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Laboratory evaluation of the virulence of *Beauveria bassiana* isolates to the sorghum shoot borer *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) and their characterization by RAPD-PCR

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Summary

Beauveria bassiana has long been used as a mycopesticide. It has a wide host range; isolates have been reported to differ in host range and virulence to a given insect species. Identification of a molecular marker linked to a virulent phenotype to a target pest would be useful in screening for isolates effective against it. Twenty *B. bassiana* isolates were tested for their virulence to the second instar larvae of *Chilo partellus* Swinhoe in laboratory bioassays and their DNA fingerprints were generated by RAPD-PCR. Three arbitrary categories of aggressiveness were chosen; isolates that caused >70%, between 70 and 40% and <40% larval mortality were grouped as highly, medium and less aggressive types, respectively. In the random amplified polymorphic DNA (RAPD) analysis a 30% variability was observed among the isolates; which clustered into three major groups. The groups based on virulence rating did not match with the RAPD clusters. One of the highly aggressive isolates clustered with less aggressive isolates in one cluster and the other grouped along with the medium aggressive isolates in a different cluster. The *B. bassiana* isolates were classified phenotypically based on the taxonomic order of the original insect host and the climatic zone (tropical/temperate) from which they were isolated. No correlation between the aggressiveness of the isolate and the relatedness of the original insect host to the tested insect was observed; both the highly aggressive isolates were from coleopteran insects. A correlation was found between the RAPD grouping and the phenotypic classification of the isolates. All the lepidopteran isolates grouped into one major cluster, most sub clusters were constituted by isolates from the same climatic zone.

Introduction

With the current urgent and conflicting goals of reduced pesticide usage while maintaining adequate agricultural production, microbial control agents with selectivity and a low environmental impact could become ideal components of integrated pest management programmes in this century (Lacey & Goettel 1995). There has been a renewed interest in the use of fungal entomopathogens in biocontrol because of the spectacular epizootics they cause and their aggressive mode of action.

Beauveria bassiana (Balsamo) Vuillemin, the most common and ubiquitous fungal entomopathogen, has perhaps the longest history as an experimental mycoinsecticide (Leathers *et al.* 1993). Virulence and host specificity are two essential elements in the selection of a suitable candidate for microbial control. *Beauveria bassiana* has a wide host range but differences in both host specificity and virulence among isolates have been reported (McCoy *et al.* 1988; Ferron *et al.* 1991). Distinctive markers that characterize individual isolates would be useful to determine their efficacy, host specificity, survival and spatial–temporal distribution in the field. Molecular diagnostics (typing) of *B. bassiana* isolates have been attempted by studying isozyme (Bridge *et al.* 1990; St. Leger *et al.* 1992; Castrillo & Brooks 1998) and esterase profiles (Varela & Morales 1996), telomeric fingerprinting (Viaud *et al.* 1996; Couteaudier & Viaud 1997), polymorphisms in internal transcribed spacer regions of rDNA (Glare & Inwood 1998), mitochondrial DNA fingerprints (Pfeifer & Khachatourians 1993) and RAPD analysis (Maurer *et al.* 1997; Berretta *et al.* 1998; Castrillo & Brooks 1998; Glare & Inwood 1998; Luz *et al.* 1998).

RAPDs, the PCR-based DNA fingerprints (produced by amplifying DNA using short arbitrarily chosen

oligonucleotide primers) provide a rapid and reliable means of identifying neutral genetic markers. It has been possible to correlate particular fungal genotypes defined by RAPD markers with particular pathogenicity groups (Goodwin & Annis 1991; Mills *et al.* 1992; Crowhurst *et al.* 1995; Cooke *et al.* 1996; Berretta *et al.* 1998). The RAPD characterization would be useful for proprietary reasons when fungal isolates are introduced into new ecosystems for pest control. It ensures a means of detecting the introduced pathogen versus the native one. It will be useful for patenting purposes when the isolates are used in commercial formulations. The sorghum shoot borer, *Chilo partellus* Swinhoe is a serious pest of sorghum, a millet widely cultivated as a food crop in the semi-arid tropics spanning Africa and the Indian sub-continent. *Beauveria bassiana* has been field-tested in various formulations on borer-infested sorghum crop with successful results (Maniania 1998). We conducted laboratory bioassays with 20 isolates of *B. bassiana* against the sorghum shoot borer to identify virulent and aggressive isolates. These isolates were genotyped by RAPD to find a DNA marker defining their virulence to *C. partellus*.

Materials and Methods

Fungal cultures

Twenty *B. bassiana* isolates obtained from national and international culture collections and a few local isolates were used in the study. The isolates were obtained from different insect hosts and geographical areas (Table 1), and were classified based on the taxonomic order of the insect from which they were isolated and the climatic zone (temperate/tropical) in which they were found (Table 2). These isolates were stored as conidial suspensions in 20% glycerol at −20 °C for 2 years. The cultures were revived from the glycerol stocks on SDAY (Sabouraud's dextrose agar yeast with 1% yeast extract, 1% peptone, 2% dextrose and 1.5% agar) slants. The cultures were maintained at 25 °C and 14/10 h day/night regime. Conidia from 14-day-old fungal cultures were used in the laboratory bioassays on insect larvae. To obtain mycelia for DNA extraction, the cultures were grown in liquid medium (Sabouraud's dextrose broth with 1% peptone and 2% dextrose) according to the method described by Pfeifer & Khachatourians (1993). The mycelium was harvested by filtration, washed three times with distilled water, freeze-dried and stored aseptically at −70 °C until needed.

Insect cultures

The second generation larvae of *C. partellus* obtained by breeding the field – collected insects in the laboratory were treated in the bioassays. Neonate larvae of *C. partellus* were transferred to and maintained for 15 days on an artificial diet developed by Seshu Reddy & Davies (1978). Bioassays were done with these 15-day-old (~10 mm long) second instar larvae.

Table 1. The accession numbers, original host, geographical origin of *B. bassiana* isolates: results of laboratory bioassay on *C. partellus*.

Accession No.	Insect host isolated from	Geographic origin	Aggressiveness to *C. partellus*	
			% Mortality ± SE[a]	Arbitrary rating[b]
NRRL 20700	*Popillia japonica*	Unknown	26.2 ± 5.1	L
ARSEF 1169	*Sitonia lineatus*	France	36.0 ± 0.9	L
NRRL 20698	*Dysdercus* spp.	Peru	36.0 ± 0.9	L
ARSEF 3286	*Spodoptera littoralis*	France	40.0 ± 1.8	M
ARSEF 1788	*Helicoverpa virescens*	Spain	42.0 ± 0.8	M
NRRL 22865	Unknown	USA	44.0 ± 0.5	M
ITCC 913	Unknown	Unknown	44.0 ± 0.5	M
NRRL 20699	Unknown	USA	44.0 ± 1.7	M
ITCC 1253	*Musca domestica*	Czechoslovakia	46.0 ± 1.3	M
ITCC 4215	*Diatraea saccharalis*	India	46.1 ± 0.5	M
UAS*	Soil	India	48.0 ± 2.3	M
ARSEF 1316	*Helicoverpa virescens*	France	48.0 ± 0.8	M
ITCC 4644	*Deanolis albizonalis*	India	50.0 ± 0.4	M
ARSEF 1166	*Helicoverpa armigera*	Spain	50.1 ± 1.4	M
ARSEF 326	*Chilo plejadellus*	Australia	52.0 ± 1.3	M
ARSEF 3387	*Myzus persicae*	USA	52.1 ± 0.7	M
NRRL 3108	*Ostrinia nubilalis*	Unknown	54.0 ± 1.4	M
BbH*	*Helicoverpa* spp.	India	54.1 ± 1.0	M
ARSEF 739	*Diabrotica paranoense*	Brazil	71.8 ± 4.5	H
NRRL 22864	*Glichrochilus quadrisignatus*	USA	77.8 ± 3.1	H

ARSEF Isolates are from USDA-ARS Collection of Entomopathogenic Fungi, Ithaca, New York; NRRL isolates are from NRRL culture collection, Peoria, Illinois; ITCC isolates from Indian Type Culture Collection, IARI, New Delhi.

* Isolates given by regional research institutes and not yet accessioned.

[a] Means of three bioassays; [b] Numerical values of % mortality converted into a qualitative character as: H (>70%), M (70–40%) and L (<40%).

Table 2. Taxonomic order of the original insect host, climatic zone of geographical origin, arbitrary rating of aggressiveness to *C. partellus* and cluster number from RAPD analysis of *B. bassiana* isolates.

Accession No.	Taxonomic order of host insect	Climatic zone of geographical origin	Arbitrary rating of aggressiveness[a]	Phenetic group from RAPD analysis[b]
NRRL 20700	Coleoptera	Unknown	L	I
ARSEF 739	Lepidoptera	Tropical	H	2A
NRRL 3108	Lepidoptera	Unknown	M	2A
NRRL 20698	Hemiptera	Tropical	L	2A
ARSEF 1169	Coleoptera	Temperate	L	2A
ARSEF 1788	Lepidoptera	Temperate	M	2B i
ITCC 4215	Lepidoptera	Tropical	M	2B ii
ARSEF 326	Lepidoptera	Temperate	M	2B ii
NRRL 22865	Unknown	Temperate	M	2C i
ARSEF 3286	Lepidoptera	Temperate	M	2C ii
ITCC 913	Unknown	Unknown	M	2C ii
ITCC 1253	Diptera	Temperate	M	2C ii
ARSEF 1166	Lepidoptera	Temperate	M	2C iii
ARSEF 1316	Lepidoptera	Temperate	M	2C iii
UAS*	–	Tropical	M	2C iv I
ITCC 4644	Lepidoptera	Tropical	M	2C iv II
BbH*	Lepidoptera	Tropical	M	2C iv II
NRRL 20699	Unknown	Temperate	M	3A
ARSEF 3387	Homoptera	Temperate	M	3A
NRRL 22864	Coleoptera	Temperate	H	3B

[a] H, M, L (Highly, Medium and Less aggressive).
[b] Phenetic groups as defined in Figure 2.
* Local isolates.

Bioassays

Aqueous conidial suspensions of each isolate (with 0.01% Tween 80, Sigma (Sigma, St. Louis, MO)) with a concentration of 1×10^9 conidia/ml were prepared. A batch of 10 second instar larvae was used in each treatment. Twenty five μl of conidial suspension was carefully applied to each larva with a micropipette. Treatment with each isolate was done in triplicate with 10 larvae/replicate. After treatment, 10 larvae which constituted one replicate was released into a 7×10 cm plastic cup with sorghum stem bits. Ten larvae treated with water (with 0.01% Tween 20) served as the control for each replicate. The cups were placed in an environmental chamber with 25 °C and 90% RH. Larval mortality was recorded at 4-day intervals until the twelfth day by which time the larvae completed the fourth instar and began to pupate. The dead insects were placed in humid chambers (Petri dishes lined with moist paper) to facilitate mycosis. The bioassay was repeated three times.

DNA extraction

DNA was extracted as described for fungal mycelia and conidia by Lee & Taylor (1990) with some modifications. Freeze-dried mycelia were powdered with a mortar and pestle. The powder was extracted with five volumes of extraction buffer (0.4 M Tris-HCl pH 8.5, 5 M NaCl, 5.0 mM EDTA pH 8.5, 4.0%(w/v) SDS, 1% β-mercaptoethanol) and kept at 65 °C for 20 min. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, by vol.) was added to the slurry. It was mixed gently and then centrifuged at 25 °C for 20 min at $12{,}000 \times g$. The aqueous phase was re-extracted with an equal volume of chloroform:isoamyl alcohol (24:1, by vol.). The separated aqueous phase was precipitated by 0.6 volume of isopropanol. DNA was spooled with a glass rod, washed twice with 70% (v/v) ethanol and dissolved in $T_{50}E_{10}$ buffer (50 mM Tris-HCl, 10 mM EDTA, pH 8.0). It was treated with RNase (50 μg ml^{-1}) at 37 °C for 2 h. An equal volume of phenol:chloroform:isoamyl alcohol was added to the solution at the end of incubation and centrifuged. The aqueous phase was transferred to another tube and was precipitated by adding 0.33 volumes of sodium acetate and 2.5 volumes of absolute ethanol. The DNA pellet was washed twice with 70% ethanol, vacuum dried, and suspended in $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0). In all cases purity and quantity of DNA in the samples were estimated using a UV-spectrophotometer and checked in ethidium bromide-stained 0.8% agarose TBE gels. DNA samples were diluted to 25 ng of DNA/μl.

DNA amplification

DNA was amplified by the RAPD–PCR technique (Williams *et al.* 1990). Twenty random decamer primers (OPE-1–20) from Operon Technologies (Operon Technologies Inc., Alameda, CA) were used for amplification. The 25 μl reaction mixture in each tube consisted of 2.5 μl of dNTP mix, 1 μl of primer (10 mM stock), 1 μl of template DNA (25 ng), 0.5 μl of *Taq* polymerase and 16 μl of sterile double distilled water. Amplification reactions were performed in a Perkin Elmer gene Amp system 9600 (Perkin-Elmer-Cetus, Connecticut)

programmed as follows: initial denaturation for 1 min at 94 °C, 45 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 36 °C and extension for 1 min at 72 °C and final extension for 5 min at 72 °C, using the fastest available temperature transitions. Amplified DNA fragments were separated in a 1.5% agarose TBE gel, stained with ethidium bromide and were photographed under UV illumination. Patterns obtained from those primers which were reproducible through duplicates of each amplification reaction for each isolate, were scored and these stable polymorphic bands were used in cluster analysis.

Data analysis

Pathogenicity

The total mortality caused in each replicate treatment was converted to percentage value. In the controls, a 10% mortality was observed in treatment 1 while no mortality occurred in the other two treatments. The percent mortality values in treatment 1 were corrected for control mortality using Abbott's formula (Abbott 1925). The mean of the percentage mortality of all the nine replicates in the three bioassays was calculated and arcsine transformed (Gomez & Gomez 1984). These values were converted into a qualitative character on an arbitrary mortality scale (Berretta *et al.* 1998) to classify the isolates. Isolates that caused >70%, 70–40% and <40% mortality were classified as highly (H), medium (M) and less (L) aggressive isolates, respectively (Table 1).

Cluster analysis

Data from RAPD experiments were recorded as a binary matrix of 0 and 1 corresponding to the absence or presence of reproducible individual bands. Similarity index matrices were generated based on the proportion of common amplification products between two accessions (Nei & Li 1979). Cluster analysis of data was carried out using the statistical software Genstat 4 (Genstat 1983) by single linkage cluster analysis, and the dendrogram was constructed. The hierarchical cluster program of Genstat was used for generating correlation value following co-phenetic correlation developed by Sokal & Rohlf (1962). The phenetic groups of isolates are denoted in Table 2.

Results

Pathogenicity assays

All isolates were infectious against the sorghum shoot borer but they differed in their aggressiveness (Table 1). Aggressiveness of the isolates expressed in percent insect mortality, ranged between 77.7 and 26.2%. When classified arbitrarily based on percent mortality, only 2 out of 20 isolates (ARSEF 739, NRRL 22864) turned out to be highly aggressive. These two isolates were initially isolated from coleopteran insects. Fifteen of the 20 isolates showed medium aggressiveness. All nine isolates from lepidopteran insects including the one isolated from a species of *Chilo* were moderately (medium) aggressive. The less aggressive group constituted two coleopteran isolates NRRL 20700, ARSEF 1169 and one hemipteran isolate NRRL 20698.

Cluster analysis

Of the 20 primers tested in this study only four – OPE-1 (5′ CCCAAGGTCC 3′), OPE-2 (5′ GAGGATCCCT 3′), OPE-4 (5′ GTGACATGCC 3′) and OPE-6 (5′ AAGACCCCTC 3′) gave consistent RAPD–PCR profiles between duplicates of reactions. The rest were excluded from further investigation because they gave either inconsistent results or numerous bands, which made the analysis difficult. RAPD analysis of the 20 isolates with four OPE primers yielded 38 reproducible bands (with an average of nine bands for each primer), which allowed complete separation of isolates. Although isolated from different hosts and from different geographical regions across both hemispheres, the 20 isolates showed close similarity and could be subclassified into different phenetic groups only at 30% dissimilarity (Figures 1 and 2). This is also well reflected in analysis with each single primer.

Cluster analysis based on the single linkage method evidenced three phenetic groups in the dendrogram (Figure 2). The correlation between the similarity matrix and dendrogram is 0.563. The isolate NRRL 20700 branched out singly as cluster 1. This is a coleopteran isolate of unknown geographic origin and is less aggressive to the sorghum shoot borer. Cluster number 2 is the largest formed by 16 isolates; it subclustered into three groups: 2A, 2B and 2C. Four isolates grouped in 2A at 72% similarity. The three isolates in 2B separated into two subgroups 2B i and 2B ii at 75% similarity. The subcluster 2C was the largest with nine isolates, it branched into four groups 2C i, 2C ii, 2C iii and 2C iv at various similarity levels. Three isolates clustered in cluster number 3. It further diverged into 3A and 3B at 78% similarity. (Figures 1 and 2, Table 2).

The subcluster 2A was formed by isolates of diverse insect and geographic origin, isolates of all the three types – low, medium and high aggressiveness towards the borer – are present in this group. The isolates NRRL 20698 and ARSEF 1169 are less aggressive types; the former is from a hemipteran insect in a tropical zone (Peru) and the latter, a coleopteran isolate collected in a temperate region (France). The isolate NRRL 3108 that showed moderate aggressiveness to the borer is a lepidopteran isolate of unknown geographic origin. The isolate ARSEF 739 is highly aggressive to the borer and was collected in the tropics (Brazil) from a coleopteran insect.

All the isolates that grouped into the subclusters 2B and 2C were medium aggressive type; except two, all

NRRL 22864	...																		
NRRL 3108	63.2	...																	
NRRL 20698	68.4	73.7	...																
NRRL 22865	52.6	68.4	68.4	...															
ARSEF 3286	55.3	71.1	65.8	65.8	...														
ARSEF 3387	78.9	68.4	68.4	68.4	60.5	...													
NRRL 20700	65.8	50.0	50.0	55.3	36.8	71.1	...												
NRRL 20699	65.8	65.8	60.5	65.8	68.4	81.6	57.9	...											
ARSEF 1788	65.8	60.5	55.3	60.5	57.9	60.5	68.4	68.4	...										
ARSEF 1169	52.6	57.9	63.2	63.2	60.5	57.9	55.2	60.5	65.8	...									
ARSEF 739	52.6	68.4	63.2	73.7	60.5	68.4	55.3	71.1	60.5	68.4	...								
ARSEF 326	63.2	63.2	73.7	68.4	65.8	57.9	60.5	65.8	71.1	68.4	68.4	...							
ITCC 4215	57.9	57.9	68.4	63.2	65.8	57.9	60.5	60.5	76.3	73.7	63.2	89.5	...						
ITCC 1253	60.5	60.5	50.0	65.8	57.9	71.1	52.6	68.4	68.4	60.5	71.1	55.3	65.8	...					
ITCC 913	55.3	71.1	65.8	76.3	78.9	60.5	42.1	68.4	63.2	65.8	71.1	71.1	71.1	78.9	...				
UAS	63.2	68.4	52.6	63.2	60.5	52.6	55.3	55.3	71.1	68.4	68.4	73.7	68.4	71.1	71.1	...			
ARSEF 1166	65.8	65.8	60.5	60.5	68.4	50.0	36.8	52.6	57.9	65.8	65.8	65.8	65.8	68.4	73.7	76.3	...		
ARSEF 1316	63.2	68.4	68.4	63.2	71.1	52.6	39.5	60.5	60.5	68.4	68.4	73.7	68.4	60.5	71.1	73.7	92.1	...	
BbH	52.6	52.6	36.8	57.9	50.0	57.9	60.5	71.1	65.8	57.9	63.2	57.9	57.9	76.3	60.5	73.7	60.5	63.2	...
ITCC 4644	57.9	63.2	47.4	57.9	60.5	57.9	55.3	65.8	65.8	57.9	57.9	63.2	63.2	76.3	65.8	78.9	65.8	73.7	89.5
	NRRL 22864	NRRL 3108	NRRL 20698	NRRL 22865	ARSE F 3286	ARSE F 3387	NRRL 20700	NRRL 20699	ARSE F 1788	ARSE F 1169	ARSE F 739	ARSE F 326	ITCC 4215	ITCC 1253	ITCC 913	UAS	ARSE F 1166	ARSE F 1316	BbH	ITCC 4644

Figure 1. Genetic similarity coefficient matrix of twenty strains of *B. bassiana*. Matrix calculated from 38 DNA bands yielded by four primers. Simple matching coefficients given in percentage similarity. Isolate details given in Table 1.

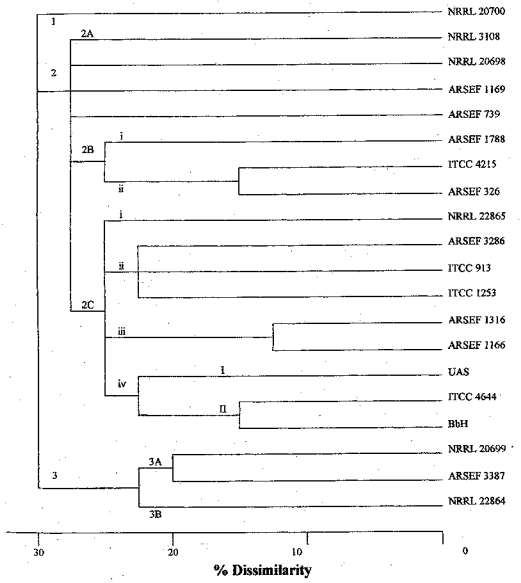

Figure 2. Relationship of twenty isolates of *B. bassiana*. The dendrogram was generated from genetic similarity coefficients (Figure 1) and is based on the single linkage method. The correlation between similarity matrix and dendrogram is 0.563. Phenetic groups are denoted as 1, 2A, 2B i, 2B ii, 2C i, 2C ii, 2C iii, 2C iv-I, 2C iv-II, 3A and 3B.

isolates of known insect origin are from lepidopteran insects. In the subcluster 2B, a single isolate ARSEF 1788 separated into 2B i from the other two isolates ITCC 4215 and ARSEF 326, which subclustered in 2B ii at 88% similarity. The subcluster 2C branched out as four sub groups 2C i, 2C ii, 2C iii and 2C iv at 75% similarity. The first three subgroups are constituted by isolates from the temperate zone and the fourth had all tropical isolates. The isolate NRRL 22865 separated out singly in 2C i. Three isolates, ARSEF 3286, ITCC 913 and ITCC 1253, clustered with 78% similarity in 2C ii; among them the second is of unknown insect origin and the third, a dipteran isolate. The isolates ARSEF 1316 and ARSEF 1166 clustered with 90% similarity in 2C iii. The three tropical isolates from India in 2C iv further diverged into 2C iv-I constituted by the soil isolate UAS and 2C iv-II with the lepidopteran isolates ITCC 4644 and BbH that clustered together at 88% similarity.

Three isolates NRRL 20699, ARSEF 3387 and NRRL 22864, all from the temperate region, grouped into cluster 3 at 78% similarity. Among them the first two are medium aggressive type and diverged into 3A from the third highly aggressive type which constituted 3B.

Discussion

Among the 20 isolates of *B. bassiana* tested, a variation in virulence to the sorghum shoot borer was found, but no isolate non-pathogenic to the borer was found. Such variation in virulence among isolates of *B. bassiana* has been reported in bioassays with the coffee berry borer, sugarcane stem borer and the darkling beetle (Varela & Morales 1996; Berretta *et al.* 1998; Castrillo & Brooks 1998). None of the lepidopteran isolates, including the isolate from *Chilo* sp., were found to be highly aggressive to the borer. Both the highly aggressive types are coleopteran isolates. A rigid specialization among isolates with respect to the host invaded has not been observed in *B. bassiana* in our present study and in others' work (Varela & Morales 1996; Beretta *et al.* 1998; Castrillo & Brooks 1998). Humber (1991) observed that entomogenous habits of *B. bassiana* may be relatively new and therefore it is rather poorly adjusted to its hosts. In fact isolates picked up during an epizootic at the same time in one area from larvae of the same insect species showed differences in aggressiveness to the same host when tested in laboratory bioassays (Berretta *et al.*1998; Castrillo & Brooks 1998).

In the RAPD analysis of the *B. bassiana* isolates, the clustering of isolates did not reflect their similarity in aggressiveness to the sorghum shoot borer. The two highly aggressive isolates fell into two different clusters and grouped with less aggressive isolates. Luz *et al.* (1998) found a similar situation in *B. bassiana* isolates tested against *Triatoma infestans*; the virulence of the isolates was not correlated to phenetic groups in cluster analysis of RAPD markers. However in the study of Berretta *et al.* (1998), *B. bassiana* isolates highly virulent (four out of six) to *Diatraea saccharalis* formed one phenetic group with 85% similarity. Similarly in plant pathogenic fungi like *Colletotrichum graminicola*, *C. gloeosporoides*, *Leptosphaeria maculence*, *Fusarium oxysporum* and *Phytophthora cactorum*, isolates pathogenic to different plant species could be clearly distinguished by RAPD analysis. Isolates infecting one plant species clustered into one group (Weising *et al.* 1994; Crowhurst *et al.* 1995; Cooke *et al.*1996). Viaud *et al.* (1996) and Couteaudier & Viaud (1997) from their work with *B. bassiana* observed that differences exist among the insect species with respect to the type of fungal isolates they are susceptible to. All the *B. bassiana* isolates found virulent to the corn borer (*Ostrinia nubilalis*) belonged to one vegetative compatibility group (VCG) and had a similar telomere fingerprint and therefore, inferred to be genetically similar. In contrast, the *B. bassiana* isolates found to be virulent to *Sitonia* weevils belonged to different VCGs and had different telomere fingerprints, and were hence identified as genetically dissimilar. Thus Viaud *et al.* (1996), Couteaudier & Viaud (1997) concluded that some insects like *O. nubilalis* were infected by only one strain (genotype) while others like *Sitonia* are susceptible to several genotypically dissimilar strains. Whether this statement stands if bioassays are done with more isolates of *B. bassiana* on these insects remains to be tested. If it does stand, then the sorghum shoot borer could be classified as of the *Sitonia* type.

The clustering of the *B. bassiana* isolates reflected the relatedness of the original hosts from which they were isolated and their geographic origin. All the lepidopteran isolates clustered in one group. Most of the subgroups are constituted exclusively by temperate or tropical isolates.

The RAPD analysis showed a high (~70%) level of similarity among the *B. bassiana* isolates though they had been isolated from different climatic zones spreading over both hemispheres and from varied hosts. Large genetic distances have been observed among *B. bassiana* isolates in the RAPD analysis of Berretta *et al.* (1998) and Castrillo & Brooks (1998). The primers used by Berretta *et al.* (1998) and Castrillo & Brooks (1998) in the RAPD analysis of *B. bassiana* are different from those used in the present study. With the same primers used in our study, high – 60% similarity was also found among 20 isolates of *Metarhizium anisopliae* (Leal *et al.* 1994). The genomic regions amplified with these four primers we used could represent conserved regions, while the highly variable regions could have been amplified with the primers used by Berretta *et al.* (1998) and Castrillo & Brooks (1998). The RAPD fragments observed in our analysis, probably being conserved regions, could serve as informative probes in RFLP analysis. Six of the seven RAPD products of *Fusarium solani* did turn out to represent unique sequences and were useful in identifying markers for different mating groups (Crowhurst *et al.* 1995).

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