

Feasible Management of Southern Corn Leaf Blight via Induction of Systemic Resistance by *Bacillus cereus* C1L in Combination with Reduced Use of Dithiocarbamate Fungicides

Yi-Ru Lai¹, Pei-Yu Lin¹, Chao-Ying Chen^{2*}, and Chien-Jui Huang^{1*}

¹Department of Plant Medicine, National Chiayi University, Chiayi 60004, Taiwan, Republic of China

²Department of Plant Pathology and Microbiology, National Taiwan University, Taipei 10617, Taiwan, Republic of China

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Dithiocarbamate fungicides such as maneb and mancozeb are widely used nonsystemic protectant fungicides to control various plant fungal diseases. Dithiocarbamate fungicides should be frequently applied to achieve optimal efficacy of disease control and avoid either decline in effectiveness or wash-off from leaf surface. Dithiocarbamates are of low resistance risk but have the potential to cause human neurological diseases. The objective of this study was to develop a strategy to effectively control plant disease with reduced use of dithiocarbamates. Southern corn leaf blight was the model pathosystem for the investigation. When corn plants were drench-treated with *Bacillus cereus* C1L, a rhizobacterium able to induce systemic resistance in corn plants against southern leaf blight, frequency of spraying dithiocarbamate fungicides could be decreased. The treatment of *B. cereus* C1L was able to protect maize from southern leaf blight while residues of dithiocarbamates on leaf surface were too low to provide sufficient protection. On the other hand, frequent sprays of mancozeb slightly but significantly reduced growth of corn plants under natural condi-

tions. In contrast, application of *B. cereus* C1L can significantly promote growth of corn plants whether sprayed with mancozeb or not. Our results provide the information that plant disease can be well controlled by rhizobacteria-mediated induced systemic resistance in combination with reduced but appropriate application of dithiocarbamate fungicides just before a heavy infection period. An appropriate use of rhizobacteria can enhance plant growth and help plants overcome negative effects caused by dithiocarbamates.

Keywords : *Bacillus cereus*, *Cochliobolus heterostrophus*, dithiocarbamate, induced systemic resistance

Dithiocarbamates maneb and mancozeb are multi-site, non-systemic, protectant fungicide belonging to the Fungicide Resistance Action Committee (FRAC) group M3. They have to be sprayed on leaf surface to inhibit fungal spore germinating and penetration into host plants. However, non-systemic protectant fungicides can be washed off the foliage by rainfall (or sprinkler irrigation). To obtain optimal protection, regularly using these dithiocarbamate fungicides once every week is recommended to control many foliar fungal diseases. It is not economically feasible because high application costs and the need for frequent applications. In addition, maneb is phytotoxic to a number of plants, especially application of maneb under hot condition (Conover, 1956). Recently, application of FRAC group M3 fungicides, especially maneb, has been limited by Environmental Protection Agency of the United States because of potential risks to human health and environments. Permanent parkinsonism has been observed in men with chronic exposure to the fungicides

*Co-corresponding authors.

CY Chen

Phone) +886-2-33665207, FAX) +886-2-23657735

E-mail) cychen@ntu.edu.tw

CJ Huang

Phone) +886-5-2717825, FAX) +886-5-2717451

E-mail) chienjui.huang@mail.ncyu.edu.tw

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mancozeb and maneb (Ferraz et al., 1988; Meco et al., 1994).

In Taiwan, maneb and mancozeb are recommended for control of many foliar fungal diseases including southern corn leaf blight. Southern corn leaf blight caused by *Cochliobolus heterostrophus* (Drechsler) Drechsler is a widespread disease throughout most of hot humid corn-growing areas of the world (Ullstrup, 1972; White, 1999). This disease was not considered an important pathogen until 1970 when *C. heterostrophus* race T became prevalent in the United States corn belt. Race T was highly pathogenic on Texas male-sterile cytoplasm (cms-T) and caused a major epidemic in 1970 and 1971 (Ullstrup, 1972). Although cms-T has been eliminated from elite germplasm since that time and effective polygenic resistance has been introduced. Southern corn leaf blight, predominantly caused by *C. heterostrophus* race O, is still a problem in sweet corn and seed production in the southern Atlantic coast area of the United States (Ullstrup, 1972) as well as in Taiwan (Tsai et al., 1993; Wu and Wang, 1987). Most effective control of Southern corn leaf blight with the recommended protectant fungicide maneb is achieved when maneb is applied as soon as disease is observed and reapplied every 4 to 7 days. As described above, frequent application of maneb has been increasingly limited due to public concerns about potential harmful effects of fungicide residues on human health and environments (Ferraz et al., 1988; Meco et al., 1994). Thus, we were interested in developing a strategy for integrated control of plant disease to reduce application of maneb or the other dithiocarbamate fungicides based on *Zea mays*—*C. heterostrophus* model pathosystem.

To find an alternative way for control of southern corn leaf blight, our previous study showed that a biocontrol rhizobacterium *Bacillus cereus* C1L can trigger systemic resistance in maize against southern corn leaf blight (Huang et al., 2010). Moreover, *B. cereus* C1L can well colonize the rhizosphere and promote growth of corn plants (Huang et al., 2010). These characteristics indicate that *B. cereus* C1L can act as a plant growth-promoting rhizobacterium (PGPR) of maize (Huang et al., 2010). Although *B. cereus* C1L-treated corn plants exhibited lower severity of southern leaf blight than untreated plants, the protection level of *B. cereus* C1L was significantly lower than that of maneb (Huang et al., 2010). We suggested that *B. cereus* C1L-mediated induced systemic resistance in maize may not provide enough protection against southern leaf blight under high disease pressure.

Thus, the aim of this study was to investigate a strategy for integrated control of southern corn leaf blight with reduced use of dithiocarbamate fungicides such as maneb and mancozeb. We found that southern leaf blight can

be effectively suppressed in *B. cereus* C1L-treated corn plants with lesser application of dithiocarbamate fungicides. The protection level of *B. cereus* C1L treatment in combination with less frequent but appropriate application of mancozeb was as effective as that of frequent application of mancozeb only. In addition, *B. cereus* C1L can significantly promote growth of corn plants whether treated with mancozeb or not.

Materials and Methods

Microorganisms, culture media, and culture conditions. *B. cereus* C1L (Liu et al., 2008) was cultured on Luria-Bertani (LB) agar plates (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% agar) at 28°C overnight. Cells of strain C1L were scraped off the plates and resuspended in sterile saline solution (0.85% NaCl). *C. heterostrophus* CYC402 (Huang et al., 2010) of race O was cultured and maintained on Difco potato dextrose agar (PDA) (BD Diagnostic Systems, Sparks, MD, USA).

Pathogen inoculation and disease rating. For sporulation, *C. heterostrophus* was cultured on autoclaved corn leaves or PDA plates at 28°C for 1 to 2 weeks (Wu and Wang, 1987). A conidial suspension of *C. heterostrophus* was prepared in 0.05% Tween-20 and adjusted to a final concentration of 5×10^4 conidia/ml for inoculation on corn plants. A spore suspension of *C. heterostrophus* (5×10^4 spores/ml) was sprayed as a fine mist until running off onto both surfaces of fully expanded leaves of 28-day-old corn plants. The inoculated corns were kept under moist condition at 25°C for 1 day and then replaced on the greenhouse bench. Two days after inoculation, disease symptoms were scored, and disease ratings were expressed on the basis of diseased leaf area using a 0–4 scale (i.e., 0, no symptoms; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 75–100% leaf area covered with lesions; Huang et al., 2010).

Induction treatments and fungicide sprays. Seeds of maize cvs. Honey Jean No. 3 and Bright Jean No. 2 (Known-You Seed Co., Ltd., Kaohsiung, Taiwan) were used in this study. Unless otherwise noted, corn plants were grown under greenhouse conditions (25°C, 16-h photoperiod) in commercial potting mixture (BVB Substrate No. 2; Bas van Buuren, Massland, the Netherlands) that had been autoclaved (at 121°C) twice on alternate days for 25 min.

Greenhouse experiment one: Induction of resistance in maize by *B. cereus* C1L was performed basically according to the method of Huang et al. (2010). Briefly, 28-day-old corn plants were treated with a bacterial cell suspen-

sion of strain C1L (1×10^7 cfu/g potting mixture) as a soil drench one day prior to pathogen inoculation. Fungicide maneb (Dithane M-22, 80% wettable powder; Rohm and Haas, Philadelphia, PA, USA) was sprayed until running off onto both surfaces of fully expanded leaves at 10% of the recommended dose (4,000 \times dilution, i.e., 0.2 g active ingredient (a. i./l) one day prior to pathogen inoculation.

One day post treatment, a spore suspension of *C. heterostrophus* (5×10^4 spores/ml) was sprayed as a fine mist until running off onto both surfaces of fully expanded leaves. The inoculated corns were kept under moist condition at 25°C overnight and then placed on the greenhouse bench. Two days after inoculation, disease evaluation was performed as described above, and each corn plant was individually assessed. Each treatment had three replications (three plants per replication) and all experiments were repeated at least one time.

Greenhouse experiment two: Induced resistance assays were performed with slight modification as described by De Vleeschauwer et al. (2006) and Huang et al. (2010). Briefly, corn seeds firstly were surface sterilized with 1% sodium hypochlorite for two min, rinsed three times with sterile water, and incubated on wet sterile filter paper for five days at 25°C to germinate. Prior to sowing in plastic pots (12 cm in diameter, three germinated seeds per pot), roots of germinated seeds were dipped in bacterial suspensions (1×10^7 cfu/ml) for 10 min. In addition, the rhizobacterial inoculum was thoroughly mixed with the potting mixture to a final density of 1×10^7 cfu/g potting mixture. Fourteen and 28 days later, the rhizobacterial inoculum was applied a second time and a third time, respectively, as a soil drench. Plants were grown at 25°C with 16 h/8 h light/dark cycle for 28 days (five- to six-leaf stage). In control treatments, sterilized saline was used

instead of bacterial suspension.

Furthermore, fungicide mancozeb (Indofil M-45, 80% wettable powder; Indofil, Maharashtra, India) was sprayed until running off onto both surfaces of fully expanded leaves at the recommended dose (400 \times dilution, i.e., 2.0 g a. i./l) at 14 and 28 days after sowing. All treatments of the experiment two are listed in Table 1.

Three days post the last treatment, corn plants were inoculated with spore suspensions of *C. heterostrophus* (5×10^4 spores/ml) and subsequent assessment of disease severity was performed as described above, and each corn plant was assessed individually. Each treatment had three replications (three plants per replication) and all experiments were repeated at least one time.

Experiment under natural conditions: The disease control experiment under natural conditions was performed according to the method of a second greenhouse experiment with slight modification. Ten germinated seeds with or without treatment of *B. cereus* C1L were sown in a plastic rectangular pot (21 \times 14 \times 7 cm), and unsterilized potting mixture was used. After sowing, plants were grown under natural conditions. The timing of rhizobacterial treatments, fungicide spray, and pathogen inoculation was as the same as that described in a second greenhouse experiment. Two weeks post inoculation, numbers of lesions per plant and plant height were measured. disease ratings were expressed on the basis of lesions per plant using a 0–4 scale (i.e., 0, no symptoms; 1, 1–3 lesions per plant; 2, 3–10 lesions per plant; 3, 11–20 lesions per plant; 4, 21–30 lesions per plant). This experiment had three replications (ten plants per replication).

Fungicide sensitivity tests. Various concentrations of mancozeb (Indofil M-45, 80% wettable powder)-water

Table 1. Treatments of mancozeb, *Bacillus cereus* C1L and the combined applications

Treatment	Applications*		
	Before planting	2 wk after planting	4 wk after planting
Control	-	-	-
M1st + 2nd	-	Foliar sprays of mancozeb	Foliar sprays of mancozeb
M1st	-	Foliar sprays of mancozeb	-
M2nd	-	-	Foliar sprays of mancozeb
C1L	Root dipping	Drench treatment	Drench treatment
C1L + M1st	Root dipping	Drench treatment with foliar sprays of mancozeb	Drench treatment
C1L + M2nd	Root dipping	Drench treatment	Drench treatment with foliar sprays of mancozeb

M, mancozeb; C1L, *B. cereus* C1L; -, applications of control treatments (sterile saline solution).

*Detailed applications of mancozeb and rhizobacterial inoculum were described in the “Materials and Methods”.

suspension were mixed with sterilized PDA to obtain the desired fungicide concentration. Mycelial disks of *C. heterostrophus* or a bacterial suspension of strain C1L (ca. 1×10^4 cfu/ml) were transferred onto unamended PDA and maneb-amended PDA. After incubation at 25°C for 1 day, fungicide sensitivities of both strains were examined.

Evaluation of root colonization by *B. cereus* C1L. Corn seeds were germinated, treated, and planted as described in the second greenhouse experiment. Three days post the last treatment, bacterial colonization of the corn roots was determined. Roots of three plants per treatment were excised and rinsed under tap water to remove most of the potting mixture. After blotting dry and weighing, roots were macerated in sterile saline using mortar and pestle and serial dilutions were plated on LB agar. Bacterial colonies showing the typical morphological characteristics of *B. cereus* C1L, and which do not appear on control plates, were counted after incubation at 28°C for 24 to 48 h.

Data analysis. Data were analyzed using either Tukey's

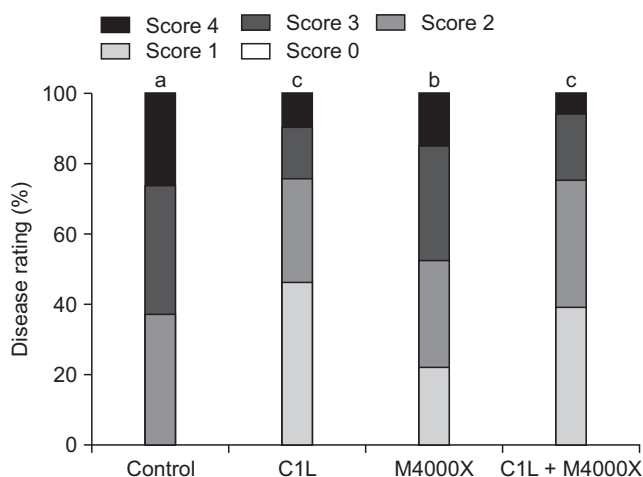


Fig. 1. Suppression of southern corn leaf blight by individual and combined applications of *Bacillus cereus* C1L and reduced use of maneb under greenhouse conditions. *B. cereus* C1L and maneb were applied as a soil drench and a foliar spray, respectively. One day after treatment, corn plants were inoculated with spore suspensions of *Cochliobolus heterostrophus*. Control, control treatment with sterile saline; C1L, *B. cereus* C1L; M4000X, 4,000-fold diluted maneb (i.e., 2.5 mg active ingredient (a. i.)/l equal to 10% the recommended dose); C1L + M4000X, a soil drench with *B. cereus* C1L in combination with a foliar spray of 4,000-fold diluted maneb. Two day post inoculation, disease evaluation was performed using a 0–4 disease severity scale as described in “Materials and Methods”. Bars indicated with the same letter are not statistically different based on non-parametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$).

multiple comparison or nonparametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$) depending on normality of data. All data of the experiments were analyzed using the PAST3 software package for Windows (<http://folk.uio.no/ohammer/past/>).

Results

Control of southern corn leaf blight by a soil drench with *B. cereus* C1L together with a foliar spray of a reduced dose of maneb. To know how *B. cereus* C1L and dithiocarbamate fungicide maneb could be applied together, the sensitivity of *B. cereus* C1L to maneb was tested firstly. Maneb at either the recommended dose (2.0 g a. i./l) or 50% recommended dose completely inhibited growth of *B. cereus* C1L, indicating that *B. cereus* C1L cannot mix with maneb. As the result, *B. cereus* C1L and maneb should be simultaneously applied as a soil drench and a foliar spray, respectively.

In the experiment one, maneb was applied at 0.2 g a. i./l (i.e., 10% of the recommended dose). At this dose, maneb slightly but significantly suppress the severity of southern corn leaf blight (Fig. 1, Table 2). Compared with the control treatment, the severity of corn leaves sprayed with maneb at the decreased dose was reduced by 14% (Table 2). *B. cereus* C1L was more successful in protecting corn plants against *C. heterostrophus* (Fig. 1) than the low maneb treatment. Moreover, the severity of southern corn leaf blight was effectively suppressed by a soil drench

Table 2. Control of southern corn leaf blight by a soil drench application of *Bacillus cereus* C1L alone and in combination with a spray of a reduced dose of maneb under greenhouse conditions

Treatment*	Disease severity [†]
Control	2.81 ± 0.79 a
M4000X	2.41 ± 0.99 b
C1L	1.87 ± 0.98 c
C1L + M4000X	1.91 ± 0.89 c

Values followed by the same letter are not statistically different based on non-parametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$).

*Detailed applications of maneb and rhizobacterial inoculum are described in the materials and methods. M4000X, 4,000-fold diluted maneb (i.e., 2.5 mg active ingredient (a. i.)/l); C1L, *B. cereus* C1L; C1L + M4000X, a soil drench with *B. cereus* C1L together with a foliar spray of 4,000-fold diluted maneb.

[†]Disease severity represents the mean disease score ± standard deviation. Disease evaluation was performed using a 0–4 disease severity scale as described in “Materials and Methods”.

with *B. cereus* C1L together with application of the reduced dose of maneb although the applied dose of maneb was at only 10% of the recommended dose. Treatments of *B. cereus* C1L alone and in combination with a foliar spray of a reduced dose of maneb provided equally effective protection in maize and caused around 30% reduction in the severity of southern leaf blight (Fig. 1, Table 2).

Control of southern corn leaf blight by programmed application of *B. cereus* C1L and mancozeb. The experiment two was conducted with dithiocarbamate fungicide mancozeb and maize cv. Bright Jean No. 2 instead of maneb and maize cv. Honey Jean No. 3, respectively, because commercial formulation of maneb and maize cv. Honey Jean No. 3 are no longer available in Taiwan. Firstly, the sensitivity of *B. cereus* C1L to mancozeb was tested. Mancozeb at either the recommended dose (2 g a. i./l) or 50% recommended dose completely inhibited growth of *B. cereus* C1L, indicating that *B. cereus* C1L can not be mixed with mancozeb.

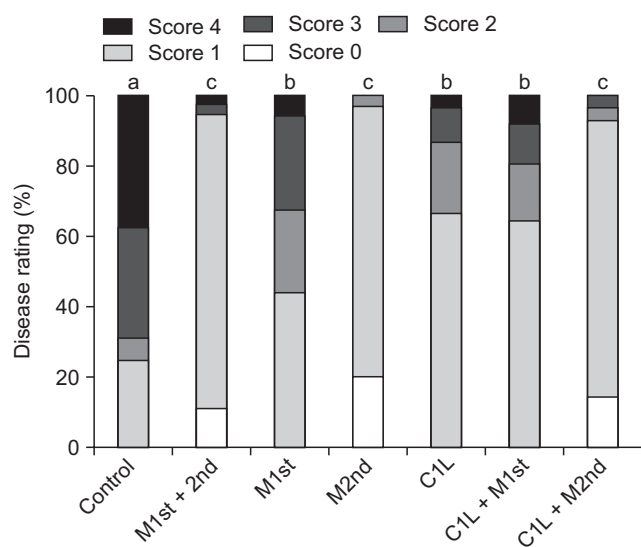


Fig. 2. Suppression of southern corn leaf blight by mancozeb, *Bacillus cereus* C1L and a soil drench with *B. cereus* C1L in combination with a foliar spray of mancozeb under greenhouse conditions. The composition and application program of each treatment is present in Table 1. *B. cereus* C1L and mancozeb (at the recommended dose of 25 mg active ingredient (a. i.)/l) were applied as a soil drench and a foliar spray, respectively. Detailed applications of mancozeb and rhizobacterial inoculum are described in the materials and methods. Three days post the last treatment, corn plants were inoculated with spore suspensions of *Cochliobolus heterostrophus*. Two day post inoculation, disease evaluation was performed as described in “Materials and Methods”. Bars indicated with the same letter are not statistically different based on non-parametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$).

A simple, sequential application program was used to further examine whether treatment of *B. cereus* C1L could reduce fungicide application in the experiment two. Programmed mancozeb applications (M1st + 2nd) provided pronounced effects on suppression of southern corn leaf blight (Fig. 2, Table 3). There was no significant difference in protection levels of mancozeb between programmed applications (M1st + 2nd) and a single application (M2nd) before infection of *C. heterostrophus* (Fig. 2, Table 3). The severity of southern corn leaf blight was reduced by around 65% in these two treatments (M1st + 2nd and M2nd). Compared with the treatments M1st + 2nd and M2nd, a single application (M1st) at 17 days before inoculation exhibited lower protection level (Fig. 2, Table 3) and caused around 30% reduction in the severity of southern corn leaf blight.

On the other hand, sequential treatments of *B. cereus* C1L provided pronounced protection in maize cv. Bright Jean No. 2 from southern leaf blight (Fig. 2, Table 3). Compared with the efficacy of programmed mancozeb applications, the treatment of *B. cereus* C1L exhibited lower effect on suppression of southern corn leaf blight (Fig. 2, Table 3). In addition, the severity of southern leaf blight in corn plants treated with *B. cereus* C1L in combination with either single mancozeb application (C1L + M1st and C1L + M2nd) was lower but not significantly different from either single mancozeb application (M1st

Table 3. Control of southern corn leaf blight by a soil-drench application of *Bacillus cereus* C1L alone and in combination with a foliar spray of mancozeb under greenhouse conditions

Treatment*	Disease severity [†]
Control	2.81 ± 1.18 a
M1st + 2nd	0.97 ± 0.50 c
M1st	1.94 ± 0.97 b
M2nd	0.83 ± 0.45 c
C1L	1.50 ± 0.81 b
C1L + M1st	1.64 ± 0.98 b
C1L + M2nd	0.96 ± 0.57 c

M, mancozeb; C1L, *B. cereus* C1L.

Values followed by the same letter are not statistically different based on non-parametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$).

*The composition and application program of each treatment is present in Table 1. Detailed applications of mancozeb and rhizobacterial inoculum are described in the materials and methods. Mancozeb was applied at the recommended dose (i.e., 25 mg active ingredient (a. i.)/l).

[†]Disease severity represents the mean disease score ± standard deviation. Disease evaluation was performed using a 0–4 disease severity scale as described in “Materials and Methods”.

and M2nd), respectively (Fig. 2, Table 3). Moreover, there was no significant difference in severity of southern corn leaf blight among treatments M1st + M2nd, M2nd, and C1L + M2nd (Fig. 2, Table 3). These three treatments caused around 65% reduction in the severity of southern corn leaf blight.

The effect of *B. cereus* C1L in combination with a foliar spray of mancozeb on suppression of southern corn leaf blight was evaluated under natural conditions. For disease assessment, numbers of lesions per plant were measured. The treatments C1L + M2nd most significantly reduced numbers of lesions per corn plant compared to the other treatments (Table 4). The effects of treatments M1st + 2nd and C1L on reduction of lesion numbers were not significantly different from those of the other treatments except for the control (Table 4).

Furthermore, the effects of mancozeb, *B. cereus* C1L and the combined application on growth of corn plants were examined. *B. cereus* C1L-treated corn plants with or without a spray of mancozeb were significantly higher than the control and mancozeb-treated corn plants when grown under natural conditions ($P < 0.05$; Fig. 3). Two times application of mancozeb slightly but significantly reduced mean height of corn plants in comparison with the control treatment. When mancozeb was sprayed once, no significant effect on height of corn plants was observed in comparison with the control (Fig. 3).

Table 4. Control of southern corn leaf blight by a soil-drench application of *Bacillus cereus* C1L alone and in combination with a foliar spray of mancozeb under natural conditions

Treatment*	Disease severity [†]
Control	1.39 ± 1.29 a
M1st + 2nd	0.34 ± 0.71 bc
M1st	0.50 ± 0.80 b
M2nd	0.30 ± 0.46 b
C1L	0.37 ± 0.78 bc
C1L + M1st	0.67 ± 0.99 b
C1L + M2nd	0.13 ± 0.61 c

M, mancozeb; C1L, *B. cereus* C1L.

Values followed by the same letter are not statistically different based on non-parametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$).

*The composition and application program of each treatment is present in Table 1. Detailed applications of mancozeb and rhizobacterial inoculum are described in the materials and methods. Mancozeb was applied at the recommended dose (i.e., 25 mg active ingredient (a. i.)/l).

[†]Disease severity represents the mean disease score ± standard deviation. Disease evaluation was performed using a 0–4 disease severity scale as described in “Materials and Methods”.

Effect of mancozeb on root colonization of *B. cereus* C1L. Our data did not show any apparently negative effect of mancozeb on induction of systemic resistance by *B.*

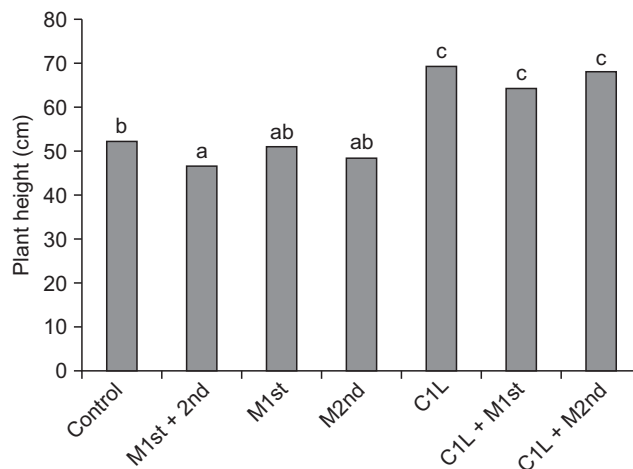


Fig. 3. Effects of mancozeb, *Bacillus cereus* C1L and a soil drench with *B. cereus* C1L in combination with a foliar spray of mancozeb on growth of corn plants under natural conditions. The composition and application program of each treatment is present in Table 1. *B. cereus* C1L and mancozeb (at the recommended dose of 25 mg active ingredient (a. i.)/l) were applied as a soil drench and a foliar spray, respectively. Detailed applications of mancozeb and rhizobacterial inoculum are described in “Materials and Methods”. Three days post the last treatment, corn plants were inoculated with spore suspensions of *Cochliobolus heterostrophus*. Plant height was measured two weeks post inoculation. Bars indicated with the same letter are not statistically different based on non-parametric Kruskal-Wallis and Mann-Whitney comparisons ($P < 0.05$).

Table 5. Root colonization by *Bacillus cereus* C1L under the absence or presence of a foliar spray with mancozeb

Treatment*	Strain C1L population (in log cfu/g of fresh root)	Significance
Control	-	-
C1L	6.17 ± 0.07	a
C1L + M1st	5.94 ± 0.24	b
C1L + M2nd	6.00 ± 0.08	ab

M, mancozeb; C1L, *B. cereus* C1L; -, indicates that no colony of *B. cereus* C1L was detected in the control treatment.

C1L population represents the mean log population size ± standard deviation.

Values followed by the same letter are not statistically different based on Tukey’s multiple comparisons ($P < 0.05$).

*The composition and application program of each treatment is present in Table 1. Detailed applications of mancozeb and rhizobacterial inoculum are described in “Materials and Methods”. Mancozeb was applied at the recommended dose (i.e., 25 mg active ingredient (a. i.)/l).

cerus C1L. Since dithiocarbamate fungicides are highly bacteriocidal against *B. cereus* C1L, we further investigated whether a mancozeb spray could affect populations of *B. cereus* C1L on corn roots. Populations of *B. cereus* C1L were able to reach $\sim 1.0 \times 10^6$ cfu/g of fresh root under the absence or presence of a mancozeb spray (Table 5). Compared to treatments C1L or C1L + M1st, the treatment C1L + M2nd did not cause a significant difference in populations of *B. cereus* C1L on the roots. Only the treatment C1L + M1st caused a significant but slight decrease in root colonization by *B. cereus* C1L in comparison to the treatment with *B. cereus* C1L alone (Table 5).

Discussion

In this study, we aimed to develop a strategy to reduce applications of dithiocarbamate fungicides such as maneb and mancozeb which are widely used in control of plant disease. Although maneb and mancozeb are of low acute toxicity in humans, chronic exposure to these dithiocarbamates may cause diseases or disorders including permanent parkinsonism (Costello et al., 2009; Ferraz et al., 1988; Meco et al., 1994), endocrine disruption (Cecconi et al., 2007; Iorio et al., 2014), and metal overload (Hoffman et al., 2016) in humans. Hence, the risks of frequent applications of dithiocarbamate fungicides are concerned. According to our investigation, programmed treatments of *B. cereus* C1L in combination with a proper foliar spray of mancozeb can provide sufficient protection in maize from southern leaf blight. Furthermore, times or frequency of mancozeb application can be properly reduced.

Soil drenches with *B. cereus* C1L were able to protect two widely planted commercial cultivars of maize from southern leaf blight (Fig. 1, 2), reflecting that treatments of *B. cereus* C1L may commonly provide effective protection in corn cultivars planted in Taiwan. Moreover, it has been demonstrated that *B. cereus* C1L-mediated induced systemic resistance in maize cv. Honey Jean No. 3 contributes to suppression of southern leaf blight (Huang et al., 2010). Thus, it is presumed that *B. cereus* C1L may commonly trigger systemic resistance in corn cultivars commercialized in Taiwan.

Sprays of dithiocarbamate fungicides near a heavy infection period can exhibited sufficient effectiveness of plant disease control. However, effectiveness of disease control by maneb and mancozeb declined when the applied dose was lower than the recommended dose or the interval between a spray of mancozeb and a heavy infection period was longer than 10 days, respectively (Fig. 1, 2). Thus, it is not economically feasible because the need for frequent applications and high application costs. Be-

sides, frequent applications also increase risks of exposure to dithiocarbamate fungicides. On the other hand, sequential treatments of *B. cereus* C1L can significantly suppress southern corn leaf blight (Fig. 2; Huang et al., 2010). As shown in Fig. 2, the application program including sequential treatments of *B. cereus* C1L in combination with a single spray of mancozeb near a heavy infection period (C1L + M2nd) was of equal effectiveness as programmed application of mancozeb (M1st + 2nd) under greenhouse conditions. Moreover, the protection level of treatment C1L + M2nd was significantly better than that of treatment M2nd under natural conditions (Table 4). Accordingly, it is suggested that regular treatments of *B. cereus* C1L are able to provide good levels of protection under moderate to low disease pressure. When disease pressure is increasing, a proper spray of mancozeb or the other dithiocarbamate fungicide on *B. cereus* C1L-treated plants is required for sufficient control of foliar disease in corn.

In addition to suppression of disease severity, application of *B. cereus* C1L significantly promoted growth of corn plants whether treated with mancozeb or not under natural conditions (Fig. 3). Growth of corn plants was slightly but significantly reduced by a frequent spray of mancozeb (M1st + 2nd). Our data is in full agreement with our previous study (Huang et al., 2010), indicating that applications of dithiocarbamates can effectively control foliar plant diseases but potentially cause certain negative effects on plant growth. Maneb was reported to be phytotoxic especially under hot conditions (Conover, 1956). Recently, Pereira et al. (2014) reported that exposure to mancozeb caused disturbance in metabolism of lettuce such as decreases in several amino acids and polyphenolics. Thus, it is suggested that frequent applications of dithiocarbamate fungicides to control plant diseases may result in disturbance/inhibition of plant growth depending on the dose of practical use or the environmental conditions. Excitingly, *B. cereus* C1L can effectively help growth of corn plants to overcome the negative effects of mancozeb on one hand. On the other hand, it is implied that a spray of mancozeb is not able to dramatically interfere plant growth promotion by *B. cereus* C1L. Thus, we suggest that appropriate uses of plant growth-promoting rhizobacteria may avoid the inhibition/disturbance of plant growth by dithiocarbamates.

Our results indicate that *B. cereus* C1L is incompatible with maneb and mancozeb, revealing that care should be taken when this biocontrol bacterium and dithiocarbamates are used together in an application program. Because induction of systemic resistance in plants contributes to the main mode of action of *B. cereus* C1L for biocontrol of plant foliar diseases (Huang et al., 2010; Liu et al., 2008), soil drenches with *B. cereus* C1L can pro-

vide its optimal protection in plants and prevent possible inhibition by foliar sprays of dithiocarbamates.

While *B. cereus* C1L was applied to the rhizosphere of corn plants once every two weeks in combination with or without a mancozeb spray, *B. cereus* C1L populations from all treatments were able to reach $\sim 1.0 \times 10^6$ cfu/g of fresh root (Table 5). Previous studies point out that populations of biocontrol PGPR should reach a concentration of $\sim 1.0 \times 10^5$ cfu/g of fresh root, a crucial level considered necessary for optimal biocontrol (Haas and D efago, 2005; Raaijmakers et al., 1999). Our data indicate that colonizing populations of *B. cereus* C1L were at a sufficiently high level for effective induction of systemic resistance and biocontrol, indicating that biocontrol *B. cereus* C1L can be applied in combination with bacteriocidal dithiocarbamate fungicides by using an appropriate method. Moreover, the data also support our hypothesis that for *B. cereus* C1L, potting mixtures (or soil in practical use) are able to provide a certain level of protection from direct contact with high doses of dithiocarbamates. Based on our findings, care should be taken when spraying dithiocarbamate fungicides at early growth stage of corn plants which are drench-treated with *B. cereus* C1L.

Therefore, our study provides the information that an intensive program of dithiocarbamate application is not absolutely necessary for sufficient control of plant disease. A regular, biweekly application of *B. cereus* C1L can reduce sprays of mancozeb and suppress southern corn leaf blight effectively. Feasible management of southern corn leaf blight can be achieved by sequential treatments of rhizobacterium *B. cereus* C1L in combination with proper sprays of dithiocarbamate fungicides. Collectively, using our strategy to control plant disease may further reduce risks of chronic exposure to mancozeb and the other dithiocarbamate fungicides. Application of PGPR can significantly promote plant growth and avoid the negative effects of dithiocarbamates on plants as well.

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