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Phytophthora Diseases of Compositae in Taiwan

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ABSTRACT

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Phytophthora diseases in the family of Compositae were surveyed in Taiwan from 1980 to 1994. Three species of *Phytophthora* were detected and seven flower plants and one vegetable crop were found to be infected. Diseases included *P. cryptogea* on *Chrysanthemum morifolium*, *Gerbera jamesonii*, *Gunura bicolor*, *Liatris spicata* (blazing-star), *Senecio cruentus* (cineraria), *P. parasitica* on *Centaurea cyanus* (cornflower) and *Senecio cineraria* (dusty miller), and *P. capsici* on *Dahlia hybrida*. All three species of *Phytophthora* caused wilt and death of affected plants due to serious basal stem rot and/or root rot. Occasionally, the fungi attacked leaves and stems and induced leaf blight and stem rot. In pathogenicity tests, each species of *Phytophthora* caused diseases on their individual host plants similar to those occurring in natural field conditions. Species of *Phytophthora* identical to inocula were reisolated from all of the diseased inoculated plants. All isolates of *P. cryptogea* and *P. capsici* obtained in this study were A^1 mating type; whereas all *P. parasitica* were A^2 mating type. *P. cryptogea* on *S. cruentus*, *C. morifolium*, *L. spicata*, and *G. bicolor*; *P. parasitica* on *S. cineraria* and *C. cyanus*; and *P. capsici* on *D. hybrida* were first reported in Taiwan.

Key words: Compositae, *Phytophthora cryptogea*, *Phytophthora parasitica*, *Phytophthora capsici*, *Centaurea cyanus*, *Chrysanthemum morifolium*, *Dahlia hybrida*, *Gerbera jamesonii*, *Liatris spicata*, *Gunura bicolor*, *S. cruentus*, *Senecio cineraria*, Taiwan.

INTRODUCTION

Wilt and death of *Gerbera jamesonii* Bolus & Hook (非洲菊) caused by *Phytophthora cryptogea* Pethybridge & Lafferty has been reported to be a very serious disease in Taiwan (6) as well as in many other countries (10). However, species of *Phytophthora* on other crops in the family of Compositae have not been formally described in Taiwan. Although a few crops including *Chrysanthemum* spp. (菊花), *cosmos* sp. (大波斯菊), and *Lactura sativa* L. (萵苣) have been observed to be infected with *Phytophthora* in the fields (4). A survey was conducted beginning from 1980 and a brief article have been reported in 1990 (2). This paper reports on the identity of *Phytophthora* species obtained from diseased tissues of several crops in the family of Compositae. The pathogenicity of each candidate *Phytophthora* to their individual host plants is also studied.

MATERIALS AND METHODS

Isolation and identification

Diseased tissues of plants in the family of Compositae were collected from fields. Pieces of tissues taken from stems or roots (*ca.* 5-10 mm long) with advanced water soaking 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 pentachloro-nitrobenzene (9). 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 prepared and used for following studies. Cultures were maintained on 5% V-8 juice agar blocks in sterile water in test tubes at 24 C.

Classification Keys described by Stamps *et al.* (11) and Waterhouse (12) were used for identification of the *Phytophthora* isolates obtained.

Production of sporangia and zoospores

The method described by Hwang *et al.* (5) was used for production of 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 (for *P. parasitica* Dastur and *P. capsici* Leonian) or 2-4 hr (for *P. cryptogea*) followed by returning them to 24 C for 30 min.

Determination of mating types and production of oospores

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 (3). Those isolates forming oospores independent of each other were designated as homothallic. Those forming oospores when paired with the A^2 tester were A^1; while those forming oospores with the A^1 tester were A^2.

The polycarbonate membrane method described by Ko (7) was used for sexual reproduction of heterothallic *Phytophthora* spp.

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 used as inocula. One agar disc inoculum was transferred onto the edge of each 5% V-8 agar plate. Inoculated agar plates were incubated at 8, 10, 12, 15, 18, 21, 24, 27, 30, 33, 36 and 38 C, respectively, 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

One isolate, able to produce a large amount of sporangia, of *Phytophthora* species obtained from each host plant was selected for pathogenicity tests. Zoospore suspension was adjusted to 10^5 zoospores/ml. Seedlings (or cuttings and bulbs) were grown in disinfested soils in pot (12 X 12 X 12 cm) in green house for 1 month. A small piece of sterile cotton boll containing 1 ml of zoospore suspension was placed around the basal stems of a tested plants. Five seedlings were inoculated for each treatment and tests were repeated twice. Seedlings inoculated with distilled water were used as controls. Inoculated seedlings were kept at 24 C for one month. Disease incidence was rated every three days. The diseased tissues were taken, disinfested and plated on selective medium to reisolate fungus for confirmation of each candidate pathogens.

RESULTS

Isolation and Plants infected with *Phytophthora* spp.

In this survey, *Phytophthora* were isolated from the diseased stems and/or roots of seven flower plants and one vegetable in the family of Compositae, including *Centaurea cyanus* L. (Cornflower) (矢車菊), *Chrysanthemum morifolium* Ramat (Chrysanthemum) (菊花), *Dahlia hybrida* Hort. (Dahlia) (大理花), G. *jamesonii* (非洲菊), *Liatris spicata* Willd. (Blazing star) (麒麟菊), *Senecio cruentus* DC. (cineraria) (瓜葉菊), *S. cineraria* DC. (Dusty miller) (銀葉菊), and *Gunura bicolor* PC. (Gunura) (紅鳳菜). Gerbera was the flower to be most frequently isolated with *Phytophthora* in the fields. Species of *Phytophthora* detected from the diseased plant tissues in the study included *P. cryptogea*, *P. parasitica* Tucker, and *P. capsici* Leonian. Table 1 lists the location, plants infected by *Phytophthora*, and number of isolates obtained. Figures 1-4 showed diseased symptoms of some important crops.

Table 1. Isolation of *Phytophthora* from diseased tissues of crops in Compositae

Name of host Scientific & Chinese	Infected sites	*Phytophthora* species	Location and No. of isolates obtained
Gerbera jamesonii (非洲菊)	Basal stem & root	*P. cryptogea*	Taichung (25A[1]), Nantow (6A[1]), Chiayi (3A[1]), Kaohsung (1A[1])
Centaurea cyanus (矢車菊)	Basal stem & root	*P. parasitica*	Chiayi (4 A[2])
Chrysanthemum morifolium (菊花)	Root	*P. cryptogea*	Taichung (10A[1]), Chiayi (9A[1])
Dahlia hybrida (大理花)	Basal stem & root	*P. capsici*	Nantow (4 A[1])
Gunura bicolor (紅鳳菜)	Leaf, stem & root	*P. cryptogea*	Taipei (5 A[1])
Liatris spicata (麒麟菊)	Root	*P. cryptogea*	Nantow (3 A[1])
Senecio cruentus (瓜葉菊)	Basal stem & root	*P. cryptogea*	Chiayi (3 A[1])
Senecio cineraria (銀葉菊)	Stem & root	*P. parasitica*	Chiayi (2 A[2])

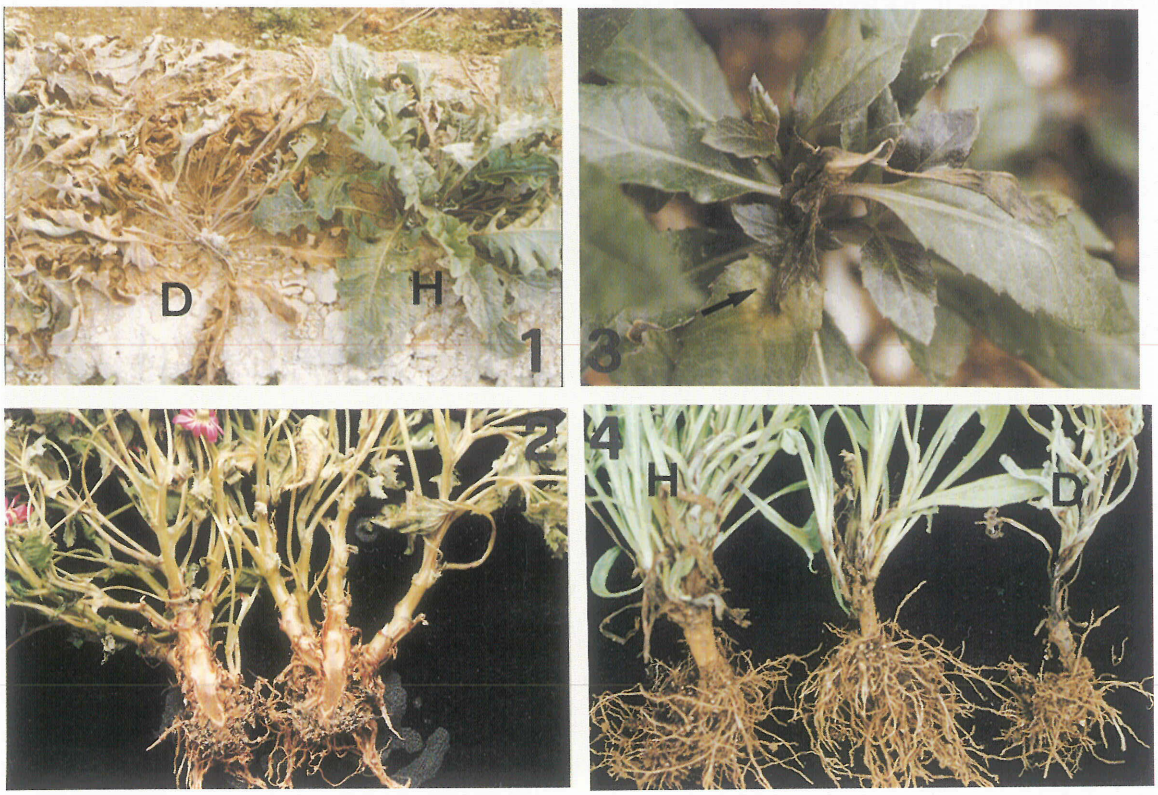

Fig.1-4. Disease symptoms caused by *Phytophthora* species on the crops in Compositae. 1:wilt of *Gerbera jamesonii* caused by *P. cryptogea*; 2:basal stem rot of *Senecio cruentus* (瓜葉菊) caused by *P. cryptogea*; 3:stem and leaf blight of Gunura bicolor (紅鳳菜) caused by *P. cryptogea*; 4:basal stem and root rot of *Centaurea cyanus* (矢車菊) caused by *P. parasitica*

Pathogenicity test

All test isolates of *Phytophthora* caused disease symptoms, as wilting and death of entire plants, on their respectively host plants similar to those occurring in field conditions. Dahlia were highly susceptible to *P. capsici*; all inoculated cuttings died 3-7 days after inoculation. *C. cyanus*, *G. jamesonii*, *G. bicolor*, and *S. cruentus* were also very susceptible to *P. cryptogea* and *P. parasitica*, respectively; more than 80% of inoculated seedlings or cuttings were killed within 2 weeks after inoculation. *L. spicata* and *S. cineraria* was moderately susceptible to *P. cryptogea* and *P. parasitica*, respectively, about 30-

50% of inoculated flowers wilted. However, chrysanthemum was less susceptible to *Phytophthora*; only 10, 15, and 5% of inoculated seedlings of three test varieties (Shiah Bair, Yellow Shuho, and Shing Lih Hong) were infected, respectively.

P. cryptogea and the diseases it caused

P. cryptogea was considered to be the most destructive pathogen of gerbera in Taiwan. In this study, *P. cryptogea* was also isolated from the diseased stems and roots of gerbera (Fig. 1A) and four other crops in Compositae, including *C. morifolium*, *L. spicata*, *S. cruentus* (Fig. 1B), and *G. bicolor*. The basal stems of affected plants appeared to have black discoloration and collapsing; the entire diseased plants eventually wilted and died. The fungus also attacked the above ground parts of *G. bicolor* during the rain. It produced black blotch on the affected leaves and stems (Fig. 1C). *Chrysanthemum* spp. were occasionally infected with *Phytophthora* in the fields. While *S. cruentus*, *G. bicolor* and *L. spicata* were very susceptible to *P. cryptogea*.

Thirty five, eighteen, three, three and five isolates of *P. cryptogea* were isolated from gerbera, *chrysanthemum* sp., *L. spicata*, *S. cruentus*, and *G. bicolor*, respectively (Table 1). All isolates of *P. cryptogea* obtained from Compositae crops belong to mating type A^1 and sexuality type S^2 (8), since they can induce the A^2 standard isolate (p731) of *P. parasitica* to produce oospores, but can not be stimulated to form oospores itself.

Isolates of *P. cryptogea* obtained from plants of Compositae were the typical type. The fungus produced a few sporangia on 5% V-8 agar medium at 24 C under light; while a large amount of sporangia were formed under liquid condition by using the method described by Hwang *et al.* (5). Sporangia were born sympodially. Usually, one to five (*av.* 2-3) sporangia distantly grew on each single sporangiophore (*av.* 100-500 μm). Sporangia were obpyriform or ovoid, non-papillate, and non-deciduous; average ranges of length and width were 49.5-59.7 X 34.2-41.5 μm; average ratios of length/width (L/W) of sporangia were ranged from 1.32-1.53 (Table 2). In distilled water isolates of *P. cryptogea* produced clusters of small spherical hyphal swellings as one of the important identification criteria, *i.e.* similar to those of the carnation isolates of *P. cryptogea* in Taiwan (3). Chlamydospores were not formed by any test isolates.

Table 2. Size of sporangia of isolates of *Phytophthora* spp. obtained from Compositae in Taiwan

Isolate no. and host	Sporangia (μm) Length X Width	Length/width	Pedicel (μm)
P. cryptogea			
PCrG5, *G. jamesonii* (非洲菊)	47.5-64 X 35-47.5 (54.0 x 40.9)[1]	1.17-1.48 (1.32)	-[2]
PCrR1, *G. bicolor* (紅鳳菜)	45.5-64 X 32-42.5 (56.8 x 37.1)	1.26-1.79 (1.53)	-
PCrCh3, *C. morifolium* (菊花)	36-63.2 X 28-40 (50.9 x 34.7)	1.18-1.63 (1.47)	-
PCrBs1, *L. spicata* (麒麟菊)	40-59 X 29.5-40 (49.8 x 34.2)	1.32-1.62 (1.45)	-
PCrY1, *S. cruentus* (瓜葉菊)	40-85 X 35-50 (59.7 x 41.5)	1.05-2.00 (1.44)	-
P. parasitica			
PPCo1, *C. cyanus* (矢車菊)	46.5 X 34-48 (53.3 x 41.7)	1.11-1.65 (1.26)	-
PPDm1, *S. cineraria* (銀葉菊)	42.5-63 X 34-47.5 (51.4 x 40.4)	1.14-1.60 (1.29)	-
P. capsici			
PCaD1, *D. hybrida* (大理花)	40-73.5 X 35-50 (55.7 x 42.8)	1.11-1.59 (1.42)	20.5-99 (44.3)

1. Data in parenthesis are in average.
2. -: sporangia are nondecidous.

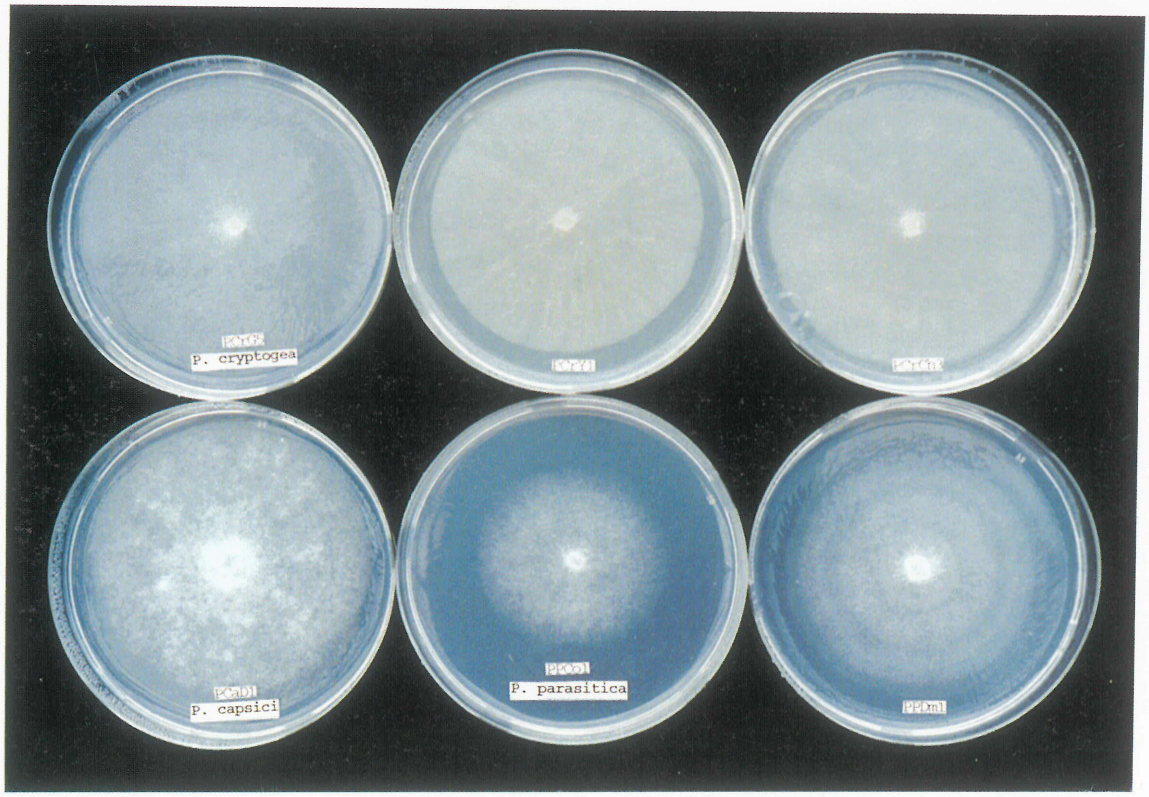

Fig. 5. Colony patterns of three species of Phytophthora isolated from crops in Compositae on 5% V-8 agar plates for 5 days at 24C.

All test isolates were grown on 5% V-8 agar from 8 to 32-33 C with a optimal growth temperature around 24 C. The colonial patterns formed by these isolates on V-8 agar were radiate without aerial mycelia (Fig. 5).

P. parasitica and the diseases it caused

P. parasitica was isolated from diseased tissues of *C. cyanus* and *S. cineraria*. The fungus mainly attacked the basal stems and roots of both host plants. The diseased tissues appeared to have a water soaked discoloration, collapsing, and shrunken init-ially; all affected plants eventually died due to serious stem rot. All Six isolates (four from *C. cyanus* and two from *S. cineraria*) obtained belong to A^2 mating type and sexuality type S^4 (8) due to that they could be induced to form oospores by the standard p991 of *P. parasitica* and vice versa.

Isolates of the fungus produced a large amount of sporangia in liquid but only a few on solid agar. Sporangia were spherical or ovoid, papillate, non-deciduous (most), non symmetrical (most), and born sympodially; average ranges of length to width were 51.4-53.3 X 40.4-41.7 μm with ratios of L/W averaging from 1.26-1.29 (Table 2). Isolates of *P. parasitica* readily produced large spherical chlamydospores on agar media and in distilled water.

The same fungus formed colonial patterns with mosaic spots and a few aerial mycelia on V-8 agar plates. The minimum, optimum, and maximum temperatures for growth of isolates of *P. parasitica* were 10-12, 24-28, and 36-37 C, respectively.

P. capsici and the disease it caused

P. capsici was only isolated from diseased tissues of *D. hybrida*. The fungus was found to attack basal stems of dahlia under natural field conditions. All affected dahlia wilted and eventually died. Dahlia was very susceptible to *P. capsici* since the fungus killed all flower plants in a garden during the rain within a very short period of time. All of the four A^1 isolates of *P. capsici* obtained from dahlia can stimulate p731 to produce oospores and vice versa; whereas belong to Sexually type S^1 (8).

Dahlia isolates of *P. capsici* produced a large amount of sporangia on V-8 agar at 24 C under light but more in liquid conditions. Sporangia were spherical, ovoid to fusiform, papillate, deciduous (30-50 %), and born 3 to 5 on the top of each single sporangiophore. About 1% of sporangia were with 2 papilla.

Length to width of sporangia ranged from 40-73.5 X 35-50 μm (*av.* 57.5 X 42.8 μm) with the ratios of L/W from 1.11-1.59 (*av.*1.42) (Table 2). Pedicel length was measured from 20.5 μm to 99 μm with a average of 44.3 μm. The minimum, optimum, and maximum temperatures for growth of dahlia isolates of *P. capsici* were 10-12, 28-30, and 36-37 C, respectively.

Pathogenicity of *P. cryptogea* to crops in Compositae

Zoospore suspension of nine isolates of *P. cryptogea* obtained from different host plants was individually inoculated on the basal stems of 24 crops of Compositae. According to these results, inoculation reaction to the same crops by the nine different isolates from different host were almost similar (Table 3). Virulent variability did exist among the test isolates in some inoculation tests, but were not significantly different. Among the 24 tested crops in Compositae *Artemisia princeps* (艾草) showed highly resistant to test isolates of *P. cryptogea* and none of inoculated cuttings were killed. *Callistephus chinensis* (翠菊) and *Wedelia trilobata* (澎蜞菊) showed moderately resistant with plants dead rates of 0-50%. Rest of the plants were highly susceptible or moderately susceptible to test isolates. This study also found the tissues culture plants of a commercial variety (Rozamunde, double type, 重瓣) of gerbera to be more susceptible to *P. cryptogea* than seedlings of a old variety (single type, 單瓣). Three other flower plants including *Dianthus caryophyllus* (carnation, 康乃馨) (3); *Euphorbia pulcherrima* (poinsettia, 聖誕紅) (1,2), and *Lobularia maritima* (香雪球) (1), which were found to be infected by *P. cryptogea* in the fields, were also susceptible to the 9 isolates of *P. cryptogea*.

Table 3: Pathogenicity of nine isolates of *Phytophthora cryptogea* to crops in Compositae and some other plants

Plants inoculated	% of plants killed by isolate of *P. cryptogea* [1]								
	PCrG5	PCrCh3	PCrR1	PCrBs1	PCrY1	PCrCa14	PCrPo5	PCrWa2	PCrL1
G. jamesonii (單瓣) (S)[2]	67	82	80	80	80	60	80	50	40
(非洲菊) (重瓣) (T)	100	100	100	100	100	100	100	100	80
S. cruentus 瓜葉菊 (C)	90	100	100	100	100	100	100	100	80
L. spicata 麒麟菊 (B)	50	50	50	25	25	50	50	50	50
C. morifolium 菊花 (C)	25	35	50	20	25	65	10	5	40
Dahlia hybrida 大理花 (C)	100	100	100	100	100	100	100	90	100
Centaurea cyanus 矢車菊 (S)	100	100	100	70	100	85	100	70	75
Cosmos bipinnatus 大波斯 (S)	100	100	100	50	100	50	80	50	50
C. sulphureus 黃波斯 (S)	85	100	100	100	100	100	100	95	95
Callistephus chinensis 翠菊 (S)	0	0	30	20	0	20	0	0	0
Bellis perennis 雛菊 (S)	100	100	100	85	100	85	100	100	90
Helichrysun bracteatum 麥桿菊 (S)	50	100	55	85	100	70	100	90	50
Gazania hybrida 勳章菊 (S)	50	100	50	65	100	65	50	35	20
Dorotheanthus bellidiformis 彩虹菊 (S)	100	100	100	100	100	100	100	100	100
Wedelia trilobata 澎蜞菊 (S)	0	0	0	0	0	5	10	0	15
Tagetes erecta 萬壽菊 (S)	65	90	90	80	90	80	65	75	90
T. patula 孔雀草 (S)	65	80	90	90	40	60	75	95	65
Calendula officinalis 金盞花 (S)	80	100	80	100	100	90	100	65	50
Zinnia elegans 百日草 (S)	80	95	90	80	95	70	65	90	95
Helianthus annus 向日葵 (S)	80	100	70	100	100	80	60	100	50
Gunura bicolor 紅鳳菜 (C)	90	75	100	80	100	100	100	90	90
C. coronarium 茼蒿 (S)	100	100	100	95	100	100	95	75	90
Lactura sativa 萵苣 (S)	100	100	100	100	100	100	100	60	85
Artemisia princeps 艾草 (C)	0	0	0	0	0	0	0	0	0
D. caryophyllus 康乃馨 (C)	60	80	100	100	100	85	75	50	85
E. pulcherrima 聖誕紅 (C)	50	50	50	50	50	50	50	25	50
Lobularia maritima 香雪球 (S)	65	95	65	95	95	100	90	50	80

1.10-20 plants (1-month-old) were inoculated, data taken after 1 month, isolates & hosts: PCrG5, *Gerbera jamesonii* 非洲菊; PCrCh3, *Chrysanthemum morifolium* 菊花; PCrR1, *Gunura bicolor* 紅鳳菜; PCrBs1, *Liatris spicata* 麒麟菊; PCrY1, *Senecio cruentus* 瓜葉菊; PCrCa14, *Dianthus caryophyllus* 康乃馨; PCrPo5, *Euphorbia pulcherrima* 聖誕紅; PCrPo2,蓮霧; PCrL1, *Lily hybridum* 百合.

2.S: seedling; C: cutting; B: bulb.

Discussion

In this survey, seven flower plants and one vegetable crop in the family of Compositae were found to be infected by *Phytophthora* in the fields (Table 1). Three species of *Phytophthora*, including *P. cryptogea*, *P. parasitica*, and *P. capsici*, were isolated from different crops. All isolates of the three fungus obtained during this study were typical types with essential morphological and physiological characteristics described in the taxonomy papers (11,12). Phytophthora diseases, including *C. morifolium*, *G. bicolor*, *L. spicata*, and *S. cruentus* infected by *P. cryptogea*, *C. cyanus* and *S. cineraria* by *P. parasitica*, and *D. hybrida* by *P. capsici*, were first reported in Taiwan.

Wilt and death of gerbera due to serious basal stem and root rot caused by *P. cryptogea* was the most serious disease in Compositae in the fields. This disease and the fungus was first found by Kao in Taiwan in 1978 (6). Henceforth, Phytophthora wilt has become the most important disease of gerbera crop in Taiwan. It has destroyed many gerbera plantations during wet seasons and caused serious economic loss each year. Native varieties of gerbera (seedlings) had been planted (without commercial cultivation) in Taiwan for a long period of times, but free from infection by *P. cryptogea*. The old varieties were also more resistant to *P. cryptogea* than the commercial varieties in inoculation test. This may indicate that the pathogen was introduced to Taiwan together with those imported commercial varieties of gerbera from European countries and established itself in Taiwan since that moment on.

Isolates of *P. cryptogea* from different host plants exhibited only a slight virulent variability in some inoculation tests. Among the 24 test crops in Compositae, *A. prinoeps* is highly resistant, whereas most test crops showed highly susceptible to all test isolates of *P. cryptogea* in present study. However, in most cases, percentages of the same kind of plants killed by different test isolates were nearly identical. This result indicated that resistance to the pathogen does exist in test crops, but host specificity among test isolates do not exist.

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摘　要

安寶貞．1996. 台灣菊科植物之疫病．植病會刊 5:146-153.
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自 1980-1994 年調查本省菊科作物疫病，發現有七種花卉與一種蔬菜罹患疫病，共分離出三種疫病菌。包括：*Phytophthora cryptogea* 危害非洲菊 (*Gerbera jamesonii*)、瓜葉菊 (*Senecio cruentus*)、麒麟菊 (*Liatris spicata*)、菊花 (*Chrysanthemum morifolium*)、紅鳳菜 (*Gunura bicolor*)；*P. parasitica* 危害矢車菊 (*Centaurea cyanus*) 與銀葉菊 (*Senecio cineraria*)；*P. capsici* 危害大理花 (*Dahlia hybrida*)。受害植物主要是莖基部或根系被感染，造成萎凋，嚴重時全株死亡；有時莖部與葉片亦會被感染，造成莖腐與葉枯。病原性測定，被接種的植株均出現與田間一致的病徵，相同的病原菌亦可自罹病組織上再分離得到。所有分離得到之 *P. cryptogea* 與 *P. capsici* 菌株均爲 A^1 配對型，而所有 *P. parasitica* 菌株均爲 A^2 配對型。*P. cryptogea* 危害瓜葉菊、麒麟菊、菊花、紅鳳菜；*P. parasitica* 危害矢車菊與銀葉菊；*P. capsici* 危害大理花均爲台灣之新紀錄。

關鍵詞：疫病、菊科植物、非洲菊、瓜葉菊、麒麟菊、菊花、矢車菊、銀葉菊、大理花、紅鳳菜、疫病菌、*Phytophthora cryptogea, P. parasitica, P. capsici.*