DOI:10.6716/JPM.202012_62(4).0003

葡萄晚腐病菌分子鑑定及對殺菌劑之感受性

段中漢[1*]、陳冠穎[1]

[1] 農業藥物毒物試驗所農藥應用組，臺灣 臺中市

[*] 聯絡作者，Email: chduan @tactri.gov.tw

摘　要

段中漢、陳冠穎。2020。葡萄晚腐病菌分子鑑定及對殺菌劑之感受性。植物醫學62(4): 23-32。

由*Colletotrichum* species 引起的晚腐病是臺灣地區為害最為普遍亦最為嚴重的葡萄病害。本研究以分生孢子形態及五種特定基因序列 (ITS, GAPDH, ACT, TUB2, ApMat) 鑑定近五年來分離自台灣地區的葡萄晚腐病菌，得知其主要菌種為 C. viniferum ，而次要菌種為C. tropicale。噴施化學藥劑並配合果實套袋是晚腐病的主要防治措施，因而藥劑效果是決定晚腐病防治成敗的關鍵因素。本研究以微量滴定盤法測試20種晚腐病防治藥劑之藥效，結果顯示各藥劑的抑菌效果對供試菌株具一致性。其有效抑制晚腐病菌孢子發芽的藥劑計有腈硫醌、克熱淨、鋅錳乃浦、免得爛、快得寧、保粒黴素 (甲) 及得恩地等7種，而有效抑制其菌絲生長的藥劑則有克熱淨、撲克拉及撲克拉錳等3種。

關鍵詞：葡萄、晚腐病菌、殺菌劑

前　言

臺灣地區葡萄 (*Vitis vinifera* L.) 栽培面積現約有二千七百餘公頃，栽培品種以鮮食用「巨峰」(*V. vinifera* × *V. labrusca*, Kyoho) 為主，產地大多分布在彰化、臺中、苗栗及南投等縣市[5]。臺灣葡萄品質優越，頗受國內消費者喜愛，又因鄰近中國大陸及東亞地區，外銷市場大，是極具發展潛力的水果。惟因臺灣地處亞熱帶，海島型氣候高溫潮濕，加以葡萄果實生長期適逢梅雨及颱風季，利於各種葡萄真菌性病害之發生與傳播，其中又以晚腐病最為普遍也最為嚴重 [14, 15, 33]。病原菌之分類及鑑定是病害研究的首要工作，早期葡萄晚腐病菌均稱 *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. [14, 31, 41]，近年因分子生物學及相關技術快速發展，炭疽病菌屬 (*Colletotrichum* spp.) 的分類系統及鑑定標準有了新的面貌 [27, 28]。葡萄晚腐病菌在國外已有許多新記錄種 (species) 被發表[30, 40, 42, 55, 58, 59, 60]；台灣地區晚腐病菌亦有數個新記錄種被報導[18, 33, 34]，但相關資訊尚欠完整，值得進一步探討。炭疽病菌可用於分類與鑑定的形態特徵很少且差異不大，如依據分生孢子 (conidium) 形態為分類標準僅能區分為*C. acutatum*、*C. boninense*及*C. gloeosporioides*等為主的11個複合種 (species complex) 及23個獨立種 (singleton species)，而每一複合種又包含數個至數十個種[28]。但如依寄主作物命名，又因炭疽病菌未必具寄主專一性而難以為據[1, 22, 23]。欲將每一複合種所涵蓋的許多個別種作區別，勢須藉助分子生物學為主的其他鑑定技術[7, 12, 28]。*Colletotrichum* spp.在種間鑑定上常用的基因序列有核糖體核酸內轉錄間隔區 (internal transcribed spacer, *ITS*)，甘油醛-3-磷酸脫氫酶 (glyceraldehydes-3-phosphate dehydrogenase, *GAPDH*)、肌動蛋白 (actin, *ACT*)、微管蛋白 (β-tubulin-2, *TUB2*)、鈣調蛋白 (calmodulin , *CAL*)、幾丁質合成酶 (chitin synthase 1, *CHS-1*)、麩醯胺酸合成酶 (glutamine synthetase , *GS*) 及超氧化物歧化酶 (manganese-superoxide dismutase , *SOD2*) 等多種基因[12, 27, 28, 35, 38, 47]。另一新的鑑定用基因Apn2-Mat1-2 intergenic spacer (*ApMat*) 已導入*Colletotrichum* spp.之鑑定，且足以替代前述多基因 (multi-gene) 鑑定之結果[49, 50]，本研究亦將之列入。運用這些基因序列與模式菌株 (type strain) 進行序列比對，將能獲致可靠的鑑定結果[28, 35, 38]。

施用化學藥劑並配合果實套袋是葡萄晚腐病現行主要的防治措施，在果實套袋前為防止感染，常須連續噴藥以資保護，因此藥劑施用關係晚腐病防治成敗，而藥劑的有效性也就成了關鍵。農委會農業藥物毒物試驗所「植物保護資訊系統」即列有20種葡萄晚腐病防治用藥[4]，然與晚腐病菌同屬的炭疽病菌在國內外卻常見抗藥性問題 [11, 25, 26, 36, 43, 46, 52, 56]，臺灣地區葡萄晚腐病菌是否亦已對其防治藥劑產生抗性，早期雖有報告[25]，現況如何，仍待評估。藥效評估的目的在運用有效率的方法針對藥劑的抑菌效果進行測試，以推斷其實際的防治作

用，並希能在實驗室進行以節約成本。葡萄晚腐病菌在人工培養基上除產生菌絲外，亦能生成大量分生孢子，菌絲生長與孢子發芽皆是良好的藥效評估指標[48]。傳統的室內藥效試驗是以計算半數抑制濃度 (half maximal effective concentration, EC_{50}) 作為評估方法，需設計一系列的藥劑濃度供試，惟此方法耗時費力[11, 52, 56]。近年來，國際學研界已發展出一種藉單一殺菌劑劑量鑑別病原菌抗感性的試驗方法，此劑量稱為鑑別劑量 (discriminatory dose)[20, 45, 57]。在臺灣，由藥毒所建構的植物保護資訊系統對作物病蟲害所列防治藥劑多有稀釋倍數之規定[4]，因而我們可就藥劑主成分 (active ingredient) 含量百分率及稀釋倍數兩參數計算出其田間實際的使用濃度 (use rate) 做為該藥劑的鑑別劑量，以測定病原菌對藥劑的抗感性，並搭配簡易的微量滴定盤法 (microtiter plate) 進行試驗[13]。本研究將以台灣地區葡萄晚腐病菌之鑑定及其防治藥劑之藥效評估為主題進行探討，以明瞭葡萄晚腐病菌菌種分布概況及其防治藥劑的有效性，供作未來田間應用之參考。

材料與方法

供試菌株之分離與鑑定

自2014年迄2018年，分別前往臺灣中部各葡萄產區地採集罹晚腐病之葡萄果實供分離病原菌。成熟的罹病果實病斑部會產生分生孢子盤 (acervulus)，上有大量分生孢子，只需以棉花棒沾取孢子並置入2-mL離心管 (Eppendorf tube)，即完成採樣。樣品攜返實驗室後，將沾有晚腐病菌分生孢子的棉花棒塗佈於2%洋菜平板 (water agar)，再以玻璃針單孢分離法獲得單孢菌株。單孢菌株培養於馬鈴薯葡萄糖洋菜培養基 (potato dextrose agar, PDA)，置於24℃及每日光照12小時之定溫箱，供後續試驗之用。另將培養7日所得之菌落 (colony) 以直徑5 mm 打孔器切取菌落周邊菌絲塊，放入內裝1 mL無菌水之2-mL冷凍小管 (cryogenic vial, Nalge Co., Rochester, NY, USA)，置16℃定溫箱作長期保存。葡萄晚腐病菌經數年之採集獲得菌株約100餘，乃逢機選取代表各縣市產區之晚腐病菌24株，作為菌種 (species) 鑑定與藥劑試驗之供試菌株 (表一)。各菌株學名之鑑定係以分生孢子形態及菌種鑑定用基因序列即分子鑑定 (molecular identification) 為依據。分生孢子形態係度量其長度與寬度並記錄其形狀。分子鑑定用基因序列則以供試菌株之5種鑑定用基因 (*ITS*, *GAPDH*, *ACT*, *TUB2*, *ApMat*) 與各菌種模式菌株之基因進行序列比對。為進行基因定序，供試菌株分別接種於馬鈴薯葡萄糖洋菜培養基平板，並於24℃及每日光照12小時之定溫箱培養7日。刮取菌絲後，以核酸萃取套組 (AllPure Plant Genomic DNA Kit；百歐生技公司，臺灣) 抽取基因組核酸 (genomic DNA)。並以聚合酶連鎖反應（polymerase chain reaction, PCR）增幅核糖體核酸內轉錄間隔區 (*ITS*, 引子對ITS1 / ITS4)[54]、甘油醛-3-磷酸脫氫酶 (*GAPDH*, 引子對GDF1 / GDR1)[24]、肌動蛋白 (*ACT*, 引子對ACT512F / ACT783R)[9]、微管蛋白 (*TUB2*, 引子對T1 / T2)[39]及Apn2-Mat1-2 intergenic spacer (*ApMat*, 引子對AM-F / AM-R)[49, 50] 等5種基因序列。聚合酶連鎖反應之引子對黏合溫度 (annealing temperature) 分別為*ITS* (55℃)、*TUB2* (52℃)、*GAPDH* (55℃)、*ACT* (55℃) 及*ApMat* (60℃)，均作用30 sec。其他聚合酶連鎖反應相關之材料與方法悉參照著者已發表報告[18]。而後，以聚合酶連鎖反應產物進行基因雙向定序，定序結果整理成序列重疊群 (sequence contig)[51] 供後續菌種鑑定之用。供試菌株之鑑定是以上述5種基因序列分別與炭疽病菌各菌種之模式菌株的相同基因序列作比對，以基因序列相同百分率 (percent identity) 表示之。綜合菌株分生孢子形態特徵及各基因序列比對結果判定菌株之學名。為圖示各菌株與模式菌株的親緣關係，復將各菌株作多基因序列分析 (multilocus sequence analysis, MLSA) ，而以系統發生樹 (phylogenetic tree) 呈現[12]。此所稱多基因序列之基因係採用與菌種鑑定用相同的5種基因，分析前，先將各菌株之單一相同基因序列並列切齊，再將同一菌株之此5種基因序列鏈結成多基因序列進行親緣性分析，分析時並納入各相關菌種之模式

表一、葡萄晚腐病菌供試菌株

TABLE 1. *Colletotrichum* isolates of grape used in this study

Isolate	Origin	Date collected
GC1	Xinshe,Taichung	Aug. 2014
GC10	Erlin, Changhua	Jul. 2015
GC12	Xihu, Changhua	Jul. 2015
GC15	Xihu, Changhua	Jul. 2015
GC18	Sinyi, Nantou	Jul. 2015
GC19	Xinshe, Taichung	Oct. 2015
GC27	Juolan, Miaoli	Jul. 2016
GC28	Xihu, Changhua	Jul. 2016
GC31	Juolan, Miaoli	Dec. 2016
GC37	Dacun, Changhua	Nov. 2017
GC39	Dacun, Changhua	Nov. 2017
GC40	Sinyi, Nantou	Nov. 2017
GC41	Juolan, Miaoli	Nov. 2017
GC42	Xinshe, Taichung	Nov. 2017
GC43	Xihu, Changhua	Jun. 2018
GC44	Erlin, Changhua	Jul. 2018
GC46	Erlin, Changhua	Jul. 2018
GC48	Dacun, Changhua	Jul. 2018
GC50	Juolan, Miaoli	Aug. 2018
GC51	Xinshe, Taichung	Oct. 2018
GC55	Dacun, Changhua	Jun. 2019
GC57	Sinyi, Nantou	Jul. 2019
GC59	Sinyi, Nantou	Jul. 2019
GC61	Xinshe, Taichung	Oct. 2019

菌株：*C. aenigma* (ICMP 18608)、*C. fructicola* (ICMP 18581)、*C. siamense* (ICMP 18578)、*C. tropicale* (CBS 124949) 及 *C. viniferum* (GZAAS5.08608)[12, 42]。分析方法是採用貝葉斯推斷法 (Bayesian inference method, TOPALi version 2.5)。

供試殺菌劑

為探討殺菌劑對葡萄晚腐病菌之抑菌效果，乃依據藥毒所網站之「植物保護資訊系統」[4]，收集其防治藥劑共20種供試，計有亞托敏 (azoxystrobin)、白克列 (boscalid)、貝芬替 (carbendazim)、賽普護汰寧 (cyprodinil + fludioxonil)、腈硫醌 (dithianon)、三氟派瑞 (fluopyram +trifloxystrobin)、克熱淨 (iminoctadine triacetate)、依普同 (iprodione)、克收欣 (kresoxim-methyl)、鋅錳乃浦 (mancozeb)、撲克拉錳 (manganese prochlorate)、免得爛 (metiram)、快得寧 (oxine-copper)、保粒黴素(甲) (polyoxins)、撲克拉 (prochloraz)、百克敏 (pyraclostrobin)、得克利 (tebuconazole)、腐絕 (thiabendazole)、甲基多保淨 (thiophanate-methyl)、得恩地 (thiram) 等 (表二)。藥劑係購自本地農藥零售店之成品農藥或藥毒所檢驗合格之成品農藥剩餘樣品。供試藥劑屬多點作用機制藥劑 (multi-site) 有6種，其餘分屬8種不同單點作用機制藥劑 (specific-site)[32]。

殺菌劑對炭疽病菌分生孢子發芽之影響

本試驗以微量滴定板法[13]測試20種葡萄腐病防治藥劑 (表二) 對逢機選取之12株晚腐病菌 (~~表一~~) 分生孢子發芽之抑制作用。供試藥劑之藥液配製是以無菌蒸餾水稀釋至其田間施用濃度 (表二)。測試時，取49 μL供試藥液滴入微量滴定板之盤穴 (well)，再加入1 μL供試菌株之孢子懸浮液 (1×10^6 spores / mL)，均勻混合。另以供試菌株孢子加入無菌水之處理為對照。處理後之微量滴定板覆以封口膜 (parafilm, PM-996) 以防水分蒸散並置於實驗室 (24~28℃)。2小時後，將盤穴內之混合液分別塗布於直徑9公分之2%洋菜平板上，洋菜平板靜置於24℃黑暗定溫箱。24小時後，於光學顯微鏡下計數孢子發芽率。每處理4重複，每重複計數200個孢子，以百分率表示孢子發芽率。各處理之發芽百分率先進行顯著性分析 (One-way analysis of variance, ANOVA)，差異達5%顯著水準，則對處理間之差異進行費雪最小顯著差異測驗 (Fisher's protected least significance test, LSD, 5%)。

殺菌劑對炭疽病菌菌絲生長之影響

為測試前述各種殺菌劑對~~供試~~葡萄晚腐病菌菌絲生長之影響，乃以同上之菌株培養於馬鈴薯葡萄糖洋菜培養基7日之菌落供試，先以直徑5 mm打孔器切取菌落周緣之菌絲塊，將之放入注有200 μL供試藥液之微量滴定板盤穴內並置室溫下。另以菌絲塊加入無菌水之處理為對照。供試藥劑之稀釋方法及濃度同於前項試驗。處理2小時後，以移植針將菌絲塊挑出，置滅菌過之吸水紙將藥液吸乾，再移置於直徑9 cm之馬鈴薯葡萄糖洋菜培養基平板中央，於24℃ 無光照之定溫箱培養5日。以通過菌落中心點之兩條垂直線為準，量取菌落直徑，並以二者平均值為該菌落直徑度量，以比較各藥劑之抑菌效果。每菌株每藥劑處理4重複。

表二、本研究供試殺菌劑之種類、劑型、作用機制代碼及使用劑量

TABLE 2. Fungicides used in this study

Fungicide	Formulation	FRAC code[1]	Use rate (μg a.i./ml)[2]
Azoxystrobin	23% SC	11	115
Boscalid	50% WG	7	333
Carbendazim	41.7% SC	1	210
Cyprodinil 37.5% + fludioxonil 25%	62.5% WG	9, 12	313
Dithianon	42.2 SC	M9	352
Fluopyram＋trifloxystrobin	50%SC	11, 7	125
Iminoctadine triacetate	25%SL	M7	267
Iprodione	23.7% SC	2	296
Kresoxim-methyl	44.2% SC	11	250
Mancozeb	80% WP	M3	550
Manganese prochlorate	50% WP	3	83
Metiram	80% WG	M3	1600
Oxine-copper	40% WP	M1	300
Polyoxins	50% SG	19	167
Prochloraz	25% EW	3	100
Pyraclostrobin	23.6% EC	11	80
Tebuconazole	25.9% EW	3	173
Thiabendazole	41.8% SC	1	420
Thiophanate-methyl	70%WP	1	700
Thiram	80% WP	M3	1000

[1] Fungicide Resistance Action Committee (2019).

[2] Use rate represents the active ingredient concentration in a label recommended on gra.

結　果

葡萄晚腐病菌之鑑定

供試葡萄晚腐菌在馬鈴薯葡萄糖洋菜培養基經7日以上之培養，以菌落產生之分生孢子作形態描述。分生孢子均為兩端頓圓形 (broadly rounded ends) 之直長柱形 (straight, cylindrical)，應屬*C. gloeosporioides*複合種。供試菌株以其5種基因序列 (*ITS*、*GAPDH*、*ACT*、*TUB2*、*ApMat*) 與模式菌株進行比對，其中各基因序列與最近模式菌株之相似百分率均達 98%以上 (表三)。經鑑定，*C. viniferum*佔22種，且在分子親緣性分析之樹狀圖上分布於同一分支群 (clade)，而僅有GC12及GC15二菌株屬*C. tropicale*，不同菌種明顯分屬不同分支群 (圖一)。

表三、葡萄晚腐病菌與模式菌株五種鑑定用基因序列之相同百分率

TABLE 3. Percent identity of the given gene sequences between *Colletotrichum* isolates from grape and the reference cultures

Isolate[1]	Species	Reference culture[2]	Percent identity (%)[3]				
			ITS	*GAPDH*	*ACT*	*TUB2*	*ApMat*
GC1	*C. viniferum*	GZAAS5.08608	99.4	98.2	98.2	99.4	98.7
GC10	*C. viniferum*	GZAAS5.08608	99.6	100.0	99.1	100.0	99.9
GC12	*C. tropicale*	CBS124949	99.5	98.2	99.6	99.7	100.0
GC15	*C. tropicale*	CBS124949	99.1	97.8	99.7	99.9	100.0
GC18	*C. viniferum*	GZAAS5.08608	99.4	98.2	98.2	99.4	98.7
GC19	*C. viniferum*	GZAAS5.08608	99.4	98.2	98.6	99.3	98.7
GC27	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.2	99.4	98.8
GC28	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.2	99.3	98.7
GC31	*C. viniferum*	GZAAS5.08608	99.4	99.1	98.6	99.2	98.7
GC37	*C. viniferum*	GZAAS5.08608	99.4	99.1	98.2	99.0	98.7
GC39	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.6	99.3	98.7
GC40	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.6	99.6	98.8
GC41	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.2	99.2	98.3
GC42	*C. viniferum*	GZAAS5.08608	99.4	98.2	98.2	99.4	98.8
GC43	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.2	99.3	98.7
GC44	*C. viniferum*	GZAAS5.08608	99.6	100.0	99.1	99.9	98.8
GC46	*C. viniferum*	GZAAS5.08608	99.6	100.0	99.1	99.9	100.0
GC48	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.6	99.3	98.8
GC50	*C. viniferum*	GZAAS5.08608	99.4	99.5	98.6	99.4	98.8
GC51	*C. viniferum*	GZAAS5.08608	99.4	99.5	98.6	99.4	98.7
GC55	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.2	99.2	98.7
GC57	*C. viniferum*	GZAAS5.08608	99.4	99.6	98.2	100.0	98.7
GC59	*C. viniferum*	GZAAS5.08608	99.4	98.2	98.63	99.2	98.7
GC61	*C. viniferum*	GZAAS5.08608	99.4	98.2	98.2	99.4	98.7

[1] Refer to TABLE 1 for isolate information.

[2] Reference culture: ex-type or authentic culture of each *Colletotrichum* species.

[3] Percent identity: identity between each gene sequence of the isolate with that of the reference culture in percentage ratio. Genbank accession numbers of reference cultures are JN412802 (*ITS*), JN412800 (*GAPDH*), JN412793 (*ACT*), JN412811 (*TUB2*), and KJ623242 (*ApMat*) for GZAAS5.08608, and JX010264 (*ITS*), JX010007 (*GAPDH*), JX009489 (*ACT*), JX010407 (*TUB2*), and KC790728 (*ApMat*) for CBS124949. *ITS*: complete rDNA – ITS; *GAPDH*: glyceraldehyde-3-phosphate dehydrogenase; *ACT*: partial actin; *TUB2*: β-tubulin; *ApMat*: Apn2-Mat1-2 intergenic spacer.

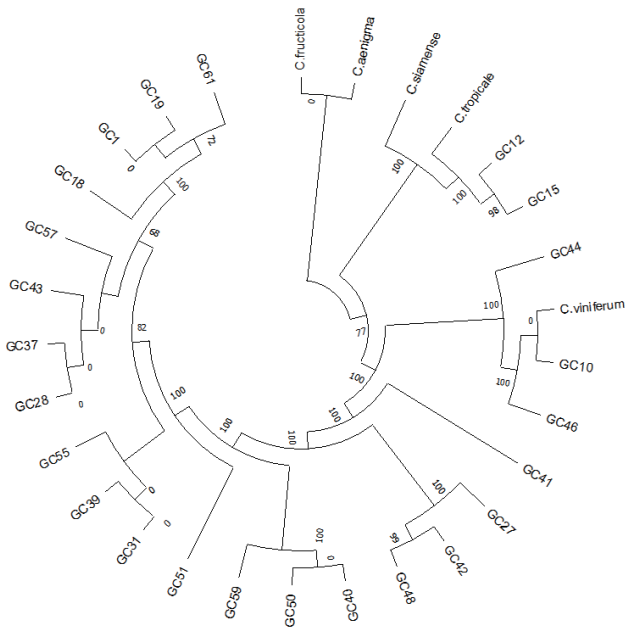
*C. viniferum*代表菌株GC1分生孢子長寬 (mean ± SE) 為 14.49 ± 0.23 (12.50-17.50) × 5.48 ± 0.08 (5.00-6.00) μm，長柱形，兩端頓圓；*C. tropicale* 代表菌株GC12長寬為14.96 ± 0.18 (12.50-17.50) × 5.85 ± 0.14 (5.00-8.00) μm，形狀與前者類似，二者度量均符合各該菌種之度量範圍 (range)[12, 30, 58]。

殺菌劑對炭疽病菌孢子發芽之影響

供試之20種殺菌劑對12株晚腐病菌之藥效基本一致，完全有效抑制孢子發芽的藥劑有腈硫醌、克熱淨、鋅錳乃浦、免得爛、快得寧、保粒黴素 (甲) 及得恩地等7種，其藥劑處理各菌株分生孢子發芽率均為0%。僅有一例外 (GC12)對克熱淨及保粒黴素 (甲) 較不具感受性，分別呈現較低 (14.0%) 及較高 (78.0%) 的孢子發芽率；而賽普護汰寧對半數供試菌株之孢子具完全抑制作用。此外，三氟派瑞尚能抑制菌株GC18 及GC19二菌株之孢子發芽，使其呈現極低 (6.3%) 的孢子發芽率。其他藥劑對供試菌株孢子發芽均不具抑制作用且發芽率皆達80%以上 (表四)。

殺菌劑對葡萄晚腐病菌菌絲生長之影響

供試20種殺菌劑對12株晚腐病菌菌絲生長之抑制作用也是一致的。有效抑制菌絲生長的藥劑有克熱淨、撲克拉及撲克拉錳等3種，供試菌株之菌絲幾無法生長。但菌株GC12卻完全不受克熱淨之抑制，其對撲克拉亦僅受局部抑制；另一例外是GC46，該菌株經撲克拉錳處理後仍有少量生長。其餘藥劑對供試菌株之菌絲生長均無完全抑制作用，且多數菌株之生長與對照處理無異 (表五)。



圖一、葡萄晚腐病菌之分子親緣關係圖。

Fig. 1. Phylogenetic analysis of the isolates of grape ripe rot pathogen and their closely related species of *Colletotrichum* based on the concatenate sequences of *ITS*, *GAPDH*, *ACT*, *TUB2* and *ApMat* genes. This analysis was performed by Bayesian inference method using TOPALi version 2.5. The numbers beside nodes are Bayesian posterior probability. Culture accession numbers for each reference species are *C. aenigma* (ICMP 18608), *C. fructicola* (ICMP 18581), *C. siamense* (ICMP 18578), *C. tropicale* (CBS 124949), and *C. viniferum* (GZAAS5.08608). Refer to TABLE 1 for grape isolate information.

表四、殺菌劑對葡萄晚腐病菌孢子發芽率之影響

TABLE 4. Effect of fungicides on conidial germination of 12 *Colletotrichum* isolates from grape

	No. of isolates with germination rate (%) of [1]			
Fungicide	0.0	1.0~39.0	40.0~79.0	80.0~100.0
Azoxystrobin	0	1	1	10
Boscalid	0	0	1	11
Carbendazim	0	0	0	12
Cyprodinil + fludioxonil	6	0	2	4
Dithianon	12	0	0	0
Fluopyram+trifloxystrobin	0	2	1	9
Iminoctadine triacetate	11	1	0	0
Iprodione	0	1	0	11
Kresoxim-methyl	0	0	0	12
Mancozeb	12	0	0	0
Manganese prochlorate	0	0	0	12
Metiram	12	0	0	0
Oxine-copper	12	0	0	0
Polyoxins	11	0	1	0
Prochloraz	0	0	1	11
Pyraclostrobin	0	0	2	10
Tebuconazole	0	1	0	11
Thiabendazole	0	0	2	10
Thiophanate-methyl	0	0	1	11
Thiram	12	0	0	0
Control (water)	0	0	0	12

[1] Germination rate on water agar plate 24-hour at 24 ˚C after fungicide treatment.

討　論

近年來，在物種鑑定上，特別是形態特徵稀少且不易辨識區別的微生物，應用分子生物學及相關技術於其分類及鑑定，頗為常見，炭疽病菌 (*Colletotrichum* spp.) 即是案例之一[12, 27]。葡萄晚腐病菌學名早期均稱 *C. gloeosporioides*，其實只是依據其形態而命名的一個複合種[12, 28]，且該種作為許多作物炭疽病菌之學名屢受質疑[44]。近年葡萄晚腐病菌之菌種在國外已有多個新記錄種被發表，例如，*C. acutatum*、*C. aenigma*、*C. capsici*、*C. citri*、*C. fructicola*、*C. godetiae*、*C. hebeiense* 及*C. viniferum*等[30, 40, 42, 55, 58, 59, 60]，台灣則已有*C. siamense*、*C. tropicale*及*C. viniferum*等菌種被報導[18, 33, 34]。其中，又以*C. viniferum*最受矚目，因該菌種學名源自葡萄學名，作為葡萄晚腐病菌別具意義。而該菌種也是近年中國大陸相關報告中所列的優勢種[30, 42, 58]，在我們的研究中亦然，其在已知菌株中佔絕大部分 (表三、圖一)。據此，我們推斷，臺灣葡萄晚腐病菌應存有數個菌種，且在數量上可能以*C. viniferum*居於多數，至於*C. tropicale*則為世界首例[19]，在國內外報告中僅見於本文及著者之前的報告(18)。*C. viniferum*代表菌株GC1 及*C. tropicale* 代表菌株GC12 亦已接種於摘離 (detached) "巨峰"葡萄果實確認其病原性 (著者，未發表)。然因本研究所用菌株尚屬有限，是否尚有其他菌種，所佔比率為何，則應當納入更多菌株才能獲知較完整的菌種分布概況。

本研究為能測試12株晚腐病菌對多達20種殺菌劑之抗感性，又同時兼以孢子發芽及菌絲生長為指標，囿於有限試驗資源，乃採用藥劑田間施用濃度之單一劑量進行殺菌劑抗感性試驗。這種試驗結果與半數抑制濃度 (EC_{50}) 均屬實驗室推估之結果，與田間實際狀況終究有別。此方法雖在檢測上可節省大量人力及時間，但對了解病原菌在不同藥劑濃度下的反應 (dose-response relationships) 則有不足，特別是欲探討田間病原菌族群對藥劑的動態反應上可能無法釐清菌株族群的即時變化。理論上，供試菌株越多越好，但受限於研究資源僅能就不同年份選出數株代表性菌株供試，但就著者近年所發表之炭疽病菌抗藥性調查報告及本研究觀之，尚未見因年份不同而有差異之現象[16, 17, 18]。

本研究證實大部分葡萄晚腐病防治藥劑已無抑制病原菌孢子發芽的效果，菌絲生長則更甚，只有含撲克拉成分之藥

表五、殺菌劑對葡萄晚腐病菌菌絲生長之影響

TABLE 5. Effect of fungicides on mycelial growth of 12 *Colletotrichum* isolates from grape

Fungicide	No. of isolates with colony diameter (cm) of [1]			
	<0.1	0.1~2.0	2.1-4.0	>4.0
Azoxystrobin	0	0	0	12
Boscalid	0	0	0	12
Carbendazim	0	7	3	2
Cyprodinil + fludioxonil	0	6	5	1
Dithianon	0	0	0	12
Fluopyram+trifloxystrobin	0	0	0	12
Iminoctadine triacetate	11	0	0	1
Iprodione	0	1	10	1
Kresoxim-methyl	0	0	0	12
Mancozeb	0	1	7	4
Manganese prochlorate	11	1	0	0
Metiram	0	3	7	2
Oxine-copper	0	0	5	7
Polyoxins	0	1	7	4
Prochloraz	12	0	0	0
Pyraclostrobin	0	1	8	3
Tebuconazole	0	5	4	3
Thiabendazole	0	8	2	2
Thiophanate-methyl	0	9	1	2
Thiram	0	0	2	10
Control (water)	0	0	0	12

[1] Diameter range of colony on potato dextrose agar plate 5-day at 24 °C after fungicide treatment.

劑及克熱淨尚能有效，餘已無藥效。事實上，國內外在近年關於炭疽病防治藥劑的抗藥性報告屢見不鮮，例如，許多臺南芒果炭疽病菌株已對苯并咪唑類殺菌劑 (benzimidazoles) 產生抗藥性[36]，且國內常用芒果炭疽病防治藥劑多數已無抑菌效果[16, 17]。在美國，多種果樹及草皮炭疽病菌對醌外抑制劑 (quinone outside inhibitors, QoIs)、固醇去甲基化抑制劑 (sterol demethylation inhibitors, DMIs) 及琥珀酸去氫酶抑制劑 (Succinate dehydrogenase inhibitors, SDHIs) 等類別之殺菌劑均已產生抗藥性[2, 3, 21, 26, 56]。日本亦有多種水果炭疽病菌*C. gloeosporioides*對甲基多保淨及克熱淨產生抗藥性[11]。巴西蘋果葉斑及苦腐病菌 (*C. acutatum*) 的部分菌株已對鋅錳乃浦及亞托敏產生抗藥性，且其多數菌株則對甲基多保淨產生了抗藥性[37]。上述藥劑中除克熱淨及鋅錳乃浦外，其他藥劑之抗藥性現象均與本研究之結果一致，顯示炭疽病菌抗藥性具有超越地域的普遍性現象。儘管炭疽病菌抗藥性現象很常見，但仍有數種藥劑即使已在臺灣已使用數十年仍保有良好的藥效，例如，4-4式波爾多液、撲克拉及撲克拉錳等藥劑抑制多種果樹炭疽病菌菌絲生長效果仍佳；而4-4式波爾多液、鋅錳乃浦、免得爛、腈硫醌、快得寧等藥劑抑制果樹炭疽病菌孢子發芽效果最好[53]。另，郭氏測試1375支菌株證實臺灣芒果炭疽病菌並未對撲克拉產生抗藥性[29]。此外，國外亦報導二硫代胺基甲酸鹽殺菌劑 (Dithiocarbamate fungicides) 是防治芒果炭疽病的有效藥劑，而屬固醇去甲基化抑制劑類 (DMIs) 之撲克拉也未見抗藥性，且該藥劑兼具感染前保護與感染後防除之功效，是唯一用於芒果採收後處理的藥劑(6)。這些報告的結論均在本研究中再一次獲得證實。

本研究顯示抑制孢子發芽的殺菌劑除保粒黴素 (甲)，其餘均屬多點作用機制藥劑，且以二硫代胺基甲酸鹽類藥劑為主(表四)。根據著者觀察，保粒黴素 (甲) 之功能並非抑制晚腐病菌分生孢子發芽，而是使發芽管前端膨大成圓形泡狀體並停止伸長及產生附著器 (appressorium)，因而是不具致病能力的無效發芽，可視同受到抑制。此外，賽普護汰寧仍對半數供試菌株之孢子具完全抑制作用，表示該藥之抗藥性發展正在進行中，在未來使用上必須謹慎，應減少施用次數。而國內有報告稱賽普護汰寧雖不能完全抑制孢子發芽，但孢子發芽後不易形成附著器，這可能是該藥劑仍受農民青睞的原因[53]。而多數菌株對三氟派瑞不具感受性，卻仍有少數菌株 (GC18、GC19) 具感受性，應屬例外，該藥劑是否繼續於田間使用實已面臨考驗。而菌株GC12是本研究中唯二鑑定為*C. tropicale*的菌種，卻也特別對克熱淨及保粒黴素 (甲) 分別表現不同程度的抗性，是否與菌種特性有關，則須待更多的*C. tropicale*供試，始能定論。

能抑制菌絲生長的藥劑種類在本研究中更少，僅有屬咪唑類 (imidazoles) 之撲克拉、撲克拉錳及屬胍類 (guanidines) 之克熱淨等3種。其中撲克拉對抑制炭疽病菌菌絲生長表現最優，此與國內外相關研究一致[8, 10, 17, 29]，但撲克拉在相同濃度下卻完全不能抑制其孢子發芽，國內亦有類似的研究結果[53]。在本研究中，與撲克拉 (imidazoles類) 同屬固醇去甲基化抑制劑類的供試藥劑尚有得克利 (triazoles類)，但該藥劑並無抑制炭疽病菌菌絲生長或孢子發芽之作用，可見相同作用機制的不同藥劑仍可能因化學結構的差異而對誘導病原菌抗藥性族群的發展具有不同效果。在田間為減緩病原菌抗藥性族群之生成，輪用不同作用機制藥劑固是優先選項，但如缺少不同作用機制藥劑供選，則輪用相同作用機制的不同藥劑亦可作為選項。而克熱淨是唯一兼具抑制晚腐病菌孢子發芽與菌絲生長的藥劑，在葡萄晚腐病的防治上是否具優良的防治效果，值得觀察。

本研究亦證實許多葡萄晚腐病菌有多重抗藥性 (multiple fungicide resistance) 現象，這些藥劑多屬單點作用機制藥劑，且有效抑制炭疽病菌分生孢子發芽與有效抑制其菌絲生長的藥劑種類幾乎完全不同。因而當進行殺菌劑篩選時，對孢子發芽與菌絲生長的藥效試驗均應為之，才能完整獲知殺菌劑的藥效功能。雖然在抗藥性管理的實務上，多認為一種藥劑連續且長期使用易導致病原菌族群對該藥劑產生抗藥性，但本研究顯示，無論是二硫代胺基甲酸鹽類藥劑、撲克拉系列藥劑 (DMIs)

或其他類別的有效藥劑均為臺灣地區長期用於防治果樹炭疽病的殺菌劑，卻未必使病原菌族群產生顯著的抗藥性，且這些藥劑涵蓋多點作用機制及單點作用機制藥劑。我們認為一種單點作用機制藥劑是否易於產生抗藥性應屬該藥劑之特性，病原菌是否能藉演化過程產生抗藥族群與藥劑之分子結構可能具關聯性，因不同藥劑有不同的分子結構，雖其抑菌的作用機制相同。有些藥劑的結構易於被病原菌克服，因而利於抗藥性族群的產生；但有的藥劑結構則否，因而產生抗藥族群的速度緩慢。但對多點作用機制藥劑而言，由於需同時累積多種抗藥基因於同一個體，自然不易發展成抗藥族群。當前，抗藥性管理的策略仍以延緩抗藥性之發生為綱，實務上則以少用藥劑與輪用不同作用機制藥劑為本，則有效單點作用機制藥劑與多點作用機制藥劑輪流使用或為葡萄晚腐病以及其他作物炭疽病抗藥性管理的務實做法。

致　　謝

本研究承行政院農業委員會108農科-8.4.1-藥-P1(3) 計畫經費補助，謹此誌謝。

引用文獻

1. Alahakoon, P. W., Brown, A. E., and Sreenivasaprasad, S. 1994. Cross-infection potential of genetic groups of ***Colletotrichum gloeosporioides*** on tropical fruits. Physiol. Mol. Plant Pathol. 44:93-103.
2. Ali, M. E., Hudson, O., Hemphill, W. H., Brenneman, T. B., and Oliver J. E. 2019. First report of resistance to pyraclostrobin, boscalid, and thiophanate-methyl in ***Colletotrichum gloeosporioides*** from blueberry in Georgia. Plant Health Prog. 20 (4):261-262.
3. Ali, M. E., Hudson, O., Waliullah, S., Cook, J., and Brannen, P. M. 2020. Sensitivity of ***Colletotrichum*** isolates collected from strawberries in Georgia to pyraclostrobin, a quinone outside inhibitor (QoI) fungicide. Plant Health Prog. 21(1):69-70.
4. ~~Anomyous,~~ 2020. Plant Protection Information System. Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Executive Yuan. Taichung. Retrieved from https://otserv2.tactri.gov.tw/ppm/ (June 10, 2020) (in Chinese).
5. Anonymous, 2018. Agricultural Statistics Yearbook. Council of Agriculture, Executive Yuan (R.O.C.), Taipei, 350 pp. (in Chinese).
6. Arauz, L. F. 2000. Mango anthracnose: economic impact and current options for integrated management. Plant Dis. 84:600-611.
7. Cai, L., Hyde, K.D., Taylor, P. W. J., Weir, B.S., Waller, J. M., Abang, M. M., Zhang, J. Z., Yang, Y. L., Phoulivong, S., Liu, Z.Y., Prihastuti, H., Shivas, R. G., McKenzie, E. H. C., and Johnston, P. R. 2009. A polyphasic approach for studying ***Colletotrichum***. Fungal Divers. 39:183-204.
8. Cao, X., Xu, X., Che, H., West, J. S., and Luo, D. 2017. Distribution and fungicide sensitivity of ***Colletotrichum*** species complexes from rubber tree in Hainan, China. Plant Dis. 101:1774-1780.
9. Carbone, I., and Kohn, L. M. 1999. A method for designing primer sets for speciation studies in filamentous Ascomycetes. Mycologia 91:553-556.
10. Chiangsin, R., Wanichkul, K., Guest, D., and Sangchote, S. 2016. Reduction of anthracnose on ripened mango fruits by chemicals, fruit bagging, and postharvest treatments. Australas. Plant Pathol. 45:629-635.
11. Chung, W. H., Ishii, H., Nishimura, K., Fukaya, M., Yano, K., and Kajitani, Y. 2006. Fungicide sensitivity and phylogenetic relationship of anthracnose fungi isolated from various fruit crops in Japan. Plant Dis. 90:506-512.
12. Damm, U., Cannon, P. F., and Crous, P. W. 2012. Studies in Mycology 73: ***Colletotrichum***: complex species or species complexes? CBS-KNAW Fungal biodiversity centre, Utrecht, the Netherlands. 215 pp.
13. Duan, C. H. 2015. Microtiter plate for evaluation of plant extracts and oils against ***Uromyces appendiculatus*** and ***Botrytis cinerea***. Plant Pathol. Bull. 24:67-75. (in Chinese).
14. Duan, C. H., and Hsieh, W. H. 1990. Pathogenicity of ***Glomerella cingulata*** on Kyoho grape. J. of Taiwan Agric. Res. 39(2):113-120. (in Chinese).
15. Duan, C. H., and Hsieh, W. H. 1991. Inoculum sources of ripe rot of grape. Plant Prot. Bull. 33:275-282. (in Chinese).
16. Duan, C. H., Chang, C. K., and Wang C. C. 2017. Fungicides and postharvest heat treatment to control anthracnose disease of mango. Taiwan Pestic. Sci. 3:65-78. (in Chinese).
17. Duan, C. H., Pan, H. R., and Wang C. C. 2019. Molecular identification and fungicides sensitivity of ***Colletotrichum*** isolates from various fruit crops in Taiwan. Taiwan Pestic. Sci. 6:71-104. (in Chinese).
18. Duan, C. H., Pang, H. R., and Wang C. C. 2018. Identification, pathogenicity and fungicide sensitivity of ***Colletotrichum*** isolates from five fruit crops in Taiwan. Taiwan Pestic. Sci. 5:91-

111. (in Chinese).

19. Farr, D.F., and Rossman, A.Y. 2019. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved from https://nt.arsgrin.gov/fungaldatabases/ (June 01, 2020)
20. Forcelini, B. B., and Peres, N. A. 2018. Widespread resistance to QoI fungicides of ***Colletotrichum acutatum*** from strawberry nurseries and production fields. Plant Health Prog. 19 (4):338-341.
21. Forcelini, B. B., Seijo, T. E., Amiri, A., and Peres, N. A. 2016. Resistance in strawberry isolates of ***Colletotrichum acutatum*** from Florida to quinone-outside inhibitor fungicides. Plant Dis. 100:2050-2056.
22. Freeman, S., and Shabi, E. 1996. Cross-infection of subtropical and temperate fruits by ***Colletotrichum*** species from various hosts. Physiol. Mol. Plant Pathol. 49: 395-404.
23. Freeman, S., Katan, T., and Shabi, E. 1998. Characterization of ***Colletotrichum*** species responsible for anthracnose diseases of various fruits. Plant Dis. 82: 596-605.
24. Guerber, J. C., Liu, B., Correll, J. C., and Johnston, P. R. 2003. Characterization of diversity in ***Colletotrichum acutatum sensu lato*** by sequences analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. Mycologia 95: 872-895.
25. Hsieh, W. H, and Duan, C. H. 1984. Investigation on fungicide tolerant strains of ***Collectotrichum gloeosporioides*** causing ripe rot of grape. Plant Prot. Bull. 26:33-39. (in Chinese).
26. Hu, M.-J., Grabke, A., Dowling, M. E., Holstein, H. J., and Schnabel, G. 2015. Resistance in ***Colletotrichum siamense*** from peach and blueberry to thiophanate-methyl and azoxystrobin. Plant Dis. 99:806-814.
27. Hyde, K. D., Cai, L., Cannon, P. F., Crouch, J. A., Crous, P. W., Damm, U., Goodwin, P. H., Chen, H., Johnston, P. R., Jones, E. B. G., Liu, Z. Y., McKenzie, E. H. C., Moriwaki, J., Noireung, P., Pennycook, S. R., Pfenning, L. H., Prihastuti, H., Sato, T., Shivas, R. G., Tan, Y. P., Taylor, P. W. J., Weir, B. S., Yang, Y. L., and Zhang, J. Z. 2009. ***Colletotrichum***-names in current use. Fungal Divers. 39:147-182.
28. Jayawardena, R. S., Hyde, K. D., Damm, U., Cai, L., Liu, M., Li, X. H., Zhang, W., Zhao, W. S., and Yan, J. Y. 2016. Notes on currently accepted species of ***Colletotrichum***. Mycosphere 7(8):1192-1260.
29. Kuo, K.-C. 2001. Sensitivity of mango anthracnose pathogen, ***Colletotrichum gloeosporioides***, to the fungicide prochloraz in Taiwan. Proc. Natl. Sci. Counc. 25(3):174-179.
30. Lei, Y., Tang, X. B., Jayawardena, R. S., Yan, J. Y., Wang, X. D., Liu, M., Chen, T., Liu, X. M., Wang, J. C., and Chen, Q. X. 2016. Identification and characterization of ***Colletotrichum*** species causing grape rot in southern China. Mycosphere 7(8): 1177-1191.
31. Leu, L.-S., and Chang, C.-W. 1985. Histological study of ***Colletotrichum gloeosporioides*** on grape fruit. Plant Prot. Bull. 27:11-18.
32. Li, M. L., Lin, Y. S., Chiang, Y. J. 2019. Categorization of Mode of Action of Pesticides. Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture, Executive Yuan. Taichung, 40 pp. (in Chinese).
33. Lin, C. P., Tsai, J. N., and Ann, P. J. 2019. Preliminary report of ***Colletotrichum viniferum*** and other new species of ***Colletotrichum*** sp. as grape ripe rot causing pathogens in Taiwan. Plant Med. 61(2/3):74. (in Chinese).
34. Lin, C. P., Tsai, J. N., Ann, P. J., and Chen, P. R. 2017. Investigation and management of grape disease in recent years. Plant Med. 59(1/2):53. (in Chinese).
35. Liu, F., Wang, M., Damm, U., Crous, P. W., and Cai, L. 2016. Species boundaries in plant pathogenic fungi: A ***Colletotrichum*** case study. BMC Evol. Biol. 16:81.
36. Lou, P. S., Huang, Y. J., Chung, W. C., Cheng, A. S., and Chung, W. H. 2010. Application of PCR-RFLP in detecting benzimidazoles-resistant isolates of ***Colletotrichum gloeosporioides*** from mango in Tainan area. Plant Pathol. Bull. 19: 255-260. (in Chinese).
37. Moreira, R. R., Hamada, N. A., Peres, N. A., and May De Mio, L. L. 2019. Sensitivity of the ***Colletotrichum acutatum*** species complex from apple trees in Brazil to dithiocarbamates, methyl benzimidazole carbamates, and quinone outside inhibitor fungicides. Plant Dis. 103:2569-2576.
38. Noireung, P., Phoulivong, S., Liu, F., Cai, L., Mckenzie, E. H. C., Chukeatirote, E., Jones, E. B. G., Bahkali, A. H., and Hyde, K. D. 2012. Novel species of ***Colletotrichum*** revealed by morphology and molecular analysis. Cryptogam. Mycol. 33:347-362.
39. O'Donnell, K., and Cigelnik, E. 1997. Two divergent intra genomic rDNA ITS2 types within a monophyletic lineage of the fungus ***Fusarium*** are nonorthologous. Mol. Phylogenet. Evol. 7:103-116.
40. Pan, F. Y., Huang, Y., Lin, L., Zhou, Y. M., Wei, R. F., Guo, W. F., Yin, L., and Lu, J. 2016. First report of ***Colletotrichum capsici*** causing grape ripe rot in Guangxi, China. Plant Dis. 100:2531.
41. Pearson, R. C., and Goheen, A. C. 1990. Compendium of Grape Diseases. APS Press, St. Paul, Minnesota, USA. 93 pp.
42. Peng, L-J., Sun, T., Yang, Y.-L., Cai, L., Hyde, K. D., Bahkali,

A. H., and Liu, Z.-Y. 2013. *Colletotrichum* species on grape in Guizhou and Yunnan provinces, China. Mycoscience 54:29-41.

43. Peres, N. A. R., Souza, N. L., Peever, T. L., and Timmer, L. W. 2004. Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. Plant Dis. 88:125-130.
44. Phoulivong, S., Cai, L., Chen, H., McKenzie, E. H. C., Abdelsalam, K., Chukeatirote, E., and Hyde, K. D. 2010. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. Fungal Divers. 44:33-43.
45. Ramdial, H., Hosein, F. N., and S. N. Rampersad, S. N. 2016. Detection and molecular characterization of benzimidazole resistance among *Colletotrichum truncatum* isolates infecting bell pepper in Trinidad. Plant Dis. 100:1146-1152.
46. Rampersad, S. N., and Teelucksingh, L. D. 2012. Differential responses of *Colletotrichum gloeosporioides* and *C. truncatum* isolates from different hosts to multiple fungicides based on two assays. Plant Dis. 96:1526-1536.
47. Rojas, E. I., Rehner, S. A., Samuels, G. J., van Bael, S., Herre, E. A., Cannon, P. F., Chen, R., Pang, J., Wang, R. W., Zhang, Y., Peng, Y. Q., and Sha, T. 2010. *Colletotrichum gloeosporioides s.l.* associated with *Theobroma* cacao and other plants in Panama: multilocus phylogenies distinguish host-associated pathogens from asymptomatic endophytes. Mycologia 102:1318-1338.
48. Secor, G.A., and Rivera, V.V. 2012. Fungicide Resistance Assays for Fungal Plant Pathogens, pp. 385-392. In: Bolton M., Thomma B. (eds) Plant Fungal Pathogens. Methods in Molecular Biology (Methods and Protocols), vol 835. Humana Press, New York, USA.
49. Sharma, G., Kumar, N., Weir, B.S., Hyde, K. D., and Shenoy, B. D. 2013. The *ApMat* marker can resolve *Colletotrichum* species: a case study with *Mangifera indica*. Fungal Divers. 61:117-138.
50. Silva, D. N., Talhinhas, P., Várzea, V., Cai, L., Paulo, O. S., and Batista D. 2012. Application of the *Apn2/MAT* locus to improve the systematics of the *Colletotrichum gloeosporioides* complex: an example from coffee (*Coffea* spp.) hosts, Mycologia 104:396-409.
51. Staden, R. 1980. A new computer method for the storage and manipulation of DNA gel reading data. Nucleic Acids Res. 8:3673-3694.
52. Torres-Calzada, C., Tapia-Tussell, R., Higuera-Ciapara, I., Martin-Mex, R., Nexticapan-Garcez, A., and Perez-Brito, D. 2015. Sensitivity of *Colletotrichum truncatum* to four fungicides and characterization of thiabendazole resistant isolates. Plant Dis. 99:1590-1595.
53. Tsai. J. N., Ann, P. J., Hu, C. Y. and Cheng, S. F. 2006. Evaluation of fungicides for suppression of mycelial growth and conidial germination of *Colletotrichum* species isolated from mango, pomelo and banana fruit. Plant Pathol. Bull. 15: 39-54. (in Chinese).
54. White, T. J., Bruns, T. Lee S., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds.), PCR protocols: a guide to methods and applications. Academic, San Diego, CA, USA. pp. 315-322.
55. Whitelaw-Weckert, M. A., Curtin, S. J., Huang, R., Steel, C. C., Blanchard, C. L., and Roffey, P. E. 2007. Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropicl Australia. Plant Pathol. 56:448-463.
56. Wong, F. P., and Midland, S. L. 2007. Sensitivity distributions of California populations of *Colletotrichum* cereale to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. Plant Dis. 91:1547-1555.
57. Wong, F. P., de la Cerda, K. A., Hernandez-Martinez, R., and Midland, S. L. 2008. Detection and characterization of benzimidazole resistance in California populations of *Colletotrichum cereal*. Plant Dis. 92:239-246.
58. Yan, J.-Y., Jayawardena, M. M. R. S., Goonasekara, I. D., Wang, Y., Zhang, W., Liu, M., Huang, J.-B., Wang, Z.-Y., Shang, J.-J., Peng, Y.-L., Bahkali, A., Hyde, K. D., and Li, X.-H. 2015. Diverse species of *Colletotrichum* associated with grapevine anthracnose in China. Fungal Divers. 71:233-246.
59. Zapparata, A., Da Lio, D., Sarrocco, S., Vannacci, G., and Baroncelli. R. 2017. First report of *Colletotrichum godetiae* causing grape (*Vitis vinifera*) berry rot in Italy. Plant Dis. 101:1051.
60. Zhang, Y., Xu, H., Jiang, S., Wang, F., Ou, C., Zhao, Y., Ma, L., and Li, Y. 2018. First report of *Colletotrichum truncatum* causing anthracnose on the berry stalk and the rachis of Kyoho grape (*Vitis labruscana* × *V. vinifera*) clusters in Hebei, China. Plant Dis. 102:2040.

ABSTRACT

Duan, C. H.,* and Chen, G. Y. 2020. Molecular identification and fungicide sensitivity of *Colletotrichum* isolates from grape in Taiwan. J. Plant Med. 62(4): 23-32.

*Corresponding author, E-mail: chduan@tactri.gov.tw

Ripe rot of grape caused by *Colletotrichum* spp. is a prevalent and severe grape disease in Taiwan. It always incurs great losses due to its fast dissemination by heavy rain in summer. Identification of the pathogens based on morphological and molecular characters indicated that *C. viniferum* was the dominant species and *C. tropicale* was the minor one in our culture collections. Chemical control followed by fruit bagging is the main measure to control this disease. Therefore, the effective fungicides are vital for the success of the disease control. In this study, we used microtiter plate method to evaluate the inhibition effect of the fungicides against the ripe rot pathogens. The results showed that dithianon, iminoctadine triacetate, mancozeb, metiram, oxine-copper, polyoxins and thiram were consistently effective to inhibit conidial germination of the pathogens, while only iminoctadine triacetate, manganese prochlorate and prochloraz were able to inhibit their mycelial growth.

Keywords: grape, *Colletotrichum*, fungicide