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由*Neopestalotiopsis rosae*引起之臺灣草莓新病害與藥劑篩選

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摘　要

徐巧芳、鄭婷文、陳冠勳、連怡婷、林政谷、黃卓君、夏凱、王惠亮。由*Neopestalotiopsis rosae*引起之臺灣草莓新病害與藥劑篩選。植物醫學62(4): 39-48。

2019年12月至2020年2月間，於苗栗大湖草莓園及南投正瀚生技公司溫室觀察到草莓葉片有紅褐色至深棕色病斑，病徵類似炭疽病，另果實亦有紫色、褐色或白色水浸狀病徵，後期有白色菌絲產生。同時亦自屏東縣林邊鄉採集紫色水浸狀病徵的蓮霧果腐病果實。兩個作物之病葉、病果經分離、純化後，進行菌落與孢子形態觀察，並進行接種試驗。此外透過增幅internal transcribed spacer、β-tubulin基因和transcription elongation factor基因之序列進行分子鑑定。初步結果認為引起草莓病害之病原菌為*Neopestalotiopsis rosae*，由其危害病徵定名為草莓葉斑病。蓮霧果腐病原菌為*Neopestalotiopsis* sp.。*Neopestalotiopsis*已於中國、西班牙及巴西等國家被報導可感染草莓葉片、果實、冠部及根部，而在台灣草莓為首次鑑定命名。本研究欲了解不同品種的草莓對*N. rosae*及蓮霧果腐病原菌之罹病程度，三個品種的草莓以離體葉片接種測試，結果顯示香水為感病品種，桃薰為中感品種，豐香不易被感染屬抗病品種。*N. rosae*於10^4 conidia/ml濃度時即可感染香水葉片，而蓮霧果腐病原菌在10^4及10^5 conidia/ml接種濃度下，皆不易感染前述草莓品種之葉片，但當接種濃度提高至10^6 conidia/ml時，三個品種的草莓葉片均會出現病徵，顯示蓮霧果腐病原菌對草莓葉片感染性較差，且罹病程度仍以香水最為嚴重。果實接種結果顯示與葉片接種結果一致。近幾年因豐香對炭疽病較感病而不易栽培，農民開始改種香水，可能就是造成*N. rosae*在這兩年感染較嚴重的原因。進一步以培養基添加藥劑測定抑制*N. rosae*菌絲生長的效果，結果顯示25%撲克拉乳劑、25.9%得克利水基乳劑、75g/L依普座乳劑、39.5%扶吉胺水懸劑、80%鋅錳乃浦可濕性粉劑及62.5%賽普護汰寧水分散性粒劑有較佳的抑制效果，抑制率均可達90%以上，僅62.5%賽普護汰寧水分散粒劑在植物保護資訊系統推薦使用於草莓灰黴病防治。

關鍵詞：草莓葉斑病、*Neopestalotiopsis rosae*

緒　言

根據行政院農業委員會農糧署107年蔬菜生產概況統計，台灣草莓栽培面積約506公頃，其中苗栗為主要產區佔九成以上[10]，主要的栽培品種為香水和豐香。豐香為桃園區農業改良場命名之桃園一號，果實之香氣成分多，果肉細緻，但植株對炭疽病的抗性較弱[31]。香水由美國加州大學育出之品種，香水比豐香果形大而重，且有抗高溫、抗病、更快採收等特點[6]。桃薰為日本品種之白草莓，由母本野生草莓 K58N7-21，父本豐香 (久留米IH1號) 雜交而成，果實顏色為淡粉紅，帶有淡淡的水蜜桃味，因果實柔軟不耐儲放[22]，在台灣主要為觀光果園少量栽培。台灣草莓在育苗期重要的病害有炭疽病 (由 *Colletotrichum gloeosporioides* species complex 引起) ，在採果期重要的病害有白粉病 (由 *Sphaerotheca macularis* f. sp. *Fragariae* 引起) ，和灰黴病 (由 *Botrytis cinerea* Pers. 引起) 等[33]。其中以炭疽病最重要，炭疽病會在花莖、走莖、葉柄和葉片上引起深色病灶，並在果實產生圓形深褐色凹陷甚至腐爛[9]。

2019年12月至2020年2月間，於苗栗縣大湖鄉草莓園及正瀚生技公司溫室發現草莓葉片有紅褐色至深棕色病斑，葉片上褐色病斑類似炭疽病，果實則呈現多變的病徵，包括紫色、褐色或白色水浸狀，後期有白色菌絲產生。將草莓葉片病害分離後發現菌落形態和蓮霧果腐病極為相似，而蓮霧果腐病最早在民國68年發現由*Pestalotiopsis eugeniae* Thuem引起而命名[17]。在泰國蓮霧果腐病原菌被命名為*Neopestalotiopsis samarangensis*，初始病徵為圓形黑色略為凹陷的斑點，後期病斑迅速擴大下陷，導致果肉腐爛[19]。*Neopestalotiopsis* spp.在近十年內已於墨西哥[26]、中國[32]、伊朗[3]、印度[2]、西班牙[6]及阿根廷[24]等國家被報導造成草莓葉枯 (leaf blight)、果腐 (fruit rot)、冠腐 (crown rot) 及根腐 (root rot) 等。

本研究欲了解(一) 確定造成草莓葉片及果實病害之病原菌種類，(二) 不同品種草莓之感病性，(三) 草莓葉斑病原菌和蓮霧果腐病原菌對草莓和蓮霧的葉片和果實發病反應，(四) 篩選可供防治之藥劑。

材料與方法

一、病原菌收集及分離純化

2019年12月至2020年2月間，在苗栗縣大湖鄉草莓園及正瀚溫室發現草莓葉片有褐色病斑，果實呈現水浸狀病徵。同時自屏東縣林邊鄉蓮霧園採集蓮霧果腐病果實，初期呈現紫色水浸狀病徵，後期擴大呈黑色病斑。將整個葉片及果實以75%酒精表面消毒後，以滅菌的解剖刀將病健部以3×3 mm大小的葉片或果肉取下，置於2%水瓊脂培養基 (water agar, WA) 平板，於28 ℃恆溫生長箱 (Chih Chin, Taiwan) 黑暗中培養三天後移至馬鈴薯葡萄糖瓊脂培養基 (potato dextrose agar, PDA) 平板，待其產孢後挑取單一孢子進行純化以供後續試驗使用。草莓葉片分離的病原菌代碼為SL，蓮霧果實分離之病原菌代碼為WF。

二、病原菌菌落及孢子形態觀察

將病原菌SL及WF培養於PDA培養基平板上，於28 ℃恆溫生長箱黑暗中培養8天後，觀察其菌落形態，待病原菌產孢後，以無菌水洗下，於顯微鏡下觀察其孢子型態，並取30個孢子測量長、寬之平均大小。

三、接種試驗

將病原菌SL和WF培養於PDA培養基，於28 ℃恆溫生長箱黑暗中培養8天，待其產孢後以無菌水洗下，以濾布 (miracloth, Merk) 去除菌絲，並以無菌水稀釋調整孢子濃度為10^5 conidia/ml，以微量吸管尖在草莓 (香水品種) 葉片、果實及蓮霧果實製造傷口後，分別接種病原菌SL及WF，以完成柯霍氏法則 (Koch's postulates)，並於4天後觀察草莓葉片與蓮霧果實發病之情形。

四、病原菌分子鑑定

將病原菌SL和WF培養於PDA培養基，於28 ℃恆溫生長箱黑暗中培養8天，以無菌細胞刮勺刮取菌絲至1.5ml微量離心管， 加入鋼珠以混合冷凍球磨機(Oscillating Mill MM400, Germany)進行細胞破碎，並以去氧核醣核酸萃取試劑套組(Qiagen DNeasy® Mini Plant Kit, Germany)進行DNA萃取。將萃取之DNA進行聚合酶連鎖反應 (Polymerase chain reaction, PCR)，增幅internal transcribed spacer (ITS) rDNA片段。選用引子對參考自Paride[25] ITS-5並作修改為(5'-GGGAAGTAAAAGGTCGTAAC-3')及ITS-4 (5'-TCCTCCGCTTATTGATATGC-3')。PCR反應試劑含有各1 µl 10 µM引子對、25 µl KAPA HiFi HotStart ReadyMix PCR Kit、100 ng DNA模板，加水至總體積50 µl。ITS增幅條件為先以95 ℃反應3分鐘，之後進行95 ℃ 30秒，56 ℃ 30秒，72 ℃ 50秒，共25個循環，最後以72 ℃ 3分鐘完成反應。增幅β-tubulin片段之引子對為BT2Fd (5'-GTBCACCTYCARACCGGYCARTG-3')和BT4Rd (5'-CCRGAYTGRCCRAARACR AAGTTGTC-3') [30]，增幅條件為先以95 ℃反應3分鐘，之後進行95 ℃ 30 秒，58 ℃ 30秒，72 ℃ 50秒，共25個循環，最後以72 ℃ 3分鐘完成反應。增幅 transcription elongation factor (TEF)引子對為EF1-526F (5'-GTCGTYGTYATYGGHCAYGT-3') 及EF1-1567R (5'-ACHGTRCCRATA CCACCRATCTT-3')[19] 增幅條件為先以95 ℃反應3分鐘，之後進行95 ℃ 30 秒，54 ℃ 30秒，72 ℃ 50秒，共25個循環，最後以72 ℃ 3分鐘完成反應。

PCR擴增產物委託源資國際生物科技股份有限公司進行核酸序列定序。以CodenCode Aligner編輯序列，並將正向和反向定序結果組成接合序列 (Contig)。使用軟體MEGA X (版本10.1.1)，將ITS、β-tubulin和TEF個別排序後，以共同區段接合，再使用軟體PAUP (版本4.0) [28]，以鄰接法 (Neighbor-joining tree) 建構演化樹，建構參數為Kimura 2-parameter model，外群為 *N. natalensis* 和 *N. steyaertii*，使用之序列取自Akinsanmi等[1]、Bezerra 等[4]、Freitas等[12]、Hyde等[13]、Jayawardena等[14]、Jiang等[15]、Kumar等[16]、Ma等[18]、Maharachchikumbura等[19, 20, 21]、Norphanphoun等[23]和Tibpromma等[29]，登錄於美國生物技術資訊中心 (National Center for Biotechnology Information, NCBI) 網站 (http://www.ncbi.nlm.nih.gov/) 之GenBank資料庫。

五、草莓品種感病性測試

為了解不同品種之草莓對病原菌SL及WF之感病性，將病原菌SL和WF培養於PDA培養基，於28 ℃恆溫生長箱黑暗中培養8天，待其產孢後以無菌水洗下，以濾布 (miracloth, Merk) 去除菌絲，並以無菌水稀釋調整孢子濃度10^4、10^5、10^6 conidia/ml，以微量吸管尖製造傷口後進行接種。將SL及WF接種於豐香、桃薰及香水的葉片及果實上。並使用葉面積分析軟體WinFOLIA 2014 (Regent Instruments Inc., Canada) 計算罹病面積。

六、防治藥劑篩選

將病原菌SL培養於9公分PDA平板至長滿，以內徑5 mm之圓形打孔器切取菌盤最外圍之菌絲，分別放置於含有農藥之培養基培養，對照組放置於不含農藥之PDA平板，每個藥劑處理4重複，培養皿置於室溫觀察，待對照組病原菌長滿 (9天) 後

紀錄各處理的菌落直徑，並以下方公式計算生長抑制率：

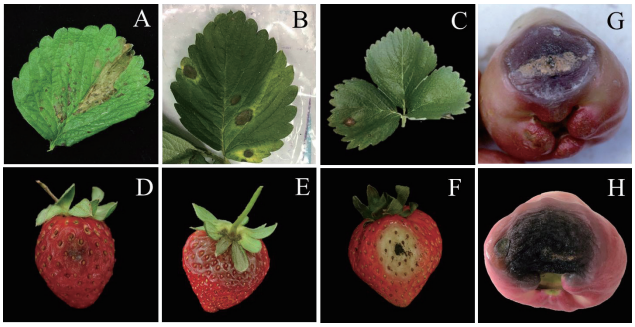
抑制率(%)=[1－(處理組菌落直徑／對照組菌落直徑)]×100

依據植物保護資訊系統選擇草莓、蓮霧推薦用藥及曾經測試於防治*Neopestalotiopsis* spp.之藥劑，測試藥劑分別為25.9% 得克利水基乳劑 (folicur, 台灣拜耳) 稀釋1,500倍、75 g/L 依普座乳劑 (epoxiconazole, 台灣巴斯夫) 稀釋1,000倍、250 g/L 待克利乳劑 (difenoconazole, 海博生技) 稀釋3,000倍、40%邁克尼可濕性粉劑 (myclobutanil, 惠光) 稀釋12,000倍、25%撲克拉乳劑 (prochloraz, 正農化學) 稀釋3,000倍、23%亞托敏水懸劑 (azoxystrobin, 日農科技) 稀釋2,000倍、500 g/L三氟敏水懸劑 (trifloxystrobin, 萬得發) 稀釋5,000倍、38%白列克敏水分散性粒劑 (pyraclostrobin + boscalid, 台灣巴斯夫) 稀釋1,500倍、62.5% w/v 賽普護汰寧水分散性粒劑 (cyprodinil + fludioxonil, 朝暘生化) 稀釋1,500倍、80% w/v 鋅錳乃浦可濕性粉劑 (mancozeb, 中華民國農會附設各級農會農化廠) 稀釋400倍、40% w/v 四氯異苯腈水懸劑 (chlorothalonil, 臺灣庵原) 稀釋1,000倍和39.5% 扶吉胺水懸劑 (fluazinam, 台灣石原) 稀釋1,500倍。

結　果

一、病原菌收集及分離純化

田間或溫室採集之草莓病葉有紅褐色至深棕色病斑 (圖一A-C)，果實則呈現多變的病徵，包括紫色、褐色或白色水浸狀，後期可能有白色菌絲產生 (圖一D-F)。蓮霧果腐病初始病徵為橢圓形紫色略為凹陷的水浸狀，後期病斑迅速擴大呈黑色，並形成黑色環狀小點，此為孢子盤構造(圖一G-H)。

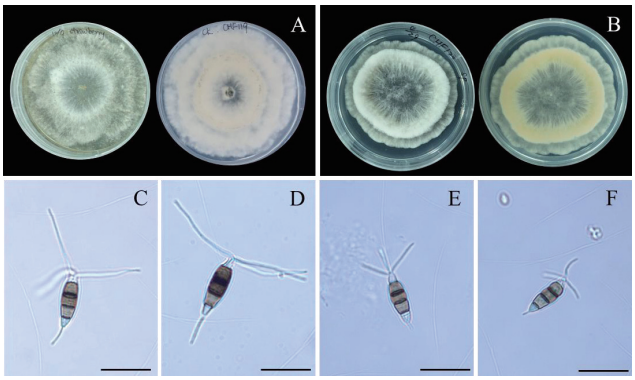


圖一、病原菌SL在草莓及病原菌WF在蓮霧的病徵，(A-C) 草莓葉片上*N. rosae*造成現紅褐色至深棕色病斑，果實上呈現多變的病徵(D) 褐色、(E) 紫色和(F) 白化，(G) *Neopestalotiopsis* sp.蓮霧果實呈現紫色水浸狀，(H) 蓮霧果實發病後期

Fig. 1. Symptoms of SL pathogen on strawberry and the symptoms of WF pathogen on wax apple. SL pathogen causes the red brown to dark brown leaf spot (A-C), and brown (D), purple (E), and bleaching (F) symptoms on fruits. *Neopestalotiopsis* sp. isolate WF causes purple water-soak symptoms (G). The symptom in late stage on wax apple (H).

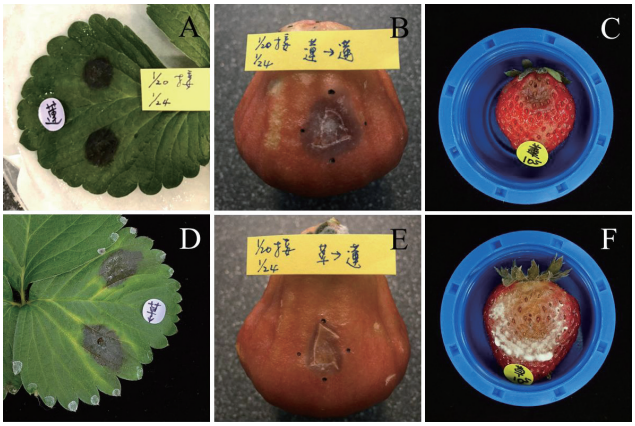
二、病原菌菌落及孢子形態觀察

將病原菌SL及WF培養於PDA培養基平板上，觀察其菌落型態，菌落邊緣呈不規則狀，具有蓬鬆的白色氣生菌絲，呈現玫瑰般層層堆疊向外擴張，背面呈淡鵝黃色，分生孢子盤為黑



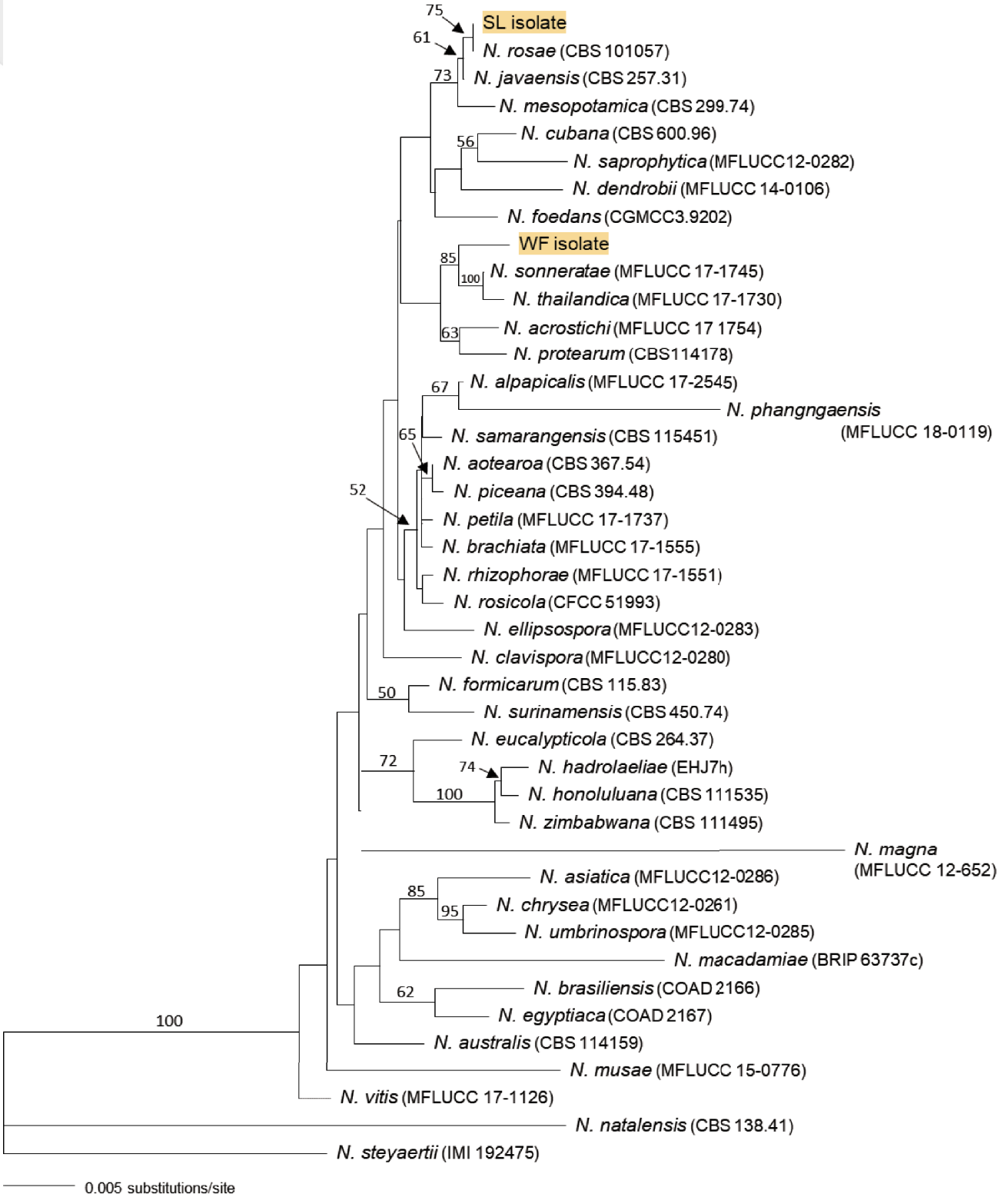
圖二、病原菌SL及WF之菌落及分生孢子型態。病原菌在PDA培養基菌落正面及背面形態，(A) SL病原菌， (B) WF病原菌；孢子形態 (C-D) SL病原菌，(E-F) WF病原菌。比例尺=20 μm。

Fig. 2. Morphology of SL and WF pathogens. Front and back sight of colony on PDA. (A) SL pathogen on PDA, (B) WF pathogen on PDA. (C-D) Conidia of SL pathogen, (E-F) Conidia of WF pathogen. *Scale Bar*：20 μm



圖三、病原菌SL及WF分別接種草莓葉片、果實和蓮霧果實結果。WF病原菌接種後第4天，(A) 草莓葉片自接種處出現黑褐色病斑，(B) 蓮霧果實出現紫色水浸狀之病徵；(C) 草莓果實接種第6天自接種處出現水浸狀。SL病原菌接種後第4天，(D) 草莓葉片自接種處出現黑褐色病斑，(E) 蓮霧果實出現紫色水浸狀之病徵；(F) 草莓果實接種第6天自接種處出現白色菌絲病兆。

Fig. 3. SL and WF pathogens were inoculated on the fruits and leaves of strawberry and fruits of wax apple. After inoculated with WF pathogen, black-brown symptom on the strawberry leaf (A) and purple water-soaked symptom on wax apple fruit were observed at 4 days post inoculation (dpi) (B). Water-soaked symptom on the strawberry fruits were observed at 6 dpi (C). After inoculated with SL pathogen, black-brown symptom on the strawberry leaf (D) and purple water-soaked symptom on wax apple fruit were observed at 4 dpi (E), while brown lesion with white mycelia was observed on the strawberry fruit at 6 dpi (F).



圖四、結合ITS、β-tubulin以及TEF的基因序列建構NJ演化樹，大於50%的Bootstrap值標示在節點。從GenBank取得40種 *Neopestalotiopsis* 屬的序列進行演化樹分析，外群為*Neopestalotiopsis natalensis* 與 *Neopestalotiopsis steyaertii* 兩菌種。菌株編號標示於括號中。

Fig. 4. Neighbor joining (NJ) tree obtained from the combined DNA sequence data of ITS, β-tubulin and TEF genes. NJ bootstrap values ≧ 50% were given at the nodes. Forty species of genus Neopestalotiopsis from GenBank were used in this consensus phylograms. *Neopestalotiopsis natalensis* and *Neopestalotiopsis steyaertii* were used as outgroups for rooting the tree. Strain names were indicated in the round brackets.

色，分子孢子呈紡錘形有4個隔膜(septa) 分成5室，中間3室為褐色，兩端無色透明，頂端有2-3根附屬絲，基部有短尖的附屬絲，SL孢子大小為16.8-26.1×5.9-7.7 μm (平均21.5×6.7 μm)，頂部附屬絲長度為13.6-28.8 μm (平均20.9 μm)，尾部附屬絲為2.8-10.8 μm (平均6.5 μm)；WF孢子大小為14.3-20.1×5.4-7.4 μm (平均17.3×6.4 μm)，頂部附屬絲長度為5.1-17.3 μm (平均11.5 μm)，尾部附屬絲為0.9-4.0 μm (平均2.2 μm) (圖二)。

三、接種試驗

將草莓葉片、果實和蓮霧果實表面製造傷口後分別接種病原菌SL及WF孢子，WF病原菌接種後第四天，草莓葉片自接種處出現黑褐色病斑 (圖三A)，蓮霧果實出現紫色水浸狀之病徵(圖三B)，草莓果實接種第六天自接種處出現水浸狀(圖三C)。SL病原菌接種後第四天，草莓葉片自接種處出現黑褐色病斑(圖三D)，蓮霧果實出現紫色水浸狀之病徵(圖三E)，草莓果實接種第六天自接種處出現白色菌絲病徵(圖三F)。

四、病原菌分子鑑定

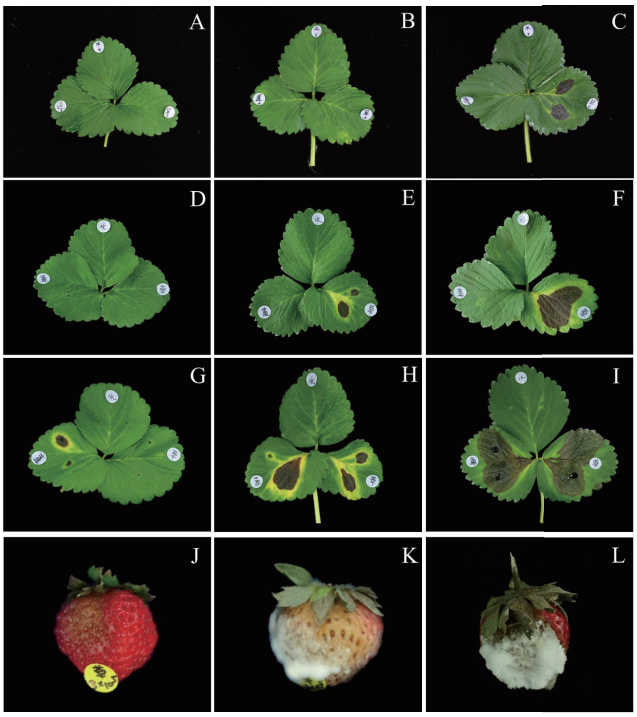
病原菌SL及WF以ITS5/ITS4、BT2Fd/BT4Rd及EF1-526F/EF1-1567R引子對增幅核苷酸個別得約600、400及1000 bp大小之序列，將所得之序列提交至NCBI並取得Accession No.，病原菌SL之ITS 序列為MT515745、β-tubulin 序列為MT515746、TEF序列為MT515747；病原菌WF之ITS 序列為MT515748、β-tubulin序列為MT515749，TEF序列為MT515750。將所得之序列進行親緣性比較繪製演化樹，*N. rosae*與*N. javaensis*分岐bootstrap值為75，病原菌SL和*N. rosae*分類群相同，病原菌WF則無相同之分類群(圖四)。

五、草莓品種感病性測試

草莓葉片接種病原菌SL 10^6 conidia/ml於香水、桃薰及豐香後第11天，以葉面積分析軟體計算得香水的病斑面積約佔葉片53.2%屬感病品種；桃薰病斑面積約為14%，且病斑周圍出現黃暈抑制病程發展屬中感品種；豐香病斑沒有擴大跡象，不易被感染屬抗病品種 (圖五G-I)。病原菌WF在接種濃度10^4、10^5 conidia/ml 的情況下，皆不易感染香水葉片 (圖五A-F)，當提高接種濃度至10^6 conidia/ml則可造成病徵。果實接種結果顯示，所有品種果實皆會呈現水浸狀病斑或產生菌絲，發病嚴重程度因品種有所差異，香水最為嚴重，其次是桃薰和豐香 (圖五J-L)。

六、防治藥劑篩選

以培養基添加藥劑測定防治效果，結果顯示25%撲克拉乳劑稀釋3,000倍、25.9%得克利水基乳劑稀釋1,500倍、75 g/L依普座乳劑稀釋1,000倍、39.5%扶吉胺水懸劑稀釋1,500倍、80%鋅錳乃浦可濕性粉劑稀釋400倍及62.5%賽普護汰寧水分散性粒劑稀釋1,500倍有較佳抑制菌絲生長的效果，平均抑制率可達90%以上 (表一)。



圖五、不同品種之草莓葉片接種病害之病徵，上方接種無菌水作為對照組，右方接種SL，左方接種WF。各接種10^4 conidia/ml，接種後8天，(A)豐香，(B)桃薰，(C)香水；各接種10^5 conidia/ml，接種後11天，(D)豐香，(E)桃薰，(F)香水；各接種10^6 conidia/ml，接種後11天，(G)豐香，(H)桃薰，(I)香水。不同品種草莓果實接種病原菌SL 3×10^5 conidia/ml，接種後6天，(J)豐香，(K)桃薰，(L)香水，後期有菌絲產生。

Fig. 5. Foliar symptoms on different varieties of strawberry after inoculation, top leaf was inoculated with sterile water, while on the right leaf was inoculated with SL isolate, and left leaf was inoculated with WF isolate. Inoculated with 10^4 conidia/ml at 8 days post inoculation (dpi). (A) 'Taoyuan No. 1', (B) 'Tokun', (C) 'Aroma'. Inoculated with 10^5 conidia/ml at 11 dpi. (D) 'Taoyuan No. 1', (E) 'Tokun', (F) 'Aroma'. Inoculated with 10^6 conidia/ml at 11 dpi. (G) 'Taoyuan No. 1', (H) 'Tokun', (I) 'Aroma'. Fruits symptom on different varieties of strawberry after inoculated with SL isolate (3×10^5 conidia/ml) at 6 dpi (J) 'Taoyuan No. 1', (K) 'Tokun', (L) 'Aroma'.

討　論

2019年12月至2020年2月間，在苗栗縣大湖鄉草莓園及正瀚溫室發現草莓葉片有紅褐色至深棕色病斑，病徵類似炭疽病。經分離純化病原菌後回接至草莓葉片，可產生相同之病徵，確認分離獲得的SL菌株為草莓病原菌。將草莓葉片病害

表一、利用培養基測試農藥對病原菌SL之菌落生長抑制率

TABLE 1. Growth inhibition rate of the fungicides against the SL pathogen

Fungicide	Dilution Rate	Inhibition Rate (%)
25% Prochloraz EC	3,000	98.8
75 g/L Epoxiconazole EC	1,000	97.5
25.9% Folicur EW	1,500	97.3
80% Mancozeb WP	4,000	95.3
39.5% Fluazinam SC	1,500	94.0
62.5% Cyprodinil + Fludioxonil WG	1,500	90.7
250 g/L Difenoconazole EC	3,000	70.3
40% Chlorothalonil SC	1,000	64.7
38% Pyraclostrobin + Boscalid WG	1,500	56.7
23% Azoxystrobin SC	2,000	40.0
40% Myclobutanil WP	12,000	35.3
500 g/L Trifloxystrobin SC	5,000	22.7

分離後發現菌落形態和蓮霧果腐病極為相似，而蓮霧果腐病最早在民國68年由陳等發現並命名為*Pestalotiopsis eugeniae* Thuem[9]，此次經由分子生物學鑑定後發現病原菌WF無相同之分類群，暫定為*Neopestalotiopsis* sp. 需再進一步確認其分類。在台灣，九成以上的草莓都種在中部地區，而蓮霧則多種在南部地區，為了解病原菌SL和蓮霧果腐病菌是否會交叉感染各自的來源寄主，因此進行草莓葉片、果實和蓮霧果實接種試驗。結果顯示病原菌SL和WF接種至草莓葉片、果實和蓮霧果實均可造成病徵。經比對菌落和孢子形態及分子生物學鑑定，確認病原菌SL為*Neopestalotiopsis rosae*。Maharachchikumbura等在2014年利用產孢細胞 (conidiogenous cell)、分生孢子的顏色和分子鑑定，將*Neopestalotiopsis*屬和*Pseudopestalotiopsis*屬自Pestalotiopsis屬獨立出來[20]。由孢子形態觀察，病原菌SL頂部和尾部附屬絲均較病原菌WF長，病原菌SL頂部和尾部附屬絲長度約各為病原菌WF的1.8倍和3倍長。Neopestalotiopsis spp.在近十年內已於墨西哥[26]、中國[32]、伊朗[3]、印度[2]、西班牙[6]及阿根廷[24]等國家被報導造成草莓葉枯 (leaf blight)、果腐 (fruit rot)、冠腐 (crown rot) 及根腐 (root rot) 等。在巴西*Pestalotiopsis longisetula* Guba經常造成草莓產量巨大損失[27]；在西班牙*Neopestalotiopsis clavispora* (syn. *Pestalotiopsis clavispora*) 主要造成根腐及冠腐[5]。2020年Rebollar-Alviter等[26]在墨西哥首次報導由*Neopestalotiopsis rosae*造成草莓根腐、冠腐及葉斑病害，在2017年部分栽培地區造成50%的損失。由其報告內容孢子形態、分子生物鑑定和藥劑篩選結果與本研究相近。而*Neopestalotiopsis rosae*在台灣草莓病害為首次鑑定命名，病徵位置主要在葉片及果實上。

草莓葉片接種病原菌SL結果顯示，香水品種發病面積最大，其次為桃薰，且病斑周圍出現黃暈抑制病程發展為中感品種；豐香屬抗病品種。果實接種結果顯示，所有品種果實皆會呈現水浸狀病斑或產生菌絲，發病嚴重程度亦不相同，香水最為嚴重，其次是桃薰和豐香，和葉片接種趨勢相同。桃薰由豐香及野生草莓雜交而成，故抗病能力介於感病之香水及抗病之豐香間。豐香及香水為台灣草莓栽培主力品種，台灣每年所需草莓種苗量約為2,750萬株，而2016年豐香因炭疽病造成缺苗達400萬株[34]，加上豐香對炭疽病抵抗力差栽培不易，農民栽培逐漸提高香水栽培面積。2014年香水種植約佔2成，豐香為8成，2019年香水種植比例更大幅增加至90%。香水對病原菌SL為感病品種，加上這兩年擴大栽培面積可能就是造成該病害在田間較嚴重發生的原因。將病原菌WF以人工接種亦可造成草莓病徵，在接種濃度10^4 conidia/ml情況下，無法感染香水、桃薰和豐香的葉片，而病原菌SL接種孢子10^4 conidia/ml即可感染香水葉片，顯示病原菌WF對草莓的感染性比病原菌SL差。

台灣草莓多以走莖跳苗或組織培養方式繁殖種苗，12月中旬可開始採收，收穫期可長達半年[7]。當溫度低於25 ℃，連續下雨且相對濕度大於80%，將造成果腐及葉片嚴重的病害[11]，在大雨過後應嚴防病害傳播。以培養基添加藥劑測定防治效果，結果顯示25%撲克拉乳劑、25.9%得克利水基乳劑、75 g/L依普座乳劑、39.5%扶吉胺水懸劑、80%鋅錳乃浦可濕性粉劑及62.5%賽普護汰寧水分散性粒劑有較佳的抑制效果，抑制率均達90%以上。由於目前僅賽普護汰寧為植物保護資訊系統推薦於草莓防治灰黴病使用，希望本研究結果能提供農政單位及農民防治本病害之參考。

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ABSTRACT

Hsu, C. F.[1], Cheng, T. W.[1], Chen, K. H.[1], Lien, Y. T.[1], Lin, C. K.[1], Huang, C. C.[1], Xia, K.[1], and Wang, H. L.[1*] 2020. Studies of a new disease on strawberry caused by *Neopestalotiopsis rosae* in Taiwan. J. Plant Med. 62(4): 39-48.
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During Dec. 2019 to Feb. 2020, the leaves of strawberry with red brown to dark brown lesions, the symptom looked like anthracnose. The fruits of strawberry with purple, brown, white and water-soaked symptoms, at later stage, it will appear white mycelium. The infected leaves and fruits of strawberry were collected from Dahu strawberry farm, Miaoli county, and the green house in CH Biotech R & D Co., Nantou city, LTD. At the same time, the fruits of wax apple with purple and water-soaked symptoms were collected from Linbian township, Pingtung county. The pathogens of leaves and fruits of strawberry and wax apple were isolated, purified and colonized to observe the spores, and inoculation. The pathogens of strawberry (SL) and wax apple (WF) were identified as *Neopestalotiopsis rosae* and *Neopestalotiopsis* sp., respectively, based on the combined sequences of internal transcribed spacer, β-tubulin and transcription elongation factor. *Neopestalotiopsis* were reported infecting the strawberry leaf, fruit, crown and root in China, Spanish and Brazil, however *N. rosae* was identified in Taiwan for the first time. In this study, *N. rosae* and the pathogen of wax apple fruit rot were inoculated on detached leaves of three different strawberry varieties. The result showed 'Aroma' was susceptible, 'Tokun' was moderate resistant, and 'Taoyuan No.1' was resistant. *N. rosae*. could infect the leaf of 'Aroma' by inoculated the concentration of 10^4 conidia/ml. The pathogen of wax apple fruit rot could not infect three different strawberry varieties with the concentration of 10^4 and 10^5 conidia/ml. If the inoculated concentration increased to 10^6conidia/ml, the pathogen of wax apple could infect three different strawberry varieties. It showed that pathogen of wax apple could not infect the leaf of strawberry easily, and 'Aroma' is the most susceptible variety. The farmers change the cultivated varieties into 'aroma'. It may cause *N. rosae*. damaged more intense. Moreover, growth inhibition rate of fungicides were

tested. The result showed that 25% prochloraz EC, 25.9% folicur EW, 75 g/L epoxiconazole EC, 39.5% fluazinam SC, 80% mancozeb WP, and 62.5% cyprodinil + fludioxonil WG had better suppression effect, and the growth inhibition rate could reach up to 90%. Only 62.5% cyprodinil + fludioxonil WG was recommended to control the strawberry Botrytis disease in Plant Protection Information System.

Keywords: strawberry leaf spot, *Neopestalotiopsis rosae*