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Effects of cyazofamid against *Plasmodiophora brassicae* Woronin on Chinese cabbage

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Abstract: Cyazofamid (4-chloro-2-cyano-*N,N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide) is a novel fungicide with high levels of activity against Oomycetes fungi and *Plasmodiophora brassicae* Woronin. The effects of cyazofamid were investigated against *P brassicae*, the causal agent of clubroot disease in Chinese cabbage. Cyazofamid at 0.3 mg litre^{-1} inhibited resting spore germination of this pathogen by about 80%. Cyazofamid at 3–10 mg litre^{-1} exhibited fungicidal activity to resting spores of *P brassicae* 1–10 days after treatment. When cyazofamid was applied to infested soil, both root-hair infections and club formation caused by *P brassicae* were strongly inhibited at 1–3 mg kg^{-1} dry soil. These results suggest that cyazofamid directly inhibits resting spore germination, thereby leading to the inhibition of root-hair infection and club formation. Cyazofamid at 3 mg kg^{-1} dry soil also exhibited complete control of clubroot disease. The effect of broadcast soil application using a dust formulation at 2 kg AI ha^{-1} (equivalent to 1.3 mg AI kg^{-1} dry soil), and plug seedling tray application by a suspension concentrate formulation at 200 and 400 mg AI tray^{-1} (30 × 60 × 4 cm^3) against *P brassicae* was also evaluated. Cyazofamid exhibited good efficacy against the pathogen. The sequential treatment including plug seedling tray application with cyazofamid and pre-plant broadcast soil application with the fungicide fluazinam also exhibited excellent levels of control. These results indicate that cyazofamid has a high potential to be an effective fungicide for the control of clubroot disease.

Keywords: cyazofamid; IKF-916; *Plasmodiophora brassicae*; clubroot; resting spore

1 INTRODUCTION

Clubroot disease caused by the pathogen *Plasmodiophora brassicae* Woronin is one of the most serious problems in crucifer cultivation world-wide.[1–4] Heavy infection by this pathogen leads to severe economic losses of Chinese cabbage, cabbage, broccoli, turnip, oilseed rape and other crucifers.[1–5] Clubroot is one of the most difficult plant diseases to control, since the pathogen is wholly confined to the soil and its resting spores are able to survive for a long period of time in the soil.[2,3,6] Although cultural practices for the control of clubroot, such as crop rotation with non-cruciferous crops, raising soil pH and improvement in drainage have long been known and used, it is difficult to achieve satisfactory control when there is a high density of resting spores and highly virulent populations of *P brassicae*.[1,2,7] Use of resistant cultivars of crucifers have been only partially successful. As a result, control strategies for clubroot in Japan often rely on application of fungicides.

Cyazofamid (4-chloro-2-cyano-*N,N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide; IKF-916; Ranman®; Fig 1) is a novel fungicide with high levels of activity against Oomycetes fungi.[8–12] The mode of action of cyazofamid is through inhibition of electron transport in Oomycete fungal mitochondria resulting from binding at a specific site on cytochrome *b*.[11] This inhibition of energy (ATP) production in the fungus affects all stages in the life cycle of *Phytophthora infestans* (Montagne) de Bary.[9]

Cyazofamid was approved for use in Japan in 2001 for the control of potato and tomato late blight and also Chinese cabbage, cucumber, grape and melon downy mildew.[10] Cyazofamid is suitable for not only foliar application but also for soil application for the control of plant pathogens, since it is relatively easily degraded in the soil (DT_{50}: 4–5 days), and has low risk of damage to fish (acute 48-h LC_{50}, rainbow trout > 100 mg litre^{-1}). Furthermore, cyazofamid has low water solubility (0.121 mg litre^{-1}, 25 °C, pH 7), and a high degree of soil adsorption (K_{oc}: 490–6300) and therefore does not leach into deeper soil layers.[8,10]

Cyazofamid has also been found to provide good control of crucifer clubroot caused by *P brassicae* (Protozoa) which is not classified as belonging to the Oomycetes (Chromista). This new fungicide has been approved in Japan for the control of Chinese cabbage clubroot since November 2001. We have previously

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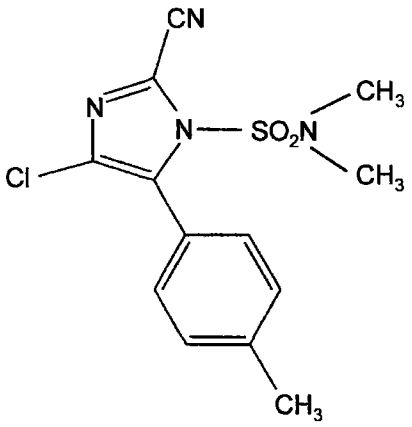

Figure 1. Chemical structure of cyazofamid.

reported the biological effects of cyazofamid against *P infestans* on tomato and *Pseudoperonospora cubensis* (Berkeley & Curtis) Rostowzew on cucumber,[12] but there are no data reported concerning activity against *P brassicae*.

This paper describes the effects of cyazofamid against *P brassicae*, leading to inhibition of the development of clubroot on Chinese cabbage. In particular the activity against resting spore germination, root-hair infection, clubroot formation and resting spore viability was studied. We also examined the effect of broadcast soil application using a dust formulation and plug seedling tray application using a suspension concentrate (SC) formulation against *P brassicae*.

2 MATERIALS AND METHODS

2.1 Fungicide, plant and soil

Cyazofamid was synthesized and formulated as a 100 g litre^{-1} SC, (the formulation for the Asian market), a 400 g litre^{-1} SC (the formulation for the European and the American markets), and as a 10 g kg^{-1} dust (the prototype formulation for the Asian market), by Ishihara Sangyo Kaisha, Ltd. The 100 g litre^{-1} SC was mainly used in this study. Fluazinam was formulated as a 5 g kg^{-1} dust (Frowncide® dust) by Ishihara Sangyo Kaisha, Ltd.

Chinese cabbage (*Brassica campestris* L [pekinensis group] cv Muso) which is susceptible to *P brassicae*, was used in the study. Seeds were sown into a sterilized clay loam soil (obtained from an upland field site in Shiga, Japan, pH 6.4), previously divided into 72- or 2000-cm^2 plastic pots (diameter 9.6 cm, depth 10 cm or width 33 × 61 cm^2, depth 20 cm). In some tests, seeds were sown into a plug seedling compost TM-1 (N:P:K = 150:560:100 mg kg^{-1} of compost, Takii Seed Co Ltd, Japan), which was previously divided into plug seedling trays (width: 30 × 60 cm^2, depth: 4 cm, 128 holes per tray, U-plug tray 128®, Nissen Chemitec Corp, Japan).

2.2 Inoculum

Inoculum was prepared as a suspension of resting spores of *P brassicae*. Resting spores were obtained from clubroot galls collected from Chinese cabbage plants grown in a field in Shiga and Nagano, Japan. The inocula were designated as strain S-1 and strain N-1, respectively. Unless otherwise described, the strain S-1 was used in the experiments. The galls were washed with water and stored at −25 °C in polyethylene bags until use. Frozen galls were then macerated and homogenized in water and the homogenate was filtered through four layers of gauze.

Artificially infested soils were prepared by adding the spore suspension to the sterilized clay loam soils to obtain 1 × 10^5 to 1 × 10^7 resting spores g^{-1} dry soil.

2.3 Cyazofamid application into the soil

Infested soil in a plastic case was treated with aqueous dispersions of cyazofamid SC (100 or 400 g litre^{-1}), to give a treatments equivalent to 0.1, 0.3, 1, 3 and 10 mg cyazofamid kg^{-1} dry soil, or with cyazofamid dust (10 g kg^{-1}) at 2 kg AI ha^{-1}, or with fluazinam dust (5 g kg^{-1}) at 2 kg AI ha^{-1} (When the soil is cultivated to 15 cm depth, 2 kg AI ha^{-1} is equivalent to 1.3 mg AI kg^{-1} dry soil). The soil was mixed thoroughly by hand 24 h (SC) or immediately (dust) after application. In some tests, 2 litres of the suspension of cyazofamid (100 g litre^{-1} SC) at 100 and 200 mg litre^{-1} (final concentration) was drenched onto a plug seedlings tray (seedlings: 5-leaf stage) using a sprinkling can 24 h before transplanting (200 or 400 mg AI per tray).

2.4 Effect on germination from resting spores

Germination of resting spores of *P brassicae* was evaluated by the method of Takahashi.[6] Resting spores were suspended with water (1 × 10^6 resting spores ml^{-1}), and incubated with roots of turnip seedlings [*Brassica campestris* L subsp *rapa* L Hook f & Anders (rapifera group), cv Wase-okabu, 15 seedling roots 100 ml^{-1} water] for 10 days at 20 °C. Cyazofamid 100 g litre^{-1} SC was added to the suspension at the start of incubation to give final concentrations of 0.1, 0.3 and 1 mg AI litre^{-1}. Germination of resting spores was observed with a microscope (BH-2, Olympus Optical Co Ltd, Japan), and the percentage of resting spore germination was calculated.

2.5 Effect on root-hair infection

Infested soil was treated with the suspension of cyazofamid (100 g litre^{-1} SC) to give treatments equivalent to 0.1, 0.3 and 1 mg AI kg^{-1} dry soil. Chinese cabbage seedlings were grown in pots (three seeds per 72-cm^2 pot) of soil infested with *P brassicae* (1 × 10^6 resting spores g^{-1} dry soil) for 10 days after sowing in the glasshouse. The root-hair infections of the seedlings were evaluated by the methods of Naiki *et al.*[13] The seedlings were removed from pots and their roots washed in water to remove soil and sand particles. The taproots of these seedlings were stained

with Cotton Blue (50 mg litre^{-1}). The number of infected root-hairs (the number of zoosporangial clusters), in each taproot was counted under a microscope.

2.6 Effect on resting spore viability

Resting spore viability was evaluated by the method of Naiki and Dixon,[14] and Suzuki *et al.* [15] Cyazofamid 100 g litre^{-1} SC was added to the resting spore suspension of *P brassicae* (1×10^6 resting spores ml^{-1}) to give final concentrations of 3 and 10 mg AI litre^{-1}. After 1, 5 and 10-day incubation at 20 °C, the resting spores were separated and removed from cyazofamid by centrifugation (3000 rev min^{-1}, 10 min). The pellets of resting spores were washed with water twice, followed by repeated centrifugation. The pellets of resting spores were resuspended in water and were added to sterile soil (1×10^6 resting spores g^{-1}). The seeds of Chinese cabbage were sown into the soil (three seeds per 72-cm^2 pot), and root-hair infection of the seedlings was observed after 10-day incubation. The percentage of root-hair infections with the washed spores was calculated and compared with the control (ie the spores without separation of cyazofamid).

2.7 Dose effect on clubroot formation

Infested soil (1×10^5, 1×10^6 and 1×10^7 resting spores g^{-1} dry soil) was treated with aqueous dilutions of cyazofamid 100 or 400 g litre^{-1} SC to give a treatments equivalent to 0.1, 0.3, 1 and 3 mg cyazofamid kg^{-1} of dry soil. Chinese cabbage seedlings were grown in pots (three seeds per 72-m^2 pot) of soil infested with *P brassicae* for 30–40 days after sowing in the glasshouse.

2.8 Effect of two application methods on clubroot formation

2.8.1 Broadcast soil application with cyazofamid dust

Infested soil (1×10^5 resting spores g^{-1} of dry soil) was treated with 10 g kg^{-1} dust of cyazofamid at 2 kg AI ha^{-1} (When the soil is cultivated to 15 cm depth, 2 kg AI ha^{-1} is equivalent to 1.3 mg AI kg^{-1} dry soil). The soil was mixed by hand just before transplanting of plug seedlings (5-leaf stage). The seedlings were grown in pots (six seedlings per 2000-cm^2 pot) for 40–50 days after transplanting.

2.8.2 Plug seedling tray application

Two litres of aqueous dilutions of cyazofamid 100 g litre^{-1} SC at 100 and 200 mg AI litre^{-1} (final concentration) were drenched onto plug seedlings trays (200 and 400 mg AI per tray, seedlings at 5-leaf stage) using a sprinkling can 24 h before transplanting. The plug seedlings were transplanted into infested soil (1×10^5 resting spores g^{-1} dry soil, six seedlings per 2000-cm^2 pot) and were grown for 40–50 days.

2.9 Effect of sequential treatment on clubroot formation

Plug seedling tray application with cyazofamid (final concentration: 200 AI mg litre^{-1}) was conducted by the methods of described above. Infested soil (1×10^6 resting spores g^{-1} dry soil), was treated with fluazinam dust (5 g kg^{-1}) at 2 kg AI ha^{-1} (When the soil is cultivated to 15 cm depth, 2 kg AI ha^{-1} is equivalent to 1.3 mg AI kg^{-1} dry soil) just before transplanting cyazofamid-treated seedlings (six seedlings per 2000-cm^2 pot). The plug seedlings were grown for 40–50 days.

2.10 Assessments of disease severity

The seedlings were removed from pots 30–40 days after sowing or 40–50 days after transplanting and their roots washed in water to remove soil and sand particles. Disease severity in a seedling was expressed in terms of disease index as follows: 0: none, 1: <25%, 2: 26–50%, 3: 51–75% and 4: >76% of root clubbed. The data were converted into disease severity of the plot (three or six seedlings per pot, two pots per plot) as follows:

$$\text{Disease severity} = 100 \times (B + 2C + 3D + 4E)/5(A + B + C + D + E)$$

where A, B, C, D and E represent the number of plants rated at 0, 1, 2, 3 and 4, respectively.

3 RESULTS

3.1 Effect on germination from resting spores

Cyazofamid (100 g litre^{-1} SC) strongly inhibited spore germination of *P brassicae* (Table 1), causing 80 and 100% inhibition at 0.3 and 1 mg AI litre^{-1}, respectively. Resting spore germination in the control (untreated) was relatively low (11%).

3.2 Effect on root-hair infection

Cyazofamid (100 g litre^{-1} SC) strongly inhibited root-hair infection of *P brassicae* (Table 2), causing 89 and 100% inhibition at 0.3 and 1 mg AI kg^{-1} dry soil, respectively.

Table 1. Effect of cyazofamid on spore germination of *Plasmodiophora brassicae*[a]

Concentration of cyazofamid (mg litre^{-1})	*Inhibition (±SD) (%)*
1	100
0.3	80(±14)
0.1	42(±25)
0	[11 (±3)][b]

[a] Resting spores (strain S-1, 1×10^6 resting spores ml^{-1}) were incubated with turnip seedlings in the presence of cyazofamid (100 g litre^{-1} SC) at 20 °C for 10 days.

[b] Percentage spore germination in the control (n=3).

Table 2. Effect of cyazofamid on root hair infection of *Plasmodiophora brassicae*[a]

Concentration of cyazofamid (mg kg^{-1} dry soil)	Inhibition (±SD) (%)
1	100
0.3	89 (±7)
0.1	68 (±23)
0	[290 (±111)][b]

[a] Infested soil (strain S-1, 1×10^6 resting spores g^{-1} of dry soil) was treated with cyazofamid (100 g litre^{-1} SC) 24 h before sowing. Chinese cabbage seeds were sown into the soil and were grown for 10 days.
[b] Number of zoosporangial clusters per taproot of plant in the control (n=3).

3.3 Effect on resting spore viability

Resting spore viability was evaluated by root-hair infection from resting spores. Inhibition of root-hair infection increased as the incubation period with cyazofamid increased. When resting spores were separated from cyazofamid (100 g litre^{-1} SC) after 1, 5 and 10-day incubation with 3 mg litre^{-1} of the compound, the inhibition of root-hair infection was 0, 51 and 80%, respectively (Fig 2). This result suggests that 1-day incubation with 3 mg litre^{-1} cyazofamid exhibited fungistatic control, whereas 10-day incubation with 3 mg litre^{-1} cyazofamid exhibited fungicidal activity to about 80% of resting spores. Fungicidal activity increased when the dose of cyazofamid increased to 10 mg litre^{-1}.

3.4 Effect on club formation

Cyazofamid (100 g litre^{-1} SC) exhibited excellent activity against clubroot disease (Table 3), giving complete control of both strains of *P brassicae* (strains S-1 and N-1) on Chinese cabbage at 3 mg kg^{-1} dry soil. Cyazofamid also gave protection from disease to plants exposed to high inoculum dosage (1×10^7 resting spores g^{-1} dry soil) of the pathogen (Table 3). We also compared the efficacy of cyazofamid 100 g litre^{-1} SC (the formulation for the Asian market) with that of cyazofamid 400 g litre^{-1} SC (the formulation for the European and the American markets). Cyazofamid 400 g litre^{-1} SC also gave good disease control and there was no statistical difference between the activities of the 100 g litre^{-1} SC and the 400 g litre^{-1} SC (data not shown).

On the basis of the outstanding activity exhibited, the effect of cyazofamid dust formulation was evaluated in order to approximate field application conditions in Japan more closely. Cyazofamid (10 g kg^{-1} dust) at 2 kg AI ha^{-1} (equivalent to 1.3 mg AI kg^{-1} of dry soil) exhibited good disease control against *P brassicae* on Chinese cabbage (Fig 3). We also evaluated the effect of cyazofamid application onto the plug seedling tray (seedlings at 5-leaf stage). When two litres of a dilution of cyazofamid 100 g litre^{-1} SC at 100 or 200 mg AI litre^{-1} were drenched onto plug seedlings trays (200 or 400 mg AI per tray) using a sprinkling can 24 h before transplanting, cyazofamid gave good disease control without any phytotoxicity (Fig 3). Sequential treatment using both plug seedling tray application with cyazofamid and broadcast soil application to the soil with another fungicide, fluazinam, significantly reduced the disease severity of clubroot (Fig 4).

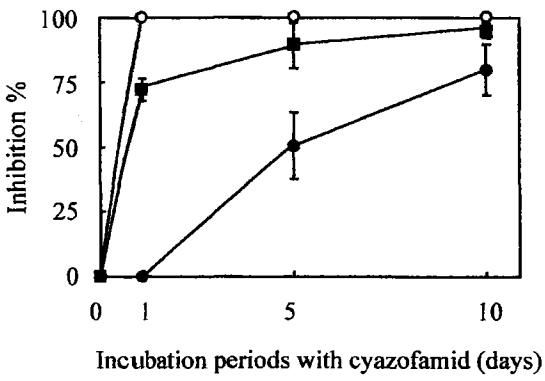

Figure 2. Effect of cyazofamid on resting spore viability of *Plasmodiophora brassicae*. Cyazofamid (100 g litre^{-1} SC) at (●) 3 mg AI litre^{-1} or at (■) 10 mg AI litre^{-1} was applied at the start of incubation of resting spore suspension (strain S-1, 1×10^6 resting spores ml^{-1}). Cyazofamid was separated and removed from resting spore suspension after 1, 5 or 10 day incubation. (○) indicates that cyazofamid at 3 mg litre^{-1} was not removed. Vertical bars represent standard deviations (SD) of the mean (n=3).

4 DISCUSSION

Resting spores of *P brassicae* in soil are the source of inoculum in natural infections of clubroot.[2,6,16] In tests, cyazofamid strongly inhibited resting spore germination (Table 1). When cyazofamid was applied to the soil infested with resting spores of *P brassicae*, both root-hair infection and club formation in Chinese cabbage were strongly inhibited (Tables 2 and 3). These results suggest that cyazofamid directly inhibits resting spore germination, thereby leading to the inhibition of root-hair infection and club formation. Cyazofamid may also inhibit primary zoospore motility. We previously reported the effect of cyazofamid on the infection process of another target fungus *P infestans*. In this case the inhibition of zoospore motility by cyazofamid was superior to that of zoospore release from zoosporangia at the same concentration.[9]

Cyazofamid at 1 mg kg^{-1} dry soil inhibited root-hair infection of *P brassicae* (Table 2). The biology of *P brassicae*, including the need for the root-hair stage in pathogenicity, is not fully understood.[13,17–21] However, it is generally considered that root-hair infection increases the *P brassicae* populations in susceptible host roots, and the root-hair infection presumably serves to cause severe damage to Chinese cabbage.[7,17]

Concentration of cyazofamid (mg kg^{-1} of dry soil)	Inhibition of club formation (%) (±SD)			
	Strain S-1			Strain N-1
	1×10^{5} [b]	1×10^{6} [b]	1×10^{7} [b]	1×10^{6} [b]
3	100	100	100	100
1	83 (±6)	77 (±10)	75 (±7)	98 (±3)
0.3	48 (±9)	25 (±7)	21 (±12)	52 (±9)
0.1	27 (±9)	13 (±18)	10 (±9)	nt[c]
0	[100][d]	[100][d]	[100][d]	[67 (±18)][d]

[a] Infested soil was treated with cyazofamid (100 g litre^{-1} SC) 24 h before sowing. Chinese cabbage seeds were sown into the soil and were grown for 30–40 days (three seeds per 72 cm^2 pot).
[b] Inoculum density: resting spores g^{-1} dry soil.
[c] nt: not tested.
[d] Disease severity in the control (n=2).

Table 3. Dose effect of cyazofamid on club formation in Chinese cabbage by *Plasmodiophora brassicae*[a]

Since cyazofamid inhibits root-hair infection, the fungicide may suppress an increase in *P brassicae* populations.

There are some reports concerning the biological mode of action of fungicides against *P brassicae*. Fluazinam and flusulfamide inhibit resting spore germination and primary or secondary infection of *P brassicae*.[5,22] In contrast, quintozene (pentachloronitrobenzene, PCNB) exhibits a limited effect on resting spore germination, but does act against *P brassicae* established within the cortical tissue of the host root.[14]

It is well known that resting spores of *P brassicae* are able to survive for long periods of time in soil.[2,3,6] There are some reports concerning the action of fungicides on the viability of resting spores. Suzuki *et al*[15] reported that fluazinam exhibited fungicidal action on resting spores of *P brassicae*, while PCNB exhibited fungistatic action, because inhibition of root-hair infection increased as the incubation period between fluazinam and resting spores was expanded, but did not increase as the incubation period between PCNB and resting spores was expanded. Naiki and Dixon[14] also reported that PCNB at 100 mg litre^{-1} had fungistatic action on resting spores. Tanaka *et al*[22] reported that flusulfamide was fungistatic to resting spores of *P brassicae* on the basis of the results of Evan's blue staining assay. On the basis of the method of Naiki and Dixon[14] and Suzuki *et al*,[15] cyazofamid at 3 mg litre^{-1} exhibited an irreversible inhibition (fungicidal action) (Fig 2). The result suggests that cyazofamid can contribute to suppressing clubroot epidemics in the soil. We have also reported that cyazofamid had fungicidal action and strong sporulation inhibitory activity against *P infestans* and *P cubensis* and therefore it can contribute to suppress tomato and potato late blight and, cucumber downy mildew epidemics.[9,12]

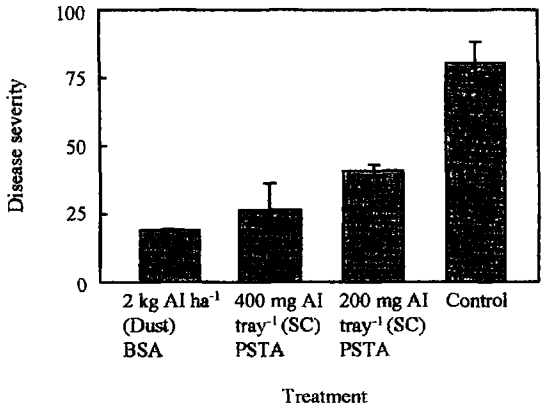

Figure 3. Effect of broadcast soil application with cyazofamid dust (10 g kg^{-1}) and plug seedling tray application with cyazofamid SC (100 g litre^{-1}) on club formation in Chinese cabbage by *Plasmodiophora brassicae*. The plug seedlings were transplanted into the infested soil (strain S-1, inoculum density: 1×10^5 resting spores g^{-1} dry soil) and were grown for 40–50 days (six seedlings per 2000-cm^2 pot). BSA: Broadcast soil application with cyazofamid, PSTA: Plug seedling tray application with cyazofamid. Vertical bars represent standard deviations (SD) of the mean (n=2).

Since cyazofamid specifically interferes with cytochrome bc_1 complex (complex III) activity of *Pythium spinosum* Sawada mitochondria,[11] the biochemical mode of action against *P brassicae* is probably a similar inhibition of mitochondrial complex III. Structural features of the cytochrome bc_1 complex may be similar between Oomycetes and *P brassicae*.

Recently the use of plug seedlings for mechanical transplanting has increased in Japan.[23,24] The seedlings are nursed in uniform plastic plug seedling trays. Cyazofamid application onto a plug seedling tray clearly exhibited a good level of control of Chinese cabbage clubroot (Figs 3 and 4). In some official tests in 1999 and 2000, carried out by the Japan Plant Protection Association, the efficacy of cyazofamid at 400 mg per tray for control of clubroot was equal to that of fluazinam at 2 kg AI ha^{-1} or flusulfamide at 0.9 kg AI ha^{-1} (broadcast soil application). Cyazofamid has low water solubility (0.121 mg litre^{-1}, 25 °C, pH 7),[8,10] and so the fungicide probably does not have systemic translocation activity from taproot and root-hairs to untreated new roots.[12] These facts indicate that taproot protection by cyazofamid is important to get good level of control of the disease.

Although the control level in the plug seedling application method is inferior to that of the broadcast

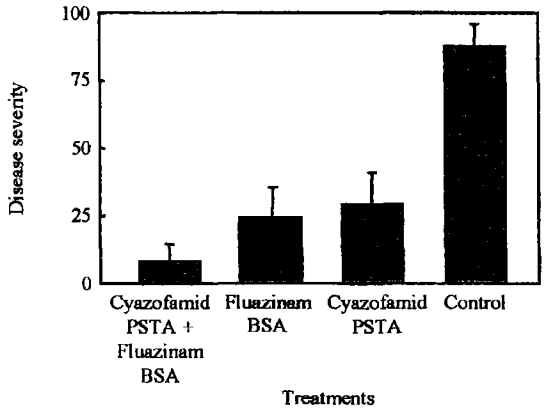

Figure 4. Effect of sequential treatment using both plug seedling tray application with cyazofamid SC (100 g litre^{-1}) and broadcast soil application with fluazinam dust (5 g kg^{-1}) on club formation in Chinese cabbage by *Plasmodiophora brassicae*. The plug seedlings were transplanted into the artificial infested soil (strain S-1, inoculum density: 1×10^6 resting spores g^{-1} dry soil) and were grown for 40–50 days (six seedlings per 2000-cm^2 pot). BSA: Broadcast soil application with fluazinam at 2 kg AI ha^{-1}. PSTA: Plug seedling tray application with cyazofamid at 400 mg AI per tray. Vertical bars represent standard deviations (SD) of the mean (n=3).

soil application (Fig 3), this method enables the farmer to save labour and money for control of the diseases. Therefore if disease pressure is low, we recommend the plug seedling tray application alone. In situations with very high disease pressure with insufficient control by pre-plant broadcast soil application with other fungicides alone, we recommend a sequential treatment including cyazofamid (plug seedling tray application with cyazofamid SC prior to transplanting, followed by soil application with other commercial fungicides such as fluazinam dust). In field tests, we compared the efficacy of sequential treatment including cyazofamid (400 mg per tray, plug seedling tray application) and fluazinam (2 kg AI ha^{-1}, broadcast soil application) with cyazofamid (400 mg per tray, plug seedling tray application) alone and fluazinam (2 kg AI ha^{-1}, broadcast soil application) alone (Shiga, Japan in 2001 and 2002). Even in the case of heavy infestation (disease severity in the control: 81–98), all the fungicides tested exhibited fungicidal activity against clubroot. Among them, sequential treatment gave the best control of clubroot on Chinese cabbage (Mitani, S, unpublished results).

Cyazofamid registration for the control of use of Chinese cabbage clubroot (400 mg AI per tray, plug seedling tray application) was approved in Japan November 2001 and will be followed by that for use on cabbage and broccoli. To our knowledge, this is the first registration for plug seedling tray fungicide application for the control of clubroot.

When plants are raised in plug seedling trays, the developing seedlings are cultivated under conditions of high humidity at a high seeding rate, encouraging the outbreak of other diseases such as seedling damping off caused by *Pythium megalacanthum* de Bary and *Pythium aphanidermatum* (Edson) Fitzpatrick.[25–27] Kubota and Abiko[27] also reported that downy mildew caused by *Peronospora parasitica* Takahashi presents a major problem in cabbage seedlings nursed in plug seedling trays. Since cyazofamid exhibits a very high level of activity against a broad spectrum of Oomycetes including *Pythium* and *Peronospora*,[8–10] this fungicide application onto the plug seedling tray prior to the application for clubroot control should provide a good level of control of these diseases. The simultaneous control of these two diseases may eliminate the need for application of other fungicides onto the plug seedling tray or in the field where application of cyazofamid is used to control clubroot. Further development work with cyazofamid is needed to establish the most efficient application and cost beneficial method.

In conclusion, cyazofamid strongly inhibits resting spore germination, root-hair infection and club formation caused by *P brassicae*. It has a strong fungicidal action on resting spores. Cyazofamid has a high potential to be a new effective fungicide for the control of clubroot disease. This disease can be controlled with a plug seedling tray application of cyazofamid prior to transplanting.

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