Plant Pathology Bulletin 1: 70-78, 1992

Peach Gummosis Disease Caused by *Botryosphaeria dothidea* in Taiwan

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Accepted for publication: July 29, 1992.

ABSTRACT

Ko, Y., and Sun, S. K. 1992. Peach gummosis disease caused by *Botryosphaeria dothidea* in Taiwan. Plant Pathol. Bull. 1:70-78.

A gummosis disease on low-land peach trees has been found occurring severely in recent years in Taiwan. The disease has seriously affected many thousands of trees in central part of Taiwan where peach was grown economically. *Botryosphaeria dothidea* (Moug. *ex* Fr.) Ces. et de Not. was always found from free hand sections and cultures made from diseased twigs, limbs and trunks of the peach trees. Inoculation of current-season shoots with the fungus isolated from diseased tree showed typical symptoms which indicated that *B. dothidea* was the causal agent. Principal symptoms including sunken necrotic lesions in bark around lenticels, from which gum exudated, brown necrotic areas in bark beneath infected lenticels, and convex warts on surfaces of infected twigs and shoots are characteristic. Anamorph stage of *B. dothidea*, *Fusicoccum* sp., is common in nature. Pycnidia are black or dark brown, 143–207 μm in diameter, with hyaline and non-septate conidia, 12.5–17.8 × 3.8–5.0 μm. Ascostroma are black, 173–248 μm in diameter, usually produced in dead wood. Asci are cylindrical, eight-spored, bitunicate, 95.0–123.5 × 12.7–18.5 μm. Ascospores are hyaline, one-celled and ovoid, 22.5–30.0 × 9.3–12.5 μm. Growth rate was greatest on potato-dextrose agar (PDA) and oatmeal agar after 72 hr, and least on V-8 juice agar and peach-shoot decoction agar. The optimum temperature for mycelial growth were 24–32 C and for conidial germination was 20–36 C. For elongations of germ tube, 28 C was the optimum (134 μm after 4 hr incubation). Conidial germination in 2% water agar reached 96% in 4 hr at 24 C and declined as water potential reduced from −1 bar to −45 bars, no germination was observed at −55 bars. Continuous light is essential for the promotion of conidial formation. Under 128 ± 25 $\mu E \cdot m^{-2} \cdot s^{-1}$ condition, approximately 2.1–3.6 × 10^5 conidia/cm^2 could be harvested from 7-day to 9-day-old PDA culture. Literatures revealed that the peach gummosis disease is a new record in Taiwan.

Key words: peach gummosis, *Botryosphaeria dothidea*.

INTRODUCTION

Although peach [*Prunus persica* (L.) Batsch.] has been grown for several hundreds of years in Taiwan, it was never considerated as an economically important crop until 1958 when large quantity of peach seedlings were introduced from Japan and planted them in Lishan areas at the altitude around 2,000–2,500 m above sea level. Peach growth was quite successful and very profitable, but diseases such as leaf curl (*Taphrina deformans* (Berk.) Tul.) and Armillaria root rot (*Armillaria mellea* (Vahl.) Quel.) were serious (3,4). A time pasts by, soil erosion in peach orchards in Lishan areas are getting serious, so peach cultivation was not encouraged. As a consequence, farmers began to grow low-land peaches in central part of Taiwan including Tung-Shih, Cho-Lan and Hsin-She counties. The acreage is still increasing and extend to Heinchu and Chiayi prefectures (1). Under the tropical warm temperature, peach leaf curl is suppressed and Armillaria root rot has not been found so far. However, by the year of 1989 (2), peach gummosis appeared to be a great problem. As farmers recalled, gummosis disease had been there several years after peach has been planted a decade ago.

Pycnidia and conidia of *Fusicoccum* sp. were found on old dead twigs and stroma embeded with perithecia and asci of *Botryosphaeria* sp. were found on old dead twigs (2). Literatures indicates that this is a new record of peach diseases in Taiwan. The study was undertaken to isolate the suspect fungus, determine its pathogenic role, and identify it.

MATERIALS AND METHODS

Isolation of the causal agent

In order to isolate fungi from peach twigs, samples were collected from 12 peach trees in each of 12 orchards in which 120 samples with apparently healthy twigs, 240 samples with diseased twigs but without gum and 240 samples with gum were collected.

Three or four years old diseased peach twigs were periodically collected from 12 orchards in Taichung, Nantou, and Miaoli prefectures. The diseased peach twigs were cutted into 1-cm pieces and surface-sterilized in 0.5% sodium hypochloride for 1 min, then rinsed in sterile water, and dried on sterile filter paper, these tissue pieces were then transferred on acidified Difco potato-dextrose agar (APDA) (2.5 ml of a 25% solution of lactic acid per liter of medium). All dishes were incubated in dark at 25 ± 1 C for 3 days. Hyphal tips were cutted off by sterilized transferred needle and transferred onto petri dishes or agar slants. Sporulation was observed periodically.

Single ascospore cultures were obtained by sticking a perithecial bearing twig on inside-top of petri dish cover, moisting it by drapping bit of sterilized water, and then covered it on bottom dish which containing water agar. Put the disher on inclined angle by overnight. Ascospores that discharged onto the agar plate could be picked up by transfer needle singly and transferred it into agar tubes.

Pathogenicity test

The preparation of conidia suspension was made from tissue culture as well as from single ascospore cultures which were grown under continuous light on Difco PDA slant culture. Conidia were obtained from 8–10 days culture by scrapping off the sporulating mycelium from surface of PDA slant culture and blending the remaining mycelia and pycnidia for 30 sec. in sterile distilled water in a Polytron blender (Kinematica, Switzerland). The resulting suspension was strained through 3 layers of cheesecloth to exclude the mycelial fragements. The spore suspension was diluted to the desired concentrations based on counts of conidia in a hemacytometer.

Pathogenicity test for the isolated fungus was carried out in greenhouse. Five local peach varieties, "Ying-Ko", "San-Yueh", "Liu-Yueh", "Pe-Feng", and "Tang-Tao", were used. Three-month old shoots were surface sterilized by 70% ethanol and wound with bunch of 4-needle head. Each inoculations were made either with one 5 mm mycelial discs or with 0.2 ml 10^5 conidia/ml spore suspension. The inoculated area was wrapped immediately with cotton saturated with sterile water, and covered with polyethylene bags to maintain high humidity. The inoculations were replicated on 2 shoots each of ten trees. Control shoots were inoculated with PDA discs or sprayed with sterile water. The polyethylene and cotton were removed from the branches 7 days after inoculation. Inoculated branches were observed at 2-wk intervals for symptoms development. The causal agent was reisolated from lesions produced by inoculation to complete the Koch's postulates.

Incidence of gummosis in field

Incidence of gummosis in 23 peach orchards, located in Taichung, Nantou and Miaoli, a major peach growing areas, were investigated during 1989–1991. A orchard had more than 100 2-3-year-old peach trees were choosen at random for observation. The incidence of disease was evaluated by the appearance of gummosis and protuberance on the bark.

Effect of temperature and culture medium on the growth of *B. dothidea*

Five kinds of media were used, *i.e.*, potato-dextrose agar (PDA), oatmeal agar (OMA), V-8 juice agar (V-8A), peach-shoot decoction agar (PSDA) and Czapek's agar (CA). Each agar plate was seeded with a 5-mm mycelial disc from the periphery of 3-day-old cultures of *B. dothidea*. All dishes were incubated in the dark at 12, 16, 20, 24, 28, 32 and 36 C for 3 days. Each set of treatments was 4 replicates. Colony diameters were recorded at 24-hr intervals.

Effect of temperature on conidial germination and germ tube development

Conidia were harvested from PDA culture as described earlier. Conidia were incubated on 2% WA in dark for 4 hr at 8, 12, 16, 20, 24, 28, 32 and 36 C. A minimum of 300 conidia per plate were counted. A conidium was considered to have germinated if the germ tube was at least one-half the length of the spore. Germ tube length was determined at 4 hr by measuring 30 germ tubes selected randomly.

Effect of osmotic water potential on conidial germination

The water potential for study was related to the NaCl molarity according to the values given by Lang (12) and Robinson *et al.* (15). Water potential at 0, −1, −5, −15, −25, −35, −45, and −55 bars were obtained by amending 2% WA with 0.02, 0.11, 0.33, 0.54, 0.76, 0.98 and 1.2 M NaCl, respectively. One drop of conidial suspension was placed in a petri dish containing 15 ml of WA amended with sodium chloride as previously described. Plates were sealed with Parafilm (American Company, Greenwich, CT) and incubated at 16, 24 and 32 C. After 4 hr, conidial germination was assessed. Each set of treatments was replicated four times.

Effect of light on sporulation

The studies were conducted under artificial light of known constant light intensity with continuous exposure. The cultures were placed 20 cm below 3 20-watt-fluorescent tubes with light intensity of 128 ± 25 $\mu E \cdot m^{-2} \cdot s^{-1}$ in

incubator at 25 C. For total darkness, the cultures were wrapped with black polyethylene bags as control. Each treatment was 4 replicates. The culture were examined at 2-day intervals for conidial estimation. Methods for collection of conidia was similar to the preparation of spore suspension as described previously. Then, the filtrated spore grindings were diluted to 10 times with sterilized distilled water and counted in a hemacytometer.

RESULTS

Symptomology

Peach gummosis is primary associated with lenticels. Limb and twig infections begin around lenticels as small, sunken lesion or sunken discolored areas at the margins of wounds. Following infection, the bark becomes depressed and then convex warts appeared (Fig. 1). These convex warts often cracked, rupture the epidermis and gum liquid exuded. When the outer surface of the warts was removed from crack and lenticels, circular or oval brown necrotic areas were found (Fig. 2). In severe diseased trees, gum deposits covered the surface of entire limbs, twigs and trunks (Fig. 3). After heavy rainfall, gum exuded abundantly and often piling the ground beneath the trees. During winter season, black stroma were produced embeded in the bark of infected lenticels of diseased trees or dead wood (Figs. 4 and 5). Although diseased trees do not die, but thrifty growth induced dieback of some small twigs.

Isolation and identification of the causal pathogen

During 1990 growing season, *B. dothidea* was isolated 10, 71 and 76% of the twig samples from healthy, diseased without and with gum, respectively. The incidence of *B. dothidea* in isolation from 2-yr-old diseased twigs was 100% and in 3- and 4-yr-old diseased twigs 96 and 93% respectively.

During the winter season, anamorph and teleomorph stages of *B. dothidea* were produced from stroma in the lenticels on the bark of diseased trees (Fig. 6). Identification of *B. dothidea* was based on morphological features of conidia from nature anamorph phase and that of artificial culture (Table 1). Ascospores from nature-infected wood were also examined. Identification was confirmed by Dr. A. E. Sivanesan, invited mycologist from Commonwealth Mycological Institute, United Kingdom.

Fusicoccum, anamorph stage of *B. dothidea*, is most common in nature. The pycnidia are black or dark brown, 143–207 μm in diameter (Fig. 7). Conidia are hyaline and nonseptate, 12.5–17.8 × 3.8–5.0 μm (Fig. 8). Ascostroma black, 173–248 μm in diameter (Fig. 9), are usually produced in dead wood. Asci are cylindrical, eight-spored and bitunicate, 95.0–123.5 × 12.7–18.5 μm (Fig. 9). Ascospores are hyaline, one-celled and ovoid, 22.5–30.0 × 9.3–12.5 μm. Single ascospore and conidial cultures on PDA were made form comparison (Figs. 10 and 11). Former records of *B. dothidea* are summarized for comparison (Table 1).

Pathogenicity tests

Small, sunken brown necrotic lesions were developed, 2 weeks after wound inoculation on 3-month-old shoots of each tested varieties, either with mycelial discs or with spore suspensions (Table 2). Exudes with gummy liquid was produced 3 wk after inoculation. Eight weeks later, the sunken brown necrotic lesion was more obvious with more gummy deposits. Within 8–12 wk after inoculation, symptoms could be observed in 1-year-old shoots. None of the controls showed symptoms. Reisolation from inoculated shoots showing symptoms yielded *B. dothidea*. Inoculation made on non-wounded twigs showed no symptoms.

Incidence of gummosis in the field

During 1990–1991, more than 75% of the plants showing gummosis in 23 orchards surveyed. In May, 1991, the occurrence of gummosis appeared more widespread than in 1990. An average of 61% of 2100 plants examined showed gummosis symptoms (Table 3). All plants in 23 peach orchards were affected.

Results of the survey did not indicate correlation of occurrence of gummosis and peach variety. Usually, infected plants were scattered over the fields, however, in some instances, they were localized in poorly managed and drained areas.

TABLE 1. Size of conidium, ascus and ascospore of *Botryosphaeria dothidea* on different host plants

Host plant	Source of culture	Conidium (μm)	Ascus (μm)	Ascospore (μm)
Currant	Grossenbacher *et al.* (9) and Punithalingam (14)	16–23×7–9	100–110×16–20	15–24×6–10
Blueberry	Witcher et al. (24)	16–22.9×4.9–9.7	83–98×16–19	16–26×6–10
Apple	Jones et al. (11)	19.8–23.1×6.4–6.6	115–135×13.5–16.5	19–23×7.5–8.5
Grape	Kuo et al. (5)	11.5–25.0×2.5–5.0	80.0–127.3×12.0–23.3	19.7–32.0×5–9.4
Peach	Weaver (22)	15.2–28.8×4.8–8.0	120×19	17.5–28×9.3–12.0
Peach	The authors	12.5–17.8×3.8–5.0	95–123.5×12.7–18.5	22.5–30.0×9.3–12.5

Fig. 1–5. Symptoms of peach gummosis caused by *Botryosphaeria dothidea*: (1) convex warts or protuberances on twigs and limbs; (2) surface of bark removed from infected twigs, showing necrotic areas; (3) deposits of gum on limbs and twigs of infected tree; (4) stroma on dead wood; (5) close-up of stroma embeded in lenticels of dead wood.

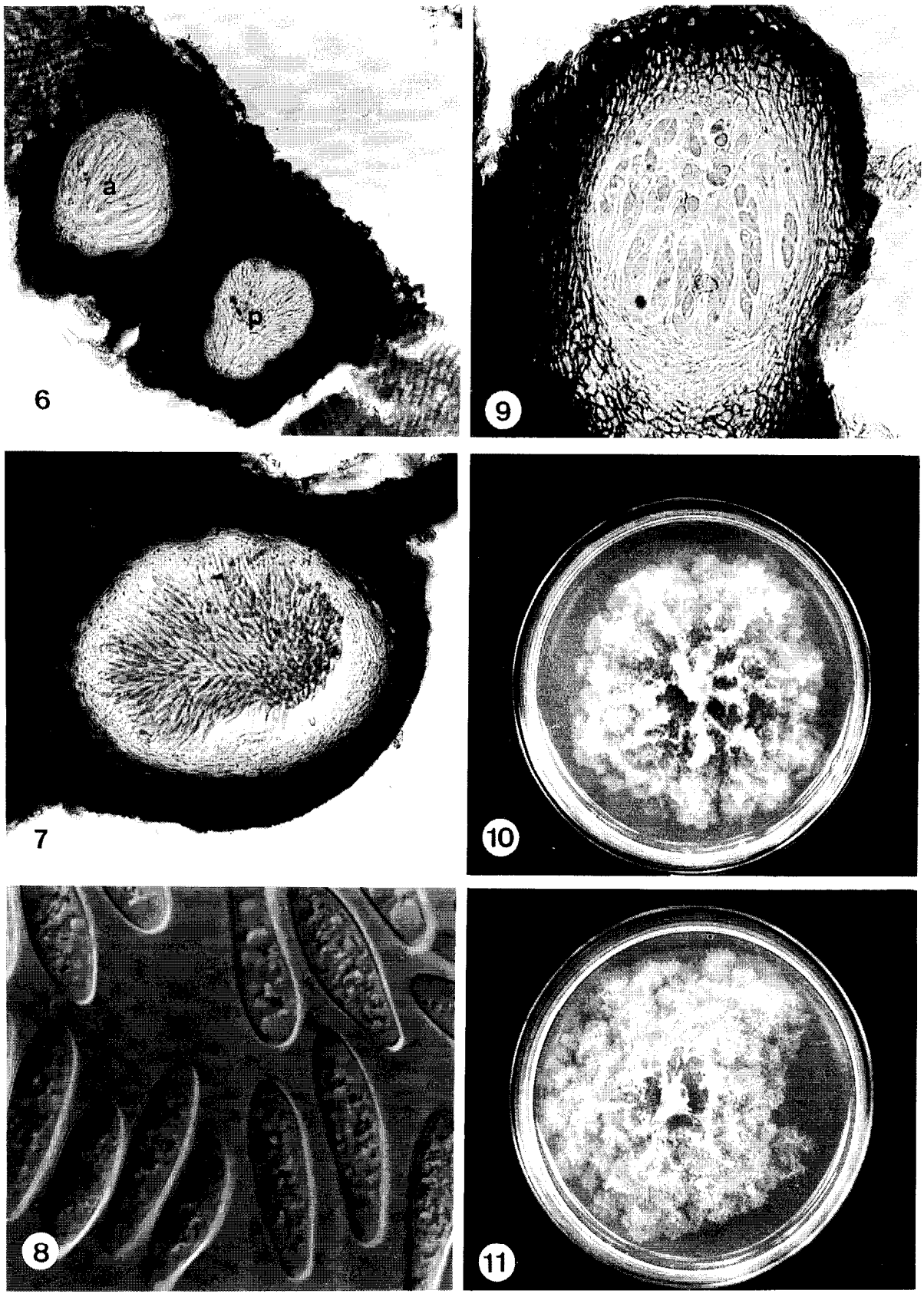

Fig. 6–11. The anamorph and teleomorph of *Botryosphaeria dothidea*: (6) ascocarp (a) and pycnidium (p); (7) pycnidium and conidia; (8) conidia; (9) perithecium containing asci and ascospores; (10) mycelial growth from ascospore; (11) mycelial growth from conidium.

TABLE 2. Development of gummosis symptoms of peach young shoots after wound inoculation with conidia of *Botryosphaeria dothidea*

Variety	No. of young shoots inoculated	No. of young shoots showed sunken lesion	% of infection with sunken lesion[1]
"Ying-Ko" Tao (鶯歌桃)	20	19	95
"San-Yueh" Tao (三月桃)	20	20	100
"Liu-Yueh" Tao (六月桃)	20	20	100
"Pe-Feng" Tao (白鳳)	20	19	95
"Tang" Tao (糖桃)	20	20	100

1. Symptoms present 2 wk after inoculation.

TABLE 3. Incidence of peach gummosis (*Botryosphaeria dothidea*) in 23 peach orchards in central Taiwan in 1990–1991

Variety	No. of orchard surveyed	No. of orchards with infection percentage				
		1–10	11–30	31–50	51–75	>75
"San-Yueh" Taur	7	0	2	3	1	1
"Ying-Ko" Taur	5	2	2	1	0	0
Flordared	5	0	0	2	1	2
Premier	6	1	1	2	2	0
Total	23	3	5	8	4	3

Effect of temperature and culture medium on the growth of *B. dothidea.*

The relative growth rate of *B. dothidea* after 72 hr was greatest on PDA and OA, and least on V-8A and PSDA at all temperatures tested (Fig. 12). The optimum temperature for mycelial growth were 24–32 C. Maximum mycelial growth occurred at 24–28 C, no growth occurred at 8 C, and sparse growth at 36 C. The average colony diameters at 24 C after 3 days incubation on PDA, OA, CA, V-8A and PSDA were 72, 67, 43, 32 and 31 mm, respectively.

Effect of temperature on conidial germination and germ tube development

Temperature favorable for both germination and germ tube development ranged from 20–36 C. Conidial germination after 4 hr at 8, 12, 16, 20, 24, 28, 32, and 36 C was 0, 20, 43, 89, 95, 98, 97 and 92% respectively (Fig. 13). Development of germ tube after 4 hr incubation showed greatest at 28 C (134 μm) with reductions in germ tube length of about 30% at 24 and 32 C, 62% at 20 C and 87% at 16 C (Fig. 14).

Effect of osmotic water potential on conidial germination

Conidial germination on 2% WA amended with various concentrations of NaCl after 3 days of incubation at 16, 24 and 32 C declined with decreasing osmotic water potentials through −55 dars (96% RH) (Fig. 15). The results also indicated 50% spore germination occurred at water potential higher than −26 bars at 24 and 32 C.

Effect of light on sporulation

Light is necessary for the sporulation of *B. dothidea* (Table 4). Conidial formation started as early as the fifth day after exposure to continuous light at 25 C. The amounts of sporulation after 7–9 days incubation were 2.1–3.6 × 10^5 conidia/cm^2.

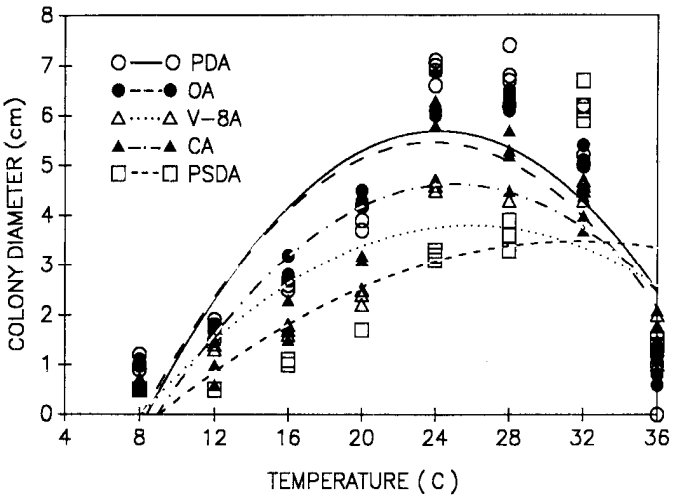

Fig. 12. Effect of culture media and temperatures on growth of *Botryosphaeria dothidea* 3 days after incubation. Lines are predicted values from the following regression equations: for PDA, $Y = -7.6 + 1.1\ X - 0.02\ X^2$ ($r = 0.90$); for OA, $Y = -7.2 + 1.1\ X - 0.02\ X^2$ ($r = 0.93$); for V-8A, $Y = -4.2 + 0.62\ X - 0.01\ X^2$ ($r = 0.90$); for OA, $Y = -6.5 + 0.89\ X - 0.02\ X^2$ ($r = 0.91$); for PSDA, $Y = -3.3 + 0.43\ X - 0.007\ X^2$ ($r = 0.84$).

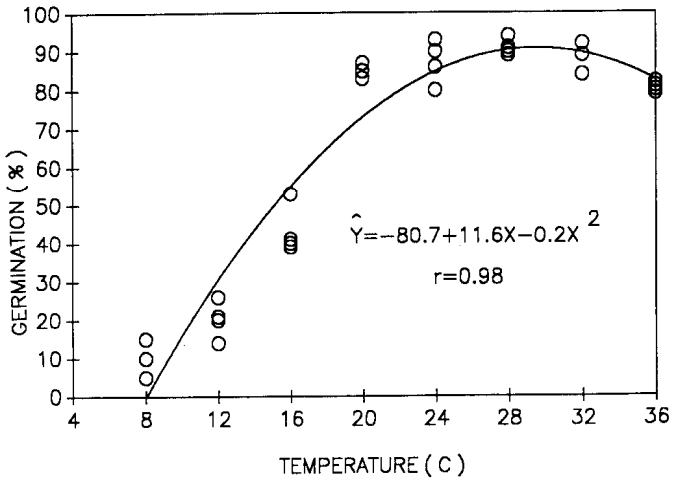

Fig. 13. Effect of temperature on conidial germination of *Botryosphaeria dothidea* 4 hr after incubation.

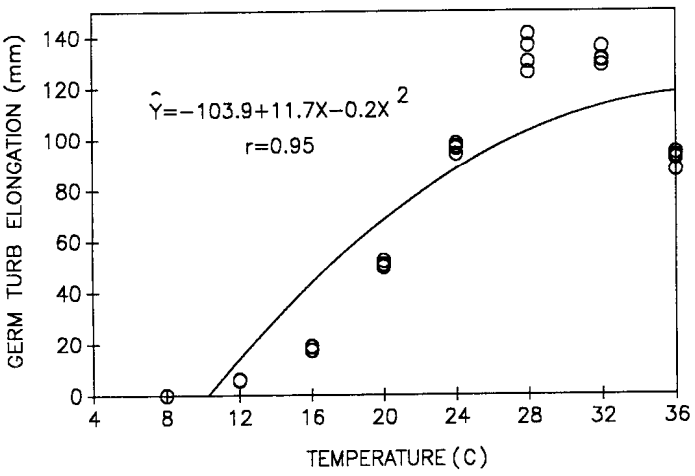

Fig. 14. Effect of temperature on conidial germ tube length of *Botryosphaeria dothidea* 4 hr after incubation.

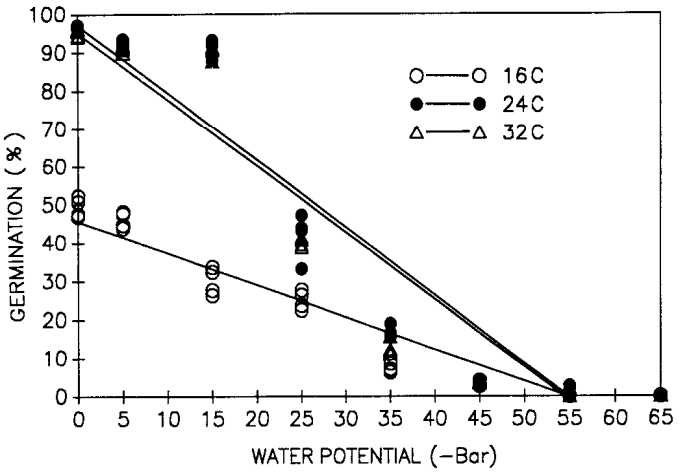

Fig. 15. Effect of water potential on conidial germination of *Botryosphaeria dothidea* 4 hr after incubation. Lines are predicted values from the following regression equations: conidia at 16 C: $Y = 4.56 - 0.83\ X$ ($r = 0.98$); at 24 C: $Y = 97.0 - 1.8\ X$ ($r = 0.97$) and at 32 C: $Y = 95.05 - 1.746\ X$ ($r = 0.97$).

TABLE 4. Effect of continuous light on the sporulation by *Botryosphaeria dothidea* on Difco PDA plate at 25 C

Treatment	Conidia ($\times 10^5$)/cm^2 at days after culture[1]				
	3	5	7	9	11
Light[2]	0	0.06	2.14	3.56	3.60
Dark	0	0	0	0	0

1. Amounts of sporulation was based on the averages of 4 replicates.
2. Light intensity was maintained at 128 ± 25 $\mu E \cdot m^{-2} \cdot s^{-1}$.

DISCUSSION

Since peach gummosis is a hitherto unrecorded disease in Taiwan, field observation and laboratory study were carried out. Disease incidence surveyed showed all orchards with disease variation lies severity among orchards as well as on age of trees. It is estimated that the gum disease will be serious as peach trees getting older if no effective control methods are practiced.

Infected plant parts consistently harbor the pycnidial of *Fusicoccum* sp. and *Botryosphaeria dothidea* was found on dead twigs as well as on the pruned twigs piled on the ground. *B. dothidea*, reported previously under the synonym *B. ribis*, caused branch and trunk cankers on different variety of plants in the temperate and tropical zones throughout the world (2,5,7,8,13,16,17,20,21,22,23, 24,25). Smith (19), in a study of the host range of *B. ribis*, showed that the fungus was pathogenic to 50 plant species representing 34 genera and 20 families. In addition to causing canker, *B. dothidea* has been reported to cause fruit rot of apple, avocardo, citrus, grape, lemon, pistachio, and tung (5,6,8,10,13,17,18,20,23).

Peach gummosis incited by *B. dothidea* was first reported in Georgia in 1974 (22) where both anamorph and teleomorph stages of *B. dothidea* were found.

In 1863, *de* Cesati and *de* Notaris established the genus *Botryosphaeria* and listed 9 species, including *B. dothidea* (9,24). In 1911, Grossenbacher and Dugger (9) offered a great description of a fungus from cane blight of currant, illustrated both sexual and asexual fruiting bodies, spores, and named the fungus *B. ribis*. Therefore, according to the International Rules of Nomenclature, the name of *B. dothidea* is valid since it antedates *B. ribis*.

The morphology of fruiting bodies of our suspected pathogen was closely coincided with *B. dothidea* of previous reports (Table 1). Inoculation tests and Koch's postulates showed that *B. dothidea* was the causal agent of peach gummosis in Taiwan.

The appearance of *B. dothidea* isolated from naturally affected trees was high during late summer and fall in central Taiwan, indicating that the fungus was continuously active. Similarly, the frequencies of the isolates of the *B. dothidea* peaked early in the summer in Georgia (6). Both

indicated *B. dothidea* was a high temperature pathogen dominant in the summer season.

Physiological tests showed that mycelial growth, sporulation, spore germination, and germ tube elongation all favored under high temperature. Another fact showed that peach gummosis was not found in Lishan area where temperature is low (16 C annual average) that inhibits the growth of *B. dothidea*.

ACKNOWLEDGEMENTS

This study was supported by Division of Plant Protection, Taiwan Provincial Department of Agriculture and Forestry. We indebted to Dr. A. E. Sivanesan for his identification of the fungus and thanks to M. Y. Liao, S. H. Huang, and W. C. Jung for their valuable assistance in the experiment.

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摘　　要

柯　勇、孫守恭. 1992. *Botryosphaeria dothidea*引起之桃流膠病. 植病會刊 1:70-78. (台中市國立中興大學植物病理學系)

近年來本省中部地區栽培之熱帶種桃樹，發生嚴重的流膠病，從罹病桃樹之主幹、主枝和側枝進行徒手切片鏡檢和病原菌之分離、培養，常發現到 *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. et de Not.之存在。以當年生之幼嫩枝條進行接種試驗，無論以 *B. dothidea*的培養菌絲塊或

以胞子懸浮液行傷口接種，都可形成典型之流膠病病徵，證實 *B. dothidea* 爲引起桃流膠病之病原菌。病害初期，罹病枝幹樹皮表面上之皮目周圍呈褐色凹陷，繼之出現疣狀凸起，每一小疣下之皮層組織呈褐色壞疽；病害中後期，疣狀凸起出現裂紋，並有膠狀液體從罹病之皮目內溢出，雨後流膠更多常垂落於地上。在田間 *B. dothidea* 之無性世代爲 *Fusicoccum* sp.，柄子殼扁球形，黑色或暗褐色，大小爲 143–207 μm（直徑）。分生胞子無色，單胞的紡綞形，其大小爲 12.5–17.8 × 3.8–5.0 μm。子囊殼燒瓶狀，大小爲 173–248 μm（直徑），內含雙膜 (bitunicate) 棍棒狀子囊，其大小爲 95.0–123.5 × 12.7–18.5 μm。每個子囊內含有 8 個無色、單胞的紡綞形子囊胞子，其大小爲 22.5–30.0 × 9.3–12.5 μm。*B. dothidea* 菌絲生長在馬鈴薯培養基和燕麥培養基上最爲良好，最適溫度在 24–32 C。分生胞子發芽之最適溫度則在 24–36 C 範圍內，而發芽管生長適溫爲 28 C，4 小時後其長度平均爲 134 μm。在 24 C 溫度下，於 2% 水瓊脂培養基上培養 4 小時，分生胞子之發芽率可達 96%，而水分潛勢從 −1 bar 到 −45 bars 範圍內，分生胞子的發芽率隨水分潛勢之降低而減少；而於 −55 bars 時分生胞子即不發芽。光照可促進分生胞子之產生，於 128 ± 25 $\mu E \cdot m^{-2} \cdot s^{-1}$ 連續光照下培養 7–9 天，產量約爲 $2.1–3.6 \times 10^5$ 個分生胞子 /cm^2。查閱文獻資料顯示桃流膠病爲本省之新病害。

關鍵字：桃流膠病，*Botryosphaeria dothidea*。