RESEARCH NOTE

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Susceptibility of the eastern tent Caterpillar (*Malacosoma americanum*) to the Entomogenous Fungus *Beauveria bassiana*

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Malacosoma americanum (Fabricius) (Lepidoptera : Lasiocampidae), the Eastern tent caterpillar, is a pest native to the United states. It is particularly injurious to apple, cherry, and other fruit trees, but can be destructive to other deciduous trees. Control of this pest is difficult, largely because of its congregation behavior. Larvae spend the day inside of their protective tents, where they are largely shielded from exposure to insecticides. At night and in rainy weather they emerge to forage, at which time a widely disseminated control would be necessary. Malathion and Bacillus theriengensis preparations have been used to control *M.americanun*, although diurnal cutting and removal of individual tents is often the recommended treatment. Efforts to develop new biocontrol agents against *M. americanum* have focused on parasitic wasps and entomogenous nematodes (Nielsen, 1989). However, because of the insect's congregative lifestyle, If even a few larvae are successfully infected by a contagious agent during a foray, they may return to the tent to die and eventually infect the entire colony.

The entomopathopathogenic fungus *Beauveria bassiana* is widely regarded as one of the most promising species known for potential development into a practical insect biocontrol agent. Unlike bacterial, viral, or protozoanent mopathogens, fungi need not be consumed by their hosts in order to be infective. Instead, germinating fungal spores are able to grow directly through the insect's cuticle. It has been suspected that entomopathogenic fungi are enabled in this novel mode of infection by the production of cuticle-degrading enzymes (Smith *et al.,* 1981). Larvae of *the Eastern tent caterpillar. (M. americanum*) were collected locally and maintained on a diet or fresh leaves from the wild choke cherry (*Prunus anginiana*).

Healthy larvae of *M. americanum* (approximately 0.1 g/larva) were placed in plastic dishes in groups of 10 and suspension containing 6 x 10^7spores in water (*i.e.*, 7.2 x 10^7spores ml). Treated larvae and water controls were then maintained on their diet and scored daily for mortality.Liquid cultures of *B. bassiana* strains were grown on a basal salts medium (0.06% $MgSo_4$, 0.05% NaCl, 1.5% KH_2PO, 0.001% $FeSO_4$, $7H_2O$, 0.001% $ZnSO_4$) containing 0.5% (w/v) insect cuticle. Cuticles from larvae of *M. americanum* were prepared by dissection. Media containing cuticle were subjected to heating at 65^0C for 15 min to inactivate any endogenous enzymes. Cultures were inoculated to a concentration of 2, 10 spores/ml and incubated on a shaker at 200 rpm 26^0C for 96 hr. Supernatants were prepared by centrifugation at 2000g for 10 min and stored at 20^0C for enzyme assays. In preliminary studies, we found no effect of dialyzing samples, so this step was omitted.

Assays of cuticle-degrading enzymes were carried out as previously described (Gupta *et al.,* 1991). One endochitinase unit is defined as the change of one optical density at 540nm per 45hr of incubation at 37^0C. Other enzymes are expressed in international units. Protein was determined by the procedure of Bradford, 1976. Using bovine serum albumin as a standard protein.

Since preliminary experiments indicated that enzyme levels were both too low to be measured in early growth cultures and relatively stable in stationery phase (96 hr). Since the insolubility of cuticle made dry weight measurements impractical, activity values were normalized by total extra cellular protein. Enzyme values are presented as the mean and standard deviation of three or more measurements and are expressed in units per milligram extra cellular protein rounded to three significant figures.

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Five strains of *B.bassiana* that were origirally isolated from widely divergent sources and or geographical sites were chosen. These strains were tested for their ability to infect *M.americanum in vivo* as well as for their production of cuticle-degrading enzymes. Then grown *in vitro* on a medium containing purifiec insect cutiele from *M.americanum* as a sole carbon and nitrogen source (Table 1).

Table 1: Selection of strains of *B. bassiana*

Strain No.	Source
ARSEF 283	"Baverin" preparation
ARSEF 1775	Leptinotarsa decemlineata
NRRL 3108	European corn borer
NRRL 20699	Soil
NRRL 20700	Japanese beetle

As shown in Table 2 all strains of *B.bassiana* were effective against *M. americanum*, resulting in the death of all treated caterpillars within 4 days. Water inoculaced control larvae showed no mortality. In some cases, *M. americanum* caterpillars exhibited signs of distress (inactivity and melanization of cuticle) within 6 hr of exposure to *B. bassiana* spores. The surface of the

Table 2 : Mortality of *Malacosoma americanum* expanded to spore suspensions of *Beauveria bassiana* strains

Strains No.	Day1	Day2	Day3	Day4
Control	0/10	0/10	0/10	0/10
283	1/10	3/10	9/10	10/10
1775	7/10	9/10	10/10	10/10
3108	2/10	2/10	10/10	10/10
20699	4/10	6/10	10/10	10/10
20700	1/10	2/10	10/10	10/10

caterpillars was thickly covered with hairs, which may enhance the adsorption and retention of spores and promote vid infection. Furthermore, the moist nature the nature surface may promote fungal growth. Although all tested strains were highly effective, strains AREF 1775 and NRRL 20699 appeared to be some what faster acting than others (Table 2). As an initial attempt to identify potential virulence factors, we cultured all fungal strains *in vitro* on a defined medium containing cuticle purified from *M. americanum* as a sole carbon and nitrogen source. Cuticle-degrading enzymes were then quantitated in the culture supernatant. All strains produced proteases, esterase, and chitinase activities, consistent with the notion that these enzymes may be necessary for fungal infection. Chymoelastase and chymotrypsin activities were particularly high, ranging from 13,900 to 48,100 IU/mg extracellular protein. Nevertheless, highly virulent strains ARSEF 1775 and NRRL 20699 were not exceptional in the production of cuticle-degrading enzymes. Although these *in vivo* conditions, results do limiting factors in viruelence, at known to produce insecticidal toxins such as Beauverin (Gillespie and Claydon, 1989) and the speed at which the *M. americanum* larvae succumbed may argue for toxins as a primary virulence factor.

A limiting factors in the practical commercialization of biological control agents has been their relatively slow action compared to synthetic chemical pesticides. Poor persistence of fungi in field environments compounds this problem. The high level of susceptibility of *M. americanum* to certain strains of *B. bassiana* suggests that this pest may be a promising target for biocontrol efforts. This is particularly important since the insect is difficult to control by conventional means, due to its congregative lifestyle. We plan to pursue field testing of strain ARSEF 1775 for control of *M. americanum* and to broaden our studies to include additional strains of *B. bassiana*. We will also examine supernatants from cuticle-grown cultures of *B. bassiana* for the presence of entomotoxins.

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