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Full Length Research Paper

A study of the efficacy of two isolates of *Metarhizium anisopliae* isolated from different geographical areas

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The efficacies of two isolates of *M. anisopliae* were evaluated against black maize beetle *Heteronychus licas* in the laboratory at 15 and 28^{o}C and relative humidity of 55 - 70% at the University of Zimbabwe in 2000. The beetles were exposed to the fungus that was suspended either in oil and in water at concentrations ranging from 1.4x10^{4} - 1.4x10^{8} conidia/ml. Mortality was observed in all concentrations and mortality increased with increase in conidia concentration. Fungal infection reduced feeding activity in beetles with most deaths occurring on the soil surface. Conidia concentration, isolate, type of medium, the interaction between temperature and medium and the interaction between isolate and medium had marked effects on beetle mortality ($p < 0.05$). The oil formulations were in general more effective than the water formulations. The most potent treatment at 15^{o}C was the oil suspension of isolate IMI098376 (LC_{50} = 4.47x10^{4} conidia/ml) with an LT_{50} at the lowest concentration (1.4x10^{4}conidia/ml) of 13.3 weeks and earliest mortality at highest and lowest concentration occurring in 3 and 4 weeks, respectively. At 28^{o}C the most potent treatment was the oil formulation of IMI098376 (LC_{50} = 3.10x10^{4} conidia/ml) with an LT_{50} at the lowest concentration of 13.3 weeks and the earliest mortality at highest and lowest concentration occurring in 4 and 5 weeks, respectively. Field trails using the oil formulations are recommended.

Key words: Formulation, media, mortality, potent, efficacy, lethal concentration, biocontrol.

INTRODUCTION

The black maize beetle (BMB**)** *Heteronychus licas* (Coleoptera: Scarabibadea) is indigenous to Africa and is an important pest in tropical regions, causing damage to crops such as maize, sugarcane, tobacco and cereal crops through feeding on the roots and buried stems (Cackett, 1992).

In Zimbabwe, *H. licas* is endemic to the Southeast lowveld, where it is considered the most important insect pest of sugarcane (Cackett, 1992; Mazodze, 1997) . Yield losses in severely affected fields are estimated at 40 -50 tonnes cane per hectare (Mazodze et al., 1999). The life cycle of *H. licas* in sugarcane under local conditions has been described by Cackett (1992).

The gains brought about by the discovery and use of pesticides, firstly the organochlorines in the 1940s and later the organophosphates, carbamates, prythroids and others in the years that followed, which were hailed by many entomologists as the ultimate victory against insect pests were short lived (Mazodze, pers. com) . Within short periods of time, insect resistance to, first the organo-chlorines and then to many other insecticides were re-ported. To date, the ever increasing resistance of pests to pesticides, inducement of secondary pest species, crop loss and plant

ABBREVIATIONS

LC_{50} – time taken for killing 50%; LT_{50} – concentration killing 50% IMI - International Mycological Institute; RH - Relative humidity.

hardening due to accumulative pesticide phytotoxicity and concern about harm to non-target species and environmental pollution has reinvigorated scientists' search for solutions in an integrated complex of control measures (Burges, 1981).

The perennial problem with chemical control in terms of cost, hazardousness and efficacy have led researchers in the sugar industry in Zimbabwe to turn their efforts to evaluating biological control using entomopathogenic microorganisms. *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes) is a promising agent for controlling insect pests because of its wide geographical range and broad host range (Mazodze, pers. com) . Biological control using this fungus, apart from being environmentally friendly is likely to be self-perpetuating (Mazodze et al., 1999). This fungus has no mammalian toxicity (Ward et al., 1981; Jevanand et al., 1995).

The major objective for carrying out this research was to compare the efficacy of two isolates of *M. anisopliae* isolated from different geographical areas, one from Australia (isolate IMI098376) and a local one, isolate IMI350395, on adult BMB from the Southeast lowveld of Zimbabwe. Both isolates were originally isolated from *H. licas*.

Very little has also been done on the differential virulence of isolates of the same species when applied in different carrier materials (formulations). This study thus also aimed at exploring the effects of water and oil based formulations on the virulence of these two isolates. Biological control using this fungus would be ideal in the sugar industry where continuous irrigation keeps the soil moist and the air relatively humid and thus suitable for establishment and perpetuation of the fungal pathogen.

MATERIALS AND METHODS

The experiment was carried out at the University of Zimbabwe, Zimbabwe in 2000.

M. anisopliae isolates

Isolate IMI098376 was obtained from International Center for Agriculture and Biosciences (CABI) and isolate IMI350394 was obtained from the Zimbabwe Sugar Association Experiment Station (ZSAES) in Chiredzi, Zimbabwe. Both isolates were streaked onto Potato Dextrose Agar (PDA) and incubated at 28^{o} C in darkness for maximum mycelial growth and sporulation. For positive identify-cation of the fungus, slides were prepared from *in vitro* material of the fungus in distilled water and examined immediately under the microscope.

Determination of spore viability

Germination tests were carried out to determine spore viability according to the method described by Hall (1976). The mycelia were scrapped using a sterile blade and suspended in a 0.003 M KH_2PO_4 solution containing 0.02 MTritonX-100 (Octyl Phenoxy Polethoxyethanol) and filtered through sterile nylon chiffon (4 threads/mm) to remove the debris and dissociate the conidia balls. A Neubar's haemocytometer was used to count the conidia. At least 100 conidia were counted per replicate using a compound microscope and showered onto water agar, covered with a cover slip and incubated at 28^{o} C. Germination counts were made before extensive growth of germ tubes. The tests were repeated using oil.

Preparation of conidia suspensions

Conidia suspensions were prepared according to the method described by Feng and Johnson (1990). The surfaces of 10-day-old cultures of each isolate were scrapped using blades to harvest both conidia and hyphal debris. The mixture was suspended in 0.003 KH_2PO_4 solution containing 0.02 M TritonX-100 and filtered through sterile nylon chiffon (4 threads/mm) to remove the debris and dissociate the conidia balls (confirmed by microscopic examination). Conidia concentration was determined with a haemocytometer. Concentrations ranging from $1.4x10^4$ to $1.4x10^8$ conidia/ml were made by serial dilution with either sterile water or sunflower oil

Rearing of *H. licas*

Adult *H. licas* were collected from ZSAES in Chiredzi, southeastern Zimbabwe in March 2000, and kept in flowerpots filled with soil also from ZSAES. The flowerpots contained pieces of cut cane stems and bagasse as food for the beetles and were kept at ambient humidity and a summer day length (about 12 h light and 12 h darkness)

Laboratory bioassays

The bioassays were performed under aseptic conditions. Sterile plastic vials, each measuring 6 cm in height and 4 cm in diameter with holes in the bottom and top lid for radiation and drainage were used. The beetles were selected at random for bioassay to keep variation to a minimum. The beetles were gently removed from the flowerpots using sterile blunt forceps to avoid inadvertent cuticle damage, which would make them more prone to infection by fungi. Each vial contained the following: heat-treated soil (sterilized at 105^{o}C for 24 h before use) or non-treated soil for the control, moisture [supplied as distilled water (5 mls) at the start and periodically as necessary], one live beetle and food was provided as fresh bagasse (10 g per vial) (sterilized in 10% Potassium Hypo chlorite). The soil used was from a *H. licas* infested sugarcane plot at ZSAES. There were 16 vials per treatment. The treatments were as follows: a control (without fungus) plus 10 ml distilled water and, for each of the two isolates (IMI350394 and IMI098376), 5 ml of conidia in water with conidia concentrations of about $1.4x10^4$, $1.4x10^5$, $1.4x10^6$, $1.4x10^7$ and $1.4x10^8$ conidia/ml added to heat sterilized soil in each vial and incubated at 15^{o}C and RH of 55 to 70% and a photoperiod of about 11 h. Each treatment was replicated three times. The same treatments were also done using conidia that were suspended in oil.

The whole procedure was repeated and the vials incubated at 28^{o}C and the same humidity and photoperiod. The following mycotic effects were considered: site of host penetration by the fungus, mycelial growth on the insect body, anatomical changes in the host (colour, size) and the place where death occurs (under soil or on the above). Beetle mortality was assessed 14 days after inoculation and thereafter every seven days for a total of 16 weeks. The assessment periods were based on earlier findings that the fungus is slow acting against the beetle with LT_{50} of between 5 and 6 weeks (Mazodze, 1997). Beetle mortalities in the fungus- Where x is the percentage mortality in the untreated control and y is the percentage survival in the treated insects. The results of the bioassays were recorded as cumulative percentage mortality as

Table 1. Probit analysis of beetle mortality data obtained from bioassays using two isolates of *M. anisopliae* against *H. licas* at 15^0C.

Isolate	Formulation	Slope	χ^2	df	p	LC_{50} (Conidia/ml) with 95% fiducial limits (range)
IMI098736	Water	0.30	33.59	3	<0.00001	$2.48x10^5(3.59x10^4$-$1.0x10^6)$
	Oil	0.22	45.85	3	<0.00001	$4.77x10^4(3.38x10^1$-$4.04x10^5)$
IMI350394	Water	0.24	76.99	3	<0.00001	$4.06x10^7(2.78x10^6$-$1.32x10^{17})$
	Oil	0.25	38.97	3	<0.00001	$7.10x10^4(1.25x10^3$-$4.26x10^5)$

Table 2. Probit analysis of beetle mortality data obtained from bioassays using two isolates of *M anisopliae* against *H. licas* at 28^0C.

Isolate	Formulation	Slope	X^2	df	p	LC_{50} (Conidia/ml) with 95% fiducial limits (range)
IMI098736	Water	0.22	4.92	3	<0.00001	$3.56x10^4(2.18x10^4$-$5.42x10^4)$
	Oil	0.21	24.49	3	<0.00001	$3.10x10^4(5.17x10^2$-$1.73x10^5)$
IMI350394	Water	0.25	28.72	3	<0.00001	$6.82x10^6(1.39x10^6$-$7.57x10^7)$
	Oil	0.14	38.14	3	<0.00001	$1.11x10^5(1.8x10^{-2}$-$1.80x10^6)$

inoculated treatments were corrected for mortalities in the control using the formula of Abbot (1925).

$$\%\ Mortality = \frac{x - y \times 100}{x}$$

well as total mortality for each concentration.

Confirmation of cause of death

Dead beetles were inspected for the presence of *M. anisopliae* fungal growth (white mycelia and conidia) and recorded as mycosed (death caused by fungus). Beetle cadavers not showing the fungus were surface sterilized for 2 min in 0.2% Mercuric chloride, then for 2min in 70% ethanol, then placed on PDA in Petri dishes and incubated at 28^0C. Incubation was done for 10 days during which the cadavers were monitored for fungal growth. Any fungal growths were scrapped and slides made for microscopic examination and those showing the fungi recorded. The proportion of beetles mycosed (confirmed by eye and after incubation) was calculated and recorded for each concentration.

Data analysis

Mortality data for each fungal isolate were Probit transformed and linearly regressed against concentration to determine LC_{50} and against time (weeks) to determine LT_{50}s for all effective concentrations with the Probit procedure (SAS, 1995). Analysis of variance (ANOVA) was performed to find out if there are any marked differences in mean mortalities between isolates, temperature and formulations. Proportion analyses were used to compare the relative effectiveness of the different treatments on the mortality of the beetles.

RESULTS

M. anisopliae was successfully cultured on PDA. It was observed to produce yellowish green colonies. Microscopic examination of the fungus showed the presence of conidiogenous cells borne at the apices of broadly branched, densely intertwined conidiophores that form a hymenium. Green conidia occurred in chains at the apical ends of conidiogenous cells and these were cylindrical in shape with rounded ends, a positive confirmation of this fungus (*M. anisopliae)*. The average spore viabilities for both isolates were 85 and 84.3% in water and oil, respectively.

Observed feeding behaviour of beetles showed that feeding was reduced prior to death as indicated by the decrease in bagasse eaten just prior to death as compared to control treatments. Most of the deaths occurred on the surface of the soil and the fungus grew on the surface of the beetle in restricted areas mainly along joints especially between the head and abdomen. Mortality was recorded in all treatments where fungal conidia were added. There were a lot of variations in mortality in the lethal time from initial exposure to conidia to time of death, the time taken for 50% of the exposed beetles' population to die and the concentration required to kill 50% of the population. In all treatments, percentage mycoses were observed to be slightly lower than percentage mortality.

Lethal concentration (LC_{50}s)

All concentration levels for both isolates were able to induce mortalities but lower concentrations generally induced lower mortalities. There was a general increase in beetle mortality with increase in spore concentration. The LC_{50}s of the two isolates against *H. licas* are shown in Tables 1 and 2. IMI098376 in oil suspension was the most potent treatment against the beetles at both temperatures (15^0C, $LC_{50} = 4.77x\ 10^{4\ and}$ at 28^0C, $LC_{50} = 3.10x10^4$) . The oil formulations of both isolates tended to be more potent than the water formulations at both temperatures ($X^2 = 37.74$, $p = 0.006$) . Water formulations of both isolates were more potent at higher temperatures 28^0C) than at lower temperature (15^0C) ($X^2 = 35.74$, $p = 0.004$). Oil formulation for isolate IMI098376 tended to be

Table 3. Mean percentage mortalities and mycoses (in brackets) induced by two isolates of *M. anisopliae* in different media at15^{o}C.

Isolate	Formulation	% Mean Mortality
IMI098376	Water	64b±33.73 (54±24.42)
	Oil	72.4b±25.2 (71.2±27.14)
IMI350394	Water	26.8a±27.22 (21±20.55)
	Oil	71.0b±27368 (65±26.8.6)

Means in the same column followed by the same letter are not significantly different at p = 0.05 (Proportion analysis).

Table 4. Mean percentage mortalities and mycoses (in brackets) induced by two isolates of *M. anispliae* in different media at 28^{o}C.

Isolate	Formulation	% Mean Mortality
IMI098376	Water	74.0b±22.68 (61.2±18.56)
	Oil	74.4b±22.99 (64.0±36.91)
IMI350394	Water	38.8a±28.23 (24.6±24.9)
	Oil	64.6b±19.82 (59.2±26.25)

Means in the same column followed by the same letter are not significantly different at p = 0.05 (Proportion analysis).

Table 5. Mean percentage mortality and mycoses (in brackets) induced by two isolates of *M. anisopliae* at 15 and 28^{o}C.

Tempe-rature (^{o}C)	Isolate	% Mean mortality.
15	IMI098376	68.2b ± 28.42 (62.6 ± 25.97)
	IMI350394	48.9a ± 34.7 (43 ± 32.35)
28	IMI098376	74.2b ± 21.53 (62.6 ± 27.58)
	IMI350394	51.7a ± 26.72 (42.1 ± 30.37)

Means in the same column followed by the same letter are not significantly different at p = 0.05 (Proportion analysis).

(more potent at higher temperature than at lower temperature while those of isolate IMI350395 tended to be more potent at lower temperature and less potent at higher temperature.

Variable influencing mortality

Analysis of variance (ANOVA) showed that beetle mortality was significantly influenced by the type of isolate ($p < 0.0001$), the medium in which the fungal conidia were added ($p < 0.0001$), the concentration of conidia added ($p < 0.0001$), the interaction between isolate and medium ($p < 0.0001$), and although temperature on its own did not have any marked effect it did interact with the medium used to influence mortality ($p = 0.008$) Analysis of the percentages mortalities using Proportions were able to highlight differences between specific treatments. This analysis also showed that suspension medium and isolate type had some influence on beetle mortality (Tables 3 to 5).

Influence of different media on the same isolate

Proportion analysis showed that there were marked differences in mortality between water and oil for isolate IMI350394 at both 15^{o}C and 28^{o}C. The oil suspension of IMI350394 proved to be more potent at 15^{o}C ($X^2 = 38.74$, $p = 0.0001$) and 28^{o}C ($X^2 = 13.54$, $p = 0.0002$) than the water suspensions (Tables 3 and 4). On the other hand, for isolate IMI098376, the medium in which the spores were applied had no influence on mortality at the two treatment temperatures.

Effects of the same medium on different isolate

Water had a differential influence on the potency of the two isolates on the beetles at both 15 and 28^{o}C (Tables 3 and 4). Water significantly enhanced the potency of isolate IMI098376 over that of IMI350394 at both temperatures.

Influence of isolate and temperature

Isolate type had a marked influence on mortality in the beetles. Isolate IMI098376 was more potent at 15^{o}C ($X^2 = 7.43$, $p = 0.006$) and at 28^{o}C ($X^2 = 10.38$, $p = 0.0001$) than isolate IMI350394 (Table 5). Temperature, however, had no differential effect on beetle mortality.

Time taken for earliest mortality to occur in relationship to inocula levels

There was a general increase in mortality with increase in time of incubation. Higher concentrations tended to cause mortality much earlier than lower concentrations.

The time at which the treated beetles died of infection by *M. anisopliae* varied between the two isolates. The highest concentration (1.4×10^8 conidia/ml) induced earlier beetle mortalities than the lowest concentration (1.4×10^4 conidia/ml) irrespective of the isolate (Table 6). At the highest concentration (1.4×10^8 conidia/ml), the effect of isolate, formulation and temperature was minimal on the earliest time taken for mortalities to occur with most of the deaths occurring in 3 to 5 weeks. Irrespective of isolate and concentration, a lower temperature (15^{o}C) induced earlier deaths than a higher temperature (28^{o}C).

At the lowest concentration (1.4×10^4 conidia/ml) the water formulation of IMI098376 was more virulent on the beetles than the water formulation of IMI350394 as the earliest mortalities occurred 9 weeks later (at 15^{o}C) and 6 weeks later (at 28^{o}C) in the latter compared to the former. At this lowest concentration, a lower temperature induced earlier mortalities than a higher temperature in the oil

Table 6. Time taken (weeks) after inoculation for earliest beetle death to occur at the highest ($1.4x10^8$ conidia/ml) and lowest concentration ($1.4x10^4$conidia/ml) at 15 and 28^oC.

Temperature	Isolate	Medium	Highest concentration	Lowest concentration
15^oC	IMI098376	Water	3	5
		Oil	3	4
	IMI350394	Water	4	14
		Oil	4	5
28^oC	IMI098376	Water	4	5
		Oil	4	5
	IMI350394	Water	5	11
		Oil	5	7

Table 7. The LT_{50} s (in weeks) of two isolates of *M. anisopliae* against *H. licas* at levels of concentrations from 0 to $1.4x10^8$ conidia/ml, at 15^oC.

Isolate	Formulation	0	$1.4x10^4$	$1.4x10^5$	$1.4x10^6$	$1.4x10^7$	$1.4x10^8$
IMI098376	Water	_	_	14.9	14.3	8.1	6.5
	Oil	_	13.3	10.2	8.3	6.3	5.0
IMI350394	Water	_	_	_	_	14.2	11.9
	Oil	_	14.3	13.1	9.3	7.7	6.5

-LT_{50} not achieved in time of observation.

Table 8. The LT_{50}s (in weeks) of two isolates of *M. anisopliae* against *H. licas* at levels of concentrations from 0 to $1.4x10^8$ conidia/ml, at 28^oC.

Isolate	Formulation	0	$1.4x10^4$	$1.4x10^5$	$1.4x10^6$	$1.4x10^7$	$1.4x10^8$
IMI098376	Water	_	15.1	15	12.3	7.2	5.1
	Oil	_	13.3	10.5	7.4	6.4	4.9
IMI350394	Water	_	_	_	_	13.4	9.1
	Oil	_	15.3	11.5	9.6	7.3	9.5

-LT_{50} not achieved in time of observation

formulation for isolate IMI098376. For isolate IMI350394 in water formulation a higher temperature induced earlier mortalities (earliest mortality in 11 weeks) than a lower temperature (earliest mortality in 14 weeks) whereas in oil formulation a lower temperature induced earlier mortalities (in 5 weeks) than a higher temperature (in 7 weeks).

At the lowest concentration, the effect of formulation on earliest mortalities varied with isolate and temperature. At 15^oC, both isolates induced earlier beetle deaths in oil formulation than in water formulation (Table 6). At 28^oC formulation had no effect on isolate IMI098376. At this higher temperature the oil formulation of isolate IMI350394 induced earlier adult deaths than the water formulation.

Lethal Time (LT_{50}s)

The LT_{50}s for the effective concentrations are shown in Tables 7 and 8. A general decrease in LT_{50} with increase in conidia concentration was noted (Tables 7 and 8). Variations in LT_{50}s were also noted between isolates, temperature and suspension medium (Tables 7 and 8).

The LT_{50}s of the two isolates of *M. anisopliae* against *H. licas* at different concentrations at different temperatures are given in Tables 7 and 8.

At the highest concentration, both isolates, irrespective of formulation were very effective against the beetles at both temperatures. At this concentration, isolate IMI098376 in oil formulation, was the most effective treatment against the beetles (LT_{50} < 5 weeks). At the lowest concentration ($1.4x10^4$ conidia/ml) isolate IMI098376 in oil was the most effective treatment against the beetles at both temperatures (LT_{50} = 13.3 weeks) Regardless of the all variables the lowest LT_{50} for the beetles at the lowest concentration was 13.3 weeks and at the highest concentration 4.9 weeks. At both tempe-ratures the oil formulations for both isolates generally performed better than the water formulations but the oil

formulation of IMI098376 was more effective than the oil formulation of IMI350394. IMI098376 in water also performed better than IMI350394 in the same medium. At the highest concentration the LT_{50}s were generally lower at higher temperature than at a lower temperature.

DISCUSSION

The two isolates of *M. anisopliae* caused mycosis in *H. licas*, which was confirmed by direct microscopic observations and by culturing the infected beetles on PDA. These observations agree with those of Mazodze (Mazodze, pers. com). The association of this fungus with the black maize beetle was further confirmed by findings of infected beetles in the sugarcane field (Mazodze, pers.com).

A reduction in food consumption is quite encouraging as this would mean that damage to sugarcane would be markedly reduced once the larvae are infected.

The occurrence of death on the soil surface could be a result of the beetles moving onto the soil surface in an attempt to 'sun' themselves and raise body temperature to curb disease development. This behavioural change is of great benefit to the fungus whose conidia can be carried away by air currents or get into contact with other insects hence infecting them and perpetuating the disease cycle. Kooyman et al. (1997) noted that *Schistocerca gregaria* infected with *M. flavoride* 'sunned', even when it was very hot, possibly to cause a fever in order to slow down the disease process. Elevated body temperature would be able to delay or reduce the impact of infectious diseases, which can cause high levels of mortality in insect populations (Steinhaus, 1949; Burges, 1981).

Maniania (1992) reported that susceptibility of insect pest to fungal pathogens depends upon the fungus species and that different levels of pathogenicity were observed towards different insect pests, when strains were isolated from the same host or from soil. Although the two isolates tested can infect *H. licas*, their virulence depended on the treatment conditions.

The time lag between the time of first exposure to fungal conidia and death in beetles indicates that the disease occurs in stages. The initial stages of insect infection by entomopathogenic fungi include the penetration of cuticle (Hajek et al., 1994). Studies have shown that cuticle solubilization and subsequent hyphal penetraion occur by action of extracellular enzymes and metabolites (Bidochka et al. 1991). Following cuticle penetration, the fungus proliferates within the insect body. Penetration is frequently followed by blackening which is a melanic reaction of the integument at the site of the infection probably caused by changes in the phenoloxidase activity caused by the fungus (Ferron, 1978).

Isolate type had a very strong influence on the mortality of beetles. Isolate IMI098376 was more potent against the beetles than IMI350394 making the former isolate a better candidate to develop for the biocontrol of the black maize beetle. Hajek et al. (1994) noted that infection of the host is achieved when the conidia recognizes the host as susceptible. This ability to recognize the host varies between isolates (Hajek et al., 1994). Recognition is dependent on chemical and topographical signals (nature of micro folds on cuticle surface) resulting in differential 'resolving powers' in the conidia of different isolates. It is thus possible that the conidia of isolate IMI098376 have greater resolving powers for the beetle surface structure than isolate IMI350394.

The correlation of total mortalities to conidia concentration agrees with Ferron's (1978) observations. Earliest mortalities and 50% mortalities occurred much earlier at higher concentrations than lower concentrations. This means that when conidia concentration is high, the chances of insect being infected and the levels of infections are also high. One important component in fungal disease development is the attachment of the infective unit (conidia) to the insect's body which in turn is dependent on whether the insect comes into contact with the unit or not (Roberts, 1981). Various laboratory application rates have thus been recommended, concentrations ranging from 10^4 to 10^8 conidia/ml (Ferron, 1971). It is also possible that the fewer the conidia the longer it will take for them to achieve adequate growth to cause mycoses. Low conidia concentrations may also not be able to overcome the healthy insects' resistance mechanisms and thus the insects can survive the infection, with only the older and unhealthy ones succumbing to the infection. Muller-Kogler (1967) noted that there is a minimum threshold required to cause a lethal infection below which there is no disease.

The medium in which the spores are applied proved to have a very strong bearing on the virulence of the fungus. Differences in mortalities between the media used can be attributed to differential level of conidia adhesion to the insect cuticle. Adhesion is facilitated by hydrophobic interactions between the mucus produced by the conidia and the lipids and waxes on the cuticle. Lipids and waxes make the epicuticle highly lipophilic and thus highly hydrophobic. Oil and water may thus contribute in different ways to this adhesion process, oil being a better facilitator than water, allowing the conidia to stick with ease on the insect body wall because of the presence of lipids and waxes on the cuticle surface, unlike those with a water film around them where a "water-off-the duck's-back" problem has to be overcome (Prior et al., 1988). This differential facilitation by oil may thus explain the general increase in virulence of the suspensions of both isolates in oil and in particular the marked difference in potency of the suspension of IMI350394 in oil as compared to the suspension in water to the beetles These results thus confirm findings of Prior et al. (1988) that some Hyphomycete fungi are more infectious when applied in oil rather than in water.

Temperature affects mortality by affecting both the fun-

gus and the insect. Temperature influences the rate of growth through interfering with enzymatic activities, with the consequence that growth only occurs within a particular range of temperature within which there is an optimum. The optimum temperature for *M. anisopliae* is 28^{o}C and it is at this temperature that maximum virulence was expected as the fungus can easily grow and overcome the insect's resistance. At this temperature the insect's activity levels are quite high and are likely to move around frequently and hence pick up the conidia. Low temperatures slow down fungal growth and insect activity and hence mortalities are expected to be low. Higher temperatures may also increase the rate at which the conidia stick to the insect body.

Conclusion

The results of the investigation show that *M. anisopliae* isolate IMI098376 and IMI350394 infect *H. licas* and cause death within reasonable time periods (earliest mortality occurring within 3 to 5 weeks at concentrations of $10x10^{8}$ conidia/ml) at temperatures between 15 and 28^{o}C and high humidities similar to the conditions prevailing in the Southeast lowveld of Zimbabwe. Infection results in reduced feeding and thus offers a potent protection to the sugarcane. The occurrence of death at the soil surface further enhances disease perpetuation as it allows infection to spread rapidly. It is important that field trials are done to determine the effectiveness of the isolates under field conditions in oil formulation.

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