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由 *Bipolaris zizaniae* 引起的文心蘭花瓣斑點病

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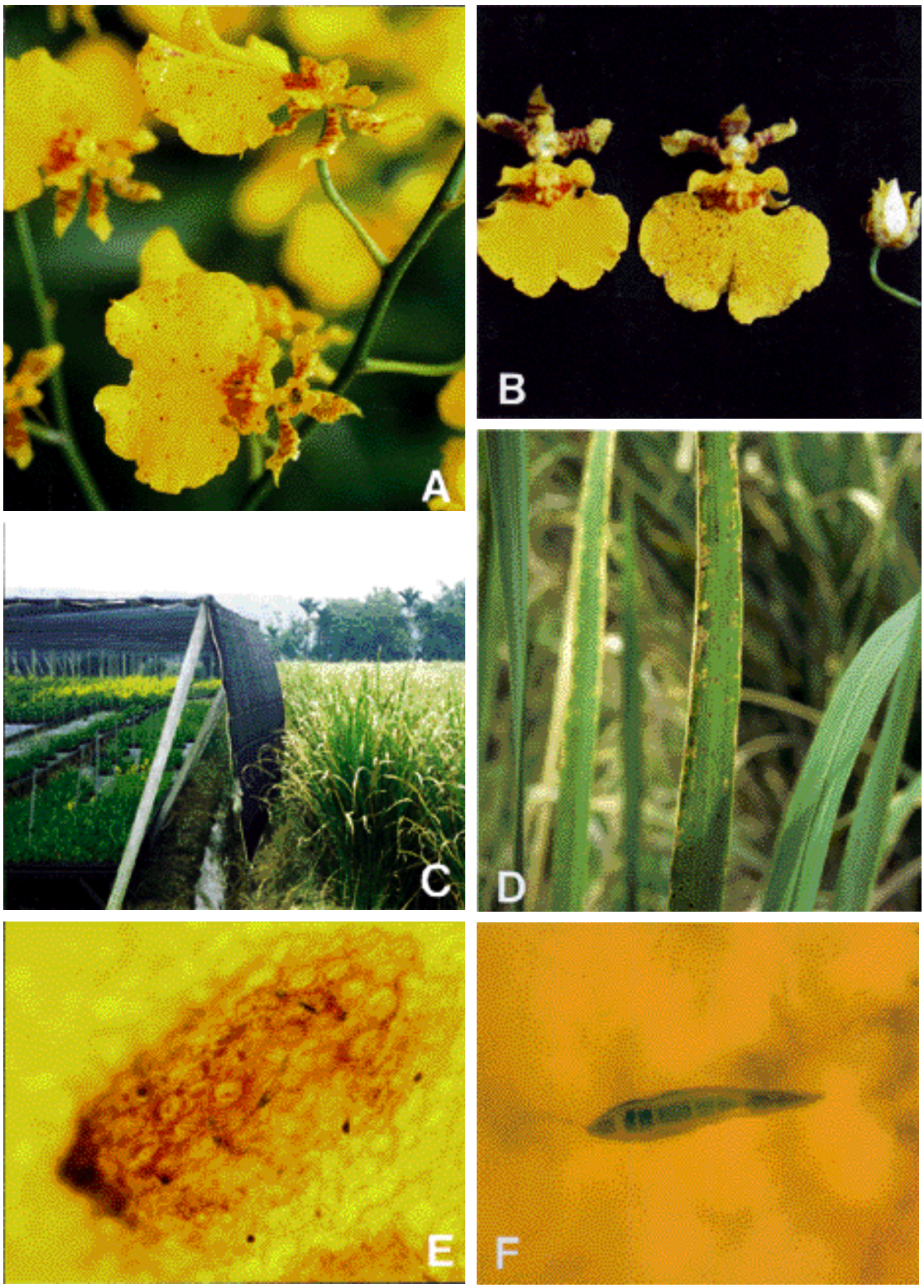
文心蘭 (Oncidiums) 原生於熱帶美洲的墨西哥、巴西、玻利維亞一帶，屬於熱帶的複莖地生蘭或著生蘭，花莖成分枝狀、多花苞，因其形狀酷似美少女穿著長禮服跳舞，故俗稱跳舞女郎蘭 (dancing lady orchids)，原生種約有七百多種。臺灣地處於亞熱帶氣候區，適合栽植文心蘭，近年來在產學雙方極力推廣種植之下，儼然已成為臺灣最大宗栽培的蘭科植物 [5]。文心蘭品種繁多，目前臺灣以種植黃色系的 Oncidium " Gower Ramsey " (南茜) 品種為主。據 1999 年台灣農業年報統計，栽植面積超過 150 公頃，主要產區分布於南投縣和屏東縣 [5]。臺灣生產之文心蘭以供應切花為主，內外銷數量各半，外銷以日本市場需求量最大。在文心蘭栽培過程中常會遭受多種病原危害，臺灣已記錄的病害包括灰黴病 (由 *Botrytis cinerea* Pers. ex Fr. 引起) [2]、炭疽病 (由 *Colletotrichum gloeosporioides Penzig* 引起 [15]、疫病 (由 *Phytophthora palmivora* (Bulter) Bulter 引起) [1]、細菌性軟腐病 (由 *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey *et al.* 引起) [7]、喜姆比蘭嵌紋病 (由 Cymbidium mosaic virus 所引起) [6] 及齒舌蘭輪斑病 (由Odontoglossum ringspot virus 引起) [3] 等。本文所述為另一種新發現的病害，曾於一九九三年植物病理學年會中論文宣讀 [4]。

近年來，南投縣埔里鎮及魚池鄉利用水稻 (*Oryza sativa,* rice) 及茭白筍 (*Zizania latifolia = Z. aquatica,* water bamboo, coba or Manchurian wild rice) 之栽培田種植文心蘭。這些轉作田之文心蘭花瓣常出現褐色之小斑點 (圖一、A 和 B)，影響切花之品質與瓶插壽命，進而降低其商品售價。一九九三年九月中旬，筆者等至該地區之多處蘭園實地觀察，並調查栽培田區所種植之文心蘭花瓣罹病之情形，發現距茭白筍田愈近，花瓣罹患褐色斑點之數目愈多 (圖一、C)。遂擇一栽培園調查園區內文心蘭花瓣斑點病與茭白筍田之位置相關性，結果顯示近茭白筍田之文心蘭罹斑點病的情形較遠離者為重；另外，茭白筍田位於文心蘭園之東南邊，期間之風向為吹東南風及南風，且當時茭白筍葉片上罹患葉枯病 (由 *Bipolaris zizaniae* (Nisikado) Shoem. (＝*Helminthosporium zizaniae* Nishikado) 引起 [9]) 的情況相當嚴重 (圖一、D)。埔里地區茭白筍的種類共有早生種、青殼種及紅殼種等，早生種於每年十二月定植，翌年四月中旬採收至十月中旬；青殼種於每年六月定植，九月初採收至十一月底；而紅殼種由每年的四月定植，於十至十一月採收完成；而葉枯病的發生時期為每年七月中旬至十一月底，尤其在九月初起，葉枯病相當嚴重，罹病葉片上產生大量胞子。由上述幾點，初步認為茭白筍葉片之葉枯病菌與文心蘭花瓣斑點病應該存有相關性。因此，於九月及十月間著手進行標本採集及鑑定病原。將所採之標本直接染色，方法為利用 0.5 % (w/v) 棉染 (cotton blue) 直接滴於花瓣罹病斑處，進行染色三分鐘，去除染劑後再滴一滴無菌水於該處，蓋上蓋玻片 (18 ×18 mm)，立即於光學顯微鏡 (BX50, Olympus Optical Co. Ltd., Tokyo, Japan) 下鏡檢。結果發現花瓣上新鮮褐化病斑處均有一個或一個以上已發芽之 *Bipolaris* sp. 胞子 (圖一、E)，檢查 200 個病斑中，尚未發現有因其他胞子感染之病斑。病斑處之胞子發芽管只稍微侵入花瓣組織之中，而發芽管則未見伸長，推測胞子發芽後可能分泌有害毒質，致使胞子周圍之花瓣細胞組織受害而呈褐化現象。植物病原菌在感染寄主之初，會分泌多種代謝物質，直接作用於寄主細胞，有些作用廣泛，未具寄主選擇性，而有些則具寄主專一性 [8]。Vidhyasekaran 氏等 [11] 報告 *Bipolaris* 或 *Helminthosporium* 胞子發芽時則會分泌專一性毒質 (host-specific phytotoxins)，以為害寄主細胞；如 *H. oryzae* (=*B. oryzae*) 在侵入部位累積毒質，以降低植物體中酚 (phenolics) 的含量及過氧化酵素 (peroxidase) 的活性，抑制寄主的防禦機制，使病原菌易於成功入侵 [10,12]。然而，*B. oryzae* 在胞子發芽時亦可分泌 ophiobolin A 和 ophiobolin B，惟前者屬非寄主專一性毒質，對寄主或非寄主不具選擇性，皆可引起葉斑 [14]。致於 *Bipolaris* sp. 在文心蘭花瓣上所造成的斑點究竟屬於非專一性毒質或是寄主本身對病原菌的過敏性反應，則尚待進一步探討。為進一步鑑定花瓣上之病原菌，在室內以組織分離技



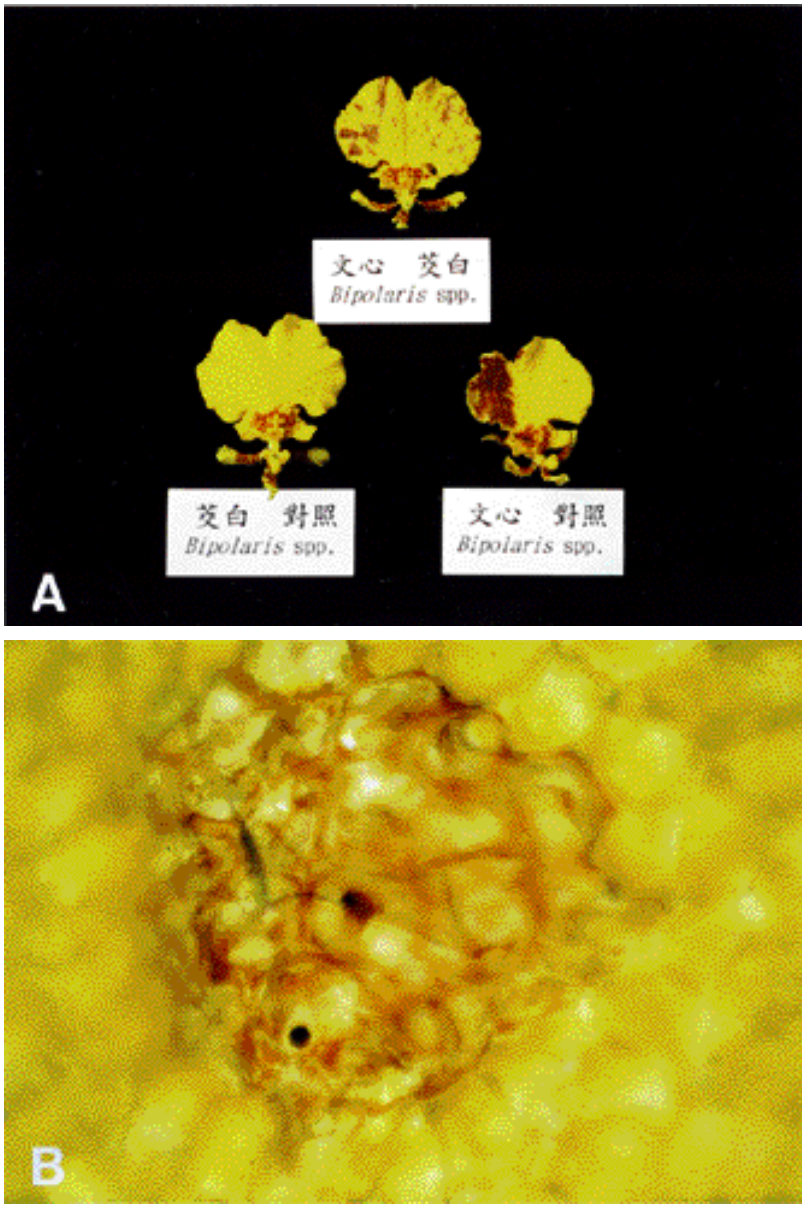
圖一、文心蘭花瓣斑點病與茭白筍葉枯病之關係。A.文心蘭花瓣斑點病之病徵，B.文心蘭花苞及花瓣上之病斑病徵，C.文心蘭園緊鄰罹葉枯病之茭白筍田，D.茭白筍葉枯病病徵近照，E.文心蘭花瓣斑點上可檢出 *Bipolaris* 的發芽胞子，F.文心蘭花瓣上之 *Bipolaris* sp. 胞子。

Fig. 1. The relationships between oncidium petal spot and water bamboo leaf blight. A. Disease symptoms of oncidum petal spot in the field. B. Brown spots on bract and petal of oncidium. C. A oncidum field adjacent a water bamboo field which was infected with serious Bipolaris leaf blight disease. D. Symptoms of water bamboo leaf blight. E. A germinating *Bipolaris* spore on the center of spot of an infected oncidium petal was observed microscopically. F. Spore of *Bipolaris* sp. on diseased oncidium petal.

術分離罹病組織上之可疑病原菌。將罹病部位之花瓣切下，在 1 % (v/v) 次氯酸鈉 (sodium hypochloride) 溶液中表面消毒 2 分鐘，再於無菌水中漂洗 3 次，以吸水紙吸乾水份後，直接置於 2 % (w/v) 水瓊脂培養基 (water agar: agar (Difco Laboratory, Detroit, MI, USA) 20 g, distilled water 1 L)平板上培養，待菌絲長出後，切取菌絲尖端，移置於馬鈴薯葡萄糖洋菜培養基 (PDA, potato dextrose agar; Difco Laboratory, Detroit, MI, USA) 斜面上，於 25℃ 定溫箱中培養與保存。由花瓣小褐斑上所分離培養之真菌，經形態鑑定確為 *Bipolaris* sp. (圖一、F)。另外，同法由罹葉枯病之茭白筍葉片上分離出 *B. zizaniae*。

為明瞭所分離之真菌與茭白筍上之 *B. zizaniae* 是否屬同一種，進行交互接種試驗。將兩種 *Bipolaris* 菌株培養於 PDA 平板上，在 25℃ 下生長三天，以口徑 0.6 公分打孔器切取菌落邊緣，將菌絲塊移置於玉米葉培養基 (2 % WA 平板上上覆上一片經高壓滅菌之玉米葉片) 上，於 25℃ 下 12 小時光照與黑暗交替定溫箱中培養三天，再加 10 ml 無菌水，以移殖環刮下胞子，經二層砂布過濾後，以血球計數器 (hemacytometer) 計算胞子濃度，並稀釋濃度成為 5×10^4／ml。以無菌之毛筆沾取胞子懸浮液，分別刷在盆栽的文心蘭花瓣及茭白筍葉片上，每處理十重覆，並以塑膠袋套封保持濕度，置於 28±4℃ 之溫室中，觀察病徵進展情形。結果發現分離自文心蘭的 *Bipolaris* sp. 與分離自茭白筍的 *B. zizaniae* 均會感染文心蘭花瓣 (圖二、A 和 B) 及茭白筍葉片，出現與田間所見相似的病徵，由罹病部位可再次分離出接種的病原菌。而且，兩種病原菌在菌落生長與胞子形態特徵並無兩樣。由此推測，埔里鎮及魚池鄉地區，水稻及茭白筍轉作文心蘭發生花瓣斑點病，係由 *B. zizaniae* 所引起。

另外，以取自農試所植病系水稻病害研究室所保存之水稻胡麻葉枯病菌 *Bipolaris oryzae* (Bred de Hann) Shoem.、玉米葉枯病菌 *B. maydis* (Nisikado) Shoem. [13] 及竹葉上分離之 *Bipolaris* sp. 等菌株，依前述方法接種至文心蘭花瓣上，亦會感染產生相類似之斑點。多數的 *Bipolaris* spp. 具寄主專一性 [8]，在本研究中發現多種 *Bipolaris* spp. 均可感染文心蘭花瓣，產生類似過敏性反應之小型病斑，病斑不隨著時間而擴大，為明瞭此種現象的真正原因，必須進一步觀察 *Bipolaris* spp. 在文心蘭花瓣上之侵染行為，以釐清病原與寄主之間的相互關係。另外，*B. zizaniae* 在茭白筍葉片上或 *B. maydis* 在玉米葉片上，均會產生大量分生胞子，鄰近栽種文心蘭之花農，應觀察茭白筍葉枯病或玉米葉枯病的發生時期，預先做適當的防治措施。



圖二、 交互接種試驗。 A.分離自文心蘭花瓣及茭白筍葉片上之 *Bipolaris* 菌株皆可為害文心蘭花瓣，B.病斑上亦可見 *Bipolaris* 之發芽胞子。

Fig. 2. Cross inoculation test. A. Both *Bipolaris* isolates from oncidium and water bamboo were able to infect the petal of oncidium and cause disease symptoms same as in the fields. B. A spore of *Bipolaris zizaniae* from water bamboo was germinating on the inoculated site of oncidium petal was observed.

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ABSTRACT

Hsieh, T. F. [1], Chang, Y. C. [1,3], and Tu, C. C. [2] 2000. Petal spot of Oncidium hybridium caused by *Bipolaria zizaniae*. Plant Pathol. Bull. 9:75-78. ([1.] Department of Plant Pathology, Taiwan Agricultural Research Institute, Wufeng 413, Taichung, Taiwan; [2.] Retired from former Department of Agriculture and Food, Taiwan provincial government, Taipei, Taiwan; [3.] Corresponding author, E-mail: ycchang@wufeng.tari.gov.tw, Fax No: 886-4-3338162)

Oncidium has become one of the most popular orchids in Taiwan in recent years. The cultivation area of oncidium cv. Gower Ramsey, used as cut flower, was over 150 ha in 1999 and was distributed mainly in the central and southern Taiwan. In the summer of 1993, a new flower disease of oncidum was found to occur at Puli and Yuche, Nantou County. The infected petals of the orchid plants appeared small brown spots. The disease shortened the life spans of the infected cut flowers and decreased flower quality. All of the diseased orchid fields were located adjacent water bamboo (*Zizania latifolia*) fields, which had serious leaf blight disease caused by *Bipolaris zizaniae*. The diseased flowers were collected, stained with 0.5% cotton blue, and examined microscopically. In the center of each young spot, one or two germinating spores of *Bipolaris* species were observed. After isolation, purification, inoculation and reisolation procedures, the *Bipolaris* fungus was conformed to be the causal agent inducing brown spots on oncidum petals. The morphological characteristics of spores of the *Bipolaris* isolates from oncidum were similar to those of *Bipolaris zizaniae* attacking water bamboo. Two isolates of *Bipolaris* from each host were selected and were cross-inoculated on both hosts in greenhouse. Test isolates were cultivated on autoclaved corn-leaves for sporulation. Spore suspension approx. $5x10^4$ spores/ml were inoculated on oncidium petals and water bamboo leaves by aid of stilled brush. Those two *Bipolaris* isolates from both hosts were pathogenic to both plants and induced disease symptoms same as those appearing in the fields. Therefore, the causal agent of petal brown spot of oncidum is identified as *Bipolaris zizaniae* and the primary inoculum sources of the disease were considered to come from *Bipolaris*-infected water bamboo nearby. Besides, *B. oryzae* from rice, *B. maydis* from corn, and *Bipolaris* sp. from bamboo also caused similar brown spot symptoms on petals of the inoculated oncidium in pathogenicity test. Based on these results, to avoid occurrence of brown spot disease on oncidium, farmers should protect their orchid from infection by these four pathogens in advance.

Keywords : *Bipolaris zizaniae*, cross infection, oncidium, water bamboo (Manchurian wild rice)