1984). Since it is well established that neuromuscular junctions in insects are also mediated by glutamate, it is reasonable to propose that the paralytic symptoms of insects with JSTX administrations as described above are ascribable to inhibition of the neuromuscular junction to the motor muscles (USHERWOOD and DUCE, 1985). Very recently various glutamate receptor antagonists were successively discovered from venom of spiders, Argiopine from *Argiope lobata* (GRISHIN et al., 1986), Argiotoxin 622 and 636 from *Argiope trifasciata* and *Araneus gemma* (USHERWOOD et al., 1987), JSTX-3, -4 and -5 from *Nephila clavata* (ARAMAKI et al., 1986), and NSTX (New Guinean spider toxin) from *Nephila maculata* (ARAMAKI et al., 1986). Although available information so far is limited, it is interesting to note that there are common features among these substances such as high solubility in water, fairly low molecular weight near 600 and, most of all, antagonistic activities against glutamate receptor. Since no activity on real insects has been reported for them, we are interested in the application of our assay on these toxins.

To summarize the present results, the *Culex* mosquito, the German cockroach and the green rice leafhopper are concluded to be feasible and

handy tools for assaying spider venom, because of the distinctiveness of poisoning symptoms detectable on respective administrations of at least 1 GE/0.2 ml, 0.1 GE, 0.5 GE/50 µl of the venom to the insect.

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Effect of Rearing Humidity of Host Insects on the Spore Types of *Entomophaga maimaiga* HUMBER, SHIMAZU et SOPER (Entomophthorales: Entomophthoraceae)¹

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In studies on entomophthoraceous fungi, it is essential to know their life cycles in order to elucidate the mechanism of their prevalences. To clarify their life cycles, an understanding of conditions influencing formation and germination of resting spores is necessary. I experienced that the

spore types of *Entomophaga maimaiga* on the gypsy moth, *Lymantria dispar*, vary from one insect rearing container to another, and this led to the hypothesis that differences in humidity of the rearing containers may affect spore types. To confirm this hypothesis, the effects of humidity on spore types in infected host insects were investigated using dry and humid rearing chambers.

Gypsy moth, *Lymantria dispar* and three-spotted plusia, *Acanthoplusia agnata* were used in this study as the experimental insects. The larvae of *L. dispar* used for the experiment were a laboratory reared strain derived from Ibaraki Prefecture. Those of *A. agnata* were kindly supplied by Dr. Kenjiro KAWASAKI of the National Institute of Agro-Environmental Sciences, and reared continuously in my laboratory. Two isolates of *E. maimaiga*, F-495 from Ishikawa Prefecture and F-541-2 from Ibaraki Prefecture were used for the

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experiment; both were isolates from *L. dispar*. The fungus was cultured in an insect cell culture medium, MGM-443 with 5% fetal bovine serum (MITSUHASHI, 1982), for 7 days at 15°C. The resulting protoplasts were harvested by centrifugation at 1,000 rpm for 5 min, and added with CARLSON's fluid (CARLSON, 1946) to prepare the protoplast suspension of $1 \times 10^5/\text{ml}$ for inoculation. The inoculum was injected to the larvae by means of a Drummond® Microdispenser with a glass capillary. Amounts of the inoculum were 5 μl for the 2nd instar and 10 μl for the 3rd to 5th instar larvae. The inoculated larvae were placed in petri dishes 6 cm or 9 cm in diameter and reared on artificial diets prepared for each species (BELL et al., 1981; KAWASAKI et al., 1987). Five larvae were reared in each petri dish and the dishes were divided into 2 groups. In one group the glass lids were replaced with gauze to keep the rearing chamber dry. In the other group, moistened filter paper was spread on the bottoms of the dishes, whose tops were then covered with glass lids and sealed with Parafilm® to maintain humidity inside the rearing chamber. All petri dishes were kept at 20°C for *L. dispar*, and at room temperature ranging from 23.8° to 16.2°C with a mean value of 20.1°C for *A. agnata*. Larval mortality was examined daily, and dead larvae were transferred to other petri dishes with moistened filter paper. Approximately 7 days later spore types were determined in the following manner. The cadavers were first examined under a dissecting microscope and conidial formation was recorded based on the surface area of the cadaver which was covered with conidiophores (+++ : fully covered, ++ : more than 1/2 covered, + : less than 1/2 covered, $\pm$: sparsely formed in a very limited area, — : not formed). After assessing conidial formation, tissues from internal parts of the cadavers were observed under a light microscope and formation of resting spores was recorded based on the area covered by the resting spores in the microscopic field (+++ : entire field, ++ : more than 1/2, + : less than 1/2, $\pm$: few, — : none).

The predominant spores formed on/in the cadaver differed depending on the humidity of the rearing vessels (Table 1 for *A. agnata* and Table 2 for *L. dispar*). In these tables, Tc (or Tr) was calculated by multiplying the number of insects showing each degree of spores by the degree coefficient (3 for +++, 2 for ++, 1 for +, 0.5 for $\pm$, and 0 for —). The fungus often formed both

conidia and resting spores on/in the same body, and in these tables numbers of insects having both types of spores were recorded in the columns of both spores. Since spore formation status was not expressed as actual number of spores but as relative amount of the spores formed on/in a cadaver, Tc and Tr could not be compared directly. Nevertheless, the ratio Tc/Tr represented the general tendency of spore formation under different conditions. A larger Tc/Tr indicated the tendency for conidial formation, whereas a smaller Tc/Tr indicated the predominance of resting spores. As is seen in the tables, Tc/Tr ratio was larger under dry than under humid conditions at each instar. Moisture seemed to affect mainly the period between infection and death, since the cadavers from each group were treated in the same way after death. Thus, content of the body before death was thought to be an important factor in determining the spore types. On the other hand, 2nd instar larvae showed a greater tendency to induce formation of conidia than 3rd or 4th instar larvae. Entomophthoraceous fungi on lepidopteran larvae have been reported to show a tendency to form conidia in younger larvae, and resting spores in older larvae (NEWMANN and CARNER, 1975; SHIMAZU and SOPER, 1986). In other words, the spore types seem to be affected by the age of the insects. A younger insect has a smaller body than an older insect, but the surface area per weight of the former is larger. From this fact, it can be speculated that a younger larva loses internal water easier than an older one. This may result in poor water content in the young larva and rich water content in the old one. If this assumption is correct, it may explain the fact that younger larvae tend to produce conidia and older larvae tend to produce resting spores. WILDING and LAUCKNER (1974) and KRAMER (1979) reported that the spore types of *Entomophthora* are determined by the days after emergence of the adult flies. SHIMAZU (1979) reported that the spore types of *Erynia radicans* (*Entomophthora sphaerosperma*) on/in *Nilaparvata lugens* varied between the wing forms of the adults. Common factors determining the spore types in those studies are not only the size but also the physiological state of the insects. Whether these factors are related to the water content of the insects should be investigated.

Up to the present, factors determining the spore types have not been discussed from the viewpoint of water content of host insects. From the present

Table 1. Spore types of *Entomophaga maimaiga* on/in *Acanthophasia agnata* larvae reared under dry and humid conditions

Instar	Rearing condition	No. of insects	Percent infection	Number of cadavers forming each spore type										T _c /Tr
				Conidia					Resting spores					
				###	##	+	±	T _c ^a	##	##	+	±	Tr ^b	
2	Dry	15	60	1	2	1	1	8.5	1	1	0	0	5.0	1.70
	Humid	18	83	1	1	2	2	8.0	6	0	0	1	18.5	0.43
3	Dry	23	69	4	9	1	0	31.0	7	2	1	1	26.5	1.17
	Humid	23	91	0	2	5	2	10.0	10	3	1	1	37.5	0.26

a,b See text.

Table 2. Spore types of *Entomophaga maimaiga* on/in *Lymantria dispar* larvae reared under dry and humid conditions

Instar	Replication	Rearing condition	No. of insects	Percent infection	Number of cadavers forming each spore type										Tc/Tr	
					Conidia					Resting spores						
					###	##	+	±	Tc ^a	###	##	+	±	Tr ^b		
2	1	Dry	20	40	1	2	0	0	0	7.0	0	0	0	0	0.0	∞
		Humid	20	0	0	0	0	0	0.0	0	0	0	0	0	0.0	—
	2	Dry	20	35	0	3	2	0	0	8.0	1	0	0	0	3.0	2.67
		Humid	20	40	2	2	0	0	0	10.0	1	1	0	0	5.0	2.00
3	1	Dry	25	52	8	4	0	0	0	32.0	1	0	0	0	3.0	10.67
		Humid	25	24	1	0	1	0	0	4.0	3	1	0	0	10.0	0.40
	2	Dry	20	45	6	0	0	0	0	18.0	1	0	2	1	5.5	3.27
		Humid	20	75	0	0	1	0	0	1.0	2	0	2	0	8.0	0.13
4	1	Dry	25	64	7	5	1	0	0	32.0	1	2	0	0	7.0	4.57
		Humid	25	8	0	0	0	0	0	0.0	0	1	0	1	2.5	0.00
	2	Dry	10	70	3	0	1	0	0	10.0	4	2	0	0	16.0	0.63
		Humid	10	40	0	0	1	0	0	1.0	3	0	0	0	9.0	0.11

a,b See text.

study, the hypothesis was developed that water content in host insects may affect the spore types. Henceforth, it is necessary to confirm when and how the water content affects the fungus to form resting spores, and whether or not this phenomenon is common in other entomophthoraceous species.

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- Effect of the Concentration of Fetal Bovine Serum in a Culture Medium on the Susceptibility of *Bombyx mori* Cells to a Nuclear Polyhedrosis Virus¹
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- The growth and survival of cultured insect cells are greatly affected by the concentration of fetal bovine serum (FBS) in the medium (SOHI and SMITH, 1970; MITSUHASHI, 1976). The optimal concentration of FBS for maximum cell growth seems to be dependent on the cell lines and the medium composition. In the present study, *Bombyx mori* (BM-N) cells in media with different concentrations of FBS were compared on the basis of their growth curve and susceptibility to a nuclear-polyhedrosis virus (NPV).
- BM-N cells, supplied by Dr. S. MAEDA, Tottori University, Tottori, were cultured in BML-TC/10 medium (GARDINER and STOCKDALE, 1975) supplemented with 5, 10 or 20% FBS and antibiotics (streptomycin, 100 µg/ml; penicillin, 100 units/ml; fungizone, 25 µg/ml, purchased from Gibco). Sixty millimeter plastic petri dishes etched on the external bottom surface with 2 mm square grids were seeded with 2×10^4 cells/ml in 3.5 ml of each medium. The dishes were kept at 26°C. The number of cells adhering loosely to the bottom surface of 10 square grids was counted with an inverted microscope every day or every two days and the cell densities (number of cells/ml) were calculated. The growth curve of cells in media supplemented with different concentrations of FBS is shown in Fig. 1.
- Good cell growth was obtained in the culture with 10% FBS, slightly less growth with 20% FBS, and a slow growth with 5% FBS. However, the logarithmic growth of these three cultures began at the same time, i.e., 2.5–3 days after seeding. The cells in cultures with 10 and 20% FBS reached the maximum cell density about the same time, 10 days after seeding, whereas those in cultures with 5% FBS reached maximum at 17 days after seeding.
- The susceptibility of cells to NPV had been reported to depend on the cell cycle phase at the time of infection; the cells at an early logarithmic

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