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Nematicidal activity of fipronil against *Pratylenchus zeae* in sugarcane

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Abstract

No nematicide has been registered for controlling plant-parasitic nematodes in sugarcane in Japan. Greenhouse and field experiments were conducted to evaluate the efficacy of the phenylpyrazole insecticide fipronil (0.3–0.45 kg a.i./ha) against the root-lesion nematode, *Pratylenchus zeae*, which is an important plant-parasitic nematode species associated with sugarcane (*Saccharum* spp. hybrids) in Okinawa, Japan. Both experiments showed a reduction of *P. zeae* population densities in sugarcane roots to 27 to 56% of the non-treated control after 7 wk in the greenhouse experiment and 3 months in the field experiment (two trials). In contrast, *P. zeae* population densities in soil were not reduced by the fipronil treatment. At harvest, sugarcane yields in the field experiment were significantly increased by 6 to 8% in the fipronil treated plots compared to the non-treated control. The data showed that fipronil reduced numbers of *P. zeae* in sugarcane roots at the early stage of sugarcane seedling growth resulting in increased sugarcane yields. This is the first report of nematicidal activity of fipronil against *P. zeae* under field conditions.

Keywords
Endoparasitic nematodes, Fipronil, Management, Nematicide, Nematode control, Pathogen, Plant-parasitic nematode, *Pratylenchus zeae*, Sugarcane, Yield loss.

Sugarcane (*Saccharum* spp. hybrids) is an economically important crop in subtropical and tropical regions. Okinawa is located in the subtropical region in Japan and half of its farmland acreage is used for sugarcane cultivation. Many remote islands in Okinawa are economically reliant on sugarcane-related industries.

Plant-parasitic nematodes are one of the major yield limiting factors of sugarcane production (Cadet and Spaull, 2005). Previous studies revealed that 20 to 30% sugarcane yield losses were observed for both plant and ratoon crops due to the root-lesion nematode, *Pratylenchus zeae* (Kawanobe et al., 2014, 2016, 2019). Despite the very significant yield losses caused by the nematode, no nematicide has been registered for the control of plant-parasitic nematodes on sugarcane in Japan. Though alternative approaches to control plant-parasitic nematodes in sugarcane fields are available, such as antagonistic plants and crop rotation, nematicides may be an effective tool for farmers to manage nematodes in sugarcane.

Fipronil (5-Amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(trifluoromethyl) -1H-pyrazole-3-carbonitrile]; CAS number 120068-37-3) is known to block the γ-aminobutyric acid (GABA)-gated chloride channel and is the first phenylpyrazole insecticide (Cole et al., 1993). Fipronil is a highly active, broad spectrum insecticide that is widely used to control sugarcane pests such as the pink stem borer and the wireworm (Kawasaki et al., 2014). Fipronil is registered, as a nematicide, for the rice white tip nematode (*Aphelenchoides besseyi*), yet, to the best of our knowledge, it is not registered for *Pratylenchus* spp. Kawanobe et al. (2014) showed that *P. zeae* submerged in a fipronil water solution became inactive within 48 hr, while fipronil treated soil did not have an observable effect on the nematode.

The result suggested that fipronil may not be effective against *P. zeae* in soil. However, this may not affect its nematicidal activity on the nematode in roots because of the systemic property of fipronil. Therefore, the objective of this study was to evaluate the effects of fipronil treatments on numbers of *P. zeae* in sugarcane roots and soil, and on the sugarcane yield.

Materials and methods

Identification of root-lesion nematodes

Before the greenhouse and field experiments, the root-lesion nematodes present in the sugarcane field soils were confirmed to be predominantly *P. zeae* using the real-time PCR method (Kawanobe et al., 2015). In the soil for the greenhouse experiment, 100% ($n = 8$) were *P. zeae* and in the field experiment, 71% ($n = 7$) and 100% ($n = 8$) were *P. zeae* for Trials 1 and 2, respectively. The other root-lesion nematodes were confirmed to be *P. coffeae* using unpublished real-time PCR primers.

Nematicide

The fipronil products used in the experiments, Prince® in granular form: 1% active ingredient (a.i.) for the greenhouse experiment and Prince® Bait: 0.5% a.i. for the field experiment, were supplied by BASF Japan (Tokyo, Japan). Prince® Bait (0.5% a.i.) has been registered for pests including the sugarcane wireworm.

Greenhouse experiment

A greenhouse experiment was conducted to test nematicidal efficacy of fipronil against the root-lesion nematodes in soil and in sugarcane roots. Additionally, initial growth of sugarcane seedlings was evaluated. The experiment was conducted in a greenhouse for 7 wk on Kitadaito Island (25°56′N, 131°17′E), Okinawa, Japan. The soil used for the greenhouse experiment was collected in May 2016 from 0 to 30 cm depth in a sugarcane field, which was known to be infested with root-lesion nematodes. On the same day of collection, the soil (sand 0.1%, silt 27.8%, clay 72.1% with total C 16.8 mg/g, total N 2.0 mg/g, pH (H_2O) 4.7, and EC 400 µS/cm) was well mixed. Then, a 2.8 kg subsample was mixed with fipronil at 0.34 g/kg soil (Prince® granular, 0.3 kg a.i./ha; equivalent to 30 kg/ha in the planting furrow) and chemical fertilizer at 0.61 g/kg soil (49-26-50 N-P-K). The soil was put into each plastic pot (inside diam. 17 cm and 15 cm height) with a hole in the bottom. Two single bud setts of sugarcane cv. Ni29, one of the commonly grown cultivars in Okinawa, were planted in each pot, watered when necessary, and grown for 7 wk. Pots without fipronil were also prepared and used as a control. There were three replicates per treatment.

After 7 wk, culm height and the number of fully extended leaves per plant were measured and each plant was removed from the pot. Roots were carefully washed and kept at room temperature for no more than a day until nematode extraction. The roots were cut into 1–2 cm pieces and homogenized in water with a blender (BL143GJP, T-fal, Tokyo, Japan) for 15 sec. From 1 g fresh root subsample, nematodes were extracted using the Baermann funnel method (Kawanobe et al., 2019), at room temperature and collected after 48 h. The soil in each pot was collected and passed through a 5 mm aperture sieve to remove rocks and debris, mixed well and kept at room temperature for no more than a day before nematodes were extracted. Nematodes were extracted from 20 g subsamples of soils using the Baermann funnel method (Ingham, 1994) at room temperature and collected after 72 h. Nematodes were counted and identified based on their morphological characters under a stereo-microscope (SZX10, Olympus, Tokyo, Japan). The root-lesion nematodes extracted from roots were randomly selected (five or more nematodes per experiment) and identified using the real-time PCR method (Kawanobe et al., 2015).

Field experiment

In total, 32 experimental plots (8 m length × 1.35 m width; sugarcane variety cv. Ni22) were established in each of the two trials. Fipronil treated and non-treated control plots were prepared in 16 replicates. The experimental fields were also known to be infested with an economically important insect, the sugarcane wireworm, and clothianidin (Dantotsu® for sugarcane wireworm control, Sumitomo Chemical, Osaka, Japan; 0.5% a.i.) was applied in a granular form at 60 kg/ha (0.3 kg a.i./ha) for controlling the wireworm in the non-treated control in Trial 1 and in the entire experimental field in Trial 2. The soils in the two field sites had the following properties: Trial 1 was a silty clay (sand 3%, silt 40%, clay 57%), with total C 13.5 mg/g, total N 1.0 mg/g, pH (H_2O) 4.3, and EC 98 µS/cm and Trial 2 was a silty clay loam (sand 3%, silt 58%, clay 39%), with total C 13.3 mg/g, total N 1.2 mg/g, pH (H_2O) 3.9, and EC 147 µS/cm.

Two-bud sugarcane setts were planted in March 2017 (Trial 1) and March 2018 (Trial 2). Sugarcane plants were grown under the conventional management practice for 11 months and harvested in February 2018 and 2019, respectively. Fipronil (Prince® Bait) was applied in a granular form at

90 kg/ha (0.45 kg a.i./ha), which is the manufacturer's recommended rate for the sugarcane wireworm (*Melanotus* sp.) in the planting furrow just before spring planting and incorporated by tillage.

Roots from a randomly selected sugarcane shoots were collected from each of 32 plots at 3- and 6-month to determine numbers of root-lesion nematodes. Root samples were kept at room temperature for no more than a day before the nematodes were extracted using the methods described for the greenhouse experiment.

Soil was collected at 0- (just before cane planting), 1-, 3-, and 6-month after sugarcane planting and 11-month (at harvest; Trial 1 only). The soil was collected with an auger (3 cm diam.) at 0 to 30 cm depth within 10 to 15 cm of the base of one or two randomly selected sugarcane plants in each plot. Four plot samples were combined to make a composite sample; thus there were four replicates per treatment rather than 16 for the soil samples. The soil was passed through a 5 mm aperture sieve to remove rocks and debris, mixed well and kept at room temperature for no more than two days before nematodes were extracted using the methods described for the greenhouse experiment.

After 11 months of growth, the number of canes in each plot was counted. Then, all the millable stalks were harvested from 4 m sections in the middle of each plot. The number of stalks and the total stalk weight per hectare were recorded and average single stalk weight was calculated. Of the stalks harvested from each plot, 12 were randomly chosen to measure length, diameter, and the value of Brix.

Statistical analysis

The statistical differences were determined by Student's *t*-test comparing control and test groups (Greenhouse experiment) or analyzed by ANOVA (field experiment) where the effect of treatment, trial, and treatment × trial on nematode densities and sugarcane yield were included in the model. Statistical analyses were conducted using Microsoft Excel and its add-in software Statcel (3rd ed., OMS, Tokyo, Japan).

Results

Greenhouse experiment

Initial root-lesion nematode densities were 21 nematodes/20 g soil. After 7 wk of sugarcane growth, root-lesion nematode densities were 63 and 70 nematodes/20 g soil in control and fipronil treatments, respectively, and no significant difference between the treatments was observed. Root-lesion nematode population densities in roots after 7 wk were greater ($P < 0.01$) in the control (1,550/g root) than the fipronil treatment (764/g root). Root-lesion nematodes (100%; $n = 5$) extracted from sugarcane roots at the end of the greenhouse experiment were confirmed to be *P. zeae* by real-time PCR. Culm height and numbers of fully extended leaves after 7 wk of sugarcane growth did not differ between the treatments.

Field experiment

Free-living, root-lesion, and spiral nematodes were found throughout 2017 and 2018 spring-planted crops (Trials 1 and 2). Population densities of free-living and lesion nematode tended to increase after planting, whereas densities of the spiral nematode tended to decrease or remain the same (Fig. 1). However, densities of these nematodes did not differ between the treatments. Root-lesion nematode densities in roots after three months of sugarcane growth in the fipronil treatment were lower ($P < 0.01$) than in the control by 44% and 73% in Trials 1 and 2, respectively (Fig. 2A). There was an interaction between treatment and trial ($P < 0.05$) for the densities of *P. zeae* in roots at three months. Nevertheless, in both trials, root-lesion nematode densities were lower ($P < 0.01$) in the fipronil treatment than in the control, although the magnitude of the difference between treatments was greater for Trial 2 than for Trial 1 (Fig. 2A). Root-lesion nematodes (100%; $n = 5$ and 9) extracted from sugarcane roots at three months in Trials 1 and 2, respectively, were confirmed to be *P. zeae* by real-time PCR. The population densities after six months of sugarcane growth were almost equivalent between the treatments (Fig. 2B).

The millable stalk weight in Trials 1 and 2 were more ($P < 0.05$) in the fipronil treatment than in the non-treated control by 6% and 8%, respectively (Fig. 3A) and these results were consistent among trials (no treatment × trial interaction). The numbers of millable stalks were greater ($P < 0.05$) in the fipronil treatment than in the non-treated control by 5% and 6% in Trials 1 and 2, respectively (Fig. 3B). The average single stalk weight in Trials 1 and 2 were greater in the fipronil treatment than in the non-treated control by 1% and 3%, respectively, but not statistically significant (Fig. 3C). Stalk length was 5% longer ($P < 0.05$) in the fipronil treatment than in the non-treated control in Trial 1 but not in Trial 2 (Fig. 3D). Millable stalk diameter was not different between treatments in both Trials 1 and 2 (Fig. 3E). The Brix in Trial 1 was higher ($P < 0.05$) in the fipronil treatment than in the non-treated control by 2%, although there was no difference in Trial 2 (Fig. 3F). For single stalk

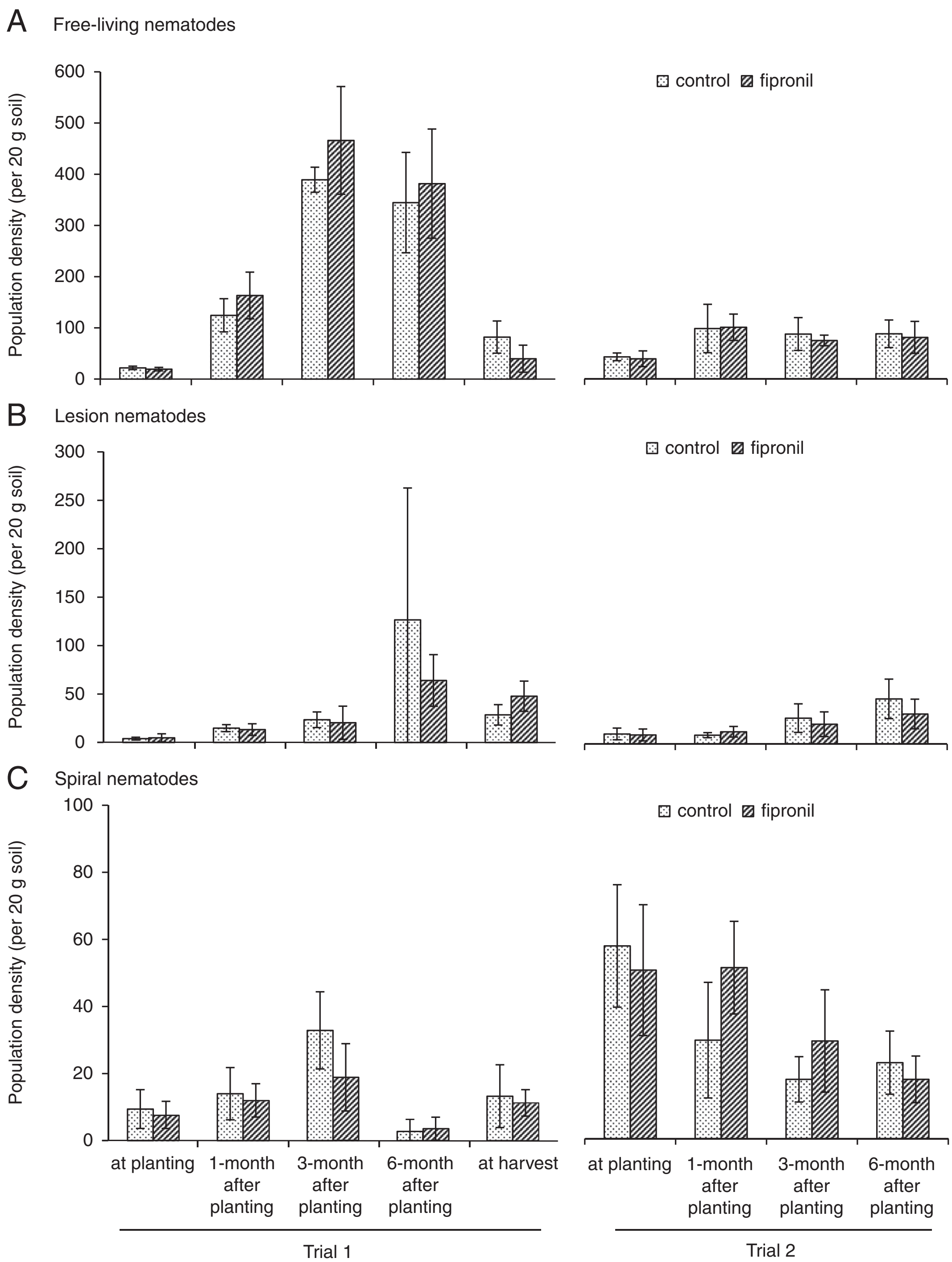

Figure 1: Nematode population densities in soil in Trials 1 and 2 of the field experiment. (A) Free-living nematodes; (B) Root-lesion nematodes (*Pratylenchus zeae* and *P. coffeae*), and (C) Spiral nematodes (*Helicotylenchus* sp.). Each bar is the mean of four replicates ± standard deviation.

weight, stalk length, diameter, and the value of Brix, no significant difference was observed between in the fipronil treatment and the non-treated control in the combined analysis of Trials 1 and 2.

Discussion

In the current study, both the greenhouse and field experiments consistently showed nematicidal

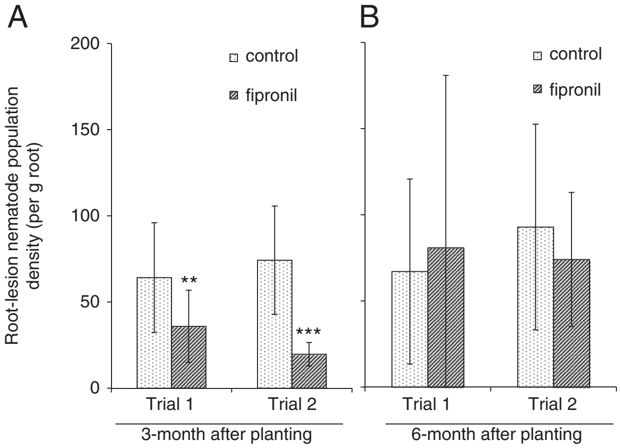

Figure 2: Root-lesion nematode (*Pratylenchus zeae*) densities in sugarcane roots in Trials 1 and 2 of the field experiment. Nematodes were extracted from roots at (A) three months after planting and (B) six months after planting. Each bar is the mean of 16 replicates ± standard deviation. Asterisks indicate significant difference from the control (**$P < 0.01$ and ***$P < 0.001$). There was an interaction between treatment and trial ($P < 0.05$) for the three-month analysis.

efficacy of fipronil against *P. zeae* in roots, while no such activity against *P. zeae* in soil was observed. In addition, the free-living and spiral nematodes in soil were not reduced by the fipronil treatment compared to the control. These results were consistent with the previous study (Kawanobe et al., 2014), which demonstrated that *P. zeae* in a fipronil water solution became immobile at 22.5 ng a.i./100 µl (0.45 kg a.i./ha) but not in soil with 0.7 times the concentration of fipronil (0.3 kg a.i/ha). In the same study, a 3.3 times higher dose of fipronil (1.5 kg a.i/ha) decreased *P. zeae* population density in the soil to half of the non-treated control. The observation that fipronil is effective in solution but not in soil may be due to its hydrophobic and high soil adsorption characteristics (Kf: 11.85; Kfoc: 727; 1/n: 0.95; IUPAC Pesticide Properties Database: https://sitem.herts.ac.uk/aeru/iupac/Reports/316.htm). Fipronil may be absorbed by soil and thereby decrease its efficacy against nematodes in soil. Other studies (Aajoud et al., 2006, 2008) showed that fipronil could be absorbed by root and transported into sunflower leaves. Fipronil may be taken up by sugarcane roots where it has activity against plant-parasitic nematodes. The exact mechanism of control of *P. zeae* by fipronil is unknown and further studies are necessary.

Fipronil's nematicidal activity was not observed in roots six months after cane planting in the field experiment. This may be due to fipronil's dissipation in soil since its half-life is 30 to 33 days in aerobic soil (Mandal and Singh, 2013). Further research is needed to determine whether fipronil can be applied later in the season or applied multiple times to the soil to extend the period of nematode control.

Fipronil is a broad spectrum N-phenylpyrazole insecticide that inhibits GABA-gated chloride channels, and has a high affinity for insects compared to mammalian GABA receptors (Mohamed et al., 2004). In addition to insects, fipronil is effective for control of nematodes including the white tip nematode (*A. besseyi*) on rice (Cuc et al., 2010). Fipronil, however, is not expected to be effective against all nematode species. For example, it was found to cause differential mortality (17–100%) of three entomopathogenic nematode species *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. arenarium* (Gunasekara et al., 2007), and it is likely that fipronil will vary in its efficacy against different plant-parasitic nematodes. To the best of our knowledge, there have been no other studies testing the efficacy of fipronil against *P. zeae*.

The 7-wk greenhouse experiment did not show clear differences in seedling growth between treatments. However, in the 11-month field experiment, sugarcane yields were greater (by 6–8%) in the fipronil treatment than in the control. This suggests that *P. zeae* population densities in roots at the early growth period of sugarcane can affect yield. The nematicide fosthiazate showed much greater reduction in the number of the root-lesion nematodes in sugarcane field soils (Kawanobe et al., 2014, 2016) and in sugarcane roots (Kawanobe et al., 2019) in the early growth period than later in the season. These studies (Kawanobe et al., 2016, 2019) showed 20% more sugarcane yield in the spring-planted crop and the ratoon crop by applying fosthiazate. Fipronil may not be as effective as fosthiazate at the dose applied in this study, yet it still suppressed *P. zeae* in sugarcane roots and resulted in greater sugarcane yields than the non-treated control. The yield response by fipronil application may be due to the greater number of millable stalks compared to other yield components. The result was consistent with the previous study on fosthiazate (Kawanobe et al., 2016). Further, fipronil has an advantage as a nematicide in sugarcane, as it is already used to control sugarcane pests in Okinawa and thus will not cause additional input costs to sugarcane farmers. This is the first report of the nematicidal

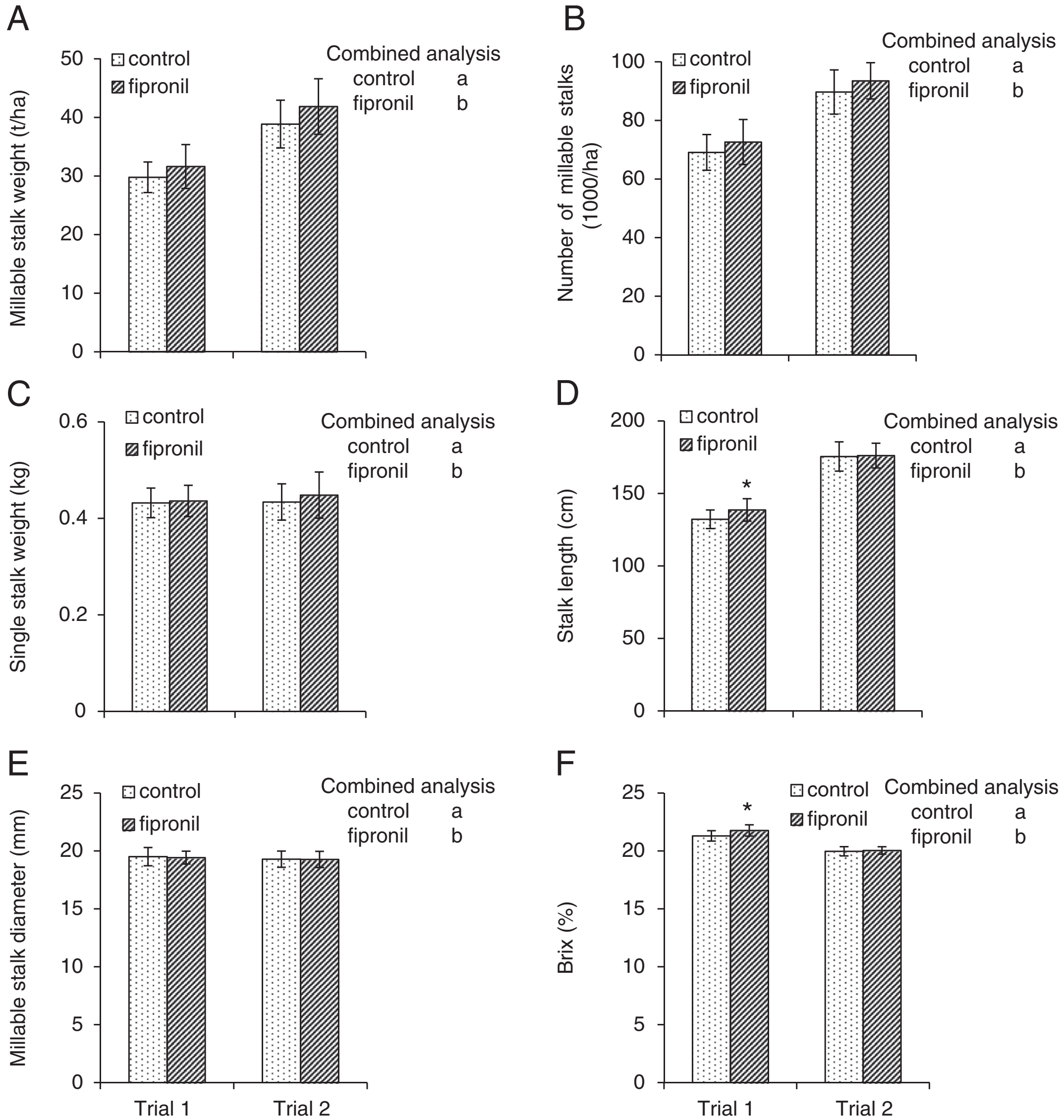

Figure 3: Effect of fipronil treatment on sugarcane yield in Trials 1 and 2 of the field experiment. (A) Millable stalk weight; (B) Number of millable stalks; (C) Single stalk weight; (D) Stalk length; (E) Millable stalk diameter; and (F) Brix. Each bar is the mean of 16 replicates ± standard deviation (*$P < 0.05$). Different letters indicate significant difference ($P < 0.05$) between the treatments when the trials were analyzed together in ANOVA. There was no interaction between treatment and trial.

activity of fipronil against *P. zeae* in sugarcane roots in the field environment.

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