PATHOGENICITY OF *METARHIZIUM ANISOPLIAE* VAR. *ACRIDU* TO THE DEVEOLPMENTAL STAGES OF BROWN PLANTHOPPER *NILAPARVATA LUGENS* STÅL AND *SOGATELLA FURCIFERA*(HORVATH)

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(Received Dec. 16, 2003; accepted Apr. 26, 2004)

Abstract The susceptibility and virulence of entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* to the stages of brown planthopper (BPH), *Nilaparvata lugens* (Stål) and whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvath) were investigated under laboratory conditions. Three dosages of *M. anisopliae* var. *acridum* ranging from 10.5, 116.3 and 1027.1 conidial/mm^2 were used in the experiment. The tested stages of host included three developmental stages, young nymphs (1–2 instars), old nymphs (3–5 instars) and adults. It was found that all tested stages of the planthoppers were susceptible to the fungal infection. The degree of virulence LT_{50} of *M. anisopliae* var. *acridum* against young nymphs of *N. lugens* are >21, 20.82 and 16.55, respectively with the 3 dosages, the LT_{50} of the fungus against the old nymphs are 17.68, 15.49 and 13.98, respectively with the 3 dosages; the LT_{50} of the fungus against the adults are 17.10, 12.57 and 9.14 respectively with the 3 dosages. The degree of virulence $LT_{50}$of *M. anisopliae* var. *acridum* on young nymphs of *S. furcifera* are >21, 17.29 and 13.13, respectively with the 3 dosages ; the LT_{50} of the fungus against the old nymphs are 16.94, 15.02 and 13.03, respectively with the 3 dosages; the LT_{50} of the fungus against the adults are 12.78, 10.16 and 7.64, respectively with the 3 dosages. Adults were more susceptible to *M. anisopliae* var. *acridum* infection than their nymphs and the young nymphs were most resistant to the fungal infection. The cumulative mortality of each stage was dosage-dependent. Of all the developmental stages, WBPH was more susceptible than BPH to *M. anisopliae* var. *acridum* infection with the same dosages.

Key words *Nilaparvata lugens* (Stål), *Sogatella furcifera* (Horvath), *Metarhizium anisopliae* var. *acridum*, susceptibility, virulence, developmental stages.

1 INTRODUCTION

The brown planthopper (BPH) *Nilaparvata lugens* (Stål) and white backed planthopper *Sogatella furcifera* (Horvath) are major rice pests throughout Asia (Dyck and Thomas 1979,

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Kuno 1979, Li *et al.* 1996, Matsumura 2001). For a long time, the strategy for controlling these pest species has relied on chemical insecticides and the introduction of BPH-resistant rice varieties. It has detrimental impact on the natural enemies of both BPH and WBP as well as on environment. In general, natural parasitoids and predators in paddy fields are more sensitive to most chemical insecticides than the planthoppers (Croft *et al.* 1975, 1990). Chemical control has thus resulted in a resurgence of BPH populations (Kiritani *et al.* 1971, Otake 1979, Heinrichs *et al.* 1982). Also, certain insecticides may stimulate BPH reproduction (Heinrichs *et al.* 1982, Reissig *et al.* 1982), a phenomenon known as *hormoligosis* (Croft 1990). Therefore, interest in ecologically sound control measures is increasing.

Metarhizium anisopliae var. *acridum* is an entomopathogenic fungus which infects insects through direct contact. It infects many pests such as sugarcane. Field trials with oil formulated conidia have proven to be effective for locusts and grasshoppers control (Lomer *et al.* 1997, Milner 1997) under natural field conditions. It is also reported as a fungal pathogen of many pest such as whitegrubs (Milner 2003), BPH (Rombach *et al.* 1986), and mound building termites (Milner 2003b).

However, the pathogenicity of *M. anisopliae* var. *acridum* to both BPH and WBPH is not well documented. Hence, the experiments were conducted to further evaluate the pathogenicity of *Metarhizium anisopliae* var. *acridum* to the different developmental stages of the planthoppers.

2 MATERIALS AND METHODS

2.1 Rice

The rice variety used in the experiment was Qidaizhan which is susceptible to BPH. All rice was prepared as follows: 10-day-old rice seedlings were transplanted in 16 cm diameter plastic pots and cultured for another 10 days. The surface of the soil was kept dry to make easy to collect the cadavers of BPH and WBPH.

2.2 Insects

Adults of BPH and WBPH, collected from paddy fields in Zhaoqing City, Guangdong Province, were reared at 25–30°C on susceptible rice variety Qidaizhan in meshed cages. Nymphs and adults were randomly collected from the meshed cages for bioassay. Three developmental stages were tested: young nymphs (1–2 instars), old nymphs (3–5 instars) and adults. All adults were selected from the group of the nymphs that had recently molted.

2.3 Fungus

The fungus, *M. anisopliae* var. *acridum* used in the experiment, was originally isolated from cadavers of the BPH collected from paddy fields in Zhaoqing City, Guangdong Province, China. The fungus was grown for 2–3 weeks on Sabouraud dextrose agar plates at (26±2)°C and conidia were harvested in 0.05% Tween-80 solution and their concentration was adjusted to the required level. Spore suspensions were kept overnight at 10°C before they were used in the bioassay. Virulence to BPH was maintained by passing the fungus through the insect (Schaeffenberg 1964).

2.4 Inoculation of different developmental stages

Three concentrations of conidia were tested against each stage by directly spraying 10 mL of a given concentration of spores onto the insects

using a spray tower. Concentrations of 1×10^6, 1×10^7 and 1×10^8 conidia/mL were used. The conidia dosage was estimated by spraying three sterile 15 mm glass coverslip the same volume and same concentration of conidia as the spray on the insect.The sprayed dosage was determined by microscopically counting the numbers of conidia from 5 fields of the coverslip (0.785 mm^2 per field). As a result, the 3 concentrations corresponds to a surface coverage area of 10.5, 116.3 and 1027.1 conidia/mm^2 respectively.

Control lots were treated with sterile distilled water containing 0.05% Tween-80. The experiment consisted of 3 replicates of 100 insects per replicate for each dosage at each developmental stage.

Dead BPH and WBPH (no movement or response to stimulus) were collected and incubated on 1% water agar at 25 °C. The presence of sporulating mycelia on hopper cadavers as an indication of fungi infect.

2.5 Statistical analysis

All analyses were performed with the DPS Data Processing System software (Tang and Feng 1997). LT_{50} was determined from mortality data by TDM model after correction for natural mortality. The recorded percentages of mortality were normalized through angular transformation after corrected with Abbott's (1925) formula.

3 RESULTS

In the viability of *M. anisopliae* var. *acridum* conidia test, more than 91% of conidia germinated. The BPH and WBPH attributed to *M. anisopliae* var. *acridum* infection was observed mostly from day 1 to day 21 after exposure, respectively. All the planthoppers killed by the fungus sporulated well and displayed typical signs of *M. anisopliae* var. *acridum* infection under microscopic examination after overnight incubation in the moist chamber.

3.1 Effect of *M. anisopliae* var. *acridum* on young nymphs of BPH and WBPH

Infection of young nymphs of both BPH and WBPH were recorded 3 to 4 days after inoculation. Young nymphs were most resistant to fungal infection. The mortality of young nymphs of BPH and WBPH reached 33% and 42%, respectively on day 21 after spray of 10.5 conidia/mm^2, reached 51% for BPH and 75% for WBPH at 116.3 conidia/mm^2, and reached 68% for BPH and 86% for WBPH on the 21st day for WBPH in 1027.1 conidia/mm^2. Mortality of the control was at very low level throughout the experiment (Fig. 1).

3.2 Effect of *M. anisopliae* var. *acridum* on old nymphs of BPH and WBPH

Old nymphs of *N. lugens* and *S. furcifera* were more susceptible than their young nymphs to *M. anisopliae* var. *acridum* infection with the same dosage. Figure 2 showed the percentages of cumulative mortality in 21 days after spraying of *M. anisopliae* var. *acridum*. The mortality of old nymphs of BPH reached 67%, 74% and 81% and of WBPH reached 76%, 88% and 90% respectively, on the 21st day after spraying of 10.5, 117.3 and 1027.1 conidia/mm^2 Mortality of control was at very low level throughout the experiment.

3.3 Effect of *M. anisopliae* var. *acridum* on adults of BPH and WBPH

Adults of both *N. lugens* were more suscep-

tible than their nymphs to *M. anisopliae* var. *acridum* infection with the same dosages.

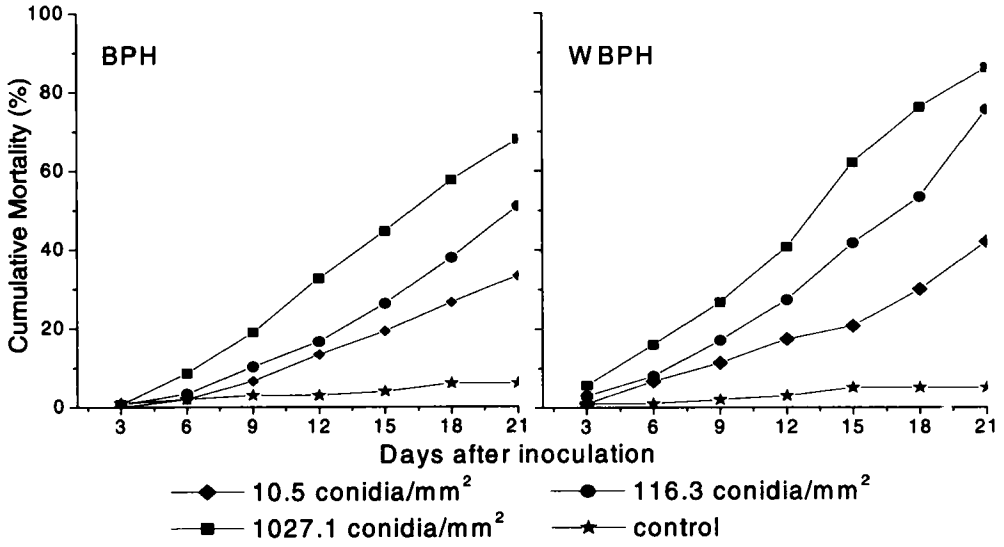

Fig.1 Effect of different concentration of *Metarhizium anisopliae* var. *acridum* on the young nymphs of BPH and WBPH.

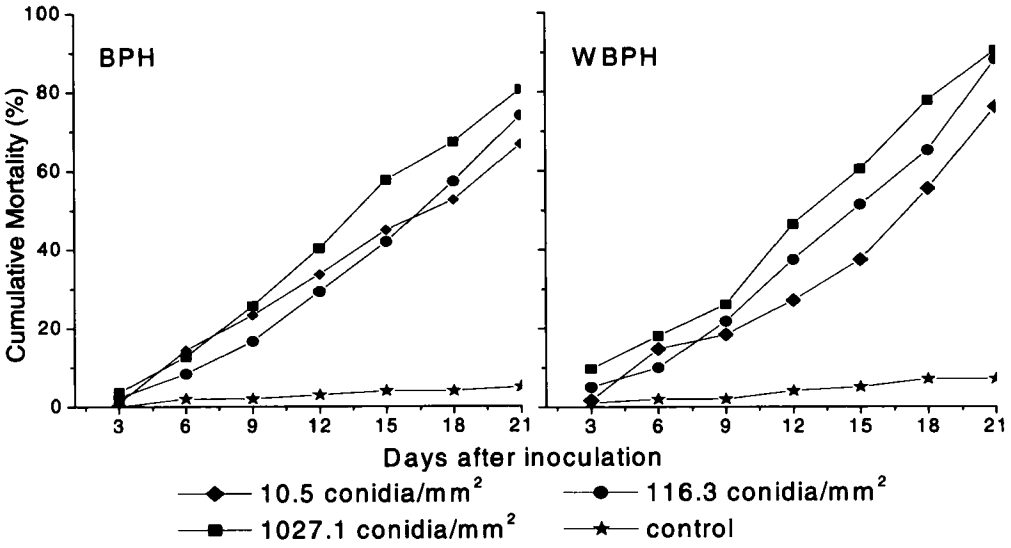

Fig.2 Effect of different concentration of *Metarhizium anisopliae* var. *acridum* on the old nymphs of BPH and WBPH.

Figure 3 showed the percentages of cumulative mortality in 21 days after spraying of *M. anisopliae* var. *acridum*. The earliest sign of infection was observed within 3 days after inoculation and was expressed as fungal colonization of adults or nymphs. The mortal-

ity of adults of BPH reached 81% and 100%, respectively on day 21 after spraying of 10.5 and 116.3 conidia/mm^2. The mortality of BPH reached 100% in 15 days after spraying of 1027.1 conidia/mm^2. Mortality of control treatment was at very low level throughout the experiment. The mortality of adults of WBPH reached 85%, respectively on day 21after spraying of 10.5 conidia/mm^2 and 100% 116.3 after spraying of conidia/mm^{2}on the 18th day. The mortality of BPH reached 100% in 15 days after spraying of 1027.1 conidia/mm^2.

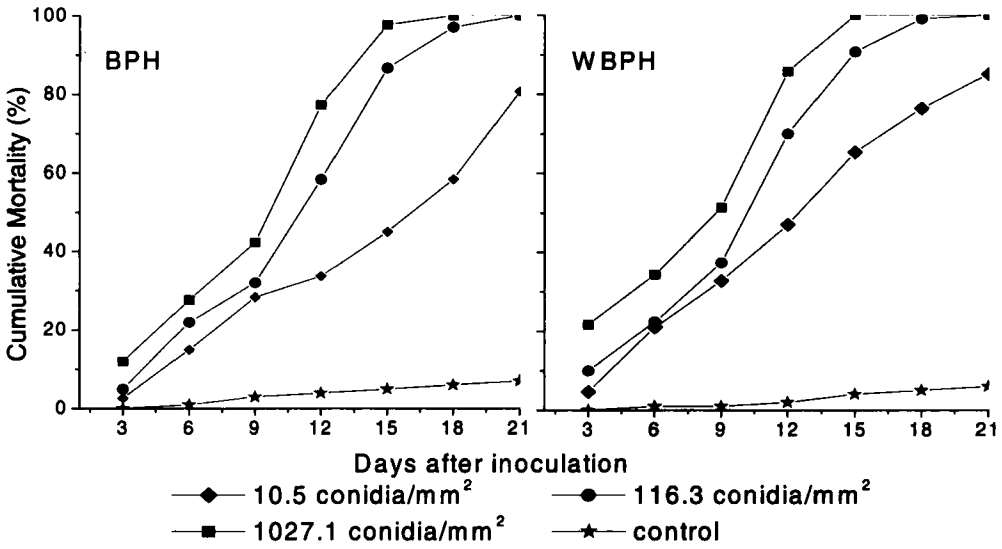

Fig.3 Effect of different concentration of *Metarhizium anisopliae* var. *acridum* on the adults of BPH and WBPH.

Of all the developmental stages, WBPH was more susceptible than BPH to *M. anisopliae* var. *acridum* infection with the same dosages because they had a higher mortality at the same time.

3.4 Virulence of *M. anisopliae* var. *acridum* on *N. lugens* and *S. furcifera*

Table 1 shows the degree of virulence of *M. anisopliae* var. *acridum* on *N. lugens* and *S. furcifera* according to the time in days required to achieve 50% mortality (LT_{50}). At different developmental stages, the susceptible sequence is adults > old nymphs > young nymphs of both *N. lugens* and *S. furcifera*.

4 DISCUSSION

The susceptibility of *N. lugens* and *S. furcifera* varied in their different developmental stages to *M. anisopliae* var. *acridum* infection. Adults were more susceptible to the infection than young and old nymph stages. That is important for the biocontrol of plathoppers because the adult stage is the most damaging stage for its high longevity and feeding rate. This observation concurred with those of Vestergaard *et al.* (1995) and Holdom *et al.* (1988). Vestergaard *et al.* (1995) reported that larval and pupal stages of the western flower thrips, *F. occidentalis* were more resistant to

Table 1. The LT_{50} (days) of *Metarhizium anisopliae* var . *acridum* against *Niloparvata lugens* and *Sogatella furcifera.*

Developmental stage		Dosages (conidia/mm^2)		
		10.5	116.3	1027.1
Sogatella furcifera	adults	12.78	10.16	7.64
	old nymphs	16.94	15.02	13.03
	young nymphs	>21	17.29	13.13
Niloparvata lugens	adults	17.10	12.57	9.14
	old nymphs	17.68	15.49	13.96
	young nymphs	>21	20.82	16.55

M. anisopliae var. *acridum* infection than adults. Different susceptibility at various life stages can be ascribed to interaction between the insect integument being penetrated by the fungus and ecdyses of larval and pupal stages. Ecdysis has been reported to be an important factor in insect resistance to fungal infection, particularly when the time interval between successive ecdysis is short (Vey and Fargues 1977). Holdom *et al.* (1988) reported that *N. lugens* nymphs infected with *E. delphacis* in Indonesia were less susceptible than adults. This could be due to the smaller surface area of the insects, resulting in fewer conidial contacts, the removal of conidia by molting before penetration of the cuticle could occur, or due to some internal resistance mechanism of the insect.

Shimazu (1977) found that only macropterous adults and old nymphs could be readily infected by another entomopathogenic fungus *E. delphacis* in the laboratory. Similar dose-mortality responses at different developmental stages were reported by several authors (Feng *et al.* 1985; Poprawski *et al.* 1985; Fransen 1987). The aberrant behavior of moving more torpidly and abdominal arching were observed in nymph and adult stages at 6-8 days post-inoculation in our experiments, similar to those of thrips species (Vestergaard *et al.* 1995). This has been attributed to toxins produced by *M. anisopliae* var. *acridum* following successful invasion of the host (Gillespie and Claydon 1989). Infected grasshoppers die with their legs wrapped around the plant stalk and heads pointed upward, this behavior was not observed in insect control.

Entomopathogenic fungi are being developed worldwide for the control of many important pests in agriculture (Ferron 1985) and some are already available commercially for the control of various species of thrips (Goettel *et al.* 1990).Their mass production is easy and does not require high-input technology (Prior *et al.* 1988). Reduction in the adult population could mean a reduction in the rate of population build-up of the insect, as fewer progeny and possibly a fewer number of generations are produced per season. Although the fungal spray is effective to BPH, its effects are not immediate. It takes take 6-10 days to die but the insects are greatly incapacitated during this time - eating and moving far less than healthy insects.

In conclusion, this study demonstrated the potential of *M. anisopliae* var. *acridum* as a

promising biological control agent for *N. lugens* and *S. furcifera.* Field tests using the fungus will be necessary in determining its place in both BPH and WBPH management program.

Secondary infection of both BPH and WBPH cadavers killed by *M. anisopliae* var. *acridum* was unknown in this study because all fresh cadavers were removed for verification of *M. anisopliae* var. *acridum* infection. Thus, further study is needed to investigate epizootiological features of both *M. anisopliae* var. *acridum* species in BPH colonies as well as their virulence.

Acknowledgements The authors are grateful to Dr. Qian Xia for her offering rice variety, to Hai-dong Chen and Ke-chen Ouyang for their technical assistance.This investigation received financial support to the fulfillment of National Basic Research and Development Program TG2000016210.

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黄绿绿僵菌对褐飞虱和白背飞虱不同发育阶段的病原性的研究

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本文报道了不同孢子浓度下黄绿绿僵菌对褐飞虱和白背飞虱不同发育阶段的易感性和毒力的研究。实验设 10.5 孢子 /mm^2，116.7 孢子 /mm^2 和 1027.1 孢子 /mm^2 三种孢子剂量，两种飞虱分为幼龄若虫（1 龄和 2 龄若虫）、高龄若虫（3、4、5 龄若虫）和成虫三个发育阶段。实验发现褐飞虱与白背飞虱的三个发育阶段对黄绿绿僵菌的不同浓度的孢子液有不同程度的易感性。黄绿绿僵菌对褐飞虱幼龄若虫的毒力指标 LT_{50} 在三种孢子剂量下依次为 >21、20.82 和 16.55；对高龄若虫的 LT_{50} 在三种孢子剂量下依次为 17.68、15.49 和 13.98；而对成虫的 LT_{50} 在三种孢子剂量下依次为 17.10、12.57 和 9.14。黄绿绿僵菌对白背飞虱幼龄若虫的毒力指标 LT_{50} 在三种孢子剂量下依次为 >21、17.29 和 13.13；对高龄若虫的 LT_{50} 在三种孢子剂量下依次为 16.94、15.02 和 13.03；而对白背飞虱成虫的 LT_{50} 在三种孢子剂量下依次为 12.78、10.16 和 7.64。二者的成虫的易感性比若虫的易感性强，高龄若虫的易感性比幼龄若虫的强。白背飞虱比褐飞虱对黄绿绿僵菌更加敏感。二者的死亡率随孢子浓度的增大而增大。

关键词 褐飞虱 白背飞虱 黄绿绿僵菌 易感性 毒力 发育阶段

致谢 本研究得到了国家基础和发展项目（TG2000016210）的支持，特此致谢。