

Free Fatty Acids on the Integument of the Striped Flea Beetle, *Phyllotreta striolata* F., and Their Effects on Conidial Germination of the Entomopathogenic Fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*

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ABSTRAK

Asid lemak bebas dari integumen kumbang lenting berjalur, *Phyllotreta striolata* F., dan kesannya ke atas percambahan konidium *Metarhizium anisopliae* (Metch.) Sorokin, *Beauveria bassiana* (Bals.) Vuill dan *Paecilomyces fumosoroseus* (Wise) Brown & Smith telah dikaji. Analisis kromatograf gas ekstrak kutikel kumbang lenting dewasa menunjukkan dua puncak yang memaparkan jangka penahanan yang bersesuaian dengan asid butirik dan asid nonanoik. Lain-lain asid lemak yang telah dikesan ialah kaproik, heptanoik dan kaprilik asid. Toksisiti asid lemak ini ke atas percambahan konidium *M. anisopliae*, *B. bassiana* dan *P. fumosoroseus* adalah bergantung kepada jenis kepekatan. Butirik ialah yang paling kurang toksik, hanya toksik ke atas *B. bassiana* dan *P. fumosoroseus* pada kepekatan melebihi 0.125%. Begitupun, pada kepekatan melebihi 0.075%, kesemua asid lemak didapati terlalu toksik. Asid nonanoik didapati paling toksik dan ia merencatkan kesemua percambahan konidium pada kepekatan terendah 0.025%, terutamanya ke atas *M. anisopliae* dan *P. fumosoroseus*. Asid heptanoik didapati amat toksik ke atas *B. bassiana* dengan membenarkan hanya 12.5% percambahan pada kepekatan terendah 0.025%. Dirumuskan bahawa asid lemak rantai lurus di atas integumen kumbang lenting melindungi kumbang itu daripada serangan kulat entomopatogen.

ABSTRACT

Free fatty acids on the integument of the striped flea beetle, *Phyllotreta striolata* F., and their effects on the conidial germination of *Metarhizium anisopliae* (Metch.) Sorokin, *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces fumosoroseus* (Wise) Brown & Smith were investigated. Gas chromatographic analysis of adult flea beetle cuticular extracts showed two peaks exhibiting a retention time corresponding to those of butyric acid and nonanoic acid. Other fatty acids detected were caproic, heptanoic and caprylic acids. Toxicity of these fatty acids to conidial germination of *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* was dependent on their type and concentration. Butyric was the least toxic—only toxic to *B. bassiana* and *P. fumosoroseus* at a concentration higher than 0.125%. However, at concentrations greater than 0.075%, all of the fatty acids were very toxic. Nonanoic acid was the most toxic and it completely inhibited conidial germination at the lowest concentration of 0.025%, especially to *M. anisopliae* and *P. fumosoroseus*. Heptanoic acid was very toxic to *B. bassiana*, allowing only 12.5% germination at the lowest concentration of 0.025%. It is surmised that straight-chain fatty acids on the flea beetle integument protected the beetles against the invasion of entomopathogenic fungi.

INTRODUCTION

Invasion of the striped flea beetle, *Phyllotreta striolata* F., by entomopathogenic fungi occur-

ring via penetration of the integument was influenced by both chemical and physical properties of the integument (Samson *et al.* 1988). Prior to

such penetration it is necessary for the fungal conidia to germinate on the surface of the beetle. Epicuticular compounds such as fatty acids, amino acids and glucosamines were thought to play a significant role in the germination process (Smith and Grula 1982; Saito and Aiko 1983; Boucisa and Pendland 1984; Kerwin 1984; Woods and Grula 1984). Successful germination of the fungi requires the presence of utilisable nutrients and that either toxic components not be present in amounts sufficient to inhibit germination, or that the organism be able to ignore or overcome them (Smith and Grula 1982).

Butt *et al.* (1984) recently reported that flea beetles and aphids are differentially susceptible to the entomopathogenic *M. anisopliae*. The aphids *Myzus persicae* and *Lipaphis erysimi* were highly susceptible and died more quickly than the mustard beetle, *Phaedon cochleariae*, and the flea beetle, *Psylliodes chrysocephala*. The striped flea beetle adult is also more resistant to *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* than the larvae (Priyatno and Ibrahim 2002). Differences in susceptibility reflect the response of the pathogen to cuticular cues. According to Butt *et al.* (1995), *M. anisopliae* conidia germinate faster on the surface of live aphids and at sites under the elytra than on the dorsal elytra and the ventral thorax of live beetles. Presence of fungistatic compounds in the dorsal elytra and ventral thorax could probably be responsible for protecting the insect against potential entomopathogenic fungi, as attributed by poor conidial germination (Butt *et al.* 1995). The most toxic components of the insect cuticle are the straight-chain saturated fatty acids such as butyric, valeric, caproic, heptanoic, caprylic and

nonanoic acids (Koidsumi 1957; Smith and Grula 1982; Saito and Aoki 1983). However, according to Samson *et al.* (1988), straight-chain fatty acids are not present in all insect orders; they are absent from some Coleoptera and Homoptera (Brey *et al.* 1985).

The aims of this study were to identify the fatty acids on the surface of the striped flea beetle adults and to determine the effects of such compounds on the conidial germination of *M. anisopliae*, *B. bassiana* and *P. fumosoroseus*.

MATERIALS AND METHODS

Fungus

The original hosts and countries of origin for the isolates of entomopathogenic fungi used in this study are listed in Table 1. All isolates were maintained at room temperature of $28 \pm 2^\circ\text{C}$ on potato dextrose agar (PDA, Oxoid, Basingstoke, UK) containing 0.5% yeast extract (Difco) which had been sterilised for 20 minutes at 121°C at 1.05 kg/cm^2 . To prepare fungal inocula, conidia from 15 day-old sporulating cultures were scraped from the surface of the plates with a sterile scalpel and suspended in a 0.05% aqueous solution of Tween 80. A Neubauer haemocytometer was used to estimate the conidial concentration and subsequent appropriate dilutions were made.

Insect

Field-collected flea beetles from Chinese mustard, *Brassica juncea*, in the vegetable plot at the UPM Research Park were used for various bioassays. They were maintained at an ambient environment of $28 \pm 2^\circ\text{C}$, 12:12 (L:D) h photoperiod and $80 \pm 10\%$ R.H. in plastic contain-

TABLE 1
Isolates of *M. anisopliae*, *B. bassiana* and *P. fumosoroseus*, their original hosts and countries of origin

| Species | Code | Insect host | Country of origin |
|------------------------|------|---|-------------------|
| <i>M. anisopliae</i> | Cy3 | <i>Cylas formicarius</i> (Curculionidae) | Indonesia |
| | MPs | <i>Phyllotreta striolata</i> (Chrysomelidae) | Malaysia |
| <i>B. bassiana</i> | Wls | <i>Leptocorisa oratorius</i> (Alydidae) | Indonesia |
| <i>P. fumosoroseus</i> | Pf | <i>Pteroma pendula</i> (Psychidae) | Malaysia |

ers and provided with the same host plant as in the field. The beetles were collected with an aspirator.

Lipid Extraction

Fatty acids were extracted from the adult beetles according to the method described by Smith and Grula (1982). A sample of 0.5 g of beetles was placed in a beaker of distilled water and continuously agitated for 15 min with a magnetic stirrer to remove any dirt. Insects were then placed in a beaker containing 5 mL methanolic-HCl (2.5% v/v, methanol/HCl) and shaken for 30 min. The extract was then filtered using a vacuum suction pump through Whatman No. 1 filter paper using a rotary evaporator. The residue was then methylated for gas chromatography by the addition of 4 ml sodium-dried benzene, 4 ml 2,2-dimethoxypropane and 0.5 ml of methanolic-HCl (2.5% v/v, methanol.HCl). The solution was kept at room temperature overnight after which time the resulting methyl esters would have evaporated.

Gas chromatography was accomplished using HITACHI 263-50 equipped with a DEGS capillary column, with hydrogen as the carrier gas. The temperature was held at 150°C for three minutes after injection and then increased to 250°C. The column was connected to a HITACHI D-2000 reader.

Bioassays

The medium used for conidial germination of the fungal pathogen was water agar (1.5% w/v, Bacto). A 5 ml sample of the medium was placed in a tube and autoclaved for 20 min at 121°C at 1.05 kg/cm². The medium was left to cool to 40°C. The hydrophobic butyric, caprylic, caproic, heptanoic and nonanoic acids as determined through gas chromatography earlier, were neutralised and made water soluble by an addition of 0.2M Na₂HPO₄. Each of these fatty acids was added to the medium in concentrations of 0.025, 0.050, 0.075, 0.10 and 0.125% (v/v). The medium was then left to cool in a Petri dish (8 cm diameter). A 50 ml of conidial suspension containing 1 x 10⁵ conidia mL⁻¹ was applied to the medium using a Gilson pipetman and spread with a bent glass rod. Conidial germination was recorded under a microscope 24 h after incubation at room temperature.

RESULTS AND DISCUSSION

Gas chromatographic analysis of adult flea beetle cuticular extract showed that several fatty acids were present in differing amounts. There were two peaks (Fig. 1). These peaks exhibited retention times corresponding to those of butyric and nonanoic acids. Three other fatty acids detected were caproic, heptanoic and caprylic acids (Table 2). According to Smith and Grula (1982), chromatography of inhibitory compounds from the surface of the corn earworm, *Heliothis zea*, showed only one peak that exhibited a retention time corresponding to caprylic acid, indicating that besides the sclerotised cuticle, fungistatic straight-chain fatty acid could play an important role in the establishment of an entomopathogenic fungal infection.

Fig. 2 shows that depending on the type and concentration, fatty acids inhibited the conidial germination of *M. anisopliae*, *B. bassiana* and *P. fumosoroseus*. Gershon *et al.* (1973) reported that toxicity of fatty acids to fungi was affected by the chain length of the fatty acid, pH of the medium and the presence in the test medium of an adsorbent such as serum albumen. At concentrations between 0.025 - 0.125, all the fatty acids demonstrated varying levels of toxicity to *M. anisopliae*. Butyric acid was the least toxic—only toxic to *B. bassiana* and *P. fumosoroseus* at concentrations higher than 0.125%, i.e. the concentration at which all the fatty acids were very toxic. Nonanoic acid was toxic to both *M. anisopliae* and *P. fumosoroseus* with no conidial germination at the lowest concentration of 0.025%, while *B. bassiana* managed to germinate at between 3 - 18%. The conidial germination of *B. bassiana* was especially inhibited by heptanoic acid with only 12.5% germination at the lowest concentration of 0.025%. Results from this study concur with the conclusion made by Smith and Grula (1982) who reported that at concentrations between 0.02 and 0.04% saturated fatty acids with 10 carbons or less were highly inhibitory to the conidial germination of *B. bassiana*.

Samson *et al.* (1988) reported that most straight-chain saturated fatty acids were toxic to the conidia of entomogenous fungi. The fatty acids detected on the surface of corn earworms identified as caprylic, valeric and nonanoic acids were also reported to inhibit the conidial germination of *B. bassiana* (Smith and Grula

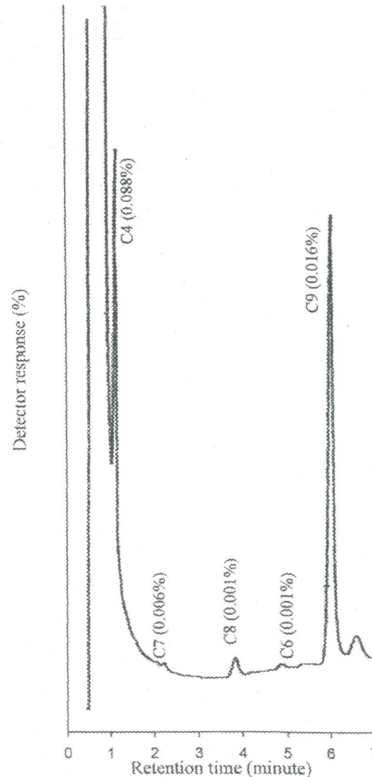


Fig. 1: GC tracing of hexane extract of adult striped flea beetle, *P. striolata* F., integument

TABLE 2
Concentration of fatty acids extracted from the integument of the adult *Phyllotreta striolata*

| Fatty acid | Concentration (%) |
|----------------|-------------------|
| Butyric (C4) | 0.0881 |
| Caproic (C6) | 0.0014 |
| Caprylic (C7) | 0.0063 |
| Heptanoic (C8) | 0.0012 |
| Nonanoic (C9) | 0.0157 |

1982), while Koidsumi (1957) reported that cuticular lipids of the silkworm and the rice stem borer, presumably caprylic or caproic acids, inhibited the germination and growth of *Aspergillus flavus*.

Smith and Gula (1982) suggested that the toxicity of fatty acids to *B. bassiana* conidia *in vitro* was dependent on the nutritional conditions present, since the fungus could grow in the presence of different carbon, nitrogen and energy sources. On the contrary, Boucias and Pendland (1984) observed that isolates of

Nomuraea rileyi could use epicuticular fatty acids to germinate. In other cases, these molecules induced penetration of germ tubes on the host cuticle (Kerwin 1984; Latge *et al.* 1987). While according to Lecuona *et al.* (1997), a pentane-extract of the cuticular lipids of *M. melolontha* larvae did not affect germination and hyphal growth of the virulent isolate of *B. bassiana*. However, it acted as an inhibitor to an avirulent isolate. Similar results were reported for the cuticular lipids of *Acyrtosiphon pisum* to the aggressive strains of *Conidiobolus obscurus* (Boucias and Latge 1988). This study has shown that straight-chain fatty acids on the flea beetle integument were inhibitory to conidial germination and thus protected the beetles against the invasion of entomopathogenic fungi. Therefore, the use of entomopathogenic fungi to control flea beetles, and perhaps other ground dwelling insect pests too, could probably be made more effective if nutrients were added into a conidial formulation of virulent strain so as to stimulate conidial germination.

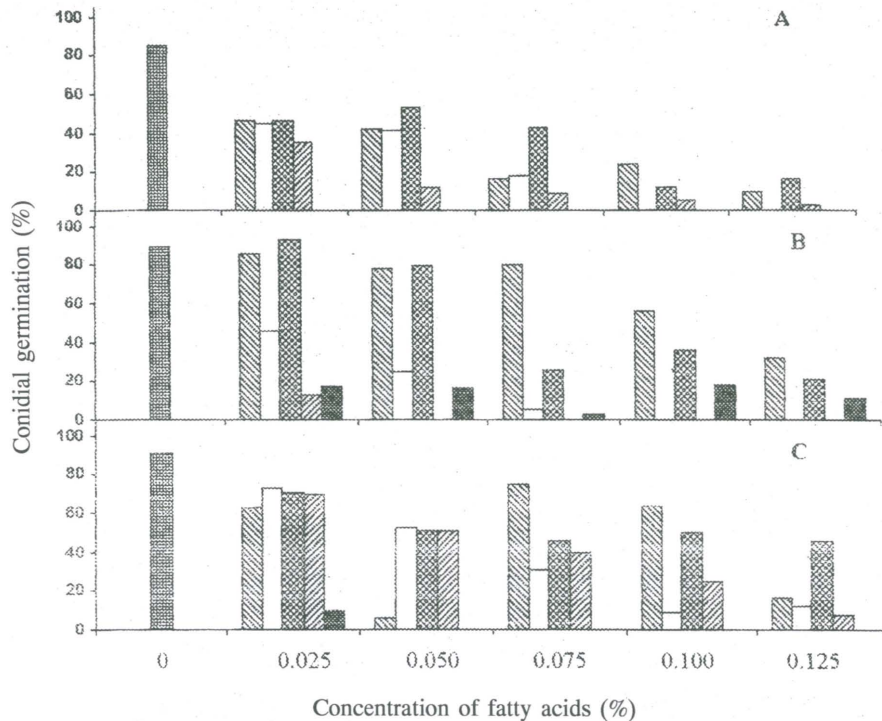







Fig.2. Effect of fatty acids (C4 , C6 , C7 , C8 , C9 ) on percentage of conidial germination of *M. anisopliae* (A), *B. bassiana* (B) and *P. fumosoroseus* (C) on water agar

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