Innovative Intercropping System to Improve Soil Health in Organic Greenhouse Cultivation

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Abstract

In a field experiment in an organic greenhouse, an innovative cropping system (the so-called 'Köver' system) was tested. In this system, planting beds are divided below ground in two physically separated strips. In one section, vegetables are cultivated at half the normal planting distance. In the other section, the soil is either left fallow or planted with crops antagonistic towards plant-parasitic nematodes. After one growing season, vegetable and fallow strips are reversed. In this way, crop rotation is broadened, with the aim of improving soil health. Compared to the normal cultivation system, the availability of space and light in the Köver system might be unfavourable for some crops (e.g., sweet pepper), which may lead to an initial production loss. However, improved soil health should ultimately lead to a healthier crop and higher yields. The Köver system was introduced in sweet pepper 'Derby' on rootstock 'Capital'. The aim was to achieve production levels of 88–92% or higher of the maximum production targets on a healthy, previously uncultivated soil, while reducing numbers of plant-parasitic nematodes, particularly *Meloidogyne incognita*. In the Köver system, the following treatments were compared: (1) Sweet pepper 'Derby' on rootstock 'Capital', (2) fallow, (3) Marigold (*Tagetes patula*) 'Single Gold' (brand name: Ground Control) and (4) the densely planted rootstock *Capsicum annuum* 'Snooker'. At the end of the season, the number of *Meloidogyne* juveniles was significantly reduced by fallow, *Tagetes* and 'Snooker' treatments, compared with the sweet pepper crop. The 'Snooker' rootstock was less effective in reducing *Meloidogyne* juveniles than fallow or *Tagetes*. Numbers of juveniles hatching from eggs increased significantly in the sweet pepper crop compared with other treatments. Due to considerable production loss in the sweet pepper crop, antagonistic plants are not recommended in neighboring strips. The fallow treatment was the most promising in combination with sweet pepper in the Köver system. However, further research is needed to confirm these results.

INTRODUCTION

In organic greenhouse horticulture, it is of key importance to develop a healthy soil as the foundation for a sound production system. Short crop rotations and little or no resting periods cause high pathogen loads in the soil system. Root-knot nematodes have especially rapid rates of multiplication on good hosts, and a relatively small population can reach economically damaging levels within one growing season. Soil-borne fungi such as *Verticillium dahliae* can also cause high crop damage at relatively low densities. Additionally, available rootstocks are not always effective and may not provide resistance to all soil diseases found on one farm.

Common crop rotations in organic horticulture in The Netherlands typically include tomato (*Lycopersicon esculentum* Mill.), sweet pepper (*Capsicum annuum* L.) and cucumber (*Cucumis sativus* L.), which are all good hosts for tropical root-knot nematodes. Most vegetables are grown in a year-round cultivation schedule, with a few

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weeks of fallow in early winter (November-January) before the start of a new growing season. Only cucumber is cultivated in three successive cycles during one year, in order to maintain plant production levels. Organic horticulture has limited means of suppressing soil-borne diseases. The main tools for organic agriculture to stimulate soil health are crop rotation, application of green manures and the use of organic fertilizers. In organic horticulture, the inclusion of green manures and crop rotation are difficult to apply for economic reasons. Organic soil applications such as composts and manure have limited possibilities to suppress pathogens and enhance disease resistance of soils (Termorshuizen et al., 2006).

During an interactive session with organic growers, the idea of an innovative intercropping system, the so-called 'Köver system' (a combination of the designers' names, Kögeler and Verbeek) was born (Blom et al., 2007). In this system, planting beds are divided underground into two strips using a thick, plastic sheet. Above ground, planted strips are alternated: on one side of the plastic sheet, strips are planted with vegetable crops, while on the other side, strips are either left fallow or planted with antagonistic crops or non-hosts for pathogenic nematodes or fungi. In order to prevent production loss, vegetable crops are planted at twice the planting density as usual. In this system, above ground competition for space and light, as well as below ground needs for nutrients and water, pose possible problems. After one or two years, planting of the strips is reversed. The reduction of nematode numbers in the fallow/non-host strips should be sufficient to prevent damage in the following vegetable crops. Pilot experiments in 2008 clearly showed differences in the ability of vegetable crops to adjust to circumstances in the Köver system. Both tomato and cucumber were able to quickly adapt to the new configuration of space and light. Cultivation of sweet pepper, however, revealed possible problems with the system, as growth is much slower and rigid plant stems adapt with difficulty to the new environment, slowly occupying the space above the fallow strips (Boonekamp, 2008).

The effects of non-hosts, antagonistic crops and fallow periods on *M. incognita* have been examined in numerous laboratory and field experiments. Antagonistic results have been obtained with different varieties of *Tagetes patula* such as 'Single Gold', 'Tangerine' and 'Dwarf Primrose' and *Tagetes erecta* 'Flor de Muerto' (McSorley and Frederick, 1994; Ploeg and Maris, 1999). When grown within a 20–30°C soil temperature range, cultivars tested by Ploeg and Maris (1999) significantly reduced root-galling and nematode infestation of the subsequent tomato crop, compared with tomato following a fallow treatment. At temperatures below 15°C, most marigolds were ineffective against *M. incognita*. The cover crops castor (*Ricinus communis*), cowpea (*Vigna unguiculata* 'Iron Clay'), crotalaria (*Crotalaria spectabilis*) and American jointvetch (*Aeschynomene americana*) have shown consistent resistance against *M. incognita* (McSorley, 1999). Organic growers in the Netherlands believe that populations of *Meloidogyne* decline when sweet pepper is cultivated on *Capsicum annuum* 'Snooker' rootstocks. Oka et al. (2004) found an intermediate resistance of 'Snooker' to *M. incognita* race 2. In their experiments, rootstocks of *C. chacoense* and *C. frutescens* showed high resistance to *M. incognita*. However, most of these rootstocks gave lower yields compared with the ungrafted cultivar. For use within the Köver system, antagonistic crops should not compete with vegetable crops for space and light to the degree that crop growth is inhibited, and should be relatively shade-tolerant, in order to provide enough biomass to exhibit an antagonistic effect. Growth of antagonistic crops in a greenhouse will be different from agricultural fields, and may depend on soil type.

MATERIALS AND METHODS

In 2009, the field experiment was conducted in an organic greenhouse with a crop rotation of sweet pepper and tomato. The Köver system was established for yellow sweet pepper (*Capsicum annuum* 'Derby' on rootstock 'Capital' (De Ruiter Seeds)). Greenhouse crops on this farm are cultivated year round, with a short fallow period (5–8 weeks) between November and January. The greenhouse was situated in the Voorne-

Putten region (Zuid-Holland, The Netherlands) on a 'Polder' vague soil in calcareous young marine clay (Gleyic Fluvisol) (de Bakker and Schelling, 1989; Krasilnikov and Arnold, 2009). The soil contained 19% lutum, 9% organic matter and 3.4% calcium in the upper layer. The greenhouse was divided into compartments (spans) of 8 x 65 m. Each span contained five 1.1 m wide planting beds, separated by harvesting paths. The Köver system was introduced in two adjacent spans, while a third span with a normal production scheme served as a control. Planting beds were divided into two planting strips over the entire length of the span, using thick plastic sheets buried in the ground as vertical dividers. In the first span, sweet pepper strips were alternated with fallow strips. In the second span, three blocks were assigned to sweet pepper strips paired with alternating antagonistic treatments, which were randomly assigned to subplots within each strip. Three antagonistic treatments were tested: fallow, *Tagetes patula* 'Single Gold' (brand name: Ground Control) (Sahin) and the rootstock *Capsicum annuum* 'Snooker' (Syngenta). Each replicate consisted of 2 subplots within a strip, which were pair-wise sampled and analyzed. In order to facilitate second year monitoring, all sweet pepper plots adjacent to antagonistic plots were sampled (resulting in 9 instead of 3 replicates). For final data analysis of soil samples, 3 replicates of sweet pepper were randomly chosen from each block, in order to obtain a balanced design.

Sweet pepper was cultivated in a two-stem system. In the Köver system, planting distances were 0.4 m instead of the standard 0.8 m. In order to optimize use of space and light, one stem was allowed to grow naturally, while the second stem was bent towards the neighboring strip using a supporting twine. *Tagetes patula* was directly sown into the plots in March. 'Snooker' rootstocks were raised in a nursery and planted in the plots at a density of 33 plants m^{-2}, to enlarge the rooting volume and possible nematode suppressing effects. When sufficiently developed, 'Snooker' rootstocks were mown every other week at waist height, in order to prevent competition with neighboring sweet pepper plants. At the end of the growing season, antagonistic crops were chopped up with a wood shredder and incorporated to a depth of about 15 cm into the plots. Fresh and dry weights of antagonistic crops were measured. One week after incorporating antagonistic crops, soil samples were taken to determine *M. incognita* survival.

Prior to planting the sweet pepper crop, the following organic amendments were incorporated into the soil: 72 ton ha^{-1} compost, together with a base dressing of 1500 kg ha^{-1} Agro Biosol (Sandoz) (NPK 7-1-1) and 1000 kg ha^{-1} Biovin (Plant Health Care) (NPK 2.5-1.5-2.5). In August 2009, equal side dressings of Monterra Malt (Monterra) (NPK 5-1-5) were applied to the control and Köver plots, at a rate of 2500 kg ha^{-1}. Water was applied using a combination of drip and sprinkler irrigation. In the Köver system strips, the amount of drip irrigation units was doubled, with each plant receiving one dripper. Sprinklers were placed in order to supply the sweet pepper with about 2/3 of the sprinkler irrigation, while 1/3 was used for the antagonistic crop plots. Antagonistic crops did not receive any drip irrigation. Fallow strips were covered with a white, perforated plastic sheet in order to minimize growth of weeds. Soil samples were collected at ca. 4-week intervals from February until November in the normal production crop and in the densely planted Köver system to monitor for soil nutrients. Forty 1.0-cm diameter cores 0–25 cm deep were taken from each section and composited.

In March and November, soil samples were taken to determine the effect of the treatments on the population of root-knot nematodes. Eighty soil cores were taken from each plot to a depth of 10 cm. Second-stage juveniles (J2) of *M. incognita* were extracted from 100 cc soil using an Oostenbrink elutriator (s'Jacob and van Bezooijen, 1984). Soil samples were incubated for 28 days in a mistifier chamber at 20°C in the dark, so that viable egg masses would hatch (Southey, 1986). For both extracted and incubated soil fractions, numbers of plant-parasitic nematodes were counted and identified to genus level. At each sampling time, twenty individuals (of plant-pathogenic species) from 4 randomly chosen samples were identified to species level. The total number of non-plant-pathogenic nematodes was counted in directly extracted soil samples. Statistical analysis using Repeated Measurements with ANOVA (GenStat Release 11.1; Lawes Agricultural

Trust, Rothamsted Experimental Station) was carried out on ln(x + 1) transformed numbers of nematode counts. In the text and tables, back transformed means are given.

Production was measured in 4 randomly chosen sweet pepper plots in (1) the conventional cultivation system, (2) the Köver system in the first span, with only fallow strips (without antagonistic crops), (3) the Köver system in the second span, with mixtures of antagonistic crops, and upright growing stems at sun and shade side, (4) the Köver system in the second span, with mixtures of antagonistic crops, and bent stems growing at sun and shade side. From 20 stems per plot, number and weight of fruit were measured over the course of 27 weeks (week 16–42). Analysis of yields under different growth conditions (stems growing in different combinations of upright/bent/shade/sun sides) were carried out the last 4 weeks of the growing season (week 43–46). Statistical analyses were carried out using ANOVA (GenStat Release 11.1; Rothamsted Experimental Station).

RESULTS AND DISCUSSION

Effects on *Meloidogyne incognita*

Sweet pepper plots were planted on January 22 and contained 69 second-stage juveniles (J2) of *M. incognita* and 63 juveniles from hatched eggs ($J2^{egg}$) per 100 cc soil in March (Table 1). The antagonistic treatments were left fallow for about 2 months and contained significantly higher numbers of *Meloidogyne*, as compared with the sweet pepper plots. No differences were found in nematode numbers among these antagonistic plots, which varied between 287-323 J2 and 175-259 $J2^{egg}$ per 100 cc soil. Differences in numbers of *Meloidogyne* J2 between the fallowed antagonistic and sweet pepper plots might stem from preferential movement of *Meloidogyne* to the pepper roots, lowering their numbers in the bulk soil of planting beds. However, as no samples were taken prior to planting sweet pepper, only assumptions can be made regarding nematode development in the first two months of the experiment.

At the end of the growing season in November, soil samples were taken one week after the incorporation of antagonistic crop residues into the soil. Sweet pepper was still in production at sampling time. Numbers of *M. incognita* J2 and $J2^{egg}$ differed significantly among treatments. The lowest numbers were found in Fallow (23 J2 and 18 $J2^{egg}$ 100 cc^{-1} soil) and *Tagetes* plots (31 J2 and 10 $J2^{egg}$ 100 cc^{-1} soil). Intermediate numbers were found in 'Snooker' plots (82 J2 and 58 $J2^{egg}$ 100 cc^{-1} soil). In the 'Snooker' treatment, numbers of J2 differed significantly from *Tagetes* and Fallow, but numbers of $J2^{egg}$ of the three antagonistic plots did not differ significantly from each other, nor from $J2^{egg}$ numbers in March. Numbers of J2 of *Meloidogyne* diminished significantly from March to November for all antagonistic treatments. For sweet pepper plots, both J2 and $J2^{egg}$ increased significantly from March to November; in March, 69 J2 and 63 $J2^{egg}$ were found, compared to 330 J2 and 1619 $J2^{egg}$ in November.

Intercropping systems have been tested with varying results on their potential to prevent damage caused by pathogenic nematodes. The advantages of the Köver system are the spatial separation of crop roots from antagonistic strips by means of a physical barrier, which prevents migration of nematodes from the antagonistic intercrop towards the susceptible crop. In the first year of the intercropping schedule, direct positive effects of antagonistic crops on the yield of subsequent vegetables cannot be measured. Considering the fact that low initial amounts of *M. incognita* can cause substantial damage in the same year, it remains unclear whether the strong reduction of *M. incognita* in *Tagetes* and fallow plots is sufficient to prevent damage of the following tomato crop. However, the Köver system offers wider possibilities for crop rotations. Not only is the sequence [Pepper – Fallow – Tomato – Fallow] possible, but also [Pepper – Tomato – Fallow – Fallow]. In the latter crop rotation, two years of fallow could reduce nematode numbers even more, and precede a less susceptible crop such as sweet pepper on a resistant rootstock, followed by a susceptible tomato crop.

Effects on Non-Plant-Parasitic Nematodes

In March, no significant differences were apparent in non-plant-parasitic nematodes in any of the treatments. Numbers of nematodes varied from 1493–2146 individuals 100 cc^{-1} soil in antagonistic plots, to 2892 individuals 100 cc^{-1} soil in sweet pepper plots (Table 2). One week after incorporation of crop residues in November, significant differences were measured in numbers of non-plant-parasitic nematodes between treatments. The lowest numbers of non-pathogenic nematodes were found in fallow plots (601 individuals 100 cc^{-1} soil). Intermediate numbers were found in sweet pepper plots (1624 individuals 100 cc^{-1} soil) and high to very high numbers were found in *Tagetes* (4486 individuals 100 cc^{-1} soil) and 'Snooker' plots (7630 individuals 100 cc^{-1} soil). A previous analysis of trophic groups of nematodes in the organic greenhouses of this grower showed that 88% (2007) to 96% (2008) of the non-plant-pathogenic nematodes belonged to bacterivorous species, mainly consisting of *Rhabditidae* (unpublished data). These results are not surprising, as high levels of organic amendments, characteristic of organic greenhouse cultivation, stimulate saprotrophic bacterial growth, which would, in turn, promote bacterivorous nematodes. Unlike *Tagetes* and 'Snooker', fallow plots did not receive any crop residues at the end of the growing season. In sweet pepper plots, soil biota were constantly supplied with pruning residues during the growing season, which quickly decomposed and stimulated the soil microflora. The ample food supply after incorporating fresh crop residues ('Snooker' rootstocks and *Tagetes* residues) stimulated the quick development of bacterivorous nematodes, which generally have a short generation time, resulting in large final population measurements. The difference between 'Snooker' and *Tagetes* might be directly related to the amounts of organic residues incorporated: dry matter incorporated was about 2.3 times higher in 'Snooker' plots. This was partially caused by *Tagetes* plants having already died-off by the end of the growing season. For *Tagetes*, an average of 11.7 (±6.6) ton ha^{-1} fresh material or 3.7 (±2.1) ton ha^{-1} dry matter was incorporated. For the rootstock 'Snooker', an average of 39.5 (±7.9) ton ha^{-1} fresh material or 8.4 (±2.0) ton ha^{-1} dry matter was incorporated. During the growing season, 'Snooker' plots also received a continuous supply of organic material, as plants were mown every other week. Roubtsova et al. (2007) found comparable effects of broccoli residues on *Meloidogyne incognita* and non-plant-parasitic nematodes, which were almost exclusively bacterivorous *Rhabditidae* in their experiments.

Sweet Pepper Yields

Within the Köver system, several factors influenced production capacity of the individual stems of sweet pepper plants. Stems were grown either upright, or bent over the neighboring strip. Also, there was a general difference between plants or stems that grew on the shade or sun side of the planting bed. According to the growers' observation, sweet pepper plants especially suffered in the initial growth period after stems were bent over the fallow plots. This resulted in unbalanced plant growth, a drop in production and even the wilting and dying of stems on the side of the fallow strip. Analysis of yields under different growth conditions showed that in this harvesting period, bent stems growing in the shade yielded only 57% of the amount produced by bent stems in the sun, or upright growing stems (Table 3). Assuming that this production loss would take place on 25% of the total area, this would result in a total production of the Köver system of 90% compared to normal production levels. However, these production losses are typical for this specific harvesting period, and may not apply to production levels in the beginning or middle of the harvesting period.

Results of the production measurements in the Köver system combined with fallow strips during weeks 16–42 were incorrect, as one of the rows with bent/sun combination, was not harvested. Omitting the measurement of the rows with bent/sun stems, the production level of the Köver system (covering 75% of the area) was 77% of the standard production levels (on 100% of the area) (Table 4). Assuming similar production losses as in the separate measurement of stems during weeks 43–46, omitting

the bent/sun stems would lead to production levels of only 72% of standard production. This suggests that total production levels for the Köver system are far more positive than the estimations of 90% which were made above.

When combining Sweet pepper with antagonistic crops in the Köver system, very low yields (51% of standard production) were measured in plots with bent stems and a combination of sun and shade sides. The presence of antagonistic crops in this compartment led to the complete wilting and disappearance of sweet pepper stems, as both *Tagetes* and 'Snooker' out-competed sweet pepper for space and light. Surprisingly, upright stems did not suffer from the presence of antagonistic crops, and rows with sun and shade sides with only upright stems gave similar yields (102%) to control plots.

Soil Nutrient Monitoring

Initial values of nutrients were very high for both the standard production system and the Köver system, especially for nitrate (10.3 and 10.6 mmol L^{-1} for standard and Köver, respectively), calcium (9.5 and 9.1 mmol L^{-1}) and sulphate (10.7 and 9.6 mmol L^{-1}). During the growing season, the lowest nitrate concentration measured was 1.7 mmol L^{-1} and application of additional side dressings was not necessary. From August until the end of the growing season, potassium concentrations were very low (<1 mmol L^{-1}), although both control and Köver plots received a side dressing with Monterra Malt (NPK 5-1-5) in August. No deficiencies were visible in crop growth, until the start of October. The plants in the Köver system started to show signs of nitrogen deficiency and/or excess soil water. As soil concentrations of nitrogen remained high (3.6 mmol L^{-1} in the Köver system at the end of the growing season), regulation of water supply might be a focus point for future experiments.

CONCLUSIONS

A nematode-suppressing effect of antagonistic crops and fallow treatments was seen in both juveniles and juveniles hatching from eggs of *Meloidogyne incognita*. Fallow and *Tagetes patula* 'Single Gold' performed best in suppression of nematodes, and gave lower numbers of juveniles than rootstocks of *Capsicum annuum* 'Snooker'. Although the numbers of J2 hatching from eggs of *Meloidogyne* did not decline in the antagonistic crop treatments, final numbers were significantly suppressed in comparison with sweet pepper. Follow-up research should examine whether the reduction in plant-pathogenic nematodes is sufficient to prevent damage in subsequent crops.

Yields of sweet pepper in Köver plots fell dramatically when antagonistic crops were cultivated in neighboring plots. In combination with fallow plots, production losses occurred at the end of the growing season only in bent pepper stems on the shaded side of the planting bed. Although no overall measurements for the entire growing season could be made, data suggest that production losses due to inefficient use of space and light by sweet pepper plants may be confined to less than 10% of normal production. However, further research is needed to confirm the results of this study.

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Tables

Table 1. Population development of *Meloidogyne incognita* juveniles and juveniles hatching from eggs in field soil with different antagonistic treatments and sweet pepper crop.

Treatment	Juvenile development[1] (no. 100 cc^{-1} soil)		Juveniles hatching from eggs development[2] (no. 100 cc^{-1} soil)	
	March	November	March	November
Fallow	295 d[3]	23 a	193 abc	18 ab
Tagetes	287 d	31 ab	175 abc	10 a
'Snooker'	323 d	82 c	259 bc	58 ab
Sweet pepper	69 bc	330 d	63 ab	1619 c

[1]Second-stage juveniles recovered by direct extraction.
[2]Second-stage juveniles recovered from hatched egg masses, after incubation in a misitifier chamber for 28 days.
[3]Means in two adjacent columns belonging to the same variable, followed by the same letter do not differ significantly at P=0.05. Means are back transformed from logarithmic transformed data.

Table 2. Population development of non-plant-pathogenic nematodes in field soil with different antagonistic treatments and sweet pepper crop.

Treatment	Non-plant-pathogenic nematodes development (no. 100 cc^{-1} soil)	
	March	November
Fallow	1493 b[1]	601 a
Tagetes	1511 b	4486 cd
Snooker	2146 bc	7630 d
Sweet pepper	2892 bc	1624 b

[1]Means in two adjacent columns belonging to the same variable, followed by the same letter do not differ significantly at P=0.05. Means are back transformed from logarithmic transformed data.

Table 3. Yields of sweet pepper stems under different growing conditions (sun/shade and bent/upright growing stems), measured at the end of the growing season (week 43–46).

Side	Yield (kg/week/20 stems)	
	Bent stem	Upright stem
Shade side	0.562 a[1]	0.988 a
Sun side	0.987 a	0.963 a

[1]Means followed by the same letter do not differ significantly at P = 0.05. Fprob =0.068. Least significant difference is 0.3471.

Table 4. Yields (kg m^{-2}) of sweet pepper (week 16–42) in different production systems.

Treatment	Yield (kg m^{-2})	Yield (%)
Control	19.6 c[3]	100
Köver (upright stems only)[1]	20.0 c	102
Köver (without sun/bent stems)[2]	15.2 b	77
Köver (only bent stems)[1]	9.9 a	51

[1]Köver system of Sweet pepper in combination with various antagonistic crops.
[2]Köver system of Sweet pepper in combination with fallow.
[3]Means followed by the same letter do not differ significantly at P=0.05.