	**s.e.**
E	pea				50.99		
	chives	0.07	0.10	0.14	0.31	0.10	0.04
	cress	2.71	-	3.59	6.30	3.15	0.62
	leek	0.47	0.19	0.45	1.11	0.37	0.16
	quinoa	23.33	30.84	15.28	69.45	23.15	7.78
	radish	22.54	22.57	31.07	76.18	25.39	4.92
	rest	27.39	24.49	25.55	77.43	25.81	1.47
	rocket	4.59	8.76	7.35	20.70	6.90	2.12
	rye	17.28	26.47	27.01	70.76	23.59	5.47
	tomato	86.61	109.13	67.72	263.46	87.82	20.73
L	pea				25.41		
	chives	0.01	0.01	0.02	0.04	0.01	0.00
	cress	1.50	2.82	0.45	4.77	1.59	1.19
	leek	0.87	2.43	1.74	5.04	1.68	0.78
	quinoa	31.01	3.05	10.70	44.76	14.92	14.45
	radish	4.40	10.48	-	14.88	7.44	4.30
	rest	5.51	-	1.65	7.16	3.58	2.73
	rocket	10.05	3.79	22.41	36.25	12.08	9.48
	rye	7.38	11.64	3.06	22.08	7.36	4.29
	tomato	41.41	42.80	46.86	131.07	43.69	2.83
M	pea				32.66		
	chives	0.07	0.09	0.27	0.43	0.14	0.11
	cress	1.24	0.89	1.95	4.08	1.36	0.54
	leek	0.41	0.86	1.91	3.18	1.06	0.77
	quinoa	28.45	18.08	3.88	50.41	16.80	12.33
	radish	14.24	10.30	6.81	31.35	10.45	3.72
	rest	6.97	6.47	11.37	24.81	8.27	2.70
	rocket	2.23	3.49	2.50	8.22	2.74	0.66
	rye	23.59	16.95	11.35	51.89	17.30	6.13
	tomato	143.18	-	170.23	313.41	156.71	19.13

Appendix 2: Seed weights and germination numbers per treatment for cress, radish and rye

species	Treatment	Seed weight (g)			Germination	
		n weighted	total	per seed	n sown	n germinated
cress	E1	25	0.049	0.00196	10	10
	E2	25	0.530	0.0212	10	10
	E3	25	0.056	0.00224	10	10
	M1	25	0.048	0.00192	10	10
	M2	25	0.033	0.00132	10	10
	M3	25	0.049	0.00196	10	9
	L1	25	0.048	0.00192	10	7
	L2	25	0.055	0.0022	10	5
	L3*	-	-	-	-	-
radish	E1	10	0.084	0.0084	10	9
	E2	10	0.097	0.0097	10	9
	E3	10	0.075	0.0075	10	9
	M1	10	0.075	0.0075	10	7
	M2	2	0.005	0.0025	2	0
	M3	8	0.060	0.0075	8	7
	L1	3	0.017	0.0057	3	2
	L2	10	0.085	0.0085	10	5
	L3*	-	-	-	-	-
rye	E1	10	0.414	0.0414	10	10
	E2	10	0.374	0.0374	10	10
	E3	10	0.367	0.0367	10	10
	M1	10	0.407	0.0407	10	10
	M2	10	0.309	0.0309	10	10
	M3	10	0.352	0.0352	10	10
	L1	7	0.217	0.031	7	7
	L2	5	0.174	0.0348	5	5
	L3	6	0.15	0.025	6	6

* There was no seed setting for cress and radish in tray 3.