

Evaluation of alternative rice planthopper control by the combined action of oil-formulated *Metarhizium anisopliae* and low-rate buprofezin

Shao-Feng Jin, Ming-Guang Feng,* Sheng-Hua Ying, Wen-Jing Mu and Jue-Qi Chen

Abstract

BACKGROUND: High resistance of brown planthopper (BPH) *Nilaparvata lugens* Stål to common insecticides is a challenge for control of the pest. An alternative control strategy based on the combined application of fungal and chemical agents has been evaluated.

RESULTS: Three gradient spore concentrations of oil-formulated *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma456) were sprayed onto third-instar nymphs in five bioassays comprising the low buprofezin rates of 0, 10, 20, 30 and 40 $\mu\text{g mL}^{-1}$ respectively. Fungal LC_{50} after 1 week at 25 °C and 14 : 10 h light : dark photoperiod decreased from 386 conidia mm^{-2} in the buprofezin-free bioassay to 40 at the highest chemical rate. Buprofezin (LC_{50} : 1647, 486 and 233 $\mu\text{g mL}^{-1}$ on days 2 to 4) had no significant effect on the fungal outgrowths of mycosis-killed cadavers at the low application rates. The fungal infection was found to cause 81% reduction in reproductive potential of BPH adults. In two 40 day field trials, significant planthopper (mainly BPH) control (54–60%) was achieved by biweekly sprays of two fungal candidates (Ma456 and Ma576) at 1.5×10^{13} conidia ha^{-1} and elevated to 80–83% by incorporating 30.8 g buprofezin ha^{-1} into the fungal sprays.

CONCLUSION: The combined application of the fungal and chemical agents is a promising alternative strategy for BPH control.

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Keywords: *Nilaparvata lugens*; fungal formulation; *Metarhizium anisopliae*; buprofezin; fungal and chemical interaction; time-concentration-mortality modelling; field control efficacy

1 INTRODUCTION

Hopperburn caused mainly by brown planthopper (BPH) *Nilaparvata lugens* Stål threatens global rice crops, particularly in Asia.¹ Long-term reliance on chemical control has caused high brown planthopper resistance to common insecticides. For instance, imidacloprid has been compromised by resistance development in BPH^{2–4} and other sucking pests^{5,6} since the 1990s and is no longer recommended for BPH control in China. This presents BPH control with the dilemma of choosing efficacious insecticides at reasonable cost. One of a few choices is buprofezin [(Z)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one], a moulting interferent.^{7–9} However, BPH resistance to buprofezin is also developing.¹⁰ Thus, cautious use of this chemical is necessary for its prolonged market life. An alternative strategy is to reduce chemical pressure on BPH by exploiting the knockdown action of chemicals and the longer effect of fungal biocontrol agents such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin.^{11,12}

Fungal candidates with desired potential for BPH control are scarce, although many isolates of *B. bassiana*, *M. anisopliae* and *Isaria fumosorosea* Wize are highly infective to other sucking pests.^{13–18} In the 1980s, a large number of fungal isolates were

bioassayed on BPH but rarely caused >70% mortality.¹² Early field trials resulted in inconsistent BPH control.^{19,20} In a recent study on 35 isolates, only two *M. anisopliae* isolates (Ma456 and Ma576) caused >50% BPH mortalities under a concentrated spray of unformulated conidia, and had the LC_{50} values of 731 and 1124 conidia mm^{-2} on day 7 and of 284 and 306 conidia mm^{-2} on day 10.²¹ In another study, the LC_{50} of a *B. bassiana* isolate highly virulent to aphids²² was 1652 unformulated conidia mm^{-2} against BPH on day 7, decreasing to 1016 conidia mm^{-2} when applied as oil formulation, and further dropping to 503, 135 and 26 conidia mm^{-2} when the formulation was sprayed together with the low imidacloprid rates of 0.5, 1.0 and 2.0 $\mu\text{g mL}^{-1}$ respectively.²³ Thus, the combined application of oil-formulated *M. anisopliae* and buprofezin is likely to be an alternative measure for BPH control

* Correspondence to: Ming-Guang Feng, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, People's Republic of China. E-mail: mgfeng@zju.edu.cn

Institute of Microbiology, College of Life Science, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China

because the chemical has little effect on the conidial viability of fungal agents^{24–26} and is less toxic to paddy predators.²⁷

This study sought to evaluate the feasibility of alternative BPH control based on selected fungal isolates and buprofezin. The effects of the fungal and chemical agents on BPH nymphs were assessed in a series of bioassays by means of time–concentration–mortality (TCM) modeling.^{28–30} Fungal effects on the potential of BPH reproduction were evaluated in the laboratory. Two field trials were carried out to assess the control efficacies of applying the *M. anisopliae* formulation alone or together with a selected low rate of buprofezin.

2 MATERIALS AND METHODS

2.1 Fungal isolates and conidial preparation

Two isolates selected for this study²¹ were *M. anisopliae* 456 (Ma456) and 576 (Ma576) (ARSEF accession numbers; RW Holley Center for Agriculture and Health, Ithaca, NY) and derived from *N. lugens* in the Philippines and Indonesia respectively.

Aerial conidia of both isolates were produced on steamed rice. For the laboratory study, rice cultures in 15 cm diameter petri dishes were incubated at 25 °C and 12:12 h light:dark photoperiod for ca 7 days using 2 day liquid culture (Sabouraud dextrose broth) as inoculum. The rice cultures were dried under ventilation at 30 °C for 24 h and harvested through a vibrating sieve. For the field study, rice cultures were produced in an upright multitray conidiation chamber (60 × 60 × 200 cm),³¹ and aerial conidia were harvested using an MK-V cyclone spore separator (CABI Bioscience, UK). All conidial preparations produced as above were further vacuum dried to ca 5% water content, followed by immediate use or storage at 4 °C for use as soon as possible in the following experiments, warranting ≥92% viability.

2.2 Assays of fungal and chemical effects on BPH nymphs

Third-instar BPH nymphs were prepared for bioassays following previous protocol.^{21,23} Five bioassays of three fungal concentrations were conducted on the nymphs in caged cups over a period of 3 months. Ma456 formulations of 2×10^{10} , 2×10^9 and 2×10^8 conidia mL⁻¹ were made by suspending conidia in mineral oil (paraffin) containing 5% (v/v) emulsifier AEO-3 (Xiaoshan Chemical Additives Co., Hangzhou, China) and diluted to 2×10^8 , 2×10^7 and 2×10^6 conidia mL⁻¹ with water. The diluted spore suspensions plus blank control (100-fold aqueous dilution of the emulsion) were separately sprayed, from low to high concentrations, onto 30–40 nymphs on 3 cm high seedlings in uncaged cups using a handheld Micro Ulva sprayer (Micron Sprayers Ltd, Herefordshire, UK). Five bioassays had the same fungal treatments and blank control but with the addition to the fungal sprays of buprofezin 200 g kg⁻¹ WP (Pu-Shi-Ling; Sanshan Pesticide Co. Ltd, Huai'an, Jiangsu, China) at the low rates of 0, 10, 20, 30 and 40 μg AI mL⁻¹ (designated as B₀, B₁₀, B₂₀, B₃₀ and B₄₀ respectively). Apart from the blank control, the corresponding chemical rate was used as the second control in each bioassay. Two more application rates of buprofezin, 100 and 200 μg AI mL⁻¹ (B₁₀₀ and B₂₀₀), and blank control were included in a separate assay to assess the lethal effect of buprofezin on BPH nymphs. Four replicates (30–40 nymphs each) were included in each treatment.

After spray, the cups were caged again and maintained in a growth chamber at 25 °C and 14:10 h light:dark photoperiod. The concentration of conidia deposited onto the nymphs and seedlings was measured as number of conidia mm⁻² using microscopic

counts of conidia collected onto four glass slips (20 × 20 mm) under each spray. All sprayed nymphs were examined daily for survival and death records. Cadavers found in the cages at each time were transferred to moist petri dishes for further incubation, and those with a layer of fungal outgrowths (i.e. mycelium and conidia) were considered to have been mycotised.

2.3 Assays of fungal effects on BPH fecundity and longevity

Brachypterous adults (≤24 h after last ecdysis) on seedlings in uncaged cups were sprayed with a suspension (1×10^8 conidia mL⁻¹) of unformulated Ma456 conidia in 0.02% (v/v) Tween-80 using the same method. The concentration of deposited conidia was 1021 (967–1055) conidia mL⁻¹, based on microscopic counts from three glass slips for spore collection. A blank control was sprayed with 0.02% Tween-80 only.

After spraying, males and females were paired, and each pair was transferred onto a single 40 day rice plant in a top-meshed glass tube (3 cm diameter × 25 cm height). The roots of each plant were wrapped with a sterile cotton ball saturated with rice nutrition solution. All paired adults (30 pairs for both fungal treatment and control) were maintained at 25 °C and 14:10 h light:dark photoperiod and examined daily for the number of eggs deposited in the sheaths of each plant and the death times of the adults. Each pair was transferred to a new plant for feeding and oviposition after daily examination, and the plant harbouring the eggs laid on the previous day was kept at the same regime for egg hatch, which was also examined daily for ca 20 days until no more eggs hatched on three consecutive days. Nymphs found at each time were removed from the plant. All unhatched eggs were counted as dead.

2.4 Field trials

Two late-season rice fields (0.14 ha each) of hybrid cultivar (*Oryza sativa* L. var. Zhongzheyou No. 1) were located at a rice farm in Jinhua, Zhejiang, for repeated trials from 17 August to 28 September 2008. The rice plants of the two fields, ca 100 m away from each other, were tillering (average 21.2 ± 4.8 tillers per hill) at the starting time. Each trial consisted of seven treatments: (1) sprayed with water (control 1); (2) sprayed with 500-fold aqueous dilution of fungus-free emulsion (control 2); (3) sprayed with the same dilution containing buprofezin 200 g kg⁻¹ WP at 40 μg AI mL⁻¹, i.e. one-fifth of its labelled application rate (B_{1/5}); (4) and (5) sprayed with 500-fold aqueous dilutions (2×10^7 conidia mL⁻¹) of oil formulation Ma456 and Ma576 (standardised to 1×10^{10} conidia mL⁻¹ with 6% emulsifier); (6) and (7) sprayed with the same dilutions of Ma456 and Ma576 in conjunction with the low buprofezin rate (Ma456 + B_{1/5} and Ma576 + B_{1/5}).

Both fields were under regular management with no chemical spray, and each was divided into 13 × 3 m plots for the treatments of three replicates in randomised block design. Edges of 2 m width and intervals between plots of 1 m were used as buffer areas (not sprayed). The first spray was conducted in the two fields on 17 and 18 August respectively, followed by two more sprays at 14 day intervals. All sprays were performed in the evening to protect the applied conidia from solar UV irradiation. Each plot was sprayed with 3 L dilution (= 769 L ha⁻¹) at each time using a gas-driven backpack sprayer 3 MF-50 (Tiandi Machinery Co., Jiaxing, Zhejiang). Thus, a rate of 1.54×10^{13} conidia ha⁻¹ was applied in the fungal treatments, while buprofezin was applied at 30.8 g ha⁻¹ in the chemical-inclusive treatments.

Initial counts of nymphs and adults of rice planthoppers were made *in situ* the day before the first spray by sampling early in the

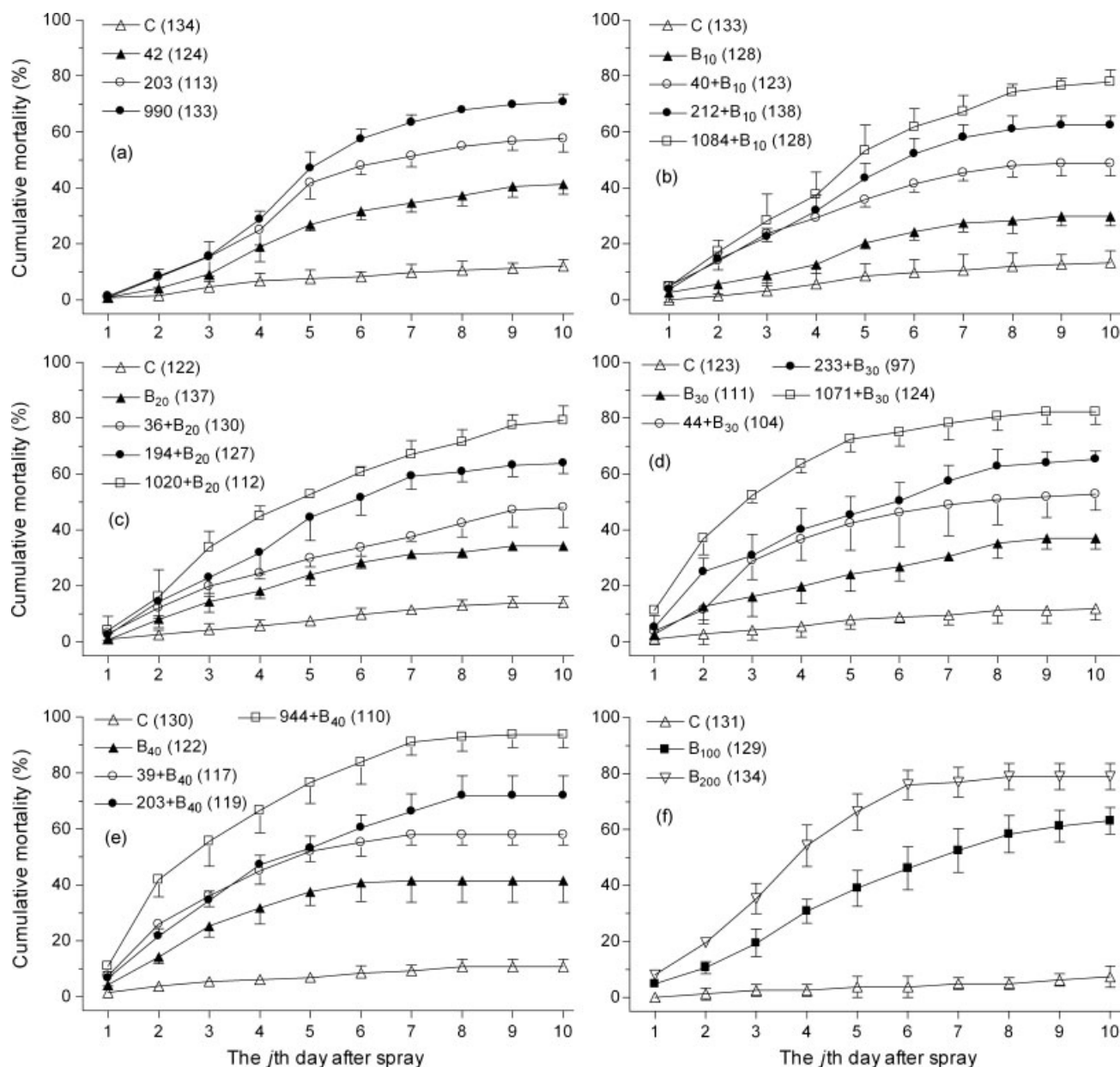


Figure 1. Mortality trends of BPH nymphs over days after spraying oil-formulated *Metarhizium anisopliae* (Ma456). (a) Fungal sprays; (b) to (e) fungal sprays containing 10, 20, 30 and 40 μg buprofezin Al mL^{-1} (B_{10} to B_{40}) respectively; (f) buprofezin sprays at 100 and 200 μg Al mL^{-1} (B_{100} and B_{200}). Symbols denote low, median and high concentrations of fungal sprays (number of conidia mm^{-2}) and blank control (C). Error bars: SEM from four replicates (with the total number of treated nymphs given in parentheses).

morning (i.e. prior to dew drying). Five sample sites were fixed at equal intervals along the middle line of each plot. At each site, all nymphs and adults on two hills were gently patted into a white tray and immediately counted. BPH adults were distinguished as much as possible from those of the whitebacked planthopper (WBPH), *Sogatella furcifera* Hovarth, at the counting time, but their nymphs were pooled. After the first spray, planthopper densities (counts per two-hill sample) were monitored weekly using the same sampling method. Field sampling was always terminated prior to 9:30 am. As mycosed insects tended to fall into the paddy field, the counts of living ones were used for computing field efficacies of all treatments.

An electronic hydrothermometer (Zheda Electric Apparatus Inc., Hangzhou, Zhejiang, China) was hung on a stick 30 cm above the

soil surface at the centre of one field to take half-hourly records of field temperature and relative humidity (RH) during the trial. Rainfall records were obtained from the local weather station ca 3 km away from the farm.

2.5 Statistical and modelling analyses

Variations in the deposits of sprayed conidia (in logarithms) and the percentages of mycotised cadavers (in arcsine square roots) were differentiated by two-way analysis of variance (ANOVA). TCM datasets were fitted to a TCM model,^{28–30} generating parameters for the effects of spore concentration and post-spray time and the interaction of both from each bioassay. The fitted parameters were then used to compute LC_{50} values and associated 95% confidence limits (CL) over days after spray and LT_{50} values declining with spore

concentrations. Both LC_{50} and LT_{50} trends were plotted to show the combined effects of the fungal formulation and buprofezin at the low application rates. The TCM data observed at buprofezin rates of $10\text{--}200\ \mu\text{g mL}^{-1}$ in all assays were also subjected to the same modelling analysis for assessing the chemical LC_{50} values over days after spray.

The counts of eggs laid female $^{-1}$ day $^{-1}$ after spraying and of those hatched day $^{-1}$ after oviposition were graphed for daily comparisons between fungal treatment and blank control and examined by a likelihood-ratio G -test. Overall means of fecundities and hatch rates based on the daily counts per female were calculated and compared by Student's t -test. Mean life spans of the males and females were also compared between the two treatments via the t -test.

For all treatments of the field trials, the planthopper densities (in logarithms) and the proportions of nymphs (in arcsine square roots) were subjected to two-way ANOVA. The efficacy (E) relative to control 1 was computed as $E = [1 - (d_{C_0} d_{T_i}) / (d_{C_i} d_{T_0})] \times 100$, where d_{C_0} and d_{T_0} are the initial densities estimated from control 1 and a given treatment, and d_{C_i} and d_{T_i} are the densities from the control and the treatment on the i th day after the first spray. All the statistical and modelling analyses were completed using DPS software.³¹

3 RESULTS

3.1 Time–concentration–mortality trends in bioassays

The gradient concentrations of oil-formulated Ma456 conidia deposited onto BPH nymphs were 41 (36–45), 208 (194–233) and 1016 (944–1084) conidia mm^{-2} respectively. These deposits differed significantly among the fungal concentrations ($F_{2,42} = 2500$, $P < 0.01$) but were similar in the five bioassays irrespective of the application rate of buprofezin ($F_{4,42} = 1.8$, $P = 0.15$).

BPH mortality trends in the bioassays are illustrated in Fig. 1. Generally, the mortalities increased with the fungal or chemical concentrations and the time length after spray. Percent means (\pm SD) of mycotised cadavers (Fig. 2a) differed significantly among the three fungal treatments ($F_{2,42} = 8.3$, $P < 0.01$) but were not affected by the low rates of buprofezin ($F_{4,42} = 1.2$, $P = 0.33$). No cadavers in the chemical and control treatments were mycotised.

The TCM data fitted the TCM model very well ($P \geq 0.13$ in homogeneity tests for the goodness of fit), generating parameter estimates for sound TCM relationships for all the bioassays. As a result, the LC_{50} and 95% CL of the fungal formulation (Fig. 2b) against BPH nymphs dropped from 1426 (853–2381) conidia mm^{-2} on day 5 to 199 (145–273) on day 10 after spray. The same estimates were greatly reduced by including a low rate of buprofezin into the fungal sprays, e.g. dropping from 113 (70–181) to 27 (13–56) conidia mm^{-2} at $40\ \mu\text{g mL}^{-1}$ during the same period. The inclusion of $\leq 20\ \mu\text{g}$ buprofezin mL^{-1} did not significantly reduce the fungal LC_{50} values, as indicated by partially overlapped 95% CL. Interestingly, the fitted TCM relationship for buprofezin alone gave LC_{50} values of 1647 (750–3614), 486 (291–811), 233 (161–338), 137 (103–183), 95 (75–121) and 79 (63–98) $\mu\text{g mL}^{-1}$ on days 2 to 7 respectively. This indicates that the knockdown effect of the chemical on BPH was mild in this study, reflecting the 100-fold dilution based on the label rate for the $200\ \text{g kg}^{-1}$ buprofezin WP (750–1125 g ha^{-1} in 750 L, i.e. $150\text{--}225\ \mu\text{g AI mL}^{-1}$). On the other hand, the LT_{50} values estimated by interpolating the fitted TCM relationships decreased with increase in fungal concentration (Fig. 2c). Taking 500 conidia mm^{-2} , for example, the LT_{50} values were 6.4 days for the fungal formulation alone and 5.7, 5.8, 3.9

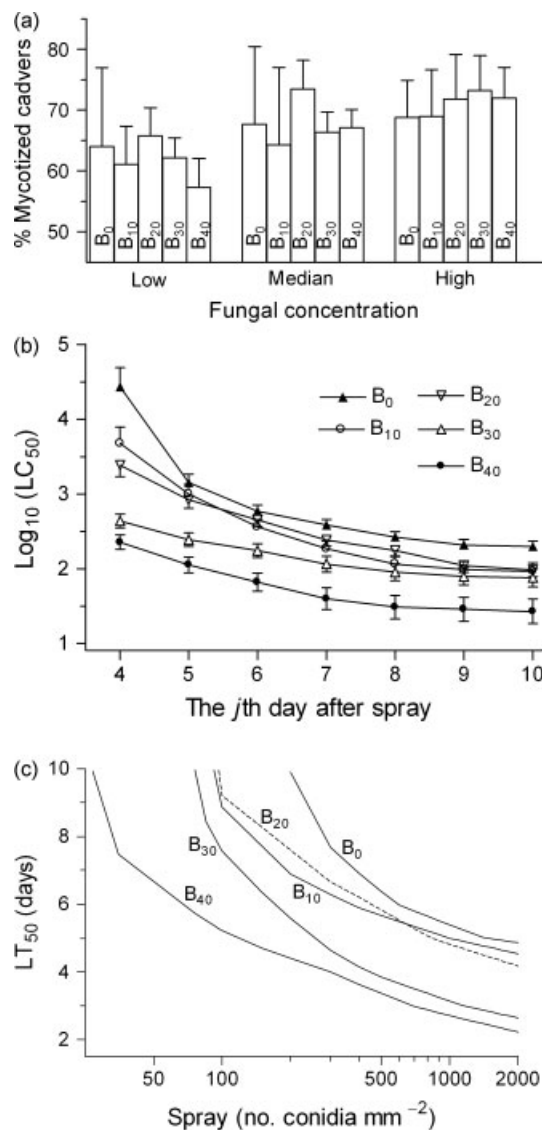


Figure 2. Lethal effects of oil-formulated *Metarhizium anisopliae* (Ma456) and buprofezin on BPH nymphs. (a) Percentages (error bars: SD) of mycotised cadavers at the low, median and high concentrations of fungal sprays containing 0, 10, 20, 30 and 40 buprofezin AI mL^{-1} (B_0 to B_{40}). (b) LC_{50} (number of conidia mL^{-1} in \log_{10} scale; error bars: SEM) trends over days after the fungal spray alone (B_0) or together with the low buprofezin rates. (c) LT_{50} trends over the fungal concentrations.

and 3.4 days for the combined action of the formulation with buprofezin at 10, 20, 30 and $40\ \mu\text{g mL}^{-1}$ respectively.

3.2 Fungal effects on BPH fecundity and longevity

The counts of daily laid eggs per female and those daily hatched in the Ma456 treatment and the control are illustrated in Fig. 3. Temporal distributions of these paired counts were significantly different in likelihood-ratio G -tests ($P \leq 0.01$). BPH adults sprayed with Ma456 survived for a much shorter time than those in the control, irrespective of females (9.6 versus 21.8 days, $t_{29} = 22.1$, $P < 0.01$) or males (11.1 versus 23.9 days, $t_{29} = 25.5$, $P < 0.01$). Thus, the fungal infection greatly reduced the overall mean fecundity of BPH adults (190 versus 653 eggs per female, $t_{29} = 25.4$, $P < 0.01$) and the egg hatch rate (58.5% versus 91.1%, $t_{29} = 17.7$, $P < 0.01$).

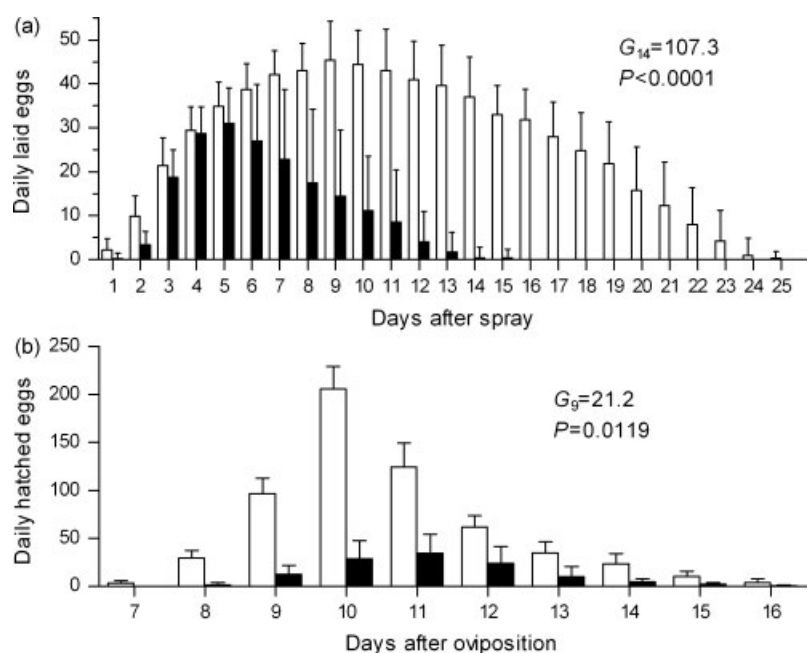


Figure 3. Effects of fungal (Ma456) infection on the fecundity of BPH females (a) and the hatch of their eggs (b). Black bars: infected. White bars: not infected (control).

3.3 Planthopper populations in two field trials

The trends of planthopper densities and the proportions of nymphs in the two field trials are shown in Fig. 4. The initial densities were similar in the seven treatments of trial 1 ($F_6 = 0.43$, $P = 0.84$) and trial 2 ($F_6 = 2.51$, $P = 0.08$). After the first spray, overall density trends varied significantly with treatment (trial 1: $F_{6,96} = 235$, $P < 0.01$; trial 2: $F_{6,96} = 223$, $P < 0.01$), sampling dates (trial 1: $F_{6,96} = 926$, $P < 0.01$; trial 2: $F_{6,96} = 732$, $P < 0.01$) or both (trial 1: $F_{36,96} = 17.8$, $P < 0.01$; trial 2: $F_{36,96} = 13.3$, $P < 0.01$), based on two-way ANOVA.

The field population was composed of BPH and WBPH based on the counts of their adults. The ratios of WBPH over BPH (data not shown) in the two fields ranged from 0.29 to 1.45 during the first two weeks, dropped drastically to only 0.07 in the following week and maintained a value of ca 0.01 thereafter. Thus, BPH dominated the population on most sample dates.

During the field trials, the proportion of nymphs was around 0.9 in most plots (Figs 4c and d). In particular, egg hatch peaked soon after the first spray in both fields. Thus, planthopper densities in all treatments increased sharply during the first week and were then differentiated from the controls to the fungal or chemical treatments (Figs 4a and b). The density differences between the controls and the treatments were further enlarged by the second and third sprays. As a result of the three sprays, mean (\pm SD) densities (number of planthoppers per two-hill sample) in the trial 1 treatments $B_{1/5}$, Ma456, Ma576, Ma456 + $B_{1/5}$ and Ma576 + $B_{1/5}$ were 702 ± 53 , 483 ± 78 , 382 ± 78 , 115 ± 14 and 95 ± 10 on day 40 respectively. The same estimates in trial 2 were 598 ± 53 , 397 ± 115 , 295 ± 25 , 113 ± 9 and 98 ± 11 . In contrast, the two controls resulted in pest densities of 1259 ± 56 and 1361 ± 46 in trial 1 and 1265 ± 49 and 1214 ± 36 in trial 2. Apparently, population trends in all treatments were similar in both trials, but no treatment suppressed the population below the initial density (12.3 ± 3.4). However, the population increases were greatly slowed down by the treatments, particularly by the combination of both agents, which reduced the pest densities 11–14-fold compared with the

two controls. These reductions were much greater than those in the pure fungal (2.6–4.1-fold) or chemical (1.8–2.1-fold) treatments.

The field conditions during the trials were generally suitable for the germination and infection of *M. anisopliae* (Fig. 4e). Daily mean RH fell in the range 92.5–98.3%, with 100% RH being recorded for ≥ 8 h on most days. The 40 day trial witnessed a total rainfall of 68.4 mm on 17 rainy days (0.2–17.2 mm per day). Daily mean temperature ranged from 18.8 to 30.1 °C, fluctuating around 25 °C on most days. However, daytime temperature exceeded 35 °C (up to 38.2 °C) on the first few days, accompanied with lower RH. This is perhaps, at least in part, why the first spray was less effective.

3.4 Field efficacies against planthoppers

The relative efficacies of the fungal and/or chemical treatments for the pest control in the two trials are listed in Table 1. The combinations of Ma456 and Ma576 with buprofezin resulted in consistently best control, followed by the use of each fungal formulation alone. The efficacies increased with the times of sprays, reaching 87–93% after the third spray. The pure fungal sprays led to higher efficacies than those of buprofezin. In contrast, spraying the aqueous dilution of the emulsion alone (control 2) failed to provide any substantial control.

4 DISCUSSION

The present results indicate that the combined application of fungal and chemical agents is an alternative strategy against rice planthoppers. An effective application rate of buprofezin, chosen for incorporation into fungal sprays in five bioassays, is as low as $40 \mu\text{g AI mL}^{-1}$, which is one-fifth of the lower limit of 750–1125 g ha^{-1} labelled for 200 g kg^{-1} buprofezin WP. This contrasts with the LC_{50} values of the chemical against BPH nymphs on days 2 to 5. Significant controls in both field trials were achieved by spraying biweekly the fungal formulation Ma456 or Ma576 in conjunction with the low buprofezin rate. Several aspects of the effects of the fungal and chemical agents are discussed below.

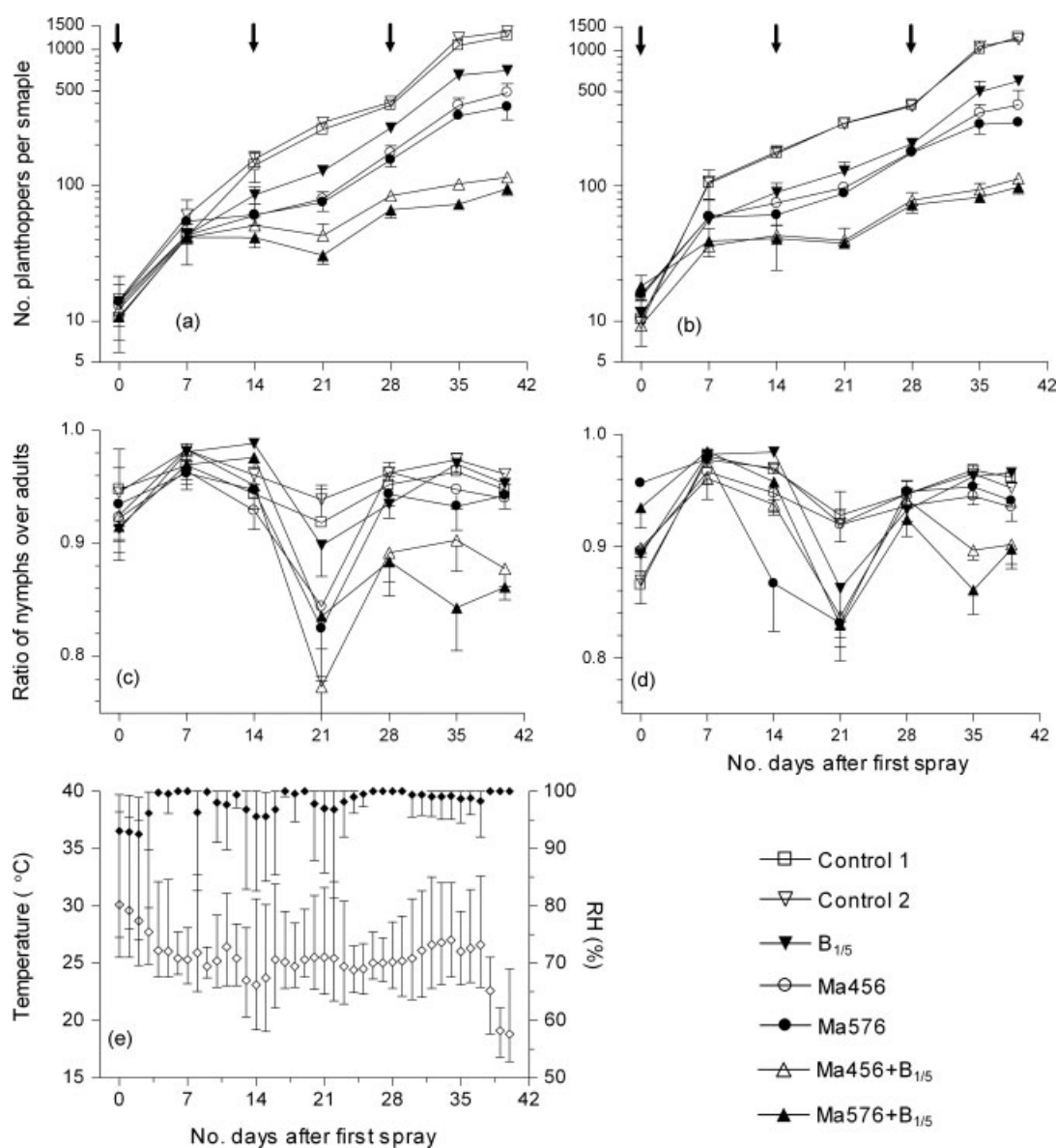


Figure 4. Trends of rice planthopper populations (mainly BPH) in trial 1 (a, c) and trial 2 (b, d). Treatments included the sprays of oil-formulated *Metarhizium anisopliae* alone (Ma456 and Ma576 at 1.54×10^{13} conidia ha^{-1}) or in conjunction with buprofezin at the low rate of $30.8 \text{ g AI ha}^{-1}$, i.e. one-fifth of its labelled rate (Ma456 + B_{1/5} and Ma576 + B_{1/5}). Control 1: water spray. Control 2: sprayed with aqueous dilution of the emulsion used for fungal formulation. Arrows indicate scheduled sprays. Daily mean temperature (\diamond) and RH (\blacklozenge) with the bars of minima and maxima (e) are based on the records of an electronic hydrothermometer under the rice canopy of one field during the trials. Error bars in (a) to (d): SD from three plots.

First of all, TCM modelling analysis, which enables elucidation not only of the effects of concentration and time but the interaction of the two,^{28–30} differentiated well the effects of the fungal and chemical sprays on BPH nymphs in the bioassays. The LC₅₀ values of the Ma456 formulation on days 7 and 10 were reduced to 386 and 199 conidia mm^{-2} from the previous estimates of 731 and 284 unformulated conidia mm^{-2} respectively.²¹ The emulsion used to formulate the fungus showed no significant effect on the pest in the bioassays and the later field trials. The enhancement of the fungal action by the oil formulation is in good agreement with the action of *B. bassiana* on BPH nymphs.²³ The LC₅₀ trends of the oil formulation declining with increased rates of buprofezin in the fungal sprays are also similar to the combined effects of *B. bassiana* and other chemical insecticides on different insects.^{22,23,32} As a result of TCM modelling, buprofezin rates of $30\text{--}40 \mu\text{g AI mL}^{-1}$

were found significantly to enhance or accelerate the fungal action on BPH (Fig. 2). Thus, a rate of $40 \mu\text{g AI mL}^{-1}$ was chosen for incorporation into the fungal sprays for field efficacies of BPH control.

Based on the efficacies of different treatments on all sample dates except the first (Table 1), overall mean efficacy in the two field trials is 54.1% for Ma456 and 59.9% for Ma576 during the trial period of 40 days. These are higher than that in the treatment with buprofezin alone (35.6%) and significant compared with the emulsion control in both trials. Incorporation of the low chemical rate into the sprays of Ma456 and Ma576 enhanced the overall efficacy to 79.7 and 82.9% respectively. Thus, the combined application of the fungal and chemical agents in both fields led to ca 25% net increase in overall mean efficacy compared with the fungal action alone, making it a promising strategy for

Table 1. Relative efficacies of *Metarhizium anisopliae* formulations (Ma456 and Ma576) alone or together with one-fifth of the labelled buprofezin rate ($B_{1/5}$) against rice planthoppers

Sample date	Mean (\pm SD) % efficacy after spraying ^a						F (P) in ANOVA
	Control 2	$B_{1/5}$	Ma456	Ma576	Ma456 + $B_{1/5}$	Ma576 + $B_{1/5}$	
<i>Trial 1</i>							
Aug 26	-36.5(\pm 25.3) a	-9.4(\pm 61.5) a	-2.3(\pm 35.9) a	-24.9(\pm 34.6) a	-9.3(\pm 76.2) a	-0.4(\pm 56.0) a	0.4 (0.819)
Sept 02	-29.9(\pm 95.6) a	39.4(\pm 19.3) a	59.0(\pm 1.9) a	58.2(\pm 2.1) a	63.7(\pm 11.4) a	70.5(\pm 9.6) a	2.7 (0.087)
Sept 09	-18.2(\pm 57.6) b	48.4(\pm 26.5) ab	67.3(\pm 18.9) a	69.7(\pm 15.2) a	83.5(\pm 5.8) a	88.4(\pm 3.6) a	5.1 (0.014)
Sept 16	-9.5(\pm 49.9) b	29.6(\pm 35.3) ab	51.9(\pm 30.2) ab	58.1(\pm 23.9) ab	77.8(\pm 10.8) a	83.2(\pm 5.8) a	4.1 (0.027)
Sept 23	-18.9(\pm 59.1) b	37.4(\pm 31.8) ab	64.1(\pm 12.9) ab	69.1(\pm 13.7) a	90.0(\pm 5.4) a	92.9(\pm 3.9) a	5.8 (0.009)
Sept 28	-15.7(\pm 64.1) b	42.5(\pm 28.9) ab	62.8(\pm 9.6) ab	70.5(\pm 8.4) ab	90.8(\pm 3.8) a	92.2(\pm 4.2) a	5.1 (0.014)
<i>Trial 2</i>							
Aug 27	-41.2(\pm 27.9) b	24.9(\pm 7.8) a	21.0(\pm 27.0) a	21.6(\pm 11.4) a	52.7(\pm 13.8) a	46.8(\pm 18.3) a	8.3 (<0.01)
Sept 03	-46.3(\pm 37.4) c	25.6(\pm 28.3) b	38.5(\pm 18.2) ab	51.3(\pm 7.7) ab	63.2(\pm 16.1) ab	68.0(\pm 13.3) a	24.0 (<0.01)
Sept 10	-40.4(\pm 40.8) c	38.6(\pm 16.5) b	52.1(\pm 17.1) ab	57.3(\pm 12.2) ab	80.8(\pm 7.2) a	81.3(\pm 7.0) a	35.4 (<0.01)
Sept 17	-35.5(\pm 27.4) c	27.5(\pm 18.1) b	37.5(\pm 12.1) b	37.6(\pm 17.8) b	72.8(\pm 4.8) a	74.8(\pm 4.7) a	49.0 (<0.01)
Sept 24	-46.5(\pm 28.4) c	33.8(\pm 7.3) b	52.7(\pm 11.2) b	60.5(\pm 12.7) ab	87.2(\pm 2.8) a	88.9(\pm 1.4) a	57.7 (<0.01)
Sept 28	-35.8(\pm 38.3) c	32.9(\pm 20.5) b	56.0(\pm 17.0) ab	67.1(\pm 9.2) ab	87.4(\pm 3.0) a	88.9(\pm 3.9) a	31.4 (<0.01)

^a Means with different letters in each line differ significantly (Tukey's HSD: $P < 0.05$ in one-way ANOVA).

Control 2: aqueous dilution of the emulsion used in fungal formulation.

$B_{1/5}$: sprayed at 30.8 g buprofezin AI ha⁻¹.

Ma456 and Ma576: sprayed at a rate of 1.54×10^{13} conidia ha⁻¹. The last sampling was ahead of schedule owing to a national vacation.

rice planthopper control. However, the first-week efficacies were not significantly different among the treatments, particularly in trial 1. This could be attributed to the slow action of the fungal formulation and the high proportions of nymphs, which hatched massively from deposited eggs in the field and escaped from the first spray. Consistently high daytime temperatures in the first week could also affect the fungal action of the first spray.

Furthermore, the fungal infection was proven not only to kill BPH nymphs and adults but also to reduce greatly the reproductive potential. BPH females infected by Ma456 exhibited significantly lower fecundity (71% reduction) than those not infected, and the hatch rate of their eggs was reduced by 36%. Both effects reduced the reproduction potential of the infected females by 81.4% (i.e. 111 versus 595 hatched eggs per female). This helps to interpret the 11–14-fold reduction in the pest densities in the combined fungal and chemical treatments in the field trials. This exceeded the sum of the density reductions in the treatments of fungal (2.6–4.1-fold) and chemical (1.8–2.1-fold) sprays. The suppressive effect of the fungal infection on the pest population tended to increase with prolonged action, with the low buprofezin rate not affecting the fungal growth and sporulation on BPH cadavers (Fig. 2a).

However, there is still a challenge to improve the field efficacy of BPH control by incorporation of a chemical into the fungal sprays. The efficacy improvement requires not only more virulent fungal candidates but more efficacious chemical insecticides. It is not easy to find a fungal candidate with higher virulence to BPH.^{12,21} The two *M. anisopliae* isolates used in this study were superior to many other isolates tested but killed only 54–64% of BPH nymphs under a concentrated spray.²¹ Their LC_{50} values were much higher than those of some desired fungal candidates against aphids and whiteflies.^{14,15,22} Moreover, from its high LC_{50} values in this study, BPH seems to have developed high resistance to buprofezin. Unfortunately, this chemical is still on a very short list of recommended insecticides for BPH control in China.

Currently, buprofezin has to be sprayed alone or together with other chemical(s) weekly or even more frequently for consistent control during the season of BPH infestation. As more effective insecticides and transgenic BPH-resistant rice varieties^{33–35} are lacking, the present study has shed light on alternative BPH control, but better fungal and chemical candidates are needed for improved efficacy, warranting future studies.

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