Mycopathologia (2006) 161: 385–394
DOI 10.1007/s11046-006-0020-2

Allee effect in the infection dynamics of the entomopathogenic fungus *Beauveria bassiana* (Bals) Vuill. on the beetle, *Mylabris pustulata*

K. Uma Devi & C. Uma Maheswara Rao
Department of Botany, Andhra University, Visakhapatnam, 530 003 AP, India

Received 16 August 2005; accepted in revised form 24 January 2006

Abstract

Successful infection by *Beauveria bassiana* as with all other entomopathogenic fungi, is accomplished only at a high conidial dose while, theoretically, a single conidium should be sufficient. Indeed, this is a major deterrent in its use as a biocontrol agent. High pathogen load for infection is required by organisms which display 'Allee' effect. In such organisms, a threshold exists for pathogen dose, below which no infection can be caused. *B. bassiana* has a semelparous life cycle and, therefore, its infection dynamics are expected to conform to the mass action principle with a linear relationship between dose and successful infection observable as mortality of the insect. Whether the need for a high conidial dose to induce insect mortality by *B. bassiana* is due to the operation of Allee effect was examined. A sample of 34 isolates was bioassayed on *Mylabris pustulata* (Coleoptera: Meloidae) at four conidial concentrations. With more than half of the isolates in the sample, the lowest dose tested (10^4 conidia/insect) did not cause insect mortality. Thus, a threshold pathogen load is required to cause successful infection. In these isolates, the dose–mortality relationship was sigmoid. Allee effect is thus identified in the infection dynamics of *B. bassiana–M. pustulata* system. The isolates that induced mortality at the lowest dose tested are concluded to be highly virulent with a lower threshold dose required for successful infection. With some isolates, at high conidial dose, the infection rate decreased either due to a decrease in the proportion of insects showing mycosis, to the speed of death, or both. Such a response could result from intra scramble competition arising from overload of pathogen at very high dose.

Key words: high effective conidial dose, semelparous life history, sigmoid dose–mortality response, threshold dose

Introduction

Beauveria bassiana, an ubiquitous insect pathogenic fungus with a very wide host range is marketed as a biopesticide. From a perusal of the several published reports of bioassays studying virulence of this fungus on insects [1, 2], and results on bioassays on nine insect species in our laboratory (unpublished), it has been observed that even under the most favorable conditions for infection, mortality is caused only when large conidial doses are applied, even though a single conidium should theoretically be sufficient to induce infection. With most hyphomycetous entomogenous fungi, the regression slopes computed from probit analysis of dose–mortality data are low [3]. Consequently, a very high dose of 10^{13}–10^{14} conidia of *B. bassiana* per hectare is used in field applications for effective knockdown of insect population [1].

Beauveria bassiana is an obligate killer. It has a semelparous life history with a single reproductive

episode. It releases all its infective propagules (conidia) in a single spell – after the death of the insect. The infection process of parasites with this type of life history is transparent – every successful infection results in host mortality and therefore can be detected. The infection dynamics of a semelparous parasite with clustering of all its (infective) propagules should conform to the 'mass action principle' [4], wherein infection rate is a linear function of the density of parasites and susceptible hosts. On the contrary, parasites with an iteroparous life history which have repeated spells of reproduction and a serial transmission of their infective units, can initiate infection only with a high dose of infective propagules. Such parasites display a so-called 'Allee effect' [5], a phenomenon that is characterized by an invasion threshold for the parasite, i.e., the parasite population can establish an infection only if its founder population size exceeds the invasion threshold [5]. In such systems that exhibit an Allee effect, the dose–infection relationship resolves into a sigmoid curve [5] in contrast to the linearity with systems in which infection dynamics follow the mass action principle. The Allee effect has been observed in infections of tree cutting ant by *Metarhizium anisopliae*, an entomogenous fungus closely related to *Beauveria bassiana* [4]. We tested whether the need for a large conidial dose to initiate infection by *B. bassiana* is also due to the existence of an Allee effect. To this end, bioassays were done on blister beetle (*Mylabris pustulata* Thunberg) (Coleoptera: Meloidae).

Materials and methods

A sample of 34 isolates of *B. bassiana* (Table 1) was bioassayed against the blister beetle. Isolates from diverse insect hosts and geographic (climatic) regions were selected to constitute the sample to have a representation of the wide diversity within the species. This sample has been characterized in our laboratory for several other phenotypes and also molecular genetically characterized to assess the reproductive biology and phylogenetic affiliation of this species [6–10, Uma Devi et al., unpublished]. The fungal cultures were initiated on Sabouraud dextrose yeast agar (SDAY) slants from conidia stored in 20% glycerol at −20 °C. The conidia from 14-day-old cultures were used in the bioassays. The conidia were harvested from the cultures by scraping with a sterile spatula, and 5 ml of an aqueous suspension with 0.02% Tween 80 (Sigma–Aldrich, India) was made by vortexing. An aliquot of the suspension was taken to check the viability of the conidia as described by Varela and Morales [11]. The remaining suspension was stored in a refrigerator (4 °C) for the treatments the next day. In a few isolates, the viability was also tested after storing in the refrigerator. No significant variation in viability was observed in the aqueous conidial suspension tested before and after storing at 4 °C. Cultures with more than 90% viable conidia were used in the treatments. The number of conidia per ml of the suspension was estimated through haemocytometer counts. Conidial suspensions of concentration of 10^5–10^8 conidia/ml were made.

Adult beetles (*M. pustulata*) were collected from a heavily infested pigeon pea field using an insect trap. Care was taken to ensure that all insects collected were of comparable size to avoid large variations in age. Beetles were placed as a batch of 10 (for each treatment) in perforated plastic boxes (10×5 cm) with lids. Fresh pigeon pea flowers were provided as feed every day to the beetles. The boxes were cleaned of insect litter daily. They were placed in an environmental chamber set at 25 ± 1 °C, 90% relative humidity and an 8 h/16 h light/dark cycle.

The insects were treated 2 days after collection. The beetles that were fatally injured during capture could be identified and removed in the 2 days before treatment. For treatment, the beetles were anaesthetized by exposing them to chloroform fumes from a cotton plug dipped in chloroform. The insects were treated singly with 100 μl of inoculum. The inoculum was dispensed with a micropipette (Gilson®) on the ventral side of the insect all over its body and head [12]. Treated beetles were put in boxes. Mortality was recorded daily for 30 days – the time up to which more than 90% of the control beetles were alive. There was no significant mortality in the treated insects after 14 days. Therefore, data up to 14 days after treatment was considered for analysis.

The boxes with the treated insects were arranged in an environmental chamber set to conditions described above. The bioassays were set up as a completely randomized block design [13] with two replicates for each treatment. The

Table 1. The original host and geographic origin of the isolates of the sample of the *entomopathogenic* fungus *Beauveria bassiana* used in the bioassay on *Mylabris pustulata*

Isolate[a]	Original host	Geographic origin
ARSEF 326	*Chilo plejadellus*	Queensland, Australia
ARSEF 501	*Ostrinia nubilalis*	China
ARSEF 652	*Ostrinia nubilalis*	Beijing, China
ARSEF 739	*Diabrotica paranoense*	Goiania, Brazil
ARSEF 1038	*Ostrinia nubilalis*	New York, USA
ARSEF 1098	*Nephotettix cincticeps*	Japan
ARSEF 1149 & 1166	*Helicoverpa armigera*	Cordoba, Spain
ARSEF 1169	*Sitona lineatus*	Senneville, France
ARSEF 1314, 1315 & 1316	*Helicoverpa virescens*	La Miniére, France
ARSEF 1491	*Diatraea saccharalis*	Lucighan, France
ARSEF 1512	*Spodoptera littoralis*	La Miniére, France
ARSEF 1788	*Helicoverpa virescens*	Spain
ARSEF 2860	*Schizaphis graminum*	Idaho, USA
ARSEF 3041	*Reticulitermus flavipes*	Toronto, Canada
ARSEF 3120	*Senecio* sp.	Yvelines, France
ARSEF 3286	*Spodoptera littoralis*	Montpellier, France
ARSEF 3387	*Myzus persicae*	Washington, USA
NRRL 3108	*Ostrinia nubilalis*	Unknown
NRRL 20698	*Dysdercus koengii*	Lima, Peru
NRRL 20699	Unknown	Illinois, USA
NRRL 22864	*Glichrochilus quadrisignatus*	Illinois, USA
NRRL 22865	Unknown	Iowa, USA
NRRL 22866	*Pachnaeus litus*	Florida, USA
ITCC 913	Unknown	The Netherlands
ITCC 1253	*Musca domestica*	Mumbai, Central India
ITCC 4521	*Diatraea saccharalis*	Karnal, North India
ITCC 4644	Oil palm larva	Ambajipeta, South India
ITCC 4688	*Helicoverpa armigera*	Hyderabad, South India
BB2	*Spodoptera litura*	Bangalore, South India
BB3	Soil	Bangalore, South India
BB4	*Helicoverpa armigera*	Warangal, South India

BB Isolates are from local (south Indian) fields and are not yet accessioned.

controls were treated with an equal volume of water with 0.02% Tween 80. The dead insects were transferred to moist chambers (autoclaved Petri dishes with a moist filter paper lining) to facilitate mycosis. The number of insects that expressed mycosis was noted. The bioassays were repeated once.

The cumulative insect mortality in each treatment was corrected for control mortality [14]. The number of insects with mycosis was estimated as percent proportion of dead insects. The mortality and mycosis values were arcsine percent square root transformed to normalize the data [15]. The mean and standard error (S.E.) of all (four) replicates for mortality and mycosis in each treatment were calculated and arcsine back transformed. Median lethal time (LT_{50}) was calculated from the cumulative mortality data on each day post treatment, using survival analysis with Weibull distribution [16]. The percent mortality, percent mycosis and LT_{50} of each isolate at the four conidial doses were subjected to principal component analysis (PCA), a data reduction statistical method, to compute relative virulence index (RVI) of the isolate at each dose. The RVI value would indicate the infection rate of the isolate having been derived from all the three virulence parameters. In results which indicated the prevalence of an Allee effect, the dose (log value) mortality data was plotted as a graph to test for fit for a sigmoid curve. Statistical analysis was done using the computer adaptive statistical software packages – SPSS ver. 7.5 and STATISTICA ver. 5.0 [17, 18].

Results

Dose–mortality relationship

With 16 of the 34 isolates tested, no mortality was observed at the lowest (10^5 conidia/ml; 10^4 conidia/insect) dose tested (Table 2). Thus, a concentration of 10^4 conidia per insect was not sufficient to induce successful infection. The mortality caused by five of these 16 isolates leveled off at values less than the theoretical maximum of 100% at the two high doses (10^7 and 10^8 conidia/ml). In the remaining isolates, 100% mortality was attained at the dose of 10^7 conidia/ml and remained the same at the higher dose (10^8 conidia/ml) (Table 2). Since no mortality was caused with these isolates at a dose of 10^5 conidia/ml, mortality is also not expected at a still lower dose (though this assumption was not tested). The dose–mortality relationship in these isolates thus resolved into a sigmoid curve (Figure 1).

With the other 18 isolates, mortality was caused at the lowest conidial dose tested (10^5 conidia/ml), and it increased with increasing dose up to a dose of 10^7 conidia/ml, and beyond this dose, it leveled off in 14 isolates, in 11 among them, a theoretical maximum (~100%) was reached by 10^7 conidia/ml; and it decreased in three isolates (Table 3). The isolates in which less than 15% mortality was caused at the lowest dose were also taken as suggestive of sigmoid response (Table 2).

Dose–mycosis relationship

One isolate, ARSEF 1038, was found to be non-mycotic at all the conidial concentrations tested (Table 1). With 14 isolates, no mycosis was induced in the dead insects at the low doses, 10^5 or 10^6 conidia/ml. With the other isolates, a positive correlation between mycosis and dose was observed up to a dose of 10^7 conidia/ml. A further increase in dose had a positive (18 isolates), negative (7 isolates) or neutral effect (8 isolates – in two of them 100% mycosis was produced at a dose of 10^7 conidia/ml) on mycosis (Tables 2 and 3).

Relationship between dose and speed of kill

The speed with which the insects were killed as indicated by LT_{50}, increased with increasing dose up to a concentration of 10^7 conidia/ml in all isolates. At a conidial concentration of 10^8 conidia/ml, the speed of kill further increased (seven isolates), decreased (18 isolates) or leveled off (8 isolates) (Tables 2 and 3). No consistent relationship between speed of kill and mycosis was observed.

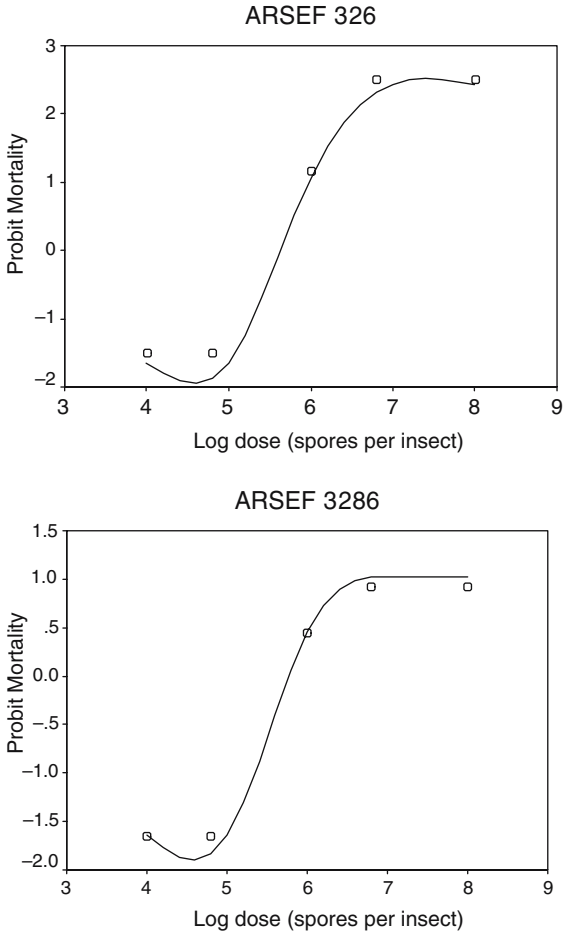

Figure 1. The sigmoid dose–mortality relationship in *Beauveria bassiana–Mylabris pustulata* system in isolates showing an 'Allee' effect (a) isolate ARSEF 326 as a representative of isolates in which leveling off of insect mortality beyond a conidial dose occurred because the theoretical maximum of 100% is reached ($y = -8.472 + 1.5281X + 0.188$, $r^2 = 0.8829$); (b) isolate ARSEF 3286 as a representative of isolates in which leveling off of mortality values beyond a conidial dose occurred even when theoretical maximum of 100% is not reached ($y = -0.851 + 0.799X + 0.56$, $r^2 = 0.8821$). The graphs have been derived from probit-derived log values of dose and mortality (uncorrected for control mortality) using SPSS ver. 7.5 [17].

Table 2. Laboratory bioassay data demonstrating an Allee effect in the infection dynamics of the entomopathogenic fungus *Beauveria bassiana* on *Mylabris pustulata*

Isolate[a]	Conc[b]	Mor	Myc	LT_{50}[c]	Rid	Response pattern[ed]
ARSEF 326, NRRL 22865	10^8	99.2 ± 0.8	50.0 ± 0.4	5.5 (4.5–6.3)	1.33	RVI: Inc
	10^7	99.2 ± 0.8	40.0 ± 1.6	8.9 (6.8–10.2)	0.79	Mor: Sig
	10^6	85.7 ± 0.4	0	9.2 (8.6–11.3)	0.08	Myc: Inc
	10^5	0	0	e	−1.14	LT_{50}: Dec
ARSEF 652, NRRL22866, ITCC 4521	10^8	62.5 ± 0.3	40.0 ± 0.4	13.1 (10.6–15.9)	0.46	RVI: Dec
	10^7	62.5 ± 1.5	37.5 ± 0.5	11.2 (9.8–2.5)	0.89	Mor: Sig
	10^6	52.2 ± 0.3	33.0 ± 0.3	11.5 (10.3–2.9)	0.61	Myc: Inc
	10^5	0	0	e	−1.28	LT_{50}: Inc
ARSEF 739	10^8	96.2 ± 3.8	45.0 ± 0.5	4.1 (3.1–4.9)	0.86	RVI: Dec
	10^7	98.3 ± 1.7	44.0 ± 1.2	2.7 (2.3–4.5)	0.96	Mor: Sig
	10^6	81.1 ± 1.6	43.0 ± 0.3	9.5 (8.5–10.6)	0.34	Myc: Lev
	10^5	0	0	e	−1.25	LT_{50}: Inc
ARSEF 1038	10^8	37.6 ± 0.2	0	e	−0.73	RVI: lev
	10^7	37.5 ± 0.1	0	e	−0.73	Mor: Sig
	10^6	37.8 ± 0.3	0	e	−0.73	Myc: –
	10^5	0	0	e	−1.33	LT_{50}: –
ARSEF 1098	10^8	100	100	6.6 (5.7–7.3)	1.04	RVI: Dec
	10^7	100	100	6.2 (4.9–7.3)	1.08	Mor: Sig
	10^6	90.0 ± 0.4	80.0 ± 0.5	9.9 (8.5–11.3)	0.57	Myc: Lev^s
	10^5	0	0	e	−0.87	LT_{50}: lev
ARSEF 1315	10^8	56.3 ± 0.4	44.0 ± 0.3	9.5 (7.7–11.9)	0.81	RVI: Dec
	10^7	56.3 ± 3.4	42.5 ± 0.5	8.3 (6.2–9.3)	1.13	Mor: Sig
	10^6	48.6 ± 0.1	40.0 ± 0.2	e	0.24	Myc: Lev
	10^5	0	0	e	−1.21	LT_{50}: Inc
ARSEF 1512	10^8	99.2 ± 0.8	40.0 ± 0.0	6.6 (5.7–7.2)	0.79	RVI: Dec
	10^7	99.2 ± 0.8	63.0 ± 2.6	7.3 (6.1–8.5)	1.02	Mor: Sig
	10^6	85.7 ± 0.4	38.0 ± 0.3	9.5 (7.5–11.3)	0.34	Myc: Dec
	10^5	12.5 ± 0.3	0	e	−1.25	LT_{50}: Dec
ARSEF 1788, ARSEF 3286, NRRL 3108, NRRL 20698	10^8	100	40.0 ± 0.4	4.2 (3.2–4.9)	0.95	RVI: Inc
	10^7	100	33.0 ± 2.4	3.4 (2.4–3.4)	0.87	Mor: Sig
	10^6	90.0 ± 0.4	33.0 ± 0.3	9.8 (7.5–11.2)	0.34	Myc: Inc
	10^5	0	0	e	−1.25	LT_{50}: Inc
ARSEF 3387, ITCC 4688	10^8	99.2 ± 0.8	10.0 ± 0.0	8.9 (7.6–9.7)	1.29	RVI: Inc
	10^7	99.2 ± 0.8	0	8.6 (7.6–9.7)	0.66	Mor: Sig
	10^6	85.7 ± 0.4	0	10.4 (8.6–12.1)	0.21	Myc: Inc
	10^5	0	0	e	−1.19	LT_{50}: Lev
NRRL 22864	10^8	99.2 ± 0.8	80.0 ± 0.4	6.3 (5.1–7.3)	0.63	RVI: lev
	10^7	99.2 ± 0.8	50.0 ± 0.4	3.2 (2.2–4.3)	0.62	Mor: Sig
	10^6	85.7 ± 0.4	89.0 ± 0.1	5.5 (3.9–7.2)	0.66	Myc: Inc
	10^5	0	0	e	−1.29	LT_{50}: Inc
ITCC 1253, BB3	10^8	100	100	5.3 (4.4–6.1)	1.70	RVI: Inc
	10^7	100	40.3 ± 1.6	9.8 (4.9–11.0)	0.74	Mor: Sig
	10^6	48.6 ± 0.1	0	e	−0.46	Myc: Inc
	10^5	12.5 ± 0.3	0	e	−0.76	LT_{50}: Dec

[a]Isolates with similar response pattern are clubbed. The values represent the actual values of the first isolate in the group.
[b]Conidia/ml: 100 μl/insect.
[c]Values in brackets indicate fiducial limits, e = error, LT_{50} could not be computed because the mortality caused did not reach 50%.
[d]Relative virulence index of an isolate at the four doses computed from mortality, mycosis and LT_{50} using principal component analysis, a data reduction statistical method. The RVI reflects the infection rate of the isolate.
[e]Response pattern over the doses: Inc – increasing with increase in conidial dose; Dec – increasing with dose up to a concentration of 10^7 conidia/ml and decreasing at 10^8 conidia/ml; Lev – increasing with dose up to 10^7 conidia/ml with no further increase at 10^8 conidia/ml; Lev^s – increasing with dose and reaching the theoretical maximum (100%) at a concentration (usually) of 10^7 conidia/ml with no scope for increase with increase in conidial dose; Sig – sigmoid, with no mortality at the lowest dose, increasing mortality with increase in dose at the next two higher doses which finally levels off even with an additional increase in dose. For LT_{50}, values with overlapping fiducial limits are taken as not significantly different.

Table 3. Laboratory bioassay data of isolates of the entomopathogenic fungus *Beauveria bassiana* on *Mylabris pustulata* in which an Allee effect was not observable*

Isolate[a]	Conc[b]	Mor	Myc	LT_{50}[c]	RVI[d]	Response pattern[e]
ARSEF 501	10^8	100	100	5.9 (5.1–6.6)	0.92	RVI: Inc
	10^7	100	100	6.2 (3.9–7.3)	0.90	Mor: Lev^S
	10^6	90.0 ± 0.4	40.0 ± 0.4	4.9 (3.9–6.2)	0.34	Myc: Lev^S
	10^5	25.0 ± 0.5	50.0 ± 0.4	e	−1.25	LT_{50}: lev
ARSEF 1149	10^8	84.7 ± 0.3	89.0 ± 0.4	3.6 (2.4–4.6)	1.10	RVI: Inc
	10^7	84.7 ± 0.8	100	5.1 (3.9–6.5)	1.08	Mor: Lev
	10^6	67.0 ± 0.1	78.0 ± 0.5	5.1 (4.2–9.4)	0.49	Myc: Dec
	10^5	50.0 ± 0.5	33.0 ± 0.3	11.3 (10.1– 14.7)	−0.89	LT_{50}: Dec
ARSEF 1166	10^8	100	20.0 ± 0.5	5.8 (4.9–6.4)	1.12	RVI: Dec
	10^7	100	20.0 ± 1.0	2.9 (1.5–3.8)	1.33	Mor: Lev^S
	10^6	90.0 ± 0.4	0	10.9 (8.5–15.6)	−0.02	Myc: Lev
	10^5	25.0 ± 0.3	0	e	−0.81	LT_{50}: Inc
ARSEF 1169	10^8	50.0 ± 0.5	50.0 ± 0.5	10.4 (8.7–12.3)	0.80	RVI: Dec
	10^7	75.0 ± 1.3	49.0 ± 1.3	7.9 (5.4–9.3)	1.40	Mor: Dec
	10^6	45.0 ± 0.3	60.0 ± 0.4	e	0.39	Myc: Lev
	10^5	0	0	e	−0.86	LT_{50}: Inc
ARSEF 1314	10^8	59.4 ± 0.1	43.0 ± 0.0	9.5 (7.7–11.9)	0.88	RVI: Dec
	10^7	59.4 ± 0.1	49.0 ± 1.3	7.6 (6.4–9.2)	0.92	Mor: Lev
	10^6	50.4 ± 0.4	57.0 ± 0.3	6.5 (4.3–8.6)	0.35	Myc: Dec
	10^5	37.5 ± 0.1	0	e	−1.25	LT_{50}: Inc
ARSEF 1316	10^8	100	10.0 ± 0.0	4.1 (3.0–4.7)	0.36	RVI: Dec
	10^7	100	33.0 ± 2.4	2.4 (1.5–3.6)	1.08	Mor: Lev^s
	10^6	90.0 ± 0.4	33.0 ± 0.3	6.7 (5.4–8.2)	0.53	Myc: Dec
	10^5	37.5 ± 0.5	20.0 ± 0.0	e	−1.25	LT_{50}: Inc
ARSEF 1491	10^8	60.6 ± 1.1	25.0 ± 0.3	8.7 (7.5–10.1)	0.02	RVI: Dec
	10^7	78.4 ± 1.2	70.0 ± 0.5	8.4 (7.2–9.8)	0.86	Mor: Dec
	10^6	90.0 ± 0.0	50.0 ± 0.0	8.5 (4.8–10.2)	0.79	Myc: Dec
	10^5	40.0 ± 0.5	0	e	−1.23	LT_{50}: Lev
ARSEF 2860, NRRL 20699	10^8	96.2 ± 3.8	90.0 ± 0.4	5.8 (4.7–6.6)	1.04	RVI: Inc
	10^7	98.3 ± 0.7	70.0 ± 0.5	3.2 (2.1–4.4)	1.02	Mor: Lev^S
	10^6	81.1 ± 1.6	44.0 ± 0.3	7.9 (5.2–9.2)	0.16	Myc: Inc
	10^5	25.0 ± 0.3	20.0 ± 0.5	e	−1.19	LT_{50}: Inc
ARSEF 3041	10^8	98.3 ± 0.7	80.0 ± 0.4	6.8 (5.6–7.7)	1.03	RVI: Dec
	10^7	98.3 ± 0.7	89.0 ± 1.6	6.7 (5.4–7.9)	1.15	Mor: Lev^S
	10^6	83.9 ± 0.8	33.0 ± 0.3	8.7 (5.9–10.2)	0.06	Myc: Dec
	10^5	25.0 ± 0.3	50.0 ± 0.5	e	−1.15	LT_{50}: Lev
ARSEF 3120	10^8	100	30.0 ± 0.5	9.8 (8.7–10.7)	1.01	RVI: Dec
	10^7	100	13.0 ± 0.5	5.6 (4.5–6.6)	1.11	Mor: Lev^S
	10^6	90.0 ± 0.4	0	9.9 (8.5–11.2)	0.11	Myc: Inc
	10^5	25.0 ± 0.3	0	e	−1.17	LT_{50}: Inc

ITCC 913	10^8	50.0 ± 0.0	87.0 ± 0.3	8.3 (6.5–10.5)	0.30	RVI: Dec
	10^7	75.0 ± 0.5	66.0 ± 0.6	3.8 (2.8–4.9)	0.61	Mor: Dec
	10^6	90.0 ± 0.5	80.0 ± 0.4	5.6 (4.5–7.8)	0.81	Myc: Inc
	10^5	12.5 ± 0.3	33.0 ± 0.3	e	−1.27	LT_{50}: Inc
ITCC 4644	10^8	50.0 ± 0.5	50.0 ± 0.4	7.5 (5.7–9.4)	0.75	RVI: Dec
	10^7	75.0 ± 0.3	50.0 ± 0.4	5.6 (4.3–6.1)	1.44	Mor: Dec
	10^6	45.0 ± 0.3	100	e	0.40	Myc: Dec
	10^5	12.5 ± 0.3	33.0 ± 0.3	e	−0.86	LT_{50}: Inc
BB2	10^8	100	85.0 ± 0.3	5.9 (4.5–7.0)	1.21	RVI: Dec
	10^7	100	75.0 ± 1.2	5.5 (4.3–6.9)	1.32	Mor: Lev^S
	10^6	45.0 ± 0.3	50.0 ± 0.5	e	−0.23	Myc: Inc
	10^5	25.0 ± 0.0	90.0 ± 0.0	e	−0.77	LT_{50}: Lev
BB4	10^8	100	60.0 ± 0.0	6.4 (4.9–7.0)	1.04	RVI: lev
	10^7	100	86.4 ± 2.9	8.8 (7.5–10.1)	1.05	Mor: Lev^S
	10^6	90.0 ± 0.0	40.0 ± 0.4	9 (7.1–11.2)	0.49	Myc: Dec
	10^5	37.5 ± 0.3	44.0 ± 0.3	e	−0.38	LT_{50}: Dec

* Bioassay at a lower concentration may reveal an Allee effect in these isolates.

[a]Isolates with similar response pattern are clubbed. The values represent the actual values of the first isolate in the group.

[b]Conidia/ml: 100 μl/ insect.

[c]Values in brackets indicate fiducial limits, e = error, LT_{50} could not be computed because the mortality caused did not reach 50%.

[e]Relative virulence index of an isolate at the four doses computed from mortality, mycosis and LT_{50} using principal component analysis, a data reduction statistical method. The RVI reflects the infection rate of the isolate.

[f]Response pattern over the doses: Inc–Increasing with increase in conidial dose; Dec– Increasing with dose up to a concentration of 10^7 conidia/ml and decreasing at 10^8 conidia/ml; Lev–Increasing with dose up to 10^7 conidia/ml with no further increase at 10^8 conidia/ml; Lev^s–Increasing with dose and reaching the theoretical maximum (100%) at a concentration (usually) of 10^7 conidia/ml with no scope for increase with increase in conidial dose For LT_{50}, values with overlapping fiducial limits are taken as not significantly different.

Dose–infection rate relationship

The overall infection rate was assessed from the relative virulence index of an isolate at the four doses tested. In 14 isolates, dose–infection rate showed a linear relationship through all the doses tested, it decreased beyond a dose of 10^7 conidia/ml in 18 isolates and leveled off at the two high doses in two isolates (Tables 2 and 3). In cases where infectivity increased through all the doses tested, when the mortality leveled off at the two high doses, the increase was either due to an increase in the speed of kill or increase in mycosis or both.

Discussion

Virulence of a pathogen or parasite is normally dose-dependant with increased virulence correlated with increased doses of the pathogen. In the present study, in a substantial number (19/34) of *B. bassiana* isolates, the linear relationship between dose and virulence was found to be restricted to only the medium doses. In these isolates, at low doses, no mortality was caused and, with increasing dose from medium to high, no further increase in mortality was observed. In some of these isolates the theoretical maximum of 100% mortality was induced at medium dose and, therefore, no further increase was possible at higher dose; in others, % mortality leveled off from the medium dose even when 100% mortality was not reached. The fact that a low conidial dose was ineffective in inducing mortality with these *B. bassiana* isolates indicates that a threshold load of infective propagules is required to be effective and dose–mortality relationship is sigmoid.

The *B. bassiana* isolates in which a sigmoid dose–mortality relationship was not detected may be highly virulent isolates in which the threshold value for effective infection is low. Bioassay with a lower conidial dose may reveal sigmoid nature of dose–mortality response in these isolates. It has been noted [4] that an Allee effect is subtle and may be detected only when sufficient range of conidial doses is tested. Bioassays with several different doses spanning a wider range of conidial concentrations in the highly virulent *B. bassiana* isolates would bring out the sigmoid nature of dose–mortality relationship characteristic of such infection dynamics.

A threshold dose to cause infection may be required due to any of the three mechanisms: (a) the probability of mortality per conidium is constant and independent of the number of conidia applied, but this probability is extremely low so that large numbers of conidia are needed to get measurable infection, or (b) the likelihood of mortality per conidium is highly variable with only a few conidia being actually infective but again this probability is independent of the number of conidia applied or (c) the possibility of mortality per conidium increases as more conidia are applied because the conidia overwhelm host defenses. Only the last of these could really be labeled an Allee effect. It might be possible to distinguish among these mechanisms with additional definitive experiments analyzing the relationship between mean and variance in mortality and number of conidia applied.

The results of the present study point to a sigmoid dose–mortality response with several isolates of *B. bassiana* on *M. pustulata*. Such a response has also been reported in *Metarhizium anisopliae*, an entomogenous fungus closely related to *B. bassiana* with a similar life history pattern [4, 19, 20] and some parasites [21–23]. The basis for the existence of Allee effect has been discussed in *Metarhizium* [4]. The entry of the infection peg from the conidium into the insect host triggers its second line of defenses – the cellular and humoral immune responses [24]. Successful infection may be possible only when the immune reaction is counteracted and completely saturated. A critical number of conidia may be required to achieve this task. When invaded by fewer conidia, the insect immune system may be successful in overtaking them either through phagocytosis or melanization or encapsulation responses. No infection is thus apparent when the conidial dose is lower than this threshold number. The actual threshold dose for successful infection may depend on the virulence of the fungal isolate. With a highly virulent isolate the threshold value may be low. A highly virulent isolate can more vigorously grow in the host and deplete its nutrients faster.

A negative effect of the highest dose tested (10^8 conidia/ml) on mortality rates was observed with three *B. bassiana* isolates in beetle bioassays. Goettel et al. [25] reported a similar negative correlation between dose and mortality at concentrations higher than 10^4 ascospores of *Ascosphaera*

aggregata on larvae of the leaf cutting bee, *Megachile rotunda*.

A negative correlation between dose and the reproductive rate of a parasite has been forecast from theoretical considerations [5]. Such a pattern was observed in lesser mealworm infected with *B. bassiana* [26]. At the highest doses tested many mealworm larvae that succumbed showed no or few overt symptoms of mycosis [26]. In the present study, such a negative effect of high conidial dose on mycosis was observed with eight isolates. This negative effect of dose on mycosis is reported to be due to premature death of the insect due to toxicosis without ample opportunity for the fungus to grow [27] so that it is rapidly out competed by saprobic antagonists on the insect cadaver [28], or due to scramble competition between the numerous parasites (hyphal bodies) when high doses are applied [29]. In the present study, at the conidial concentrations tested, with 16 *B. bassiana* isolates, there was a linear increase in the proportion of insects that showed mycosis with increase in dose though mortality levels flattened off from the lower conidial dose. In organisms with semelparous life history, the number of progeny released is a direct reflection of the number of progeny produced at the time of death of the host [4]. In the present bioassays, no count of conidia produced on insect cadavers was made. Only the occurrence or absence of mycosis was scored. The mere occurrence of mycosis does not indicate the number of progeny produced, but it points to the efficiency of the invaded fungal mycelia in the insect host to cross the cuticular barrier effectively and grow and sporulate on its cadaver. Despite scramble competition, when higher doses of parasites are applied, the number of pathogen progeny produced in the host insect at the time of death may be greater than when lower pathogen doses are applied. A larger number may produce more of the enzymes (e.g., chitinase) facilitating emergence from the cuticle after the death of the insect host and external growth of the fungus on the cadaver.

Regoes et al. [5] predicted that the life span of the infected hosts should be negatively correlated with dose. Such a trend was observed with seven of the 34 isolates tested here. In a majority (26) of *B. bassiana* isolates a negative correlation was observed up to a dose of 10^7 conidia/ml. Above this dose, the life span of the infected host increased (LT_{50} increased) over the lower concentration (19 isolates) or remained similar (seven isolates). High-dose treatments were found to slow down the growth of *B. bassiana* isolates [30]. The intra (between individuals of the same isolate) scramble competition intensified at high dose must have contributed to the slower growth resulting in slower death of the insect.

From these bioassay studies of *B. bassiana* on *Mylabris pustulata*, an Allee effect with a sigmoid relationship between dose and mortality was evident explaining the need for applying high conidial dose to achieve effective results in insect management.

Acknowledgements

We are thankful to Dr. R.A. Humber (ARSEF culture collection: Ithaca, New York); Dr. Kerry O' Donnell (NRRL culture collection; Peoria, Illinois) and the Indian Type Culture Collection (ITCC, IARI, New Delhi) for providing the cultures of *B. bassiana*. We are thankful to DST, New Delhi, Project No SP/SO/A-10/97, for financial support. CUM is thankful to CSIR for a research fellowship.

References

1. Bartlett MC, Jaronski ST. Mass Production of entomogenous fungi for biological control of insects. In: Burge MN, ed. Fungi in Biological Control Systems, Manchester University Press, Manchester, UK, 1988: 61–85.
2. Wraight SP, Jackson MA, de Kock SL. Production, stabilization and formulation of fungal biocontrol agents. In: Butt TM, Jackson C, Magan N, eds. Fungi as Biocontrol Agents: Progress. Problems and Potential, CAB International, Wallingford, UK, 2001: 253–287.
3. Wraight SP, Caruthers RI. Production, delivery and use of mycoinsecticides for control of insect pests of field crops. In: Hall FR, Menn JJ, eds. Methods in Biotechnology, Vol. 5, Biopesticides: Use and Delivery, Humana Press, Totowa, NJ, 1999: 233–269.
4. Hughes WOH, Petersen KS, Ugelvig LV, Pedersen D, Thomsen L, Poulsen M, Boomsma JJ. Density-dependence and within-host competition in a semelparous parasite of leaf-cutting ants. Evol Biol 2004; 4: 45.
5. Regoes R, Ebert D, Bonhoeffer S. Dose-dependent infection rates of parasites produce the Allee effect in epidemiology. Proc R Soc Lond B 2002; 269: 271–279.
6. Uma Devi K, Sridevi V, Murali Mohan C, Padmavathi J. Effect of high temperature and water stress on *in-vitro* germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. J Invert Pathol 2005; 88: 181–188.

7. Butters JA, Uma Devi K, Murali Mohan C, Sridevi V. Screening for tolerance to bavistin, a benzimidazole fungicide containing benzimidazol-2-yl carbamate (MBC), in *Beauveria bassiana:* Sequence analysis of the β tubulin gene to identify mutations conferring tolerance. Mycol Res 2003; 107: 260–266.
8. Padmavathi J, Uma Devi K, Uma Maheshwara Rao C. The optimum and tolerance pH range is correlated to colonial morphology in isolates of the entomopathogenic fungus *Beauveria bassiana* – a potential biopesticide. World J. Microbiol Biotechnol 2003; 19: 469–477.
9. Padmavathi J, Uma Devi K, Uma Maheswara Rao C, Nageswara Rao Reddy N. Telomere fingerprinting for assessing chromosome number, isolate typing and recombination in the entomopathogen, *Beauveria bassiana* (Balsamo) Vuillemin. Mycol Res 2003; 107: 572–580.
10. Uma Devi K, Padmavathi J, Sharma H C, Sitarama N. Evaluation of the virulence of 20 strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.)Vuillemin to the sorghum shoot borer *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in a laboratory bioassay: their characterization by RAPD-PCR. World J Microbiol Biotechnol 2001; 17: 131–137.
11. Varela A, Morales E. Characterization of some *Beauveria bassiana* isolates and their virulence toward the coffee berry borer *Hypothenemus hampei*. J Invertebr Pathol 1996; 67: 147–152.
12. Butt TM, Goettel MS. Bioassays of entomogenous fungi. In: Navon A, Ascher KRS, eds. Bioassays of Entomopathogenic Microbes and Nematodes, CAB International, Wallingford, UK, 2000: 141–195.
13. Goettel MS, Inglis GD. Fungi: Hyphomycetes. In: Lacey LA, ed. Manual of Techniques in Insect Pathology, Academic Press, London, 1997: 213–249.
14. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925; 18: 265–267.
15. Gomez AK, Gomez AA. Statistical Procedures for Agricultural Research. Singapore: John Wiley & Sons, Inc, 1984.
16. Robertson JL, Preisler HK. Pesticide Bioassay with Arthropods. Boca Raton, Florida: CRC Press, 1992.
17. SPSS Inc. SPSS Standard Version Copyright (c), SPSS for windows Release 7.5.1 (1989–1996), 1996.
18. StatSoft. Stastistica for Windows (Computer Programme Manual) Tulsa, OK: StatSoft, Inc., 1995.
19. Milner RJ, Prior C. Susceptibility of the Australian plague locust, *Chortoicetes terminifera*, and the wingless grasshopper, *Phaulacridium vittatum*, to the fungi *Metarhizium* spp. Biol Control 1994; 4: 132–137.
20. Vestergaard S, Gillespie AT, Butt TM, Schreitter G, Eilenberg J. Pathogenicity of the hyphomycete fungi *Verticillium lecanii* and Metarhizium anisopliae to the western flower thrips, *Frankliniella occidentalis*. Biocontrol Sci Technol 1995; 5: 185–192.
21. Agnew P, Koella JC. Life-history interactions with environmental conditions in a host–parasite relationship and the parasite's mode of transmission. Evol Ecol 1999; 3: 67–89.
22. Little TJ, Ebert D. The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): The role of host genetics. Proc R Soc Lond B 2000; 267: 2037–2042.
23. Mc Lean AR, Bostock CJ. Scrapie infections initiated at varying doses: an analysis of 117 titration experiments. Philos Trans R Soc Lond B 2000; 355: 1043–1050.
24. Gillespie JP, Bailey AM, Cobb B, Vilcinskas A. Fungi as elicitors of insect immune responses. Arch Insect Biochem Physiol 2000; 44: 49–68.
25. Goettel MS, Vandenberg JD, Duke GM, Schaalje GB. Susceptibility to chalkbrood of alfalfa leafcutter bees, *Megachile rotundata,* reared on natural and artificial provisions. J Invertebr Pathol 1993; 64: 71–73.
26. Steinkraus DC, Geden CJ, Rutz DA. Susceptibility of lesser mealworm (Coleoptera: Tenebrionidae) to *Beauveria bassiana* (Moniliales: Moniliaceae): Effects of host stage, substrate, formulation, and host passage. J Med Entomol 1991; 28: 314–321.
27. Glare TR, Milner RJ. Ecology of entomopathogenic fungi. In: Arora DK, Mukerji KG, Drouhet E, eds. Handbook of Applied Mycology, Vol. 2: Humans, Animals and Insects, Marcel Dekker, New York, 1991: 547–612.
28. Fargues J, Remaudière G. Considerations of the specificity of entomopathogenic fungi. Mycopathologia 1977; 62: 31–37.
29. Nowak MA, May RM. Superinfection and the evolution of virulence. Proc R Soc Lond B 1994; 255: 81–89.
30. Luz C, Tigano MS, Silva IG, Corderio CM, Aijanabi SM. Selection of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to control *Triatoma infestans*. Memorias do Instituto Oswaldo Cruz 1998; 93: 839–846.

Address for correspondence: K. Uma Devi, Department of Botany, Andhra University, Visakhapatnam, 530 003 AP, India
Phone: +91-891-2525582, +91-891-2844563; Fax: +91-891-2755547
E-mail: kumadevi@eth.net