Suppression of the soybean cyst nematode, *Heterodera glycines*, by short-term field cultivation and soil incorporation of mung bean

Seiya CHIKAMATSU[1], Ai TAKEDA[2], Kazuhiro OHTA[3], Takeo IMURA[4], Roland N. PERRY[5] and Koki TOYOTA[1,*]

[1] *Graduate School of Bio-Applications and Systems Engineering, Tokyo University Agriculture and Technology, 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan*

[2] *Chiba Prefectural Agriculture and Forestry Research Center, 808 Daizenno, Midori, Chiba 266-0006, Japan*

[3] *Kanagawa Agricultural Technology Center Miura Peninsula Regional Office, 3002 Shimomiyada, Hasse-machi, Miura, Kanagawa 238-0111, Japan*

[4] *Nara Prefecture Agricultural Research and Development Center, 130-1 Ikenouchi, Sakurai, Nara 633-0046, Japan*

[5] *University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK*

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***** Corresponding author, e-mail: kokit@cc.tuat.ac.jp

Summary – Our previous study using pots reported that short-term growth of mung bean (*Vigna radiata*) may be useful to decrease the density of soybean cyst nematode (SCN), *Heterodera glycines*, in soil. The objective of this study was to determine whether short-term growth of mung bean and its incorporation by ploughing decreased the SCN density in infested fields. Firstly, we did pot experiments to evaluate the optimum temperature and moisture for hatching in soil. SCN hatching was stimulated at 25°C and 30°C and not at 20°C; however, it was stimulated at alternating temperature conditions between 20°C and 25°C. Soil moisture levels with pF 2.76 or less were required to stimulate SCN hatch in soil. Field experiments were done in Saitama, Kanagawa and Nara Prefectures, Japan. SCN density was reduced by nearly half even in control plots, in which mung bean was not cultivated and ploughed, in Saitama and Nara Prefectures. However, SCN density was reduced by nearly 80% or more in the three prefectures, except for one plot in Kanagawa, and the soil temperature and moisture conditions were kept around 20°C to 30°C and at < pF 2.8. Increase in yield of green soybean by SCN control was estimated 350 kg $(1000\ m)^{-2}$. Overall, the present study revealed that short-term field cultivation of mung bean and ploughing was a profitable method to decrease SCN density in infested fields and thereby to increase yield of green soybean.

Keywords - field experiment, green manure, Japan, real-time PCR, soil moisture, soil temperature, *Vigna radiata*.

Soybean (*Glycine max* (L.) Merr) is a major and important crop around the world. However, yield losses are caused by many pests, *e.g*., soil fungi *Rhizoctonia solani* Kuhn (Ajayi-Oyetunde & Bradley, 2018) and *Peronospora manshurica* Syd (Da Silva *et al*., 2016), insects such as soybean aphid (*Aphis glycines* Matsumura) (Tilmon *et al*., 2011) and bean leaf beetle (*Cerotoma trifurcate* Forster) (Smelser & Pedigo, 1992), and soybean cyst nematodes (SCN), *Heterodera glycines* Ichinohe. In the USA, yield losses due to SCN were 1.9 million tonnes in the major production regions in 2005, and were greater than losses caused by any other disease (Wrather & Koenning, 2006). In Japan, SCN mainly causes yield losses of soybean and green soybean, which is immature soybean. The damage has spread not only in Hokkaido (Aiba, 2001), which is the main prefecture for soybean production, but also in urban areas such as Chiba and Saitama (Chikamatsu *et al*., 2017), which are the main prefectures for green soybean production.

To control nematodes including SCN, there are several biological and non-biological methods. As non-biological control, nematicides are effective but economic and environmental issues have increasingly limited their use (Rich *et al*., 2004). Of the biological control methods, resistant soybean cultivars have reduced the density of SCN and increased soybean yields (Chen, 2007), and crop rotation with non-host crops reduced the density of SCN (Sasser & Uzzell, 1991). However, in urban areas in Japan, it is difficult to adopt crop rotation and to use nematicides, especially fumigant types, because the land prices are high and fields and residential zones are close. Thus, we need to develop environmentally friendly management strategies for SCN that have limited negative environmental impact (Grabau & Chen, 2014).

The invasive second-stage juveniles (J2) of *H. glycines* hatch more in response to stimulation by exudates from host roots. If hatch can be caused in the absence of hosts the J2 will die and the population will decrease, providing a good control strategy. Many studies have reported on hatch and density reduction of SCN. For example, glycinoeclepin A, which was extracted and identified from dried roots of kidney beans (*Phaseolus vulgaris* L.) (Masamune *et al*., 1982), promotes hatch of SCN and may be effective in reducing the density of SCN, but is expensive to synthesise and is not feasible for field application (Shiina *et al*., 2010). Sunn hemp (*Crotalaria juncea* L.) and red clover (*Trifolium pratense* L.) are known to stimulate hatch and thereby to reduce SCN populations (Kushida *et al*., 2002, 2003). However, they often do not match the crop

system, particularly in urban areas, because it takes several months to cultivate these green manures. Recently, attention on SCN control has increased. Extracts from camellia (*Camellia oleifera* Abel) and *Paeonia* sp. inhibited hatch of SCN (Wen *et al*., 2019). The density of SCN in soil was decreased by growing winter camelina (*Camelina sativa* L. Cranz) for 35 days in a growth chamber (Acharya *et al*., 2019), or growing cereal rye (*Secale cereale* L.) and rapeseed (*Brassica napus* L.) for several months in fields and their subsequent incorporation into the soil (Wen *et al*., 2017).

We previously reported that incorporation of bean sprout residue or its water extract to soil stimulated hatch of J2 and decreased SCN density (Toyota *et al*., 2013; Ito *et al*., 2015). However, it is difficult to apply the residue of bean sprout (*Vigna radiata* (L.) Wilczek) to fields because the residue contains more than 90% water and the application rate (5,000 kg (1000 m)$^{-2}$ is too high. Therefore, we developed an alternative method using mung bean, which is the raw material of bean sprouts. In this method, mung bean was sown in soil, grown for 2 to 4 weeks, and then incorporated into the soil. The benefit of mung bean growth and its incorporation requires only a short-term growth period, while other green manures require more than 1 month (Wen *et al*., 2017; Acharya *et al*., 2019; Kushida *et al*., 2002, 2003). By this process, hatch of J2 was stimulated and SCN density was decreased (Chikamatsu *et al*., 2017). All these experiments were done in pots. Thus, the objective of this study was to evaluate the effects of growing and incorporating mung bean on SCN density in SCN infested fields.

Materials and methods

Soils

Four soils naturally infested with SCN were collected from farmlands and their basic data are shown in Table 1. Soil moisture release curves were constructed using a Multi-Fold pF Meter (Daiki Rika Kogyo Co., Ltd). For S and K soils, soil passed through a 5 mm sieve was packed tightly in 100-ml cores in three and seven replicates, respectively. For N soil, a 100 cc core was collected from a field (Supplementary Fig. S1).

To use a moisture sensor (5TM, Decagon Device) in fields, we evaluated the

relationship between values of 5TM (m^3 m^{-3}) and the actual soil moisture contents, that were measured by oven-drying. Soil (800 g) was mixed with 30 to 120 ml of water and tightly packed into a pot with 100 cm^2 surface area and the moisture content was measured with 5TM operated by a measuring instrument (ProCheck, Decagon Device). Then, 20 g of the soil was collected and dried at 105˚C overnight to measure the absolute water content. Water was re-added to the soil remaining in the pot. The soil was mixed well and tightly packed in the pot and the moisture content was measured with 5TM. This process was repeated several times for each soil. The relationship between values of 5TM and soil moisture content is shown in Supplementary Figure S2.

In the following pots and field experiments, soil moisture content was measured with 5TM and its pF value was calculated according to the calibration curves of Supplementary Figures S1 and S2.

Effect of temperature on SCN hatching

The optimal temperature range for hatching was determined. Firstly, to prepare hatching stimulus, 12 to 15 seeds (about 8 to 9 g) of mung bean seeds (‘Green Mappe’, Nakahara Seed Co.) were sown in a plastic cup with a 9 cm diameter containing C soil. A total of nine cups were grown in a growth chamber at 25°C with 16 h : 8 h light : dark cycle for 1 week. All the mung bean plants including the roots were collected from the pots, cut into 3 cm pieces with scissors, and shaken in 500 ml distilled water at 80 rpm for 6 h. The resulting solution was filter-sterilised (0.2 µm pore size) and stored at 4°C until use.

Air-dried C soil (100 g) was put in four replicates in a plastic cup with a 9 cm diameter and 30 ml of the mung bean solution prepared above was added. The pots were incubated at 20°C, 25°C, and 30°C and 20 g soil was taken in duplicate after 0, 2, 4, 7 and 11 days. To measure the number of hatched SCN J2, nematodes were extracted with the Baermann funnel extraction method (3 days incubation at 25°C).

Next, to simulate actual temperature fluctuations in field, we evaluated the effect of temperature change between 20°C and 25°C. Pots containing 100 g of air-dried C soil were incubated in triplicate: *i*) at 20°C for 24 h; *ii*) at 20°C for 12h and at 25°C for 12 h; and *iii*) at 25°C for 24 h. After 1, 3, 6, 10 and 14 days, 20 g of soil was taken in duplicate

from the pots for the Baermann funnel extraction to observe the number of hatched J2 using a stereoscopic microscope. In both experiments, pots were covered with a plastic bag to avoid water evaporation during the incubation period.

Effect of soil moisture on SCN hatching

S soil (36 kg dry soil basis) was put into a container with a volume of 35 l. Half of the container was sown with mung bean (11 seeds, corresponding to 18 g seed m^{-2}) and the other half was not sown. The mung bean was grown for 18 days in a glasshouse. The soil moisture was pF 1.9 at sowing and decreased to pF 3.0 after 18 days. Soil was separately collected from the non-mung bean part (control) and mung bean part (MB) and mixed well to homogenise the density of SCN. Then, the mixed soil was divided into 18 bags (1 kg fresh soil (= 740 g of dry soil each bag)). All the mung bean plants, including roots, were gently removed from the container and cut into 1 cm pieces with scissors. Since the plant sample weighed 11.2 g, 0.62 g (11.2 g divided by 18 bags) was added to each bag of MB soil. The control and MB soils were mixed well after adding different amounts of water to adjust their moisture content to between pF 2.0 and pF 3.0. Six water levels were prepared in three replicates. Each soil in plastic bags was put into a pot with 100 cm^2 surface area and then the soil moisture levels were measured with 5TM. The pots were wrapped to avoid moisture evaporation and put on a heating mat. At 0 and 4 days, the soil was mixed well and then 20 g was taken in duplicate for the Baermann funnel extraction method (2 to 3 days incubation at room temperature) to determine the number of hatched J2 of SCN using a stereoscopic microscope. The average soil temperature during 4 days was 23.6˚C.

Field experiments

Saitama Prefecture

In a field covered with plastic film (5 m × 50 m) in Saitama Prefecture, we conducted the following experiment consisting of five treatments: 1, control (no mung bean and no irrigation); 2, MB9 (sowing rate of mung bean was 9 g m^{-2} , an amount recommended by

Chikamatsu *et al*. (2017), and irrigation was done only at sowing); 3, MB9i (sowing rate, 9 g m^{-2} and irrigation was done both at sowing and ploughing); 4, MB18i (sowing rate, 18 g m^{-2} and two times irrigation); and 5, MB27i (sowing rate, 27 g m^{-2} and two times irrigation). The treatment size of control and MB9 were each 25 m^2 (5 m × 5 m), and MB9i, MB18i and MB27i were each 65 m^2 (13 m × 5 m). Nine plots (0.36 m^2, 0.6 m×0.6 m) were set up at even intervals in each treatment. On 6 July 2018 (before sowing), tillage was done with a tractor and then soil in each plot was manually thoroughly mixed with a shovel. Five soil cores (0-20 cm) were sampled from each plot with a root auger and a composite sample was made for each plot. Mung bean seeds were sown and the soil surface was raked to stimulate germination. Watering was done using irrigation tubes at 20 l m^{-2} to the whole field except for the control treatment. On 7, 8 and 9 July 2018, watering was done at a rate of 20 l m^{-2} to the whole field except for the control treatment, and in 10 July 2018, watering was done at 10 l m^{-2} to the whole field except for the control treatment, in order to stimulate mung bean growth. On 26 July 2018, plant densities of mung bean were measured in each plot and then the mung bean plants were ploughed with a tractor. To stimulate SCN hatch, treatments MB9i, MB18i, and MB27i were irrigated at a rate of 40 l m^{-2}. Summary of the managements is shown in Table 2. In August 25, 2018, soil samples were taken to measure the SCN density using real-time PCR, as described below. Sensors (5TM) were inserted in duplicate to a depth of 10 cm to 15 cm in MB9 and MB9i and soil temperature and moisture were measured at intervals of 1 h with a data logger (Em50, Decagon).

Kanagawa Prefecture

Four naturally infested fields were used for the field experiment. Three plots (1 m^2, 1 m × 1 m) were set up in each field (Table 2). Before sowing mung bean seeds, the soil was collected from five points in each plot and a composite sample made for each plot. Two to five days later, seeds were sown at 9 g m^{-2} in each field. After growing for 25 to 44 days, mung bean was ploughed into each field with a tractor and soil was collected from each plot. Two weeks after ploughing, soil was again collected from each plot. The management schedule is shown in Supplementary Table S1. Sensors (5TM) were inserted

to a depth of 10 cm to 15 cm in each field and soil temperature and moisture were measured at intervals of 1 h with a data logger (Em50, Decagon).

Nara Prefecture

In Saitama and Kanagawa Prefectures, green soybean is generally cultivated from March to July. Thus, mung bean was grown in July to September after harvest of green soybean in the prefectures. The maximum temperature for SCN hatch is 25˚C (Ito *et al*., 2015) and therefore July to September is a suitable period for SCN hatch. By contrast, green soybean is generally cultivated from July to October in Nara Prefecture. An available period for mung bean cultivation is May, when soil temperature is generally lower than 25˚C. To increase the soil temperature, mulching was used in Nara Prefecture. A naturally infested field was divided into two parts; control (no mung bean) and mung bean part (MB). In each part, three plots (1 m^2, 1 m × 1 m) were set up. In the mung bean part, mung bean seeds were sown at 9 g m^{-2} on 22 May. On 4 June 2018, mung bean plants were ploughed by a tractor. After ploughing, the mung bean part was mulched with black polyethylene films (Table 2). Soil was collected from five points in each plot on sowing, 5 days before ploughing, the day after ploughing, and three weeks after ploughing. Sensors (5TM) were inserted to a depth of 10 cm to 15 cm in a control plot and in a mung bean plot and soil temperature and moisture were recorded at intervals of 1 h with a data logger (Em50, Decagon).

QUANTIFICATION OF THE DENSITY OF *HETERODERA GLYCINES*

The densities of SCN in soils were estimated using the real-time PCR method (Toyota *et al*., 2013) with some modifications. Composite samples from five points in each plot were passed through a 2-mm sieve to make homogeneous samples and oven-dried at 60˚C for 1 to 2 days. Ten g of the dried soils were taken in three replicates from each plot and pulverised into powder for 2 min at 45 m s^{-1} in a ball-mill (Fast Prep; MP Biomedicals). The total of 30 g was combined for each plot and then used for DNA extraction. DNA was extracted in duplicate from 5 g using a modified method reported by Cheng *et al*. (2018). Briefly, DNA was extracted from the ball-milled soils with

phosphate buffer and purified with a commercially available column. Purified DNA extracts were used as templates in real-time PCR after ten-fold dilution. Real-time PCR was performed using a Step One Real-Time PCR System (Life Technologies Japan) in a final volume of 10 µl containing 5 µl of a Fast SYBR Green Master Mix (Life Technologies Japan), 5 mM of each primer (SCNnew-f (ITS1, 5'- CTG CAC ATG TGA AAG CCT GTG TA-3') and SCNnew-r (ITS1, 5'-GAG CGT GCA TCC CAC ATT G-3')) (Shirai & Toyota, 2019) and 2 µl of template DNA under the manufacturer's recommended conditions (95°C for 10 s, (95°C for 5 s and 60°C for 20 s) × 40 cycles). Ct values (y) obtained with the primer set were converted to the densities (x: $\log_{10}$ eggs equivalent (20 g dried soil)$^{-1}$) of SCN using the calibration curve (y = –2.82x + 34.5) (Shirai & Toyota, 2019).

Statistical analysis

Significant differences among mean values were analysed by Fisher's test at 5% with one-way ANOVA using Excel Statistics (Social Survey Research Information) software. Homogeneity of variances was testing using Levene's test and normal distribution of residuals was assessed using Statcel 3rd ed (OMS publication). Suitable transformations (log: Fig. 1A and Supplementary Figs S3, S4), square root: Fig. 1B) were used when data did not meet assumptions.

Results

Effect of temperature on SCN hatching

There was no significant difference ($P < 0.05$) in the 20°C treatment during the incubation period, indicating no hatch at 20°C (Fig. 1A). On the other hand, the number of SCN J2 increased in the 25°C and 30°C treatments and reached a maximum on day 4, indicating that SCN hatched both at 25°C and 30°C. The increasing numbers of SCN J2 from 0 to 4 days were higher at 25°C (45 J2 (20 g soil)$^{-1}$) than at 30°C (33 J2 (20 g soil)$^{-1}$), although there was no significant ($P = 0.56$) difference.

Under fluctuating temperature conditions between 20°C and 25°C, the maximum number of SCN J2 that hatched was similar to the numbers at constant 25°C, although the period to reach the maximum number was longer (Fig. 1B), being 3 days at 25°C and 10 days at fluctuating temperatures.

Effect of soil moisture on SCN hatching

There was no difference in the number of J2 that hatched in control among soils with different pF values. By contrast, the number of J2 in MB increased with decrease in pF, *i.e.*, with higher moisture content. There was no significant difference ($P = 0.21$) in soil with > pF 2.9 between control and MB. However, the number of J2 was significantly higher ($P < 0.05$) in MB than in control, when soil moisture was < pF 2.76 (Fig. 2).

Field experiments

Saitama Prefecture

When mung bean was grown for 20 days and ploughed, the above-ground biomass was 587 g m^{-2} for MB9, 736 g m^{-2} for MB9i, 1,310 g m^{-2} for MB18i and 1,706 g m^{-2} for MB27i at fresh weight (Supplementary Fig. S3). It was significantly higher ($P < 0.05$) in MB18i and MB27i than in MB9 and MB9i, and there was no significant difference between MB18i and MB27i ($P = 0.07$).

The density of SCN decreased by nearly 50% in the control between before sowing and 30 days after mung bean ploughing (Fig. 3). By contrast, it decreased by nearly 80% in all MB treatments. There was significant difference ($P < 0.05$) between control and all MB treatments.

Immediately after sowing mung beans, MB9, MB9i, MB18i and MB27i were irrigated between 6 to 10 July to enhance mung bean germination. During this period, the moisture level in MB9 ranged between pF2.2 and pF2.4, and in MB9i between pF2.3 and pF2.8. The soil moisture 2 days after ploughing ranged from pF2.0 to pF2.4 in MB9i for irrigation and gradually deceased (Fig. 4). On the other hand, the soil moisture in MB9 increased to a similar level in MB9i 2 days after ploughing. The average soil temperature

for 30 days after ploughing was 30.5°C for MB9 and 28.5°C for MB9i (Supplementary Fig. S4).

Kanagawa prefecture

The ratio of SCN density 2 weeks after ploughing to that before sowing was less than 20% in two plots in fields 1 and 2 and in all plots in field 4 (Fig. 5). In field 3, SCN density decreased in two plots; however, it increased from 171 to 436 eggs equivalent 20 g^{-1} soil in one plot. SCN density was significantly lower ($P < 0.05$) 2 weeks after ploughing than before sowing in fields 1, 2, and 4, except for field 3 ($P < 0.05$).

The average soil moisture and temperature for 14 days after mung bean ploughing were < pF 2.8 and nearly 25°C (Supplementary Table S2).

Nara Prefecture

The initial density of SCN, 5 days before ploughing, was similar between the control and MB. The ratio of SCN density 22 days after ploughing to that at five days before ploughing was 42% in control, while it was only 5% in MB, and these values were significantly different ($P < 0.05$) (Fig. 6). From two days after ploughing, soil moisture increased in MB and consistently remained at < pF 2.8, while in the control it sometimes dried to near pF 2.8 (Supplementary Fig. S5). Daily changes in soil temperature after ploughing were smaller in MB than in the control and the soil temperatures remained higher in MB than in the control (Supplementary Fig. S6). The average soil moisture and temperature were higher in MB (23.1°C and pF 1.8) than in control (21.3°C and pF 2.2).

Discussion

We previously reported that short-term growth and incorporation of mung bean in pots stimulated SCN hatching and then decreased SCN density (Chikamatsu *et al*., 2017). This study was conducted to show whether short-term cultivation of mung bean and its ploughing in fields decreased the population density of SCN in the soil. Two climatic factors are mainly involved in the efficacy to decrease SCN density: temperature and

moisture (Chikamatsu *et al*., 2017). In previous studies, different optimal temperatures were reported for SCN hatching. It was between 25°C and 30°C in studies by Okada (1971, 1977), whilst Ito *et al*. (2015) reported an optimum of 25°C, with only a few SCN hatching at 30°C. When mung bean cultivation is implemented in fields, it is necessary to consider a cropping period based on temperature. Therefore, the optimum temperature was again evaluated in this study. The results showed that hatching was stimulated at 25°C and 30°C, but not at 20°C. However, hatching was stimulated by alternating temperature conditions at 20°C and 25°C, although hatching was delayed compared with that at a constant temperature of 25°C. These results suggest that periods of temperatures of 25°C and 30°C are most favourable for stimulating SCN hatch, but that hatch stimulation may occur even in much cooler temperature conditions, *e.g*., night time temperatures of 20°C, with day time temperatures reaching 25°C.

Chikamatsu *et al*. (2017) showed the importance for hatch stimulation of ploughing, rather than continuous growth of mung beans, and the soil moisture content at ploughing. Wallace (1954) showed that SCN J2 rarely hatch in soil with a moisture level of pF 3.0. However, it was still not known how soil moisture level affects hatching after mung bean incorporation. The present study suggested that a soil moisture level with least pF 2.76 or less was required to stimulate SCN hatch.

In the present study, pot experiments revealed the optimal soil temperature and moisture conditions, so we tested the biocontrol method for SCN consisting of short-term mung bean cultivation and its subsequent ploughing of mung bean in SCN infested fields in Saitama, Kanagawa, and Nara Prefectures. The density of SCN decreased even in the control soil not sown with mung bean in both Saitama and Nara Prefectures. According to Perry (2002), SCN hatch moderately in water when soil temperature conditions are suitable for hatching. Thus, it was considered that some SCN hatched without the effect of mung bean and then, in the absence of host plants, starved to death. However, the density of SCN in soil sown with mung bean decreased much more than that in the control soil. The density of SCN decreased even in MB9, which was not irrigated after mung bean ploughing and was over pF 3.0 at ploughing, indicating that few J2 hatch in dry moisture conditions of pF 2.8. There was heavy rainfall of 48 mm 2 days after ploughing. The experiment using field soil was in a glasshouse covered with plastic film and there was no direct rain on the soil; however, soil moisture of MB9 was increased by water

supply through underground water and reached a similar level 6 days after ploughing to that in MB9i which was irrigated after ploughing. Thus, it was considered that the increased soil moisture in MB9 may stimulate SCN hatch and thereby decreased the SCN density.

In Kanagawa Prefecture, the density of SCN was decreased by more than 80% by growing mung bean and ploughing, except for Field 3. In Kanagawa, no control plot was set up and it was impossible to separate mung bean effect from natural phenomena. However, the soil moisture and temperature for 14 days after mung bean ploughing showed values closer to the optimal conditions (pF 2.0 to 2.6, soil temperature 24 to 25˚C), suggesting that mung bean cultivation and ploughing may stimulate SCN hatch and thereby decrease SCN density. The reason why SCN density increased in one plot in Field 3 was unknown.

In Nara prefecture, the density of SCN decreased to almost zero in mung bean plots. The density reduction was greater than that in the Saitama and Kanagawa Prefectures. This was considered to be due to the black mulch covering the plot in which mung bean was grown and ploughed, which maintained the high water content (pF 2.1 or less, from 1 to 22 days after ploughing) and appropriate hatching temperatures (23.1˚C, for 22 days after ploughing). In Saitama Prefecture, soil moisture exceeded pF 2.8 for nearly a half of the 30 days after mung bean ploughing, indicating less favourable conditions for SCN hatch. Thus, the remaining population of SCN was higher in Saitama Prefecture than in Nara Prefecture.

In Saitama Prefecture, the effect of sowing rate of mung bean was evaluated, ranging from 9 g m^{-2} to 27 g m^{-2} and there was no apparent change in the decreasing effect on SCN density. This result suggests that a sowing rate of 9 g m^{-2} was appropriate. Profitability in 1,000 m^{-2} was estimated based on the following: an expected increase in yield of green soybean due to a 80% reduction of SCN density was 350 kg, based on Ito *et al*. (2017) and the following assumption that the initial density of 2,000 eggs equivalent (20 g soil) $^{-1}$, and yield of green soybean of 1,470 kg (1,000 m)$^{-2}$, green soybean price in Saitama Prefecture of 500 yen kg^{-1}, fuel costs for tillage (×2) (3,642 yen), labour cost: 1000 yen h^{-1}, seeds (7,200 yen for 9 kg), water irrigation and associated tube cost (9,920 yen). From these data, the yield increase by reducing SCN density was determined to be

more than the necessary costs, and the profit was calculated to be 153,000 yen (1,400 US$) (1,000 m)$^{-2}$.

Conclusions

The present study found in pot experiments that hatching of *H. glycines* was stimulated under temperature conditions of 20°C to 30°C and moisture conditions of < pF 2.8. We demonstrated in field experiments that short-term field cultivation of mung bean and ploughing was a profitable method to decrease SCN density in infested fields and thereby increase yield of green soybean.

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Table 1. Basic data of the soils used in this study.

Soil	Location	Soil taxonomy	Sand (%)	Silt (%)	Clay (%)	Soil texture
C soil	35º18’ N, 139º53’ E Chiba Pref.	Haplic andosol	38	59	3	Silty loam
S soil	35º50’ N, 139º51’ E Saitama	Low humic andosol	42	28	30	Light clay
K soil	35º12’ N, 139º39’ E Kanagawa	Haplic andosol	19	41	40	Light clay
N soil	34º29’ N, 135º55’ E Nara Pref.	Grey lowland soil	52	22	26	Light clay

Table 2. Summary of the field experiments.

Site	No. of field	Treatment	Sowing rate (g m^{-2}) of mung bean	Irrigation		Plastic mulch	Soil sampling	
				after sowing	after incorporation		Plot	Plot size (m^{-2})
Saitama	1	Control	0	×	×	×	9	0.36
		MB9	9	○[1]	×	×	9	0.36
		MB9i	9	○[1]	○[2]	×	9	0.36
		MB18i	18	○[1]	○[2]	×	9	0.36
		MB27i	27	○[1]	○[2]	×	9	0.36
Kanagawa	4	MB9	9	△	△	×	3	1
Nara	1	Control	0	△	△	○[3]	3	1
		MB	9	△	△	×	3	1

△: In open fields, irrigation was not conducted.

[1] After sowing, watering was done using irrigation tubes at a rate of 20 L m^{-2} every day for 3 days and at a rate of 10 L m^{-2} 5 days after.

[2] After incorporation of mung bean by a tractor, irrigation at a rate of 40 L m^{-2} was done in these treatments.

[3] After incorporation of mung bean by a tractor, mung bean plot was mulched with black polyethylene films

Control = no mung bean sowing; MB9 = sowing rate of mung bean was 9 g m^{-2} and irrigation was done only at sowing; MB9i = sowing rate (9 g m^{-2}) and irrigation were done both at sowing and ploughing; MB18i = sowing rate (18 g m^{-2}) and irrigation (×2); and MB27i = sowing rate (27 g m^{-2}) and irrigation (×2).

Figure legends

Fig. 1. Effect of constant (A) and alternating (B) temperature conditions on the number of *Heterodera glycines* second-stage juveniles in Chiba soil supplemented with mung bean solution. Values in A = mean ± SD (n = 3); values in B = mean ± SD (n = 4).

Fig. 2. Relationships between soil moisture contents and the number of *Heterodera glycines* second-stage juveniles (J2) in Saitama soil adjusted with different moisture levels, with incorporation of mung bean plants and incubation for 4 days. Control = soils with no mung bean added; MB = soils with mung bean added. Each value is the mean of two replicates.

Fig. 3. Effect of mung bean cultivation for 20 days and its ploughing on the density of *Heterodera glycines* in soil in a field at Saitama Prefecture 30 days after ploughing. Different letters indicate significant differences at $P < 0.05$. control = no mung bean sowing; MB9 = sowing rate of mung bean was 9 g m^{-2} and irrigation was done only at sowing; MB9i = sowing rate (9 g m^{-2}) and irrigation was done both at sowing and ploughing; MB18i = sowing rate (18 g m^{-2}) and irrigation (×2); and MB27i = sowing rate (27 g m^{-2}) and irrigation (×2). Centre lines of boxes are median values and whiskers show 1.5 × IQR (Interquartile Range) (n = 9). × mean outliers. *The relative value of the *H. glycines* density after 30 days of ploughing to that at sowing (100%). When the density increased from sowing to after ploughing, the value is shown as 100 (one replicate in control and MB27i).

Fig. 4. Temporal changes in soil moisture of MB9 and MB9i at depths of 10 cm to 15 cm. A line for pF 2.8 is shown by a horizontal black line. MB9 = sowing rate of mung bean (9 g m^{-2}) and irrigation were done only at sowing; MB9i = sowing rate (9 g m^{-2}) and irrigation were done both at sowing and ploughing.

Fig. 5. Effect of mung bean cultivation and its ploughing on the density of *Heterodera glycines* in four different fields in Kanagawa prefecture. Each field had three replicate plots and values in each plot are shown. Each value is mean of two replicates. *The relative value of the SCN density after 14 days of ploughing to that at sowing (100%). When the density increased from sowing to after ploughing, the value is shown as 100 (one replicate in Field Number 3).

Fig. 6. Effect of mung bean cultivation and its ploughing on the density of *Heterodera glycines* in a field in Nara Prefecture. Values are mean ± SD (n = 3). Control = no mung bean; MB = mung bean was sown at a rate of 9 g m^{-2}. *The relative value of the SCN density after 22 days of ploughing to that 5 days before sowing (100%). Different letters indicate significant difference at $P < 0.05$.

Supplementary Fig. S1. Relationships between soil moisture content and pF values in (a); Saitama, (b); Kanagawa, (c); Nara-soils. Equations show their relationship (x = pF value, y = soil moisture content) between two points in pF values.

Supplementary Fig. S2. Relationships between soil moisture content as measured by oven-drying and those measured with the moisture sensor 5TM.

Supplementary Fig. S3. Aboveground biomass of mung bean plants grown for 20 days after sowing. Different letters indicate significant differences at $P < 0.05$. MB9 = sowing rate of mung bean was 9 g m^{-2} and irrigation was done only at sowing; MB9i = sowing rate, 9 g m^{-2} and irrigation was done both at sowing and ploughing; MB18i = sowing rate; 18 g m^{-2} and irrigation (×2); and MB27i = sowing rate, 27 g m^{-2} and irrigation (×2).

Supplementary Fig. S4. Temporal changes in soil temperature on MB9 and MB9i at depths of 10 cm to 15 cm. MB9 = sowing rate of mung bean was 9 g m^{-2}, and irrigation

was done only at sowing; MB9i = sowing rate was 9 g m^{-2} and irrigation was done both at sowing and ploughing.

Supplementary Fig. S5. Temporal changes in soil moisture in control (no mung bean) and MB (mung bean sown at a rate of 9 g m^{-2}) at a soil depth of 10 cm to 15 cm.

Supplementary Fig. S6. Temporal changes in soil temperature in control (no mung bean) and MB (mung bean sown at a rate of 9 g m^{-2}) at a soil depth of 10 cm to 15 cm.