韓蠶學誌 39(2), 146~153(1997)
Korean J. Seric. Sci.
<論 文>

Improvement of Predictive and Corrective Inspection Methods to Control *Nosema bombycis* Infection in the Silkworm, *Bombyx mori*

Mau Tuan Nguyen, Jong Sung Lim* and Myung Sae Han**
Research centre, VISERI, Bao Loc, Lam Dong, Vietnam
**AGSI, FAO of the United Nations, via delle Terme di Caracalla, Rome, Italia*
***College of Agriculture, Kyungpook National University, Daegu, 702-701 Korea*

ABSTRACT

The microsporidian infection with *Nosema bombycis*, reconfirmed its high virulence and transovarial tranmissibility, however, the characteristic symptom of the spots like pepper grains on the diseased larval skin was no more recognized by present varieties of the silkworm. Transovarial transmission rate detected from moth was above 90% in dead eggs or dead larvae in the rearing by mulberry leaves, 80% in the newly hatched larvae starved to death. Transovarially transmitted N. bombycis was easily observed from dead eggs and larvae, and were suggested an individual inspection of a few of dead eggs for detection of the pathogenic spores. The progeny population provided indicative factors on the sampling of predictive and corrective inspection. The higher concentration of *N. bombycis* spores included in the hind-abdormal part of infected moth, applicative on the simple method of individual moth inspection. For the predictive inspection of growned 5th-instar larvae, *N. bombycis* infection was detectable without microscopic observation by the unique symptom of turbid milky-white spots on the silk gland. Inspection of the meconia artificially discharged from silkworm moth, was also succesful of microscopic observation before crossing, without killing or homogenize the moths. The results provided a basis of rational methods for the inspection of *N. bombycis* infection of the silkworm.

Key words : *Nosema bombycis*, Transovarial transmission, Microsporidia

INTRODUCTION

Pebrine is the name most frequently used to designate the microsporidian disease of the silkworm, *Bombyx mori*, caused by *N. bombycis* (Pasteur, 1870). Transovarial transmission of *N. bombycis* to the silkworm progeny and the destructive effect on the sericultural industry were clarified by Pasteur (Han, 1994; Ishihara & Fugiwara, 1965). At present, for production of *N. bombycis*-free silkworm eggs used in sericultural industry, female moths of the silkworm supposed to be inspected for pebrine after oviposition (Han & Watanabe, 1988). However, the practice of current moth inspection system is insufficient, still prevalent pebrine in the developing countries lacking in facilities and methodology. For the control of pebrine, their pathogenicity against original and hybrid silkworm races (Kim *et al.*, 1972), some experimental designs of sampling by early eclosion of the diseased silkworm (Oshima, 1965), and detection of indicative factor to forecast infected progeny populations (Oshima, 1968) have been studied. The method of immunoperoxidase-staining was one of the practical methods elevated the efficiency for discrimination of *N. bombycis* spores from those of other microsporidia observable on the course of moth inspection (Han & Watanabe, 1987). Other immunoassay techniques of indirect fluorescent antibody (Sato *et al.*, 1981) or antibody-sensitized latex

Supported in part by sericultural development project from FAO of the United Nations

(Hayasaka and Ayuzawa, 1987) were also investigated their application for the same purpose. Present research will serve essential data for the improvement of efficiency on the current system of predictive and corrective inspection to prevent the disease by *N. bombycis*.

MATERIALS AND METHODS

1. Preparation of the pathogens

Nosema bombycis of standard type was maintained in the laboratory condition, the wild type of which collected from sericultural farm was identified as *N. bombycis*. Those microsporidian spores were preserved at 5°C were preinoculated, and reproduced before experiment. *N. bombycis* spores isolated by homogenation of infected larvae or moths of the silkworm in 0.8% NaCl solution, and filtered through 3 to 4 layer of gauze. The spore suspension from the filtrate was sedimented at 3 to 5°C, repeated simple purification by separatory funnel for 3 to 4 times.

2. Assay of the symptom and transovarial transmission

Commercial silkworm varieties and other silkworm strains of genetic stock were introduced, and reared the larvae by mulberry leaves or artificial diet. Microsporidian inoculum was smeared on mulberry leaves or dropped on the artificial diet, and fed on the silkworm soon after molting at 5th instar larvae. The progeny populations from infected moths were investigated transovarial infection in the dead eggs, dead larvae on the rearing, and the newly hatched larvae starved to death. The ratio of transovarial infection was calculated as the following formula : Transovarial transmission rate (%)=(a × b+c × d)/100 : a, % of dead eggs ; b, % infection of a ; c, % of hatched larvae ; d, % infection of c.

3. Investigation of indicative factors for inspection

The infected moths dried before inspection were cut into 4 parts, thorax with head and wings, anterior abdomen includes the 1st and 2nd segment from the fore abdominal part, mid part of abdomen consists the 3rd and 4th segments, and the posterior abdomen consists the remained posterial segments. The number of microsporidian spores per dried boby weight was investigated on each part of the moths, and analyzed. Death of silkworms by infection with transovarial infection was investigated on the dead eggs, dead larvae at every larval stage of the progeny population. At the same time, the evidance of detectability for microsporidian infection of the silkworm was clarified. A specific symptom by infection was investigated on the larvae pupae and moths with egg productivity, the simple and predictive moth inpection was practiced by the artificially discharged meconium.

RESULTS AND DISCUSSION

1. Revision of larval symptom on the *N. bombycis* infection

The silkworms became small or dwarfed by infection with *N. bombycis* as a general symtom of other disease, but nether of a dark brown coloration nor dull milky-white appearance was observable through the experimental infection of silkworms consist 6,030 individuals of 22 strains through the investigation during 17 years from 1979 to 1996 (Table 1). *N. bombycis* infection of the silkworm has probably existed in the sericultural farm since very remote times, but the typical symptom has recognized at the time of its first outbreaks occurred in 1845 in France (Pasteur, 1870). The external appearance of *N. bombycis* infection may vary according to the degree and extent of infection, changes in color, size, form, and activity may be evident separately or together. However, the specific symptom such as dark pepperlike spots on the larval skin was not recognizable in the silkworm strains of Japanese, Chinese, European, and tropical races following infection with *N. bombycis* or other *Nosema spp.* (Table 1). Although, the disease is still prevelent in the tropical countries, any of color alternation from transparency nor specific spot was observable in the infection with *Nosema sp.* or other microsporidiosis investigated during the year of 1979 to 1996, in different environmental condition of Korea (1979-1984, 1992-1996), Japan (1984-1989), Philippines (1988), and Vietnam (1991).

The name "pebrine" was given to the disease in 1860 by Quatrefages, because a characteristic symp-

Table 1. *Investigation of the indicative symptom of dark pepperlike spots on the integment of the silkworm by infection with N. bombycis or other microsporidia*

Nosema sp. Infection	Silkworm Varieties	No. Larvae Investigated	No. larvae with Pepperlike Spots
Standard Type *N. bombycis*	Jam107	200	0
	Jam108	200	0
	Jam107 × Jam108	300	0
	Jam125	300	0
	Jam126	400	0
	Jam125 × Jam126	100	0
	Jam113 × Jam114	100	0
	Shunrei	200	0
	Shogetsu	200	0
	Shunrei × Shogetsu	300	0
	E-16 Shin	100	0
	Kuroko	100	0
	C-137	100	0
	Sekko	100	0
	N4	50	0
	Ringetsu	100	0
	Baghdad	100	0
Wild Type *N. bombycis*	Jam107	100	0
	Jam108	100	0
	Jam107 × Jam108	100	0
	Jam125	300	0
	Jam126	300	0
	Jam125 × Jam126	200	0
	Jam113 × Jam114	100	0
	Jam123 × Jam124	100	0
	N7	80	0
	Baghdad	100	0
Nosema sp. M11	Shunrei	200	0
	Shogetsu	200	0
	Shunrei × Shogetsu	200	0
Microsporidia M12	Shunrei × Shogetsu	200	0
	Jam119 × Jam120	100	0
	Jam117 × Jam118	200	0
Microsporidia K79	Jam117 × Jam118	300	0
	Jam119 × Jam120	200	0
Total	22 strains	6,030	0

*Data from the studies on the silkworm microsporidiosis carried out during 1979 to 1996

tom of the spots on diseased silkworm skin. It was, infact, rather like pepper grains, expressive as another word of "corpuscular speckles" (Pasteur, 1870). In these days, the "corpuscle" is generally understood as alternated meaning as the spores of microsporidia. Because they might be impossible to find any other "corpuscles" than the spores observable under microscopic field in the case of *N. bombycis* infection of the silkworm.

2. Effect of *N. bombycis* infection on egg productivity

The average number of eggs from the moths infected with standard type pathogen was less than

Table 2. *Infection of the microsporidia, N. bombycis of standard type or wild type strains, and their effect on the egg productivity in the female moth of silkworm, Bomyx mori*

Silkworm Moths Infection with *N. bombycis*	Number of Eggs		No. Total Eggs
	Fertilized	Non Fertile	
Infected with Standard Type[1)]	349.7±17.56	35.0±6.00	384.7±20.47
Infected with Wild Type[1)]	380.5±20.75	19.8±4.66	400.3±27.31
Non Infected Against Inoculation of Wild Type[2)]	480.1±15.97	10.3±3.55	490.4±19.38
Control[1)]	480.1±17.36	7.9±1.29	488.0±20.22

[1)]Each value is mean±SE from 100 moths, [2)]mean±SE from 30 moths,

those from wild type pathogen, however, appeared no significant level (Table 2). There appeared also similar tendancy on the egg productivity when compared the control of non inoculated healty moth with non infected moths against inoculation. Inoculation of *N. bombycis* could not influence on the egg productivity when it failed the infection. Egg production was significantly decreased by infection of the pathogens in the both cases of standard or wild types, and non fertilized eggs significantly increased by infection. The heavy infection appears apparent decrease of egg production (Han & Watanabe, 1988), the case of light infection with *N. bombycis* was not distinguished by egg productivity. The high infectivity with transovarial transmissibility of the standard and wild type *N. bombycis* still preserved despite the typical symptom of infection disappeared (Table 3).

Propagation of spores displays their virulence, and spore concentration per dried body weight of host represented the advance of pathogenesis. Further investigation introduced the egg batches collected from the data counted total spore numbers per moth, and each egg batches from the highest and the lowest 10 were assayed transovarial transmission for the standard and wild types of the pathogens in the silkworm (Table 3). Moths of the highest 10 contained the number of *N. bombycis* spores as many as 1.06×109 for standard type, 1.26×109 for wild type. The lowest 10 contained the less spore amount of 1/3 to 1/6 as compared with those of the highest spore content. None of the significant difference was distinguished from the assay of mortal eggs, hatchability, transovarial infection of dead eggs or newly hatched

Table 3. *Transovarial transmission investigated from the infected moth groups with standard type or wild type of N. bombycis ranked high and low in total spore content in their bodies : spore content represented as the number× 10⁸/moth*

Progeny Stage Assayed	Infection with standard type		Infection with wild type	
	Poor in Spore[1)]	Spore Rich[2)]	Poor in Spore[1)]	Spore Rich[2)]
The Range of Spore Content	1.7~3.2	9.9~11.3	2.5~4.0	10.8~15.8
Average No. Spores per Moth	2.6±0.16	10.6±0.15	3.5±0.15	12.6±0.50
% Eggs Died	27.4±6.42	19.3±6.56	17.9±0.58	10.7±1.32
% Infection of Dead Eggs[3)]	92.5±2.14	93.5±1.68	94.5±1.74	96.0±1.95
% Larvae of Hatched	72.6±6.42	80.7±6.56	82.1±2.58	89.4±1.32
% Infection of Progeny Larvae[4)]	73.5±5.53	79.0±3.15	76.5±4.09	83.5±3.58
%. Transovarial Transmission[5)]	78.7±4.62	8.19±2.16	80.3±3.09	84.8±3.21

[1)]Data from the highest 10 of moths ranked in total spore content
[2)]Data from the lowest 10 of moths ranked in total spore content
[3)]Dead eggs examined at most 20 individuals by a simple method
[4)]Newly hatched larvae examined 20 individuals per replicate
[5)]Transovarial transmission rate (%)=(a×b+c×d)/100 : a,% of dead eggs ; b, % infection of a ; c, % of hatched larvae ; d, % infection of c.

larvae. However, distinguished infection of dead eggs as high as 92 to 96%, suggested as the rational prediction and corrective inspection at egg stage for the prevalence of *N. bombycis* in the sericultural farm.

3. Spore concentration of *N. bombycis* in the moths

The change of symptom regardless of preservative pathogenicity showed the same tendency in the two strain. Although both type of *N. bombycis* strains repeated their generation for several decades in the different environment of the field and laboratory condition. The similarity between wild type and standard type was a noticeable problem suggesting an ecologically unique phenomenon (Table 3, 4). Individual female moths infected with standard type *N. bombycis* contained average number of $(7.7\pm0.60)\times10^8$ spores in the silkworm varieties of Daeseongjam, and those of Saseongjam was $(6.2\pm0.23)\times10^8$ spores. Silkworm varieties of Daeseongjam was characteristically heavier than that of Saseongjam, which resulting in the more spore amount by the difference of body weight between silkworm variety (Table 4).

Productivity of the microsporidian spores in the female moths was higher than the cases of the male moths, and the average number of spores per female moth was as high as 1.5 to 1.6 times than the male moth. The microsporidian of wild type *N. bombycis* found similar spore productivity to the infection with standard type in the commercial silkworm varieties. Remarkable reduction of weight in female moths was resulting in the reversed phenomenon of male and female in the body weight after oviposition. Female moths, heavier than males in the ordinal condition, recorded at the rate of 64.8% of those in male because of the lost in weight by the eggs laid. The higher spore content of female moths, in the face of remarkably reduced body weight after oviposition, represented the vigorous propagation of *N. bombycis* specifically in the female moths (Table 4). The high concentration of pathogenic spores provide the first principle for the revision of methodology on the current moth inspection system.

4. Revision of the predictive and corrective Inspection

Transovarial transmission of the *N. bombycis* strains of standard type and wild type were assayed in the silkworm varieties of the Saseongjam and/or Daeseongjam (Table 5). Transmission rate of *N. bombycis* in the Daeseongjam was higher than that of

Table 4. *Population of N. bombycis spores contained per dried body weight in the infected moths after crossing and ovipositon*

N. bombycis Strains	Silkworm Host		No. Moth Examined	Average Weight per Moth(mg)	No. Spore per Weight ($\times10^8$/mg)
	Varieties	Sex			
Standard Type *N. bombycis*	Saseongjam	Female	50	91.3±2.47	8.4±0.32
	Saseongjam	Male	20	141.0±3.91	3.2±0.23
Wild Type *N. bombycis*	Saseongjam	Male	33	109.4±4.44	3.5±0.24
	Daeseongjam	Male	16	124.1±6.00	4.0±0.34

Table 5. *Transovarial transmission of the N. bombycis investigated from the newly hatched larvae and dead eggs of the the silkworm*

Progeny Stage Assayed	Standard Type *N. bombycis*		Wild Type *N. bombycis*
	Saseongjam	Daeseongjam	Saseongjam
% Eggs Dead[a]	12.4±2.52	14.2±1.83	10.2±1.42
% Infection of Dead Eggs[b]	90.5±1.74	99.0±0.67	9.4±1.95
% Infection of Larvae[c]	78.8±1.60	91.0±1.84	85.4±2.45
Transovarial Transmission Rate	80.6±1.60	91.2±1.18	86.3±2.19

*100 of the newly hatched larvae inspected after starved to death

*each data Mean±SE from 10 egg batches, dead eggs were inspected at most 50 per batch.

*transovarial tranmission rate (%)=[a×b+(100-a)×c]/100

Saseongjam, while the difference between two types of pathogens was not recognizable in the silkworm varieties introduced in this study. Detectability of transovarial infection to be representative of transovarial transmission appeared very high by the inspection of dead eggs, all the cases recorded over 90 to 99%, and the transmission rate detected from the newly hatched larvae starved to death was 80 to 91%. For the corrective inspection, several of dead eggs should be collected to raise the accuracy as well as efficiency, not more than 5 eggs might be enough for 100% detection.

The death of progeny populations by infection with transovarially transmitted *N. bombycis* occurred 12.62% at the egg stage, 3.2% at 1st instar, 19.86% at 2nd instar, 11.86% at 3rd instar, 41.72 at 4th instar, and 26.56% at 5th instar at this stage reached the 100% death of accumulation. These phenomena could be reconfirmed their appearance in other commercial silkworm varieties reared on mulberry leaves, the larvae died during at the 4th instar considered as useful for predictive inspection of transovarially infected larvae with *N. bombycis* as the high lethality and proper size for individual investigation. Mortality curve of the transovarially infected progeny showed chronic, and their death during 4th instar occupied the highest proportion (Fig. 1). While, those of transovarially infected progeny populations appeared accute death during egg stage and the earlier larval stage reared on artificial diet (Han and Watanabe, 1988). An expective factors influenced on the occurrence of transovarially infected larvae and different mortality curves were suggested as the effect of feeding artificial diet on the larvae, and the different susceptibility of silkworm varieties. Histopathology reported numerous spores propagated in the body of the silkworm (Han, 1994), the life span of silkworm moth was not shortened significantly. Non of specific predictive symtom was observable by the infection (Table 6). The early eclosion of moth by infection with *N. bombycis* (Oshima, 1965 ; 1968) was also denial as a predictive symptom. On the base of the transovarial transmissibility detectable from the progeny, inspection of dead individuals on the rearing provide a useful character for the sampling on the predictive inspection. Especially, most of dead eggs in each batch would provide evidence for the transovarial inspection, individiual eggs on the slideglass as modified methods of corrective inspection, transovarial infection might be detectable at most 2 or 3 of dead eggs inspection per batch.

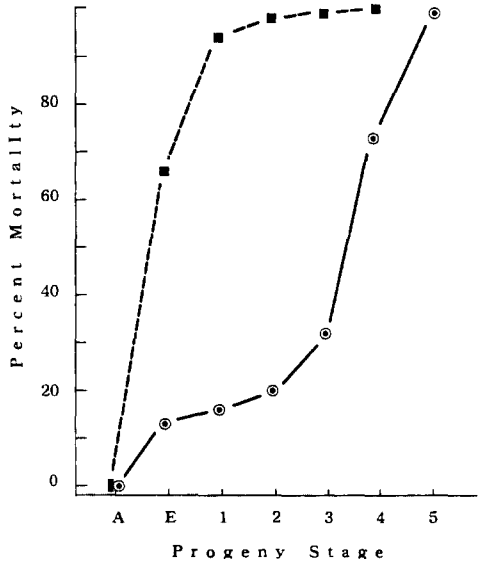

Fig. 1. *Mortality curve of the progeny populations of the silkworm reared on mulberry leaves (—⊙—), and artificial diet (--■--) following transovarial infection with N. bombycis : A, eggs soon after fertillization; E, egg stage during embryogenesis; 1-5, 1st to 5th instar larvae.*

5. Simple methods of inspection for individual silkworm

For the predictive inspection of growned 5th-instar larvae, *N. bombycis* infection was detectable without microscopic observation by the unique symptom of turbid milky-white spots on the silk gland. Inspection of the meconia artificially discharged from silkworm

Table 6. *The life span of female moths on their infection with N. bombycis in the silkworm, Bombyx mori*

Silkworm Strains	Healthy Moths	Infected Moths
Jam107	11.9±1.06	11.7±0.94
Jam126	9.4±0.77	10.2±0.92

*Each value of Mean±SE from 30 mother moths for egg production.
*Life span of the moths checked daily from the date of emergence.

moth, contained 4.6×10^6 spores/m*l* (Han, 1996), and 100% of detectability for the infected moths were comfirmed. Practice of microscopic inspection of living moths by those meconium was successful to earn the results before crossing and without killing or homogenization.

While, the moth populations were made clear their high content of pathogenic spores (Table 4), and able to conduct inspection without centrifuge. As this point of view, an establishment of the more simplistic method for the moth inspection was attempted. For the investigation of the moths with their spore content in various parts of the bodies, infected moths divided into 4 parts; the part I includes head and thorax together with the wings and legs, part II for the 1st and 2nd abdominal segment, part III for the 3rd and 4th segment, and part IV for the remained portion of hind abdomen from 5th segment (Fig. 2). Dried body weight investigated from 42 of female moths (Saseongjam) was on the average of 91.8± 2.80 mg, 41.4± 0.74% of which was occupied by thorax, and the other parts from II to IV were 18.2± 0.48%, 23.3±0.61%, 15.9±0.74%, respectively. Among the total spore content, 69% was shared by the abdominal part in spite of those weight proportioned 59%. Particulary the posterior segment of abdomen showed the higher spore concentration than anterior parts, the highest number per weight was found on the part IV as those number of $1.1\pm0.06\times107$ spores/mg. Which made contrast with the less numbers contained in thorax, $6.2\pm0.39\times10^6$ spores/mg.

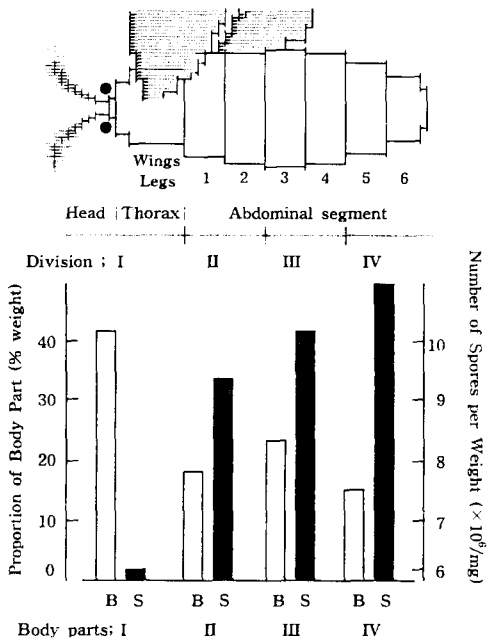

Fig. 2. *Proportion of dried body weight shared by each part and the concentration of N. bombycis spores per weight of female moth : B, proportion of body weight; S, average number of spores per weight; * division of body I=head, thorax, wings, legs; II=1st and 2nd segment; III=3rd and 4th segment; IV= 5th and the posterior segments.*

Any of the biopsy or dried sample from the hind abdominal part suggested to be introduced for the microscopic observation for the inspection of individual moth. The result provides a new method omittable the process of homogenation of the moths for inspection, the preparatory step for microscopic observation was shortened by picking the moths with clean toothpick or needles to catch the biopsy to move on slideglass. Inspection of individual moth or eggs will promote efficiency and save the facillities and labor on the predictive and corrective inspection as well as on the moth inspection for original silworm egg production to prevent *N. bombycis* infection.

적 요

새로운 집단 모아검사 체계와 수반된 예지검사 및 보정검사 방법의 개선을 시도하였다. 현대의 장려잠품종에 대하여도 표준형 및 야생형 *Nosema bombycis*는 강한 병원성과 경란전염성을 나타내었으나, 미립자병의 전형적인 증상으로 알려진 누에 피부의 미립자 병징은 현행 누에품종에서 인정되지 않았다. 그리고, 차대잠의 집단 상엽육에서는 인공사료육의 경우와는 달리 완만한 치사곡선을 나타내었다. 모아체내에 존재하는 병원포자의 부위별 분포는 頭胸部보다 後腹部가 단위체중당 포자량이 많았으며, 모아의 감염 여부를 간편하게 조사할 수 있는 개별검사에 유효하였다. 건조된 감염모아 체중당 함유된 *N. bombycis* 포자의 수는 가장 적은 경우에도 2×10^6 개/mg 이므로, 원잠종 및 원원잠종의 1아 검사에서는 시야당 최소한 300 개 이상의 포자가 관찰되었다. 따라서, 원심분리에 의한 集胞子 조작은 물론 마쇄과정을 생략하여도 감염 나방의 검출이 용이하였다. 또한, 蛾尿검사법은 마쇄조작이 불요한 동시에 살아있는 나방

의 감염 여부도 교미 산란 전에 조기진단이 가능함을 입증하였다. 경란전염된 경우 치사한 卵은 90% 이상 감염이 확인되므로 보정검사에서는 死卵을 아구당 2~5개 정도만 검사하여도 감염 아구를 판별할 수 있었고, 절식 치사한 갓 깬 누에의 경우 74~84%의 감염이 확인되었다. 큰누에때의 중증 감염은 견사선의 白濁이 특징적이므로 간이 해부법에 의한 미립자병의 진단은 현미경 검사가 불가능한 경우의 예지검사에도 유용한 것으로 인정되었다.

REFERENCES

Han, M. S. (1994) Transovarial transmission and histopathology on the gonad infection with *Nosema bombycis* in the silkworm, *Bombyx mori*. *Korean J. Seric. Sci.* **36** : 69-75.

Han, M. S. (1996) Maternal-mediated transmission of *Nosema bombycis* via the surface and internal site of silkworm eggs. *Korean J. Seric. Sci.* **38** : 48-52.

Han, M. S. and Watanabe, H. (1987) Immunoperoxidase-staining methods for discrimination of microsporidian spores in the pebrine inspection of silkworm mother moths. *J. Seric. Sci. Jpn.*, **56** : 431-435.

Han, M. S. and Watanabe, H. (1988) Transovarial transmission of two microsporidia in the silkworm, *Bombyx mori*, and disease occurrence in the progeny population. *J. Invertebr. Pathol.*, **51** : 41-45.

Hayasaka, S. and Ayuzawa, C. (1987) Diagnosis of microsporidian, *Nosema bombycis* and closely related species by antibody-sensitized latex. *J. Seric. Sci. Jpn.*, **56** : 169-170.

Ishihara, R. and Fujiwara, T. (1965) The spread of pebrine within a colony of the silkworm, *Bombyx mori Linnaeus*. *J. Invertebr. Pathol.*, **7** : 126-131.

Kim, J. S. *et al.* (1972) Studies on the pathogenicity of *Nosema bombycis* Naegeli to the parents and hybrid silkworms, *bombyx mori, in Korea. Seric. J. Korea,* **14** : 81-93.

Oshima, K. (1965) Experimental design on a sampling inspection method of Pebrine control by utilizing the characterized by early eclosion of diseased silkworm moths with pebrine of a lot. *Jpn. J. Appl. Ent. Zool.*, **9** : 83-88.

Oshima, K. (1968) Experimental design on a sampling inspection method of Pebrine control by utilizing the characterized by early eclosion of diseased silkworm moths with pebrine of a lot, VI. *J. Seric. Sci. Jpn.*, **37** : 408-419.

Pasteur, L. (1870) Etudes sur lar maladie des vers a soie ; Tom. 1, La pebrine et la flacherie, Gauthier-Villars, Paris. 322 pp.

Sato *et al.* (1981) Serological discrimination of several kinds of microsporidian spores isolated from the silkworm, *Bombyx mori*, by an indirect fluorescent antiboby techniques. *J. Seric. Sci. Jpn.*, **50** : 180-184.