Mycol. Res. **106** (2): 239–244 (February 2002). © The British Mycological Society
DOI: 10.1017/S0953756201005500 *Printed in the United Kingdom.*

Somatic incompatibility of *Rosellinia necatrix* on avocado plants in southern Spain

Rosa M. PÉREZ JIMÉNEZ[1], Rafael M. JIMÉNEZ DÍAZ[2,3] and Carlos J. LÓPEZ HERRERA[2]*

[1] *Centro de Investigación y Formación Agraria, Consejería de Agricultura y Pesca, Junta de Andalucía, 29140 Churriana (Málaga), Spain.*
[2] *Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas* (*CSIC*), *Av. Alameda del Obispo s/n, Apartado 4084, 14080, Córdoba, Spain.*
[3] *Escuela Técnica Superior de Ingenieros Agrónomos y Montes, Universidad de Córdoba, Apartado 3048, 14080 Córdoba, Spain.*
E-mail: *lherrera@cica.es*

Received 14 February 2001; accepted 24 November 2001.

A somatic incompatibility system was demonstrated in *Rosellinia necatrix* (anamorph *Dematophora necatrix*) through barrage formation in pairings among mass fungal isolates from infected avocado trees in commercial orchards in southern Spain, as well as among single ascospore isolates derived from perithecia formed in infected roots. Results revealed the occurrence of high diversity in somatic incompatibility in the *R. necatrix* population in the study. The occurrence of *R. necatrix* perithecia in naturally infected avocado roots in the field suggests that such a diversity might originate from sexual reproduction. However, the possibility that other parasexual mechanisms demonstrated in this fungus may contribute to the genetic diversity can not be discounted.

INTRODUCTION

In many fungal species, the identity of individuals is maintained by means of somatic compatibility systems (Rayner 1991). These mycelial interactions can be determined by the direct assessment of heterokaryon formation, usually through complementation of recessive auxotrophic or pigmentation markers (Buxton 1956, Puhalla 1984, 1985); as well as by the direct assessment of inability to form a heterokaryon, usually through barrage formation (Anagnostakis 1977). The barrage phenomenon is conceptually the opposite of a prototrophic vegetatively compatible heterokaryon. Barrages occur between vegetatively incompatible strains and result when hyphae of incompatible strains grow into each other and interact in an antagonistic manner (Leslie 1993).

Mycelial incompatibility has been a useful tool in studies to identify intraspecific diversity within field populations of a fungal plant pathogen (Woudt *et al.* 1995, Cortesi, Milgroom & Bisiachi 1996, Bisseger, Rigling & Heiniger 1997, Rizzo, Whiting & Elkins 1998, van Zyl *et al.* 1998). In ascomycetes, determining the frequency of mycelial interactions in an ascoma, on a tree, on nearby trees, or throughout an orchard or geographical area, might provide valuable information about the reproductive strategy of the pathogen and of its dispersal process, i.e. whether or not the disease is spreading primarily through infection by means of conidia or ascospores (Adams, Hammar & Proffer 1990).

White root rot, caused by *Rosellinia necatrix* (anamorph *Dematophora necatrix*), together with *Phytophthora* root rot (*Phytophthora cinnamomi*), are the major diseases of avocado trees (*Persea americana*) in the coastal area of southern Spain (López-Herrera 1989). Avocado was recently introduced in this area, the first plantations being established in the late sixties.

The fungus invades the root system of a tree by means of white mycelial fans. Infection by the pathogen causes wilting, chlorosis and defoliation and under conductive environmental conditions it may result in rapid death of a tree. *R. necatrix* spreads from an infected tree by means of mycelial agregates or strands which growth through soil and may contact roots of a healthy tree. Chemical control of avocado white rot was not fully successful (López Herrera, Pérez Jiménez & Zea Bonilla 1996) but efficient control of the disease was achieved by soil solarization in established avocado orchards (López Herrera *et al.* 1998, 1999a). Use of avocado rootstocks tolerant to the disease could be an

* Corresponding author.

important component in integrated control (López Herrera *et al*. 1999b).

Previous studies have demonstrated the occurrence of a somatic incompatibility system in *Rosellinia desmazieresii* (Sharland *et al*. 1988). However, no information was available as to whether a similar system would occur in *R. necatrix*. Such marker systems are of use in the identification of different individuals in the pathogen population. The main objective of this study was to determine if a somatic incompatibility system occurs in *R. necatrix*. In addition we aimed to preliminary asses the genetic variability among isolates of *R. necatrix* from avocado trees in southern Spain. To this aim, we used cultures of monoascosporic isolates obtained from perithecia produced under field conditions and mass isolates obtained from different host plants, including avocado trees.

MATERIALS AND METHODS

Fungal isolates

Preliminary disease surveys were carried from 1990 through 1992 to determine prevalence and distribution of white root rot in avocado orchards of the coastal area of southern Spain.

Isolates of *Rosellinia necatrix* were obtained from affected trees arbitrarily sampled in every disesase patch found in an orchard, during the surveys. In this period, 63 samples from 56 trees of 23 avocado orchards were collected along some 250 km of the coastal avocado growing area (Tables 1–2). Isolates from a tree were designated by a number, with those from same tree being differentiated by different letters. Small pieces of roots with white root rot symptoms were excised, surface-disinfested in 2% NaOCl for 3 min, and blotted dry between sterile filter papers. These pieces were plated onto acidified (50% lactic acid, 2.5 ml l^{-1}) potato dextrose-agar (PDA) (Difco Laboratories, Detroit, MI) in Petri dishes and incubated at 20 °C for 3–4 d. Fungal cultures growing from root pieces were characterized to species based on hyphal morphology (pear-shaped swellings) and conidial size and morphology (Sivanesan & Holliday 1972, Carrillo-Fonseca & Romero 1989).

Characterization of somatic incompatibility

Isolates of *Rosellinia necatrix* were mass-transferred to PDA and grown at 22–24 ° in the dark for 6–7 d (Table 1). Somatic incompatibility among isolates was determined by pairing of mass cultures in all possible combinations. For the pairing experiments, 6-mm diameter agar plugs with mycelia from the colony margin of two isolates were placed 4 cm apart on malt-extract agar (2% agar, MA 2%) (Difco Laboratories, Detroit, MI), in a 9 cm diam Petri dish. Cultures were incubated at 22–24 ° in the dark for 2 months. Cultures were examined every 15 d to detect zones of mycelial interaction (barrages) characteristic of vegetative incompatibitily reactions (Anagnostakis 1977).

Diversity in somatic incompatibility among mass isolates

Mass isolates of *Rosellinia necatrix* collected from avocado trees at different locations in southern Spain during the 3 yr of surveys were paired in three different sets, one per year (Table 2). Isolates from one yr were paired with each other in all possible combinations and with themselves as described above, and the development of barrage zones was determined after 2 months incubation. *R. necatrix* isolates from other hosts affected by white root rot in same area as avocado trees were also included in the study, with pairings being carried out as described above (Table 3).

Diversity in somatic incompatibility among single ascospore isolates

In the course of our study, perithecia of *Rosellinia necatrix* were found just once while observing roots of a dead, 25 yr-old avocado tree at an orchard in Málaga (southern Spain). These roots were 10 cm deep in an irrigated soil. Infected root pieces were observed under the dissecting microscope for the occurrence of perithecia of *R. necatrix*. Ten selected stromata were removed from root tissues, and perithecia, one per stroma, were suspended in 2 ml of 5% lactic acid for 15 min. Then, 10 ml of water were added to the suspension to reduce the lactic acid concentration and the mixture was filtered through four layers of sterile

Table 1. Collection information of isolates of *Rosellinia necatrix* from avocado trees used in the study of somatic incompatibility[a].

Location		
Town	Orchard	Isolate[b,c]
Almuñécar	Muro Largo	277-a, 277-b, 277-c
Almuñécar	El Molino	278, 279-a, 279-b, 280-a, 280-b, 281-a, 281-b
Fuengirola	Avocado Export	287-a, 287-b
Vélez-Málaga	La Alegría	289, 290-a, 290-b

[a] Mass cultures of two isolates were paired on MA 2% at 22–24 ° in the dark for 2 months.
[b] Isolates with same number followed by different letters were obtained from the same tree.
[c] Isolates used are preserved in the culture collection of the Centro de Investígatión y Formacíón Agraria.

Table 2. Collection information of isolates of *Rosellinia necatrix* from avocado trees collected from 1990 to 1992 and used in a study of diversity in somatic incompatibility in the fungus[a].

Location			
Town	Orchard	Year	Isolate[b]
Vélez-Málaga	La Alegría	1990	289, 290
Almuñécar	Rancho California		309, 310, 312
Motril	Vista Alegre		314
Motril	Finca Chamorro		316, 317, 318
Motril	P. Cuevas		319, 320, 321
Fuengirola	La Ventilla		332, 333, 334, 335, 336
Vélez-Málaga	La Noria		343
Jete	Los Banquillos	1991	354
Motril	El Francés		359, 360, 361
Algarrobo	La Mayora		366
Almuñécar	El Molino		367, 368, 390, 391, 400
Motril	La Esperanza		378, 379, 380, 381
Vélez-Málaga	M. Téllez		397
Vélez-Málaga	La Alegría		423, 424, 425
Estepona	P. Romano		429
Motril	F. Alvarez	1992	433, 434, 435
Almuñécar	Citasol		436
Estepona	Unknown		445, 447, 448, 449
Estepona	B. Caballero		463, 464
Motril	F. Molina		471
Benamargosa	La Huerta		472
Estepona	Las Abejeras		487

[a] Mass cultures of two isolates were paired on MA 2% at 22–24 °C in the dark for 2 months.
[b] Isolates used are preserved in the culture collection of the Centro de Investigación y Formación Agraria.

Table 3. Collection information of isolates of *Rosellinia necatrix* from different hosts used in a study of diversity in somatic incompatibility in the fungus [a].

Location			
Town	Orchard	Host	Isolate[b]
Almuñécar	Unknown	Mango (*Mangifera indica*)	009
Benamargosa	La Huerta	Mango (*Mangifera indica*)	050, 051
Jete	Los Banquillos	Loquat (*Eriobotrya japonica*)	355
Algarrobo	S. Mandillo	Loquat (*Eriobotrya japonica*)	386
Jete	Unknown	Loquat (*Eriobotrya japonica*)	467
Almuñécar	El Molino	Custard apple (*Annona squamosa*)	399
Estepona	Guadalmasa	Custard apple (*Annona squamosa*)	490

[a] Mass cultures of two isolates were paired on MA 2% at 22–24 °C in the dark for 2 months.
[b] Isolates used are preserved in the culture collection of the Centro de Investigation y Formación Agraria.

cheesecloth. One milliliter of the filtrate was spread onto hard PDA (3% agar) in a Petri dish and incubated at 22–24 ° for 24 h (Hansen *et al*. 1937). Single germinated ascospores were isolated under the dissecting microscope and transferred to PDA. Single ascospore isolates were designated as originating from a specific perithecium (i.e. Per1-1 = single ascospore 1 from perithecium 1). Eight single ascospore isolates were obtained from Per1, five from each of Per 2 and Per 4, 10 from each of Per 3 and Per 7, 12 from each of Per 6 and Per 8, four from Per 9 and nine from Per10. No viable ascospores were isolated from Per 5. Pairings were made between single ascospore isolates derived from a perithecium, as well as between single ascospore isolates derived from different perithecia.

RESULTS

Characterization of somatic incompatibility

Two types of mycelial interactions were differentiated after pairing mass isolates of *Rosellinia necatrix* on MA 2% for 14–21 d: (a) intermingling mycelia, whereby the two colonies merged and the colonies surface had an uniform aspect, which was interpreted as a somatic compatibility reaction; and (b) a mycelial interaction whereby a barrage zone was formed at sites of contact between colonies, which was considered a somatic incompatibility reaction (Fig. 1). In this latter case, the intensity of barrage reaction varied from slight to strong. Reactions of slight intensity were characterized by narrow lines of mycelial tufts being formed at the

Fig. 1. Somatic compatibility and incompatibility reactions between *Rosellinia necatrix* isolates. Upper left plate, compatibility reaction; remaindly plates, incompatibility reaction with formation of barriers differing in intensity.

Table 4. Somatic incompatibility among avocado *Rosellinia necatrix* isolates from diverse origins[a].

Nature of isolate and pairing	Number of pairings	Type and frequency (%) of mycelial interaction		
		Compatible	Incompatible	
			Narrow line	Wide line
Mass isolates				
Between themselves	63	100	0	0
Among isolates from a tree	8	100	0	0
Among isolates from different trees in:				
Same orchard	89	24	48	28
Different orchard	356	0	55	45
Total	517			
Single-ascospore isolates				
Between themselves	75	100	0	0
Among isolates from:				
Same perithecium	312	0	92	8
Different perithecia	36	0	83	17
Total	423			

[a] Isolates were paired on MA 2% at 22–24 °C in the dark for 2 months.

site of contact between mycelia. Conversely, reactions of strong intensity were characterized by wide lines of dense mycelial tufts, which were initially slightly pigmented and later became dark brown to black. Microscopic observations of barrage zones showed that hyphae in them were highly melanized and contained single cells.

Diversity in somatic incompatibility among mass and single-ascospores isolates

The two types of mycelial interactions described above were formed in pairings between *Rosellinia necatrix* isolates irrespective of their nature, i.e. mass isolates derived from the same or different trees as well as single-ascospore isolates derived from the same or different perithecia. The range in frequency of these interactions is shown in Table 4.

All *R. necatrix* mass isolates from avocado in the study were somatically self-compatible. Isolates from the same tree also demonstrated somatic compatibility. When isolates from different trees in an orchard were considered, a relatively high frequency (24%) of somatic compatibility occurred among isolates from contiguous trees. In this case, the somatic incompatibility reaction occurred among isolates from trees that were separated

by at least 150 m. Of the 76% incompatible reactions found, 48% were characterized by formation of a narrow line and the remaining 28% formed a wide barrage line. Isolates from different trees in different orchards were somatically incompatible, with narrow and wide lines being formed in 55% and 45% of cases, respectively.

Single-ascospore isolates were somatically self-compatible. Pairings between single-ascospore isolates from a perithecium were always incompatible, with the barrage zone formed being a narrow line formed in 92% of cases. Reactions among single ascospore isolates from different perithecia were also incompatible, but showed a higher frequency of wide line barrage (17%) compared to that (8%) occurring among single ascospore isolates from a perithecium (Table 4).

Mass isolates of *R. necatrix* from hosts other than avocado were somatically self-compatible but interincompatible, with wide line barrage being the predominant reaction (96% of cases).

DISCUSSION

Characterization of somatic incompatibility among isolates of a fungal pathogen may provide valuable information about genetic diversity in the pathogen as well as the epidemiology of the disease. The occurrence of a somatic incompatibility system has been demonstrated in *Rosellinia desmazieresii* (Sharland *et al*. 1988). The goal of this study was to determine whether or not a similar system could be demonstrated in *R. necatrix* from avocado. Results from the study support that hypothesis. The phenotype of somatic incompatibility in *R. necatrix* was similar to that described for other fungal species, e.g. *Cryphonectria parasitica* (Anagnostakis 1977, Causin *et al*. 1995, Cortesi *et al*. 1996, Bisseger *et al*. 1997), *R. desmazieresii* (Sharland *et al*. 1988), *Leucostoma persoonii* (Adams *et al*. 1990) and *C. cubensis* (van Zyl *et al*. 1998). In our study, isolates of *R. necatrix* either from an avocado tree or from nearby trees were somatically compatible, whereas isolates from distant trees were somatically incompatible. Van Zyl *et al*. (1998) described a similar distribution of somatic compatibility for *C. cubensis* in eucalyptus trees. This suggests that infection of a tree, or of nearby trees, might be caused by a single type of isolate differentiated through its compatibility reaction, and also that a degree of genetic diversity occurs in this *R. necatrix* population. These results indicate that the somatic incompatibility reaction could be of use to distinguish clonal sub-populations, individuals or 'genets' (Rayner 1991) or, in a more general meaning Mycelial Compatibility Groups (MCG) (Kohn *et al*. 1991), in *R. necatrix*. The latter authors demonstrated that different MCGs in *Sclerotinia sclerotiorum* are genotypically different by molecular techniques and found an exact correlation between RFLP haplotypes and groups of isolates characterized by the somatic incompatibility reaction. An adaptative meaning has been conferred to the somatic incompatibility phenomenon, which relates to a reduction in the possibilities of acquiring physical or genetic modifications and the maintenance of the genetic integrity of the fungal individuals (Rayner 1991).

The structure of the *R. necatrix* population in our study, as indicated by the distribution of somatically compatible and incompatible isolates, was similar to that found for *R. desmazieresii* (Sharland *et al*. 1988). These authors concluded that individual isolates, subcultures of an isolate, and different isolates from a 'ring of disease', were somatically compatible, whereas isolates from different 'rings of disease' were always incompatible. Similarly pairings between single-ascospore isolates derived from a perithecium, or from different perithecia located in a 'ring of disease', were always somatically compatible, but pairings involving single-ascospore isolates from perithecia in distant substrates were somatically incompatible. Our field results are in agreement with those of Sharland *et al*. (1988) since mass isolates from nearby trees were compatible; in contrast the incompatibility between single-ascospore isolates from the same perithecium of *R. necatrix* disagrees with the results on *R. desmazieresii*. Sharland *et al*. (1988) concluded that outcrossing in *R. desmazieresii* does not occur during ascospore production, leading to the formation of somatically compatible homokaryons. Because of the infection cycle and dispersal mechanisms operating in *R. necatrix*, our results on somatic incompatibility in this fungus could be explained in a similar way as that for *R. desmazieresii* (Sharland *et al*. 1988). Thus, assuming that a 'disease patch' is caused by a single individual or compatibility type, the amount of disease would increase through root contact between adjacent trees, growth of mycelial strands across the soil, or by dispersal of conidia. In these foci, the pathogen inoculum potential may increase by means of the cooperative interactions that would occur within the compatible population. In ecological terms, the 'new encounter' of *R. necatrix* with avocado, a highly susceptible host, would have facilitated an increase in the prevalence of virulent genets that might have been within a dynamic of routine selection in a stable ecosystem.

The origin of the high genetic diversity found in the population of *R. necatrix* in the present study could be due to a sporadic development of sexual reproduction. In this way, the establishment of the fungus in a tree would occur by infection of different ascospores. Mycelia derived from these ascospores would interact in an antagonistic way until one became established through the exclusion of the others, or until some stable limits could be established by means of somatic incompatibility reactions. The formation of *R. necatrix* ascomata under natural conditions has seldom been described (Prillieux 1902, Hansen *et al*. 1937) and the occurrence of a teleomorph has even been questioned (Francis 1985). In our study, perithecia of *R. necatrix*

were found only once on roots of an infected tree during 12 yr of sampling. If sexual reproduction would occur normally, this variation would originate by outcrossings. On the contrary, if a teleomorph develops only rarely it would be necessary to consider whether the genetic variation in *R. necatrix* is acquired by parasexual recombination, as has been suggested for *R. desmaziresii* (Sharland *et al*. 1988).

The relative number of MCGs in populations of ascomycetes has been used in several studies as an indicator of whether a pathogen has been recently introduced into an area or whether it has been present there from an extended period of time (Adams *et al*. 1990). A great diversity of MCGs can be due to multiple introductions or sexual outcrossing. The considerable genetic diversity within the population of *R. necatrix* on avocado crops in southern Spain suggests that this population might be endemic and diverse and that success in the selection of avocado rootstocks tolerant to this pathogen (López Herrera *et al*. 1999b) may not be straightforward. Because of the potential importance of genetic diversity in *R. necatrix* in terms of use of tolerant rootstocks for disease control, more research should be carried out using a large sample of isolates and additional techniques to measure the genetic diversity in populations of the pathogen.

REFERENCES

Adams, G., Hammar, S. & Proffer, T. (1990) Vegetative compatibility in *Leucostoma persoonii*. *Phytopathology* **80**: 287–291.

Anagnostakis, S. L. (1977) Vegetative incompatibility in *Endothia parasitica*. *Experimental Mycology* **1**: 306–316.

Bisseger, M., Rigling, D. & Heiniger, U. (1997) Population structure and disease development of *Cryphonectria parasitica* in european chestnut forests in the presence of natural hypovirulence. *Phytopathology* **87**: 50–59.

Buxton, E. W. (1956) Heterokaryosis and parasexual recombination in pathogenic strains of *Fusarium oxysporum*. *Journal General of Mycrobiology* **15**: 133–139.

Carrillo-Fonseca, C. L. & Romero, C. S. (1989) Identificación y estudio de algunas características fisiológicas del hongo causante de la pudrición blanca de la raíz del manzano en Zacatlan, Puebla. *Agrociencia* **77**: 163–172.

Causin, R., Frigimelica, L., Montecchio, L. & Mutto Accordi, S. (1995) Vegetative compatibility and conversion to hypovirulence among Italian isolates of *Cryphonectria parasitica*. *European Journal of Forest Pathology* **25**: 232–239.

Cortesi, P., Milgroom, M. G. & Bisiachi, M. (1996) Distribution and diversity of vegetative compatibility types in subpopulation of *Crpyphonectria parasitica* in Italy. *Mycological Research* **100**: 1087–1093.

Francis, S. M. (1985) *Rosellinia necatrix* – fact or fiction? *Sydowia* **38**: 75–86.

Hansen, H. N., Thomas, H. E. & Thomas, H. E. (1937) The connection between *Dematophora necatrix* and *Rosellinia necatrix*. *Hilgardia* **10**: 561–565.

Kohn, L. M., Stasovski, E., Carbone, I., Royer, J. & Anderson, J. B. (1991) Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. *Phytopathology* **81**: 480–485.

Leslie, J. F. (1993) Fungal vegetative compatibility. *Annual Review of Phytopathology* **31**: 127–151.

López Herrera, C. J. (1989) Podredumbres radiculares del aguacate en la Costa del Sol Años 1987–88. In *Estudios de Fitopatología* (J. del Moral, ed.): 172–176. SEF/DGIEA, Badajoz.

López Herrera, C. J. Pérez Jiménez, R. M. & Zea Bonilla, T. (1996) Control quimico de *Dematophora necatrix* con fungicidas sistémicos. In *Resúmenes del VIII Congress Nacional de la Sociedad Española de Fitopathologia*: 196. Sociedad Española de Fitopatologia, Córdoba.

López Herrera, C. J., Pérez Jiménez, R. M., Basallote Ureba, M. J., Zea Bonilla, T. & Melero Vara, J. M. (1998) Soil solarization in established avocado trees for *Dematophora necatrix*. *Plant Disease* **82**: 1088–1092.

López Herrera, C. J., Pérez Jiménez, R. M., Basallote Ureba, M. J., Zea Bonilla, T. & Melero Vara, J. M. (1999a) Loss of viability of *Dematophora necatrix* in solarized soils. *European Journal of Plant Pathology* **105**: 571–576.

López Herrera, C. J., Pérez Jiménez, R. M., Barceló Muñoz, A. & Zea Bonilla, T. (1999b) Evaluación de patrones de aguacate por su tolerancia a la podredumbre blanca. *Revista Chapingo*, *serie horticultura* **5**: 267–270.

Prillieux, E. (1902) Les périthèces du *Rosellinia necatrix*. *Comptes Rendu des Academie de Science* **135**: 275–278.

Puhalla, J. E. (1984) Races of *Fusarium oxysporum* f. sp. *apii* in California and their genetic interrelationships. *Canadian Journal of Botany* **62**: 546–550.

Puhalla, J. E. (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* **63**: 179–183.

Rayner, A. D. M. (1991) The phytopathological significance of mycelial individualism. *Annual Review of Phytopathology* **29**: 305–323.

Rizzo, D. M., Whiting, E. C. & Elkins, R. B. (1998) Spatial distribution of *Armillaria mellea* in pear orchards. *Plant Disease* **82**: 1226–1231.

Sharland, P. R., Rayner, A. D. M., Ofong, A. U. & Barrett, D. K. (1988) Population structure of *Rosellinia desmazieresii* causing ring-dying of *Salix repens*. *Transactions of the British Mycological Society* **90**: 654–656.

Sivanesan, A. & Holliday, P. (1972) *Rosellinia necatrix*. *Descriptions of Pathogenic Fungi and Bacteria* **352**: 1–2.

van Zyl, L. M., Wingfield, M. J., Alfenas, A. C. & Crous, P. W. (1998) Population diversity among Brazilian isolates of *Cryphonectria cubensis*. *Forest Ecology and Management* **112**: 41–47.

Woudt, L. P., Neuvel, A., Sikkema, A., van Grinsven, M. Q. J. M., de Milliano, W. A. J., Campbell, C. L. & Leslie, J. F. (1995) Genetic variation in *Fusarium oxysporum* from cyclamen. *Phytopathology* **85**: 1348–1355.

Corresponding Editor: R. F. Park