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TABLE I

Host distribution of different pathotypes of biotypes of *Beauveria* sp. tested for genetic difference and pathogenicity to *Artipus floridanus* larvae in the laboratory

Pathotype	Insect host	Location
754	Coleoptera	Ceara, Brazil
783	Tenebrionidae (adult)	Ceara, Brazil
758	*Diabrotica paranoense*	Brazil
DA-79-(YCM)	*Diaprepes abbreviatus*	Florida, USA
809	*Diabrotica paranoense*	Brazil
DA-79-3	*Diaprepes abbreviatus*	Florida, USA
5506	*Scapteriscus vicinus* (adult)	Florida, USA
757	Tenebrionidae	Ceara, Brazil
DA-79-9	*Diaprepes abbreviatus*	Florida, USA
A-34	*Lygus* sp.	Burgos, Spain
739	*Diabrotica paranoense*	Ceara, Brazil
5505	*Scapteriscus vicinus*	Florida, USA
AF-15A	*Artipus floridanus*	Florida, USA
AFL	*Artipus floridanus*	Florida, USA
169	*Sitona humeralis*	Lusignan, France
DA-79-2	*Diaprepes abbreviatus*	Florida, USA
CBW-79	*Araecerus fasciculatus*	Florida, USA
AFA	*Artipus floridanus* (adult)	Florida, USA
252	*Leptinotarsa decemlineata*	Maine, USA
139	*Sitona lineatus*	South England, U. K.
736	*Chalcodermus aeneus*	Ceara, Brazil
785	*Cerambycidae* (adult)	Ceara, Brazil
F-77	*Pachnaeus litus*	Florida, USA
733	*Chalcodermus aeneus*	Ceara, Brazil
DA-79-1	*Diaprepes abbreviatus*	Florida, USA
AF-42A	*Artipus floridanus*	Florida, USA
142	*Sitona dicoideus*	Montpelier, France
Jam	*Araecerus fasciculatus*	Kingston, Jamaica
WCRW	*Diabrotica virgifera*	Illinois, USA
737	Pentatomidae	Ceara, Brazil
738	*Chalcodermus aeneus*	Ceara, Brazil
804	Elateridae (adult)	Ceara, Brazil
143	*Sitona* spp.	Australia
AF-4	*Artipus floridanus*	Florida, USA
784	*Elateridae*	Ceara, Brazil
309	Unknown	
787	Tenebrionidae	Ceara, Brazil
735	*Chalcodermus aeneus*	Ceara, Brazil
734	*Chalcodermus aeneus*	Ceara, Brazil
ARG	Unknown	Argentina
56A	*Melolontha melolontha*	–
B. brongniartii	Phyllophaga	Switzerland

Pathogenicity of fungal isolates – For most entomogenous fungi, pathogenicity differs widely from isolate to isolate but can be maintained after repeated subculture by passage through their natural or an alternate host (Fargues, 1972; Latch, 1976; Daoust & Roberts, 1982). Lappa (1978) reported that the virulence of indigenous isolates of *B. bassiana* is usually much less than the activity of introduced or exotic isolates. In view of these results and to identify the most virulent pathotypes to citrus root weevils, the majority of the pathotypes tested for genetic similarity were also assayed quantitatively for pathogenicity and sporulation on the larvae host (Table I). The bioassay technique utilized two conidial concentrations (1 x 10^5 and 1 x 10^7 conidia/mg) and laboratory reared neonate larvae of *A. floridanus* in tissue culture wells (McCoy et al., 1985b). Subsequently, the most virulent pathotypes were further assayed at five viable conidial concentrations ranging from 5 x 10^4 to 5 x 10^8 conidia/ml.

Pathogenicity and sporulation on the host differed significantly among the 34 *B. bassiana*

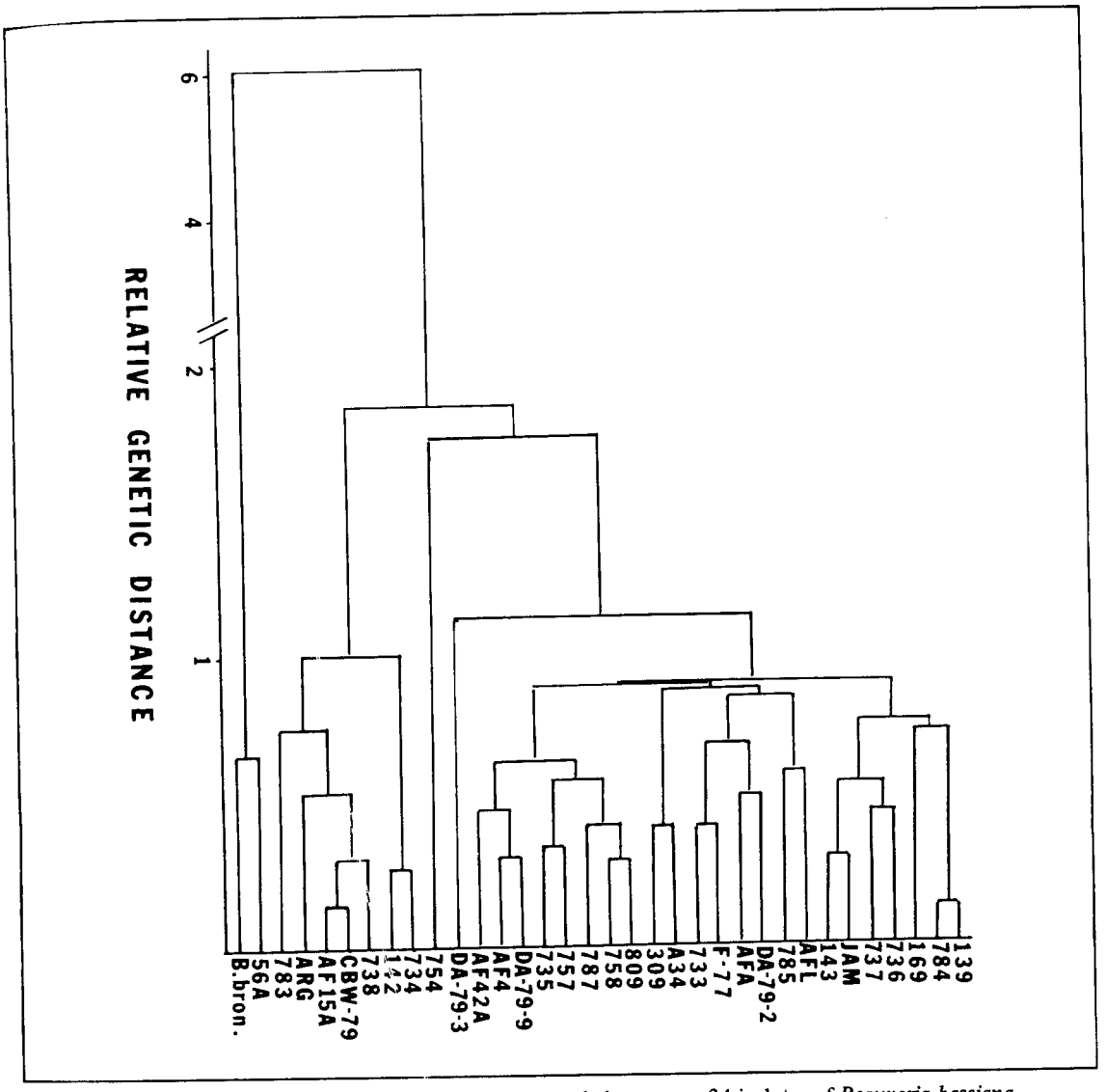

Fig. 1: dendrogram showing the relative genetic distance existing among 34 isolates of *Beauveria bassiana*.

isolates ranging from 0 to 92% and 8 to 100% at 1 x 10^5 and 1 x 10^7 conidia/ml, respectively (McCoy et al., 1985a). Differences in pathogenicity between fungal types was more pronounced at the lower conidial concentrations. Relative numbers of progeny conidia produced on cadavers increased with an increase in pathotype virulence. No consistent differences in pathogenicity to neonatal larvae *A. floridanus* could be detected between exotic and indigenous isolates, original insect host, and site of isolation.

Of the seven highly virulent isolates of *B. bassiana*, 252, AF-4, and 143 exhibited the highest potency at five dosage levels in laboratory bioassay (Table II). LT_{90} values at 5 x 10^8 CFU's/ml were 3.8, 4.3, and 6.4 days for 252, AF-4, and 143 assayed at 27 °C, respectively.

Host specificity of virulent isolates – Virulent pathotypes of *B. bassiana* from a specific host can exhibit different capacities for pathogenicity to other hosts (Champlin et al., 1981). Since four distinct weevil species *(Artipus floridanus, Pachnaeus opalus, Pantomorus cervinus,* and *Diaprepes abbreviatus)* are important pests in Florida citrus groves, it was imperative to select a virulent pathotype of *B. bassiana* that was highly active against all species. Bioassays performed against the neonatal stage of all species at 1 x 10^7 conidia/ml showed conclusively that AF-4 was most pathogenic to the larvae of all weevil species.

TABLE II

Comparative relative potencies for different pathotypes of *Beauveria bassiana* to neonate larvae of *Artipus floridanus*

Pathotype	Dosage (CFU's/ml)				
	5×10^4	5×10^5	5×10^6	5×10^7	5×10^8
252	1.000	1.000	1.000	1.000	1.000
AF-4	0.982	1.004	1.156	0.909	0.888
143	1.041	0.887	0.748	0.952	0.603
804	0.000	0.788	0.782	0.577	0.530
WCRW	0.000	0.000	0.874	0.667	–
738	0.000	0.000	0.730	0.677	0.507
737	0.000	0.000	0.807	0.532	0.359
783	0.000	0.000	0.855	0.418	0.275

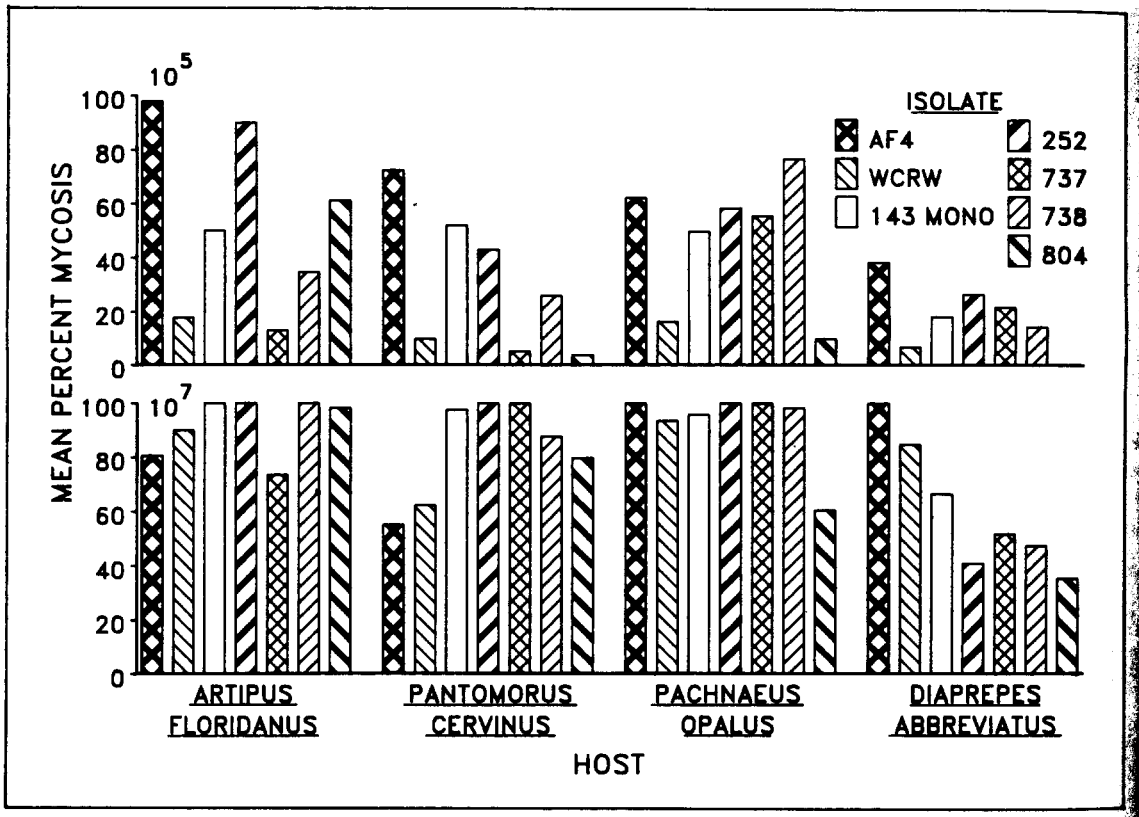

Fig. 2: mean percent mycosis for neonatal larvae of four species of citrus root weevil exposed to two concentrations of conidia from eight pathotypes of *Beauveria bassiana*.

Effect of temperature and soil moisture on survival and infectivity in the soil – In water saturated Candler soil (20% soil moisture, v/v), conidial survival for *B. bassiana* isolates 252, AF-4, 143, and 738 was 100% at 30 °C and lower (Fig. 2). In most cases at these temperatures, the various fungal isolates grew and the number of fungal propagules per area of soil increased in time. At 30 °C and above, survivorship of 252, AF-4, and 143 was less than 24 hr in dry soil (0.5% soil moisture, v/v), while 738 maintained its viability beyond 72 hr (Fig. 2). At 50 °C, survivorship of the various isolates was virtually lost after 8 hr at both moisture levels (Figs 3-4). At 30 °C, all isolates of *B. bassiana* were highly infectious to 30-day-old larvae of *A. floridanus*. Isolates AF-4, 252, and 738 appeared to be most infectious in dry soil.

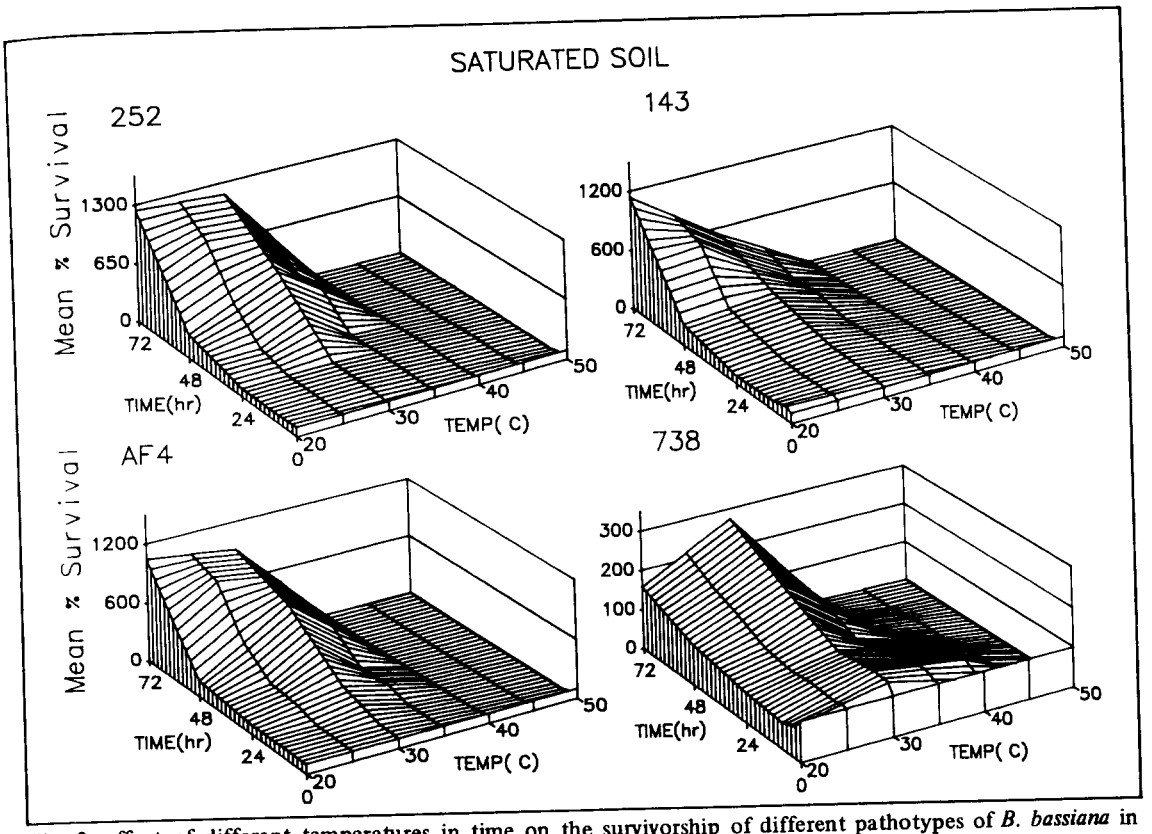

Fig. 3: effect of different temperatures in time on the survivorship of different pathotypes of *B. bassiana* in water-saturated soil (note the reversal of values on the time axis compared to Fig. 4).

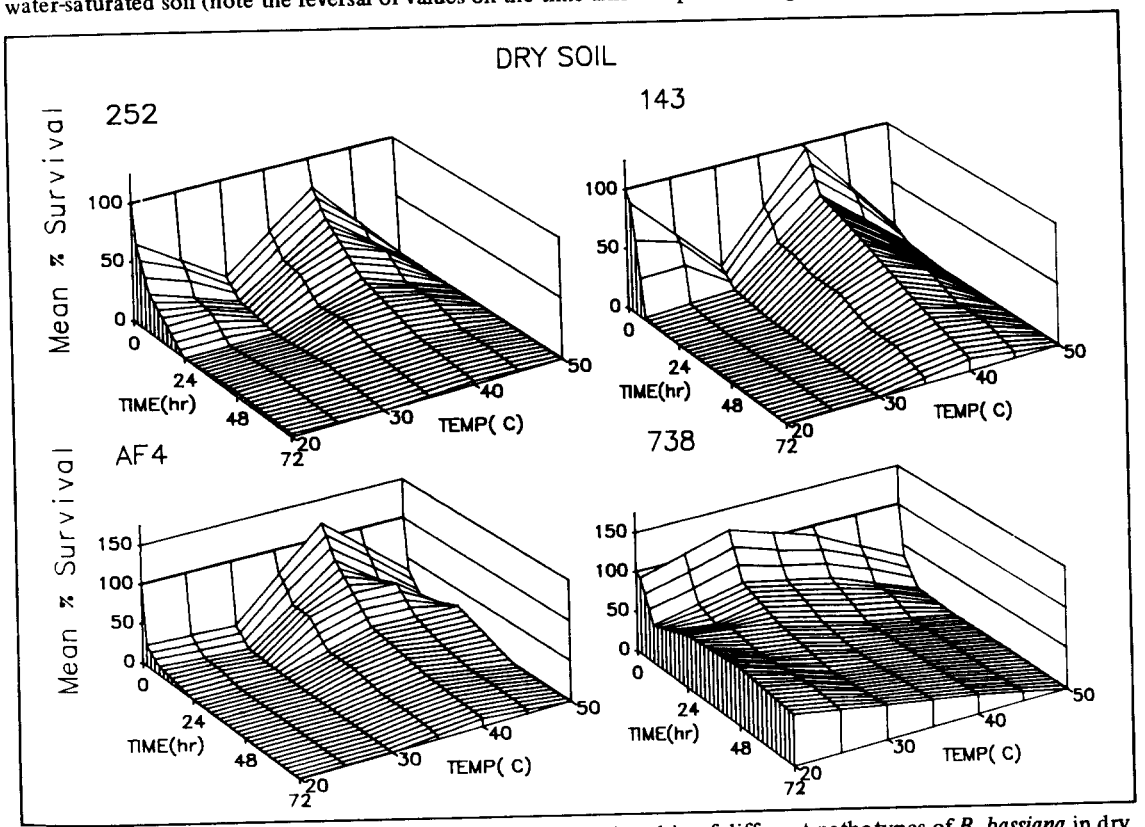

Fig. 4: effect of different temperatures in time on the survivorship of different pathotypes of *B. bassiana* in dry soil.

According to these biological studies, it would appear that pathotypes AF-4, 738, and 252 in descending order express the most favorable characteristic as candidates for microbial control. Pathotype AF-4 is highly virulent with the broadest host range while type 738 appears to have a better tolerance for high temperature particularly in dry soil. These studies suggest that environment parameters such as temperature tolerance may be equally important to pathogenicity for the selection of fungal pathotypes for microbial control. Possibly, different pathotypes of a given fungal species expressing favorable characteristics for a given agro-ecosystem and host complex can be blended into a specific mycoinsecticide to maximize efficacy.

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