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Customized Composting for Organic Vegetable Production

Use of compost and different *Pseudomonas fluorescens* inoculation techniques to reduce root-knot nematode damage in organic greenhouse cucumbers

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Abstract

A 6-week glasshouse experiment was conducted to assess the influence of different compost inoculation moments (early vs late vs non-inoculated compost) and techniques (none vs compost vs direct soil application vs root drench of transplant plugs) with *Pseudomonas fluorescens* (KBPL-2059). Inoculation treatments were combined with two compost dosages (3.2% and 9.6%), and pots received a solution with 7×10^4 counts of second stage juveniles of *Meloidogyne incognita* right after transplanting.

Use of higher compost rate improved overall plant growth while the lower compost rate plants had higher leaf chlorophyll concentrations and reduced galling. Use of low compost rate with direct soil inoculation (L × DSI) treatment was the least effective in reducing damage by *M. incognita* and had the lowest plant growth overall. Early compost inoculation with high compost (H × ECI) was the best combination for improving plant growth.

Keywords: Compost; Cucumber; *Meloidogyne*; *Pseudomonas*; organic; glasshouse

1. Introduction

Worldwide cucumber production in 2012 amounted to 53.3 million tons (FAO, 2012). It is one of the most important vegetable crops although its production in organic glasshouses in The Netherlands represents only a small percentage of the entire production. This highly specialized and intensive agricultural system (year-round and soil bound cultivation) requires intensive crop and soil care from producers. Due to the unique nature of this farming system, the growers face very specific challenges that they have to overcome.

A 2007 survey among greenhouse producers of organic vegetables resulted in development of a so-called top-10 list of pests and diseases the industry is facing. The root-knot nematode (RKN) *Meloidogyne incognita* Kofoid & White was being listed by growers as the number one pest in organic greenhouse systems (Van der Wurff et al., 2010). The persistence of RKNs in this particular production system might be explained by the broad host range of RKNs while most cultivated crops being susceptible to RKN. But, it is primarily the elevated soil temperature within the glasshouse that is thought to contribute to the persistence and proliferation of RKNs (Davies et al., 2011).

In order to effectively control RKNs and other soil-borne plant pathogens such as *Fusarium oxysporum* and *Verticillium dahliae*, most growers resort to soil steaming at 70 – 100°C. To ensure steam penetration into deeper soil layers flexible polypropylene drainpipes are installed at a depth of 80cm, these pipes act as a partial vacuum and draw the steam applied from the top to the bottom (Ludeking et al., 2012). However, this practice next to being costly, destroys soil life indiscriminately.

Another less drastic and perhaps more obvious measure to control RKNs might be the use of crop rotations. Growing non-host crop species can cause a considerable decline in RKN populations (Van der Wurff et al., 2010). However, due to the specialization in a limited number of vegetables and the marketability of those vegetables, narrow rotation schemes (e.g. 1:2, 1:3) have become the norm rather than the exception in the organic glasshouse vegetable industry. In addition to the limited number of crops that are rotated and that are economically viable, most of these crops belong to the same genus or family (e.g. *Solanacea* spp.), i.e. are susceptible to RKN infestation.

To minimize yield losses due to RKNs, grafting of desirable scions unto RKN-resistant rootstocks has been a strategy that was recently introduced in The Netherlands. However, it is not a stand-alone tool for soil-borne pest control that could be relied upon by growers. Furthermore, due to the relatively small size of the industry of organic glasshouse producers, the development and commercialization of resistant rootstocks was deemed economically unattractive and is being discontinued (Amsing et al. 2003; Van der Wurff et al. 2010).

The phenomenon of disease suppression (soil suppressiveness) in agricultural soils is described by Van Bruggen and Semenov (2000) as a reduction in disease promotion in the presence of a susceptible host and a virulent pathogen, in a physical environment conducive for infection. Several mechanisms are thought to be responsible for soil suppressiveness: antagonism, food competition, physical protection of (crop)roots, production of antibiotic substances and inducing systemic resistance (Van der Wurff et al., 2010).

Microorganisms have been proven to be effective by direct soil inoculation or via use of so-called carrier material which usually consist of an organic amendment (e.g. compost or tree bark). However, these interventions concern mostly laboratory trials under controlled conditions that do not take into account the full array of interactions so characteristic of the soil-food web. However, these complex and competitive multitrophic interactions tend to prevent biological control agents (BCAs) from effectively establishing themselves after their introduction in an organic glasshouse soil (Davies et al., 2011). Regardless, the scientific and producers communities have generated various options for control of RKN. In this context, their focus is not solely on developing a “silver bullet” organism, but more on combining promising methods using an integrative and more holistic approach. It is envisioned that by stimulating naturally occurring predators through soil amendments such as compost, a certain balance in the soil-food web can be restored, where no single organism is allowed to dominate and multiply beyond damaging threshold levels (Van der Wurff et al., 2010; Davies et al., 2011).

The preparation of composts (or derivatives thereof, e.g. extracts such as compost tea) to specifically suppress soil-borne pathogens has been subject of several studies (Hoitink et al., 1999; Everts et al., 2006; Xu et al., 2012). Growers of soil-bound organic greenhouse vegetables commonly apply substantial amounts of compost on an annual basis, using both on-farm produced as well as off-farm purchased composts. Hoitink and Boehm (1999), Nobel and Coventry (2005), and Collange et al. (2011), have all extensively reviewed the composting process in relation to microbial dynamics, including the inoculation of BCAs. Hoitink and Boehm (1999) in particular emphasized in their description of the various composting phases a point in time when the so-called thermophillic phase (i.e. heat-peak, where sterilization of pathogens and weeds occurs) starts to subside. This moment during the composting cycle is characterized by low competitive pressure from mesophillic microorganisms, the group to which most BCAs belong.

In this paper we will explore and put into practice the concept of antagonism by utilizing a bacterial antagonist that may serve as a BCA of RKNs. A particularly novel concept of lacing compost with a BCA during the composting process will be investigated in relation to disease suppressive properties once the laced compost is applied to a glasshouse soil. Finally, this research attempts to answer the following questions, whether: a) BCA inoculation timing, as related to compost temperature at inoculation, affects compost potency to minimize RKN damage in soil-bound glasshouse systems and b) BCA inoculation technique has an effect on RKN galling and overall crop performance.

2. Materials and Methods

The root-knot nematode *M. incognita* (Kofoid & White) was used as a test pathogen. A single strain of *P. fluorescens* KBPL-2059 and compost were applied alone and in combination to cucumber (*Cucumis sativus* L. cv. 'Hoffman's giganta'). The effect of the different treatments on plant growth and galling were assessed in a 6-week glasshouse pot experiment.

2.1. Compost

At the Nergena Composting site of Wageningen University in The Netherlands, four separate compost heaps of 1 m^3 each were formed from partially composted material. The feedstock material consisted mostly from crop residues (70%), grass clippings (25%) and wood chips (5%). The composting method used was aerobic windrow composting where weekly agitation was performed by a tractor mounted windrow-turner. Using a digital temperature probe, heap core temperature was monitored daily during composting (see Appendix B) and moisture content kept at around 40%. The finished compost had an N-content of 0.62% and a C-content of 10.51%.

2.2. BCA inoculum preparation and application

The BCA, *Pseudomonas fluorescens* KBPL-2059, an indigenous strain from India (Pers. Comm. Mr. Bryan Sait) was used in a talc-based formulation marketed as Sudo-Shield™ and obtained from NTS Australia. The colony forming units (CFUs) were determined per g^{-1} dry product (Hiddink *et al.* 2005).

The inoculum was applied to all four compost heaps, differing only in application time: Early (September 2nd 2013) and Late (September 9th 2013) and concentration of inoculum: 20 g^{-1} m^3 vs 60 g^{-1} m^3. Compost was either applied at low (L) vs high (H) compost application rates to the growth substrate. Half of the replicate pots for all treatments (in total8 replicates/treatment) contained either a high (H) or low (L) compost application. On a weight basis this translated to 9.6% vs 3.2% compost pot^{-1}, respectively. To ensure equal amounts of CFUs were inoculated, the L application received 60 g^{-1} m^3 inoculum compared to 20 g^{-1} m^3 for the H application.

2.3. Experimental design

The overall experimental design consisted of two main factors, being two compost-rates and five inoculation treatments (non-inoculated control vs use of early vs late compost inoculation vs direct soil vs root drench application techniques). Treatments were arranged using a complete randomized block design with 8 replicated blocks. To avoid edge effects, the first and last pot in each row of plants consisted of a border pot while there were two rows of extra pots placed at the beginning and end of the experimental set-up.

Since it was expected that compost rate may affect predation rate of nematodes by the fluorescent pseudomonads two different compost application rates were used (high vs low rate) which translated to the addition of 816 vs 272 g compost per pot, respectively. For each of these compost treatments five different inoculation techniques were used: 1. Control; 2. DSI: Direct Soil Inoculation; 3. ECI: Early Compost Inoculation; 4. LCI: Late Compost Inoculation and 5. RDI: Root Drench Inoculation.

2.4. Plant culture

Cucumber plants (*Cucumis sativus* cv. 'Hoffman's giganta') were propagated from seed and 2-week old seedlings were transplanted in 7L black PVC "Rosebush" pots (Ø 21 cm). The soil was kept at field capacity (24% moisture) in a controlled environment at 20 – 22 °C. As for the light regime, 16-hour day lengths were maintained for the experimental period and when necessary supplemental lighting was provided using High Pressure Sodium lamps (Son-T Agro, Philips®, Netherlands) emitting a light intensity of approx. 110W m^{-2}.

The potted plants were placed on 1.96m x 0.96m growing tables which were 1 m in height. Spacing of the plants during the first 3 weeks of the experiment was such that each table contained 4 rows of 7 pots (15 plants m^{-2}) and there were 140 pots in total. During the second half of the experiment the spacing was increased with 50% (i.e. 2 rows of 7 pots/table or 7.5 plants m^{-2}). Pelleted Lucerne (N: 2.48%, P: 0.35%, K: 3.31%) was used as an organic fertilizer and was applied twice at a rate of 75 g pot^{-1}, at the start (0 Days After Transplanting: DAT) and halfway through the experiment (28 DAT).

During the pot experiment plant height and leaf number were measured once every week. After 6 weeks the pot experiment was terminated, chlorophyll concentrations of the first mature leaf were determined using a SPAD-502Plus meter (Konica Minolta Optics, Inc. Japan), and all plants were harvested to assess dry matter of roots, stems including petioles, leaves and fruits. Leaf area was determined using a leaf area meter (LI3100, Li-Cor, Lincoln, NE, USA). Also, galling of roots was scored according to a modified Root Galling Index (see Appendix A). In this index we expressed the average number of galls per root dry weight of corresponding treatments.

2.5. Soil preparation & sterilization

The aim was to replicate actual soil conditions as they would be encountered at an average organic vegetable grower facing root-knot nematode infestation. The bulk of the soil mix for all treatments therefore, consisted of a steam-sterilized fine sandy soil with a soil organic matter (SOM) content of 25 g kg^{-1}. Compost was added in two different application rates (816 g vs. 272 g pot^{-1}). Also sterile potting soil (128 g pot^{-1}) was added to all treatments. All components were thoroughly mixed using a cement mixer to get a homogeneous soil mixture. This composition was formulated to simulate the average SOM content (50 g kg^{-1}) which is representative of targeted organic greenhouse systems in The Netherlands.

2.6. Nematodes

The eggs and egg masses of *Meloidogyne incognita* were harvested from heavily infected roots of tomato (*Lycopersicon esculentum* cv. 'Moneymaker') and hatched during incubation at room temperature for 48 hours. The resulting second-stage juveniles (J_2) were then collected by sieving and stored in a flask containing 100 ml^{-1} demineralized water, this suspension was then aerated by an air-compressor. The initial concentration of the J_2 (7×10^4) was determined microscopically by counting three separate 1 ml^{-1} suspensions on a gridded counting plate. This 100ml^{-1} suspension (7×10^4 J_2) was topped up by 3900 ml^{-1} of demineralized water to prepare the final suspension (4 liters) which was applied proportionally to the experimental pots in 50 ml^{-1} pot^{-1} using a 60 ml^{-1} syringe.

2.7. Statistical analysis

Statistical analysis was carried out by two-way ANOVA using Duncan's multiple range test to evaluate significant differences between means at ≥95% level of confidence. The analysis was performed using the statistical software package GenStat® version 16.

3. Results

3.1. Plant height

The effect of treatments on plant height is presented in Table 1. During the first two weeks, compost had no effect on plant height, whereas starting 21 DAT plants were taller for the high compost rate. The effect of different inoculation techniques was significant except at 14 and 42 DAT. Initially, the direct soil inoculation (DSI) treatment appeared to have slightly taller plants whereas between 21 and 35 DAT plants for this treatment were significantly shorter compared to the non-inoculated control with the other treatments having intermediate values.

Table 1
Cucumber plant height during the 6-week pot trial as affected by compost and *P. fluorescens* inoculation.

	Plant Height (cm)					
	7 DAT[x]	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
Compost (C)						
Low	10.8	21.1	51.1	90.2	118.7	132.4
High	11.3	22.9	59.7	101.6	142.2	166.6
Sig.[y]	ns	ns	**	***	***	***
Inoculation (I)[z]						
Control	10.0[b]	23.7	62.4[a]	102.4[a]	138.2[a]	152.3
DSI	13.1[a]	22.8	51.9[b]	89.9[b]	119.9[b]	138.1
ECI	10.6[b]	22.0	57.5[ab]	96.8[ab]	132.2[ab]	152.9
LCI	10.[3b]	20.8	53.4[ab]	97.9[ab]	135.3[ab]	157.4
RDI	11.2[ab]	20.7	51.7[b]	92.5[ab]	126.6[ab]	146.7
Sig.	*	ns	*	*	*	ns
C × I						
Sig.	ns	ns	ns	ns	ns	ns

[x] Days after transplanting

[y] ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: not significant ($P \geq 5\%$)

[z] Superscripted letters indicate significantly ($P < 5\%$) different means as established by Duncan's multiple range test.

3.2. Leaf number

Leaf number was significantly affected by compost rate (Table 2). With the exception of the first measurement (i.e. 7 DAT), the high compost rate had greater number of leaves.

Only during the first 3 weeks of the experiment (7, 14 and 21 DAT) was the influence of inoculation technique significant. Growth reductions (i.e. lower leaf numbers) over time were most pronounced in the DSI treatment when compared to the rest of the treatments in general and the control in particular. At 21 DAT the ECI treatment performed best among inoculated treatments yet still ECI plants had formed one leaf less compared to the control.

Table 2
Cucumber leaf number as influenced by compost and *P. fluorescens* inoculation.

	Leaf number					
	7 DAT[x]	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
Compost (C)						
Low	3.38	6.03	9.48	13.45	13.83	14.73
High	3.68	6.35	10.25	14.57	15.28	16.24
Sig.[y]	ns	*	**	*	**	***
Inoculation (I)[z]						
Control	3.94[a]	6.81[a]	11.13[a]	14.69	15.25	15.80
DSI	3.13[b]	5.88[b]	9.25[c]	13.94	13.94	15.56
ECI	3.69[ab]	6.25[b]	10.13[b]	14.31	14.94	15.86
LCI	3.50[ab]	6.06[b]	9.44[ab]	13.69	14.62	15.50
RDI	3.38[ab]	5.94[b]	9.38[ab]	13.44	14.00	14.70
Sig.	*	**	***	ns	ns	ns
C × I						
Sig.	ns	ns	ns	ns	ns	ns

[1] Days after transplanting
[2] ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: not significant ($P \geq 5\%$)
[3] Superscripted letters indicate significantly ($P < 5\%$) different means as established by Duncan's multiple range test.

3.3. Leaf area and SPAD

As shown in Table 3, use of a high compost rate significantly increased leaf area compared to the plants that received the low compost dosage. For the SPAD the inverse was true, namely that the low compost rate resulted in a significantly higher SPAD value when compared to the high compost rate. For the leaf area data a significant interaction between compost rate and inoculation technique was found (Fig. 1.). At the low compost rate, DSI performed relatively poorly while late compost inoculation did relatively well, while at the high compost treatments early compost inoculation and DSI performed relatively well.

Table 3
Cucumber leaf area (cm^{-2}) and SPAD as affected by compost and *P. fluorescens* inoculation.

	Leaf Area (cm^{-2})	**SPAD**
Compost (C)		
Low	3196	55.93
High	5414	51.48
Sig.[x]	***	***
Inoculation (I)[y]		
Control	4136	51.76
DSI	3926	54.11
ECI	4825	53.61
LCI	4440	53.54
RDI	4197	55.51
Sig.	ns	ns
C × I		
Sig.	*	ns

[x] ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: not significant ($P \geq 5\%$)

[y] Superscripted letters indicate that means differed significantly ($P < 5\%$) as established by Duncan's multiple range test.

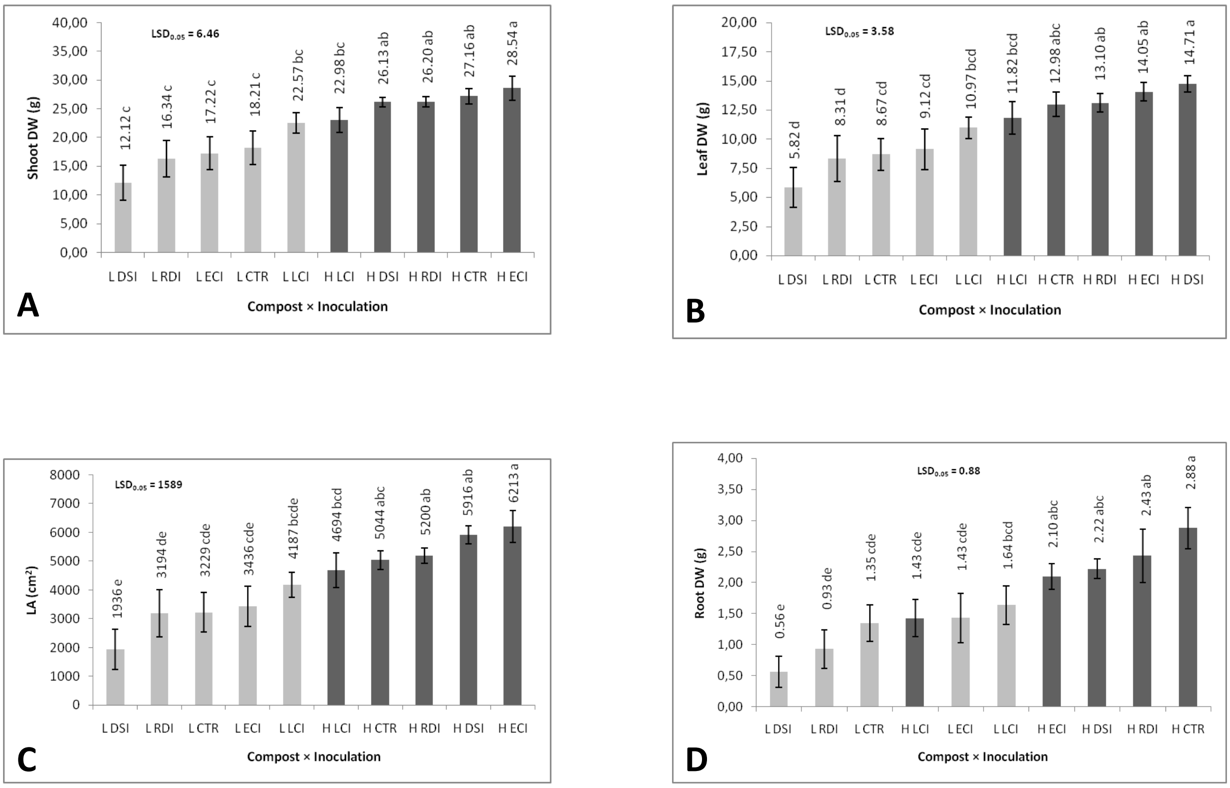

Fig. 1. Shoot (A) and leaf (B) dry weight; Leaf area (C) and Root dry weight (D). The interaction effect (compost rate × inoculation technique) on plant growth performance indicators.

3.4. Leaf, stem, fruit and shoot dry matter

The higher compost rate resulted in greater dry weights for most plant organs except for fruit, while inoculation treatments had no effect on plant growth (Table 4).

However, the interaction between compost rate and inoculation technique was significant for leaf and shoot dry weight (Table 4). For leaf dry weight it was found that the low compost-DSI plants differed significantly with the high compost-ECI and DSI plants (Fig. 2A). Likewise for shoot dry weights, the low compost DSI treatment performed worse and had significantly lower shoot DW as compared to the ECI, Control, RDI and DSI treatments for the high compost treatments (Fig. 2B). Among low compost treatments, LCI performed relatively well, whereas within the high compost rate group, it performed rather poorly (Fig. 2B).

Table 4
Cucumber leaf, stem, fruit and shoot dry weight (g^{-1}) as influenced by compost and *P. fluorescens* inoculation.

	Plant dry weight			
	Leaf DW	Stem DW	Fruit DW	Shoot DW
Compost (C)				
Low	8.58	7.04	1.62	17.29
High	13.33	9.79	3.08	26.20
Sig.[x]	***	***	ns	***
Inoculation (I)[y]				
Control	10.83	9.22	2.59	22.68
DSI	10.27	7.33	1.46	19.12
ECI	11.59	8.65	2.64	22.88
LCI	11.39	8.66	2.72	22.77
RDI	10.70	8.23	2.33	21.27
Sig.	ns	ns	ns	ns
C × I				
Sig.	*	ns	ns	*

[x] ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: not significant ($P \geq 5\%$)

[y] Superscripted letters indicate that means differed significantly ($P < 5\%$) as established by Duncan's multiple range test.

3.5. Plant-Pseudomonas-Parasite interaction

Table 5 outlines key rhizosphere interactions and how they affected the overall plant root system. It is evident that treatments with a high compost rate had significantly higher root dry weights and a corresponding lower shoot/root ratio. So at high compost application favored shoot growth to a larger extent compared to root growth. However, plants treated with the high compost dosage were found to possess a significantly lower incidence of galls of their root system compared to low compost plants. Although not significantly different, the DSI plant roots showed less pronounced root galls.

The interaction between compost rate and inoculation technique was found to significantly influence both root dry weight as well as the shoot/root ratio (Table 5). For the root dry weight it was found that the low compost-DSI differed significantly with the Control, RDI, DSI, ECI and LCI treatments within the high compost group. As for the shoot/root ratio the low compost-DSI treatment had a significantly higher shoot/root ratio, which may point to poorly developed root system.

Table 5. Effect of compost and *P. fluorescens* inoculation on cucumber susceptibility to the RKNs, as measured by root dry weight g^{-1}, Shoot:Root ratio and Root Galling indices (RGI).

	Root DW	Shoot:Root ratio	RGI[x]
Compost (C)			
Low	1.18	29.7	3.58
High	2.21	14.3	2.37
Sig.[y]	***	**	*
Inoculation (I)[z]			
Control	2.11^{a}	13.94^{a}	2.39
DSI	1.39^{b}	37.21^{b}	3.11
ECI	1.77^{ab}	15.79^{a}	2.63
LCI	1.53^{ab}	20.29^{ab}	3.28
RDI	1.68^{ab}	22.73^{ab}	2.75
Sig.	ns	ns	ns
C × I			
Sig.	*	*	ns

[x] Number of galls/root dry weight

[y] ***: *P < 0.001*; **: *P 0.001 - 0.01*; *: *P = 0.01 - 0.05* and ns: (*P* ≥ *5%*) not significant

[z] Superscripted letters indicate that means differed significantly (*P < 5%*) as established by Duncan's multiple range test.

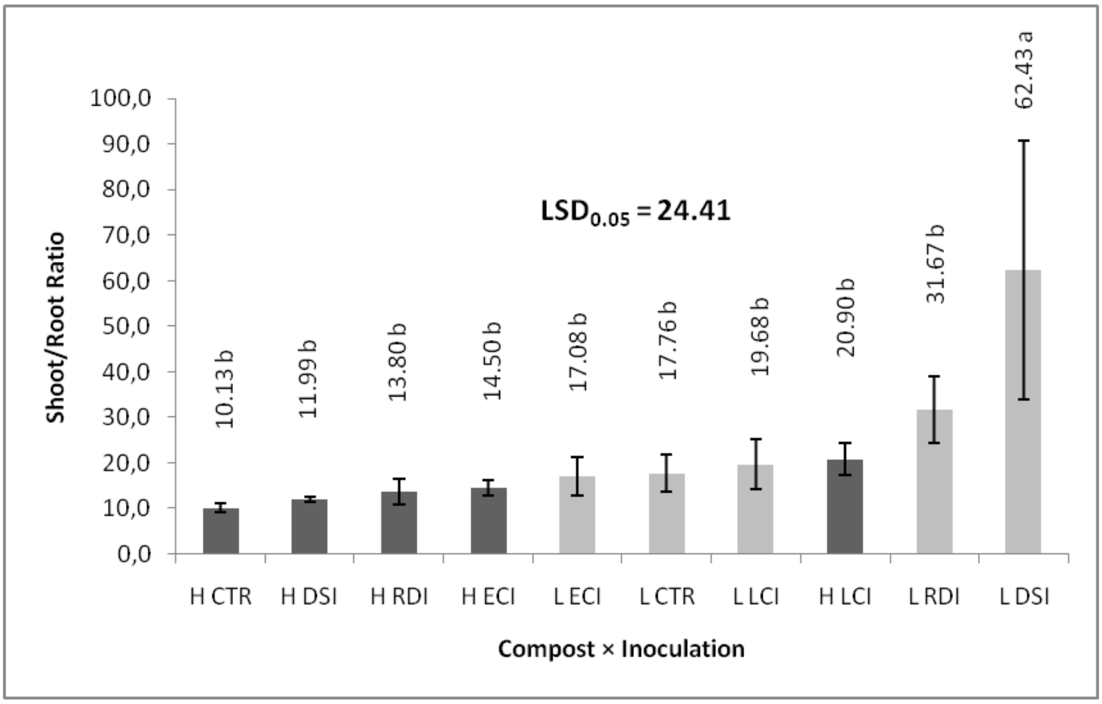

Fig. 2. Shoot/Root Ratio. The interactive effect of compost rate and inoculation technique.

4. Discussion

Regardless of inoculation technique, the higher compost rate was found to increase plant height much more profoundly over time, compared to the low compost treated plants. This effect might be due to the improved retention of water and supply of nutrients associated with higher compost rates (Oka, 2010).

However, over time plant height was adversely affected in the direct soil inoculation (DSI) treatment, this particular inoculation technique proved to be the poorest performing compared to other inoculation techniques used. The control, which only received RKNs, but no *Pseudomonas,* was expected to possess the lowest plant height, while the reverse was found to be true. The objectives of this work was to estimate the effect of *Pseudomonas* and compost on biological control of RKNs in cucumber production and to determine whether the timing or technique of *Pseudomonas* inoculation contributed to reduction or aggravation of RKN damage. Since the giant feeding cells formed by the RKNs on the roots of the host plant may cause substantial nutrient deficiencies (Perry et al. 2009), it may be argued that in the case of the DSI plants the Pseudomonads were not able to establish and colonize the roots as they have done with the other treatments (Table 1). However, when looking at the galling index (Table 5), it appears that the DSI treatment had numerically lower values so there is no evidence that root galls were larger for the DSI treatment so it appears that root systems may have been relatively poorly developed instead, as Shoot/Root ratios for the L-DSI treatment being particularly high (Fig. 2).

The control plants consistently had the highest number of leaves, which is unexpected and difficult to explain. Increased leaf development rates are usually the result of high nitrogen levels, but in this experiment all plants received the same amount of fertilizer simultaneously. The only source of additional nitrogen would have to be the compost, and obviously the higher compost rate would supply more nitrogen compared to the lower compost dosage. Alternatively, it may be possible that inoculation with *Pseudomonas* adversely affected overall stem elongation, although the underlying mechanism causing this remains unclear.

Based on values shown in Table 1, stem elongation rates were 1.5 cm vs 6.0 cm d^{-1} at 7 vs 28 DAT, respectively). Part of this difference may also be related to transplant shock right after transplanting which may be reducing initial shoot growth until the root/shoot equilibrium is being restored. So once root growth is restored difference among treatments in terms of growth may decline as is evidenced by differences among inoculation treatments after 21 DAT no longer being significant (Table 2).

The compost inoculation treatments, especially the ECI, seem to perform better than the DSI and RDI although the control plants still performed better which may be related to control plants having greater initial leaf number (Table 1, 2).

The effect of a higher compost rate in increasing leaf area is most pronounced for the ECI treatment which performed relatively well compared to the control (Fig. 1.). There seems to be a synergistic effect for this particular interaction because ECI plants with the low compost rate had a significantly lower leaf area. The plant growth promoting *Pseudomonas* that was inoculated early during the composting process might have established itself much better in the compost compared to the other treatments, and consequently capable of having a positive impact on leaf area, especially with a higher compost rate.

Robinson and Decker-Walters (1996) found that total leaf area per plant was primarily affected by a change in plant density. However, in our case all treatments were spaced at equal distances so the very low leaf area for the L-DSI treatment seems to point out that initial growth with this treatment may have been inhibited as this treatment had consistently relatively low leaf, shoot, and root dry weights (Figs. 1 and 2).

Pseudomonas belongs to a group of denitrifying bacteria, responsible for the process of denitrification. Thus we do not rule out the possibility that a fraction of total nitrogen was denitrified by *Pseudomonas* into N_2. This would mean that the net plant N availability for low compost plants may be lower compared to non-inoculated control. However, this argument is supported by the higher SPAD value in the low compost treatment. This since SPAD values corresponds to leaf chlorophyll concentration, giving a general indication of the overall crop N status in terms of nutrient acquisition. An interesting correlation exists between RGI and SPAD between the high and low compost plants. It seems that for the higher compost plants a lower chlorophyll concentration might be the result of increased root galling (Tables 3 and 5). Next to reducing overall plant growth and causing root galling, RKNs can also cause nitrogen deficiency-like symptoms in infected plants. In this case the galls or giant feeding cells of *M. incognita* are preventing the transport of nutrients which would become apparent in the leaves by a reduction in chlorophyll concentration (Siddiqui et al. 2001).

The low compost DSI plants did not perform as well, in terms of accumulating biomass, compared to other treatment combinations (Figs. 1 and 2). According to our data it seems that low efficacy of this particular inoculation technique is the primary cause. As Siddiqui and Mahmood (1998) stated, it is possible that rhizosphere and root colonization and subsequent biocontrol of the host could not be achieved by the remoteness of their introduction into the soil. A different inoculation technique that increased growth of cucumber shoot substantially was the high compost ECI treatment. The aim of this research has been to investigate the enrichment of compost with *Pseudomonas* during the composting process as opposed to enrichment at the end of the composting cycle. We have now reason to believe that the resulting shoot growth in the high compost-ECI treatment is due to the synergistic action of high compost rates and *Pseudomonas*. This also proves that responses to inoculation have to be placed in the context of overall compost status of the soil and that also governs the optimal timing of *Pseudomonas* inoculation during the composting process (Fig. 2A and B).

The compost used in this work had a C/N ratio of approximately 17:1 which can be considered a low to average C/N ratio for finished compost. Rodriguez-Kabana and associates (1987) confirmed that low C/N composts are able to substantially reduce RKN numbers by releasing nematicidal compounds such as ammonia during decomposition. Our results regarding the effect of compost rate on disease suppression are not consistent with findings by Noble and Coventry (2005). In their review they concluded that the disease suppressive effect of compost increases with the rate of application, while in our analysis of RKN pressure, represented by the Root Galling Index (Appendix A), which is consistent with our finding provided that gall index was expressed on a per unit of root weight (Table 5). In the current study it is evident that high compost rates resulted in more vigorous shoot, and to a lesser extent root growth (Figs 1, 2). Therefore, it may be argued that this larger root volume appears to foster root not gall growth since total gall number increased, which may be related to substrate availability for parasitic root not nematodes.

Another explanation for this might lie in the fact that in the study by Nobel and Coventry (2005) the focus had been on fungal pathogens which behave differently in varying levels of compost. Root dry weight (Fig. 2) was highest for the high compost RDI plants (after the high compost Control) which is easy to comprehend since this inoculation technique is the most intimate in terms of plant-BCA interaction. Introducing the *Pseudomonas* inoculum by drenching the entire 2-week old root system and thus exposing the bacterium to the rhizoplane, *Pseudomonas* is able to reside and colonize the host root easily without much competition. *Pseudomonas* in the RDI plants is thus in a more advantageous position to feed on plant exudates and possess a competitive edge to the rest of the microbial life which is to develop (Haas and Défago, 2005; Mercado-Blanco and Bakker, 2007).

5. Conclusions

From the findings of the current study, it can be concluded that compost increased plant growth considerably, while effects of inoculation techniques where not always consistent across compost rate treatments. For certain plant growth parameters there was a clear synergy between the amount of compost applied and the *Pseudomonas* inoculation technique used. It seems from the experiment that not a single inoculation technique is conducive for all plant performance indicators. However, the early compost inoculation (ECI) plants that received the higher compost rate were found to have the highest leaf sizes and weight, and highest shoot dry weight. The inoculation of *Pseudomonas* in general and early during composting in particular should be explored further as a way to control RKN in organic greenhouse systems. Additional studies are required to further elucidate the underlying mechanisms governing differential responses to inoculation techniques as related to compost application rate.

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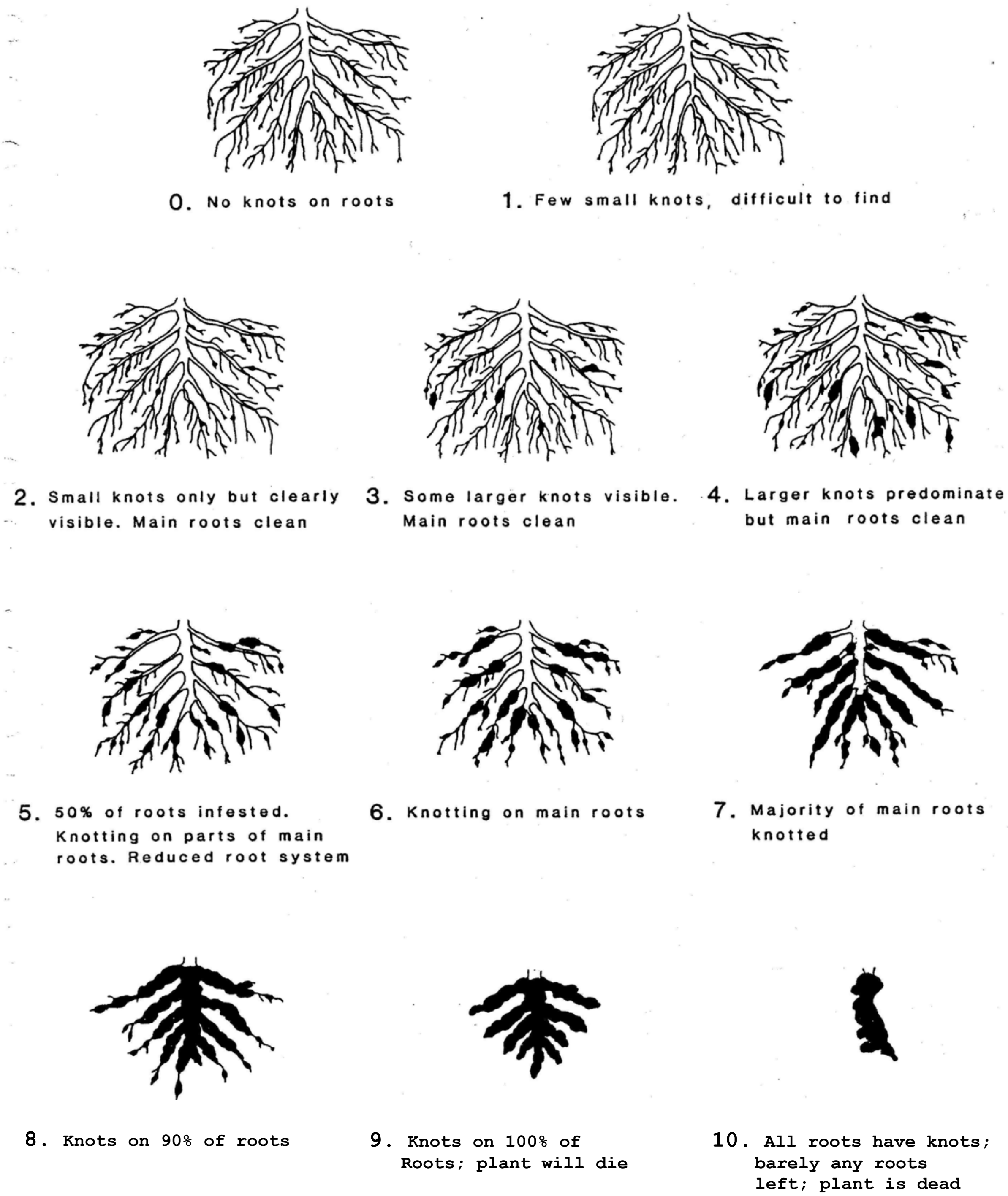
Appendix A. RGI: Root Galling Index[1]

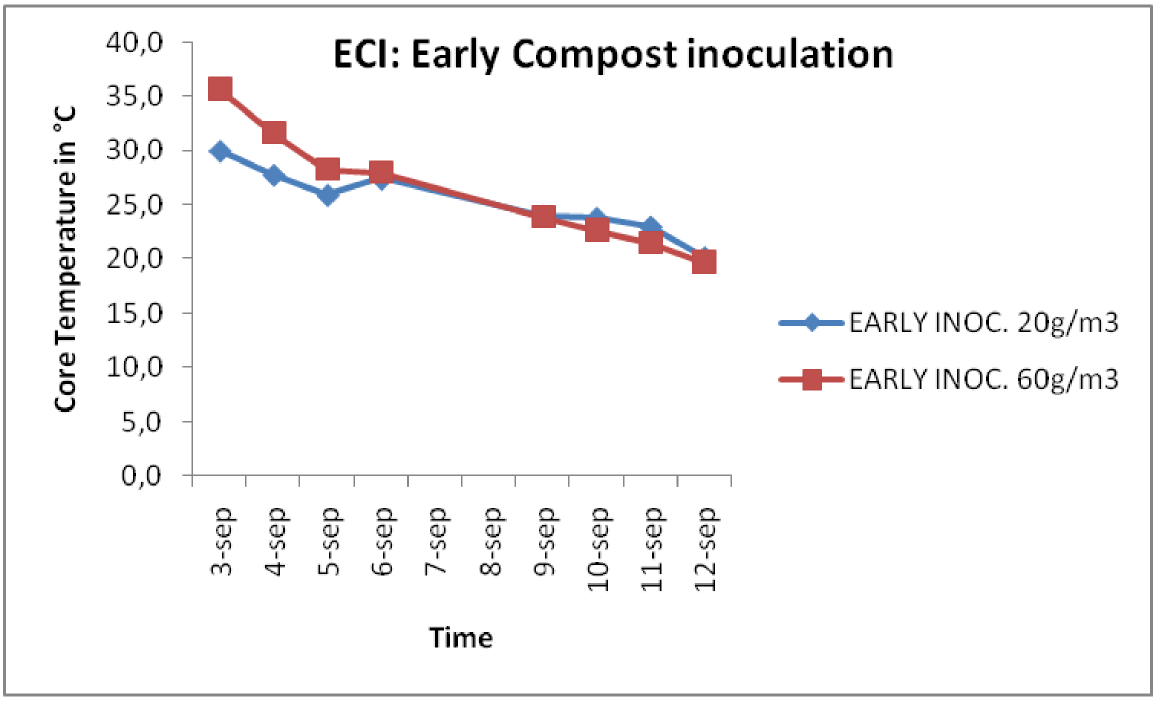


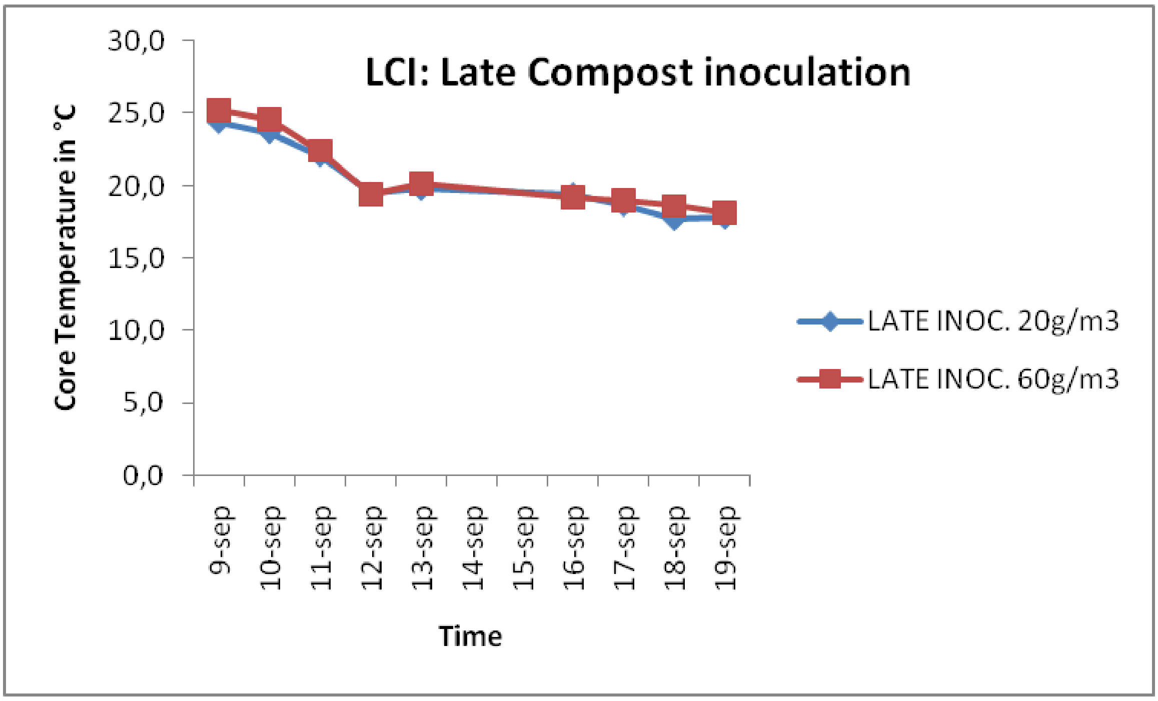
Approximate quantification of RKN galls:

1 = 1-4; **2** = 5-15; **3** = 15 -30; **4** = 30 -60; **5** = 60-125;
6 = 125-250; **7** = 250-500; **8** = 500-1000; **9** = > 1000

[1] Adapted from the original index developed by van der Wurff et al. (2010); quantification and the corresponding gall ranges per root system (gall no./root dry weight) has been the work of current authors.

Appendix B. Compost temperatures[2] and Turning Operations





[2] These curves are based on core temperatures taken on a daily basis and immediately following the inoculation for both the early as well as the late inoculated compost heaps.

	EARLY INOC. 20g/m^3					EARLY INOC. 60g/m^3				
Date	Left	middle	right	Avg		Left	middle	right	Avg	
3-sep	31,4	28,7	29,9	30,0		34,2	35,5	37,5	35,7	
4-sep	28,3	27,5	27,5	27,8		30,4	31,8	32,8	31,7	
5-sep	26,4	25,8	25,5	25,9		26,5	29,2	29	28,2	
6-sep	27	29	26,3	27,4	<-- turned	27,4	28,7	27,8	28,0	<-- turned
9-sep	24,5	23,9	23,5	24,0		23,9	24	23,8	23,9	
10-sep	23,9	24,5	22,9	23,8		22,5	22,8	22,7	22,7	
11-sep	23,5	23,5	21,9	23,0	<-- turned	22	21,7	20,8	21,5	<-- turned
12-sep	20,1	20,3	20	20,1		19,7	19,8	19,5	19,7	
13-sep	20,3	20,5	20,2	20,3		20,1	20,2	19,8	20,0	
16-sep	20,1	19,5	19,2	19,6		18,9	19,4	19	19,1	
17-sep	19,5	18,8	19,3	19,2		18,6	19	18,5	18,7	
18-sep	19,7	18,2	18,4	18,8		17,7	18,2	17,4	17,8	
19-sep	18,3	18,7	17,7	18,2		17,2	17,7	17,6	17,5	

	LATE INOC. 20g/m^3					LATE INOC. 60g/m^3				
Date	Left	middle	right	Avg		Left	middle	right	Avg	
9-sep	24,3	24,8	24	24,4		25,4	25,8	24,5	25,2	
10-sep	23,8	23,9	23,3	23,7		24,5	25,3	23,9	24,6	
11-sep	22,5	22,5	21,2	22,1	<-- turned	22,7	23,3	21,3	22,4	<-- turned
12-sep	19,7	19,3	19,4	19,5		19,6	19,4	19,3	19,4	
13-sep	19,8	19,9	19,6	19,8		20,3	20,2	20	20,2	
16-sep	19,2	19,2	19,9	19,4		19,5	19,3	19	19,3	
17-sep	18,5	18,5	19	18,7		19,1	19	19	19,0	
18-sep	17,6	18	17,5	17,7		18,7	18,5	18,8	18,7	
19-sep	17,9	17,5	18	17,8		18,2	18	18,3	18,2	

[3]

[3] Compost heap core temperatures were taken from three different locations; a single measurement from each side of the heap and a "true-core" (i.e. middle) measurement.

Appendix C. Experimental layout of greenhouse pot trial