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Folsomia hidakana (Collembola) prevents damping-off disease in cabbage and Chinese cabbage by *Rhizoctonia solani*

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Summary

This study demonstrates a method for propagating the Collembola species, *Folsomia hidakana* Uchida et Tamura and the usefulness of this species in preventing damping-off in cabbage (*Brassica oleracea* var. *capitata*) and Chinese cabbage (*Brassica rape* var. *pekinensis*) in a greenhouse. A new rearing method using 'Koji', boiled rice on which *Aspergillus oryzae* (Ahlburg) Cohn flourished, was developed for *F. hidakana* allowing population doubling in about 8.3 days. An experiment was carried out using a lysimeter in a greenhouse to evaluate the efficacy of *F. hidakana* in preventing damping-off disease in cabbage and Chinese cabbage caused by *Rhizoctonia solani*. The treatments were : 1) Control, 2) *R. solani* (soil inoculated with *R. solani*), and 3) *R. solani* + *F. hidakana* (120,000 individuals m^{-2} to the soil inoculated with *R. solani*). Two weeks after the plots were set up, the germination of three cultivars of cabbage and three of Chinese cabbage was tested. The severity of damping-off was reduced by 82–87 % in the *R. solani* + *F. hidakana* plot, in comparison to that of the *R. solani* plot, regardless of the plant cultivars and species. *R. solani* was not isolated from washed plant roots in the *R. solani* + *F. hidakana* plot, suggesting that *F. hidakana* suppressed the disease by feeding on *R. solani*.

Key words: *Folsomia hidakana*, greenhouse, cabbage, Chinese cabbage, damping-off, *Rhizoctonia solani*

Introduction

Many recent studies have focussed on the biological control of plant diseases for the low-input and sustainable agriculture in Japan (Tsuchiya 1997). Furthermore, it is well known that soil animals play important roles in changing the microbial, chemical, and physical properties of soils in agroecosystem (e.g. Lee & Pankhurst 1992). For instance, it has been demonstrated that soil fauna, in particular Collembola contribute to suppression of root diseases (Curl et al. 1979; Lootsma and Scholte 1997b).

Our laboratory has studied Collembola for their application in the control of soil-borne diseases. *Folsomia hidakana* Uchida et Tamura, *Proisotoma minuta* (Tullberg), *Sinella curviseta* Brook, *Lepidocyrtus cyaneus* Tullberg, and *Hypogastrura communis* (Folsom) were collected from farmland in the Fukushima Prefecture in 1990. These species were then tested to determine whether they eat fungal pathogens and/or suppress vegetable diseases caused by *Rhizoctonia solani* Kühn, *Fusarium oxysporum* f. sp. *cucumerinum* Owen

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and *Rosellinia necatrix* Prillieux (Matsuzaki 1991; Nakamura et al. 1991a, b; Nakamura et al. 1992; Shiraishi et al. 1993; Shiraishi & Nakamura 1994).

We propose the use of *F. hidakana* for biological control of *R. solani* for the following reasons: 1) *F. hidakana* most effectively suppressed damping-off in cabbage and Chinese cabbage of the Collembola species tested (Nakamura et al. 1991b), 2) *F. hidakana* was easily propagated on artificial food, 3) *F. hidakana* actively grazed on fungal hyphae, and 4) short-term activity of *F. hidakana* was expected to be sufficient for the suppression of *R. solani*.

This study demonstrates a method for propagating *F. hidakana* and the usefulness of *F. hidakana* in preventing damping-off in cabbage (*Brassica oleracea* var. *capitata*) and Chinese cabbage (*Brassica rape* var. *pekinensis*) in a greenhouse.

Materials and Methods

Method for propagating *Folsomia hidakana*

F. hidakana used in this study was collected from a field near our institute. The animals were reared in standard petri dishes in which *R. solani* grown on potato-dextrose agar served as the diet (Nakamura et al. 1991a). Animal numbers increased to only a few hundred individuals using this culturing method. In order to improve this method, animals were reared in polycarbonate containers (8 cm diameter, 7.5 cm height) with lids containing a hole (Ø 10 mm) sealed with a membrane filter. Each container was supplied with 45 ml of deionized water and 100 g of air-dried commercial soil (Kureha Inc., Japan). The soil is processed to form aggregates > 2 mm in diameter. The containers were autoclaved at 121 °C for 20 minutes and after cooling, a piece of paper filter (4 cm in diameter) was placed on the soil surface. Five g of 'Koji' was placed on the filter paper as food for *F. hidakana*. 'Koji', a boiled rice inoculated with *Aspergillus oryzae* (Ahlburg) Cohn and cultured for several days, is a Japanese traditional food.

Initially, five hundred *F. hidakana* adults were placed in each container and the lid was sealed with vinyl tape. The incubation continued for six weeks at 23 °C, and the 'Koji' was changed every two weeks. The propagated *F. hidakana* were extracted from the soil container with Tullgren funnels and placed into a 200 ml vessel containing a mixture of solidified gypsum and activated carbonized charcoal on the vessel bottom (Goto 1961). The vessel was subsequently used for the following experiments.

Optimization of experimental conditions for a plot trial

R. solani (AG-4; anastomosis group, cf. Parmeter et al. 1969) was aseptically cultured in 200 ml vessels using a medium of barley-grain powder (10 g), air-dried Andosol collected from the lysimeter in a greenhouse (90 g), potato-dextrose broth (DIFCO Inc., USA) (0.84 g), and deionized water (35 ml). The containers were inoculated with *R. solani* by cutting a piece of agar (1.5 × 1.5 cm) from the edge of an actively growing colony *R. solani*, which was cultured at 25 °C for four weeks.

To determine the amount of *R. solani* inoculant necessary for establishment of the disease, a pot experiment was performed in triplicate. The 200 ml containers were supplied with 35 ml of deionized water and 100 g of air-dried Andosol after mixing with 0, 1, 3, 5, 10, 20 and 30 g of the *R. solani* inoculant. After two weeks, seven seeds of cabbage ('Greenball' Sakata Seed, Japan) or Chinese cabbage ('Muso' Takii Seed, Japan) were sown. All pots were kept at 25 °C and a day/night regime of 12h/12h. Two weeks after sowing, germinated seedlings with and without symptoms of the disease were counted.

To determine the activation time of *F. hidakana* before sowing, containers with 5 g of the *R. solani* inoculant and 300 individuals of *F. hidakana* were used. Cabbage ('Taibyo-rokuju-nichi' Sakata Seed, Japan) or Chinese cabbage 'Muso' was sown 0, 3, 5, 7,10, 15, 20 and 30 days after the preparation of the containers.

Seedling experiments under greenhouse conditions

A 2.7 × 2.7 m lysimeter was used in a greenhouse. The lysimeter was filled with a low-humic Andosol to a depth of 1 m. The greenhouse was kept at a day/night regime of 11h/13h and 25/20 °C with a humidity of 70%. The soil was divided into 9 subplots (0.9 × 0.9 m) to a depth of 20 cm with plastic boards. The height of the board above the ground was 10 cm.

The following experimental plots were prepared in triplicate: 1) Control, 2) with *R. solani*, and 3) with *R. solani* + *F. hidakana*. All plots received fresh barley-grain powder and potato-dextrose broth. The *R. solani* and *R. solani* + *F. hidakana* plots received the *R. solani* inoculant at 2.3 kg subplot^{-1}, equivalent to 5 % of the dry soil of the upper 10 cm. The *R. solani* inoculant was mixed well with the surface soil to a 10 cm depth. Following the inoculation with *R. solani*, about 1.0×10^5 individuals of *F. hidakana* were transferred to the soil surface of the *R. solani* + *F. hidakana* plot.

Plots were maintained for two weeks before sowing cabbage and Chinese cabbage. Eight 90 cm lines were drawn on each subplot, which was further divided by three 30 cm sub-lines that totaled 24 sublines. Three cultivars of cabbage 'Kinkei No. 201', 'Tyuwase No. 2' and 'Greenball' (all seeds; Sakata Seed, Japan) and three cultivars of Chinese cabbage 'Muso', 'Kenshun' and 'Taibyo-rokuju-nichi' (all seeds; Takii Seed, Japan) were tested in four replicates in the subplots. Fifty seeds of each cultivar were sown on a sub-line after surface sterilization of seeds for one hour with sodium hypochlorite containing 0.1 % active chlorite. The plot was sprinkled with tap water every day. Two weeks after sowing, all seedlings (shoots and roots) were collected, counted, and checked for brown-colored and slightly wilted lesions on the stem near the soil surface, which are the symptoms of the disease caused by *R. solani*. The germination ratio was square-root-transformed and analyzed by ANOVA and Tukey's maximum significant difference test.

When symptoms of the disease were observed in a treatment, plants were collected from each plot. Five 1 cm long root segments were selected at random, washed vigorously with tap water and three times with sterilized water. Washed root segments were placed on a 1.5 % water agar in a petri dish. Hyphae that extended from the root segments were observed under a light microscope.

Soil samples at a depth of 0–5 and 5–10 cm were collected from each subplot using a 100 ml core sampler with five replicates to estimate the number of Collembola after the seedling experiment. Collembola were extracted from the soil with Tullgren funnels and were subsequently counted.

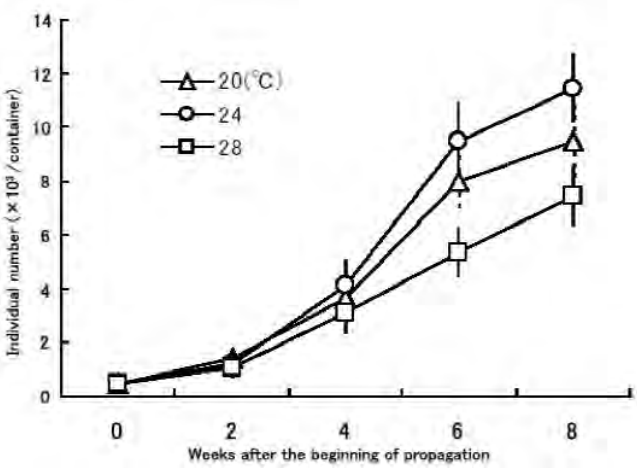

Fig. 1. Increase in the number of *Folsomia hidakana* in 200 ml polycarbonate containers at three different temperatures (Bar means S.D. in all figures)

Results

Method for propagating *Folsomia hidakana*

The number of *F. hidakana* individuals increased from 500 to about 10,000 using the improved culturing method in a period of six weeks (Fig. 1). The time required for doubling the number of *F. hidakana* was estimated to be 8.3 days at an optimal temperature of about 24 °C.

Optimization of experimental conditions

Most of the cabbage and Chinese cabbage seeds germinated with no symptoms of the disease as the volume of *R. solani* inoculant was increased to 3 % of the

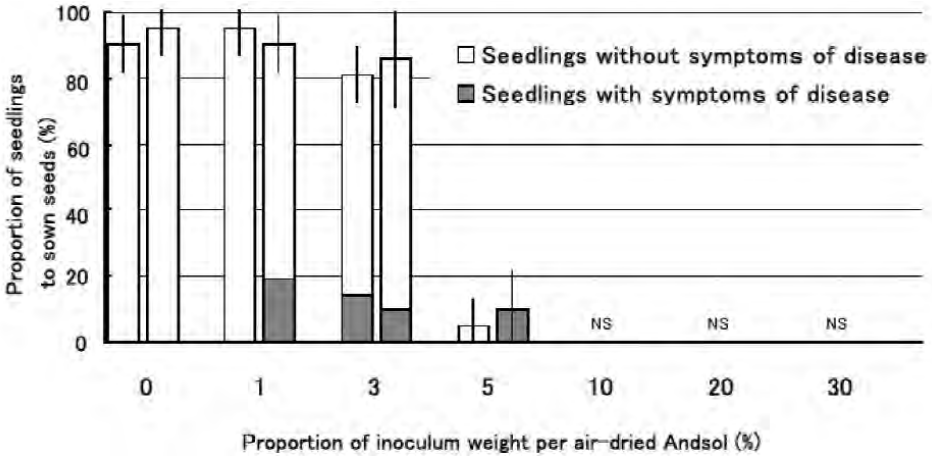

Fig. 2. Relationship between the severity of damping-off disease in cabbage (*Brassica oleracea* var. *capitata*) and Chinese cabbage (*Brassica rape* var. *pekinensis*) and the volume of the *Rhizoctonia solani* inoculant. Left, cabbage; right, Chinese cabbage in a set of two bars. * 'NS' indicates no seedlings in both plots

dry soil per container (Fig. 2). However, the germination rates suddenly decreased when the *R. solani* inoculant was applied at a rate of 5 % of the dry soil.

Most of the cabbage and Chinese cabbage seeds did not germinate when sown immediately into the containers of *R. solani* inoculant and *F. hidakana* (Fig. 3). The germination rates gradually increased with increasing time between the date of introduction of Collembola and the sowing of the cabbage. With a seed sowing delay of 15 days, 90 % of the seeds had germinated disease-free.

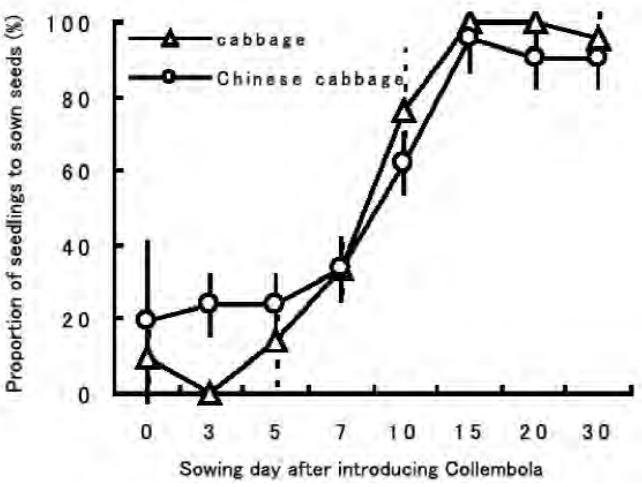

Fig. 3. Relationship between the severity of damping-off disease in cabbage (*Brassica oleracea* var. *capitata*) and Chinese cabbage (*Brassica rape* var. *pekinensis*) and the period after *Folsomia hidakana* was transferred

Seedling experiments

Most of the cabbage seeds germinated with no symptoms of the disease in the control plot (Fig. 4). There was no significant difference in the ratio of germinated seeds among the three varieties of cabbages. In contrast, while no symptoms of damping-off disease were observed, the germination rates of all three varieties of Chinese cabbage fluctuated but, on average, were lower than those of cabbage. *R. solani* was not separated from the root segments of the control plot.

The *R. solani* inoculant caused severe disease symptoms in all varieties of cabbage and Chinese cabbage. The germination ratio decreased below 14 % on all cultivars in the *R. solani* plot. Symptoms of the damping-off disease occurred in 28 % and 40 % of the cabbage and Chinese cabbage seedlings, respectively. *R. solani* was separated from all washed root segments of seedlings from the *R. solani* plot. The hyphal anastomosis criteria (Parmeter et al. 1969) proved the separated *R. solani* to be the same type as that used for inoculation.

In contrast to the *R. solani* plot, the number of germinated seeds was dramatically higher in the *R. solani* + *F. hidakana* plot. Furthermore, damping-off in the *R. solani* + *F. hidakana* plot was reduced by 82–87 %. The average germination rates were 89 % for cabbage and 84 % for Chinese cabbage, which were comparable with those of the control plot. *R. solani* did not grow on root segments from the *R. solani* + *F. hidakana* plot, although some seedlings had symptoms of the disease (Fig. 4).

The number of Collembola gradually decreased in the *R. solani* + *F. hidakana* plot and few were present four weeks after sowing (Table 1). No Collembola individuals were observed on the soil surface of the control and *R. solani* plots.

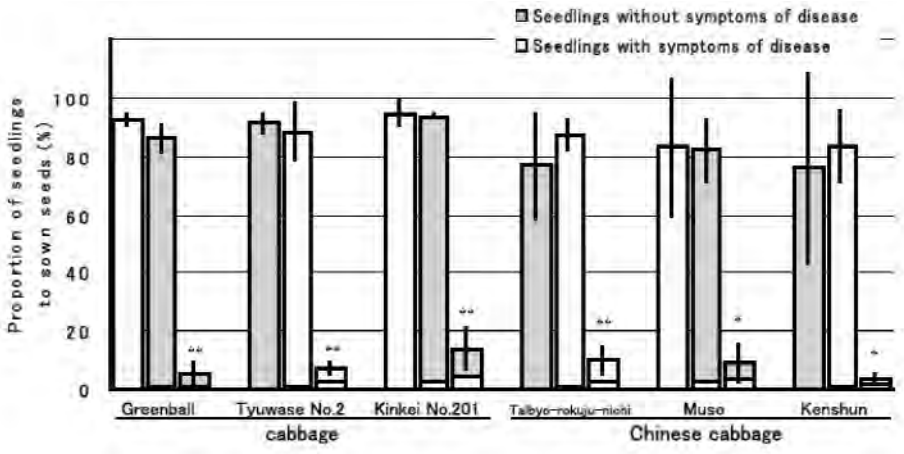

Fig. 4. Effects of *Folsomia hidakana* in suppressing damping-off in cabbage (*Brassica oleracea* var. *capitata*) and Chinese cabbage (*Brassica rape* var. *pekinensis*) in the experimental plots. Left, control plot; center, *Rhizoctonia solani* + *F. hidakana* plot; right, *R. solani* plot in a set of three bars. Bars marked with ** and * differ significantly at $P<0.01$ and $P<0.05$, respectively, from the control and *R. solani* + *F. hidakana* plots.

Table 1. Changes in densities of Collembola (ind.·m^{-2}) in the *Rhizoctonia solani* + *Folsomia hidakana* plot

		Mean ± S.D.
Beginning of experiment	(0 day, animals added)	120,600 ± 32,300
End of experiment (28 days)	(0–5 cm soil depth)	35,600 ± 22,700
	(5–10 cm soil depth)	3,800 ± 3,000
14 days after end of the experiment	(0–5 cm soil depth)	4,100 ± 2,200
(42 days)	(5–10 cm soil depth)	1,000 ± 1,100

Discussion

Collembola have been used to control the damping-off disease caused by *R. solani* (Curl 1979; Nakamura et al. 1991a, 1992; Lootsma & Scholte 1997a, b; Lartey et al. 1994); however, these experiments were limited to pots kept in the laboratory. Our new method to propagate *F. hidakana* made it possible to transfer a total of 3×10^5 individuals of *F. hidakana* to 2.4 (0.81×3) m^2 plots and demonstrated on a relatively large scale the usefulness of *F. hidakana* for the prevention of damping-off in cabbage and Chinese cabbage. Prior to the development of this method, *F. hidakana* had been cultured in petri dishes using hyphae and sclerotia of *R. solani* growing on agar (Nakamura et al. 1991b). The latter rearing method limits the use of *F. hidakana* on a large scale because *F. hidakana* may spread hyphae of *R. solani* thereby contaminating experimental systems (Visser et al. 1987), and only a limited number of *F. hidakana* can be reared. The new method overcomes these disadvantages.

When *R. solani* was cultured using only barley-grain in the medium and was subsequently used as an inoculant, the percentage of diseased cabbage and Chinese cabbage was very variable. In contrast, *R. solani* consistently infected cabbage and Chinese cabbage when soil was mixed with the medium.

Fungal hyphae increased visibly on the soil surface in the *R. solani* plot whereas no hyphae were observed in *R. solani* + *F. hidakana* plot at sowing, two weeks after *F. hidakana* was transferred. This observation supports the hypothesis that *F. hidakana* fed on *R. solani* and suppressed the disease.

The sclerotia that were produced by *R. solani* in the present experiment were investigated further. Curl (1979) concluded that *Onychiurus encarpatus* Denis (Collembola) could not suppress the disease of *R. solani* because *O. encarpatus* does not feed on sclerotia. In contrast, *F. hidakana* fed on the sclerotia of *R. solani* in petri dishes after the hyphae were consumed. The density of *F. hidakana* decreased to below 50 % of the initial number at the end of the two-week seedling test (Table 1). More than two weeks after the test, few Collembola individuals were observed in the soil. The decrease in density of *F. hidakana* may have resulted from the depletion of its food source, *R. solani* and other fungi. Environmental factors were unlikely to have caused this decrease since the temperature and water content of the top 10 cm soil were maintained at a constant level. Furthermore, predatory animals were not found. During this period, the sclerotia would have been consumed.

The current propagation method and the experiments conducted indicate the potential for using *F. hidakana* as a biological control agent against *R. solani* on a larger scale than previously thought possible. Some Japanese farmers sow small plots with cabbage or Chinese cabbage and subsequently transplant the seedlings to the field after plowing. Although it would be difficult to use *F. hidakana* to control damping-off disease in an entire field, it might be used in such small plots for seedling cultivation

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