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重要植物病原真菌抗藥性現況與檢測技術 P. 229-245.

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摘要

植物病原真菌抗藥性研究首須建立其對殺菌劑感受性基準線，以作為抗藥性偵測的參考依據，此基準線為未接觸該藥劑的病原菌菌株的半抑制有效濃度(EC_{50}) 所構成的分布圖，可作為農藥登記的藥效資料之一，而 EC_{50} 也最常用於表示病原菌的抗藥性。此外，抗藥性檢測技術尚包括分子檢測法，但因其與 EC_{50} 數值常有落差且試驗成本高，尚難實用化。微量滴定盤法用單一標稱劑量進行菌株抗藥性測定，其優點為易於處理大量菌株及多種藥劑。以微量滴定盤法測定台灣數種重要植物病原真菌對防治藥劑的感受性顯示，病原菌對多點作用機制藥劑均未產生抗藥性，其中尚包含三種酸性化食品防腐劑：苯甲酸鈉、丙酸鈣及己二烯酸鉀；單點作用機藥劑亦有部分表現良好藥效。綜合上述試驗結果得知，殺菌劑對抗藥性的影響大於病原菌，而屬相同作用機制的不同藥劑其抗藥性風險也可能互異，可作為輪用藥劑的選項。植物保護手冊關於殺菌劑之「施藥方法」，混合劑之應用及農民常提高施藥劑量等對抗藥性管理造成的影響均需務實應對。

關鍵字：殺菌劑、抗藥性、半抑制有效濃度、感受性基準線

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前言

本文旨在論述植物病原真菌對化學藥劑抗藥性的一些問題，共分兩部分，第一部分是簡介在實驗室測定病原真菌抗藥性的方法，第二部分則是列舉作者曾調查之芒果炭疽病菌、葡萄晚腐病菌、草莓灰黴病菌及稻熱病菌等四種重要病原真菌抗藥性的現況，以提供抗藥性研究及農友選用藥劑的參考。病原真菌抗藥性的產生是因病原菌族群在殺菌劑選汰壓力下，抗藥菌株存活，而感藥菌株被殺死，歷經多代後，抗藥菌株逐漸成為病原菌族群的主要部份，病原菌抗藥性於焉產生，並因此造成藥效降低的結果。抗藥性的管理的主要作法是輪用不同藥劑及減少藥劑使用，以延緩抗藥性發生，爭取開發新藥的時間 (4)。

抗藥性的主體是病原菌，而藥效的主體是殺菌劑，二者實為一體的兩面，抗藥性決定藥效，而藥效則是抗藥性發生的指標。依照標稱劑量及使用方法施藥的情況下，殺菌劑如具藥效，表示病原菌尚未產生明顯抗藥性；反之，殺菌劑藥效降低甚或完全不具藥效，則表示病原菌已產生抗藥性。由於藥劑施用劑量在不同藥劑間存有很大差異，使抗藥性量化難有統一的標準。一般是以最感藥菌株作為對照，其他菌株與其比較來決定抗藥性的程度，菌株間半抑制有效濃度 (effect concentration for 50% inhibition, EC_{50}) 比值是最常用的方法。但是作為比較基準的感藥菌株卻常因田間已無法獲得，而須以實際所得最感藥菌株的 EC_{50} 為準，但這又使得不同研究者因感藥菌株不同而難以互相比較。為解決此問題，可在殺菌劑引入國內市場時，由廠商提供標的病原菌對該殺菌劑感受性

基準線分布圖 (22, 26)，作為送審藥效資料的一部分，以為日後追蹤抗藥性發展的基準。

近年來，分子生物學及其相關技術快速發展，抗藥性研究亦常導入分生技術。由於病原菌的抗藥性屬遺傳現象會代代相傳，抗藥基因的研究因而成為抗藥性研究的課題之一，其學術價值與實際應用值得探討。此外，病原菌抗藥性研究是針對龐大的微生物族群，不同時間及空間的病原菌族群可能存有極大差異，故在相關的研究中必須廣泛且大量採樣，以獲知病原菌族群抗藥性的全貌。如何在有限的資源下，偵測大量菌株對各種藥劑的抗感性反應是抗藥性研究的巨大挑戰，應用簡便且快速的試驗方法以獲知病原菌族群的抗藥性資訊乃成為影響抗藥性研究的關鍵因素。本文對這些問題都將依作者的研究結果略作說明。

抗藥性檢測技術

一、半抑制有效濃度 (effect concentration for 50% inhibition, EC_{50})

抗藥性檢測多在實驗室進行，其所獲結果是實驗室抗藥性 (laboratory resistance) 其與田間或實際的抗藥性 (field or practical resistance) 可能存有落差，因而有學者主張使用「感受性」 (sensitivity)，但「抗藥性」仍獲部分學者支持 (4)。傳統測定病原真菌抗藥性的方法是使用半抑制有效濃度 (EC_{50})。當試驗不以死亡作爲供試菌株對殺菌劑的反應指標，而是觀察殺菌劑對真菌的某一生物

特性的影響，例如孢子發芽率或菌絲生長率，故以有效濃度 (effective concentration, EC) 來表示殺菌劑對病原菌的毒性。一種殺菌劑的半抑制有效濃度 (EC_{50}) 是指該殺菌劑造成某種病原菌孢子發芽率或菌絲生長率減半的濃度 (3, 6, 9)。這是個理論值，它可以反映藥劑的抑菌效果。實際操作這個方法是將供試殺菌劑以一系列稀釋濃度並含無藥劑之對照來處理病原菌的孢子或菌絲，經一段時間作用與培養後，依試驗結果獲得量效曲線 (Dose response curve)，可據以求出直線迴歸方程式，以獲得供試菌株對該藥劑的 EC_{50}。抗藥性是比較出來的，通常以 EC_{50} 數值最低者作為比較基準 (分母)，而以供試菌株對應各藥劑所獲取的 EC_{50} 數值作為分子，這個比值稱為抗性比 (resistance ratio) (20)，此數值愈高表抗藥程度愈嚴重。

二、感受性基準線 (baseline sensitivity of fungal pathogen to fungicide)

感受性基準線是指當一種殺菌劑引進某一地區前，未曾接觸該藥劑的病原菌菌株 (baseline isolates) 對此藥劑的半抑制有效濃度 (EC_{50}) 分布圖 (baseline sensitivity distribution) (22, 23, 24)。因病原菌的個體或族群之前並未接觸該藥劑，此分布圖是為該病原菌對新藥劑的感受性基準線。以此一基準線作為參考點，可以評估該藥劑在使用後病原菌抗藥性的發展。後續評估方法為在該藥劑施用地區進行病原菌採集，並以同於感受性基準線的試驗方法，測定病原菌對該藥劑的 EC_{50}。與基準線分布圖相較，當藥劑使用後所建立的感受性分布圖向高劑

量偏移，表示抗藥性開始發展。實務上，在藥劑引進前，低 EC_{50} 數值菌株應較多，而高 EC_{50} 數值菌株較少；但當藥劑使用後，如反是，就是抗藥性出現的徵兆。

三、分子檢測 (molecular detection)

抗藥性是可遺傳的。當病原菌對某殺菌劑產生抗藥性時，該藥劑作用於病原菌的標的酵素或蛋白質可能改變，以肆應或緩解藥劑對菌株生理上的壓力以利其存活。此種改變係源自該酵素或蛋白質基因在特定位點的核酸序列改變，導致其轉譯的胺基酸隨之改變。藉由抗感藥菌株特定基因核酸序列比對，我們可以偵知改變的位點及改變的結果，並以之作為檢測抗藥性發生的依據，這就是分子檢測。已知可供分子檢測的病原菌基因不多，常見者有 *cytochrome b*、*β-tubulin*、*Bos1*、*SdhB* 及 *Erg27* 等數種 (3, 6, 7, 8, 18, 21, 25)。實際案例如炭疽病菌 (*Colletotrichum* spp.) 對貝芬替產生抗藥性，將使微管蛋白 b (*β-tubulin*) 基因第 198 密碼子 (codon) 由轉譯的麩胺酸 (glutamic acid) 變為丙胺酸 (alanine) 及第 200 密碼子由轉譯的苯丙胺酸 (phenylalanine) 變為酪胺酸 (tyrosine) (3, 7, 25) 。但在作者的研究中，僅高度抗藥性菌株 (EC_{50}>500 μg/mL) 有此改變 (表一)。另有報告稱炭疽病菌對苯醌外部抑制劑 (quinone-outside inhibitors, QoI) 類藥劑產生抗藥性，其细胞色素 (cytochrome b, *cytb*) 基因的第 129 密碼子轉譯的胺基酸由苯丙胺酸變為白胺酸 (leucine)，第 137 密碼子轉譯的胺基酸由甘胺酸

(glycine) 變為精胺酸 (arginine)，第 143 密碼子轉譯的胺基酸由甘胺酸變為丙胺酸 (alanine) (3, 6, 21) 。但在作者的試驗中僅 129 密碼子轉譯的胺基酸由苯丙胺酸變為白胺酸，且無分藥劑感受性差異而全然發生 (表二)，這使得我們難以藉由此分子檢測結果判斷菌株基因變異與抗藥性的關聯性。

四、微量滴定盤法 (microtiter plate method)

EC_{50}需要一系列劑量方足以建立病原菌對殺菌劑感受性的估值，耗時且費力。如能以單一劑量作為菌株抗感性的區別劑量 (discriminatory dose)，將可節省大量的人力與時間。運用單一區別劑量結合微量滴定盤所建立的微量滴定盤法是一種簡便且快速的方法，可於短時間 (約 1 至 5 天) 處理大量菌株及多種藥劑 (10)。實際進行試驗時，每一種藥劑僅使用一種劑量，即該藥劑的標稱劑量 (label rate)，此亦即該成品農藥在田間的使用劑量。此劑量數值 (藥劑濃度) 得自該成品藥劑有效成分含量 (% active ingredient, a.i.) 及稀釋倍數的乘積，單位為 μg/mL，與 EC_{50}相同。在微量滴定盤進行藥劑處理的時間為 2 小時，此係模擬藥劑在田間噴灑後，約經 2 小時自然風乾。藥劑處理後，如為真菌孢子，則塗布於 2%水瓊脂 (water agar) 平板，經 10 餘小時後，鏡檢孢子發芽率；如為菌絲塊，則需先將藥劑處理後的菌絲塊移至滅菌過的吸水紙 (可用擦手紙) 吸乾藥劑，再移至可供菌絲生長的洋菜培養基。菌絲生長需時較長，約數日至一周，且限能在人工培養基生長的病原菌 (10, 11, 12, 13, 14, 15, 16, 17) 。由此試

驗所得結果可直接反映病原菌對殺菌劑施用於田間劑量的感受性，是抗藥性產生與否的直接證據。

重要植物病原真菌對殺菌劑感受性概況

一、芒果炭疽病菌

微量滴定盤法測試 37 種殺菌劑對芒果炭疽病菌分生孢子之抑制試驗顯示，腈硫醌、腈硫克敏、扶吉胺、福賽快得寧、克熱淨、鋅錳乃浦、滅特座、免得爛、快得寧、保粒快得寧、甲基鋅乃浦、腐絕快得寧、得恩地等 13 種殺菌劑可完全抑制本病原菌孢子發芽。而貝芬撲克拉、賽普護汰寧、撲克拉錳、保粒黴素(甲)及撲克拉等 5 種藥劑雖不能抑制病原菌孢子發芽但可抑制其附著器形成(表三) (11)。

二、葡萄晚腐病菌

微量滴定盤法測試 20 種葡萄晚腐病防治用藥之藥效，結果顯示藥劑的抑菌效果對供試菌株具一致性，菌株間反應差異小。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 (表四)，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 (表五) (14)。

三、草莓灰黴病菌

微量滴定盤法測試殺菌劑對草莓灰黴菌之抑菌結果顯示，灰黴菌對藥劑感受性普遍偏低 (較抗藥)。在供試的 19 種殺菌劑中僅有得恩地能抑制全部供試菌株孢子發芽，但白克列、賽普護汰寧、三氟派瑞、氟克殺及亞派占等藥劑尚能抑制部分菌株之孢子發芽，且大多為暫時性之靜菌作用；惟全部供試藥劑均不能抑制供試菌株之菌絲生長 (表六、七)。食品防腐劑苯甲酸鈉、丙酸鈣或己二烯酸鉀分別製備為濃度 2000 μg/ml 溶液，再以醋酸調降酸鹼值至 4，其對供試灰黴病菌之孢子發芽均可完全抑制且為殺菌作用；此外，苯甲酸鈉可抑制全部供試菌株之菌絲生長，己二烯酸鉀可抑制大多數菌株之菌絲生長，而丙酸鈣則對菌絲幾無抑制作用 (表六、七) (15)。

四、稻熱病菌

以微量滴定盤法測試稻熱病菌對殺菌劑及酸性化食品防腐劑之感受性，結果顯示，各種藥劑對抑制孢子發芽、菌絲生長及附著器形成等功效存有明顯差異。在抑制孢子發芽方面，鋅錳乃浦及三種酸性化食品防腐劑：苯甲酸鈉、丙酸鈣及己二烯酸鉀，均能完全抑制各菌株之孢子發芽；依普座效果亦佳，丙基喜樂松及撲殺熱也能抑制半數以上菌株的孢子發芽 (表八)。在抑制菌絲生長方面，三種酸性化食品防腐劑、依普座、鋅錳乃浦及撲克拉等藥劑均具完全抑制作用；貝芬替、克熱淨及得克利也能抑制大部分菌株之生長，護粒松、丙基喜

樂松及百克敏能抑制約三分之一菌株的生長。三賽唑及芬諾尼對稻熱病菌已發芽孢子的附著器形成則具完全抑制作用 (表九) (17)。

結語

抗藥性研究必須把握時間因素，因為抗藥性的發展是一隨時間而不斷改變的動態現象。特別是在藥劑開始進入作物栽培系統之前，此時病原菌對新藥劑的感受性狀況至關重要，是抗藥性發生的起始點，常被忽略且事後難以補救。建立新藥劑的感受性基準線是抗藥性管理及研究的基礎工作，可列為農藥登記備審藥效資料的一部分或做為藥劑延伸使用的佐證資料。且這份資料須符合科學性報告的要求，日後相關的研究必須遵照相同的試驗方法，方能作比較。

為能幫助農民選擇有效防治藥劑，即時監測田間病原菌對殺菌劑的感受性是必要工作。面對各種作物病原菌及不同作用機制與分子結構的殺菌劑，必須建立一套快速且能處理大量樣本的抗藥性偵測技術，作者以微量滴定盤搭配洋菜平板可滿足此需求。半抑制有效濃度 (EC_{50}) 雖具學理基礎且易與其他研究結果互相比較，但試驗成本高。分子偵測的試驗成本與進入門檻更高，雖具學術價值，但病原菌基因序列的變異常與藥劑感受性不具關聯性，尚難有實用價值 (7, 20)。

抗藥性管理是涉及多個層面的工作，有科學研究也有法規執行。政府發行的植物保護手冊或網路版植物保護資訊系統是農民執行植物保護工作的依據，

這些資訊應納入抗藥性管理的指引 (guidelines)，同一藥劑連續施用的「施藥方法」實有檢討必要。在抗藥性管理上，輪用不同作用機制藥劑固是最佳方案，但相同作用機制的不同藥劑因分子結構互異，如無交互抗藥性 (cross resistance)，也可納為輪用藥劑，以增加防治藥劑選項。依據作者研究結果，某些藥劑雖經數十年之施用，仍保有良好的藥效，此類藥劑尚且具廣效特性，雖然有些藥劑屬多點作用 (multi-site activity)，但也有部分為單點作用 (specific site)。如何開發低抗藥性風險的多點作用機制藥劑，是解決抗藥性問題的最佳方法。結構簡單的銅劑、硫磺劑以及作者發表之數種酸性化食品防腐劑均具價廉且廣效的優點，如無毒理顧慮應可廣為應用。

依據殺菌劑抗藥性行動委員會 (FRAC) 的資料，病原菌及殺菌劑對抗藥性的產生均有高、中、低風險分級 (1, 5)，當病原菌發生抗藥性時應歸因於藥劑風險 (fungicide risk)，抑歸因於病原菌風險 (pathogen risk)，或包含兩者的綜合風險 (combined risk) 是一值得探討的課題。根據我們的研究結果，抗藥性低風險的多點作用機制藥劑足以完全抑制抗藥性高風險的病原菌，表示殺菌劑對抗藥性風險的影響大於病原菌。農民常提高施藥劑量以對抗抗藥性，但能提高劑量的額度有限，但抗性比常為十數倍至數十倍，不但無以對抗抗藥性，且衍生加速抗藥性發展與環境污染的不良後果。農藥業者常推出混合藥劑防治病害，所持理由為減緩抗藥性發生，此亦為殺菌劑抗藥性行動委員會所肯定，但前提是各單劑均需保有良好藥效 (2)。設若其所含二單劑均具良好藥效，則輪用此二

單劑可降低其與病原菌接觸的頻率，似更能符合抗藥性管理的原則。

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表一、果樹炭疽病菌對貝芬替不同感受性菌株之 β 微管蛋白基因突變表

Table 1. Response of *Colletotrichum* spp. isolates to carbendazim and deduced amino acid substitution in *β-tubulin* gene sequence at 2 codons (source: authors, unpublished)

			Sequence in codon[3]	
Isolate	EC_{50} (μg/mL)[1]	Response[2]	198	200
AC1	<1	S	Glu	Phe
AC2	>500	HR	Ala	Phe
BC1	<1	S	Glu	Phe
BC2	<1	S	Glu	Phe
FC1	<1	S	Glu	Phe
FC2	<1	S	Glu	Phe
GC12	224	R	Glu	Phe
GC18	<1	S	Glu	Phe
GC27	223	R	Glu	Phe
GC39	<1	S	Glu	Phe
GC40	<1	S	Glu	Phe
GC46	230	R	Glu	Tyr
GC48	<1	S	Glu	Phe
GC51	<1	S	Glu	Phe
JC1	<1	S	Glu	Phe
JC3	<1	S	Glu	Phe
MC2	>500	HR	Ala	Phe
MC3	<1	S	Glu	Phe
MC4	>500	HR	Ala	Phe
MC6	<1	S	Glu	Phe
MC9	>500	HR	Ala	Phe
MC10	>500	HR	Ala	Phe
MC14	<1	S	Glu	Phe
MC17	>500	HR	Ala	Phe
MC23	>500	HR	Ala	Phe
MC26	>500	HR	Ala	Phe
SBC1	>500	HR	Ala	Phe
SBC5	>500	HR	Ala	Phe
SBC7	>500	HR	Ala	Phe

[1]EC_{50} was obtained by cultivating each isolate on half-strength PDA for 3 days.

[2]Classification of response according to Chung et al. (2006).

[3]Ala: alanine, Glu: glutamic acid, Phe: phenylalanine, Tyr: tyrosine.

表二、果樹炭疽病菌對百克敏不同感受性菌株之細胞色素 b 基因突變表

Table 2. Response of *Colletotrichum* spp. isolates to pyraclostrobin and deduced amino acid substitution in *cytochrome b* gene sequence at 3 codons (source: authors, unpublished)

			Sequence in codon[3]		
Isolate	EC_{50} (μg/mL)[1]	Response[2]	129	137	143
AC1	0.031	S	Leu	Gly	Gly
AC2	4.992	R	Leu	Gly	Gly
BC1	0.001	S	Leu	Gly	Gly
BC2	0.081	S	Leu	Gly	Gly
FC1	0.767	S	Leu	Gly	Gly
FC2	0.063	S	Leu	Gly	Gly
GC12	11.854	R	Leu	Gly	Gly
GC18	15.124	R	Leu	Gly	Gly
GC27	16.472	R	Leu	Gly	Gly
GC39	9.266	R	Leu	Gly	Gly
GC40	15.800	R	Leu	Gly	Gly
GC46	25.591	R	Leu	Gly	Gly
GC48	16.555	R	Leu	Gly	Gly
GC51	10.242	R	Leu	Gly	Gly
JC1	0.001	S	Leu	Gly	Gly
JC3	0.040	S	Leu	Gly	Gly
MC2	6.214	R	Leu	Gly	Gly
MC3	0.001	S	Leu	Gly	Gly
MC4	12.191	R	Leu	Gly	Gly
MC6	0.011	S	Leu	Gly	Gly
MC9	17.266	R	Leu	Gly	Gly
MC10	0.257	S	Leu	Gly	Gly
MC14	0.039	S	Leu	Gly	Gly
MC17	33.411	R	Leu	Gly	Gly
MC23	13.444	R	Leu	Gly	Gly
MC26	9.952	R	Leu	Gly	Gly
SBC1	0.013	S	Leu	Gly	Gly
SBC5	0.001	S	Leu	Gly	Gly
SBC7	0.208	S	Leu	Gly	Gly

[1]EC_{50} was obtained by cultivating each isolate on half-strength PDA for 3 days.

[2]Classification of response according to Forcelini et al. (2016).

[3]Leu:leucine, Gly: glycine.

表三、殺菌劑對芒果炭疽病菌分生孢子發芽及附著器形成之影響

Table 3. Effects of fungicides on spore germination and appressorium formation of *Colletotrichum* spp. that cause anthracnose disease of mango[1)]

(excerpted from Duan et al., 2017)

Fungicide	*C. asianum* (MC-45)		*C. siamense* (MC-46)	
	Spore germination (%)	Appressorium formation	Spore germination (%)	Appressorium formation
Azoxystrobin	95.3±0.5 a	＋	94.5±0.6 ab	－
Azoxystrobin 20%+ difenoconazole 12.5%	100.0±0.0 a	＋	100.0±0.0 a	－
Bordeaux mixture	100.0±0.0 a	＋	100.0±0.0 a	－
Boscalid + pyraclostrobin	80.0±2.9 b	＋	83.3±2.6 bc	－
Carbendazim	99.0±0.6 a	＋	97.3±1.4 ab	－
Carbendazim + hexaconazole	100.0±0.0 a	＋	100.0±0.0 a	－
Carbendazim + Prochloraz	98.0±0.7 a	－	99.8±0.3 a	－
Cyprodinil+fludioxonil	100.0±0.0 a	－	100.0±0.0 a	－
Difenoconazole	36.5±1.0 d	＋	55.5±3.9 de	－
Dithianon	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Dithianon + pyraclostrobin	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Fluazinam	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Fluopyram + trifloxystrobin	100.0±0.0 a	＋	100.0±0.0 a	－
Fosetyl-aluminium	100.0±0.0 a	＋	100.0±0.0 a	－
Fosetyl-aluminium +oxine-copper	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Iminoctadine triacetate	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Iprodione	99.5±0.3 a	＋	100.0±0.0 a	＋
kasugamycin hydrochloride hydrate + carbendazim	99.3±0.5 a	＋	98.8±0.8 a	－
Kresoxim-methyl	98.0±0.8 a	＋	100.0±0.0 a	－
Mancozeb	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Manganese prochlorate	99.8±0.3 a	－	90.8±1.4 ab	－

表三、殺菌劑對芒果炭疽病菌分生孢子發芽及附著器形成之影響 (續)

Table 3. (continued)

Fungicide	*C. asianum* (MC-45)		*C. siamense* (MC-46)	
	Spore germination (%)	Appressorium formation	Spore germination (%)	Appressorium formation
Metconazole	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Metiram	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Myclobutanil	92.8±2.1 a	＋	82.5±1.6 bc	＋
Oxine-copper	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Polyoxins	48.0±2.3 c	－	49.5±5.4 e	－
Polyoxins + oxine-copper	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Prochloraz	98.3±1.2 a	－	69.5±3.5 cd	－
Propineb	0.0±0.0 e	N/A	0.0±0.0 f	N/A
pyraclostrobin	95.3±1.4 a	＋	92.3±1.3 ab	－
Tebuconazole	92.0±1.4 a	＋	99.8±0.3 a	＋
Tetraconazole	100.0±0.0 a	＋	100.0±0.0 a	－
Thiabendazole	98.3±0.6 a	＋	100.0±0.0 a	＋
Thiabendazole + oxine-copper	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Thiophanate-methyl	99.8±0.3 a	＋	96.0±2.2 ab	－
Thiram	0.0±0.0 e	N/A	0.0±0.0 f	N/A
Trifloxystrobin	100.0±0.0 a	＋	100.0±0.0 a	－
Control (Water)	100.0±0.0 a	＋	100.0±0.0 a	＋

[1]Mean±standard error (n=4). Values in each column followed by the same letter are not significantly different at the 5% level by Fisher's protected least significant difference test.＋: appressorium formation, －: no appressorium, N/A: not applicable.

表四、殺菌劑對葡萄晚腐病菌分生孢子發芽之影響

Table 4. Effects of fungicides on conidial germination of 12 *Colletotrichum* isolates from grape (excerpted from Duan and Chen 2020a)

	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]Means of germination rates (n=4) on water agar plates after-24-hour (24° C) following treatments.

表五、殺菌劑對葡萄晚腐病菌菌絲生長之影響

Table 5. Effects of fungicides on mycelial growth of 12 *Colletotrichum* isolates from grape (excerpted from Duan and Chen 2020a)

	No. of isolates with colony diameter (cm) of [1]			
Fungicide	<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 after-5-day-growth (24° C) on potato dextrose agar plate following treatments.

表六、殺菌劑對草莓灰黴病菌孢子分生發芽之影響

Table 6. Effects of fungicides on conidial germination of *Botrytis cinerea* isolates collected from strawberry (excerpted from Duan and Chen 2020b)

	Germination rate (%)[1]					
Fungicide	S2	G1	D23	W1	GX1	ST1
Azoxystrobin	100.0	100.0	100.0	100.0	96.5	100.0
Benomyl	100.0	100.0	97.5	100.0	95.3	92.3
Boscalid	100.0	100.0	0.0	0.0	97.3	0.0
Carbendazim	100.0	100.0	97.5	100.0	99.0	91.8
Cyprodinil + Fludioxonil	100.0	0.0	0.0	96.0	95.5	100.0
Fluopyram +Trifloxystrobin	100.0	0.0	96.0	100.0	89.3	0.0
Fluxapyroxad	100.0	100.0	90.5	0.0	91.8	83.8
Iprodione	100.0	100.0	91.8	96.3	98.8	100.0
Isopyrazam	88.3	97.8	97.0	0.0	90.8	93.3
Mepanipyrim	100.0	97.2	100.0	99.3	98.3	98.5
Metiram	53.0	27.3	38.0	72.3	22.8	19.3
Myclobutanil	100.0	90.5	100.0	97.8	53.8	98.3
Polyoxins	98.8	97.8	100.0	100.0	100.0	100.0
Procymidone	100.0	98.0	95.5	82.0	100.0	97.5
Pyraclostrobin	100.0	100.0	99.0	98.0	100.0	92.5
Pyrimethanil	100.0	100.0	96.3	96.8	100.0	100.0
Thiabendazole	100.0	98.8	97.8	98.0	94.3	100.0
Thiophanate-methyl	100.0	98.8	97.8	99.3	96.5	96.0
Thiram	0.0	0.0	0.0	0.0	0.0	0.0
Calciun propionate	0.0	0.0	0.0	0.0	0.0	0.0
Potassium sorbate	0.0	0.0	0.0	0.0	0.0	0.0
Sodium benzoate	0.0	0.0	0.0	0.0	0.0	0.0
Control (water)	100.0	100.0	97.0	100.0	100.0	100.0

[1]Isolate numbers and means of germination rates (n=4) on water agar plates after-24-hour (24° C) following treatments.

表七、殺菌劑對草莓灰黴病菌菌絲生長之影響

Table 7. Effects of fungicides on mycelial growth of *Botrytis cinerea* isolates collected from strawberry (excerpted from Duan and Chen 2020b)

	Mycelial growth[1]					
Fungicide	S2	G1	D23	W1	GX1	ST1
Azoxystrobin	+++	+++	+++	+++	+++	+++
Benomyl	+++	+++	+++	+++	+++	--
Boscalid	+++	+++	+++	+++	+++	+++
Carbendazim	++	+++	+++	+++	+++	--
Cyprodinil + Fludioxonil	++	+	+++	+++	+	+
Fluopyram+Trifloxystrobin	++	++	++	++	++	+
Fluxapyroxad	++	+++	+++	++	+++	+++
Iprodione	++	++	+++	+	++	+
Isopyrazam	++	+++	++	++	++	+
Mepanipyrim	+++	+++	+++	+++	+++	+++
Metiram	+++	+++	+++	+++	++	+++
Myclobutanil	++	++	++	++	+	++
Polyoxins	+++	+++	+++	++	+++	+++
Procymidone	+++	+++	+++	+	+++	+
Pyraclostrobin	+++	+++	+++	+++	+++	+++
Pyrimethanil	+++	+++	+++	+++	+++	+++
Thiabendazole	+++	+++	+++	+++	+++	--
Thiophanate-methyl	+++	+++	+++	+++	+++	--
Thiram	++	+	+++	+++	+	+++
Calcium propionate	+++	++	+++	+	+++	++
Potassium sorbate	--	--	+++	--	--	--
Sodium benzoate	--	--	--	--	--	--
Control (water)	+++	+++	+++	+++	+++	+++

[1] Isolate numbers and diameter range of colony after 3-day-growth on potato dextrose agar; +++, more than 4.0 cm; ++, 2.1 to 4.0 cm; +, 0.1 to 2.0 cm; -, no growth.

表八、殺菌劑對稻熱病菌分生孢子發芽之抑制率

Table 8. Fungicide activity against *Pyricularia oryzae* conidial germination in *in vitro* evaluations (excerpted from Duan and Chen 2022)

	No. of isolate in each inhibition range (%)[1]					
Fungicide	0.0	0.1~ 25.0	25.1~ 50.0	50.1~ 75.0	75.1~ 99.9	100.0
Benomyl	0	12	7	6	1	2
Carbendazim	0	14	8	5	0	1
Cartap hydrochloride	2	25	1	0	0	0
Edifenphos	0	3	4	9	5	7
Epoxiconazole	0	0	1	3	3	21
Fenoxanil	3	23	2	0	0	0
Iminoctadine tris (albesilate)	0	20	2	0	1	5
Iprobenfos	0	0	0	1	11	16
Isoprothiolane	2	24	2	0	0	0
Kasugamycin	0	16	4	3	3	2
Mancozeb	0	0	0	0	0	28
Probenazole	1	5	1	3	3	15
Prochloraz	2	20	4	2	0	0
Pyraclostrobin	0	21	2	1	0	4
Tebuconazole	0	22	4	1	1	0
Tecloftalam	6	21	0	0	0	1
Thiophanate-methyl	3	25	0	0	0	0
Tricyclazole	3	18	3	0	2	2
Validamycin A	5	23	0	0	0	0
Calciun propionate	0	0	0	0	0	28
Potassium Sorbate	0	0	0	0	1	27
Sodium benzoate	0	0	0	0	0	28

[1]Inhibition rate (%)=1- (control-treatment / control) ×100%. The treatment or control of conidial germination percentages were averages of 4 replications on water agar plates after-24-hour (24°C) following treatments.

表九、殺菌劑對稻熱病菌菌絲生長之抑制率

Table 9. Fungicide activity against *Pyricularia oryzae* mycelial growth in *in vitro* evaluations (excerpted from Duan and Chen 2022)

	No. of isolate in each inhibition range (%)[1]					
Fungicide	0.0	0.1~ 25.0	25.1~ 50.0	50.1~ 75.0	75.1~ 99.9	100.0
Benomyl	0	6	9	4	7	2
Carbendazim	0	0	0	1	4	23
Cartap hydrochloride	8	19	0	1	0	0
Edifenphos	0	0	2	5	10	11
Epoxiconazole	0	0	0	0	0	28
Fenoxanil	7	20	1	0	0	0
Iminoctadine tris (albesilate)	0	0	0	0	3	25
Iprobenfos	0	0	4	3	10	11
Isoprothiolane	1	15	9	3	0	0
Kasugamycin	8	15	5	0	0	0
Mancozeb	0	0	0	0	0	28
Probenazole	6	21	1	0	0	0
Prochloraz	0	0	0	0	0	28
Pyraclostrobin	0	1	3	6	10	8
Tebuconazole	0	0	0	1	8	19
Tecloftalam	5	23	0	0	0	0
Thiophanate-methyl	10	18	0	0	0	0
Tricyclazole	10	18	0	0	0	0
Validamycin A	9	19	0	0	0	0
Calciun propionate	0	0	0	0	1	27
Potassium Sorbate	0	0	0	0	0	28
Sodium benzoate	0	0	0	0	0	28

[1]Inhibition rate (%)=1- (control-treatment / control) ×100%. The treatment or control of colony diameters were averages of 4 replications after-5-day-growth (24°C) on PDA plates following treatments.

Detection and Monitoring of Fungicide Sensitivities of Important Plant Pathogenic Fungi

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Abstract

Fungicide resistance and its management are of great importance to crop protection. Baseline sensitivity of the pathogen to a fungicide is a point of reference used in a decision making process of resistance management. This comprehensive profile of the baseline sensitivity is based on using biological techniques to assess the response of previously unexposed fungal strains or populations to the fungicide. The fungicide sensitivity evaluation using 50% effective concentration (EC_{50}) is the most common method for constructing a baseline sensitivity profile and detection of resistance despite its time-consuming and costly. Thence, we developed a microtiter plate method to assay fungal sensitivity to a fungicide with its label rate. Resistance to fungicides can occur as a result of point mutations in any of the following fungal genes: *β-tubulin*, *bos1*, *CYP51,* and *sdhB*. Because the levels of resistance were not always consistent with the gene mutations, the method is currently not practical to inspect fungicide resistance. Our investigations on fungicide sensitivity in vitro indicated that multi-site fungicides and acidified solutions of calcium propionate, potassium sorbate, and sodium benzoate (2 g/L, pH=4) were very effective against fungal pathogens such as *B. cinerea*, *Pyricularia oryzae*, and *Collectotrichum* spp. isolated from mango and grape. In addition, some specific site fungicides also had very good inhibition effects, although most of them were not. Furthermore, we have found that fungicides with the same mode of action but different molecular structures may have different risks to develop resistance. These fungicides could be applied in rotation in the field. Restricting the number of application of each fungicide per season and applying only when necessary would be crucial to curb fungicide resistance. Besides, to prevent the occurrence of fungicide resistance, it is imperative to apply the fungicide at a rate recommended by the manufacturers. Rotating each single fungicide may be more advantageous than using a mixture of them when coping with resistance.

Key words: baseline sensitivity, EC_{50}, fungicide resistance

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