Selection of global *Metarhizium* isolates for the control of the rice pest *Nilaparvata lugens* (Homoptera: Delphacidae)

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Abstract

BACKGROUND: This study was initiated to search for fungal candidates for microbial control of brown planthopper (BPH) *Nilaparvata lugens* Stål, to which little attention has been paid in the past two decades.

RESULTS: Thirty-five isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin and *M. flavoviride* Gams & Rozsypal from different host insects worldwide were bioassayed for their lethal effects against third-instar BPH nymphs at 25 °C and a 14:10 h light:dark photoperiod at ca 1000 conidia mm^{-2}. On day 9 post-treatment, mortality attributable to mycosis ranged from 6.5 to 64.2% and differed significantly among the tested isolates with no apparent relationship to their host origin. Only two BPH-derived *M. anisopliae* isolates from the Philippines (ARSEF456) and Indonesia (ARSEF576) killed >50% of the nymphs. Both isolates were further bioassayed for time–concentration–mortality responses of the nymphs to the sprays of 19–29, 118–164 and 978–1088 conidia mm^{-2} in repeated bioassays. The resultant data fitted a time–concentration–mortality model very well. Their LC_{50} values were estimated as 731 and 1124 conidia mm^{-2} on day 7 and fell to 284 and 306 conidia mm^{-2}, respectively, on day 10.

CONCLUSION: The two *M. anisopliae* isolates are potential biocontrol agents of BPH for further research. This is the first report of the lethal effects of global *Metarhizium* isolates on the rice pest.

Keywords: brown planthopper; *Nilaparvata lugens*; *Metarhizium anisopliae*; *Metarhizium flavoviride*; fungal biocontrol agents; bioassays; time-concentration-mortality response

1 INTRODUCTION

The brown planthopper (BPH) *Nilaparvata lugens* Stål (Homoptera: Delphacidae) is a rice insect pest that has frequent outbreaks in Asia, causing severe rice damage called 'hopperburn'.[1] Generally, rice varieties lack sufficient resistance to BPH, in spite of long-term efforts towards transgenic plants.[2–4] Thus, BPH control has long relied upon chemical insecticides, particularly imidacloprid in China, since the early 1990s.[5] However, the efficacy of this neonicotinoid insecticide has been compromised by the development of resistance in BPH[5–7] and other sucking pests, such as aphids[8] and spider mites.[9] As high levels of imidacloprid resistance are suspected of being causative of severe BPH outbreaks during 2005–2007, the neonicotinoid has been replaced by combinations of more expensive, but not necessarily more efficacious, insecticides for control of this pest in China. Therefore, alternative measures are needed for BPH control and also for use in insecticide resistance management programmes.

Entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo-Crivelli) Vuellemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, are well-known biocontrol agents of phloem-feeding arthropod pests.[10–13] These fungal agents have been developed as mycoinsecticides for the control of aphids,[14,15] whiteflies,[16–18] leafhoppers[19,20] and spider mites.[21] However, little attention has been paid to microbial control of BPH in the past two decades, in spite of some early efforts.[22,23] A large number of fungal isolates were bioassayed in the 1980s, but none caused BPH mortality of more than 70%.[24] In a recent study, a *B. bassiana* isolate that had been shown to be highly virulent to aphids[11] killed only 43–61% of BPH nymphs at the high concentration of 1298 conidia mm^{-2} 7–12 days after treatment.[25] Interestingly, the LC_{50} of the *B. bassiana* isolate against BPH on day 7 after spray application decreased from 1652 unformulated conidia mm^{-2} to 1016 conidia mm^{-2} when applied as an oil formulation, and further fell to only 503, 135 and 26 conidia mm^{-2} when the formulation was applied together with imidacloprid at the sublethal rates of 0.5, 1.0 and 2.0 μg AI mL^{-1} respectively. This has shed light on the potential combination of selected fungal biocontrol agents alongside chemical components for integrated BPH control.

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The paddy field ecosystem, dependent on routine irrigation, may provide the high moisture that is required for the successful use of fungal biocontrol agents.[24,26] However, high temperatures in the rice-growing seasons of Asia could be a limiting factor for efficacy. Moreover, possible drift of fungal spray into mulberry gardens that support silkworm cultures in southern China may cause public concern. With these issues in mind, caution must be taken to select fungal candidates for microbial control of the rice-specific BPH. Since *B. bassiana* often causes natural mycosis of silkworm cultures and is less tolerant of high summer temperatures than *M. anisopliae* in conidial germination and hyphal growth,[27,28] new efforts should be made to explore the potential of *M. anisopliae* isolates for BPH control rather than *B. bassiana*. Another consideration is that the candidate *M. anisopliae* isolates should have minimal adverse effects on non-target insects that act as prey for predators in the paddy field. The fungal species, however, is very unlikely to pose any hazard to aquatic organisms.[29] The present study searched for potential biocontrol candidates of BPH from 35 global isolates of *M. anisopliae* and *M. flavoviride* Gams & Rozsypal by comparing their lethal effects on BPH nymphs, initially at a highly concentrated spore spray rate. The most promising candidates from these were subsequently evaluated for their time–concentration–mortality (TCM) responses against the rice pest by means of TCM modelling analysis.[30,31]

2 MATERIALS AND METHODS

2.1 Fungal isolates and conidial preparations

Global isolates of *M. anisopliae* (variety unknown, denoted as Ma), *M. anisopliae* var. *acridum* (Maac), *M. anisopliae* var. *majus* (Mam), *M. anisopliae* var. *anisopliae* (Maan), *M. flavoviride* (variety unknown, denoted as Mf) and *M. flavoviride* var. *minus* (Mfm) with different hosts and geographic origins (Table 1) were requested from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF, US Plant, Soil and Nutrition Laboratory, Ithaca, NY).

Table 1. The origins of the ARSEF and local (asterisked) isolates of *Metarhizium anisopliae* (Ma), *M. anisopliae* var. *acridum* (Maac), *M. anisopliae* var. *majus* (Mam), *M. anisopliae* var. *anisopliae* (Maan), *M. flavoviride* (Mf) and *M. flavoviride* var. *minus* (Mfm) for bioassays

Isolate	Original host insect	Geographic origin
Ma0201*	*Empoasca* sp. (Homoptera: Cicadellidae)	Zhejiang, China
Ma456	*Nilaparvata lugens* (Homoptera: Delphacidae)	Manila, Philippines
Ma576	*Nilaparvata lugens* (Homoptera: Delphacidae)	Celebes, Indonesia
Ma727	Unknown species (Orthoptera: Tettigoniidae)	Goiás, Brazil
Ma759	*Deois flavopicta* (Homoptera: Cercopidae)	Goiás, Brazil
Ma1055	*Nezara viridula* (Hemiptera: Pentatomidae)	Londrina, Brazil
Ma1548	*Carpocapsa pomonella* (Lepidoptera: Olethreutidae)	Palawan, Philippines
Ma2510	*Atta* sp. (Hymenoptera: Formicidae)	Goiás, Brazil
Ma2786	Species unknown (Orthoptera: Gryllotalpidae)	Kishinev, Moldova
Ma2949	Species unknown (Isoptera: family unknown)	Goiás, Brazil
Ma2951	Species unknown (Isoptera: family unknown)	Goiás, Brazil
Ma5197	*Diaprepes abbreviata* (Coleoptera: Curculionidae)	Florida, USA
Maac3391	*Zonocerus elegans* (Orthoptera: Pyrgomorphidae)	Tanzania
Maac3612	*Kraussaria angulifera* (Orthoptera: Acrididae)	Benin
Maac3614	*Kraussaria angulifera* (Orthoptera: Acrididae)	Benin
Maac5734	Species unknown (Orthoptera: Acrididae)	Madagascar
Maac5735	Species unknown (Orthoptera: Acrididae)	Madagascar
Maac5736	*Locusta migratoria capito* (Orthoptera: Acrididae)	Madagascar
Maac6421	*Kraussaria angulifera* (Orthoptera: Acrididae)	Khelcom, Senegal
Mam978	*Oryctes rhinoceros* (Coleoptera: Scarabaeidae)	France
Mam1946	*Oryctes rhinoceros* (Coleoptera: Scarabaeidae)	Quezon, Philippines
Mam4566	*Anoplognathus sp.* (Coleoptera: Scarabaeidae)	Leighlands, Australia
Maan2080	*Nilaparvata lugens* (Homoptera: Delphacidae)	Java, Indonesia
Maan3332	*Popillia japonica* (Coleoptera: Scarabaeidae)	New York, USA
Maan3619	*Oxya multidentata* (Orthoptera: Acrididae)	Pakistan
Maan4132	*Aphodius tasmaniae* (Coleoptera: Scarabaeidae)	Australia (south)
Maan4822	*Otiorhynchus ligustici* (Coleoptera: Curculionidae)	New York, USA
Maan5628	*Schistocerca gregaria* (Orthoptera: Acrididae)	Shelsela, Ethiopia
Maan6910	*Coptotermes formosanus* (Isoptera: Rhinotermitidae)	Louisiana, USA
Mf606	*Zonocerus variegatus* (Orthoptera: Pyrgomorphidae)	Benin
Mfm1271	*Nilaparvata lugens* (Homoptera: Delphacidae)	Manila, Philippines
Mfm1547	*Ornithacris cavroisi* (Orthoptera: Acrididae)	Bicol, Philippines
Mfm2948	Species unknown (Homoptera: Cicadellidae)	Campinas, Brazil
Mfm3341	*Ornithacris cavroisi* (Orthoptera: Acrididae)	Niamey, Niger
Mfm5748	*Schistocerca piceifrons* (Orthoptera: Acrididae)	Colima, Mexico

All of the 34 ARSEF isolates plus a local isolate (Ma0201) were preserved at −76 °C and recovered on plates of Sabouraud dextrose agar plus 1% yeast extract (SDAY) at 25 ± 1 °C before use.

The method of Ye *et al*.[32] was slightly modified to produce aerial conidia of each isolate on steamed rice, which was inoculated with 2 day shaking culture (consisting of blastospores and mycelia) of Sabouraud dextrose broth (glucose 40, peptone 10 and yeast extract 10 g L^{-1}). Briefly, the rice cultures were incubated in 15 cm diameter petri dishes (100 g rice per dish) at 25 ± 1 °C and a 12:12 h light:dark photoperiod for 7–9 days, dried under ventilation at 32 ± 1 °C for 48 h and then harvested through a vibrating sieve. Recovered conidia were further dried to a water content of ca 5% at ambient temperature in a vacuum drier and then used immediately or stored in sealed glass vials at 4 °C for subsequent use in bioassays, ensuring ca 95% viability of the conidia.

2.2 Planthopper stock

BPH nymphs for use in bioassays were prepared using a tray system of rice seedlings described by Feng and Pu.[25] Briefly, a laboratory BPH population initiated from field-collected adults was maintained on caged rice seedlings grown in plastic trays (22 × 30 cm) and provided with a nutrient solution under a regime of 25 ± 1 °C and a 14:10 h light:dark photoperiod. Brachypterous adults (ca 40) taken from this population were transferred onto a tray of high-density seedlings (4 cm tall) and allowed to lay eggs for 48 h. The adults were then removed and the eggs laid on the seedlings were allowed to develop into third-instar nymphs under the same conditions (ca 20 days after adult removal). At this time, about 30 nymphs were transferred to ca 30 seedlings (3 cm tall) individually growing upwards from the pores of a sponge board floating in a plastic cup (7 cm diameter × 9 cm height), in which a nutrient solution was introduced for the growth of rice roots. Nymphs that were either too large or too small were discarded to minimize age variation among the nymphs.

2.3 Bioassays

Two different series of bioassays were performed. The first series included all 35 *Metarhizium* isolates. For each of the isolates, a high concentration of spore suspension (1×10^8 conidia mL^{-1}) was prepared in 0.2 g L^{-1} Tween-80. The spore suspension was sprayed onto the BPH nymphs among the seedlings in uncaged cups. To reduce the escape of the nymphs from the seedlings, a hand-held Micro Ulva sprayer (Micron Sprayers Limited, Herefordshire, UK) was used. The sprayer was held 1 m above the bottom of a bucket (25 cm diameter), on which a cup of seedlings infested with BPH nymphs was centrally placed, and used to generate a mist (droplets ca 50–60 μm) at 11 000 rev min^{-1} (according to the manufacturer's guide). After 25 s spraying followed by a 3 min deposition period, the seedlings were gently covered with a top-meshed cage, removed from the bucket and maintained at 25 ± 1 °C and a 14:10 h light:dark photoperiod for daily recording of BPH mortality. The concentration of conidia deposited onto the nymphs and seedlings under each spray was measured as the number of conidia mm^{-2} using microscopic counts of conidia collected by three glass coverslips (20 × 20 mm), which were triangularly placed at the base of the bucket during the spraying. The bioassay of each fungal isolate, including a blank control treatment (sprayed with 0.2 g L^{-1} Tween-80), was repeated 3 times within 6 months. All BPH cadavers recovered were transferred into saturated moisture chambers to allow fungal growth and sporulation. Those showing visible infection symptoms under microscopic inspection, or to the naked eye, were considered as being killed by the isolate under test.

The second series of bioassays included only those isolates that caused greater than 50% BPH mortality in the first series of experiments and showed desirable fungal growth and sporulation on cadavers. Spore suspensions of the selected isolates (Ma456 and Ma576) that had been passed through the host BPH 3 times before use were prepared using the methods described above. Three concentrations (1×10^6, 1×10^7 and 1×10^8 conidia mL^{-1}) of each selected isolate, plus a blank control, were sprayed onto the BPH nymphs to generate low, median and high levels of spore deposits (measured as number of conidia mm^{-2}). These experiments were undertaken to quantify the TCM responses of BPH to the tested isolates. The bioassays were repeated 4 times during 3 months using the same protocol.

2.4 Data analysis

Percent BPH mortalities (M_1) observed in the first bioassays were corrected with those in blank controls (M_2) using Abbott's formula $M_C = (M_1 - M_2)/(100 - M_2)$.[33] The corrected mortalities transformed as $\sin^{-1}\sqrt{M_C}$ and the associated log-transformed conidial deposits were subjected to analysis of variance (ANOVA) between the tested isolates.

Data from the second bioassays were fitted to a TCM model[30,31] that generated estimates for the effects of spore concentration (number of conidia mm^{-2}) and post-spray day. The estimated parameters were used to compute median lethal concentrations (LC_{50}) and associated 95% confidence limits over days after application and median lethal time (LT_{50}) depending on the concentration. All the analyses were performed using updated DPS software.[34]

3 RESULTS

3.1 BPH mortalities attributed to 35 isolates

The results from the first bioassays are summarized in Table 2. After spraying, the concentrations of the conidia deposited onto the BPH-infested seedlings ranged from 706 (Maan3332) to 1496 (Mfm1547) conidia mm^{-2} and averaged 1008 conidia mm^{-2} for

Table 2. Corrected mortalities of BPH nymphs attributed to the sprays of 35 *Metarhizium* isolates[a]

Fungal isolate[b]	Conidial spray (conidia mm^{-2})	Mortality (%)	Fungal isolate[b]	Conidial spray (conidia mm^{-2})	Mortality (%)	Fungal isolate[b]	Conidial spray (conidia mm^{-2})	Mortality (%)
Ma576	827 (±75)[98]	64.2 (±7.3)	Maac5735	900 (±198)[90]	34.0 (±4.4)	Maan3332	706 (±73)[74]	25.0 (±12.7)
Ma456	991 (±173)[83]	53.8 (±15.3)	Mam978	775 (±60)[80]	33.8 (±5.3)	Ma2510	1005 (±53)[87]	24.8 (±6.6)
Mam1946	899 (±161)[90]	46.7 (±6.9)	Mfm1547	1496 (±329)[71]	32.7 (±8.6)	Ma2786	757 (±42)[75]	23.9 (±14.4)
Maac3391	926 (±19)[108]	44.9 (±5.7)	Ma727	1048 (±93)[94]	32.1 (±6.1)	Maan5628	1191 (±333)[87]	23.8 (±4.5)
Maac3614	899 (±65)[100]	43.7 (±1.6)	Mam4566	807 (±108)[89]	30.6 (±5.6)	Ma2949	1084 (±293)[92]	22.0 (±12.0)
Ma5197	959 (±31)[83]	43.1 (±15.3)	Maan4822	1081 (±365)[78]	30.4 (±4.6)	Maan3619	1368 (±422)[83]	21.8 (±2.6)
Mfm5748	950 (±175)[84]	41.7 (±9.2)	Ma1548	1258 (±70)[98]	29.5 (±8.1)	Maan2080	1020 (±121)[101]	20.1 (±3.8)
Mfm1271	971 (±67)[70]	38.9 (±8.3)	Mfm3341	1098 (±156)[85]	26.9 (±6.8)	Maac5734	1097 (±118)[103]	17.1 (±1.8)
Ma0201*	936 (±258)[87]	38.2 (±2.2)	Maan4132	955 (±32)[103]	26.6 (±3.6)	Mfm2948	976 (±210)[76]	15.0 (±6.4)
Ma1055	1105 (±415)[86]	37.6 (±3.6)	Ma2951	916 (±157)[97]	26.0 (±1.5)	Maan6910	1159 (±198)[96]	12.5 (±6.6)
Ma759	1048 (±97)[73]	36.3 (±6.0)	Maac3612	921 (±54)[94]	26.0 (±0.8)	Maac6421	857 (±124)[89]	6.5 (±5.3)
Maac5736	1218 (±232)[118]	35.2 (±5.3)	Mf3606	1062 (±131)[87]	25.0 (±7.6)			

[a] Each mean (±SD, given in parentheses) was estimated from three repeated bioassays (the total number of BPH nymphs sprayed is given in square brackets).
[b] See Table 1 for fungal identities.

all isolates. The deposits differed significantly among the tested isolates ($F_{34,68} = 2.84$, $P < 0.01$), although all the sprayed suspensions were standardized to 1×10^8 conidia mL^{-1}. However, the deposits were not significantly different among the replicates ($F_{2,68} = 0.81$, $P = 0.45$).

On average, 89 (70–118) BPH nymphs were treated under the concentrated spray for all the fungal isolates and observed for 10 days. The BPH mortalities corrected with the control mortalities (5.0–19.7%) ranged from 1.7 to 41.7% on day 5 after spray applications, from 6.1 to 58.1% on day 7 and from 6.5 to 64.2% on day 9. The final corrected mortalities differed significantly among the 35 isolates ($F_{34,68} = 7.41$, $P < 0.01$) but were not significantly different between the replicates ($F_{2,68} = 0.22$, $P = 0.80$). Only two isolates, Ma456 and Ma576, killed more than 50% of the nymphs in the first-run bioassays and were originally derived from BPH in the Philippines and Indonesia respectively. The observed mortalities were not conspicuously related to the host origins of the tested isolates. For instance, the BPH-derived isolate Maan2080 killed only 20% of the sprayed nymphs, while some isolates from grasshoppers (Maac3391, Maac3614 and Mfm 5748) and beetles (Ma5197 and Mam1946) killed 42–47% (Table 2).

Almost all of the cadavers placed in the moist chambers were mycotized when examined individually under a microscope. However, the proportions of fully mycotized cadavers (covered with a heavy layer of fungal mats visible to the naked eye) were only 27–82% among the tested isolates. Those causing high levels of BPH mortality tended to grow better and sporulate more abundantly. This was illustrated by isolates Ma456 and Ma576. Taking Ma456 as an example, fully mycotized cadavers produced on average 1.44 (±0.33) $\times 10^6$ conidia per insect ($n = 15$) on day 6 or 7 after their death. By contrast, the percentages of fully mycotized cadavers among those killed by the isolates Ma759, Maac5736, Maac6421, Mam978, Mam1946, Mam4556, Maan6910 and Mfm2948 were less than 40%, although, of these isolates, some caused relatively high mortalities.

3.2 Time–concentration–mortality responses of BPH to selected isolates

The two isolates, Ma456 and Ma576, that killed more than 50% of BPH nymphs in the first series of bioassays were chosen for further tests in order to ascertain the TCM responses of BPH nymphs to these isolates. Cumulative BPH mortalities increased with the spore concentration for each fungal isolate and for the number of post-spray days (Fig. 1). Fungal sprays resulted in final mortalities of 29.1, 53.8 and 68.8% for Ma456 at the mean (±SD) deposits of 29 (±3.5), 164 (±46.9) and 1088 (±150.7) conidia mm^{-2} respectively (Fig. 1A). Isolate MA576 resulted in 30.6, 55.9 and 67.4% mortality at the deposits of 19 (±3.2), 118 (±22.1) and 978 (±156.3) conidia mm^{-2} respectively (Fig. 1B). By contrast, the mortalities observed in the blank controls averaged 13.6% for Ma456 and 14.0% for Ma576, both values being significantly lower than those caused by the fungal sprays (Ma456: $F_{3,9} = 49.1$, $P < 0.01$; Ma576: $F_{3,9} = 76.7$, $P < 0.01$).

For both isolates, the observations of the BPH mortalities (Fig. 1) corrected with those from the blank controls fit the TCM model well,[30,31] with no significant heterogeneity being detected using Hosmer–Lemeshow tests for the goodness of fit (Ma456: $C = 8.69$, df $= 9$, $P = 0.47$; Ma576: $C = 8.28$, df $= 8$, $P = 0.41$). Based on the fitted parameters for the effects of time and concentration, the LC_{50} values (with associated 95% confidence limits) of Ma456 and Ma576 against the rice pest (Fig. 2) were estimated as, respectively, 731 (405–1319) and 1124 (546–2311) conidia mm^{-2} on day 7 after spray, dropping to 284 (172–472) and 306 (188–498) on day 10. However, the differences of the LC_{50} estimates were not significant between the two isolates, as their 95% confidence limits overlapped (Fig. 2A). The LT_{50} values of both isolates estimated by interpolation[30,31]

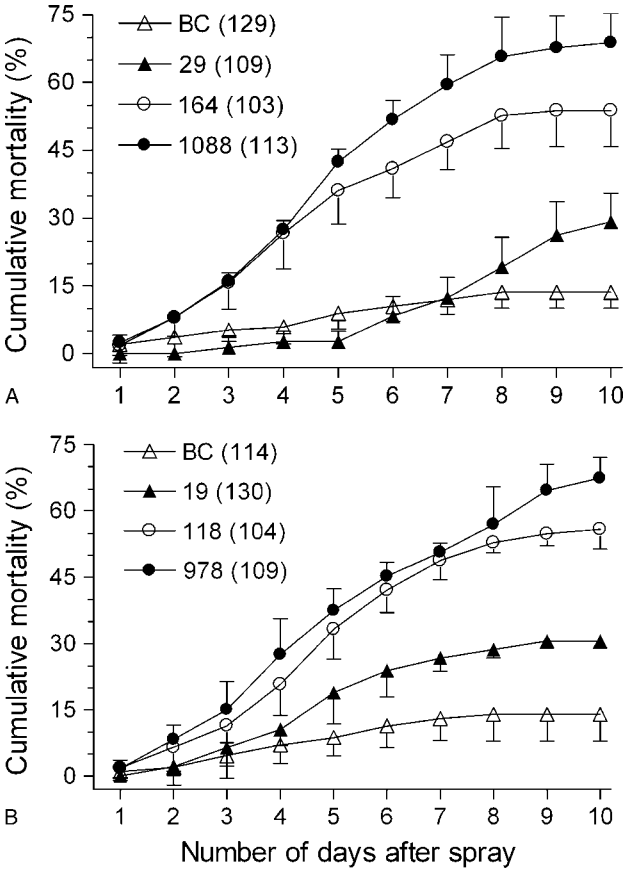

Figure 1. Cumulative mortalities of *Niloparvata lugens* nymphs after exposure to fungal sprays (number of conidia mm^{-2}; BC: blank control) of the selected isolates Ma456 (A) and Ma576 (B). Each value in parentheses is the total number of nymphs exposed to a given application. Error bars represent the standard deviation (SD) for the means of four replicates.

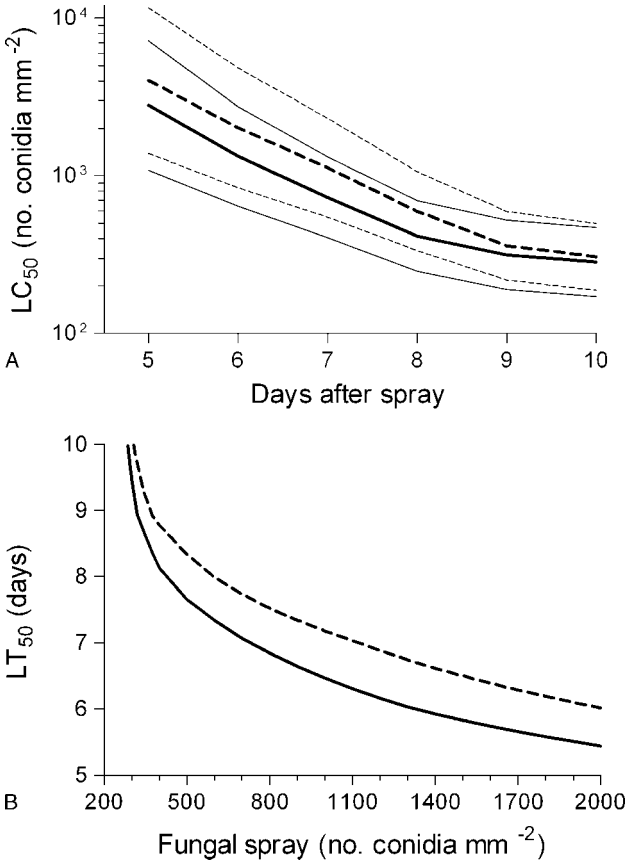

Figure 2. Virulence of the selected isolates Ma456 (solid) and Ma576 (dashed) towards *Niloparvata lugens* nymphs. (A) LC_{50} values (bold) and associated 95% confidence limits (non-bold) over days after spray. (B) LT_{50} values decreased with fungal spray concentration (number of conidia mm^{-2}).

generally decreased with the elevated spore concentration (Fig. 2B), e.g. 7.7 and 8.3 days at 500 conidia mm^{-2}, and 6.5 and 7.2 days at 1000 conidia mm^{-2}.

4 DISCUSSION

As presented above, the two isolates Ma456 and Ma576 were found to be the most promising fungal candidates for biocontrol of BPH among the 35 fungal isolates tested. This is the first report on the lethal effects of global *Metarhizium* isolates on BPH and the virulence of the selected candidates to the target pest.

Selection for potential fungal biocontrol candidates of an insect pest is generally based on their virulence in laboratory bioassays under controlled conditions. Previously, a standard bioassay protocol was developed to compare the virulence of 41 isolates of *B. bassiana* and *Paecilomyces* spp. to whiteflies.[10] With this protocol, whiteflies were exposed to the low, median and high concentrations of 20–40, 100–200 and 500–1000 conidia mm^{-2}, and their concentration–mortality responses were used to estimate LC_{50} values by probit analysis. While this protocol is technically ideal, it is too laborious to be used to assay large numbers of isolates. There is a major reason to reject an isolate that does not cause acceptable mortality of target pests at the high concentration of ~1000 conidia mm^{-2}, which is equivalent to a reasonable field application rate of $\sim 1 \times 10^{13}$ conidia ha^{-1}. This is that current technology for production of the fungal agents[24,26,32] does not support the costs of higher application rates for pest control in the field. Thus, field application rates beyond this limit would make it unattractive to develop a mycoinsecticide for practical use. Therefore, the protocol was modified by examining the BPH mortalities caused by a large number of fungal isolates at the high concentration only in the first series of bioassays, and then quantifying the virulence of the most promising isolates to the target pest in a second series of bioassays, which included the treatments of low, median and high concentrations to generate the TCM observations for modelling analysis. This modification saved time and resources, but yielded sufficient information for evaluating a large number of isolates (Table 2). Moreover, the modelling of the TCM data is much more robust than conventional probit analysis because it provides not only the effects of fungal spray and post-spray time but also the interaction of both variables.[30,31] Thus, the trends of the LC_{50} values declining with post-spray time and the LT_{50} values decreasing with the fungal concentration

can be generated to evaluate thoroughly the potential of the more promising isolates.

Control of conidial deposits on the sprayed nymphs is another important concern. In the present study, a 25 s spray time of a standard suspension of 1×10^8 conidia mL^{-1}, followed by 3 min deposition, was used to generate expected deposits of ~1000 conidia mm^{-2}. However, the resultant deposits varied greatly among the tested isolates (Table 2). This was likely to arise mainly from the large variation in conidial sizes among the tested isolates of *Metarhizium* spp. For instance, the conidial sizes of *M. flavoviride* var. *minus* are $4–7 \times 2–3\,\mu m$, whereas those of *M. anisopliae* var. *majus* are $10–16 \times 3–4\,\mu m$.[35] Another possible source of the variation could arise from the time control of conidial spray and deposition by hand, which was difficult to keep uniform for all the sprays, in spite of being carefully operated. High conidial deposits beyond the expected limit, if needed, can be achieved readily by extending the spray time of the standard suspension in laboratory bioassays or by the use of low- or ultralow-volume application methods in the field.[20]

None of the 35 *Metarhizium* isolates tested in this study killed more than 70% of BPH nymphs. This is in accordance with the results of unpublished BPH bioassays of many fungal isolates that were undertaken in the early 1980s.[24] A *B. bassiana* isolate selected from 17 fungal isolates caused 50–73% mortality in three leafhopper species.[12] It seems quite difficult to find fungal isolates with high virulence to planthoppers or leafhoppers on the basis of these studies. When more extensively examined, the two isolates Ma456 and Ma576 caused BPH mortalities close to 70% at the highest concentration of ~1000 conidia mm^{-2}. The TCM modelling indicates that the two *M. anisopliae* isolates were superior to a *B. bassiana* isolate against BPH nymphs[25] because of lower LC_{50} values on days 7–10 after spray applications and shorter LT_{50} values at 500–1000 conidia mm^{-2}. However, the LC_{50} values of Ma456 and Ma576 against BPH nymphs were relatively high compared with those of 14 out of 22 *P. fumosoroseus* and four out of 13 *B. bassiana* isolates tested against whiteflies (50–150 conidia mm^{-2}).[10] A very virulent *B. bassiana* isolate killed 50% of aphids at concentrations as low as 9–85 conidia mm^{-2} on days 5–7 after treatment.[11] Importantly, the LC_{50} of this *B. bassiana* isolate against BPH (1652 unformulated conidia mm^{-2}) was reduced by 38% (estimated to be 1016 conidia mm^{-2}) when conidia were prepared as an emulsifiable formulation, and further reduced by 70–98% (estimated as 26–503 conidia mm^{-2}) when the formulation was used in conjunction with imidacloprid at sublethal spray rates of 0.5–2.0 μg AI mL^{-1}.[25] Thus, both Ma456 and Ma576 are promising candidates for BPH control because only 731 and 1124 conidia mm^{-2} are needed to kill 50% after 7 days. Current techniques of fungal formulation and positive interactions with selected chemicals have enhanced field control of a number of phloem-feeding insect pests.[14–21] Such approaches can be utilized to increase the potential for successful exploitation and integration of fungal pathogens for BPH control. The evidence presented here indicates that the potential for the development of a mycoinsecticide for the biological control of BPH warrants further studies.

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