植保會刊 25：157～166 (1983)
Plant Prot. Bull. (Taiwan, R.O.C.) 25：157～166 (1983)

SWOLLEN FRUIT OF MULBERRY IN TAIWAN: THE PATHOGEN, DISEASE CYCLE AND FACTORS AFFECTING THE APOTHECIUM FORMATION

Y. S. Lin and J. Y. Lo

Department of Plant Pathology, Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan, R. O. C.

（接受日期：民國 72 年 5 月 20 日）

摘　　要

林益昇　羅靜儀 (1983) 臺灣之桑腫果病：病原菌、病害圈及影響子囊盤形成之因子
植保會刊 25：157～166
（臺灣省農業試驗所研究報告第 1098 號）

Ciboria shiraiana 引起桑 (*Morus australis*) 腫果病，僅爲害果實，不侵害枝條或葉片。被害果實外表先呈灰白色，內部形成一個菌核，最後木乃伊化掉落地上，越冬菌核於翌春形成子囊盤 (1～21子囊盤/菌核) 產生子囊胞子感染桑花。子囊胞子在8～28°C間皆可在水瓊脂上發芽，但是發芽管在十四種供試培養基中皆不能長成菌落，終於崩解。*C. shiraiana* 對環境條件的要求甚嚴，僅在充足的水分及 12～16°C 之條件下才能形成正常的子囊盤，而且形成的時機要符合桑之開花期，才能引起病害。倘若二月間之氣溫過超 16°C，則會抑制子囊盤的形成並減少病害。在實驗室中 atrazine 及 diuron 等殺草劑和 benomyl，PCNB 及 ronilan 等殺菌劑，皆可抑制子囊盤的形成。

（關鍵字：桑，腫果病 *Ciboria shiraiana*，病害圈）

ABSTRACT

Swollen fruit of mulberry (*Morus australis* Poir.) is caused by *Ciboria shiraiana* (Henn.) Whetzel. It attacks the fruit only, not leaves or twigs. Characteristically, the affected fruit developed a single sclerotium inside and had a grayish white appearance at the beginning. Then, they became mummified on the tree and eventually dropped. The overwintered sclerotia formed 1-21 apothecia each from which ascospores were produced for causing infection the next spring. Ascospores germinated in a temperature range of 8-28°C on water agar. However, the germ tubes failed to grow on the 14 media tested, and finally lysed. *C. shiraiana* appeared to have very narrow environmental requirements. Normal apothecia were produced only at 12-16°C, with adequate moisture. For infection the formation of apothecia should match the blooming period of mulberry during Feburary in central Taiwan. A temperature above 16°C in Feburary would inhibit apothecial formation and lessen the disease

incidence. Herbicides such as atrazine and diuron, and fungicides such as benomyl, PCNB, and ronilan inhibited the formation of apothecia in the laboratory.

(Key words: mulberry, swollen fruit, *Ciboria shiraiana*, disease cycle)

INTRODUCTION

A swollen fruit disease causes more than 90% yield reduction on accession 19 of mulberry (*Morus australis* Poir.) released from the Sericulture Improvement Station, PDAF, in central Taiwan. The disease has not been fully described since no fruiting structure of the fungus has been observed (S. K. Sun & L. S. Leu, personal communication). The swollen fruit disease of mulberry in Asia has been reported in China[3], Taiwan[4], and Japan[3] on *M. alba* and *M. acidosa*. The causal organism was described as *Sclerotinia shiraiana* by Hennings[2], based on Japanese specimens. However, Whetzel[7] thought that the Asiatic mulberry fungus should also belong to *Ciboria shiraiana*, and should be distinguished from *C. carunculoids*, which causes popcorn disease on American mulberry[5,6]. In this paper, we describe the pathogen, disease cycle and factors affecting apothecium formation.

MATERIALS AND METHODS

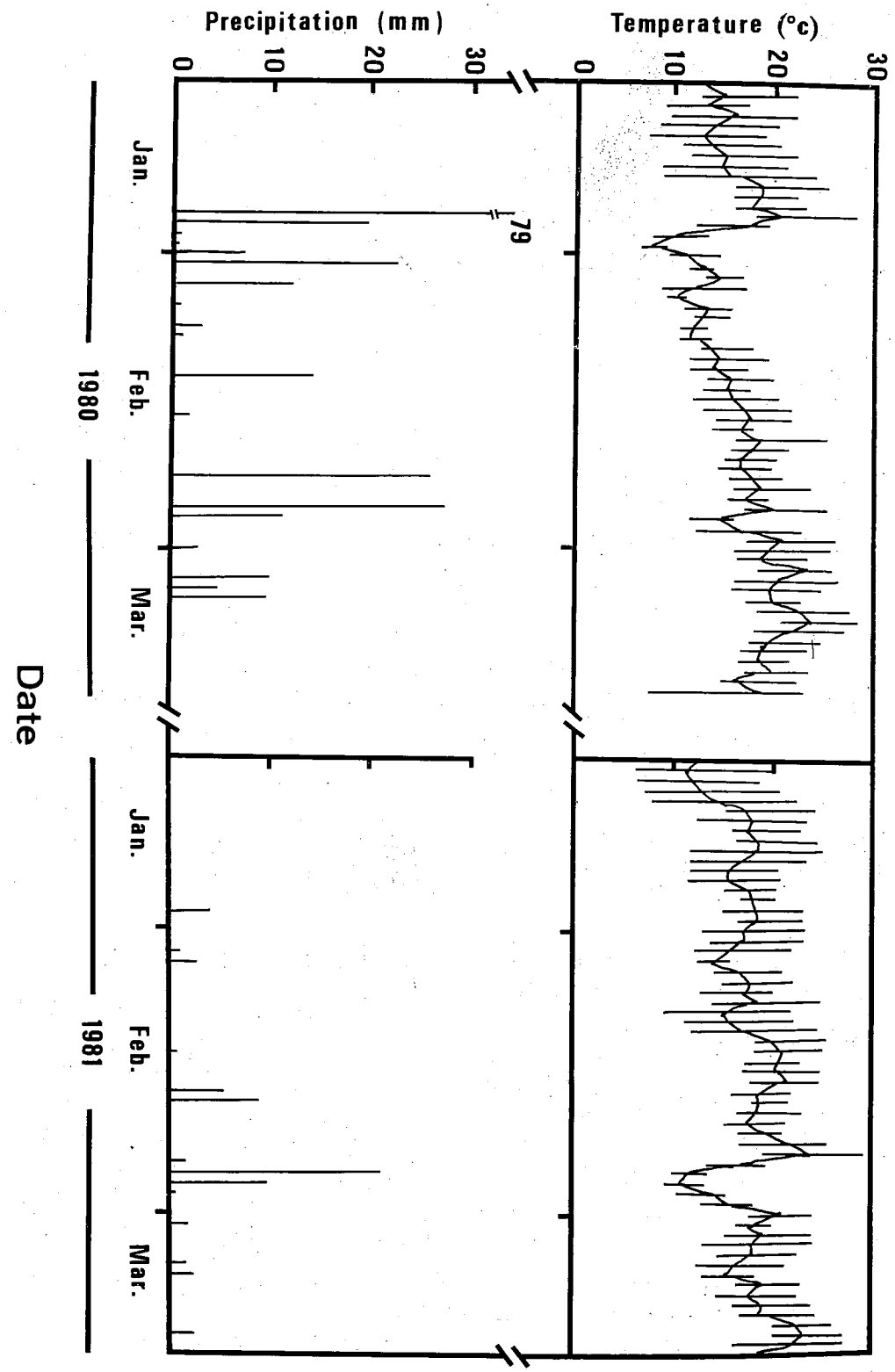
Field observation and isolation of the pathogen

Field observations and climate data, including temperature and precipitation, were conducted and recorded from 1980 to 1982 in the farm of the Taiwan Agricultural Research Institute (TARI) and in the Nantou area of central Taiwan. Isolation was made from sclerotia or ascospores using 2% water agar (difico agar), potato dextrose agar (PDA), acidified PDA, V-8 agar, yeast extract agar, radish dextrose agar, PDA plus 2-3% of mulberry fruits, autoclaved mulberry fruits, PDA plus extract of young catkins (5 g young catkins plus 20 ml of distilled water, ground, centrifuged for 15 min (3000 rpm), and then filtered through a millipore filter), autoclaved or fumigated (by propylene oxide) young catkins and basal medium[1] (NH_4NO_3 3 g, KH_2PO_4 2 g, $MgSO_4 \cdot 7H_2O$ 0.75 g, $FeSO_4$ 0.002 g, dextrose 10 g, difco agar 20 g, distilled water 1000 ml) added different concentrations of vitamins (ascorbic acid 0.1 ppm, biotin 0.005 ppm, inositol 5.0 ppm, pyridoxin hydrochloride 0.1 ppm, riboflavin 0.1 ppm or thiamine hydrochloride 0.1 ppm) or indoacetic acid (IAA) (0, 0.1, 1 or 10 ppm). All treatments were incubated at 8, 12, 16, 20, 24 or 28°C.

Pathogenicity tests

The development of stigmata was divided into 3 stages: (1) the stigma is purple and closed; (2) the stigma is white and open; and (3) the stigma is black and wilted. All three stages were inoculated by ejecting ascospores from apothecia or by spraying ascospore suspension onto the stigma. All inoculated flowers were wraped with a plastic bag for 24 hr, and then moved to a screen house at 10-16°C. Besides, *Botrytis cinerea* isolated from overwintered sclerotium was also used as inoculum in the pathogenicity tests as check.

Effects of temperature and moisture


Precipitation (mm)
Temperature (°c)
0
10
20
30
79
Jan.
Feb.
Mar.
1980
1981
Date

on the formation of apothecia

Sclerotia collected from the field in the springs of 1980 and 1981 were put on moist sand (field capacity) in petri dishes (12 cm in diamenter) and then incubated at 8, 12, 16, 20, 24 or 28°C, under 12-hr alternative photoperiod (2,400 lux). Each temperature had 3 replicates. Moreover, another set of sclerotia incubated at 12°C until initials of apothecia had developed and were moved to 20 or 24°C for 5 days, then moved back to 12°C.

Effect of herbicides and fungicides on the formation of apothecia

Herbicides, including alachlor (2-Chloro-2′-6′-diethyl-N-(methoxy methyl)-acetanilide), atrazine (2-Chloro-4-ethylamino-6-isopropylamino-5-triazine), dalapon (2,2-Dichloropropionic acid), diuron (3-(3,4-Dichlorophynel)-1,1-dimethylurea), glyphosate (Isopropylamine salt of *N*-(phosphonomethyl) glycine, paraquat (1,1'-Dimethyl-4,4′-bipyridinium Dimethyl sulfate or Dichloride compounds), treflan (a,a,a-Trifluoro-2,6-dinitro-*N*,*N*-dipropyl-p-toluidine) and fungicides including benomyl (Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate), dicloran (2,6-Dichloro-4-nitroaniline), PCNB (Pentachloronitrobenzene), sumilex (*N*-(3′,5′-dichlorophenyl)-1,2-dimethyl cyclopropane-1, 2-dicarboximide) were tested for inhibiting the development of apothecia in the laboratory. Overwintered sclorotia collected from the field were put on sand in petri dishes (12 cm in diameter) and moistened by each chemical solution at concentrations of 10, 100 or 500ppm and incubated at 12°C. The development of apothecia from these sclerotia (10 dishes per treatment) were recorded.

RESULTS

Description of the disease and the pathogen

Field observations were conducted from 1980 to 1982 in the farm of TARI and the Nantou area of central Taiwan. The fruits showed diagnostic swollen symptoms in March (Fig. 1). They had a grayish white appearance at the beginning,



Fig. 1. Symptoms of swollen fruits (arrow) of mulberry caused by *Ciboria Shiraiana*.



Fig. 2. Apothecia (A) produced from overwintered sclerotia (S) on the ground.

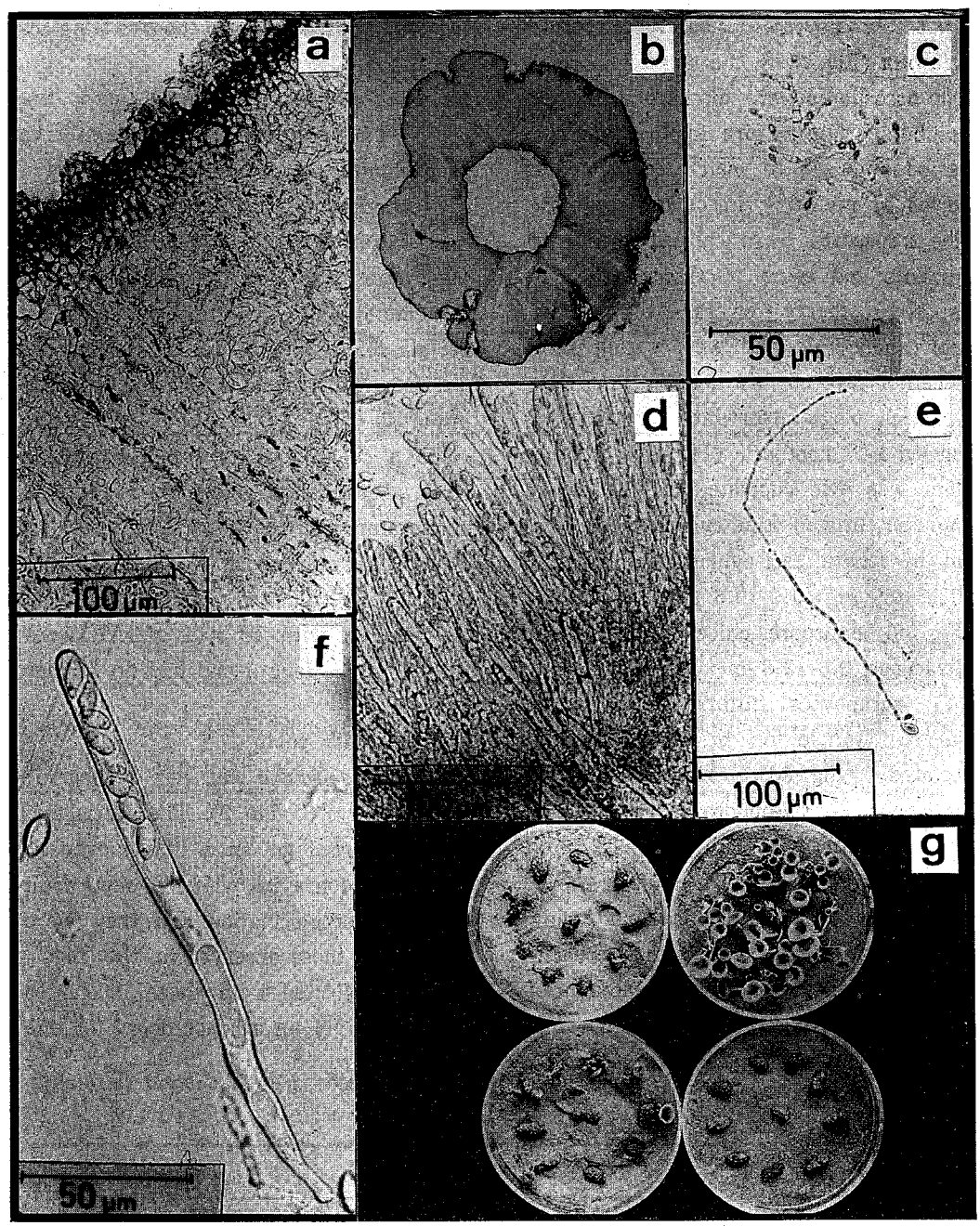


Fig. 3. *Ciboria shiraiana*. a, b··· Cross section of sclerotium. c··· Microconidia on spermatiophores. d··· Hymnium. e··· Germination of ascospores. f··· Ascus and ascospores. g··· Effect of temperature on the apothecium development on moist sand, from left to right: 8°C, 12°C (upper row), 16°C and 20°C (bottom row).

then became mummified when still on the tree in April and finally dropped. Apothecia developed on overwintered sclorotia (Fig. 2) in the next February. The ascospores were discharged in great numbers from mature apothecia over a period of 3 wk, to match the blooming period of mulberry in central Taiwan. The ascospores were carried up by air currents and some of them lodged on exposed mulberry flowers. At this time, cool (about12°C) and wet (spring rain) weather favored infection.

Each single overwintered sclerotium formed 1-21 apothecia (Fig. 2). Apothecium was disc cupulate, 0.5-2.3 cm in diameter, brown; stipe cylindrical, 0.5-1.8 cm in length; asci cylindrical to clavate, 102.6-159 μm×3.9-10.3 μm, 8 spored (Fig. 3f); ascospores unicellular, ellipsoidal, olivaceous, 9.0-12.8 μm×3.9-6.4μm (Fig. 3 f); paraphyses filiform to cylindroclavate, simple or branched, usually septated (Fig. 3d); microconidia on branched spermatophores in matrix, hyliane, ovate, 2.1-4.6 μm×1.8-3.8 μm (Fig. 3c); sclerotium black, mulberry fruit shape with depressed surface and hollow inside (Fig. 2, 3b); rinds with sclerenchmatious cells; medulla with remnants of host tissue (Fig. 3a); without imperfect stage. Infection occurs only on flowers, not on leaves or twigs of mulberry, by inoculating ascospores on the live stigmata of catkins.

Isolation of the pathogen

No pure cultures of *C. shiraiana* have been obtained from any medium used in the experiments. Sometimes, a few *B. cinerea* grew out from vulnerable sclerotia. Most ascospores ejected from a piece of hymenium (Fig. 3 d) onto media did germinate. However, the germ tubes did not branch and could only grow up to 300 μm or so (Fig. 3e). They did not develop in to a colony and but lysed within one week, especially when the incubated temperature was above 20°C (Table 1). Germ tubes on media with 0, 0.1, or 1 ppm of IAA collapsed within 3 days, and the medium with 10 ppm of IAA were only 10 μm in length and had no further growth.

Pathogenicity tests

Ciboria shiraiana was very pathogenic on mulberry (Table 2). The inoculated flowers started to show the diagnostic symptoms of grayish white swollen fruit 45 days after inoculation and they were identical to those observed in the field. Sclerotia collected from those inoculated fruits were put on sand outdoors for one

Table 1. Effect of temperature on the germination of ascospores of *Ciboria shiraiana* on agar medium

Temp.	Germination (%)	Lysis (%)
8	52	11
12	56	19
16	61	11
20	68	94
24	44	99
28	28	100

Table 2. Percentages of diseased fruits of mulberry inoculated with ascospores of *Ciboria shiraiana* onto three stages of developing stigmata

Inoculation method	Diseased fruit (%)		
	Stage I Stigma not spread	Stage II Stigma spread	Stage III Stigma wilted
Ejection of ascospores	100	100	—
Spray of ascospore suspension	5	24	0

Table 3. Percentages of sclerotia fromating initials (I) or mature apothecia (MA) on moist sand incubated at different temperatures

Temperature (C)	Incubation (day)				
	30	45	60	75	90
8	90(I)	90(I)	90(I)	90(I)	90(I)
12	100(I)	100(I)	100(MA)	100(MA)	0
16	60(I)	60(MA)	0	0	0
20	0	0	0	0	0

Table 4. Percentges of apothecium developed from sclerotia which were treated with different chemicals.

Chemical	Concentration (ppm)			
	0	10	100	500
CK	100			
Herbicide				
Alachlor		90	70	90
Atrazine		0	0	0
Dalapon		90	80	70
Diuron		90	80	10
Glyphosate		90	100	100
Paraquat		90	90	60
Treflan		90	80	—
Fungicide				
Benomyl		90	—	30
Dicloran		50	90	90
PCNB		90	0	0
Ronilan		70	0	70
Sumilex		70	90	70

year. They developed apothecia in the next spring, except in 1981. On the other hand, no swollen fruits were observed when the stigmata were inoculated with *B. cinerea*. Some inoculated flowers only showed early blight symptoms. The sclerotia of *B. cinerea* collected from PDA cultures could not develop any apothecium in the next spring. Therefore, *B. cinerea* was proven not to be the imperfect stage of *C. shiraiana*.

Effect of temperature and moisture on the apothecium formation

In the filed, many sclerotia were observed on the ground in the winter and many apothecia arose from all the visible sclerotia in the springs of 1980 and 1982, but no one in 1981. The data of temperature and precipitation from Janurary 15 to March 15 of 1980 and 1981 are presented in Fig. 4. Both temperature and precipitation had vital effects on the formation of apothecia.

The laboratory results (Fig.3g, Table 3) showed that moist sand favored the development of apothecia and 12°C was the optimum temperature. Dry sand inhibited apothecial formation. Sclerotia incubated at 12°C could develop initials after 30 days, mature apothecia after 60 days, and collapsed after 90 days. Sclerotia incubated at 16°C could also develop a few mature apothecia after 60 days, but collapsed after 75 days. Nothing grew out from sclerotia incubated at or above 20°C, and only initials could be obtained when incubated at 8°C (Table 3). In another experiment, sclerotia incubated at 12°C until initials of apothecia had developed, were moved to 20 or 24°C for 5 days, then moved back to 12°C. The results showed that only 24°C had a lethal effect on the formation of apothecia from such treated sclerotia.

Effect of chemicals on the apothecium formation

The results (Table 4) showed that atrazine completely inhibited the formation of normal apothecia at or above 10 ppm and ronilan and PCNB at or above 100 ppm, and that diuron and benomyl could only inhibit most treated sclerotia at 500 ppm.

DISCUSSION

Our pathogen only attacks live mulberry flowers, failed to grow on agar media or other artificial media and imperfect stage has not been found yet, although *B. cinerea* was isolated from diseased fruits and overwintered sclerotia occassionally. All druplets are merged into one stroma which includes host remnants. The spores are ellipsoid to olive shape. Therefore, the fungus was identified as *C. shiraiana* (Henn.) Whetzel.

Soil moisture and temperature were important in sclerotial germination and apothecial development. In the laboratory, sclerotium germination was favorable by a cool temperature and apothecial development favored by a little bit higher temperature with adequate soil moisture, although the minimum requirements of soil moisture were not determined. A temperature of 8°C is sufficient to stimulate germination, but too cool for apothecial development, while 12 to 16°C was optimum and 20°C was too high for both germination and apothecial development. Ascospores could germinate under 8-28°C. However, temperatures above 20°C increased the autolysis of the germ tube. In the field,

during late Janurary to Feburary, there usually was low temperature (below 16°C), and high moisture (spring rain) in Taiwan as in 1980 (Fig. 4). In 1982, weather condition was very similar to those of 1980. However, the abnormal climate conditions in 1981 with high temperature (above 16°C), and low moisture (Fig. 4) might account for the fact that no apothecia and disease were observed in the field. The environmental requirements of *C. shiraina* are quite narrow. The time of apothecial development should match the blooming period of mulberry, with cool temperature and adequate moisture in February in Taiwan. Otherwise, no disease would be observed.

Chemical tests showed that herbicides of atrazine, diuron and fungicides of benomyl, PCNB and ronilan might inhibit the formation of normal apothecia (Table 4). For practical use in the future, atrazine may be the first choice for applying in early spring, for both disease and weed control.

LITERATURE CITED

1. Batra, L. R. 1963. Habitat and nutrition of *Dipodascus* and *Cephaloscus*. Mycologia 55:508-521.
2. Hennings, P. 1900. *Sclerotinia Shiraiana*. In Engler's Jahrb. 28:278.
3. Kimura, K. 1979. Mulberry Diseases of Japan. 163p. (In Japanese).
4. Sawada, K. 1919. Descriptive catalogue of the Formosan fungi. Part I. p 246-247. Agric. Expt. Sta. Goverment of Formosa, Special Bull. No. 19 (In Japanese).
5. Siegler, E. A. and A. E. Jenkins 1922. A new *Sclerotinia* on mulberry. Science (N.S.) 55:353-354.
6. Siegler, E. A. and A. E. Jenkins 1923. *Sclerotinia carunculoides*, the cause of a serious disease on the mulberry (Morus alba) J. Agr. Res. 23:833-836.
7. Whetzel, H. H. 1945. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate discomycetes. Mycologia 37:698-714.
8. Whetzel, H. H. and F. A. Wolf. 1945. A cup fungus, *Ciboria carunculoides*, pathogenic on mulberry fruits. Mycologia 37:476-491.