Eur J Plant Pathol (2010) 127:399–405
DOI 10.1007/s10658-010-9606-0

Benomyl sensitivity assays and species-specific PCR reactions highlight association of two *Colletotrichum gloeosporioides* types and *C. acutatum* with rumple disease on Primofiori lemons

Manuel Valero · Santiago García-Martínez · Manuel J. Giner · Aranzazu Alonso · Juan J. Ruiz

Accepted: 15 March 2010 / Published online: 29 March 2010

Abstract Rumple is a serious peel collapse of Primofiori lemons in the southeast of Spain with an unresolved aetiology. Symptoms typically occur on fruits at ripening under wet conditions as dark sunken lesions producing premature fruit drop and damaged fruits unacceptable for fresh commercialization. A total of 16 *Colletotrichum* spp. isolates established from rumple-affected lemons collected during the autumn of 2007 from two different orchards were characterized by molecular and phenotypic assays and compared with reference isolates. Species-specific PCR reactions using β-tubulin 2 nucleotide sequences showed *Colletotrichum gloeosporioides* to predominate (81.5%) with limited occurrence of *C. acutatum* (18.75%). Among the *C. gloeosporioides* isolates, five (38.5%) showed benomyl resistance and eight (61.5%) were highly sensitive to the fungicide. The limited occurrence of *C. acutatum* could be related to factors such as the presence of both species on the same fruit, unfavourable meteorological conditions and low disease incidence. This work reveals an association of *C. gloeosporioides* and *C. acutatum* isolates with rumple disease of lemons and expands the range of *C. acutatum* on citrus.

Keywords *Colletotrichum acutatum* molecular diagnosis · *C gloeosporioides* molecular diagnosis · Diagnostic PCR · Fungicide sensitivity · Rind collapse of lemons · *tub 2* gene species-specific primers

M. Valero (✉) · M. J. Giner
Departamento de Producción Vegetal y Microbiología, Universidad Miguel Hernández (UMH) - Campus de Orihuela. Escuela Politécnica Superior de Orihuela (EPSO),
Carretera de Beniel, km 3.2,
03312-Orihuela Alicante, Spain
e-mail: m.valero@umh.es

S. García-Martínez · A. Alonso · J. J. Ruiz
Departamento de Biología Aplicada, Universidad Miguel Hernández (UMH) - Campus de Orihuela.
Escuela Politécnica Superior de Orihuela (EPSO),
Carretera de Beniel, km 3.2,
03312-Orihuela Alicante, Spain

S. García-Martínez
e-mail: sgarcia@umh.es

J. J. Ruiz
e-mail: juanj.ruiz@umh.es

Introduction

Rumple disease of lemons [*Citrus limon* (L.) Burm. f.] is an alteration, anomaly, disorder or collapse of the peel of the fruit. It was first described in Florida and Sicily, and was given names such as ‘rumple’ (Knorr 1963), ‘wrinkle rind’ (Russo and Klotz 1963) and ‘raggrinzimento della buccia’ (Salerno 1963). In the early 1970s, it had already been detected in Corsica, Italy, Turkey, Syria, Lebanon, Cyprus, Israel and Spain (Del Rivero 1967; Knorr and Koo 1969; Ozbek

et al. 1976). In Spain the peel alteration of lemons was known by different names such as 'lepra', 'mancha', 'viruela', 'corteza arrugada', 'ajado', and 'chafado'. In Florida the Lisbon and Eureka varieties of lemon are affected, whilst in Sicily rumple has been observed mainly in Femminello lemons. In the citric fruit-growing areas of the provinces of Murcia and Alicante (Spain), the main variety affected is Primofiori, Fino or Mesero (Pinilla 1991). Disease incidence on others varieties such as Eureka, Lisbon or Verna is minimal, although it has also been detected (Alarcón et al. 1996). Year on year, the occurrence of rumple in the Levante region of Spain has been increasing since the early 1980s (Alarcón et al. 1996; Lucas 1999). In particular, in 2001 rumple affected more than 80% of the harvest in the majority of orchards.

La Vega Baja is the southernmost and warmest geographical area along the coast in the province of Alicante. It is marked by the course of the river Segura which determines the mainly agricultural economy of the area. In La Vega Baja, primary rumple begins at the end of summer, between the end of August and the beginning of September, just as the early fruits are beginning to change colour. The change in the rind is noticeable at first as a slight discolouration. This chlorotic colouration affects to 4 or 5 essential oil glands of the rind. The affected areas then extend gradually and irregularly, increasing in size and slowly they assume a darker colour. Simultaneously, the essential oil glands undergo a series of similar colour changes until finally they collapse and take on the appearance of smallpox vesicles. At this stage of rumple, the unaffected area of the fruit is completely yellow.

The secondary necrotic rumple lesions are hard, and can affect areas as large as 5 cm in diameter. Pith undergoes a slow process of darkening and reduction of the thickness of the peel until it is only 2 mm thick in the necrosis area, without reaching the pulp. When this stage of development has been reached, lemon suffers abscission and drops to the ground usually about the middle of October.

Symptoms typically occur on fruits at ripening under wet autumn conditions, with a higher incidence in lemon trees with symptoms resembling wither-tip on twigs and branches and/or water-stressed (drought, use of saline water, soil salinity). It seems that a low density of branches and leaves in the tree favours the production of a large number of lesions in the peel of its fruits. Also, the zones of the fruit more exposed to dew and sunlight can form fissures and epidermal splits due to the absorption of water by the tissues of the rind and the subsequent desiccation.

Until now the aetiology of rumple has remained unresolved (Whiteside 1988a; Del Rivero 1997). The influence of physiological, nutritional or water imbalances seems to be secondary, whilst the annual fluctuation of rumple incidence suggests that climatic factors might be involved (Knorr and Koo 1969; Alarcón et al. 1996; Del Rivero 1997). Considering these established facts, a research programme was initiated to analyse the possibility that rumple could be caused by an infectious fungal agent, with the aim to isolate and identify the agent.

Material and methods

Plant material

Primofiori lemons from two separate commercial orchards located in Orihuela (Alicante, Spain) were used. Lemons were surface disinfected by wetting briefly (30 s) in 95% ethanol and immersing for 1 min in 1% sodium hypochlorite solution, followed by rinsing in sterile distilled water. Peel sections from either healthy or rumple-affected lemons were placed with the outer layers of pith in contact with Potato-Dextrose Agar (PDA, Scharlau Chemie, S.A., Barcelona, Spain) on 9 cm Petri plates (5 fragments per plate and lemon). Plates were incubated at 25°C in darkness and 90% relative humidity for 3–4 days.

Isolation of fungi

Fungi were isolated from secondary rumple lesions on Primofiori lemons from cultures grown on PDA. Agar 0.5 cm-diameter plugs from the margins of the actively growing mycelia were cut out with a sterile cork borer and placed on central point of Petri dishes containing new PDA medium. Presumptive *Colletotrichum* spp. isolates were selected on the base of colony colour (white aerial mycelium which later became salmon or grey in colour). Cultures were grown at 25°C in darkness for production of mycelia and conidia. Fungal samples were mounted on slides

and examined microscopically with a bright-field microscope Olympus CH30.

Benomyl sensitivity assay

The effect of benomyl on the colony area of 16 isolates of *Colletotrichum* spp. in PDA culture was determined. Mycelial plugs from 7-day-old cultures were transferred to PDA plates supplemented with benomyl (Benomilo AGRO 50, Fitogal, S.L., Valencia) at 1 μg a.i. ml^{-1}. Plates were incubated for 1 week as described above. Then two diagonal measurements of mycelium were made on each plate and the colony area calculated. Means of the colony diameter were used to calculate the percentage of growth inhibition of mycelium in relation to the control. The fungicide-sensitive assay was performed in triplicate. Data were analyzed using analysis of variance and the Ficher´s least significant difference (LSD) procedure with Statgraphic software (Statistical Graphic Corp. and Graphic Software Systems Inc., Rockville, MD).

DNA extraction and diagnostic PCR with species-specific primers

Thirty-mg of fresh mycelium from PDA cultures were used in DNA isolation protocol. DNA was extracted from freeze mycelium with the DNeasy plant kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. DNA concentration was assessed by electrophoresis with known quantities of λ-DNA (Fermentas, Paisley, UK). PCR primers TBCA (5′-CGGAGGCCTGGTTGGGTGAG-3′) specific for *Colletotrichum acutatum* and TBCG (5′-CGGAA GCCTGGGTAGGAGCG-3′) specific for *C. gloeosporioides* (Talhinhas et al. 2005) designed from the β-tubulin 2 (*tub* 2) sequences were each used in conjunction with the conserved primer TB5 (5′-GGTAACCAGATTGGTGCTGCCTT-3′) (Talhinhas et al. 2002) for diagnostic PCR of *C. acutatum* and *C. gloeosporioides*. Reference isolates CECT 20120 (*C. acutatum*) and CECT 2860 (*C. gloeosporioides*) from the 'Colección Española de Cultivos Tipo' (Universitat de València, Spain) were used as positive controls. Previously characterized isolates CAO-41025-G and CAO-41025-O from olive fruits affected by anthracnose disease (kindly provided by Dr. Francesc García-Figueres, Laboratori de Sanitat Vegetal, Servei de Sanitat Vegetal, Direcció General d'Agricultura i Ramaderia, Departament d'Agricultura, Alimentació i Acció Rural, Generalitat de Catalunya, Barcelona, Spain) were also included in this study.

Each PCR (25 μl) contained 25 ng of DNA, 1 μM of each primer, 0.333 mM dNTPs, 2 mM $MgCl_2$, 0.6 Units of Netzime® DNA Polymerase and 1x PCR buffer. Amplifications were performed in a Mastercycler (Eppendorf) programmed to run the following temperature profile: a denaturising step for 5 min at 94°C, then 30 cycles consisting each of a denaturising step for 30 s at 94°C; an annealing step for 30 s at 62°C; an extension step for 2 min at 72°C and the final extension for 7 min at 72°C. PCR products (10 μl) were visualized by electrophoresis in 1.5% (w/v) agarose gels.

Results

Fungal isolates and morphological study

In 89% (16/18) of rumple-affected lemons used and in 34% (31/90) of peel sections cultured on PDA, fungal growth was initiated and white-orange to grey mycelia developed. A total of 16 presumptive *Colletotrichum* spp. isolates were selected on the base of colony colour to perform physiological and molecular studies. In healthy lemons there was sporadic development of some contaminating colonies of *Alternaria* spp., observed microscopically.

On PDA, the reference isolate of *C. acutatum* CECT 20120 from strawberry (*Fragaria* x *ananassa*) produced whitish mycelium, while isolates of this species CAO-41025-G and CAO-41025-O from olive (*Olea europaea* cv. Morrut) showed grey and orange coloured mycelia, respectively. The isolate of *C. gloeosporioides* from orange (CECT 2860) produced a sparse white mycelium. Most of the isolates from rumple-affected lemons showed a sparse white to grey mycelia with in some cases orange zones due to the presence of abundant conidia. Isolates 12, 13, and 14 developed a deep orange pigmentation.

Benomyl-sensitive assay

Inhibition of mycelium growth by the fungicide was significant (Table 1). *Colletotrichum* spp. isolates showed different levels of benomyl sensitivity. Colo-

Table 1 Effect of benomyl (1 μg ml^{-1}) on the colony area of *Colletotrichum* spp. isolates from rumple-affected lemons grown on PDA

Isolates	Colony parameters		Colony area (% of control)
	Diameter (mm)	Area (mm^2)	
1	69.33 a*	3,775.50	100
2	70.00 a	3,848.45	100
3	0	0	0
4	69.00 a	3,739.28	100
5	70.33 a	3,885.19	100
6	0	0	0
7	0	0	0
8	68.67 a	3,703.24	100
9	0	0	0
10	0	0	0
11	0	0	0
12	26.33 b	544.63	14.7
13	28.33 b	630.50	17.0
14	29.00 b	660.52	17.8
15	0	0	0
16	0	0	0

[a] Means followed by the same letter are not significantly different ($P \leq 0.05$)

ny area of isolates 12, 13, and 14 was reduced to about 16.5% of the control, while mycelium growth of isolates 3, 6, 7, 9, 10, 11, 15, and 16 was completely inhibited at 1 μg ml^{-1}. In contrast, the colony area of isolates 1, 2, 4, 5, and 8 was unaffected by this benomyl concentration.

PCR analysis

Figure 1 shows the results obtained in the diagnostic PCR tests using species-specific primers. In the PCR test with the *C. gloeosporioides*-specific primer TBCG used in conjunction with the conserved primer TB5, almost all the isolates from rumple-affected Primofiori lemons yielded the same positive product as the reference CECT 2860 *C. gloeosporioides* isolate from orange. The *C. acutatum*-specific primer TBCA in combination with TB5 yielded positive products only with isolates 12, 13, and 14 and with the reference *C. acutatum* isolates CECT 20120 from strawberry, and CAO-41025-G and CAO-41025-O from olive.

Discussion

Colletotrichum spp. were frequently found on rumple-affected Primofiori lemons collected during the autumn of 2007 from two different orchards: a total of 16 isolates were established. Molecular and phenotypic characterization of *Colletotrichum* spp. isolates associated with rumple of lemons and comparative analysis with reference *C. acutatum* and *C. gloeosporioides* isolates from other hosts identified 13 isolates as *C. gloeosporioides* and only 3 isolates as *C. acutatum*.

Benomyl sensitivity revealed two different types of isolates corresponding to benomyl-sensitive or benomyl-resistant *C. gloeosporioides* and a third type belonging to *C. acutatum*. Both species differed greatly in their sensitivity to benomyl. Most isolates of *C. gloeosporioides* are highly sensitive, but some isolates have developed resistance after more than 30 years of benomyl usage in agriculture. In contrast, *C. acutatun* is moderately resistant to this benzimidazole fungicide (Adaskaveg and Hartin 1997; Kuramae-Izioka et al. 1997) and isolates from groves with different histories of benomyl use show similar levels of sensitivity (Peres et al. 2004). Eight (61.5%) isolates of *C. gloeosporioides* were highly sensitive to the fungicide, but five (38.5%) isolates had high level of resistance even though we sampled a fewer number of isolates. Benomyl usage histories of the two orchards were not known. No benomyl-resistant or benomyl-sensitive isolate of *C. acutatum* was recov-

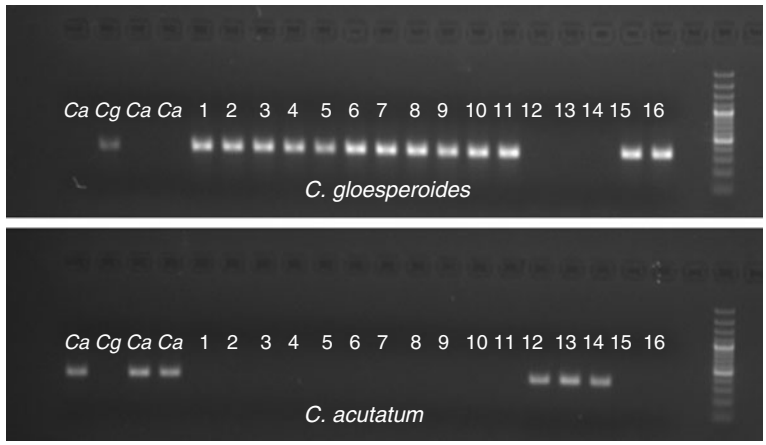

Fig. 1 Agarose gel electrophoresis of PCR products obtained with DNA of some of the *Colletotrichum* spp. isolates from infected lemon fruits (1 to 16) and controls (Ca correspond to *C. acutatum* and Cg to *C. gloeosporioides*). A: *C. acutatum*-specific products generated with β-tubulin 2 primers TB5 and TBCA. B: *C. gloeosporioides*-specific products generated with β-tubulin 2 primers TB5 and TBCG. M: molecular marker GeneRuler^{TM} 100pb (Fermentas, Paisley, United Kingdom)

ered, which was in agreement with results published earlier (Peres et al. 2004; Talhinhas et al. 2005).

Application of species-specific PCR using the *tub2*-based primers for *C. acutatum* and *C. gloeosporioides* provided rapid and reliable diagnosis of isolates belonging to *C. acutatum* and *C. gloeosporioides* from Primofiori lemons. Both species have been reported previously on citrus (Timmer et al. 2000). Conflicting reports exist in the literature on the causal agent of rumple of lemons (Ozbek et al. 1976; Majorana and Continella 1982, 1983; Davino et al. 1996; Valero et al. 1999; Beltrán et al. 2004) and this is the first report that highlights an association between the presence of the two *Colletotrichum* species and the rumple symptoms. According to our data *C. gloeosporioides* (81.25%) seems to be prevailing, while occurrence of *C. acutatum* is low (18.75%), but more isolates have to be studied to confirm this result. In order to complete Koch's postulates, re-inoculations should be conducted with these selected *C. gloeosporioides* and *C. acutatum* isolates on intact fruits in the tree.

C. gloeosporioides (Penzig) Penzig & Saccardo (anamorph of *Glomerella cingulata* (Stoneman) Spaulding & von Schrenk) is prevalent on many angiosperm hosts and is ubiquitous on citrus species. It is considered a common orchard saprophyte (Brown et al. 1996) and a postharvest pathogen (Whiteside 1988b; Zulfiqar et al. 1996) which can produce serious losses as result of anthracnose symptom development on the fruit surface. More recently, *C. gloeosporioides* was reported to be associated with wither-tip on twigs and tear stain on fruits of *Citrus sinensis* (L.) Osbeck in Morocco (Benyahia et al. 2003).

C. acutatum causes two diseases on citrus, post-bloom fruit drop (PFD) and Key lime anthracnose (KLA) and wither-tip (Peres et al. 2008). Rumple of lemons might be a completely new disease associated with this fungus, but *C. acutatum* has never been isolated from citrus outside of the Americas. However, it is not clear whether *C. acutatum* is indeed absent on citrus in Europe or remains unrecognised due to the difficulty of distinguishing it from the widespread saprophyte *C. gloeosporioides*.

In contrast with the rumple-associated *Colletotrichum* populations established in the current study, diverse *C. acutatum* groups and a low level of *C. gloeosporioides* have been associated with olive anthracnose in Mediterranean countries such us Portugal (Talhinhas et al. 2005) and Spain (Martín and García-Figueres 1999; Martín et al. 2002). Talhinhas et al. (2005) reported that the symptoms on olive fruit from which *C. gloeosporioides* was isolated were indistinguishable from those caused by *C. acutatum* and also pointed out that the *C. gloeosporioides* isolate showed the same virulence rating as the most virulent *C. acutatum* isolate from group A2. Furthermore, these authors established a correlation between high disease incidence along with favorable environmental conditions and the widespread prevalence of *C. acutatum* group A2.

Although pathogenicity tests have not been made, this work has revealed an association between the presence of *C. gloeosporioides* and *C. acutatum* isolates and the rumple symptoms of Primofiori lemons in La Vega Baja of the river Segura (Alicante, Spain). This report considerably expands the range of *C. acutatum* on citrus. Prevalence of *C. gloeosporioides* has not been clearly established because (i) a very limited number of isolates was studied, (ii) co-occurrence of *C. gloeosporioides* and *C. acutatum* has been observed in subsequent PCR analyses (García-Martínez, unpublished data), (iii) presence of benomyl-resistant *C. gloeosporioides* makes difficult the cultural distinction of species, and (iv) preliminary experiments based on colony characteristics, conidial morphology, growth rate and benomyl sensitivity suggested higher occurrence of *C. acutatun* in years with a high disease incidence (Giner, unpublished data). Further investigations on the pathogenicity of selected *C. acutatum* and *C. gloeosporioides* isolates on Primofiori lemons, the dynamics of populations in relation to rumple spread and severity, the phylogenetic lineage of *C. acutatum* isolates, and chemical control methods are essential.

Acknowledgements This study was financed by the Programa de Desarrollo y Diversificación Económica de Zonas Rurales (PRODER) through the Grupo de Acción Local Vega Baja Desarrollo Rural (VEBADER), Expedient 7.4.060. The authors are grateful to Enrique Galbis Biosca from FITOGAL, S.L for the supply of fungicide.

References

Adaskaveg, J. E., & Hartin, R. J. (1997). Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. *Phytopathology, 87*, 979–987.

Alarcón, A. L., Madrid, R., & Egea, C. (1996). El rumple del limón: descripción y factores influyentes. *Levante Agrícola, 337*, 356–364.

Beltrán, C., Lucas, A., Hermosilla, A., Fernández, A., Martínez, F. J., & Romero, S. (2004). El colapso de la corteza del limón (rumple) en la Región de Murcia, ensayo de inoculación de hongos. Murcia 2002. *Levante Agrícola, 370*, 171–177.

Benyahia, H., Jrifi, A., Smaili, C., Afellah, M. & Timmer, L.W. (2003). First report of *Colletotrichum gloeosporioides* causing withertip on twigs and tear stain on fruit of citrus in Morocco. *New Disease Reports*, 7. Retrieved July 3, 2009, from http://www.bspp.org.uk/publications/new-disease-reports/ndr/php?id=007020

Brown, A. E., Sreenivasaprasad, S., & Timmer, L. W. (1996). Molecular characterization of show-growing orange and Key lime anthracnose strains of *Colletotrichum* from citrus as *C. acutatum*. *Phytopathology, 86*(5), 523–527.

Davino, M., Caruso, A., D'Urso, F., Marino, R., Terranova, G., & Starrantino, A. (1996). dsRNA analysis of Adamo lemon affected by 'rumple' disorder. *Abstracts VIII Congress of the International Society of Citriculture*, South Africa, pp. 23.

Del Rivero, J. M. (1967). El 'rumple' de los limones. *Levante Agrícola*, Noviembre, pp. 17.

Del Rivero, J. M. (1997). Rumple una alteración de la corteza de los limones. *Agrícola Vergel, 184*, 187–194.

Knorr, L. C. (1963). Rumple—a new disease of lemon fruit. *Plant Disease Reporter, 47*(5), 335–339.

Knorr, L. C., & Koo, R. C. J. (1969). Rumple—a serious rind collapse of lemons in Florida and Mediterranean countries. *Proceedings First International Citrus Symposium, 3*, 1463–1472.

Kuramae-Izioka, E. E., Lopes, C. R., Souza, N. L., & Machado, M. A. (1997). Morphologycal and molecular characterization of *Colletotrichum* spp. from citrus orchards affected by postbloom fruit drop in Brazil. *European Journal of Plant Pathology, 103*, 323–329.

Lucas, A. (1999). Incidencia de las plagas y enfermedades en la Región de Murcia. *Phytoma España, 107*, 44–54.

Majorana, G., & Continella, G. (1982). Rumple of lemon fruits an infectious-type disease. *Tecnica Agricola, 34*, 109–119.

Majorana, G., & Continella, G. (1983). Trasmissione di sintomi di 'raggrinzimento della buccia' a cloni nucellari di limone. *Tecnica Agricola, 35*(2), 143–151.

Martín, M. P., & García-Figueres, F. (1999). *Colletotrichum acutatum* and *C. gloeosporioides* cause anthracnose on olives. *European Journal of Plant Pathology, 105*, 733–741.

Martín, M. P., García-Figueres, F., & Trapero, A. (2002). Iniciadores específicos para detectar las especies de *Colletotrichum* causantes de la antracnosis de los olivos. *Boletín de Sanidad Vegetal Plagas, 28*, 43–50.

Ozbek, N., Ozsan, M., Tuzcu, O., & Danisman, S. (1976). A preliminary study of rumple a serious rind disorder of lemons in Turkey. *Proceedings Seventh Conference of the International Organization of Citrus Virologists*, Riverside, 157–166.

Peres, N. A., Souza, N. L., Peever, T. L., & Timmer, L. W. (2004). Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. *Plant Disease, 88*, 125–130.

Peres, N. A., MacKenzie, S. J., Peever, T. L., & Timmer, L. W. (2008). Postbloom fruit drop of citrus and Key lime anthracnose are caused by distinct phylogenetic lineages of *Colletotrichum acutatum*. *Phytopathology, 98*(3), 345–352.

Pinilla, M. P. (1991). *'Viruela o corteza arrugada' en frutos de limonero*. CRIA, La Alberca, Murcia, 31 pp.

Russo, F., & Klotz, L. J. (1963). Wrinkle rind of lemons in Italy. *California Citrograph, 48*, 264.

Salerno, M. (1963). Il 'raggrinzimento della buccia' grave alterazione dei frutti di limone. *Tecnica Agricola, 15*, 507–511.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J., & Oliveira, H. (2002). Genetic and morphological character-

isation of *Colletotrichum acutatum* causing anthracnose of lupins. *Phytopathology, 92*, 986–996.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J., & Oliveira, H. (2005). Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Applied and Environmental Microbiology, 71* (6), 2987–2998.

Timmer, L. W., Garnsey, S. M., & Graham, J. H. (Eds.). (2000). *Compendium of citrus diseases* (2nd ed.). St. Paul: APS.

Valero, M., Fernández, P. S., & Salmerón, M. C. (1999). El arrugamiento de la corteza del limón o rumple, una enfermedad de origen fúngico. *Phytoma España, 110*, 26–34.

Whiteside, J. O. (1988a). Rumple of lemons. In J. O. Whiteside, S. M. Garnsey, & L. W. Timmer (Eds.), *Compendium of citrus diseases* (p. 67). St. Paul: APS.

Whiteside, J. O. (1988b). Anthracnose. In J. O. Whiteside, S. M. Garnsey, & L. W. Timmer (Eds.), *Compendium of citrus diseases* (pp. 9–10). St. Paul: APS.

Zulfiqar, M., Brlansky, R. H., & Timmer, L. W. (1996). Infection of flower and vegetative tissues of citrus by *Colletotrichum acutatum* and *C. gloeosporioides*. *Mycologia, 88*(1), 121–128.