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New Diseases and Records of Some Important Flower Plants Caused by *Phytophthora parasitica* in Taiwan

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ABSTRACT

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Phytophthora parasitica was isolated from diseased basal stems and roots of important potted flower plants including African violet, gloxinia, Christmas cactus, poinsettia, *Dianthus barbatus*, *Dianthus japonicus* and sweet alyssum in Taiwan. Phytophthora diseases of these plants were very serious in certain areas under moist condition. Disease symptoms on all of the seven kinds of flower plants in fields were wilting due to infection of the basal stems or main root systems. The infected tissues appeared water soaking initially and turned dark brown later. All affected plants died eventually. Similar diseases were reproduced when seedlings of each host were inoculated with zoospore suspension of their respective isolates of *P. parasitica* in pathogenicity tests. *P. parasitica* was reisolated from all artificially infected tissues. All seven Phytophthora diseases caused by *P. parasitica* were reported for the first time in Taiwan. Diseases caused by *P. parasitica* on the two species of *Dianthus* and sweet alyssum have not been described previously.

Key words: New records, *Phytophthora parasitica*, African violet (*Saintpaulia ionantha*), gloxinia (*Sinningia speciosa*), Christmas cactus (*Schlumbergera bridgesii*), poinsettia (*Euphorbia pulcherrima*), sweet willium (*Dianthus bartatus*), *Dianthus japonicus*, sweet alyssum (*Lobularia maritima*), Taiwan.

INTRODUCTION

Ornamental plants have become an important economic commodity in Taiwan in recent years due to improve living standard. Therefore, many famous varieties of ornamental flower plants were introduced and grown in the entire island. New diseases of these flower plants occurred subsequently and caused substantial losses to farmers. The associated pathogens may come along with imported plants, or they may be endemic but become devastating due to susceptibility of the introduced plants. According to previously reports, many ornamental plants were very susceptible to Phytophthora diseases (10). Frequent heavy rainfall and the moist environmental conditions in Taiwan are favorable to the spread of sporangia and zoospores of *Phytophthora* species in the fields (4,9,11). For this reason, survey of new diseases of flower plants associated with *Phytophthora* species was conducted recently in the island. New Phytophthora diseases of carnation (5), lily (6), and Araceae ornamental plants (1) have been published. Basal stem rot and root rot of seven kinds of important potted flower plants caused by *Phytophthora parasitica* Tucker are reported herein *via* Koch's postulate.

MATERIALS AND METHODS

Isolation

Diseased tissues of affected plants were collected from fields. Pieces of tissues taken from stems or roots (*ca.* 5-10 mm long) with advanced disease symptoms were disinfested with 0.5% NaClO for 3 min. The treated tissues were plated onto selective medium at 24 C for one to seven days. Selective medium consisting of 5% clarified V-8 juice agar (5% V-8 juice plus 0.2% $CaCO_3$ centrifuged at 1500 rpm for 5 min and 2% Bacto agar) was supplemented with 200 ppm Ampicillin, 50 ppm mycostatin, and 10 ppm pentachloronitrobenzene (17). After incubation at 24 C for 1-7 days, mycelial mats of *Phytophthora* spp. growing out of diseased tissues on selective medium were transferred to 5% V-8 juice agar. Single-zoospore cultures were

obtained using the method described by Ko (16). Cultures were maintained on 5% V-8 juice agar blocks in sterile water in test tubes at 24 C (7).

Production of sporangia and zoospores

The method described by Hwang *et al* (12) was used to produce large amount of sporangia for morphological studies and pathogenicity tests. Zoospore suspension was prepared by chilling the mycelial mats with sporangia at 15 C for 30 min.

Determination of mating types

Each isolate of *Phytophthora* was grown on 10% V-8 juice agar (10% V-8 juice, 0.02% $CaCO_3$, 2% Bacto agar) at 24 C in darkness for 10 days. Isolates which did not form oospores individually were paired with the standard A^1 (p991) and A^2 (p731) mating type of *Phytophthora parasitica* individually for determination of their mating types (2). Those isolates forming oospores in single cultures were designated as homothallic. Those forming oospores when paired with the A^2 tester were A^1; while those forming oospores with A^1 tester were A^2.

Production of oospores

The polycarbonate membrane method described by Ko (14) was used for study of sexual reproduction of heterothallic species of *Phytophthora*, and for determination of their sexuality types (15).

Growth of *Phytophthora*

Phytophthora isolates were grown on 5% V-8 juice agar for 3–5 days. Agar discs (5 mm diam.) cut from the periphery of the colonies with a sterile cork borer were each placed on the edge of a V-8 agar plate and incubated at 8, 10, 12, 15, 18, 21, 24, 27, 30, 33, 36 or 38 C in darkness. Colonies were measured daily until the mycelia reached the opposite edge of the plates or 10 days after inoculation. Four plates were used for each temperature and the experiment was repeated twice.

Pathogenicity tests

Two isolates of *P. parasitica* obtained from each host species were selected for pathogenicity tests. Zoospore suspension used as inoculum was adjusted to 10^5–10^6 zoospores/ml with a microliter pipette (18). Seedlings grown in disinfested soils in 9 cm pots were inoculated by placing a small piece of sterile cotton containing 1 ml of zoospore suspension around the basal stem of each tested plant. Five seedlings were inoculated for each treatment and tests were repeated twice. Controls for each test were similarly treated with distilled water. After incubation at 24 C for 3 days, disease incidence was rated every 3 days. The pathogens were reisolated from artificially infected tissues as described above.

RESULTS

Plants infected with *Phytophthora* spp.

Phytophthora parasitica were frequently isolated from diseased tissues of some important potted flower plants in many areas in Taiwan when they were floriculturally grown in greenhouse or directly planted in the fields. These flower plants included African violet (*Saintpaulia ionantha* Wendl.), gloxinia (*Sinningia speciosa* Thunb), Christmas cactus (*Schlumbergera bridgesii* Loffr.), poinsettia (*Euphorbia pulcherrima* Willd), sweet willium (*Dianthus bartatus* L.), *Dianthus japonicus* L., and sweet alyssum (*Lobularia maritima* Desv.).

Phytophthora diseases of these flower plants were very serious under moist conditions. *Phytophthora parasitica* usually attacked the basal part of stems and major roots of the affected plants. Occasionally it also infected flowers, stems and/or leaves. All affected plants appeared wilt and died eventually. Sometimes, more than 80% of the susceptible flower plants in a field were killed within few months during raining period.

The names of affected flower plants, infection sites, numbers of isolates obtained and locations associated with each Phytophthora disease were listed on Table 1 & 2 or showed in Fig. 1 to 6. Each Phytophthora disease and the characteristics of the responsible causal agent were described as follows:

African violet (非洲蓳)

Serious Phytophthora wilt was frequently found on potted plants or cuttings of African violet under moist conditions. Currently, *P. parasitica* appears to be the most destructive pathogen of this flower plant species in Taiwan. The fungus infected every portions of the plants including leaves, stems, flowers and roots at all growing stages. It mainly attacked basal stem and major roots under natural field conditions. The affected tissues showed water-soaked discoloration initially and turned dark brown or black later. The disease lesions extended to leaf petioles and blades resulting in droopy of leaves and flowers, and death of whole plants eventually (Fig. 1). African violet is very susceptible to *P. parasitica*. Two-month-old cuttings (with about 10 leaves) showed wilting within 2 to 5 days after dispensing 1 ml of zoospore suspension containing 10^5 to 10^6 zoospores on the unwounded basal stem. All of the inoculated seedlings died within two weeks (Table 1). All plants inoculated with distilled water remained healthyduring the test periods.

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TABLE 1. Diseases of seven ornamental potted flower plants caused by *Phytophthora parasitica* and its pathogenicity tests.

Host (寄主)	Diseases in the fields		Pathogenicity[2]	
	Infection sites	Correlation[1] (%)	Incidence	Death (%)
African violet (非洲菫)	Whole plant	100	20/20	100
Gloxinia (大岩桐)	Whole plant	55	20/20	100
Christmas cactus (螃蟹蘭)	Stem, root & flower	100	20/20	100
Poinsettia (聖誕紅)	Root & basal stem	67	a:20/20[3]	100
			b:16/16	0
			c: 6/6	33
Dianthus bartatus (美國石竹)	Basal stem & root	80	15/20	75
D. japonicus (日本石竹)	Basal stem root	50	7/10	50
Sweet alyssum (香雪球)	Basal stem & root	60	10/10	100

1. Percentage of wilted plants with *P. parasitica.*
2. Each plant inoculated with 10^5–10^6 zoospores of *P. parasitica* on the basal stem except poinsettia. Incidence = No. of plants showing symptoms / No. of plants inoculated.
3. Roots of tested cuttings (a) or flowering plants (b) immersed in zoospore suspension for one hour or over night, respectively. Inoculated flowering plants (c) submersed with water for one week.

TABLE 2. Isolation of *Phytophthora parasitica* from diseased tissues of seven ornamental potted flower plants

Host	No. of isolates obtained	Location
African violet	12 A^1 : 6 A^2	Chiayi, Puli, Taitung
Gloxinia	4 A^1 : 4 A^2	Chungpu, Holi, Puli
Christmas cactus	4 A^1 : 0 A^2	Chiayi, Tienwei,
Poinsettia	6 A^1 : 0 A^2	Chiayi, Tienwei, Taitung
Dianthus bartatus	0 A^1 : 6 A^2	Tienwei
Dianthus japonicus	0 A^1 : 2 A^2	Chiayi
Sweet alyssum	0 A^1 : 2 A^2	Tienwei

P. parasitica identical to the cultures used for inoculation was reisolated from the affected tissues.

A total of 12 A^1 and 6 A^2 isolates of *P. parasitica* were obtained from diseased tissues of potted African violet in five gardens. The A^1 isolates were found at Chiayi, Puli (Nantow) and Taitung, while the A^2 isolates were found at Taitung and Puli (Table 2). All A^1 and A^2 tested isolates obtained from African violet have essentially all the major characteristics of typical *P. parasitica.* The characteristics of sporangia, sexuality types, and growth rates of tested isolates were described on Table 3.

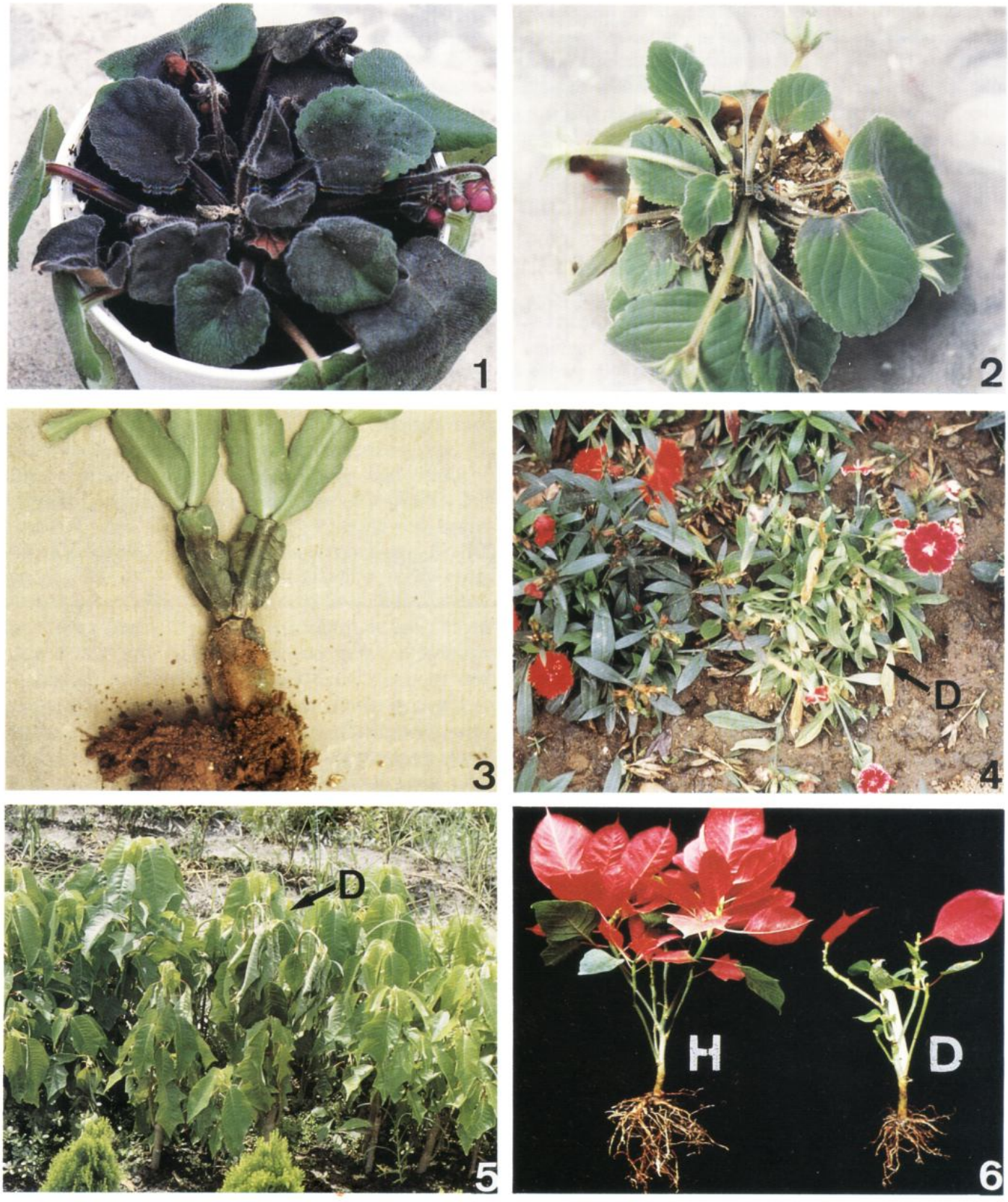
Gloxinia (大岩桐)

About one and half of wilting gloxinia plants in the fields was incited with *P. parasitica.* Gloxinia plants from young seedlings to flowering stage are very susceptible to *P. parasitica.* The fungus mainly attacked basal stem of the potted plants. The affected stem tissues showed black discoloration and appeared shrunk. Disease lesions also expanded along with leaf petioles to leaf blades. Eventually, the affected plants wilted and died (Fig. 2). Disease symptoms similar to those occurring in fields were reproduced on inoculated plants and the same fungus was reisolated from all affected disease tissues. Three- to four-month-old seedlings (with 10 to 20 leaves) wilted within 2 to 4 days after inoculation with 10^5 to 10^6 zoospores on the unwounded basal stems of tested plants. All inoculated seedlings died within 1–2 weeks (Table 1).

Four A^1 and four A^2 isolates of *P. parasitica* were obtained from diseased stem tissues of gloxinia flowering plants grown in pots in 3 gardens distributed at Chungpu (Chiayi), Holi (Miaoli) and Puli (Table 2). All cultures belong to typical *P. parasitica.* The main characteristics of the gloxinia isolates were listed on Table 3.

Christmas cactus (螃蟹蘭)

Phytophthora blight probably is the most important disease of Christmas cactus in Taiwan, today. The disease was frequently found on the plants grown in pots or tied onto plates, which were made of tree fern stems, during the rain season or under wet conditions. Flower and young green stem tissues were very susceptible to *P. parasitica,* while corked old mature stems were moderate resistant to the same fungus. The affected stems showed watersoaked discoloration in early stage of infection, and turned



Figures 1 to 6. Disease symptoms caused by *Phytophthora parasitica* on African violet (Fig. 1), gloxinia (Fig. 2), Christmas cactus (Fig. 3), *Dianthus bartatus* (Fig. 4), and poinsettia in field (Fig. 5) and potted poinsettia with necrotic roots (right) and a healthy plant (left) (Fig. 6). (D = diseased plant, H = healthy plant)

TABLE 3. Characteristics of sporangia and growth of mycelia of *Phytophthora parasitica* isolated from seven potted flower plants

Host	Mating type	Sexuality type	Size of sporangia (μm)			Growth temperature of mycelium (C)			Linear growth rate at optimum temperature (mm/day)
			Length	Width	L/W	Minimum	Maximum	Optimum	
African violet	A^1	S^4	20–65[1] (50.4)	30–44 (37.0)	1.18–1.73 (1.36)	8–10	37	27–30	9.3
	A^2	S^1	36–80 (61.1)	30.5–60 (51.3)	1.0–1.60 (1.20)	8–10	36	24–30	8.1
Gloxinia	A^1	S^4	29–85 (55.8)	25–64.5 (47.7)	1.0–1.89 (1.18)	8–10	37	24–30	8.6
	A^2	S^1	35–55 (45.9)	29–45.9 (38.0)	1.01–1.6 (1.21)	8–10	36	24–27	8.8
Christmas cactus	A^1	S^4	30–57.5 (43.1)	25–46 (34.6)	1.1–1.5 (1.25)	8–10	37	24–27	8.0
Poinsettia	A^1	S^4	35–60 (46.0)	25–40 (34.6)	1.06–2.0 (1.34)	8–10	36	27–32	8.1
Dianthus bartatus	A^2	S^1	43–65 (50.7)	38.5–52 (43.4)	1.06–1.34 (1.18)	8–10	36	24–27	8.8
D. japonicus	A^2	S^1	27–52.5 (38.9)	21–38.5 (29.7)	1.07–1.53 (1.32)	8–10	37	24–27	9.9
Sweet alyssum	A^2	S^1	50–91 (66.9)	40.5–70 (55.9)	1.11–1.55 (1.27)	8–10	37	24–27	9.0

1. The figures in parenthesis refer to average values.

light brown and fell eventually (Fig. 3). The infected flowers turned brown and droopped. Young stems of Christmas cactus showed disease symptoms which were similar to those appearing in natural field conditions, when each was inoculated without wounding with 1 ml of zoospore suspension containing 10^5–10^6 zoospores of *P. parasitica* for 2 to 4 days. All inoculated plants died and fell within 1–2 weeks (Table 1). The same fungus was reisolated from the diseased tissues.

All six isolates of *P. parasitica* obtained from diseased stems of Christmas cactus in three gardens at Chiayi and Tienwei (Changhua) belong to A^1 mating type (Table 2). All Christmas cactus isolates have the major characteristics of typical *P. parasitica* (Table 3).

D. bartatus (美國石竹) & *D. japonicus* (日本石竹)

In Taiwan, these two species of *Dianthus* were generally used as potted or garden flower plants. Phytophthora diseases were found on the two flowers when they were planted under both cultivated conditions. *P. parasitica* mainly attacked the basal stems and the major roots of the plants. Tissues of affected plants became bleached, water soaking and collapsed, which were similar to the symptoms on carnation (*Dianthus caryophyllus* L.) caused by *Phytophthora* spp. (5). Eventually, all affected Plants wilted, drooped and died (Fig. 4). The fungus could kill plants at every growing stages from young seedlings to flowering plants. Two cultures of *P. parasitica* isolated from each host plant were selected and used for inoculating their respective host plants. One mililiter of zoospore suspension containing 10^5 to 10^6 zoospores of each tested isolate was dispensed on cotton placed around the unwounded basal stem of an one-month-old seedling. Disease symptoms similar to those occurring under natural conditions were reproduced on inoculated seedlings. About 75% of *D. bartatus* and 50% of *D. japonicus* wilted 14 days after inoculation (Table 1). All seedlings inoculated with distilled water remained healthy. *P. parasitica* was reisolated from all infected tissues.

Six and two isolates of *P. parasitica* were obtained from *D. bartatus* and *D. japonicus*, respectively (Table 2). All of them belong to A^2 mating type. All isolates from the two flower plants have essentially all the majorcharacteristics of standard *P. parasitica* (Table 3).

Poinsettia (聖誕紅)

Phytophthora wilt caused by *P. parasitica* was one of the most important soil-borne disease of poinsettia in Taiwan, today. The fungus was frequently isolated from the stem and root tissues of wilted cuttings or seedlings. *P. cryptogea* Pethybridge & Lafferty, one undescribed *Phytophthora* sp., and *Pythium splendens* Braun were also detected from disease roots during this study. Young seedlings and cuttings were very susceptible to *P. parasitica*. The fungus also reduced survival rates of cuttings. Generally, poinsettia seedlings and cuttings became more and more resistant to *P. parasitica* when they grew bigger and stronger. For mature and flowering plants, only their fibrous roots could be attacked by the pathogen. However, serious wilt and death of elder plants of poinsettia occasionally occurred when they were grown in the fields after continuously rain.

The affected stems of poinsettia cuttings turned brownish and shrunk, leaves drooped and fell, and the whole plants died eventually (Fig. 5). Infected root systems showed dark brown necrotic lesions (Fig. 6). Serious root rot also resulted in wilting and death of the affected seedlings. Young cuttings of poinsettia did not survive when the new cuttings were dipped in the zoospore suspension of *P. parasitica* (containing 10^5–10^6 zoospores) for one hour before planting. Flowering poinsettia plants dipped in zoospore suspension of the same fungus under the same concentration over night produced diseased symptoms of root rot and necrosis after replanting in pots for six months. About one third of inoculated poinsettia wilted within one month after the potted soils were continuously flooded with water for one week (Table 1). All controls without inoculation with pathogen remained alive when they were treated with the same procedures. Non-inoculated plants also showed slight wilt with flooding but turned stronger after one month. All disease symptoms produced by artificial inoculation were similar to those appearing in natural field conditions. *P. parasitica* was detected from the inoculated diseased tissues *via* reisolation.

All six isolates of *P. parasitica* obtained from diseased cuttings of Christmas cactus in three gardens distributed in Chiayi, Tienwei and Taitung belonged to A^1 mating type (Table 2).

Sweet alyssum (香雪球)

Sweet alyssum is a pretty annual herbaccous flower plant used as potted plant in the cool season, but it has not been widely cultivated in Taiwan. Wilt of sweet alyssum incited by *Phytophthora* spp. was found recently. *P. parasitica* was detected from diseased stem and root tissues of infected sweet alyssum in the fields. The stem tissues attacked by *P. parasitica* showed brown to black discoloration and appeared shrunk. The affected plants wilted and died eventually. Both young seedlings and flowering plants were very susceptible to *P. parasitica*. Similar disease symptoms were reproduced on all plants inoculated with 10^5 to 10^6 zoospores on the unwounded basal stems. All one-month-old seedlings wilted and died two weeks after inoculation (Table 1). The same fungus was reisolated from all affected tissues.

Two A^2 isolates of *P. parasitica* were obtained from diseased sweet alyssum in one garden at Tienwei (Table 2). Both cultures belong to typical *P. parasitica* (Table 3).

DISCUSSION

Phytophthora parasitica (= *P. nicotianae*) has been reported as the cause of serious root and stem rot of African violet (8,10,20), gloxinia (8,20), Christmas cactus (13), and poinsettia (23) in many countries. However, wilt and death of these floriculturall important plants caused by the same fungus were reported for the first time in Taiwan. Symptoms of Phytophthora diseases of these potted flower plants found in Taiwan were similar to those reported from other countries. *Phytophthora capsici* Leonien inciting wilt of *D. bartatus* was first published by Leu & Kao in 1981 (19). However, *P. parasitica* but not *P. capsici* was detected on this flower plant during this study. Besides, diseases of *D. bartatus*, *D. japonicus* and sweet alyssum caused by *P. parasitica* have not been described previously.

Based on the survey data, all diseased African violet and Christmas cactus, and more than 50% of gloxinia, poinsettia and the two species of *Dianthus* associated with root and basal stem rot, were caused by *P. parasitica* (Table 1). All of these flower plants have been cultivated in Taiwan for a long time, but Phytophthora diseases of these plants were noticed and become serious recently. The climate factors in Taiwan were favorable to develop Phytophthora diseases. The pathogens - *Phytophthora* may come along with imported plants or may be endemic. Besides, most popular varieties of the introduced plants were very susceptible to the fungui. According to previous reports, *P. parasitica* is the most common and destructive species in the genus of *Phytophthora* in Taiwan (4,9,11). Most tested isolates of *P. parasitica* obtained from different host plants could cause severe diseases on other hosts in pathogenicity studies (1,5, and unpublished data). This diverse sources of inoculum, susceptible varieties and moist climate conditions may be the principal factors contributing to the seriousness of Phytophthora diseases of ornamental plants in Taiwan.

The morphological and physiological characteristics of all tested cultures of *P. parasitica* obtained from

diseased tissues of the seven potted flower plants in Taiwan were similar each other (Table 3). All Taiwanese isolates produced spherical chlamydospores on agar medium and formed unsymmetrically spherical to ovoid, papillate and nondeciduous sporangia with a shorter L/W (length/width) ratios of about 1.2–1.3. All isolates belong to cross-inducing (Heterothallic) type which do not form oospores when cultured individually. They were either A^1 mating type of the sexuality type S^4, which can be stimulated by A^2 and induce A^2 of *P. parasitica* to form oospores, or A^2 of S^1 type, which can be stimulated by A^1 and induce A^1 to form oospores (14,15). All tested isolates grown on 5% clarified V-8 juice agar plates formed white colonies with patterns of scatter mosaic spots and a few scantly aerial mycelia. The maximum growth temperatures for all tested isolates on 5% clarified V-8 juice agar plates were about 36–37 C. Therefore, all Taiwanese cultures of *P. parasitica* causing diseases of the seven potted flowers have essentially all the characteristics of typical *P. parasitica* (Table 3) (21) based on Tucker's descriptions in 1931 (22).

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摘　　要

安寶貞．1992．*Phytophthora parasitica* 在台灣引起一些重要花卉疫病之新記錄．植病會刊 1:166-173．(嘉義市　台灣省農業試驗所嘉義分所)

Phytophthora parasitica 自本省七種重要盆花植物(非洲菫、大岩桐、螃蟹蘭、美國石竹、日本石竹、聖誕紅、及香雪球)之罹病莖基部與根部分離得到。在高濕的環境下，花卉疫病在管理不佳之花園中發生十分嚴重。由於莖基部與主根受到疫病菌侵染，被害花卉在田間均出現萎凋病徵，被感染之部位最初出現水浸狀病斑，而後轉爲黑褐色，病斑擴大蔓延，導致罹病花卉萎凋死亡。將分離得到之疫病菌菌株之游走子，分別接種於原來寄主花卉的莖基部或根系，均出現與田間一致的病徵。*P. parasitica* 均可自接種之罹病組織上再分離得到。所有七種由 *P. parasitica* 引起之花卉疫病均是第一次在台灣被正式報導；而 *P. parasitica* 危害兩種石竹與香雪球，在世界其他地區亦尚未有正式記錄。

關鍵字：新記錄、台灣、非洲菫、大岩桐、螃蟹蘭、聖誕紅、美國石竹、日本石竹、香雪球、*Phytophthora parasitica*。