BIOLOGICAL CONTROL OF *MALACOSOMA NEUSTRIA* L. POPULATION WITH LATVIAN ISOLATE OF NUCLEAR POLYHEDROSIS VIRUS

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ABSTRACT

The Latvian isolate of *Malacosoma neustria* NPV was used as a source for production virus insecticide. In recent years novel virus insecticide formulations have been developed and tested using environmently - friendly matrix materials. Field trials with the new virus insecticide compositions indicated that high levels of mortality (89-96 %) of second and third instar larvae could be achieved 10 days after spraying. The persistence of NPV in the agroecosystem was measured. The results of bioassays demonstrated that the new virus insecticide formulations retained activity 3 weeks after spraying. Additives used: lysine KKL, polyglucine, a by-product of citric acid production and molasses of peat, increased persistence of polyhedra 7, 6, 4, and 3 times, respectively. The mean percentage of larval mortality in test variants was 2.5 to 3 times higher than that in the controls.

INTRODUCTION

Baculoviruses including nuclear polyhedrosis viruses (NPVs) are insect pathogenic viruses that cause diseases of insects and may control the population of their hosts. They are considered to be safe bioinsecticides and therefore have great potential in integrated pest control. They can substitute for chemical insecticides diminishing the amount of pests. NPVs in particular have been formulated and applied as biological insecticides against pest populations (Huber, 1986). The Latvian isolate of *Malacosoma neustria* nuclear polyhedrosis virus (Mn NPV) is a highly active and selective agent for the control of European tent caterpillar *M. neustria* population (Zarins & Eglite, 1993). Mn NPV was used as a basis of 'VIRIN-KS' which is the virus preparation registered and produced in the Soviet Union.

In recent years we have been working on problems involved in the development of microbiological methods for plant protection. Initially we analyzed possibilities of using new biological plant protection methods both for ecological agriculture as well as for conventional agriculture. Biopreparations based on NPVs may be considered for the following reasons: i) high host - specificity, ii) no evidence of occurrence in non-arthropod hosts; no observations of harmful effects on the rest of the entomofauna including beneficial insects, or on other bioagents in the agroecosystem (Grôner, 1986), iii) spread and multiplication of these viable microbial preparations in pest populations leading to the possibility of control of pest populations several years after initial spraying (Bird, 1961), iv) local virus strains and isolates have high activity in the climatical conditions of Latvia. The nuclear polyhedrosis viruses described are inactivated by different environmental factors such as sunlight, summer temperature, humidity and rain. The inactivation rate may be slowed down by using various additives to the virus preparations. It has been shown that formulated viral preparations are

more or less easily washed out from foliage, depending on the formulation used (Mohamed et al., 1982).

The aim of our studies was to test new virus insecticide formulations developed in our laboratory with high potency and desirable physical characteristics and to compare the persistence of Mn NPV activity in the different formulations.

MATERIALS AND METHODS

The Latvian isolate of *Malacosoma neustria* nuclear polyhedrosis virus (Mn NPV) strain KS-5 (Patent SU 1022350 A 01, 1983) isolated in the Institute of Biology, Latvia was used as the basis of the virus insecticide. The method of producing the virus formulation was developed in our laboratory (Patent SU 1314492 A1, 1987). Environmently-friendly matrix materials were used. The four additives used in the formulations were selected from 20 tested: polyglucine, molasses of peat (experimental product, produced in the Institute of Wood Chemistry, Latvia), lysine KKL (produced in the factory for producing lysine, Latvia), a by-product of citric acid production (produced in Institute of Microbiology, Latvia). Formulations containing bentonite were used as positive controls. All of the tested additives gave good wettability of dispersible dry formulations (Table 1), as well as promoting adhesion to the plants.

Table 1. Physical characteristics of virus preparations containing different additives

Additive in the virus preparation	Stability: concentration of virus suspension, polyhedra/ml		Dispersion, (sec)	Adhesion	Viscosity,
	before sedimentation	after 30 min of sedimentation			
Polyglucine	$2.8x10^7$	$2.7x10^6$	300-360	good	1.06
Molasses of peat	$6.9\ x10^6$	$6.3x10^6$	15	satisfactory	1.03
Lysine KKL	$2.4x10^6$	$2.4x10^6$	30	satisfactory	1.01
By-product of citric acid production	$5.1x10^6$	$3.2x10^6$	40	satisfactory	1.25

Stability against u.v. light was measured after exposing working solutions for 20 and 60 min. to u.v. light. Apple leaves were sprayed with virus preparations using a hand sprayer. The virus concentration in the working solution was 10^7 polyhedra ml^{-1}. *M. neustria* larvae reared on natural food in special cages (0.5 x 0.35 x 0.35 m) were fed on sprayed leaves for one day. Experiments were repeated 4 times (30 larvae in each replica). The efficiency of virus preparation was expressed as the percentage mortality caused by the virus, ussing the method of Abbott (Abbott, 1925) and LT_{50} (Finney, 1971). Persistence of virus viability after storage of the virus formulations for two years was tested using bioassay, as described previously. Virus insecticide formulations were stored as pastes as well as dry powders. Dry formulations were developed by means of lyophilization.

The suitability of the additives used in the virus formulations was examined in field trials

from 1990 to 1995. Apple trees infested with second instar of *M. neustria* were sprayed with virus preparations (working solution $5x10^6$ polyhedra ml^{-1}, 50 to 70 litres/ha). Backpack air-blast sprayer (Yamar-10, Japan), nozzle type 1.6 mm, spray angle 50°, operating pressure 1.6 was used.

The persistence, distribution and accumulation of NPV in the agroecosystem was determined to optimise the successful biological control of the *M. neustria* population. An experiment was performed to study the influence of environmental factors on virus viability after its application. Apple trees were sprayed with virus preparations ($2x10^7$ polyhedra ml^{-1}). Virus was exposed on foliage. After 1, 8, 15, 22 days leaves were randomly collected for bioassays and 200 discs (10 mm diameter) were cut for DNA-DNA hybridization. Third instar larvae of *M. neustria* reared on artificial diets were used for biotest. Experiments were repeated 5 times (20 larvae in each replica). Virus sprayed leaves were homogenized and added to diet. Leaves sprayed with water were added to diet and used as a control.

RESULTS AND DISCUSSION

The new tested dry formulations produced by lyophilization showed very good efficiency after two years storage (Table 2). New additives protected the virus from u.v. light. Virus exposed to u.v. light for 60 min retained on 15 % of its efficiency compared to values 70-90 % for virus in preparations containing additives.

Table 2. Efficiency of virus preparation depending on additives used and conditions of storage

Additive in virus preparation	Concentration of additive in working solution, (%)	Form of storage	Efficiency after two years of storage, (%)
Polyglucine	0.4	dry powder	92.5
Molasses of peat	0.5	dry powder	95.0
Lysine KKL	0.5	dry powder	95.0
By-product of citric acid production	0.5	dry powder	78.0
		paste	89.3

Field trials with the new virus insecticide formulations indicated that high levels of mortality 89 to 96 % of second and third instar larvae could be achieved 10 days after spraying.

The results of biotest demonstrate that the new virus insecticide formulations secure the persistence of virus activity 22 days after spraying (Table 3).The weekly loss of polyhedra determined by specific DNA-DNA hybridization on apple leaves varied between 20 to 60% in variants with additives, in the control (virus in water suspension without additives) the loss was 80%. The use of the additives: lysine KKL, polyglucine, by-product of citric acid production and molasses of peat increased the persistence of the polyhedra 7, 6, 4, and 3 times, respectively. The mean percentage of larval mortality after 15 and 22 days was 2.5 to 3 times higher than that in the controls.

Table 3. Persistence of virus activity of preparation after its application

Additive in the virus preparation	Mortality corrected after Abbot, (%)	
	Time exposed on foliage, (days)	
	1	22
Polyglucine	98.0	73.4
Molasses of peat	80.0	51.8
Lysine KKL	88.1	66.0
By-product of citric acid production	86.6	50.0
Bentonite	84.4	58.0
Virus water solution	80.5	19.0

It may be concluded that the new virus insecticide formulations can be used to control populations of the European tent caterpillar. The additives used in the preparation of the virus insecticides strongly reduced the harmful effect of weather conditions on the persistence of viral activity.

ACKNOWLEDGMENTS

Our investigations were supported by a grant from the Latvian Science Council (93.252). We acknowledge Dr. Skujane for providing *M. neustria* larvae reared on diet for bioassay and Dr. hab. G. Ievins for constructive criticism in translation.

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INCREASING THE SUSCEPTIBILITY TO NUCLEAR POLYHEDROSIS VIRUSES BY SYNERGISTIC ADDITIVES

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ABSTRACT

Additives, such as fluorescent brighteners, GV enhancin protein and neem extract, were assessed for potential effects on susceptibility of various Lepidoptera to a range of baculoviruses. A greater level of enhanced susceptibility was demonstrated by addition of fluorescent brightener to hosts challenged by heterologous rather than homologous viruses, when up to 460 fold increases in potency were demonstrated. Increases in potency of up to 3.4 fold and 2.9 fold were demonstrated with *T. ni* GV enhancin and neem extract (Neemazal-T) respectively.

INTRODUCTION

Recent research has indicated that various additives can enhance the effectiveness of baculoviruses to both homologous and heterologous hosts. Among the natural additives, both the enhancin protein from granulosis virus (GV) (Lepore *et al.* 1996) and the insecticide azadirachtin from the neem plant (Shapiro *et al.* 1994) have been shown to synergise the effects of nuclear polyhedrosis virus (NPV). There has also been considerable interest in the use of optical brighteners, initially as uv protectants and more recently as synergistic additives in their own right (Dougherty *et al.* 1996).

The aim of the present study was to investigate the effects of various additives (fluorescent brightener, enhancin and neem extract) on the susceptibility of certain lepidopterous neonate larvae when challenged by homologous (high susceptibility) or heterologous (low or nil susceptibility) viruses.

MATERIALS AND METHODS

Standardised procedures were employed to administer baculoviruses with additives to neonate larvae of a range of test Lepidoptera.

Additives

1. Fluorescent brightener 28 (Sigma) was used at concentrations of 0.05%, 0.075% and 0.1% of the applied virus suspension volume.

2. Enhancin protein from *Trichoplusia ni* GV was used at concentrations of $1x10^7$, $3.16x10^7$ and $1x10^8$ capsules per ml of semi-synthetic diet. The GV was made up in Tris buffer (pH8).

3. Sub-lethal doses of Neem extract (Neemazal-T, Trifolio, Germany) were used at concentrations of 1, 5, 10, and 50 ppm of the applied virus suspension volume.

Bioassay methods

Virus plus additives were incorporated into semi-synthetic diet for all assay tests except for those against *Plutella xylostella* when leaves dipped in the additive/virus suspensions were used. Virus concentrations were added in logarithmic dilutions. Larvae were fed on the diet and observed for 16 days. All deaths due to virus were recorded and results expressed as relative potencies.

Tested viruses

The following NPVs were tested:

Autographa californica (*AcNPV*), *Spodoptera littoralis* (*SlNPV*), *Spodoptera exigua* (*SeNPV*), *Agrotis segetum* (*AsNPV*), *Mamestra brassicae* (*MbNPV*), *Lymantria dispar* (*LdNPV*) (BBA, Darmstadt, Germany) and *Anagrapha falcifera* (*AfNPV*) (biosys, USA).

Test insect species

Neonate larvae of the following species were tested:

Agrotis segetum, Autographa gamma, Spodoptera exigua, Spodoptera littoralis, Plutella xylostella and *Mamestra brassicae*.

RESULTS

Fluorescent brighteners (fb)

The results of the bioassays employing fbs and a range of NPVs against six species of Lepidoptera are shown in Table 1. The results show that the rate of enhancement was higher in the case of the less susceptible, heterologous systems (*S. littoralis* and *A. segetum*) than in the case of the high susceptibility, homologous systems (*A. gamma* and *S. exigua*). There was no mortality in the non-susceptible hosts. However, some mortality was noted in the case of neonate larvae of *A. gamma*, *P. xylostella* and *A. segetum* when challenged by *SlNPV*, and for *LdNPV* against *S. littoralis* and *MbNPV* against *P. xylostella*. The DNA sequences, determined by REN, indicated that *AcNPV* was expressed when *SlNPV* was tested against *A. gamma* and that *AsNPV* was expressed when *SlNPV* was tested against *A. segetum*.

Table 1: Relative potencies of 6 nuclear polyhedrosis viruses applied with 3 concentrations of fluorescent brighteners against 6 lepidopterous hosts.

Tested		Potency for tested fb		
		Concentration of fb per ml of diet		
Insect species	Virus	0.05	0.075	0.1
A. gamma	*AcNPV*	9.971	38.528	37.184
	AfNPV	3.994	24.981	33.896
	MbNPV	24.416	69.848	77.26
A. segetum	*AsNPV*	3.242	23.384	132.051
	MbNPV	1.671	63.64	221.849
	AfNPV	1.017	27.999	89.55
	AcNPV	6.431	246.07	461.21
S. exigua	*SeNPV*	5.932	20.995	28.788
	SlNPV	4.188	6.975	34.055
	AfNPV	2.825	13.054	43.624
	MbNPV	1.664	15.227	30.254
	AcNPV	4.064	210.039	74.329
S. littoralis	*SlNPV*	11.525	53.798	143.99
	MbNPV	0.711	9.917	39.243
	AcNPV	9.862	70.077	123.25
	AfNPV	9.515	102.87	271.14
M. brassicae	*MbNPV*	3.922	52.132	147.86
P. xylostella	*AcNPV*	2.945	2.884	4.092

Enhancin

Results for the bioassays for tests of enhancin are shown in Table 2. The results indicate that *A. gamma* neonate larvae, which are susceptible to *T. ni* GV, showed the highest rate of synergism amongst the tested insects, as indicated by a 3.6 fold increase in potency.

Table 2: Relative potencies of 4 nuclear polyhedrosis viruses applied with 3 concentrations of *T. ni* GV enhancin against homologous hosts.

Tested		Potency for tested *T.ni* GV		
		Concn. of GV capsules per ml of diet		
Insect species	Virus	$1x10^7$	$3.16x10^7$	$1x10^8$
A. gamma	*AcNPV*	1.5107	3.295	3.364
S. exigua	*SeNPV*	1.517	1.297	1.39
A. segetum	*AsNPV*	0.7	1.0	1.673
S. littoralis	*SlNPV*	1.246	0.93	0.823

Neem additive

The results of tests employing Neemazal-T are shown in Table 3. There was a dose-dependent increase in potency when Neemazal-T was included in the virus mix. The effect was greatest for *S. littoralis* NPV, in which the effectiveness of the virus was increased 2.9 fold relative to normal NPV inoculum.

Table 3: Relative potencies of 4 nuclear polyhedrosis viruses applied with 4 concentrations of Neemazal-T against homologous hosts.

		Potency for tested Neemazal-T			
Tested		Concn. of Neemazal-T (ppm of diet)			
Insect species	Virus	1	5	10	50
S. littoralis	*SlNPV*	1.448	2.579	2.908	-
S. exigua	*SeNPV*	1.676	2.033	2.155	-
A. gamma	*AcNPV*	0.989	1.764	2.03	-
A. segetum	*AsNPV*	-	0.0956	1.286	1.727

CONCLUSIONS

The studies reported here indicate that the addition of various additives can increase the effectiveness of baculoviruses in both homologous and, particularly, heterologous virus systems. The greatest effects were observed with the addition of fluorescent brighteners when increases in potency up to 460 fold were demonstrated for the heterologous interaction between *AcNPV* and *A. segetum* as the host. These findings have implications for pest management of these important agricultural and forest pests and may enable viruses to be used more efficiently and cost-effectively in the future.

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DISTRIBUTION AND TAXONOMY OF ENTOMOPATHOGENIC FUNGI FROM KOREA, WITH SPECIAL REFERENCE TO THE GENUS *CORDYCEPS*

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ABSTRACT

Thirty five species of entomopathogenic fungi from 14 genera were collected at 16 sites from 1990 to 1995, of which 14 were previously unrecorded in Korea. The occurrence of different species varied with the time of year, for example, *Cordyceps nutans, C. sphecocephala* and *Paecilomyces tenuipes* occurred from early June to late September, while *C. militaris, C. kyushuensis* and *C. pruinosa* were mainly found from mid-July to mid-August, when relative humidity was higher. Nine species of the genus *Cordyceps,* including *C. bifusispora* and four Deuteromycetes were isolated. Following cultural tests with six *Cordyceps* species, the anamorphs of *C. militaris* and *C. kyushuensis* were identified as *Verticillium* sp, *C, pruinosa* as *Acremonium* sp., *C. sphecocephala* as *Hymenostilbe* sp. and *C. scarabaeicola* as *Beauveria* sp, respectively.

INTRODUCTION

Entomopathogenic fungi of genus *Cordyceps* (Ascomycotina) form fruiting bodies or sporocarps on their insect hosts. The earliest description of the genus can be traced to AD 800 (Kobayasi and Shimizu, 1983; Shimizu, 1994). *Cordyceps* species are distributed globally and 75 species have been recorded as pathogenic to insects (Arora et al, 1991). The genera have potential for biocontrol and medical value. Since *Cordyceps* was described as *Clavaria* by Linnaeus, the taxonomy of this fungus has been reviewed by Kobayasi (1940, 1982), Mains (1937, 1940, 1957, 1958) and Sung (1996)

The anamorphs of *Cordyceps* include species of *Akanthomyces, Beauveria, Cephalosporium, Hirsutella, Hymenostilbe, Nomuraea, Paecilomyces, Paraisaria, Pseudogibellula, Sporothrix, Stilbella,* and *Verticillium.* The taxonomy of *Cordyceps* is based on morphological characteristics. The aim of this study was to collect and classify *Cordyceps* in Korea to obtain candidate strains for our research programme, although data on other species identified coincidentally are included

MATERIALS AND METHODS

Collection and isolation : Entomopathogenic fungi were collected from diseased insects from 16 sites in Korea from July 1990 to October 1994. Data were recorded on site conditions and

insect host etc. *Cordyceps* and another entomopathogenic fungi were isolated using water agar and maintained on PDA(Potato dextrose agar)

Identification and analysis: The geographical and temporal distribution of six *Cordyceps* species was analysed. Fungi were identified according to morphological characters such as fruiting body size, colour, immersed degree of perithecium, fertile part size, existence of appendage and fine structure. Identification of collected specimens was based on the criteria of Kobayasi (1982) and Samson (1988).

Identification of anamorphs: The anamorphs of six species of *Cordyceps (C. militaris, C. kyushuensis, C. pentatomi, C. pruinosa, C. sphecocephala),* were identified by following cultures on water agar for seven days. Conidia, formed on the water agar, were observed by scanning electron microscope (SEM)

RESULTS

Distribution of entomopathogenic fungi: Entomopathogenic fungi from 12 genera and 33 species were identified. Among the species collected, *Cordyceps bifusispora, C cochlidicola, C. isariodes, C. oxycephala, C. paludosa, C. pentatomi, C. rosea, C. ryogarniensis, Akanthomyces aculeatus, Hirsutella clavispora, Polycephalomyces ramosus, Tilachlidiopsis nigra* and *Verticillium lecanii* were previously unrecorded in Korea. Entomopathogenic fungi were mainly distributed where relative humidity was high and broadleaved forest was predominant. Fig. 1. shows examples of 14 species of entomopathogenic fungi..

MORPHOLOGICAL CHARACTERS AND CLASSIFICATION

Ascomycetes

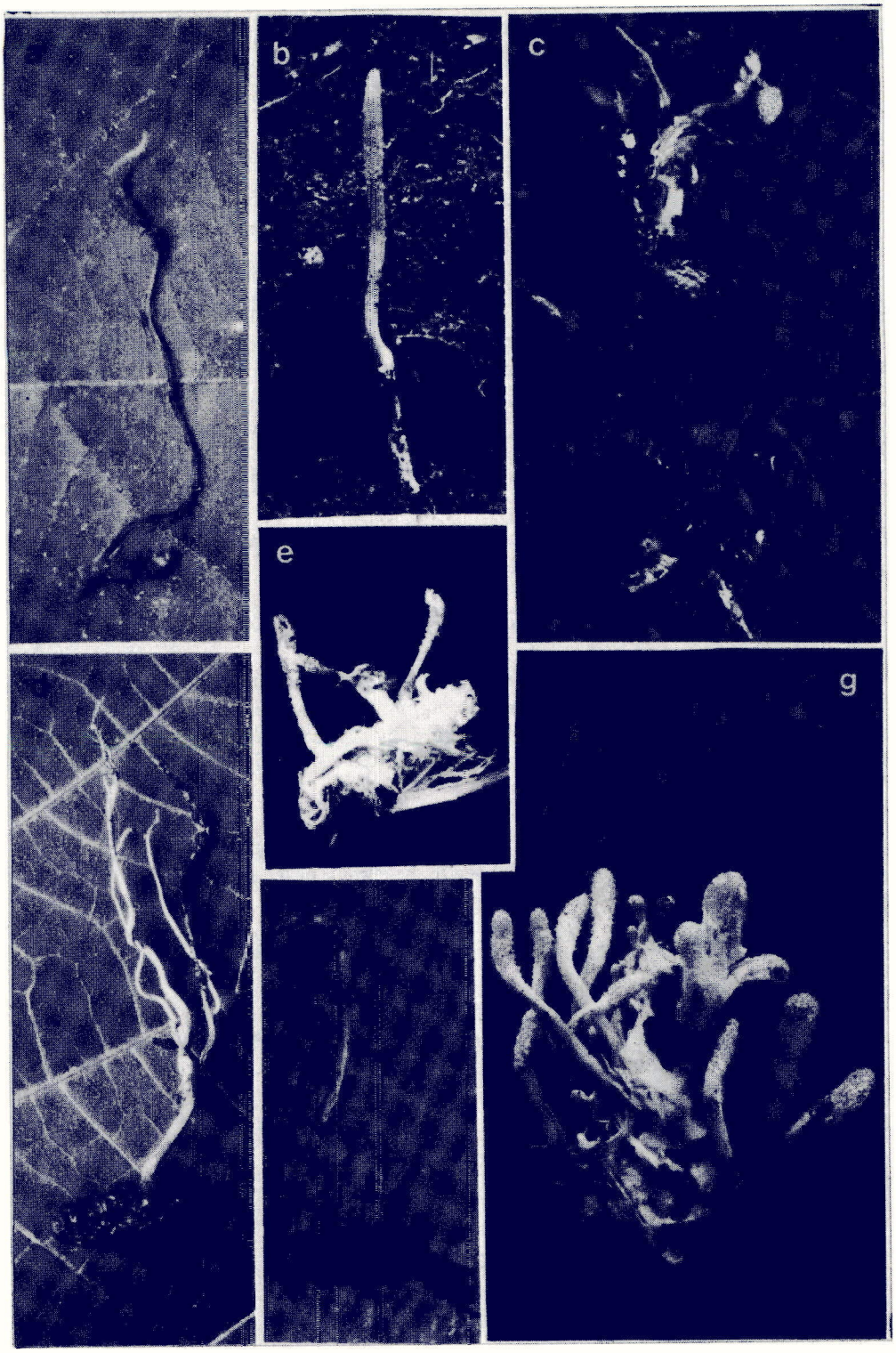
Cordyceps

Subgenus *Ophiocordyceps*. Sect. Epicarposoma.

C. paludosa Mains: On larvae of Lepidoptera. Stromata solitary or a few together, up to 50 x 1.5 mm, which is yellowish brown, with a sterile appendage. Perithecia superficial, ovoid, 550 x 330 μm.; ascospore not divided.

Fig. 1. Fruiting bodies of entomopathogenic fungi previously unrecorded in Korea

a. *Cordyceps paludosa* Mains, b. *C.* rosea Kobayasi et Shimizu, c. C. *pentatomi* Koval, d. *C. cochlidiocola* Kobayasi et Shimizu, e. *C. isarioides* Curtis et Massee, f. *C. ryogamiensis* Kobayasi et Shimizu, g. *C. bifusispora* Ove Eriksson, h. *C. oxycephala* Penz et Sacc, i. *Shimizuomyces paradoxa* Kobayasi, j. *Akanthomyces aculeatus* Lebert, k. *Hirsutella clavispora* Spease, l. *Polycephalomyces ramosus* Kobayasi, m. *Tilachlidiopsis nigra* Yakusiji et Kumazawa, n. *Verticillium lecanii* Viegas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C. rosea Kobayasi et Shimuzu: On larvae of Lepidoptera. Stromata solitary, club-shaped, up to 36 mm, which is reddish brown. Perithecia completely immersed, 240-270 x 140-170 μm ; ascospore not divided.

Subgenus *Eucordyceps*. Sect. Laterals
C. pentatomi Koval: On adult Heteroptera. Stromata solitary, up to 30 mm, high, with black stalk, borne laterally. Fertile head ellipsoid, pale yellow. Perithecia completely immersed, pear-shaped 680-820 x 330-390 μm. Ascocarp thread-like, divided into secondary spores.

Subgenus *Eucordyceps*. Sect. Racemella. subsect. Sparsae.
C. cochlidiocola Kobayasi et Shimizu: On pupae of Lepidoptera. Stromata gregarious, up to 65-70 x 0.5-1.5 mm, yellowish-orange stalk, fertile head yellowish brown. Perithecia superficial, ovoid, 400-450 x 200-250 μm ; asci 250-280 x 5-7 μm; ascospore -like.

C. isarioides Curtis et Massee: On adult Lepidoptera. Stromata gregarious, up to 10-12 mm, yellowish orange or pale orange. Perithecia superficial, thick, 450-500 x 200-280 μm; asci 250-280 x 5-7 μm ; ascospore thread-like.

C. ryogamiensis Kobayasi et Shimizu: On larvae of Coleoptera. Stromata solitary, up to 17 x 1 mm, yellowish orange. Perithecia ovoid, 300-450 x 250-300 μm.

Subgenus *Eucordyceps*. Sect. *Racemella*, subsect. Pseudoimmersae

C bifusispora Ove Eriksson: On pupae of Lepidoptera. Stromata club-shaped, borne fasciclely, up to 10-20 x 1-2 mm. Perithecia semi-immersed, ovoid, 550-620 x 250-330 μm.; ascospore thread-like, the apex thick-walled, divided by septum.

Subgenus *Neocordyceps*.

C. oxycephala Penz et Sacc: On adult Hymenoptera. Stromata solitary or few together, pale yellow. Perithecia immersed but lying inclined to the surface of the head. 700-370 x 220-280 μm; ascospore similar to *C. sphecocephala*, part-spores fusiform

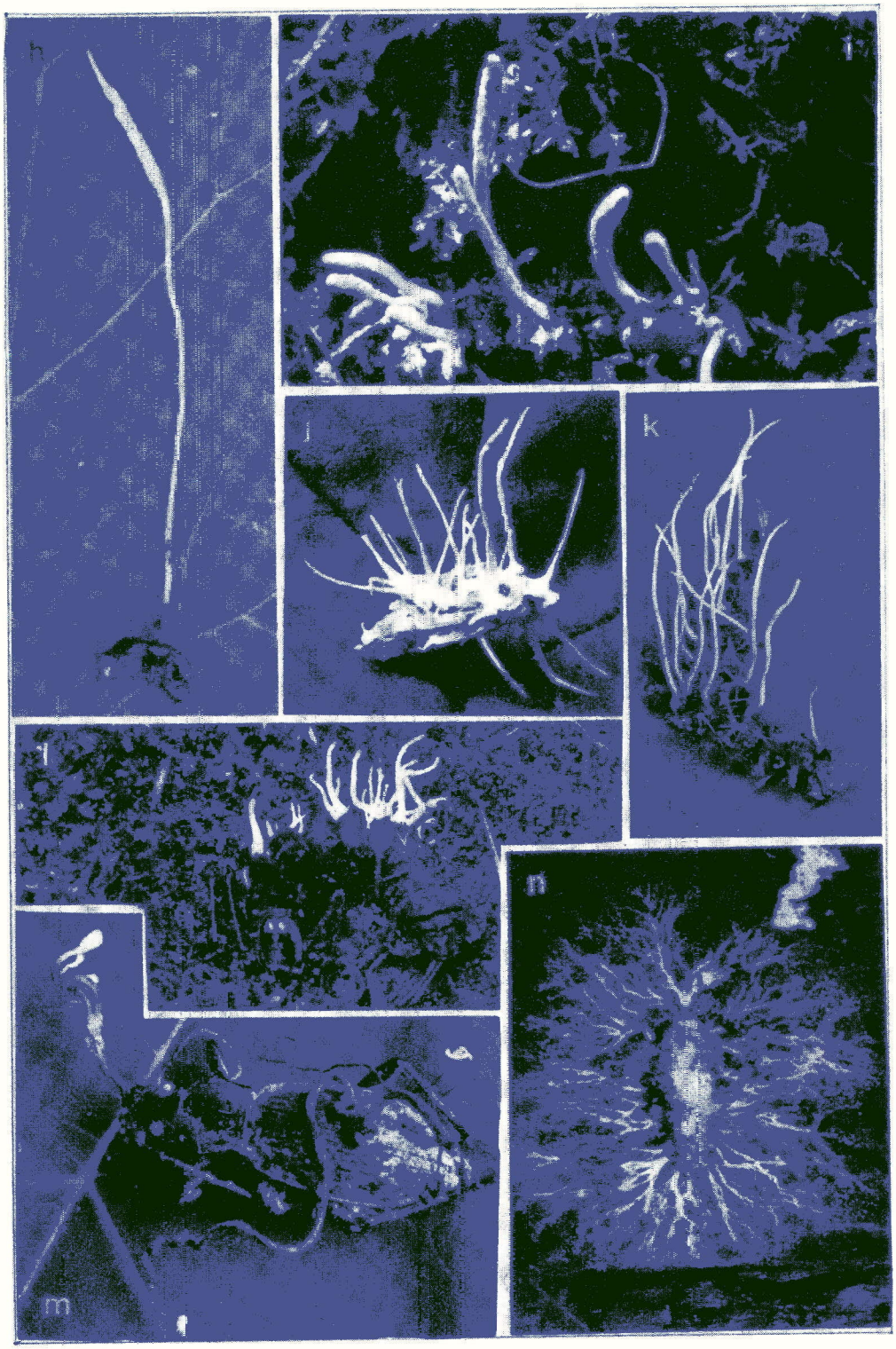
Shimuzumyces

S. paradoxa Kobayasi: Parasite on fiuit of *Smilax sieboldii.* Stromata solitary or a few together, club-shaped, pale grey. Host surface covered by whitish mycelia. Perithecia immersed, pear-like, 350-370 x 160-200 μm.; ascospore oblong, 4-8-spored.

Deuteromycetes.

Akanthomyces aculeatus Lebert: On adult. Lepidoptera. Stromata thread-like, bome fasciclely, up to 10-35 x 1 mm, white.

Hirsutella clavispora Spease: On larvae of Lepidoptera. Stromata thread-like, borne fasciclely, up to 21-32 x 1 mm, white.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Polycephalomyces ramosus Kobayasi: On larvae. Stromata up to 7-10 x 1 mm, with a yellowish orange stalk, fertile head greyish brown, which is formed out of synemmata.

Tilacchlidiopsis nigra Yakusijji et Kumazawa : On adults of *Damasier smaragdinus* CF. Stromata up to 20-40 mm, with a black needle shaped stalk, white club-shaped fertile head. Conidia bound apex round

Verticillium lecanii Viegas: Conidiophore usually verticilliated, with a gimlet-shaped stipe. Conidia unicellular, with a hyaline and soft wall, and sometimes formed in chain.

Telemorphs and anamorphs of *Cordyceps* species.

As a result of observation on the conidial stage of six *Cordyceps* species, anamorphs were identified, as follows. (Table 1. Fig. 2)

Table 1. Telemorph and anamorph associations of *Cordyceps* species

Telemorph	Anamorph
Cordyceps militaris	*Verticillium* sp.
Cordyceps kyuensis	*Verticillium* sp.
Cordyceps scarabaeicola	*Beauveria* sp.
Cordyceps pentatomi	*Hirsutella* sp.
Cordyceps pruinosa	*Acremonium* sp.
Cordyceps sphecocephala	*Hymenostilbe sphecocephala*

DISCUSSION

Thirty three species in 12 genera were collected at 16 sites from 1990 to 1994 in Korea. Among these, fourteen species, which belong to the Ascomycetes and Deuteromycetes, were unrecorded in Korea. Identification of these fungi was based on the classification system of Kobayasi, Petch, Mains, Shimizu and Samson. To date, identification of fungi has been based on morphological characters. Kobayasi (1982) subdivided *Cordyceps* into three subgenera; *Ophiocordyceps*, *Eucordyceps*, and *Neocordyceps*. According to this classification, we identified two species of *Cordyceps as Ophiocordyceps*, five species as *Eucordyceps* and one species as *Neocordyceps*, respectively.

Cordyceps were mainly collected from damp ground, usually within a valley, and close to fallen leaves, although the occurrence of these fungi is probably influenced by many factors such as habitat enviromnent, climate and availability of hosts, etc. On

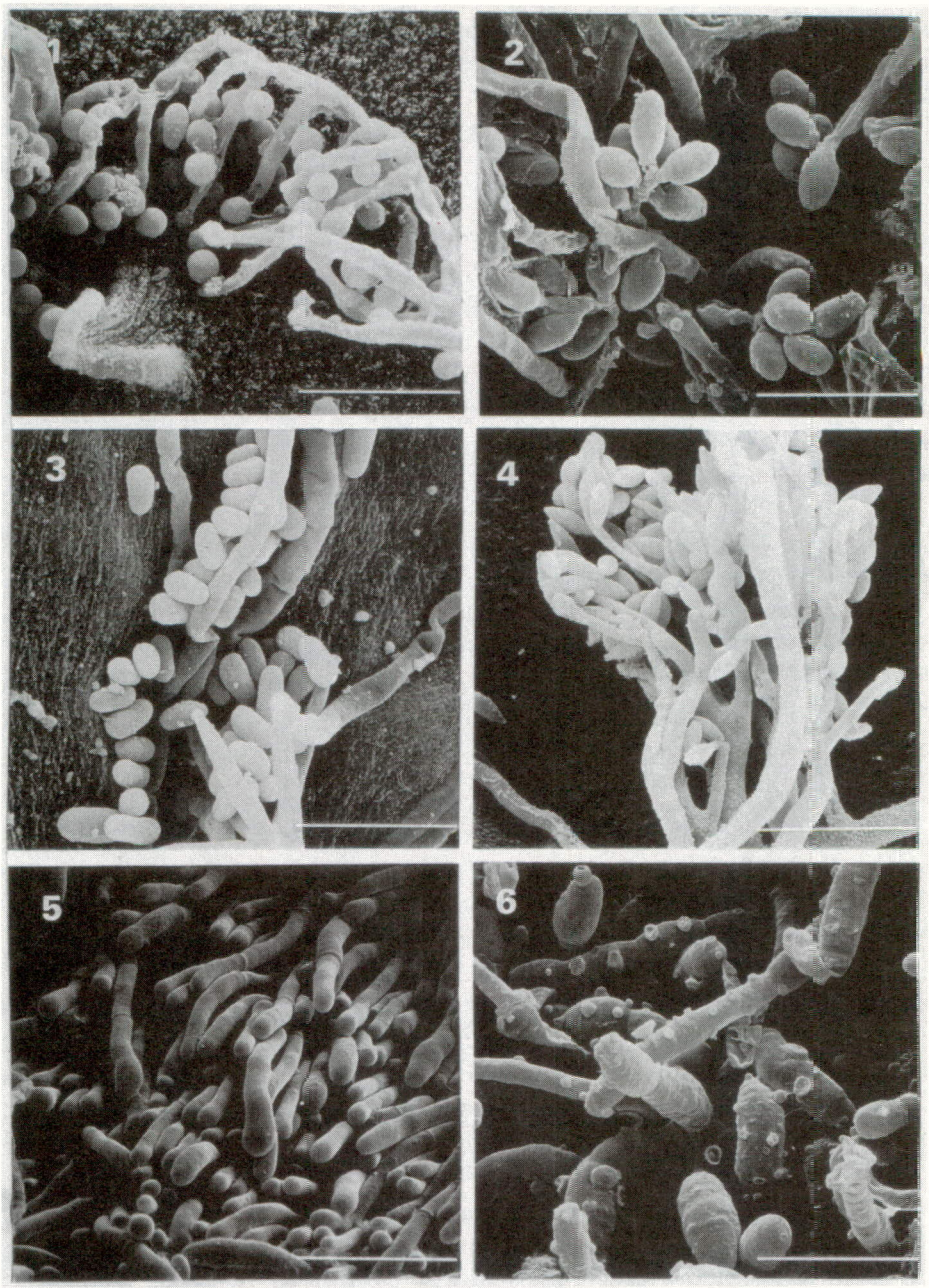

Fig. 2. Scanning electron microscope of entomopathogenic species. 1. *Verticillium sp.* (anamorph of *C. militaris*, x3000, bar; 10㎛), 2. *Beauveria sp.*(anamorph of *C. scarabaeicola*, x4000, bar; 7.5㎛), 3. *Acremonium sp.*(anamorph of *C. pruinosa*, x3500, bar; 8.6㎛), 4. *Hirsutella sp.* (anamorph of *C. pentatomi*, x3000, bar; 10㎛), 5. Sclerotia of *C. sphecocephala* (x1500, bar; 20㎛), 6. *Hymenostilbe sphecophila* (anamorph of *C. sphecocephala*, x1700, bar; 17.6㎛)

the other hand, preliminary analysis of our data indicated that Deuteromycetes were not influenced so much by their environments.

In nature, the occurrence of entomopathogenic fungi was from June to September in Korea. The species such as *C. nutans, C. specocephala, Paecilomyces tenuipes,* etc. were collected and distributed equally throughout the time that fruiting bodies were little influenced by enviromnents. However, some other species such as *C. militaris, C. kyushuensis, C. pruinosa,* etc. were collected mainly during the rainy season. The formation of their fruiting body may be influenced strongly by air humidity.

Among the entomopathogenic fungi collected, nine species of *Cordyceps* and 13 species of Deuteromycetes were isolated, but fourteen species were not isolated. So inducement of spore germination for unisolated species in artificial media may be necessary.

As a result of identification of conidial stage for *Cordyceps*, the *anamorph of C. kyushuensis and C. scarabaeicola* were newly identified as *Verticillium and Beauveria,* respectively. Like these, inducement of anamorphs from ascospore had been accomplished, but that of teleomorphs from conidia had been not accomplished. So in nature, teleomorph and anamorph association of Ascomycetes might be influenced by a range of complex factors.

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