REVIEW OF LITERATURE

Termites and their Microbial Symbionts

Termites belong to the order Isoptera, which includes over 2000 described species whose biology; behaviours, and nutritional ecology are remarkably diverse. Although frequently thought of as feeding on wood, their diet (depending on particular species) includes a variety of lignocellulosic plant material (either sound or in various stages of decay), as well as materials derived from it (e.g. animal dung), and soil rich inorganic matter (i.e. humus) as stated by Wood and Johnson (1986).

According to Wood and Sands((1978), roughly 2/3 of the Earth's land surface that lying between 45^0N and S latitude is inhibited by one or more termite species. However, their populations are greatest in tropical and subtropical regions, where their numbers can exceed 6000 m^{-2} and their biomass densities ($>50gm^{-2}$) often surpass that of grazing mammalian herbivores (0.013-l7Sgm^{-2}) as stated by Collins and Wood (1984) and Lee and Wood (1971). It has been estimated that termites from all ecological regions (approximately $2x10^{17}$ individuals) together consume about $3\text{-}7x10^{15}g$ of lignocellulose annually and mineralize a significant portion of it as stated by Collins and Wood (1984) and Khalil and Rasmussen (1990).

According to Krishna (1969) and (1970), termites are divided into two groups. The so-called lower termites (families Masto, Kalo, Hodo, Rhino and Seritermitidae) harbor in their alimentary tract a dense and diverse population of bacteria and cellulose digesting, flagellate protozoa. The latter represent unique genera and species found virtually nowhere else in nature as stated by Honigberg (1970). Higher termites (family Termitidae), comprising three fourths of all species, also harbor a dense and diverse array of gut bacteria, but they typically lack protozoa and have a more elaborate external and internal anatomy and social organization than do the lower termites.

The termite gut consists of the foregut (which includes the crop and muscular gizzard), the tubular midgut (which, as in other insects, is a key site for secretion of digestive enzymes and for absorption of soluble nutrients), and the relatively voluminous hindgut (which is also a major site for digestion and for absorption of nutrients, and which may be further subdivided into various regions). Malpighian tubules, which transport urine and urinary metabolites for excretion, empty at or near the junction of the mid-and hindgut. Many higher termites also possess a so-called mixed segment, which is a portion of the gut consisting on one side of cuticle lined hidgut tissue as stated by Noirot and Noirot(1969).

Owing to the relatively small size of most termites, and microenvironmental conditions existing within the gut have been difficult to define precisely, especially with techniques that impose little or no invasiveness. Nevertheless, anoxic conditions become more apparent as one moves from the foregut to the hindguts typically have a relatively low redox potential (ranging from - 50 to 270mV in different species) and a pH around neutrality (6.2 to 7.6), but in soil-feeding termites some portions of the hindgut (i.e. the relatively large P1 region, immediately posterior to the mixed segment, but anterior to the enteric valve) may have a pH as high as 11 as indicated by Bignell (1984), Bignell *et. al.* (1980), Veivers *et. al.* (1980) and Veivers *et. al.*(1982).

The morphological diversity of the termite gut microbiota is remarkable and has been documented in recent years for both lower, Breznak and Pankratz (1977) and To *et. al.*(1980); and higher termites, Bignell, *et. al.* (1980), (l980a) and (1983), Czolij *et. al.* (1985). Although some bacteria colonize the fore-and midgut [some rather distinctive morphotypes are situated in the midgut between microvilli, Breznak, *et. al.* (1977); in the ectoperitrophic space Bignell *et. al.*(l980); and in the mixed segment, Bignell *et. al.* (1980a), Bignell *et. al.* (1983) and Kovoor (1968)], the bulk of the intestinal microbiota is found in the hindgut, especially in the paunch, the region immediately posterior to the enteric valve.

Gut microbes are important for termite vitality and much of the termites nutrition derives from the products of microbial metabolism. Acetate, propionate, and other organic acids produced during microbial fermentation of carbohydrate in the hindgut are important oxidizable energy sources for termite, Odelson and Breznak (1983), as well as carbon skeletons for biosynthesis, Blomquist *et. al.*(1979) and (1982), Gou *et. al.* (1991), Mauldin (1982) and

Presturich *et. al.* (1981).

Gut bacteria are important to nitrogen economy. They enhance the ability of termites to acquire new N through N_2 fixation, Bentley (1984) and Breznak (1984), and to conserve N by helping to recycle excretory N (uric acid) back to the termites for biosynthesis, Potrikus and Breznak (1981).

Spirochetes, a group of highly motile, spiral or undulate bacteria defined by distinctive ultrastructural features, Canale-Parola (1984), are a major component of the gut microbiota of all termites examined. Spirochetes rarely occur in nature in as great a density and morphological diversity as in the gut of termites, Breznak (1984) and Margulis and Hinkle (1992). The range in size from 0.2x3mm, Breznak (1982), to 1.0 x 100mm, Hollande and Gharagozlou (1967), with analogous variations in wavelength and amplitude of the spiral cell body. Comparative morphometry prompted Bermudes *et. al.* (1988) to create, or revive, generic and specific epithets for a number of distinctive spirochetes in the hindgut of termites.

Spirochetes exist free in the gut fluid of all termites. In the lower mites they also occur within the cytoplasm, or they are attached to the surface of hindgut protozoa, Breznak, J.A. (1984) and participate in the motility of some of these protozoa, Cleveland and Grimstone (1964). Little else is know about termite gut spirochetes and their effect on termite vitality, as they have never been isolated and studied in pure culture. A reduced lifespan, Eutick *et. al.* (1978) and transient colonization of the gut by nonindigenous bacteria, *Serratia marcescens* was observed by Veivers *et. al.* (1982) when spirochetes were eliminated from *Nasutitermes exitiosus* by feeding the termites metronidazole, or by incubating them under pure oxygen. Such results are ambiguous, however, because elimination of spirochetes might have been accompanied by elimination of other, less morphologically conspicuous bacteria. In any case, there is no reason to believe that spirochetes are pathogenic to termites. They do not invade the gut epithelium, and termites harboring them appear vigorous and healthy.

According to Breznak and Brune (1994), higher termites in the subfamily Macrotermitinae have established an intriguing symbiotic, relationship with external basidiomycete fungi of the genus *Termitomyces*. The fungi are cultivated within the nest in convoluted, greyish-brown combs, consisting of plant material undergoing partial digestion by the fungal mycelium that permeates it, and on which the fungus develops round white nodules, or mycoteles, composed of masses of asexual spores (conidia).

The fungus combs are dynamic. New comb is formed by deposition of fresh termite feces containing finely comminuted (but only partially digested) plant material, which then becomes infiltrated with *Termitomyces* spp. Termites then feed upon older (i.e. more seasoned) parts of the comb, including the fungal nodules. Wood and Thoma (1989) have reviewed the biology of this fascinating association; however, a key question has always been the role of the fungus in termite nutrition. Evidence indicates that at least a partial digestion of plant polysaccharides and lignin by *Termitomyces* spp. can occur within the comb, Rohrmann and Rossman and Veivers *et. al.* (1991).

There is a widespread perception among biologists that cellulases are restricted to microbes. Although this restriction doesnot appear to be true, production by metazoans of cellulase components capable of depolymerizing polyglucan i.e. endo and exoglucanases) is relatively rare, Martin (1983) and (1987). A reasonably strong case can be made for symbiont-independent production of cellulases by termites, Slaytor (1993).

Lower Termites

Studies of axenic cultures of the protozoa (*Trichomitopsis termopsidis* and *Trichonympha sphaerica*), have shown the presence of CMC ase (i.e. an enzymatic activity capable of releasing reducing sugar from carboxy methylcellulose (CMC)], b-glucosidase, and CHA (cellulose hydrolyzing activity) in cell extracts, Odelson and Breznak (1985) and (1985a), Yamin (1978), (1980), and (1981), Yamin and Trager (1979).

According to Hogan *et. al.* (1985), O' Brien and Braznak (1984) and

Odelson and Braznak (1983), in termite hindgut fluid, acetate occurs at concentrations up to 80mM and can constitute over 90 mol% of the volatile fatty acid pool. It is taken up from the hindgut and oxidized to CO_2 and H_2O by termite tissues, a process that can support up to 100% of the respiratory requirement of some species.

Despite the critical role of hindgut protozoa in cellulose digestion by lower termites, evidence has also mounted that the termites themselves can produce cellulase components. Some workers suggested that some cellulase components' were produced by lower termites, Mishra (1980), Mishra and Sen Sarma (1987), Retief and Hewitt (1973), Yamaoka and Nagatani (1975) and Yokoe (1964).

Veivers *et. al.* (1982) demonstrated the presence of CMCase, CHA, and cellobiase in salivary glands, foregut, midgut, and hindgut of *Mastotermes darwiniensis.* Although most of the cellulase activity, Cookson (1992), was generally found in the hindgut and could be attributed largely to protozoa, the other enzyme activities were presumably of termite origin. Amylase and maltase activities were also found in salivary glands and midgut, indicating that *M. darwiniensis* should be able to utilize starch as a nutrient. Indeed, it was later discovered that *M. darwiniensis* could survive on a diet of starch, although this led to a loss of the large protozoa from the hindgut and, with them, the bulk of the hindgut cellulase activity, Veivers *et. al.* (1983). Such termites could no longer survive on a diet of wood, unless they were refaunted with cellulolytic protozoa. Presumably, when the termites were fed starch, a substrate more readily hydrolyzable than cellulose, the protozoa no longer had a competitive advantage in the hindgut and were displaced by bacteria, whose numbers increased. Curiously, starch and carbohydrates other than cellulose are also poor substrate for the *invitro* growth of the termite gut-protozoan *Trichomitopsis termopsidis,* Odelson and Breznak (1985) and Yamin (1978), despite the fact that such protozoa possess xylanase and amylase activities, Odelson and Breznak (1985). Even celluloses whose degree of polymerization is low will cause full or partial loss of the large cellulolytic protozoan *Pseudotrichnympha grassi* when fed to *Coptotermes formosanus*, with consequent decreased survivorship of the host Yoshimura *et. al.* (1993).

A similar picture emerged in studies of *Coptotermes lacteus*, wherein a termite origin was attributed to enzymes that were found in regions anterior to the hindgut and that persisted following removal of protozoa, spirochetes, and other bacteria by treatment with oxygen or antibacterial drugs, McEwen *et. al.* (1980) and O'Brien *et. al.* (1979). Column chromatography of enzyme activities of putative termite origin (i.e. those from pooled extracts of salivary glands, foregut, and midgut) revealed a multiplicity of activities as stated by Hogan et. at (1988). Enzymes of apparent termite origin included an exoglucanase capable of releasing glucose from cellulose or CMC, two endoglucanases (each with activity on CMC, but not on cellulose), and at least one glucono-d-lactone-inhibitable b-glucosidase (=cellobiase). Enzymes from protozoan extracts included an exoglucanases with gluconolactone - inhibitable cellobiases.

According to Bignell and Anderson (1980) and Noirot and Noirot (1969), in higher termites, the anatomy of the gut is more elaborate and multicompartmentalized than that of lower termites, with some regions possessing cuticular spines protruding into the lumen. Such specializations may be sufficient for increasing the exposure time of lignocellulose food to hydrolytic enzymes, making the protozoa unnecessary.

Higher Termites

Kovoor (1968) and Potts and Hewitt (1973) were among the first to suggest that higher termites synthesize their own cellulases. They found CMCase and cellobiase activities in regions of the gut (midgut and midgut wall) that were virtually of microscopically observable bacteria. A partially purified CMCase behaved like a true endonuclease, but with some hydrolytic activity on xylan as well, Potts and Hewitt (1974) and (1974a), although the starting material used (extracts of whole termite abdomens) compromised the strength of the conclusion that the enzyme was truly of termite origin.

A stronger case for a termite origin ofcellulases has come from slaytors group, working with *Nasutitermes exitiosus* and *Nasutitermes walkeri*, Hogan *et. al.* (1988), McEwen *et. al.* (1980), O'Brien *et. al.* (1979), and Schutz et. at (1986). They showed that (a) the bulk of the cellulase activity, including a CHA (referred to as exoglucanase, based on its ability to liberate glucose from microcrystalline cellulose), an endoglucanase, and a b-1, 4-glucosidase were present in the midgut, with a significant amount of endoglucanase and b-glucosidase associated with the midgut epithelium of *N. walkeri*; (b) the cellulase activity of *N. exitiosus* showed little or no change when gut bacterial populations were drastically reduced by starving the termites or feeding them tetracycline; and (c) attempts to reveal putatively occult, cell-bound cellulases from hindgut bacteria by sonication or by treatment with lysozyme ± EDTA were fertile. Importantly, the CHA activity of *N. walkeri* was significant in terms of the termite's respiratory activity, assuming that the CHA activity *in vitro* was comparable to that *in vivo*, and that oxidation of glucose (and/or a metabolite(s) derived from glucose) accounted for most of the oxygen consumption by this termite as stated by Breznak (1990).

The mechanism of cellulose digestion in the Macrotermitinae is not yet completely clear. The first evidence that ingestion of fungal comb contributed enzymes for cellulose digestion came from Martin and Martin's studies of *Macrotermes natalensis*, Martin and Martin (1978) and (1979). They found that most of the cellulase activity was located in the midgut and consisted of a C_1-cellulase activity (assayed as CHA), a C_x-cellulase activity (assayed as CMCase), and a b-glucosidase activity. The latter two were produced, in part, by the salivary glands and midgut. However, the C_1 -cellulase activity (which conferred upon midgut contents the ability to degrade crystalline cellulose) appeared to be derived only by ingestion of nodules present on the fungal comb. Evidence supporting this conclusion came from analysis of enzyme activities present in midguts of termites that were starved, or were fed normal or nodule-free comb material, Martin and Martin (1978), and from isoelectric focusing of enzyme activities present in midguts and in fungal nodules, Martin *et. al.* (1979).

The C_x(i.e. CMCase) components were presumably endoglucanases, because they had no activity on crystalline cellulose. However, it is not possible to tell whether the critical C_1-cellulase activities of fungal origin were endo-or exoglucanases, because substrate-product relationships were not established. These studies led to the hypothesis that mycophagy (i.e. the ingestion of fungal tissue) may result in the acquisition of enzymes that permit an animal to increase efficiency of digestion, and perhaps to expand its range of natural substrates, Martin (1987). Abo-Khatwa (1978) reached a similar conclusion for *Macrotermes subhyalinus* on the basis of a somewhat limited study.

Rouland and co-workers achieved the first purification of cellulase components for *Macrotermes mulleri* and its associated *Termitomyces* sp. They were able to distinguish two b-glucosidases, one of termite origin and one from fungal nodules, Rouland *et. al.* (1986). After being characterized with respect to catalytic and physical properties, they were judged to be identical and of fungal origin, i.e.$L_T=I_F$ Rouland *et. al.* (1988). I_F and I_T were both monomeric glycoproteins of M_r 34,000 KDa and showed respectable hydrolytic activity on microcrystalline cellulose, liberating reducing sugar at a rate roughly 5-10% of that observed with CMC as substrate. However, in contrast to results with *M. natalensis* the enzyme showing greatest activity on pure celluloses (enzyme II) was entirely of termite origin and appeared to be a cellobiohydrolase, Rouland *at. al.* (1988).

Enzyme II, a monomeric protein of M_r 52,000 KDa, also had relatively high activity on CMC, about 2/3 of that of component I. Synergism in CHA was observed between component I_F and enzyme II, and the synergism was further enhanced by the additional presence of the termite b-glucosidase, Rouland *et. al.* (1988a). This synergistic interaction between cellulase components may explain, in part, the enhanced survivorship of *M. mulleri* in the presence of its symbiotic *Termitomyces* sp., Rouland *et. al.* (1988b).

From the recent studies of Veivers *et. al.* (1991)*Macrotermes michaelseni* and *M. subhyalinus* another scenario for cellulose digestion has emerged. Cellulase activity in this species was greatest in the four worker castes (young and old, major and minor) and, again, most of the cellulase in the termites was found in the midgut, and in the fungus mainly in the nodules. Each cellulase complex comprised endoglucanase and b-glucosidase activities, both of which were multicomponent as judged by column chromatography. When column chromatographic elution profiles of midgut and nodule endocellulases were compared, only about 90% of the endoglucanase activity in the midgut could be attributed to nodule enzymes. Thus, for *M. michaelseni* and *M. subhyalinus*, it seemed as though fungal enzymes were essentially irrelevant to cellulose hydrolysis in the midgut.

Cellulose digestion

Despite various reports on the isolation of cellulolytic bacteria from gut contents of lower or higher termites, there has never been a convincing demonstration that such bacteria are quantitatively significant to cellulose hydrolysis in situ. Much of the early literature on this issue has been reviewed previously. Breznak (1982) and (1984), O'Brien and Slaytor (1982). Current reports include one of the isolation of cellulolytic actinomycetes (*Streptomyces* spp. and *Micromonospora* spp.) from wood feeding, soil-feeding and fungus-cultivating termites, Pusti and Belli (1985) and on isolation of the cellulolylic *Clostridim termitidis* from *Nasutitermes lujae*, Hethener *et. al.* (1992).

Most of the energy available to termites from cellulose digestion appears to come from oxidation of the acetate derived from cellulose, Odelson and Breznak (1983). Although higher and lower termites have all the enzymes necessary for converting glucose to pyruvate, and for oxidizing acetate to CO_2 and H_2O via the tricarboxylic acid (TCA) cycle, they appear to lack pyruvate hydrogenase or any other enzyme capable of converting pyruvate to acetyl CoA or acetate to feed into the TCA cycle, as said by O'Brien and Breznak (1984). Thus, production of acetate in the hindgut, from glucose or other intermediates of lignocellulose degradation, may be a major, critical role of many of the microbes harbored there. The bacteria capable of fermenting glucose and / or cellobiose to acetate are present in guts of higher and lower termites as refered by Eutick *et. al.* (1978) and Schutz *et. al.* (1986). The bacteria are capable of forming acetate from the reduction of CO_2 and removal of gut bacteria from higher or lower termites usually results in death of the termites within a relatively short time as indicated by Eutick *et. al.* (1978) and O'Brien *et. al.* (1979). If the mentioned hypothesis is true, one has to wonder why both higher and lower termites have apparently turned the process of acetate formation over to their gut microflora. At this moment there is still no clear answer. However, considering that termites thrive on diets relatively poor in nitrogen, and that many bacteria can use inorganic N or even N_2 as sole N source, one might speculate that this represents an evolutionary trade-off in order to maintain a population of microbes capable of upgrading poor sources of N to nutritious ones.

Hemicelluloses hydrolysis

Xylanase activity has been demonstrated: (a) in the hindgut of lower termites, Mishra (1980), where it is produced, at least in part, by hindgut protozoa, Odelson and Breznak (1985); (b) in the gut of soil-feeding termites, Rouland *et. al.* (1989); and (c) in the gut contents and fungal nodules of fungus-cultivating termites, Martin and Martin (1978) and Rouland *et. al.* (1988). According to Potts and Hewitt (1974a), partial purification of a cellulase from the higher termite *Trinervitermes trinervoides* revealed that it was also capable of hydrolyzing xylan. However, the most detailed information on xylanases so far has come from a single report by Rouland *et. al.* (1988d). These investigators purified two xylanases : one from workers of *M. mulleri* (X), and one from nodules of its symbiotic *Termitomyces* (X_F). Both were glycoproteins and proved to be identical with respect to physical and catalytic properties, including molecular weight (28.5KDa), pH optimum (5.2), and substrate specificities. Hence, they were judged to be the same enzyme, whose origin was the fungus and which was acquired by the termites through ingestion of nodules. Given the quantitative significance of xylan in lignocellulosis plant material, and its high digestibility by termites, the xylanases constitute an enzyme system sorely in need of further study.

Lignin Degradation

Studies on lignin degradation in termites are scarce. Early estimates according to Breznak (1982), based on chemical analysis of lignin in food versus feces, range from virtually no degradation to astonishingly high values (83%).

These discrepancies are not surprising, given the problems and potential pitfalls of accurate determination of the lignin content of sound wood, Lai and Sarkanen (1971), let alone of fecal materials. Approaches using radiolabeled lignin preparations are better suited to address the question of lignin digestibility in termites. The release of $^{14}CO_2$ as a final respiration product is a sure indication of complete mineralization of those subunits canying the lable.

Entomopathogenic fungus

Hallsworth and Magan (1999) evaluated the effects of temperature (5-50°C), water availability (0.998-0.88 water activity, aw), and aw X temperature interactions (15-45°C) on growth of three entomogenous fungi, *Beauveria bassiana, Metarhizium anisopliae,* and *Paecilomyces farinosus*, on a sabouraud dextrose-based medium modified with the ionic solute KCl, the non-ionic solute glycerol, and an inert solute, polyethylene glycol (PEG) 600. The temperature ranges for growth of *B. bassiana*, *M. anisoplae* and *P. farinosus* were 5-30, 5-40, and 5-30°C and optimum growth temperatures were 25, 40 and 20°C, respectively. All three species grew over a similar aw range (0.90-0.998) at optimum temperatures for growth. However, there were significant interspecies variations in growth rates on media modified with each of the three aw-modifying solutes. Growth aw optima ranged between 0.99 and 0.97 on KCl^-glycerol, and PEG 600-modified media for *M.anisopliae* and *P. farinosus*. *B. bassiana* grew optimally at 0.998 aw, regardless of aw. Comprehensive two-dimensional profiles of aw x temperature relations for growth of these three species were constructed for the first time. There results were discussed in relation to the environmental limits that determine efficacy of entomogenous fungi as biocontrol agents in nature.

De-Croos and Bidochka (1999) assessed the effects of temperature (8°C, 15°C and 22°C) on germination, growth rate and conidia production in thirty-two isolates of the entomopathogenic fungus *Metarhizium anisopliae*. Most isolates were obtained from various locations in Ontario, Canada. Ten out of thirty-two (31-3%) isolates were deemed cold-active because of their ability to grow at 8°C. Growth rates in all isolates increased as the incubation temperature was increased. There were significant differences in growth rates and conidia production among isolates. They observed that conidia production among isolates. They observed that conidia production had no relationship with a growth rate. With respect to the geographical origin, there was no general relationship between latitude and growth rates. However, they found that all the cold-active isolates were isolated from the more northern sites and no isolate originating below 43.5 degree latitude showed cold activity. Cold-active germination and growth of this biocontrol fungus have implications for strain selection and application in Canadian insect control efforts.

Jeffs *et. al.* (1999) studied the surface properties of aerial conidia from 24 strains of entomopathogenic fungi and compared using the salt-mediated aggregation and sedimentation (SAS) assay, electron microscopy, FITC-labelled lectins, and spore dimensions. Spores with rugose surfaces were hydrophobic, where as hydrophilic spores had smooth surfaces. Correlation analysis found no link between spore dimensions and either hydrophobicity or surface carbohydrates. However, they observed a strong positive correlation between spore hydrophobicity and surface carbohydrates .The three spore types of *Beauveria bassiana* were all shown to possess discrete surface hydrophobicities which were also strongly linked to surface carbohydrate profiles. Various chemical treatments had pronounced effects on spore surface properties, with sodium dodecyl sulfate (SDS) and formic acid reducing both lectin binding and surface hydrophobicity. When formic acid-protein extracts were separated and analysed using SDS-PAGE, only the hydrophobic spores had low molecular weight hydrophobin-like peptides that were unglycosylated and contained disulfide bonds. The strains with hydrophilic aerial conidia had much lower levels formic acid extractable protein per spore dry weight compared to their more hydrophobic counterparts.

The differentiation of a Brazilian isolate of *Metarhizium flavoviride*, a promising candidate for the biocontrol of grasshoppers, was investigated by Xavier *et. al.* (1999). Conidia were spread onto solid medium (1% yeast extract, 2.8% agar, 96.2% distilled water), incubated at 28°C and observed during 26h.

Germination initiated as conidia size increased from 5.3 (± 0.6) x 3.1(± 0.3) mum (Oh incubation). Germ tubes started to appear after 10h incubation showing a high degree of multipolarity. Twenty six hours after inoculation, hyphal differentiation and anastomosis among hyphae from adjacent conidia were recorded. Appressoria were formed only from conidia incubated in liquid medium containing minimum concentration of yeast extract (0.06% w/v). Appressoria were firmly adhered to the bottom plastic dishes.

Strain of hydrocarbon-degrading microorganisms (bacteria and fungi) were isolated from an agricultural soil in France by Chaineau *et. al.* (1999). In a field, a portion was treated with Oily cuttings resulting from the drilling of an onshore well. The cuttings which were spread at the rate of 600g HC m-2 contained 10% of fuel oil hydrocarbon (HC). Another part the field was left untreated. Three months after HC spreading, HC adapted bacteria and fungi were isolated at different soil depths in the two plots and identified. The biodegradation potential of the isolated strains was monitored by measuring the degradation rate of total HC, saturated hydrocarbons, aromatic hydrocarbons and resins of the fuel. Bacteria of the genera *Pseudomonas*, *Brevundimonas*, *Sphingomonas, Acinetobacter, Rhodococcus, Arthrobacter, Corynebacterium* and fungi belonging to *Aspergillus, Penicillium, Beauveria, Acremonium, Cladosporium, Fusarium* and *Trichoderma* were indentified. The most active strains in the assimilation of saturates and aromatics were *Arthrobacter* sp., *Sphingomonas spiritvorum, Acinetobacter baumanii, Beauveria alba,* and *Penicillum simplicissimum.* The biodegradation potential of the hydrocarbon utilizing microorganisms isolated from polluted and unpolluted soils were similar according to them. They indicated that, in laboratory pure cultures, saturated HC were more degraded than aromatic HC, where as resins were resistant to microbial attack. They concluded that on an average, individual bacterial strains were more active than fungi in HC biodegradation.

Smits *et. al.* (1999) say that *Metarhizium flavoviride*, a fungal pathogen of grasshoppers and locusts, appears to be an effective, nop-chemical insecticide (mycoinsecticide) for control of grass hoppers and locusts. They conducted a study during June and July, 1997. and examined the pathogenic potential of this entomopathogenic fungus to non-target avian species that encounter infected insect prey items or contaminated food sources. Ring-necked pheasant (*Phasianus colchicus*) chicks were exposed to one of three diets, (Spore-coated feed, infected insects, or untreated feed), either from 4 to 9 days of age, or, from 35 to 40 days of age. Necropsies were conducted on birds 10 days and 46 days old, respectively. They observed that neither consumption of infected insects, nor of spore-coated feed, resulted in pathological changes, or significant changes in weight growth rate, behaviour, or mortality rate. Histological examination of organs indicated either no changes related to treatment, or normal tissue responses to antigenic challenge.

Kmitowar *et. al.* (2000) performed laboratory experiments and assessed on effect of plant extracts from stingin nettle (*Urtica dioica* L.) wormwood (*Artemisia absinthium* L.) and field horsetail (*Equisetum arvense* L.) on the colony growth, sporification, germination and pathogenicity level of the four species of entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill., *Paecilomyces farinosus* (Dicks) Brown et. Smith, *P. fumosoroseus* (Wize) Brown et. Smith and *Metarhizium anisopliae* (Metsch.) Sorok. The pathogenicity was tested on *Galleria mellonella* (L.) larvae. A reaction to extract added to the medium was species - specific. The smallest changes of studied parameters under the influence of the extracts were observed in *P. fumosorseus*, and the largest in *M. anisopliae*. Studied extracts, those from nettle and wormwood in particular, influenced colony growth and germination of the spores while a horsetail extract influenced spore production from the unit area.

Wagner and Lewis (2000) used light and electron microscopy to describe the mode of penetration by the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin into corn, *Zea mays* L. After inoculation with a foliar spray of conidia, germinating hyphae grew randomly across the leaf surface. Often a germtube formed from a conidium and elongated only a short distance before terminating its growth. Not all developing hyphae on the leaf surface penetrated the cuticle. However, when penetration did occur the penetration sites were ramdomly located, indicating that *B. bassiana* doesnot require specific topographic signals at an appropriate entry site as do some phytopathogenic fungi. Long hyphal structures were observed to follow the leaf apoplast in any direction from the point of penetration. A few hyphae were observed within xylem elements. This is because bundles are interconnected throughout the corn plant, this may explain how *B. bassiana* travels within the plant and ultimately provides overall insecticidal protection. Virulency bioassays demonstrate that *B. bassiana* does not lose virulence toward the European cornborer, *Ostrinia nubilalis* (Hubner), once it colonizes corn. This endophytic relationship between an entomopathogenic fungus and a plant suggests possibilities for biological control, including the use of indigenous fungal inocula as insecticides.

A novel Chitinase was detected by Kang *et. al.* (1999) in extracellular culture fluids of the entomopathogenic fungus *Metarhizium anisopliae* (ATCC 20500) grown in liquid medium containing Chitin as a sole carbon source. They purified Chitinase to near homogeneity from culture broth of *M. anisopliae* by DEAE-sephadex, CM-Sepharose CL-6B ion-exchange chromatography, and gel filtration with Superose 12 HR. The molecular mass of the enzyme determined by SDS-polyacrylamide gel electrophoresis was approximately 60 KDa and the optimum pH of the enzyme was 5.0. This molecular mass is different from values of 33, 43.5, and 45 KDa for endochitinases and 110 KDa for an exochitinase (N-acetylglucosaminidase) from *M. anisopliae* ME-1 published previously. In addition they found that N-terminal sequences of 60-DKa Chitinase are different from those of 43.4 and 45-KDa endochitinases. The purified enzyme showed high chitinolytic activity against colloidal, crystalline chitin of crab shells as well as against p-nitrophenyl-beta-D-N-acetylglucosamide, pnitrophenyl-beta-D-N, N'-diacetylchitobiose, and p-nitrophenyl-N, N'-N''-triacetylchitotriose, indicating that tins enzyme has both endo-and exochitinase activity.

The biohydroxylation of a series of amides and related amino, Keto and hydrocarbon substrates by the fungal biocatalyst Beauveria bassiana ATCC 7159 has been examined by Holland *et. al.* (1999). The product distributions, together with data obtained from selective inhibition experiments using Cyt. P-450 inhibitors isosafrole, l-amino-benzotriazole and phenylacetylene, suggest that *B.bassiana* contains a range of hydroxylase enzymes with different substrate specificities. They presented a paradigm for the interpretation of the results of microbial hydroxylation and for the application of existing active site models for *B.bassiana*.

Beauveria sp. BTMF S10 isolated from marine sediment produced extracellular L-glutaminase as observed by Keerthi *et. al.* (1999). Maximal L-glutaminase yield (46.8 U/ml) was obtained in a medium supplemented with 1% (w/v) yeast extract and sorbitol, 9% (w/v) sodium chloride and 0.2% (w/v) methionine, initial pH 9.0 and at 27 degree C after 108h. This enzyme was inducible and growth-associated as stated by Keerthi *et. al.* (1999).

St. Leger *et. al.* (1999) stated that ambient pH regulates the expression of virulence of *Metarhizium anisopliae*, but it was unknown if *M. anisophae* can regulate ambient pH. Mutants of *M anisopliae* altered in production of oxalic acid were evaluated for the interrelationship of ambient pH, buffering capacity added to media, growth, and generation of extracellular proteases and ammonia. Wild-type and acid-overproducing mutants (Acid(+)) grew almost as well at pH 8 as at pH6, but acid, non producing (Acid (-)) mutants showed limited growth at pH 8, indicating that acid production is linked to the ability to grow at higher pH. Production of ammonia by *M.anisopliae* was strongly stimulated by low levels of aminoacids in the medium when cells were derepressed for nitrogen and carbon. Likewise, although *Aspergilus fumigatus* and *Neurospora crassa* produced some ammonia in minimal media, addition of low levels of amino acids enhanced production. Ammonia production by *A .fumigatus*, *N. crassa* and *M.anisopliae* increased the pH of the medium and allowed production of subtilisin proteases, whose activities are observed only at basic pH. In contrast, protease production by the Acid (+) mutants of *M.anisopliae* was greatly reduced because of the acidification of the medium. This suggests that alkalinization by ammonia production is adaptive by facilitating the utilization of proteinaceous nutrients. Collectively, the data imply that ammonia may have (functions related to regulation of the microenvironment and that it represents a previously unconsidered virulence factor in diverse fungi with the potential to harm tissues and disturb the hosts immune system.

Epoxide hydrolase activity was produced during the exponential and stationary growth phases of the fungus *Beauveri bassiana* ATCC 7159 as observed byMoussou *et. al.* (2000). They said that it was completely cell-associated. After cell disruption epoxide hydrolase activity was recovered in both the cell debris (EH "A") and the soluble fraction (EH "B"), but not in the membrane fraction. Activity assays of these fractions with two different substrates indicated that this substrate specificity, as well as the corresponding E value and, to a lesser extent,their regioselectivity, were different. Also, they could observe that the absolute configuration of the residual epoxide was opposite. This indicates that these two epoxide hydrolase activities are substantially different and are, therefore interestingly complementary biocatalysts for the preparation of the corresponding epoxides and or vicinal diols in nearly enantiopure form.

Mass production, Formulation and Application

Aerial conidia of *Metarhizium anisopliae* (flavoviride) var. acridum strain IMI 330189 were used by Cherry *et. al.* (1999) for the inundative biological control of grasshoppers and locusts in sub-Saharan Africa. Those aerial conidia were produced in a purpose-built facility at the International Institute for Tropical Agriculture in Benin using a standard, two-stage mass production system. The yields average 31. lg of dry conidia powder /kg of rice substrate, the production capacity was 300-350 kg of conidial year and the production costs were estimated at US dollar sign 21/100g (their recommended dose for l ha). The production process parameters varied within narrow limits established during optimization, but the yield was characterized by a high level of variation over time. The incubation period and temperature were identified as key factors, although they accounted for less than 40% of the yield variation. The variation in conidial viability and contamination were correlated with several parameters, but none could adequately explain that variation. They said that the handling time, a principal limiting factor, could be reduced by increasing the substrate quantity unit of production. They concluded that an awareness of these factors presented the opportunity to fine tune production, although the options for increasing or improving production efficiency were limited within the constraints of the system.

Four fungicides were used for controlling foliar diseases of potato (*Solanum tuberosum*) and they were evaluated under field and laboratory conditions for their effects on the colorado potato beetle, *Leptinotarsa decemlineata* (CPB) by Jaros *et. al.* (1999). They investigated the direct effect of time between fungicide and *B. bassiana* application. Effects of fungicide on conidial survival in soil and on foliage were examined by them in the field. Jaros *et. al.* (1999) observed significantly more larval mortality when larvae were sprayed with *B. bassiana* than with water control. Fungicide had no significant effect on larval mortality in the field. In the laboratory, survival of larvae was significantly lower among larvae fed fungicide-treated foliage. They observed that *B. bassiana*-induced mortality in the laboratory was only when larvae were fed foliage treated with copper hydroxide or water. Larvae fed mancozeb or chlorothalonil-treated foliage experienced high mortality regardless of *B. bassiana* treatment. They said that, while there was no significant effect of fungicide on *B.bassiana* sporulation on cadavers in the field, a pattern emerged that indicated higher proportions of cadavers producing conidia in plots sprayed with water or copper hydroxide than in plots sprayed with chlorothalonil or mancozeb. They concluded that survival of *B. bassiana* conidia in the soil and on foliage was significantly greater in plots treated with copper hydroxide or water than in plots treated with macozeb or chlorothalonil. According to them fungicides such as copper hydroxide may be less deleterious to the fungus than mancozeb and chlorothalonil.

The entomopathogenic fungus *Metarhizium anisopliae* was formulated into a bait for assessment against *Musca domestica* (housefly) by Renn *et. al.* (1999). They tested the efficacy of the formulated bait by placing either one, two or four baits on the floor of a 10m^3 polythene cubicle and releasing 100 female and 50 male *M.domestica.* They observed that one, two or four baits had equal effects and, between 95.2 and I00% of flies were killed after 10 days. In a second experiment, the durations of visits by single or pairs of flies to the baits and subsequent pick-up of conidia were recorded by them. Single flies remained in the baits for tip to 33min and picked up a mean of 38235 + 8291 conidia. When pairs of flies were exposed, the individuals picked up a mean of 95879 + 23838 conidia, which was significantly more ($P<0.05$) than the single flies, although the mean duration of the visits was not significantly different ($P>0.05$). However they observed that the numbers ofconidia picked up by the flies didnot correlate with the duration of the visit. Lastly they exposed 25 female and 25male flies in $0.027m^3$ arenas, to I0 *M.anisopliae* - killed house flies, showing sporulating hyphae. After 8 days, they noticed that 97.3% of female flies and 100% of male houseflies had succumbed to infection with *M.anisopliae*.

Lopez *et. al.* (1999) used six plant waste substrates Palm leave (*Phoenix dactyhfera, Phoenix canariensis, Washingtonia filifera* and *Chamaerops humilis*), *Phoenix dactylifera,* seed and almond mesocarp, to produce entomopathogenic (*Verticillium lecanii, Paecilomycesfarinosus, Metarhizium anisopliae,Beauveria bassiana)* and mycoparasitic *(Trichoderma harzianum* and *Gliocladium virens*) fungi.

Uninoculated plant waste substrates had very little microbial colonization. In inoculated substrates, germination conidia of *T. harzianum* were found on *P. canariensis* leaves. Widenings of hyphal apex, resembling appressoria, were found for *T. harzinum* and *B. bassiana* on palm leaves. Hyphae of *G. virens* produced adhesives on the surface of palm leaves. The development of antagonistic fungi on plant waste substrates depended on the combination fungus-substrate tested. Of the entomopathogens *P. farinosus* did not grow on almond mesocam. This fungus grew well on both *P. dactylifera* seed and *P canariensis* leave *P. farinosus* grew extensively on the edges of the leaf fragments and then started colonizing the leaf surface. *V lecanii* colonized and sporulated on almond mesocarp. Of all the plant waste substrates tested *M. anisopliae* only did grow on almond mesocarp. *C. humilis* leaves were excellent substrates for the growth and sporulation of both *V. lecanii* and *B. bassiana. B. bassiana* grew best on *P. daetilyfera* seed. The mycoparasitic fungi were, in general terms, faster and better colonizers of plant waste substrates than the entomopathogens. *T. harzianum* and *G. virens* sporulated well on *P. dactilyfera* seed *C. humilis* leaves also supported abundant growth of *T. harzianum*. *P canariensis* leaves were also readily colonized by the fungus. So their results show that plant waste has potential as substrate for production and perhaps formulation of important biocontrol fungi.

Sharma *et. al.* (1999) selected molasses yeast broth as synthetic medium for mass production for all the three pathogens which produced 8 x10^7, I x 10^9 and 2 x 10^9 conidia ml^{-1} in the slurry of *M. anisopliae*. *B. bassiana* and *B. brongniartii,* respectively. Amongst grain media, crushed maize grains for *M. anisopliae* and whole cowpea grain for *Beauveria* spp. were employed for mass multiplication using 2kg high density polypropylene bags each containing hundred grams of grains moistened with 60ml of distilled water. *M. anisopliae, B. bassiana* and *B. brongniartii* and their respective temperature yielded a grain spore dust of 2 x 10^9, 1.5 x 10^9 and 1.8 x 10^9 conidia g^{-1} dry grain weight. Fungal slurry and dried grain spore mass, after blending in electric mixture for 30 sec was incorporated in sterilized talc powder (carrier) in the proportion of 1:2 to 1:5 depending on density of spore in order to achieve 4 – 5 x 10^8 conidia g^{-1} in formulation.

They observed that *Maladerainsanabilis* was more susceptible as compared to *H. consanguimea* as it took less time to cause mortality, when exposed to the different doses of the tested pathogens by soil inoculation method. *M. anisopliae* and *B. brongniartii* showed high virulence against both the target insects with LT_{50} of 7.95-16.20 and 9.93-13.98 days, respectively with third instar larvae exposed to their highest doses of inoculum. *B. bassiana* was found to be weak pathogen against both insects.

According to *Gillespie et. al.* (2000) topical application of *Metarhizium anisopliae* var. acridum to the desert locust *Schistocerca gregaria* in changes in the biochemistry and antimicrobial defenses of the haemolymph. *M. anisopliae* var acridum colonized the host haemolymph from day two post application. The haemocytes did not attach to, phagocytose or nodulate elements of the fungus. However, the presence of the fungus appeared to stimulate haemocyte aggregation over the first few days of mycosis though the number of aggregates declined subsequently. The total hemocyte count increased two days after application, indicating an overall stimulation of the immune system, but declined to a value below that for uninoculated controls by day four. The differential haemocyte count showed that the initial increase in total haemocyte count was primarily due to a larger number of coagulocytes. After day two consistent declines in cell number were observed for all haemocyte classes in mycosed insects. The activity of the enzyme, phenoloxidase, decreased during the course of infection. However, the converse was true for prophenoloxidase. Lysozyme levels were significantly smaller in infected than control locusts. They observed a significant correlation between lysozyme and PO activities when data from mycosed and control insects were combined. The total protein content of the haemolymph decreased during the course of infection.

Hong *et. al.* (2000) harvested conidia of the entomopathogenic *Metarhizium flavoviride,* 8,12, or 15 d after inoculation at 25°C and then (as conidiated rice) dried rapidly (10-12% r.h. and 17-20 degree for 17h to about 15-22% moisture content) or slowly (50-60 % rh. and 27 degree for 5 d to about 27-32% moisture content initially). The subsequent survival of these conidia in air-dry storage at 50 degree with 8.1% moisture content was then assessed. Conidia longevity (assessed by the duration of storage until conidia viability was reduced to 50%, P^{50}) was maximal when conidia were harvested 10d after inoculation, and was much greater following slow rather then rapid drying. The substantial beneficial effect of slow desiccation to subsequent conidia survival is consistent with that detected in other propagules in anhydrous biology, and is also of considerable practical utility for the biological control of insects by entomopathogenic fungi.

The efficacy of mycoinsecticide formulated vegetable oil was tested in Brazil against the grasshopper *Rhammatocerus schistocercoides* by Magalhaes *et. al.* (2000) A set of experiments was conducted in the Chapada dos Parecis region (Mato Grosso state), a permanent zone of out breaks for this pest. Experiments were performed in zones of natural vegetation, against grasshopper bands in the third nymphal instar. Three nymphal bands were treated with a mycoinsecticide formulation based on conidia of the entomopathogenic fungus *Metarhizium anisopliae* Var. acridum (=*M. flavoviride*), strain CG 423. Three non-treated bands were used as control. The application was made with the aid of a hand held ULV sprayer adjusted to deliver 2l of the formulation ha^{-1}, containing 1 x 10^{13} conidia. Treatments were limited to the surface of the grasshopper bands and this immediate borders(5-10m), The efficacy of the mycoinsecticide was evaluated through band survival after treatment (grasshopper numbers, surface, density, behaviour and daily movement of the band), allowing the insects to move freely in their natural environment. They regularly surveyed and maintained the insects in the laboratory, allowing estimates of the infection rate. Field and laboratory studies showed a clear effect of the product 10 days after treatment. At 14 days post-spraying, mortality caused by the mycoinsecticide in the field was approximately 88%.

Inyang *et. al.* (2000) investigated the effect of simulated rain on the persistence of oil and water formulations of conidia of the entomogenous fungus *Metarhizium anisopliae* when applied to oilseed rape foliage, using third instar larvae of the mustard beetle (*Phaedon cochleariae*) as the target host. Rain significantly ($p < 0.01$) reduced the susceptibility of the beetle larvae to *M. anisopliae* but the amount of inoculum removed was influenced by the formulation. Larvae exposed to plants treated with conidia formulated in aqueous Tween, shellsol T, or sunflower oil/Shell sol T resulted in 55,82.5 and 72.5%, mortality, respectively. The mortality for these respective formulations was reduced by 42,57 and 51% if the plants were exposed for I h to simulated rain. Laboratory and field studies showed that more inoculum collected beneath plants sprayed with conidia formulated in shellsol T or aqueous Tween than in the more viscous sunflower/ shellsol T mixture. Mortality studies on leaves taken from field plots suggested that conidia on leaf surfaces could be replenished by repeated application. The number of conidia isolated from field plots was greater where inoculum was applied bi-weekly than once weekly.

Vanninen *et. al.* (2000) studied in 1988-1991 the persistence and penetration into soil of surface-applied informulated conidia of two isolates of *Metarhizium anisopliae* and one of *Beauveria bassiana* at sites with clay, peat and two kinds of sand as their soil types and at depths of from 0 to 20 cm under conditions characterised by permanent snow cover and frozen soil in the winter time. At 0-5 cm depth, *M. anisopliae* persisted throughout the experiment at all sites clay being most and peat the least favourable soil for persistence. Clay and one of the sandy soil were the least and peat the most conducive soil to penetration of *M. anisopliae* from the surface to deeper soil layers and persistence therein. Differences in persistence were evident between the two *M anisopliae* is lates in the sandy and peat sites, but not in clay site. Three years post- application there were still enough infections propagules of *M anisopliae* in soil of all sites to infect over 80% of the *Tenebrio molitor* larvae used as baits in samples taken the cylinders of all soils. All the augmented propagules of *B. bassianc* disappeared during the first winter after application in clay and one of the two sandy sites, but some persistence one year post-inoculation was evident at 0-5 depth in one of the sand soils and at 0-5 and 0.-JO cm. depths in peat.

Booth *et. al.* (2000) demonstrated that a dried mycelium formulation of an indigenous strain of *Metarhizium anisopliae* has good potential as an effective biopesticide against the black vine weevil (BVW), *Otiorhynchus sulcatus* (F.), and the cranberry girdler (CG), *Chrysoteuchia topiaria* (Zeller). The formulation was produced at moderate sized batches scales (approx. 100g product per batch) using inexpensive and easily obtainable equipment. It was observed that the levels of conidiation were consistent among production batches and storage intervals with the product remaining viable for long time periods at 4°C in one case for more than a year. Levels of contamination by yeasts and other benign fungi were also consistent. Fewer BVW and CG were found in small plots treated with the dried mycelium formulation than in untreated plots, but differences were not always significant. A waxmoth *(Galleria mellonella)* bait viable in the soil over long time periods (>7 months). Percentage of *M. anisopliae* infected waxmoth larvae was signficantly affected by rate.

According to Nankinga and Moore (2000) one of the major constraints for banana production in Uganda is the banana weevil, *Cosmopolites sordidus* (Germar), (Coleoptera : Curculionidae). Investigations were carried out to evaluate the efficacy of maize, soil-based and oil formulations of an indigenous isolate of *Beauveria bassiana* for the control of the banana weevil. Weekly trapping of weevils over a 9 month monitoring period showed significant reduction in unmarked and marked weevil population in *B. bassiana treated plots.* Application of maize formulation at 2×10^{15} conidia ha^{-1} proved most effective, reducing the weevil populations by 63-72% withing 8 weeks after a single application. They observed that the soil based formulation at 2×10^{14} conidia ha^{-1} was intermediate while the oil formulation at 6×10^{15} conidia ha^{-1} was least effective. Trapping efficiency declined in *B. bassiana* treated and untreated banana plots but was greatest in the latter.

Reduction in grasshopper population density between treated and untreated plots was significantly different, but did not convince farmers, as full grown L5 larvae had emerged as adults and began migrating. However, cadavers collected from cags containing insects from treated plots allowed farmers to follow how the fungus sporulates and contaminates healthy grasshoppers. They observed that farmer were interested in the product but were reluctant to buy a slow-acting insecticide.

Gurusubramanian et. al. (1999) evaluated the pathogenic effects of *Beauveria bassiana* (Bals) *Vuil. (Deuteromycotina : Hyphomycetes)* against workers of the termite *Odontotermes obesus (Rambur)* (Isoptera : Termitidae). Termite mortality ranged from 41-91% in treatments, compared with 19-21% in controls. Both lethality and lethal time varied significantly with conidial concentration and age of culture. Mycelial growth was recorded on the leg joint, abdomen, antenna, thorax and head regions of treated cadavers after 24h. Also observed in treatments were body surface shrinkage, colour change, and hardened and brittle appendages.

Laboratory evaluation of the pathogenicity of three isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin on the American cockroach (*Periplaneta americana*) were performed by Mohan *et. al.* (1999)

where they treated the insects in three different ways, i.e. by direct contact with spore mass, a spore-wheat flour mixture and a spray of an aqueous spore suspension. They observed a mortality of 100% in the first treatment, 57-100% in the second treatment and 17- 7500 in the third treatment. These results suggest that *B. bassiana* spore formulations in food baits can be developed for cockroaches. They concluded that mycopesticides can be ideal for the biocontrol of cockroaches because the habitat of these insects promotes initial fungal infection and its subsequent spread.

Rica and Cogburn (1999) evaluated a *Beauveria bassiana* isolate from the rice water weevil, *Lissorhoptrus oryzophilus* his Kuschel (Coleoptera: Curculionidae), as a conidial powder against 3 colepteran pests of stored grain: the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae); the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera : Bostrichidae); and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). They assessed the bioactivity of *B. bassiana* isolate 22292A on 3 types of food substrates (red flour beetle media, medium grain brown rice, and long grain rough rice) at 27 degree C and 60% RH. They observed that adult mortality was 80-100% at the higher dosage levels for all insects tested on all media types at 21d after treatment. Emergence of adult progeny on brown and rough rice was reduced by 83-99% at the 2 highest dosage level. They concluded that the abrasive nature of rice hulls on the rough rice may contribute to the accelerated mortality seen on this medium.

Sharma *et. al.* (1999) selected molasses yeast broth as a synthetic medium for mass production for all the three pathogens which produced 8 x 10^8, 1x10^9 and 2x10^9 conidia ml^{-1} in the slurry of *M. anisopliae, B. bassiana* and *B. brongniartii*,, respectively. Amongst grain media, crushed maize grain for *M. anisopliae* and whole cowpea grain for *Beauveria* spp. were employed for mass multiplication using 2kg high density polypropylene bags each containing hundred grams of grains moistened with 60m1 of distilled water. *M. anisopliae, B. bassiana* and *B. brongniartii* at their respective temperature yielded a grain spore dust of 2x10^9, 1.5x10^9 and 1.8 x 10^9 conidia g^{-1} dry grain weight. Fungal slurry and dried grain spore mass, after blending in electric mixer for 30 sec was incorporated in sterilized talc powder (carrier) in the proportion of 1:2 to 1:5 depending on density of spores in order to achieve 4-5x10^8 conidia g^{-1} in formulation. *Maladera insanabilis* was more susceptible as compared to *H. consanguinea* as it took less time to cause mortality, when exposed to the different doses of the tested pathogens by soil inoculation method. *M anisopiiae* and *B. brongniaritti* showed high virulence against both the target insects with LT_{50} of 7.95-16.20 and 9.93-13.98 days, respectively when third instar larvae exposed to their highest does of inoculum. *B. bassiana* found to be weak pathogen against both insects.

Zurek and Keddie (2000) tested a new isolate of the entomopathogenic fungus, A *Beauveria bassiana* by laboratory bioassays for potential use as a microbial control agent of the early (third) and last (seventh) instars of the satin moth, *Leucoma salicis* L. Results demonstrate that this fungus is very effective against last instars of this lepidopteran pest. Although *B. bassiana* was cultured successfully from internal contents of all cadavers examined, vegetative hyphae emerged from intact cadavers of *L. salicis* infected as early (third) instar caterpillars only.

The susceptibility of *Megalurothrips sjostedti* to *Metarhizium anisopliae* when reared on susceptible, tolerant, and moderately resistant varieties of cowpea at different constant temperatures was evaluated in the laboratory by Ekesi *et. al.* (2000). Insects were exposed either to direct spray of the conidia or to fungus-treated floral tissues. Mortality was significantly higher on the moderately resistant variety at all temperatures compared to the susceptible and tolerant varieties. Corresponding, lethal time and lethal concentration values were significantly shorter and lower, respectively on the moderately resistant variety compared to the other varieties, thus indicating that the two control methods are compatible as part of an integrated pest management strategy. Thrips raised on the tolerant variety incurred an exceptionally low level of mortality when the inoculum was sprayed directly on the insects or when the insects were exposed to fungus-treated floral tissues. Observations on the effects of airborne volatiles and crude extracts of this variety revealed in inhibitory effect on fungal germination, colony forming units and growth. This suggests the existence of anti-fungal substances in the tolerant variety.

The pathogenicity of *Vespula vulgaris* wasp workers and larvae to a range of fungi was determined by Harris *et. al.*, (2000). All fungi were isolated in New Zealand and included islates from Vespula, known generalist insect pathogens, and isolates generally nonpathogenic to insects. Workers and larvae were highly susceptible to pathogenic isolates at high spore concentrations (>1.75x10^5 cfu / individual). Eight isolates, two of *Metarhizinum anisophiae,* five of *Veauveria bassiana,* and one of *Aspergillus flavus* were pathogenic while a single isolate of M. flavouiride var. *novazealandicum, Cladosporium* so. and *Paecilomyces* sp. were not. The transfer of spores between workers, and between workers and larvae, was also investigated using several different application methods. Transfer of spores occurred between treated and untreated individuals, and for some of the application methods sufficient spores were transferred to cause mortality of the nontreated individuals. These findings are related to the potential of fungi for the control of wasps.

Beauveria bassiana (Balsamo) Vuillemin is a fungus with broad spectrum insecticidal activity as stated by Martin *et. al.* (2000). As a biological control agent used against Colorado potato bettles (*Leptinotarsa decemlineata*), this fungus has been attributed to formulation problems, UV sensitivity, and humidity. They observed that in a multisite test, *B. bassiana* controlled Colorado potato beetle larvae in both Poland and the Czech Republic, but not in Maryland. Control was measured by reduction in populations of beetle larvae. One of the major differences among these sites was temperature. In Poland, the mean temperature ranged from 5ºC to 23ºC, in the Czech Republilc the average temperature ranged from 6.7ºC to 18.7ºC; and in Maryand, temperatures at time of applicatin exceeded 45ºC at canopy level. This led them to examine *B. bassiana* growth *in vitro*. While *B. bassiana* grew in the laboratory from 16 to 30ºC, the *B. bassiana* from a formulated product (Mycotrol TM, Mycotech, Butte, MT) did not germinate at temperatures above 37ºC. Germination and subsequent development of this entomopathogenic fungi are critical factors in the infection and control of the Colorado potato bettle. As a consequence of the inabiity to germinate at high temperatures, *B. bassiana* would not be expected to effectively control pest insects in climates with hot summers. This fungus, however, may be for insect control in early spring or in cool temperature climates during the growing season.

Edgington *et. al.* (2000) considered *Beauveria bassiana* to the one of the few natural enemies available for use against the coffee berry borer. In an attempt to enhance to efficany of this pathoge, a range of concentrations of 22 substances was tested in simple laboratory tests using natural sunlight or a UV light source. Unprotected *B. bassiana* spores were almost copletely inactivated by exposure to 60mim of direct sunlight or 20s of UV ight of 302nm wavelength. Seven of the 22 substances tested were egg albumen an skimmed milk powder which could extend the persistence of *B.bassiana* spores by a factor of almost three. A mixture of 3% (w/v) albumen and 4% (w/v) milk powder gave the highest degree of spore protection per unit cost. Young coffee plants sprayed with this mixture did not suffer any significant phytotoxic effects. A field trial, involving two applications of spores with or without the milk and albumen mixture, failed to show that improved spore persistence resulted in increased coffee berry control. Very low levels of pest infestation was observed in field plots together with unusual, unfavourable weather condiditons may have accounted for this unexpected result.

The laboratory studies of Rath (2000) have shown that termit species are highly susceptible to entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana.* According to his obseervations there appears to be very little host specificity among fungal isolates with many isolates being highly virulent to many species of termites. The grooming and other social intractions between termites are seen to have the potentional to spread the fungus through the colony, allowing for colony control by the treatment of remote feeding sites. However, factors such as avoidance of the fungus conidia by the termites, the removal and burial of fungus-killed termites, together with defensive secretions and inhibitory components in termites, together with defensive secretions and inhibitory components in termite frass and the possibility of humoral resistance may limit the spread of the disease in the colony. Field studies have shown mixed resuts. Direct application of fungus to nests has resulted in complete colony mortality, but studies where feeding is ites or bait stations have been treated with fungus have yet to show similar success. The effectiveness of termite *control in urban pest management, particularly in structural timber and dwelings,* has yet to be reported in details, as indicated by Rath (2000). Such studies require the examination of the complex relationships between does, speed of kill, virulence. horizontal transmission and ultimate colony death, combined with aboidance and recognition factors, and survival of the fungi under field conditions.

The susceptibility of immature stages of the legume flower thrips, *Megalurothrips sjostediti,* to the entomopathogenic fungus *Metarhizium anisopliae,* was investigate under laboratory conditions by Ekesi *et. al.* (2000). The adult stage was found to be more susceptible to infection than the larval and pupal stages. Mortality at all stages was does-dependent, with the highest concentration of $1x10^8$ conidia ml^{-1} producing the highest mortality (256, 46 an 100% for larvae,l plupae and aduts, respectively) at 8 days post- inoculation. At the same concentration, daily pollen consumption was significantly reduced 2 days after treatment in infected adults but more slowly in infected larvae. Fecundity, egg fertility and longevity in adults surviving infection as larvae were significantly reduced compared to the control.

Noma and Strickler (1999) evaluated the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, in alfalfa seed plots for control of lygus bug (primarily *Lygus hesperus* knight) in comparison with conventional chemical insecticides and a water control. *B. bassiana* applications reduced lygus bug mymphs relative to the water control during one of three prebloom (June) trials but not during any bloom (July) trials. A single applications of conventional insecticides reduced ygus bug populations more frequently and to a greater extent compared to three applications of *B. bassiana*. The noticed that infection rates were relatively high in treated plotsdespite lower spray coverage than expected. Reduced *B. bassiana* infection during the bloom trial compared to the prebloom trial has been attributed to reduced spray penetration within alfalfa as the canopy closed. Faster nymphal growth rates relative to growth of *B. bassiana* hyphae at field temperatures could also explain seasonal differences in the effectiveness of *B. bassiana*.

The impact of relative humidity (RH) on the infective potential of the isolate BbINRA 297 of *Beauveria bassiana* (Bals) Vuillemin (Deuteromycotina Hyphomycetes) against first instar nymphs of *Rhodnius prolixus* Stal.l (Hemiptera Reldluviidae) was determined by Luz and Fargues (1999). Fungus-treated insects were exposed to RHs ranged from 75 to 100% at 25ºC. Results clearly showed a threshold of humidity at ca. 96% for high and rapid mortality. After initial exposure to increasing periods of 97% (4, 8, 16, 24, 36 and 48h) and subsequent transfer to constant lower RHs (43, 53, 75, and 86%) at a constant 25ºC, an incubation of atleast 48h at 97% RH was necessary to kill all insects. On changing RHs of 97/75% and different regimes of temperature (15/28ºC, 20/25ºC and 25/35ºC), at least 72h of initial exposure to favourable moisture condition (97% RH), significantly affected infection for up to a 3-day delay within the various temperature humidity regimes tested.

The efficacy of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuilemin was tested as a control agent by Mulock and Chandler (2000) for adult western corn rootworn, *Diabrotica virgifera* virgifera LeConte, in walk in fied cages. Suspensions of *B. bassiana* conidia were applied to corn plants in cages into which laboratory-reared beetles had been released. Beetles were collected at 3 and 5 days post- application and evaluated in the laboratory for mortality. Mortality was 101.29 and 50%, at rate equivalents of $7x10^{12}$, $2x10^{13}$ conidia / ha, respectively. They observed no significant difference in mortality of beetles collected at 3 days compared with 5 days post-application. Mortality due to *B. bassiana* was 24% when beetles were released into field cages 24h post-application ($5x10^{13}$ conidia/ha) compared with 50% when beetles were present during the application. Beetle mortality declined significantly with increasing time from application. Beetle mortality declined significantly with increasing time from application in feeding assays carried out with leaf samples removed from plants at 0, 12, 24 and 72h post application. Mortality of beetles collected from treated plants within cages and maintained is the laboratory was bound to overestimate the population decline by approx. 10% when compared with beetle estimates from treated plants within field cages.

Wraight *et. al.* (2000) assessed the microbial control potential of *Beauveria bassiana* against Bemisia white flies. Laboratory assays demonstrated the capacity of the pathogen to infect *Bemisia argentifolii* nymphs on excised hibiscus leaves incubated at relative humidities as low as 25% at 23+2 degree C (ca. 35% infection by *B. bassiana* resulted from applications of 0.6–1.4x10^3 conidia / mm^2 of leaf surface). In small-scale field trials using portable air-assist sprayers, applications at a high rate of 5x10^{13} conidia in 180 liters water / ha produced conidial densities of ca. 1–2.5x10^3 conidia / mm^2 on the lower surfaces of cucurbit leaves. Multiple applications of four isolates of *B. bassiana* made at this rate at 4 to 5 day intervals provided >90% control of large (third and fourth instar) nymphs on cucumbers and cantaloupe melons. The same rate applied at 7 days intervals also provide > 90% control in zucchini squash, and a one-fourth rate (1.25x10^{13} conidia/ha) applied at 4 to 5 day intervals reduced numbers of large nymphs by >85% in cantaloupe melons. In contrast to the high efficacy of the fungal applications against nymphs, effects against adult whiteflies were minimal. The results indicated that *B. bassiana* have strong potential for microbial control of nymphal whiteflies infecting cucurbit crops.

In insects, fungal disease are common and widespread and often decimate insect populations in spectacular epizootics. Entomogenous fungi infect insects by breaching the host cuticle; they are the principal pathogens among sucking insects because these host cannot ingest other pathogens that infect through the gut wall.

The use of newly available biochemical and molecular markers has facilitated other studies on species structure and geographical spread. For example, in recent allozyme studies of St. Leger *et. al.* (1992a), the level of genetic distance observed between cluster groups of *Beauveria bassiana* and Metarhizium anisopliae indicated that each represents a species aggregate, components of which isplay overlapping genetic variability, with some isolates currently assigned to other rarer species in the same genus. Except for isolates of *Metarhizium anisopliae* var. *majus*, most *Metarhizium* isolates are homozygous at each locus in a manner consistent with hapoidy. In spite of the maintenance of high diversity in *B. bassiana* and *M. anisopliae,* the majority of isolates are contained in a few geographically widespread genotypic classes.

According to Kulye *et. al.* (2002), fungal agents act on insect pest by contact and the cuticle degrading enzymes (CDEs), mainly chitinase, protease, and lipase contribute significantly in the process of entomo-pathogenesis. The control of *Helicoverpa armigera* infested on chickpea crop was studied using insect pathogens such as *Metarhizium, Beauveria,* and *Nomuraea* as well as with CDE complex produced by another fungus *Myrothecium verrucaria.* The percent efficacy of *Metarhizium anisopliae* and the chemical control agent, endosulfan was highest (70%). *Beauveria* showed percent efficacy in the range 45-62%.

Niranjana (2002) made and attempt to use an entomopathogenic fungus *Beauveria bassiana* in managing Coffee Berry Borer (CBB). Soil, infested berries, leaf materials, coffee twigs, and gleanings were collected from different coffee plantations and screened for presence of infected and dead CBB. These were plated on PDA and the expressed fungi were isolated and identified. *B. bassiana* isolates were grown on YPD media and conidial suspension (10^6 conidia / ml) was prepared. The borers were dipped in the suspension and maintained at ambient temperature and humidity. Mortality was recorded daily and the dead insects were transferred to sterile petriplates with moist blotters and incubated at 25 ± 2^0C for 5 days. Only those insects, which were colonised by *B. bassiana* and showed sporulation, were considered for calculation of percent mortality. The tested isolates of *B. bassiana* obtained from naturally dead CBB showed pathogenicity towards CBB with varied mortality rates. Isolate Bb2 recorded high mortality Of 93%, while isolates Bb4 and Bb1 stood next to Bb2 with 64.25% and 59.25% borer mortality rates respectively. *B. bassiana* was mass multiplied in Roux bottles and the culture was vacuum dried at – 40°C which was then suspended in sterile distilled water containing 0.02% Tween 20. The conidial concentration was adjusted to $1x10^6$ conidia/ml using a haemocytometer and the same was used as mycopesticide. Large-scale experiments were conducted at Green Hill estate of Virajpet from 1995-2002. Mycopesticide was applied and evaluation was done after 24 days of spraying. Percent insect mortality was calculated. The mean insect mortality after spraying with *B. bassiana* calculated from five different plots was 75.5% against zero mortality rates in unsprayed plots. In *robusta* insect infestation was ranging from 1.46-9.65%. The mean insect mortality after 1.33% and robusta 1.76% insect infestation.