Pathogenicity of *Beauveria bassiana* to *Riptortus linearis* (Hemiptera: Coreidae), a Pest of Soybean

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Pathogenicity of the white muscardine fungus, *Beauveria bassiana* (BALSAMO) VUILLEMIN, to a coreid bug, *Riptortus linearis* (F.), a pest of soybean, was investigated in a series of laboratory tests. *Beauveria bassiana* was pathogenic to 3rd, 4th and 5th stadium nymphs and adults of *R. linearis*. The 4th and 5th stadium nymphs were more susceptible than adults and 3rd instars. Within the range of 4.5×10^3 to 4.5×10^8 conidia/ml, there was a highly significant linear relationship between the dose of *B. bassiana* and the mortality of *R. linearis*. Isolates recovered from cadavers of *R. linearis* killed by *B. bassiana* were virulent to *R. linearis*. At 25°C and above, pathogenicity of *B. bassiana* decreased with increases in temperature. This was due to the adverse effect of high temperatures on the germination of conidia. The longer the fungus-treated insects were maintained at high temperatures, the lower the insect mortality. Ultra-violet (UV) irradiation of conidia reduced pathogenicity of *B. bassiana* to *R. linearis*.

Key words: *Riptortus linearis*, *Beauveria bassiana*, pathogenicity, temperature effect, UV irradiation

INTRODUCTION

Riptortus linearis (F.) (Hemiptera: Coreidae) and a closely related species *Riptortus clavatus* (THUNBERG) are two of the most common pests of soybean, *Glycine max* (L.) MERRILL, in Asia (KOBAYASHI, J., 1972; KOBAYASHI, Y., 1976; TALEKAR, 1987). Adults and nymphs of these coreid bugs pierce soybean pods with their proboscises and suck juices from developing tender green seeds. As a result, seeds either do not develop or remain shriveled and small. Such damage reduces yield and quality, especially germination, of the soybean seed (AVRDC, 1988).

At present, farmers in Asia use only insecticides to combat these pests. However, frequent insecticide use, in addition to being hazardous, is becoming more and more uneconomical and, thus, unsustainable. Research at the Asian Vegetable Research and Development Center (AVRDC) in southern Taiwan, therefore, aims at developing an integrated pest management strategy based on host-plant resistance and the use of natural enemies of these and other pests. The white muscardine fungus, *Beauveria bassiana* (BALSAMO) VUILLEMIN (Deuteromycotina: Hyphomycetes), has proven to be pathogenic to several insect species and attempts have been made to use this fungus to control a wide range of insect pests (FERRON, 1978; CHEUNG and GRULA, 1982; IGNOFFO et al., 1983; DAOUST and PEREIRA, 1986). This fungus also occurs in Taiwan's soils (SU et al., 1988), and, since its effects on coreids have never been investigated, various aspects of pathogenicity of *B. bassiana* to *R. linearis* were investigated here.

Fig. 1. Illustration of apparatus used for testing pathogenicity of *B. bassiana* to *R. linearis*.

MATERIALS AND METHODS

Insect source. Several wooden frame cages (50 × 50 × 50 cm), each with four sides, including one that could be closed and opened, a top made of a fine mesh nylon net and a wooden board for a bottom were prepared. One soybean plant in the R6 growth stage (FEHR and CAVINESS, 1977) was placed inside each cage. Several 20-cm long cotton threads were hung inside the cage. A 50-ml Erlenmeyer flask filled with water and closed tightly with a cotton wool plug was placed in the cage in an inverted position. Soybean seeds (about 200 g) were placed on the top of the cage. Several *R. linearis* adults, collected from soybean fields, were released inside the cages. The adults fed on the green soybean pods and dry seeds placed on the top of the cage. They laid eggs on plant and cotton threads. The eggs were then collected and placed in a new insect-rearing cage to allow them to develop into nymphs and adults.

Fungus source. A culture of *B. bassiana* (strain CH1) was obtained from National Chung Hsing University, Taichung. The fungus was grown on autoclaved potato dextrose agar. For field and greenhouse studies, the fungus was grown on autoclaved rice seeds and maintained at $25 \pm 2^\circ\text{C}$ for 15 days. A hemocytometer was used to determine the concentration of conidia in the suspension.

Pathogenicity of *B. bassiana* to various stages of *R. linearis*. Twenty individuals of 3rd, 4th, 5th stadium nymphs or adults were confined in each of three 15-cm-diameter Petri dishes, each dish being one replicate. The insects were immobilized by CO_2 gas. A conidial suspension, 2.2×10^8 conidia/ml, was then sprayed on the insect with an atomizer. The treated and untreated control insects of each replicate were transferred to individual acrylic cylinders (30 cm tall, 15 cm diameter) containing soybean seedlings and dry seeds (Fig. 1). Insect

mortality was recorded daily for up to 40 d and the LT_{50} values were calculated.

Pieces of *B. bassiana*-infected cuticle were excised and prefixed for 24 h in 25% glutaraldehyde/0.1 M cacodylate buffer. The samples were rinsed twice in 0.1 M phosphate buffer (pH 7.2) and postfixed for 2 h in 1% OsO_4 in glutaraldehyde/cacodylate buffer, followed by two rinses in phosphate buffer. The samples were then dehydrated in acetone, dried to the critical point utilizing CO_2 , gold-coated by an ion coater and examined under a Hitachi model S-450 scanning electron microscope.

Dose mortality response of R. linearis to B. bassiana infection. A series of *B. bassiana* conidial suspensions ranging from 4.5×10^3 to 4.5×10^8 conidia/ml were prepared. These were individually sprayed on 5th stadium *R. linearis* nymphs. The treated and untreated control insects were then placed in individual acrylic cylinders provided with soybean seedlings and dry seeds. Each treatment and control had three replicates. The insects were observed daily for mortality until they became adults. From the mortality data, LT_{50} and LC_{50} values were calculated.

In a related study 20 5th stadium *R. linearis* nymphs, soybean seedlings and dry seeds were placed in each of 21 acrylic cylinders. We then introduced 0.5, 1, 3, 5, 10 or 15 g of *B. bassiana* culture, grown on rice-seed medium, into each of three cylinders. Each gram of rice-seed medium had approximately 5.4×10^7 conidia. Three cylinders were maintained as untreated controls. We observed the insects daily and recorded the mortality until all nymphs became adults. In another study, conducted in an identical fashion, instead of various quantities of rice-seed medium, 1, 3, 5, 10 or 15 fresh cadavers of *R. linearis* nymphs killed by *B. bassiana* were used. Each cadaver had approximately 4.8×10^6 conidia. Insect mortality was recorded daily as described earlier.

Effect of temperature on pathogenicity of B. bassiana to R. linearis. Two sets of experiments were conducted. In the first set, the effects of constant temperatures of 15°C, 20°C, 25°C, 30°C and 35°C and alternating temperatures of 32°C during the day (12 h) and 23°C during the night (12 h) were studied. Twenty 5th instar nymphs were confined in each of 36 15-cm-diameter Petri dishes. Insects in 18 Petri dishes were sprayed with a conidial suspension containing 3.4×10^8 conidia/ml; the remaining 18 dishes were maintained as controls. Soon after inoculation, insects in the 36 Petri dishes were transferred to 36 acrylic cylinders containing soybean seedlings and dry seeds. Sets of three cylinders containing treated insects were maintained at constant temperatures of 15°C, 20°C, 25°C, 30°C or 35°C. Simultaneously, untreated control insects confined in an equal number of cylinders were also maintained at each of these temperatures. One set of three cylinders of treated as well as untreated insects was maintained at 32°C for 12 h of light and at 23°C for 12 h of darkness. Insects were observed daily, and the number of dead insects was recorded until all insects became adults. LT_{50} values were calculated from the mortality data.

In the second set, 5th instar *R. linearis* nymphs were sprayed with a conidial suspension containing 1.2×10^8 conidia/ml. They were then transferred to acrylic cylinders containing soybean seedlings and dry seeds. Each cylinder contained 20 insects. Three cylinders were maintained at a constant 25°C and another three at 35°C. Three cylinders were maintained at 35°C for 1, 2, 3, 4 or 5 d. After each interval the treated insects were held at 25°C. All insects were observed daily for mortality until they became adults.

Effect of UV irradiation on pathogenicity. *Beauveria bassiana* conidia powder was irradiated under 254 nm ultraviolet (UV) light for 0, 3, 6, 12, and 24 h by placing the fungus at 10 cm below the UV source, which provided a dose of 825 $\mu W/cm^2$. After each exposure period conidial suspensions containing 6×10^8 , 2×10^7 and 4×10^6 conidia/ml were prepared and

Fig. 2. *Beauveria bassiana* hyphae spreading out from antenna (a), and integument (b), and sporulation of the fungus on the integument (c) of *R. linearis*.

Table 1. Comparison of pathogenicity of *B. bassiana* conidial suspensions^a to nymphs and adults of *R. linearis*

Insect stage	No. insects tested	Corrected mortality (%) ^{b,c}	LT ₅₀ (days)
Adult	60	52.1 ± 27.8	38.0
5th stadium	58	78.2 ± 22.2	5.4
4th stadium	60	70.2 ± 19.9	4.9
3rd stadium	60	32.1 ± 16.5	—

^a Dose: 2.2×10^8 conidia/ml.

^b Corrected mortality (%) = (No. insects killed by *B. bassiana*/No. insects tested – No. insects that died from unknown causes) × 100%.

^c Data are means (± standard deviation) of 3 replicates.

sprayed on 5th stadium *R. linearis* nymphs, and insect mortality was recorded daily until adult emergence.

RESULTS AND DISCUSSION

Pathogenicity of B. bassiana to various stages of R. linearis

All three nymphal instars and adults were infected by *B. bassiana*. Fungal hyphae were observed spreading out of the antennae (Fig. 2a) and thoracic and abdominal integuments (Fig. 2b). In the later stages of infection, *B. bassiana* conidia arose from the hyphae on integuments (Fig. 2c). In all cases, insects with these symptoms died. The 4th and 5th stadium nymphs were far more susceptible than both adults and third instars (Table 1). It is possible that the cuticle of the nymph is not as completely sclerotized as that of adults. This would make the nymphal cuticle much more susceptible to the entry of *B. bassiana* hyphae than the adult cuticle. This probably caused greater mortality in the nymphs. For the 3rd stadium nymphs, we could not compute LT₅₀ because at the 2.2×10^8 conidia/ml dose, the highest corrected mortality was only $32.1 \pm 16.5\%$, even 40 days after inoculation. The lower mortality was probably due to the shorter duration of this stadium as compared to

Fig. 3. Dose/mortality response of *R. linearis* to *B. bassiana* infection. $Y = -0.938 - 7.134X + 2.254X^2$, $r = 0.976$.

later stadia (AVRDC, 1990). This causes the 3rd stadium nymphs to caste off the cuticle—along with the *B. bassiana* conidia adhering to it—relatively quickly which, in turn, probably reduces the pathogenicity. Similarly, early stadium nymphs of the brown planthopper, *Laodelphax striatellus* (FALLÉN) were found to be more resistant to *Metarhizium anisopliae* (METSCHNIKOFF) than the older instars (LEE and HOU, 1989).

Dose mortality response of R. linearis to B. bassiana infection

Results of the dose-mortality study are presented in Fig. 3. Within a dosage range of 4.5×10^3 to 4.5×10^8 conidia/ml, there was a highly significant linear relationship ($r = 0.976$, $df = 5$) between concentration of *B. bassiana* conidia and mortality of *R. linearis*. The LC_{50} value was 1.1×10^6 conidia/ml. The LT_{50} values were directly correlated to the concentration of conidia used. The values at 4.5×10^6 , 4.5×10^7 , and 4.5×10^8 conidia/ml were 9.2, 7.0, and 3.7 d, respectively.

When we confined the nymphs in a cage where fungal spores emanating either from cadavers of *B. bassiana*-killed insects or from fungus cultured on the rice-seed medium were presumably present, we observed an almost identical linear dose-mortality response. These data indicate that *B. bassiana* is, indeed, responsible for *R. linearis* mortality. The fact that insect mortality was proportional to the number of *B. bassiana*-infected cadavers used demonstrates that fungus from cadavers is recoverable in virulent form. This satisfies Koch's postulates.

Effect of temperature on pathogenicity of B. bassiana to R. linearis

Results of the effects of one variable and five constant temperatures on the mortality of *R. linearis* due to *B. bassiana* are presented in Table 2. Mortality was similar at 15 and 20°C; all insects died before reaching the adult stage. However, at temperatures of 25°C and above, insect mortality decreased with increasing temperatures. At a constant 35°C, mortality was too low to calculate LT_{50} . Higher temperatures exceeding 30°C are apparently detrimental to the development of *B. bassiana*. In a laboratory study, the growth of the colony on potato dextrose agar was drastically reduced at temperatures of 30°C and 32°C, and at 35°C the fungus failed to grow at all (HU, 1992). The retarded growth was related to reduced germination of conidia at these temperatures. At variable temperatures of 32°C for

Table 2. Susceptibility of *R. linearis*^a to *B. bassiana* under various temperature regimes

Temp. (°C)	No. insects tested	Corrected mortality (%) ^{c,d}	LT ₅₀ (days)
15	58	100.0 ± 0.0	6.5
20	60	100.0 ± 0.0	5.3
25	59	98.1 ± 3.2	4.1
30	60	31.6 ± 20.4	7.4
35	60	0	—
32/23 ^b	60	53.9 ± 7.0	7.1

^a Dose: 3.4×10^8 conidia/ml.

^b Light 32°C, dark 23°C (L : D = 12 h : 12 h).

^c Corrected mortality (%) = (No. insects killed by *B. bassiana*/No. insects tested – No. insects that died from unknown causes) × 100%.

^d Data are means (± standard deviation) of 3 replicates.

Fig. 4. Effect of temperature on *B. bassiana*-induced mortality in *R. linearis*. * 35(*n* d)-25 indicates that the inoculated insects were first exposed to 35°C for '*n*' days (d) and then to 25°C. Same letters at the top of bars indicate no statistical significance at the 5% level, according to DUNCAN's multiple range test.

Fig. 5. Effect of exposure of *B. bassiana* culture to UV light on its pathogenicity to *R. linearis*.

12 h of light and 23°C for 12 h of darkness, insect mortality was the same as it was with constant temperatures of 25 and 30°C. The low temperatures during darkness appear to overcome the detrimental effect of high temperatures during the day and increase pathogenicity of the fungus. Similarly, increasing the duration of exposure of fungus-treated insects

to high temperatures reduced the pathogenicity of *B. bassiana* to *R. linearis* (Fig. 4). The shorter the fungus-treated insect was maintained at high temperatures, the greater the pathogenicity. It is likely that high temperatures reduced conidial germination. The longer the exposure to high temperature, the greater are the chances of reduction in conidial germination and pathogenicity. Previous studies indicated that the ideal temperature for growth of this fungus is 24 to 27°C (STUDDERT and KAYA, 1990; FERNANDES et al., 1989 a, b; HU, 1992; JAYARAMAIAH and VEERESH, 1984). In the tropics, temperatures can remain above 30°C for a long period of time. This might limit the usefulness of the fungus as a bio-control agent in this region.

Effect of UV irradiation on pathogenicity

Irradiation of *B. bassiana* conidia by UV light caused a precipitous reduction in the infectivity of the fungus to *R. linearis* only at low conidial density (Fig. 5). At 4×10^6 and 2×10^7 conidia/ml, UV light drastically reduced the pathogenicity, presumably by killing the *B. bassiana* conidia. The longer the exposure to UV, the greater was the reduction in fungus-induced insect mortality. However, at the 6×10^8 conidia/ml concentration, a number of the conidia in the irradiated spore powder apparently survived and were able to kill practically all the insects on which the irradiated medium was applied. Ultraviolet rays are important constituents of common incidental light of the sun. Since the actual UV content of sunlight reaching the earth varies considerably with the prevailing atmospheric conditions (SANDERSON and HULBURT, 1955; COULSON, 1975), precise and consistent results concerning the ability of fungal spores to withstand a natural UV dose are impossible to obtain. Ultraviolet radiation is known to reduce infectivity of other fungi, bacteria and viruses (IGNOFFO et al., 1977; SHAPIRO, 1985). In general, exposure of viruses at 280 to 320 nm, which is present in natural sunlight reaching the earth, is nearly as effective as exposure to shortwave UV (254 nm) which does not reach the earth (JAQUES, 1977). Since the effect of UV radiation on infectivity of *B. bassiana* had not been investigated, we used a 254 nm wavelength and $825 \mu\text{W}/\text{cm}^2$, a dose routinely used in most laboratories to irradiate biological samples. Although this dose could be higher than what normally occurs in sunlight reaching the earth, the information generated is, nonetheless of significance in the process of conidial preparation while developing a fungal insecticide based on *B. bassiana*.

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