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# SELECTION OF *BEAUVERIA BASSIANA* PATHOTYPES AS POTENTIAL MICROBIAL CONTROL AGENTS OF SOIL-INHABITING CITRUS WEEVILS

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The ubiquitous fungus, *Beauveria bassiana* (Bals.) Vuill., has long been known to be the most common causative agent of disease associated with dead and moribund insects in nature (Macleod, 1954) and has been scrutinized worldwide as a microbial control agent of particularly hypogeous species (Ferron, 1981). Many curculionid weevils with a subterranean larval stage are highly susceptible to this white muscardine disease (Peirson, 1921; Petch, 1932; Muller-Kogler & Stein, 1970; Beavers et al., 1983) and researchers, on occasion, have applied high dosages of *B. bassiana* conidia artifically to the soil for microbial control of weevils such as *Sitona lineatus* L. (Muller-Kogler & Stein, 1970), pecan weevil (Tedders et al., 1973; Gottwald & Tedders, 1983), pales weevil (Walstad & Anderson, 1971), *Artipus floridanus* Horn (McCoy et al., 1984), and *Otiorrhynchus suleatus* (Tillemans & Coremans-Pelseneer, 1987).

Like many species of entomogenous fungi, *B. bassiana* is composed of many genetically distinct variants associated with geographical location and host (Poprawski et al., 1988; Shimizu & Aizawa, 1988) which differ substantially in their ability to produce pathogenesis in a susceptible host (Lappa, 1978; Daoust & Roberts, 1982; McCoy et al., 1985a). In a search for highly virulent pathotypes of *B. bassiana* to develop as a microbial control agent of the larvae of citrus root weevils, sixty-three isolates of *B. bassiana* and one isolate of *B. brongniartii* were received from cooperative scientist as pure cultures from Europe, Brazil, Argentina, and England or field-collected in Florida. The majority of the isolates were from larval and adult stages of Curculionidae and other Coleoptera. Thirty-four isolates representative of a broad host range and wide geographical distribution were characterized both biochemically to detect electrophoretic isoenzyme variation and by bioassays to determine relative pathogenicity.

The most virulent pathotypes of *B. bassiana* to neonatal larvae of *Artipus floridanus* (McCoy et al., 1985a) were also evaluated for host specificity and survivorship at different soil temperatures and soil moisture conditions. This paper addresses each of these biological parameters.

*Biochemical characterization* – Three species of *Beauveria* have been recognized taxonomically (de Hoog, 1972) and two color morphs (white and yellow) for *B. bassiana* (Aoki & Kusida, 1975). Recently, electrophoresis (Poprawski et al., 1988) and serological procedures (Shimizu & Aizawa, 1988) have been successfully used to detect polymorphism among different populations of *B. bassiana* from the same host. Heterogeneity or wider adaptive capacity by a pathotype should favor possible redistribution as a microbial control agent.

Thirty-four pathotypes of *B. bassiana* and one pathotype of *B. brongniartii* listed in Table I were characterized biochemically to identify pathotypes in terms of isoenzyme variation. As previously mentioned, the majority of isolates were from larval and adult stages of the Curculionidae and other Coleoptera. Mycelial homogenates of the 34 isolates of *B. bassiana* were electrophoresed on polyacrylamide gel slabs and stained with various histochemical systems. Pairwise coefficients of similarity calculated from the electromorphs were used to construct dendograms estimating the genetic distance among the various fungal isolates. Results demonstrated that many isolates possessed distinct electromorphs which could be used to group or separate individual isolates (Fig. 1). However, broad genetic differences among isolates were detected only between *B. bassiana* and *B. brongniartii*.

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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 |
| [illegible] | *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 ($1 \times 10^5$ and $1 \times 10^7$ 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 $5 \times 10^4$ to $5 \times 10^8$ conidia/ml.

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

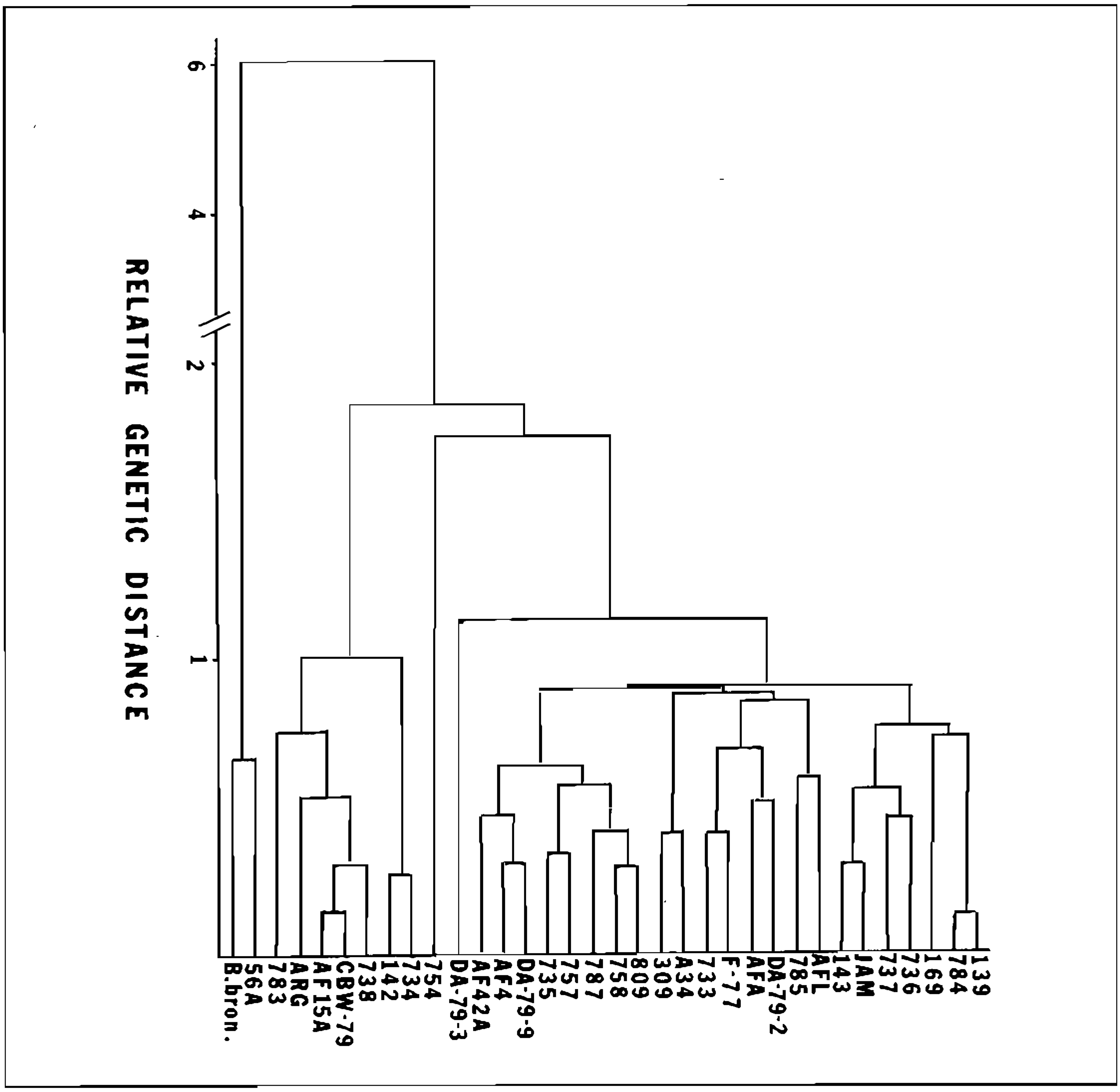


Fig. 1: dendrogram showing the relative genetic distance existing among 34 isolates of *Beauveria bassiana*.

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 x $10^4$ | 5 x $10^5$ | 5 x $10^6$ | 5 x $10^7$ | 5 x $10^8$ |
| 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 |

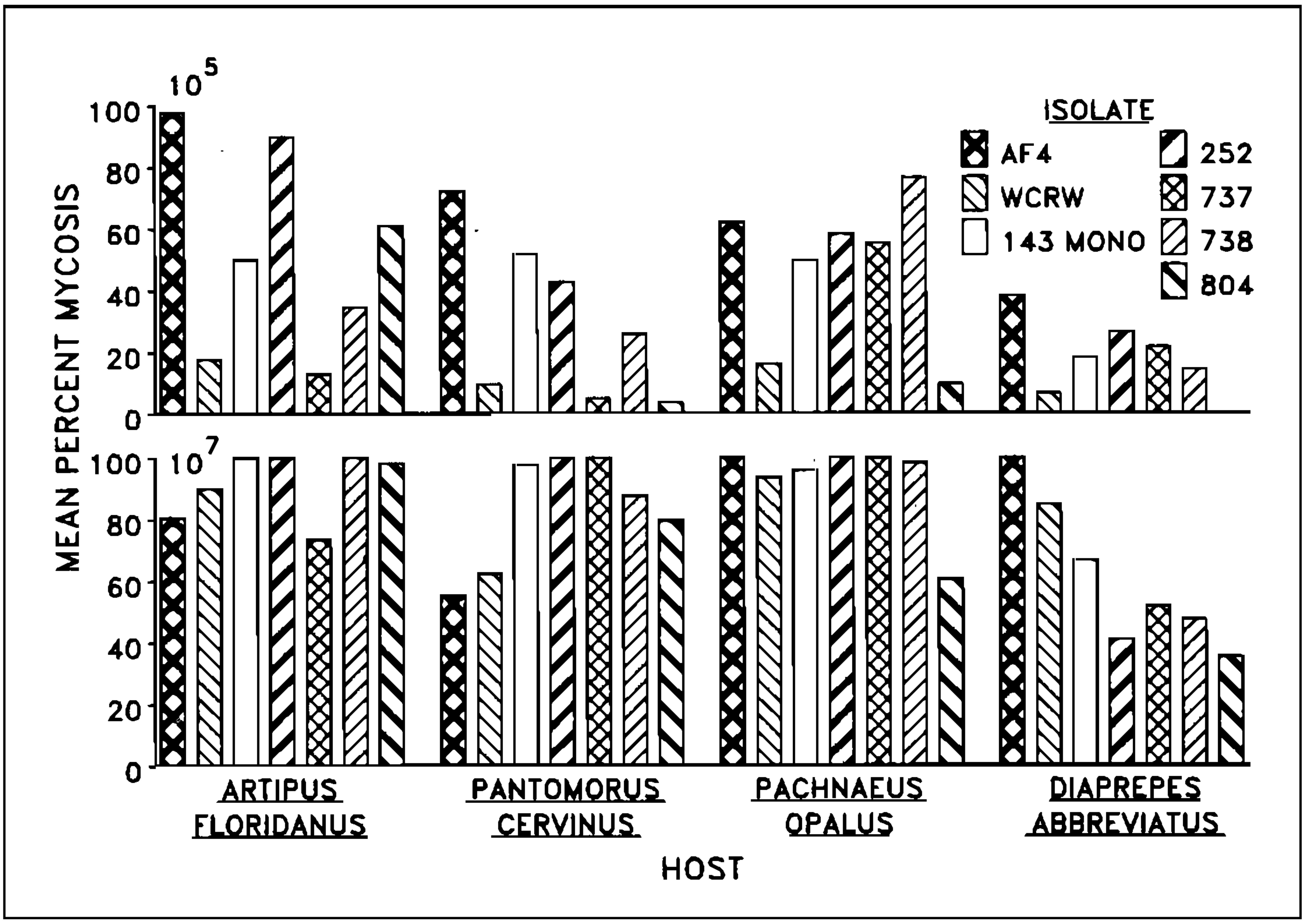


Fig. 2: mean percent mycosis for neonatal larvae of four species of citrus root weevil exposed to two concentrations of conidia from eight pathotypes of *Beauveria bassiana*.

*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.

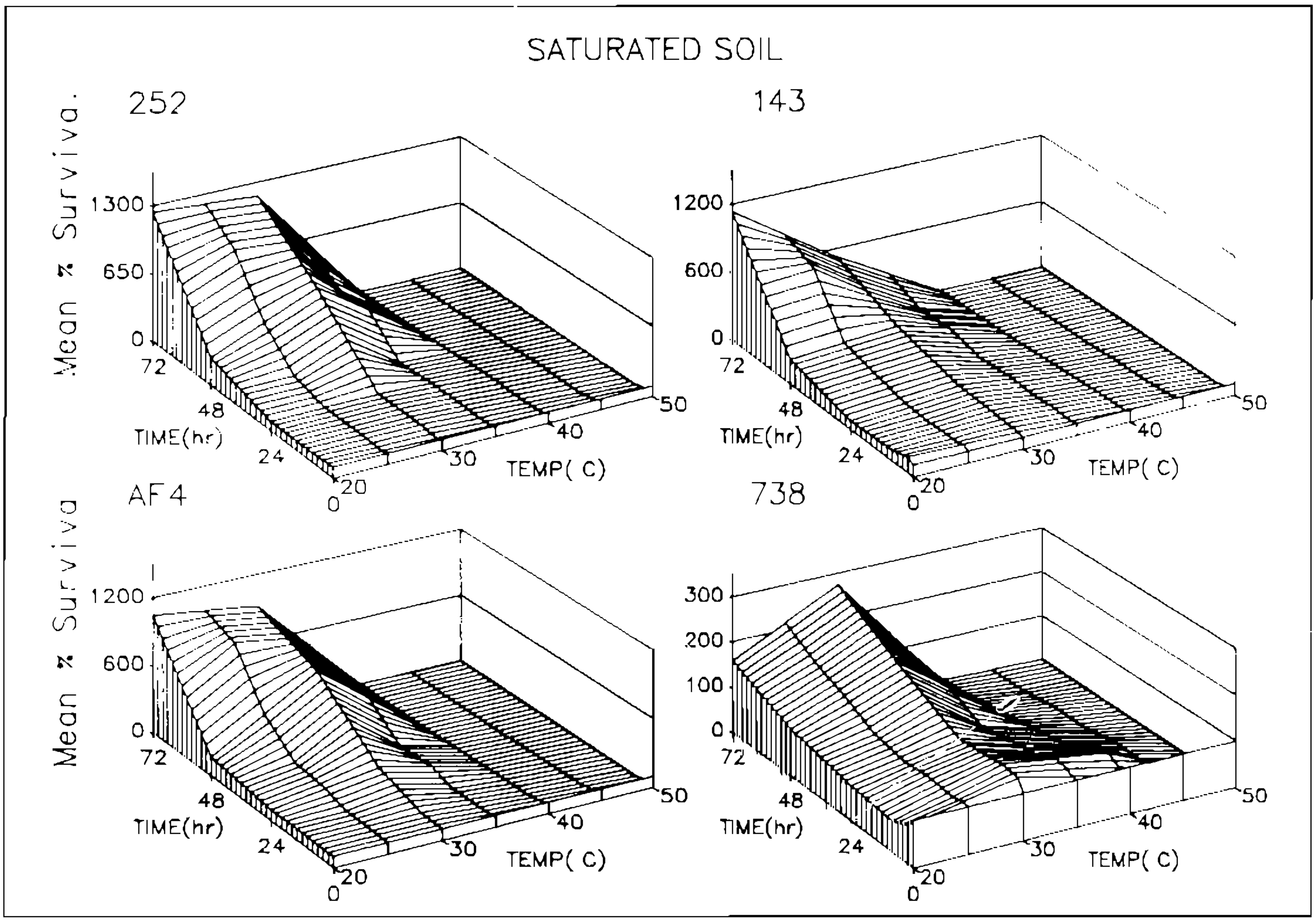


Fig. 3: effect of different temperatures in time on the survivorship of different pathotypes of *B. bassiana* in water-saturated soil (note the reversal of values on the time axis compared to Fig. 4).

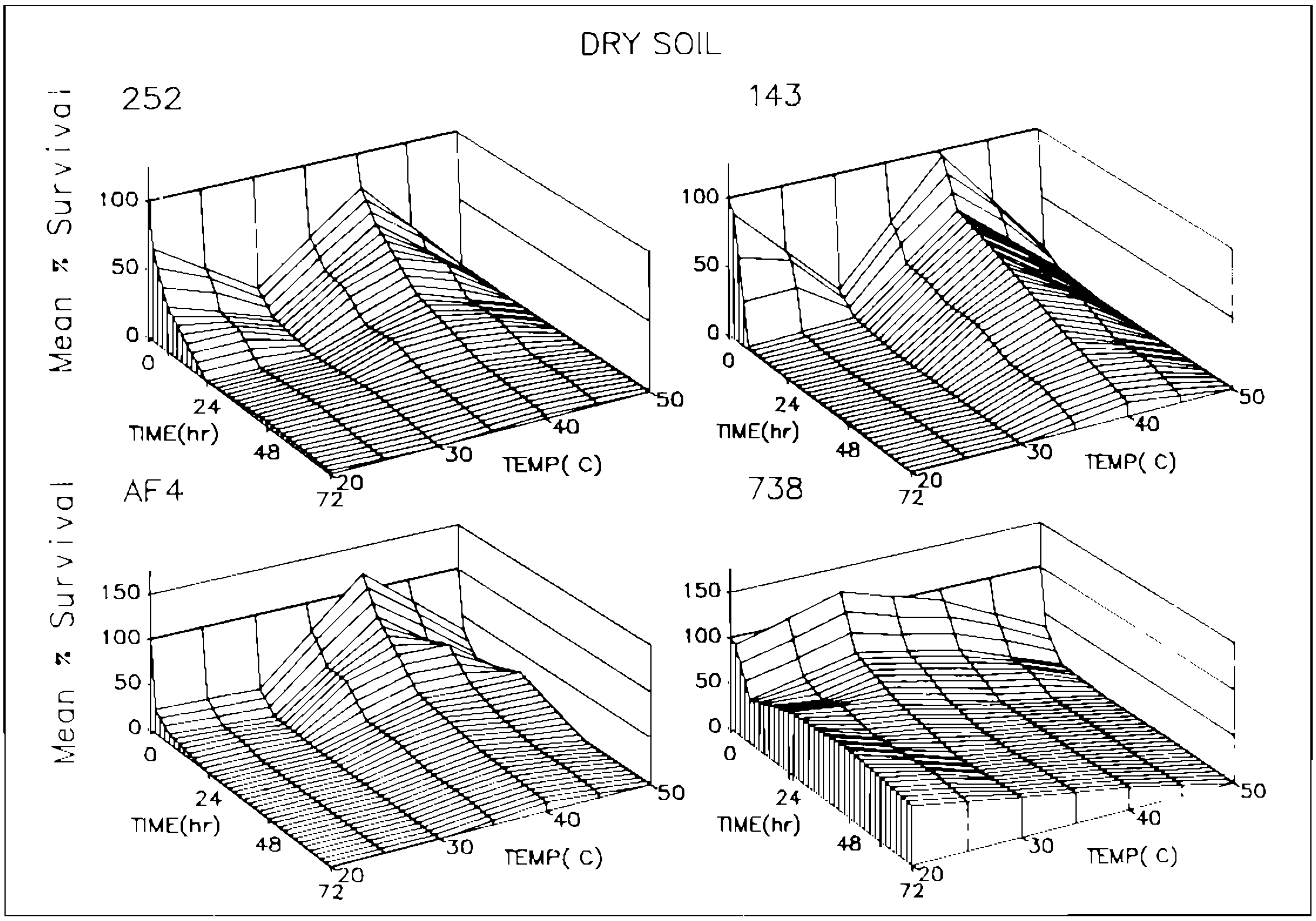


Fig. 4: effect of different temperatures in time on the survivorship of different pathotypes of *B. bassiana* in dry soil.

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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