Plant Stress ©2007 Global Science Books

Melon Roots under Stress: Melon Vine Decline

Ana Fita • Belén Picó • Fernando Nuez*

Centro de Conservación y Mejora de la Agrodiversidad (COMAV), Polytechnic University of Valencia, Camino de Vera, 46022, Valencia, Spain

Corresponding author: * **fnuez@btc.upv.es**

ABSTRACT

Melon Vine Decline is a severe root rot disease of increasing worldwide importance, characterized by a sudden plant collapse at harvest time. *Monosporascus cannonballus* Pollack *et* Uecker is the main causal agent, but other soilborne pathogens, certain stressful cultural practices and environmental conditions enhance disease symptoms. Due to its complexity, etiological studies as well as methodologies that detect and assess the disease (ranging from visual lesion scoring to real-time PCR) have been needed to select the best control methods. As vine decline depends on the soil inoculum, on the susceptibility of the plant and on the hydraulic balance of the plant, control methods have been focused on the following three main directions: I) Reducing/inactivating the soil inoculum: achieved only partially through chemical control, biological control and cultural practices; II) Increasing the resistance to root lesions: certain cultivars suffer fewer root lesions, and some breeding lines derived from the resistant accession Pat 81 (*C. melo* subsp. *agrestis)* are about to be released; III) Improving the hydraulic balance of the plant: Pat 81 has shown to be useful through grafting or by breeding for improved root systems due to its large and branched root system. Improving root systems is crucial not only in overcoming melon vine decline but also in overcoming many soil stresses. New *in vitro* culture techniques along with root image analysis allow accurate *in vivo* studies of root development. The combined use of these technologies has led to an adequate control of the disease. In addition, the generation of new genomic tools for melon is allowing a deeper knowledge of the biological/genetic mechanism involved in the disease.

Keywords: melon collapse, soilborne pathogen, sudden wilt
Abbreviations: CFU, colony-forming units; **EST**, expressed sequence tags; **ITS**, internal transcribed spacer; **PCR**, polymerase chain reaction

CONTENTS

INTRODUCTION

Significant efforts are made every year to avoid economic losses derived from plant stresses that reduce the production and quality of vegetables worldwide. Nevertheless, below-ground stresses such as salinity, drought or soilborne diseases are particularly challenging because of the complex processes involved therein. The soil environment is extremely complex: soil type, texture, pH, moisture, temperature and nutrient levels influence not only the root growth, but also the activity of soil organisms. Among all the soil stresses, soilborne diseases are particularly difficult to predict, detect and overcome. Management of soilborne diseases depends on a thorough knowledge of the pathogen, the host plant, and the environmental conditions that favor the infection. In addition, soilborne pathogens often survive in soil for many years, they may reproduce in diverse host plants, and often simultaneous infections of diverse pathogens in the same plant occur, which results in a complex disease (Koike *et al*. 2003).

This is the case of melon vine decline, also known as sudden wilt, vine decay, collapse or root rot. It is a major root-rot disease in melon crops in hot, arid and semiarid regions of the world (Martyn and Miller 1996; Cohen *et al*. 2000; Beltrán *et al*. 2007). This disease is characterized by reduced growth of the plant as well as gradual leaf decline, followed by progressive defoliation. By late in the season, about 1 or 2 weeks prior to harvest, partial or complete canopy collapse occurs, resulting in fruit sunburn and total crop loss. Aboveground symptoms, similar to those caused by drought stress, are the consequence of root damage caused by soilborne pathogens (García-Jiménez *et al*. 1989; Mer-

Received: 28 March, 2007. Accepted: 15 May, 2007.

Invited Review

tely *et al*. 1991; Miller *et al*. 1995; Martyn and Miller 1996; Cohen *et al*. 2000).

In 1974, Pollack and Uecker described *Monosporascus cannonballus* as a new species, discovering it in association with *Rhizoctonia solani* and *Verticillium albo-atrum* in rotted cantaloupe roots in Arizona (Troutman and Matejka 1970; Pollack and Uecker 1974). Since then, *M. cannonballus* has been related to root rot declines of melon and watermelon in many areas of the world such as USA (Troutman and Matejka 1970; Mertely *et al*. 1991), Japan (Watanabe 1979; Uematsu *et al*. 1985), Spain (Lobo-Ruano 1990), Tunisia (Martyn *et al*. 1994), Taiwan (Tsay and Tung 1995), Mexico (Martyn *et al*. 1996), Guatemala (Bruton and Miller 1997a), Honduras (Bruton and Miller 1997b), Saudi Arabia (Kartlatti *et al*. 1997), Italy (Gennari *et al*. 1999; Infantino et al. 2002), Korea (Kwon *et al*. 2001), Egypt (El-Desouky and El-Wakil 2003) and Brazil (Sales *et al*. 2004). In Israel, *Monosporascus eutypoides* (Petrak) won Arx was reported as the causal agent of vine decline (Reuveni and Krikun 1983; Reuveni *et al*. 1983). However, nowadays *M. cannonballus* is considered the causal agent of the disease in Israel (Pivonia *et al*. 1997; Cohen *et al*. 2000). In addition, it is possible that *M. eutypoides* is conspecific of *M. cannonballus* (Martyn and Miller 1996). However, many other causal agents, mainly fungi, but also viruses and bacteria, have been associated with vine declines in melon (reviewed in Iglesias 2000). Usually, several pathogens, such as *M. cannonballus*, *Macrophomina phaseolina* (Tassi) Goidanich, *Fusarium solani* (Mart.) Sacc., *Pythium* sp., *Acremonium cucurbitacearum* Alfaro-García, W. Gams *et* García-Jiménez, and *Rhizopycnis vagum* DF Farr (previously reported as a Stangonospora-like fungus) are isolated at the same time from plants affected by vine decline, and it is for that reason that it is considered a complex disease in which several soil pathogens are involved (Mertely *et al*. 1991; Bruton *et al*. 1995; Martyn and Miller 1996; Gwynne *et al*. 1997, Pivonia *et al*. 1997; Aegerter *et al*. 2000; García-Jiménez *et al*. 2000). The most studied fungi of all these are *M. cannonballus* and *A. cucurbitacearum*. However, the most aggressive causal agent for vine decline seems to be *M. cannonballus* (Mertely *et al*. 1991; Pivonia *et al*. 1997; Aegerter *et al*. 2000; Iglesias *et al.* 2000a, 2000b; Biernacki and Bruton 2001). Many studies refer to the wilt syndrome caused by *M. cannonballus* as "melon Monosporascus root rot/vine decline" (MRR/VD) (Mertely *et al*. 1991; Martyn *et al*. 1994; Tsay and Tung 1995; Martyn *et al.* 1996; Martyn and Miller 1996; Wolff and Miller 1998; Cohen *et al*. 2000; Stanghellini *et al.* 2003) to avoid confusions with wilts caused by other fungi.

On top of the difficulty involved in working with different soilborne pathogens, collapse symptoms, which are characteristic of vine decline, can be enhanced or totally hidden by environmental conditions. It is the result of the sum of several stresses on a weakened plant. Factors such as high soil temperature, hot winds, transplants that prevent a proper development of the root system, excessive fruit load or inadequate irrigation, increase the severity of vine collapse. Even so, the lack of these conditions could prevent collapse symptoms even when roots are damaged by the pathogens (Krikun 1985; Martyn and Miller 1996; Pivonia *et al*. 1997; Cohen *et al*. 2000; Merghany 2006; Martyn 2007).

MRR/VD syndrome has been reviewed previously both by Martyn and Miller (1996), who focused on the biology, pathology and epidemiology of *M. cannonballus* and on molecular methods for detecting variation in the pathogen, as well as Cohen *et al*. (2000), who intensively checked the control methods available at that time, pointing out the importance of an integrated management. In this review, we describe the evolution of the knowledge of this complex disease, and would like to especially highlight the impact of new methodologies in the study of soilborne diseases. We will refer mainly to MRR/VD, but will also refer to the vine decline produced by *A. cucurbitacearum*.

WHAT ARE WE FACING?

In order to define a suitable control strategy for vine decline it is necessary to have a broad knowledge of the biology of the fungus, the infection processes and the conditions that favor the pathogen. This knowledge gives important clues about possible weak links in the vital cycle of the pathogen. In addition, recognizing the symptoms, creating methodologies to reproduce the disease and assessing the infection level are crucial for its diagnosis, the screening of the efficacy of certain control methods and to evaluate plant material in breeding programs.

Biology and host range of *M. cannonballus* and *A. cucurbitacearum*

M. cannonballus is an Ascomycete (Pyrenomycete), a homothallic fungus which produces fertile perithecia both in host roots and in culture. Its main characteristic is that it produces only one ascospore per ascus instead of the expected eight; this ascospore is a large (30-50 µm), black, spherical (resembling a cannonball), thick-walled and multinucleate spore (Pollack and Uecker 1979; Watanabe 1979; Martyn and Miller 1996). Perithecia, which produce the ascospores, appear on the root cortex late in the growing season and can be seen as black spots in the root surface (Mertely *et al*. 1991; Martyn and Miller 1996). Ascospores function as the only known survival structures and inoculum for root infection under field conditions, and is therefore a monocyclic pathogen (Stangellini *et al*. 1996 and 2000). Waugh *et al*. (2003) studied the reproductive potential of *M. cannonballus*, reporting that a root system of a single mature cantaloupe plant is capable of supporting the production of approx. 400,000 ascospores, equivalent to 10 ascospores per gram of soil.

Ascospores germinate readily in the rhizosphere, producing 2-3 germ tubes that attach firmly to the root (Stanghellini *et al*. 2000; Waugh *et al*. 2001). After the penetration of the germ tubes into the epidermis, the hyphae grow radially almost directly to the xylem and establish themselves there. Eventually, the hyphae grow back out into the cortical cells where the perithecia are produced. Perithecia production occurs late in the growing season, and the induction of sporulation under field conditions appears to coincide with root death. The mode of parasitism of *M. cannonballus* is very similar to that of vascular wilt pathogens, with predominant intracellular hyphae growth, but it does not spread via the vascular system to aboveground plant tissues (Waugh *et al*. 2003).

M. cannonballus is adapted to high temperatures with no growth nor ascospore germination below 20ºC. It reaches optimum vegetative growth between 25 and 35ºC (Watanabe 1979; Martyn *et al*. 1991; Uematsu and Sekiyama 1990; Bruton *et al*. 1999; Pivonia *et al*. 2002a; Waugh *et al*. 2003) and optimum ascospore germination between 25 and 30ºC (Stangellini *et al*. 2000; Pivonia *et al*. 2002a; Waugh *et al*. 2003). The onset of root infection can occur anywhere from 9 days to 65 days after planting depending on the cropping season. High soil temperatures not only accelerate the germination of the ascospores, but also the appearance of root lesions (Stanghellini *et al*. 2004a). Perithecia production is also affected by soil temperature, reaching its optimum between 25 and 30ºC.

Despite being characteristic of hot, arid and semiarid melon-growing regions of the world (Martyn and Miller 1996; Bruton *et al*. 1999; Pivonia *et al*. 2002a), and being indigenous to the desert soil of Arizona (Stanghellini *et al*. 1996), *M. cannonballus* ascospores can survive in marsh soils flooded for several months in the Mediterranean littoral of Spain (Beltrán *et al*. 2005).

Much less information is available about the biology of *A. cucurbitacearum* Alfaro-García, W. Gams and J. García-Jiménez, a Deuteromycete (Hyphomycete) with unknown teleomorph. It produces cottony colonies with an optimum vegetative growth between 25 and 28ºC. *A. cucurbitace-*

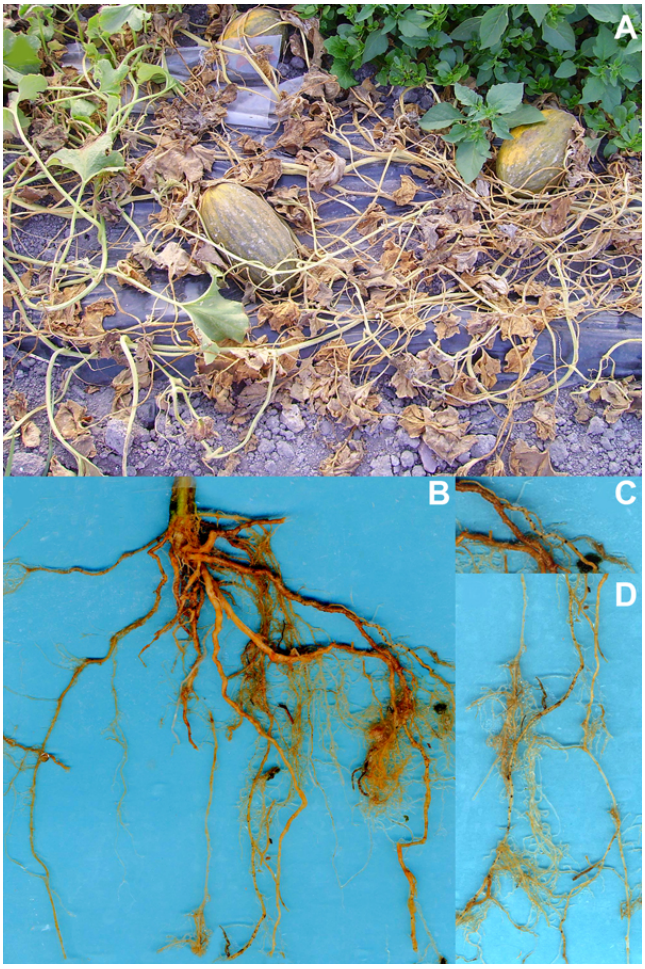

Fig. 1 Melon vine decline: above- and below ground symptoms. (**A**) Collapsed melon, (**B**) Damaged root by *M. cannonballus* and *A. cucurbitacearum*, (**C** and **D**) close-up of roots bearing perithecia of *M. cannonballus*.

arum has monophialidic conidiophores and chlamydospores are the specialized resting structures of this fungus in the soil (García-Jiménez *et al*. 1994; Alfaro-García *et al*. 1996; Armengol *et al*. 1999).

Soil temperature exerts a considerable influence on root diseases. Bruton *et al*. (1999) analyzed the relationship between temperature and the vine decline caused by *A. cucurbitacearum* and *M. cannonballus*. The optimum growth of each fungus was compared with the daily temperatures of the main melon growing areas with vine decline problems. The prevalence of either one or the other fungi seemed to be greatly influenced by the temperature. This was confirmed by Aegerter *et al*. (2000) who found greater importance of the disease caused by *A. cucurbitacearum* in northern California than in the south where *M. cannonballus* was predominant.

M. cannonballus and *A. cucurbitacearum* have been reported as pathogenic to all members of the *Cucurbitaceae* family (Mertely *et al*. 1993a; Armengol *et al*. 1998). *M. cannonballus* can infect and produce perithecia in different non-cucurbit species such as wheat, corn, bean and sorghum (Mertely *et al*. 1993a; reviwed by Martyn and Miller 1996). This situation must to be taken into account in culture rotations and in the possible build-up of the inoculum in the soil. However, other crops and weed species common to melon production areas are not likely to be involved in the perpetuation of *A. cucurbitacearum* propagation (Armengol *et al*. 1998).

Diagnosis

Although some leaf decay or yellowing can appear before plant death, the main symptom of vine decline is a fast decline of the plant and death in the harvest period. This premature death of the plant gives rise to a great deal of unmarketable fruits due to small size, low sugar content or scald damage (**Fig. 1**) (Martyn and Miller 1996). This aboveground symptom, similar to those caused by drought stress, is produced by a large hydraulic disequilibrium. Vine decline is a sudden nonvascular wilt, therefore the lesions caused by the fungi can only be observed on the roots.

Wilt symptom is highly dependent on environmental conditions. High soil temperatures and hot winds propitiate the occurrence of vine collapse. In addition, intensive agricultural practices have been pointed to as causes of the increased importance of vine decline in the last 30 years (Martyn 2007). For instance, drip irrigation can lead to disequilibrium in the plant. Plants supplied with constant water and nutrients produce large top growth and a small root system, which creates an abnormal shoot:root ratio. In this situation, any damage to the root, especially under high evapo-transpiration conditions, leads to stress with which the plants cannot cope. Other practices that enhance the presence of vine decline are: the use of mulch, which increases the temperature of the soil and may favor the growth of certain pathogens, constant humidity of the soil that increases root rots, transplants that prevent the proper growth of the tap root, and insufficient crop rotation (Krikun 1985; Martyn 2007). Another feature that increases the wilt symptom is the fruit load of the plant (Merghany 2006). Muskmelon genotypes with a concentrated fruit set are, in general, more susceptible to vine decline (Wolff 1996). Pivonia *et al*. (2002b) observed that the presence of maturing fruits reduced the root growth, and the high water and nutrient demand of the fruits keeps the stomata open even in high temperature conditions. This leads to a water deficit because of the poor water-absorbing capacity of the roots. Moreover, *M. cannonballus* promotes the occlusion of xylem vessels by tyloses mainly in the roots, resulting in a restricted water flow (Pivonia *et al*. 2002b). All of these elements together indicate the importance of the hydraulic balance in the main vine decline symptom. Improving the hydraulic balance of the plant with various methods could represent an effective control method. Another important conclusion based on the high dependence on environmental conditions of the wilt symptom is that evaluations of resistant materials using percentages of wilted plants as resistance criteria would be misleading. In fact, contradictory results have been reported in many cases (Cohen *et al*. 2000).

As the primary cause of vine collapse is root damage, diagnosis must be done by combining evaluation of the aboveground symptoms with examination of the roots. *M. cannonballus* causes root rot and necrosis in smaller feeder roots (**Fig. 1**). As the infection advances and smaller roots die, reddish-brown lesions are formed on larger roots. The fungus continues to colonize the root tissue throughout the growing season, and perithecia characteristics are formed most abundantly late in the season (Mertely *et al*. 1991; Martyn and Miller 1996; Aegerter *et al*. 2000). These perithecia are visible in the root cortex and contain an average of 66 ascospores (Waugh *et al*. 2003). *A. cucurbitacearum* causes lesions which are slightly different. It affects the hypocotil and lateral roots, which then show light yellow-brown discolorations that later develop into dry, corky brown areas; the rootlets also become discolored and then necrotic (García-Jiménez *et al*. 1994; Aegerter *et al*. 2000).

However, the similarity of the symptoms that both fungi produce and the number of other pathogenic fungi capable of colonizing melon roots confound diagnosis based on the appearance of the root. Therefore, the isolation of the pathogens in culture from root sections and their taxonomic identification is needed. These identifications are based on the appearance of the colonies and the identification of reproductive structures, such as perithecia for *M. cannonballus* and conidiophores and conidia for *A. cucurbitacearum*. Although this method is very precise, it lacks rapidity as the appearance of these structures requires several weeks of

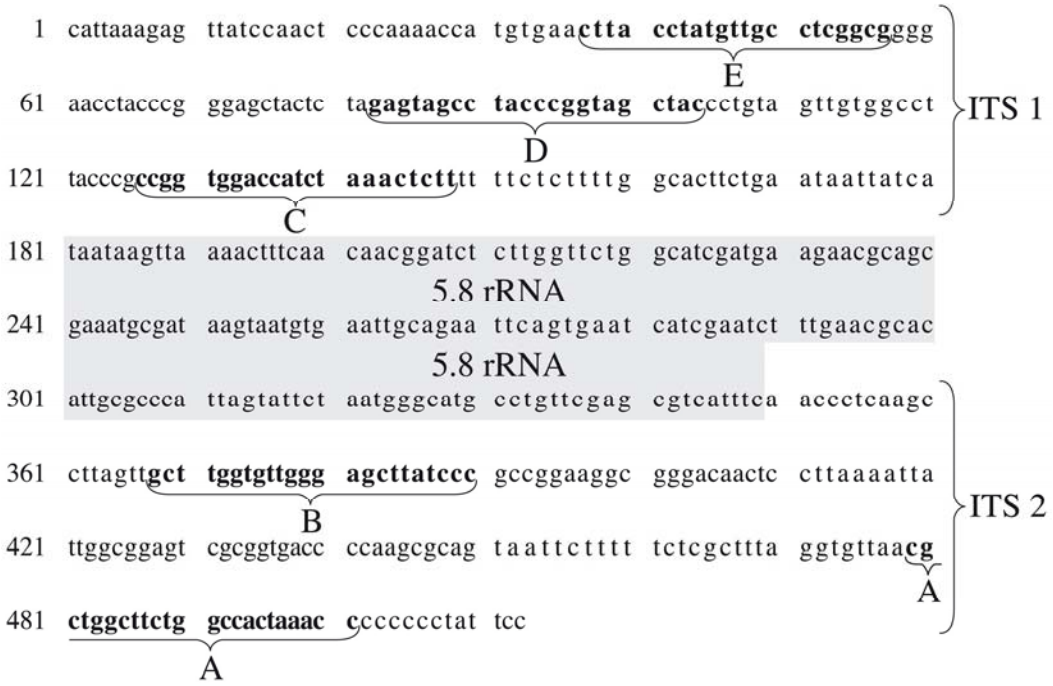

Fig. 2 Sequence of the genomic rDNA sequence (ITS1-5.8S-ITS2) of *M. cannonballus* (genebank accession AB097099), showing the position of different primers employed in the detection of this fungus. Primers are in bold (A-E), 5.8S sequence is shaded. Primer pair A+D (430 bp) was recommended for fungal detection by conventional PCR on infected root tissue, whereas a nested PCR using A+E (465 bp) followed by C+D (68 bp) was suggested as more suitable for *M. cannonballus* detection on infested soils (Lovic *et al*. 1995a, 1995b). Primers pair C + E (112 bp) was employed by Picó *et al*. 2005 to quantitatively detect *M. cannonballlus* in root tissues by real-time PCR.

culture. PCR identification methods, based on ITS regions of the rDNA, have been developed to detect the DNA extracted from infected roots not only of *M. cannonballus* (Lovic *et al*. 1995a, 1995b) (**Fig. 2**), but also of other vine decline related pathogens such as *A. cucurbitacearum* (Martínez-Culebras *et al*. 2004) and *R. vagum* (Ghignone *et al*. 2003). These PCR methods detect fungal DNA in roots of symptomless infected melons and can be used as tools for rapid, large-scale diagnosis, thereby avoiding the problems associated with isolations in roots.

Not only can *M. cannonballus* be detected in infected roots but also in the soil. The characteristics of the ascospores – their relatively large size (30-50 µm), round shape, and specific gravity – permitted the development of a relatively simple method for their direct extraction from the soil (Stangellini and Rasmussen 1992), which is based on recovering the ascospores using a gradient of sucrose. To reduce the cost of the method, it has been proposed to use commercial cane sugar instead of sucrose (Sales Jr. *et al*. 2006). The sucrose gradient technique has been used to evaluate the number of ascospores in soils (Mertely *et al*. 1993b; Stanghellini *et al*. 1996; Aegerter *et al*. 2000; Beltrán *et al*. 2005; Beltrán *et al*. 2007). However, no direct relationship between the number of ascospores and the severity of the infection has been reported. This could be explained by a minimum threshold at which only a minimum level of inoculum can cause a great damage (Mertely *et al*. 1993b) or by the fact that not all the ascospores must be viable (Aegerter *et al*. 2000). The first hypothesis is likely, bearing in mind that fields with reported problems of MRR/VD contained as few as 2 ascospores per gram of soil (Waugh *et al*. 2003); the second is also possible, since there are no reliable methods to assess the viability of these ascospores, as they rarely germinate *in vitro* (Martyn and Miller 1996; Stangellini *et al*. 2000).

DISEASE SEVERITY ASSESSMENT

Disease severity assessment methods are required in order to determine the aggressiveness of different pathogen strains, to evaluate the efficacy of different control methods and to screen potential resistant materials. The first disease assessments were made by recording the number of wilted plants (Reuveni *et al*. 1983; Esteva and Nuez 1994; Wolff 1995; Cohen *et al*. 1995; Wolff and Miller 1998). As we pointed out previously, wilt symptom is highly dependent on environmental conditions. Therefore, disease severity assessment based on root inspection is a more precise indicator of the infection. It is less dependent on environmental stresses and allows the observation of the primary cause of vine decay, even when aboveground symptoms do not appear. As the recovery of a complete root system from the field is very difficult, the study of root lesions has been done extensively in pot-grown plants. Methods routinely employed involve the artificial inoculation of the soil with the fungal pathogen and the assessment of root lesion severity (Martyn and Miller 1996; Aegerter *et al*. 2000; Bruton *et al*. 2000; Crosby *et al*. 2000; Iglesias *et al*. 2000a, 2000b; Biernacki and Bruton 2001; Dias *et al*. 2004).

Artificial inoculations

In the first pathogenic assays of *M. cannonballus* and *A. cucurbitacearum*, pieces of colonized agar were used directly, mixed with soil or added to the soil as mycelium suspension. The seeds were then planted, and generally no control of the level of inoculum was made (Watanabe 1979; Reuveni *et al*. 1983; Uematsu *et al*. 1985; Mertely *et al*. 1991, García-Jiménez *et al*. 1994; Kim *et al*. 1995; Tsay and Tung 1995; Pivonia *et al*. 1997; Wolff and Miller 1998). Other inoculation techniques, such as fungal colonized grain sorghum seeds incorporated into soil (Lovic *et al*. 1994), as well as a hydroponic system with inoculated water (García-Jiménez *et al*. 1994), have been used to test the pathogenicity of the fungi involved in vine decline. However, the most-employed method has been the use of a sand/oat hull mixture as substrate for fungal colonization that is subsequently mixed with soil in pots. This inoculation method permits the control of the inoculum densities added to the soil by calculating the number of colony-forming units (CFU/g) (Mertely *et al*. 1993a; Bruton *et al*. 1995; Karlatti *et al*. 1997; Pivonia *et al*. 1997; Armengol *et al*. 1998; Bruton *et al*. 2000; Iglesias *et al*. 2000a, 2000b; Picó *et al*. 2005, 2007). Knowledge of the range of virulence of a pathogen is essential in order to define the inoculum densities needed to reach a certain level of infection. Bruton (1995) determined that a range of 20 CFU/g for *M. cannonballus* and 10,000 CFU/g for *A. cucurbitacearum* would be enough for screening assays. However, studies of the virulence of different isolates demonstrated a wide range of virulence between isolates of the same species (Martyn and Miller 1996; Biernacki and Bruton 2001). In general for *A. cucurbitacearum*, Spanish isolates were more aggressive than American ones (Bruton *et al*. 2000; Igles-

ias *et al*. 2000b). However, there is no agreement on the virulence of *M. cannonballus* isolates, as some authors consider American isolates more aggressive than Spanish ones (Bruton *et al*. 2000), while others report the opposite (Lovic *et al*. 1996; Iglesias *et al*. 2000b). Due to the variability of the isolates' virulence and the lack of exhaustive studies testing a considerable number of isolates, it is common to adjust the inoculum level needed in each study.

Although artificial inoculations are useful in controlling the infection process, they do not cover all the possible diversity of soil microorganisms and their potential interactions. The importance of these microorganisms in infection processes was pointed out by Stangellini *et al*. (2000), who found that the germination of the ascospores of *M. cannonballus* in the melon rhizosphere occurred only in the presence of actinomycetes and never in sterile autoclaved soils. In addition, preparing large volumes of inoculated soil, as is required for a breeding program, is laborious and difficult. For that reason, naturally infested soil has been used successfully in screening assays and in breeding programs (Dias 2003; Dias *et al*. 2004). Therefore, the use of artificially inoculated soils or naturally infested soil will depend on the objectives of the research.

Lesion scoring

Methodologies for accurately assessing root lesions are essential not only in evaluations of the virulence of the pathogen but also in screening for resistant plant materials. Assessment of root lesion severity has been traditionally done using visual scoring systems based on assigning different scores to discolorations, browning, rots and necrosis in the root. The scores usually range from 0, referring to a healthy root or tissue, to 4, for extremely damaged roots (**Fig. 3**). These scores could refer to the whole root (Mertely *et al.* 1991, 1993a, 1993b; Aegerter *et al*. 2000; Batten *et al*. 2000) or to various parts, such as the hypocothyls, the taproot, the primary/secondary root system (mainly composed of mature roots involved in plant anchorage and in the support of the tertiary root system), and the tertiary root system (composed of fine roots that condition the functionality of the mature root systems) (**Fig. 3**) (Bruton *et al*. 2000; Iglesias *et al*. 2000a, 2000b; Biernacki and Bruton 2001; Dias *et al*. 2004). It is also common to use indices that combine the scoring of the lesion with other manifestations of the disease, such as root mass loss or decrease in leaf area.

The score systems are time- and labor-intensive, and require skilled expertise to identify the lesions and to score them. The visual scoring of root lesions and the evaluation of the effect of the infection upon the root system can be complemented by using image analysis software. Some studies have used this software successfully (Crosby *et al*. 2000; Crosby 2000; Biernacki and Bruton 2001; Fita *et al*. 2005a, 2006a). This software permits the identification and quantification of root colors associated with root lesions. They also allow the study of difficult-to-measure root traits such as total root length, surface area and diameter (**Fig. 4**). Even though they do not avoid the most time-consuming task in a root characterization, which is the root extraction from the soil/pot and the root spread for adequate observation, they can accelerate the selection process and also avoid some subjective measurements that depend on the observer. Obviously, the accuracy of the measurement with this image analysis is based on the correct assignment of the color classes, on the correct recovery of the root from the soil/pot, and on the correct spread of the root for image acquisition (Fita *et al*. 2006a).

Another drawback of evaluation based on the visual scoring of lesions is that the detection and evaluation must be done in the late stages of infection, when the root is clearly damaged. As we have seen in a previous section, detection of *M. cannonballus* is possible in the early stages by PCR techniques (Lovic *et al*. 1995b), although this technique does not allow the measurement of the infection level. New methods based on real-time PCR allow not only an early specific detection and identification, but also an accurate quantification of *M. cannonballus* in symptomless

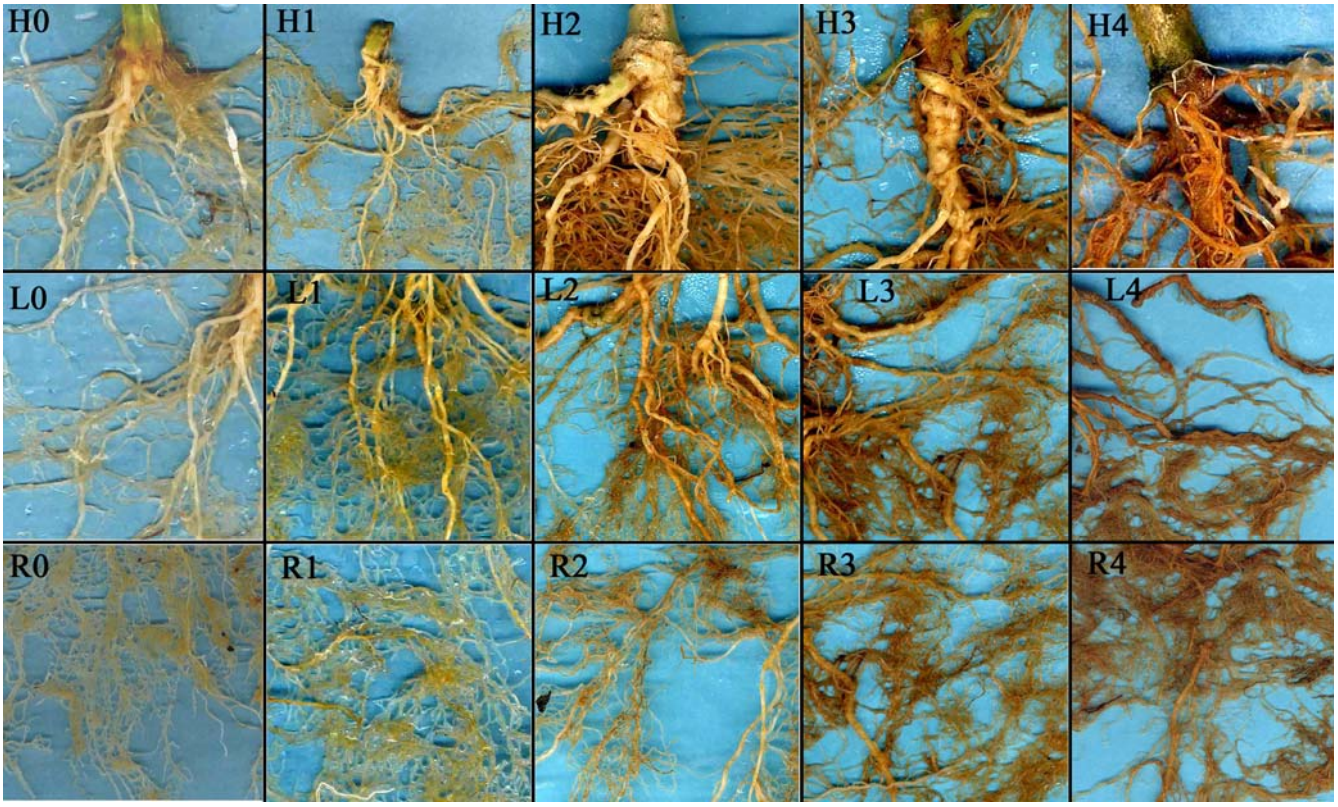

Fig. 3 Visual scoring of root lesions. First line, index for evaluation of lesions in the hypocotyl (H); second line, index for evaluation of lesions on the secondary root system and laterals (L); third line, index for evaluation of lesions in rootlets and fine roots (R). From left to right: from healthy to extremely damaged: (**H0, L0, R0**) healthy, (**H1, L1, R1**) slightly damaged, (**H2, L2, R2**) moderately damaged, (**H3, L3, R3**) severely damaged, (**H4, L4, R4**) extremely rotted and necrosed.

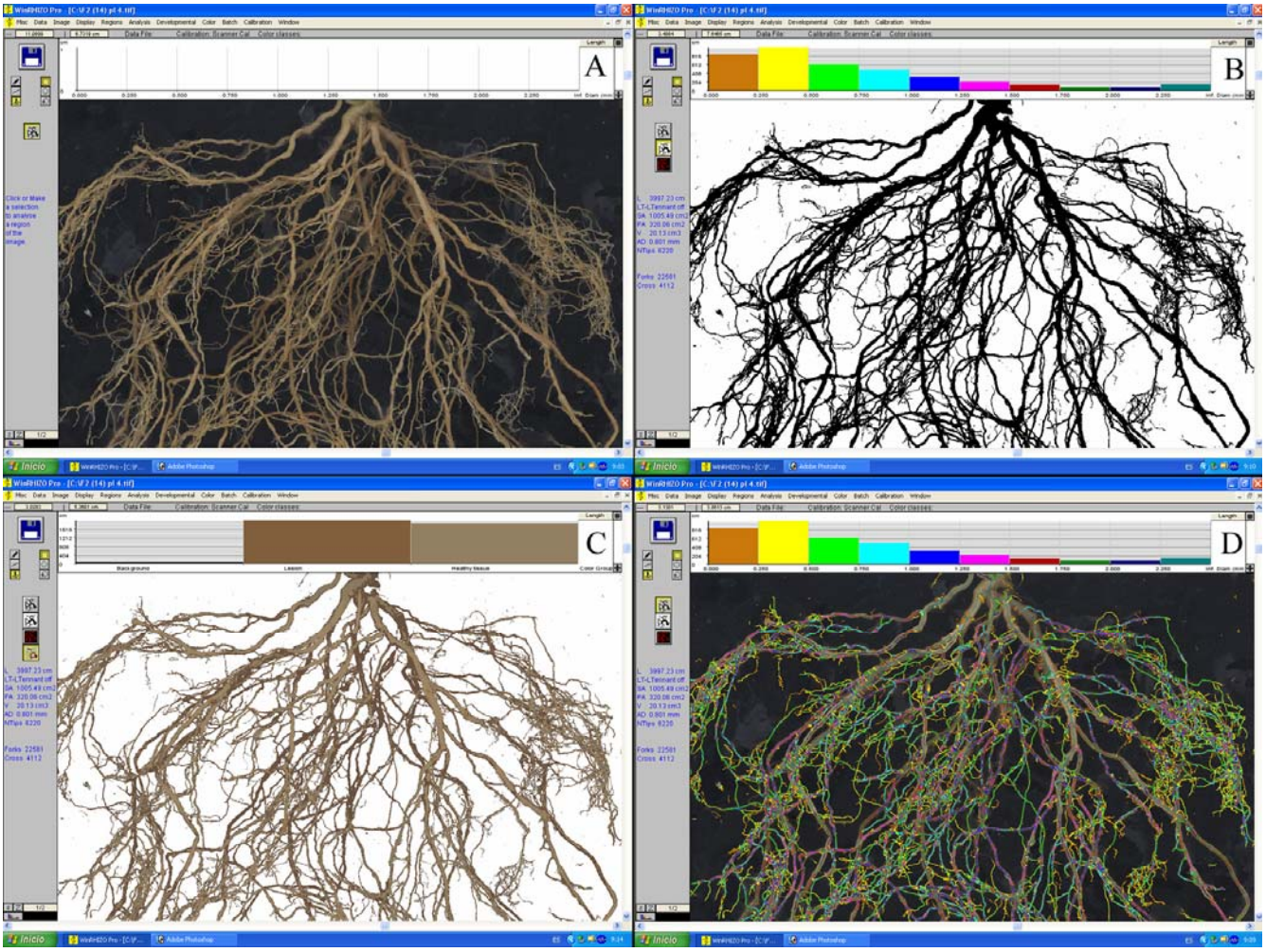

Fig. 4 Root image analysis using Winrhizo Pro software (Regent Instruments Inc. Canada). (**A**) Acquisition of the image of a root, (**B**) Shown in black is the part that the program considers to be a root, (**C**) Color analysis, colored in dark brown are the areas considered by the program to be damaged areas, colored in light brown are the areas considered by the program to be healthy areas, (**D**) Structure analysis, the skeleton of the root is marked by different colors depending on the diameter of the root.

infected melon roots (Picó *et al*. 2005). The method is reliable and highly sensitive. Using primers showed in **Fig. 2**, monitoring the reaction on an ABI Pism 7000 Sequence Detection System (Applied Biosistems) with SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) as a label system for PCR products, less than 1 pg of pathogen DNA can be detected. In addition, *M. cannonballus* can be first detected at 2 days after inoculation in roots of a susceptible cultivar, before symptoms appearance.

The combination of these methods (image analysis systems, visual scoring systems and real-time PCR) is an effective and accurate way to determine the susceptibility/ resistance of different plant material.

DISEASE CONTROL

As vine decline depends on the level of inoculum and the pathogenicity of the fungi, the susceptibility of the plant and its hydraulic balance, control methods have been focused on three main areas: I) reducing/inactivating the soil inoculum, II) increasing the resistance to root lesions, III) improving the hydraulic balance of the plant, mainly by modifying the root system.

Inoculum reduction

Reducing or eliminating the pathogens is the principal purpose of the chemical control. Pre-plant treatment of infested soils with methyl bromide or combinations with chloropicrin (Reuveni *et al*. 1983; Martyn and Miller 1996; Cohen *et al*. 2000) have so far been the most effective technique in reducing the presence of wilt symptoms, but the phasing out of methyl bromide has led to a search for chemical and non-chemical alternatives. Other pre-plant soil fumigants like methyl iodide and chloropicrin, combinations of 1-3 dicloropropene + chloropicrin and metam-sodium + formalin have shown to be effective in reducing MRR/VD severity (Martyn and Miller 1996; Stanghellini *et al*. 2003; Peretz-Alon *et al*. 2005). Metam sodium in pre-plant fumigation is not effective alone in reducing vine decline (Reuveni *et al*. 1983; Marty and Miller 1996; Cohen *et al*. 2000). However, it is effective in postharvest application via drip irrigation. This differential response is due to the lack of an effect of the metam sodium on the ascospores, whereas it kills the hyphae of *M. cannonballus* in postharvest applications preventing the formation of perithecia (Radewald *et al*. 2004). Cohen *et al.* (1999) assayed 29 fungicides *in vitro* against *M. cannonballus*, but only fluazinam and kresoxin-methyl totally inhibited the mycelium growth. Fluazinam was assayed in field and reduced the number of collapsed plants by 87% (Cohen *et al*. 1999). Fludioxonil also reduced lesion severity (Kim *et al*. 2001; Miller and Amador 2001). Traditional soil solarization, which is a feasible approach in many areas where MRR/VD is significant, is not effective by itself in controlling a thermotolerant pathogen like *M. cannonballus* (Reuveni *et al*. 1983). However, some improved solarization methods which increase the temperature reached in the solarization (Pivonia *et al*. 2002c), or solarization in combination with soil fumigants at a reduced dosage (Cohen *et al*. 2000), could be useful in controlling the disease. The persistence

of the inoculum and the difficulties in applying pesticides into the soil make the use of complementary measures necessary to avoid the disease.

Some cultural practices could greatly reduce the reproduction of *M. cannonballus* in the soil. Perithecia are formed primarily after the crop has been terminated. They produce the only survival structures of the fungus in the soil, the ascospores (Waugh *et al*. 2003; Stanghellini *et al*. 2004a). Therefore, any strategy to avoid the formation of perithecia would be valuable in reducing the level of ascospores of the soil. However, practices that kill living plants, such as foliar application of herbicides and mechanical destruction of vines, are counterproductive because they enhance rather than inhibit the production of perithecia (Stanghellini *et al*. 2004b). These treatments kill the plant but do not kill the hyphae which have a saprophytic growth after the death of the plant, allowing reproduction at even greater rates relative to the untreated controls. Conversely, exposing the infected roots by postharvest cultivation kills the pathogen hyphae in infected roots, thus preventing the formation of perithecia (Radewald *et al*. 2004).

Biological control of *M. cannonballus* using hypovirulent strains has been suggested since the earliest studies. A great variability in aggressiveness among isolates was reported as being mainly associated with the presence of some double-stranded RNA ($dsRNA^+$) and the development of yellow to brown pigments (Mertely *et al*. 1991; Lovic *et al*. 1993; Martyn and Miller 1996). Batten *et al*. (2000) studied these hypovirulent $dsRNA^+$ isolates. It was possible to reduce the aggressiveness of a virulent $dsRNA^-$ isolate by co-inoculating it with a $dsRNA^+$ hypovirulent isolate (10:1 hypovirulent:virulent). However, the presence of dsRNA did not ensure hypovirulence, and further studies in this direction are needed. In recent studies of the melanization in *M. cannonballus*, some compounds responsible from the yellow to brown colours of the degenerated cultures have been identified and related with the loss of fungal melanization and loss of virulence. However, the relationship of these pigments with the dsRNA remains unclear (Stipanovic *et al*. 2004; Wheeler *et al*. 2004). Preliminary studies with *Trichoderma virens* (Miller, Giddens and Foster) Arx and other species of the same genera that inhibit root colonization by certain soilborne pathogens have given good results in reducing infection with *M. cannonballus* and *A. cucurbitacearum* (Bruton *et al*. 1998; Sanz *et al*. 1998).

Resistance to root lesions

The use of resistant cultivars is one of the best alternatives in controlling plant diseases. Traditionally, wild relatives of *Cucumis melo* have provided a wide range of variation for resistance to several diseases (Robinson and Decker-Walters 1997), but this variation is impossible to use, since sexual crosses among *C. melo* and wild *Cucumis* species do not produce fertile hybrids (Chen and Adelberg 2000). Fortunately, genetic variability within *C. melo* sp. is broad enough to find certain tolerant cultivars. The first screening assays to find sources of resistance were made in infested fields, recording the number of wilted plants (Esteva and Nuez 1994; Cohen *et al*. 1995; Wolff 1995; Cohen *et al*. 1996; Wolff and Miller 1998; Iglesias *et al*. 2000c).

These screenings were useful in selecting several resistant/tolerant materials in the main countries affected by vine decline. In the USA, tolerance to the collapse symptom has been reported in 'Doublon,' a Charentais type also resistant to MNSV, and 'Deltex', an Ananas type (Wolff 1995; Wolff and Miller 1998). In Israel, the P6a line was resistant in field and an additive mode of gene action was suggested for the resistance (Cohen *et al*. 1995, 1996). The resistance found in the cultivar 'Black Skin', from Taiwan, is being introduced into Galia type melons (Cohen *et al*. 2000). In Spain, the accession *Cucumis melo* subsp. *agrestis* Pat 81 was selected because of its resistance under field conditions (Esteva and Nuez 1994; Iglesias and Nuez 1998; Iglesias *et al*. 2000c; Dias 2003). The genetics of the resistance derived from Pat 81 was studied under field conditions, using several families obtained from the cross of Pat 81 with susceptible cultivars 'Amarillo Canario'- and 'Piel de Sapo'-type (parents, F1, F2, BC1 and BC2) (Iglesias *et al*. 2000c). However, in field assays, the erratic effect of uncontrolled environmental factors made the study of the trait's genetics difficult. In this study, the percentage of symptomless plants scored at individual moments during the infection process in the field was an imprecise indicator of the resistance level of each genotype. It was necessary to analyze disease progress curves to minimize the stochastic fluctuations caused by environmental factors. Using these curves, data were fitted to an additive/dominance model without epistatic effects using a scaling test. The method of analysis also allowed for the characterization of the incomplete penetrance of resistance (Iglesias *et al*. 2000c).

Even though the selection of some resistant cultivars was successfully done in field experiments, the fact that the vine decline is highly influenced by many environmental factors prevents the use of this kind of evaluation for breeding programs. To accurately select the best materials, methodologies based on artificial inoculations and visual lesion scoring were proposed for breeding programs (Iglesias *et al*. 2000a, 2000b). Crosby (2000) studied the inheritance of the tolerance to *M. cannonballus* based on the effect of the artificial infection on the root system. Several crosses between tolerant and susceptible cultivars were performed and data of total length, root surface area, root tips, etc. were obtained from the image analysis of the root systems. The cultivars that showed the least-affected root systems were 'Doublon' and 'Deltex.' A high heterosis was found in some traits that led to estimates of heritabilities that were higher than 100%. A double mechanism of tolerance was suggested: morphological (improved root system) combined with a specific resistance to fungal lesions.

Accession Pat 81, which was evaluated as resistant in field, displayed less widespread and less severe lesions than susceptible cultivars in inoculated soils. These mild lesions were not severe enough to produce root mass losses, contrary to the significant losses observed in susceptible cultivars (Iglesias *et al*. 2000b; Dias *et al*. 2004). The genetics of its resistance to root damage caused by *M. cannonballus* was studied (Dias *et al*. 2004). Estimates of the broad- and narrow-sense heritabilities indicated that most of the variation found for lesion resistance in both primary/secondary and tertiary root systems could be explained by additive effects. Lower heritabilities and higher importance of dominance effects were found for lesions in hypocothyls, which shows that lesion severity in this area is more dependent on environmental factors (deep sowing or uneven distribution of inoculum in the soil). Therefore, scoring systems based on damage in the lateral roots and rootlets seem to be the most appropriate for selecting resistant material in segregant populations. Due to the high level of resistance found in Pat 81, it was selected to initiate a breeding program that would allow introgression of its resistance into the most important Spanish melon types, such as 'Piel de Sapo' and 'Amarillo Canario' (Iglesias *et al*. 2000c; Dias 2003).

Hydraulic balance, modifying the root system

The hydraulic balance of the plant depends on the vine and fruit demand as well as the capacity of the root to support this demand. It has been pointed out that some intensive cultural practices (such as mulch and drip irrigation) along with the high productivity of the melon hybrids have led to a great shoot:root disequilibrium (Krikun 1985; Cohen *et al.* 2000; Pivonia *et al*. 2002b; Martyn 2007). As a high production is desirable, it is more appropriate to modify the water absorption capacity of the root. Roots of plants developed under a high frequency drip irrigation system are shallow and dense, and are highly sensitive to water fluctuations. A deeper and more extended root system would likely support better high water demand despite having les-

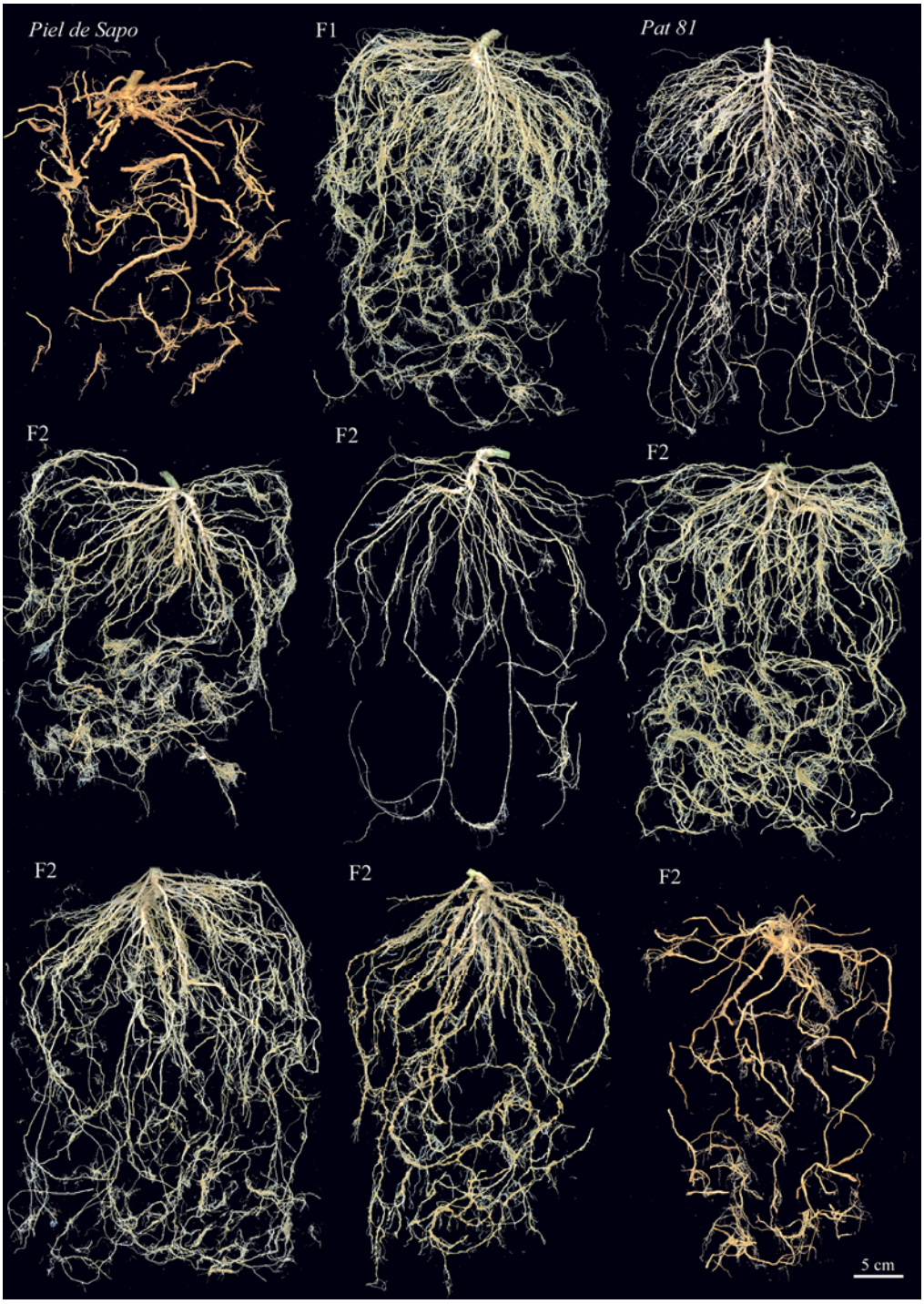

Fig. 5 Segregation for root characters and resistance to root lesions in melon plants grown in 8L pots filled with naturally infested soil (*M. cannonballus* and *A. cucurbitacearum*) and analysed 60 days after planting. Above: (**'Piel de sapo'**), highly damaged, (**F1**) derived from the cross between 'Piel de Sapo' and Pat 81, a certain degree of heterosis can be observed, (**Pat 81**), resistant to root lesions and with an improved root system. The other 6 roots are **(F2)** derived from selfing F1, segregation for root structure and lesions can be observed.

ions. The size and structure of the root can be manipulated by changing the irrigation system. Low irrigation systems have been shown to postpone the onset of plant collapse and reduce disease incidence. However, these systems also reduce the number and quality of the fruits (Cohen *et al*. 2000; Pivonia *et al*. 2004; Merghany 2006).

The response of melon plants to vine decline may be attributed in part to the size and structure of the root system. In fact, the tolerance of the Ananas-type melons has been previously attributed in part to its larger and more vigorous root system, which is better adapted to dry-land cropping (Cohen *et al*. 2000). Surprisingly, the comparative study of susceptible ('Magnum' and 'Caravelle') and tolerant ('Deltex', an Ananas type, and 'Doublon') melon cultivars did not show significant differences between them in sterile soil. The differences appeared after the infection, suggesting that tolerance was closely related to the integrity of the root system (Crosby *et al*. 2000). In this respect, a greater root system would not confer tolerance *per se*, but would be valuable in increasing the favorable response against vine decline (Dias 2004, unpublished data). During the root analysis of the breeding populations, the structure of the root system of *C. melo* subsp *agrestis* Pat 81 was clearly different from that of the susceptible cultivars, which belong to the subspecies *melo* (Iglesias *et al*. 2000b; Dias 2003). These differences in structure were observed in healthy soils and remained after infection with *M. cannonballus*. Root system differences between cultivated and wild taxa have also been reported for other species, since the roots of wild plants are usually adapted to exploit more unpredictable and stressful soil environments (Jackson and Koch 1997; Johnson *et al*. 2000). The root structure of Pat 81 may enhance its tolerance to MRR/VD by conferring a higher capacity for soil exploration.

Using Pat 81 as a donor of valuable genes for breeding root systems of cultivated melons is not an easy task. Firstly, it is necessary to define which favorable characteristics from Pat 81 determine the "ideal" root in meeting our breeding objectives. Difficulties in root analysis can condition the root traits which are evaluated. A general tendency

in root research has been to study easily measured traits related to root biomass. However, root studies based on these traits have been only marginally successful. Selecting plants with a greater overall root biomass would likely impact yield negatively and would not ensure a higher capacity for soil exploration (Clarke and McCaig 1993; Zobel 1995; Jonhson *et al*. 2000). Our results support this statement, as 'Piel de Sapo', a highly susceptible Spanish melon, displays a higher root biomass than Pat 81, and yet does not have an improved ability to overcome the disease (Fita *et al*. 2006b). Various colleagues have suggested that current root research should focus on other root traits, such as those related to root length and architecture (Jonhson *et al*. 2000; Wells and Eissenstat 2003). However, these are parameters which are difficult to measure. In our first studies, we evaluated root length and architecture by employing different visual scores. With these indices, we consistently observed a more branched root system in Pat 81 in comparison with different types of cultivated melons (Dias *et al.* 2002). Recently, the use of root image analysis, with specific software (WhinRhizo, Regent Instruments, Canada), and the use of more specific measurements of the architecture, such as the branching order or number of lateral roots, have facilitated the accurate characterization of root structure. Using these methods, the results demonstrated that both seedling and adult plants of Pat 81 display significantly higher values than 'Piel de Sapo' for all length and architectural traits assayed (maximum and average length of lateral roots, number of lateral roots, root order, total length of fine roots, branching pattern, etc.) (Fita *et al*. 2004, 2006b).

Studies of the root systems have been traditionally delayed because of the difficulty in extracting roots from the soil/pots, the challenging process of properly defining root parameters and their complex plastic responses that complicate the analysis. Many of the difficulties which arise during the analysis of root structure can be overcome by combining root image analysis with *in vitro* culture techniques (Fita *et al*. 2005a). These systems allow us to perform *in vivo* studies of root development and are now being used in a population of introgression lines that are segregant for root structure traits to identify molecular markers linked to the genes involved in desired root traits.

The genetics of root traits have been studied in an F2:3 families derived from the cross of Pat 81 and 'Piel de Sapo' (**Fig. 5**) (Fita *et al*. 2004, 2006b). Broad-sense heritabilities were low to moderate, which indicates the importance of environmental effects on the variation observed in most of the traits examined. The narrow-sense heritabilities were also moderate, the additive effects being more important than the dominant. These parameters are being successfully used to select melon plants with a root architecture that exploits the largest possible soil volume. This is not only useful in improving the resistance to vine decline, but also in overcoming other biotic and abiotic soil stresses. Deeper root systems are able to reach lower layers of the soil profile which can be richer in water and nutrients. In

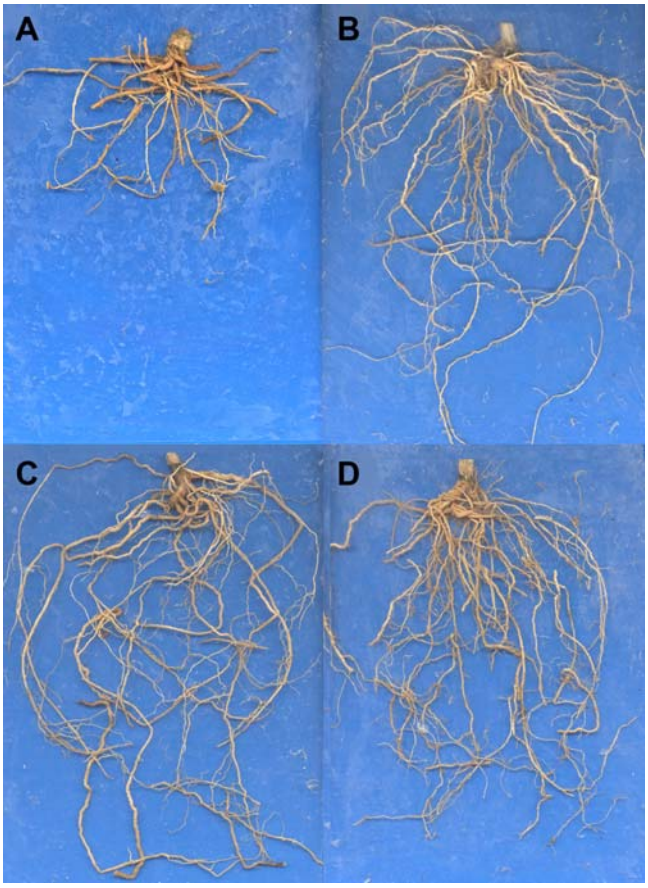

Fig. 6 Roots of plants grown in *M. cannonballus* naturally infested soil for 90 days (the fine rootlets have been removed for a better observation of the root structure). (**A**) Piel de Sapo susceptible cultivar, (**B**) Pat 81, resistant cultivar, (**C**) BC_2 derived from the cross of PS and Pat 81 plus two backcrosses to PS, (**D**) BC_4 derived from the cross of PS and Pat 81 plus four backcrosses to PS.

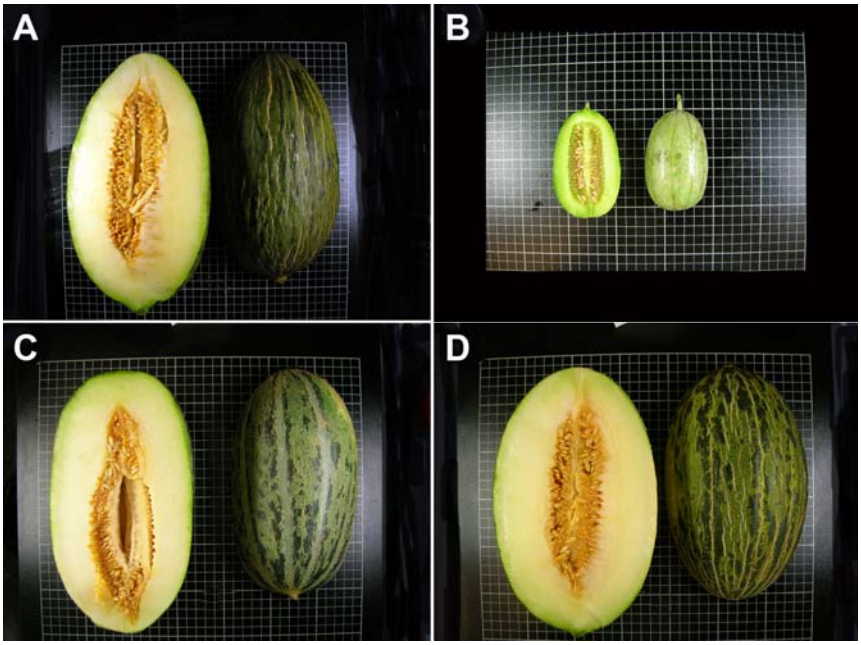

Fig. 7 Fruits of plants whose roots are shown in Fig 6. (**A**) Piel de Sapo (**B**) Pat 81, resistant cultivar, (**C**) BC_2 derived from the cross of PS and Pat 81 plus two backcrosses to PS, (**D**) BC_4 derived from the cross of PS and Pat 81 plus four backcrosses to PS.

Fig. 8 Grafting technique. (**A**) Tongue approach technique, Pat 81 used as a rootstock is on the left (the first leave have been removed), PS is the scion. (**B**) Incompatibility problems in PS grafted onto RS841 rootstock (*Curcurbita* hybrid highly employed in grafting watermelon in Spain).

fact, root length and the branched structure of the root system has been largely used to improve vegetables against drought stress (Clarke and McCaig 1993; Jonhson *et al*. 2000). Crosby (2000) also studied the heritability of some root traits in several crosses between tolerant and susceptible cultivars, finding high heritabilities for total root length and root surface area (measured with Rhizo 3.8 Pro) and great heterosis in certain crosses.

The two resistance mechanisms derived from Pat 81 – a high resistance to root lesions and a long and branched root system – are currently being exploited in a breeding program. A combined selection for resistance to *M. cannonballus* and for a favorable root structure is being conducted. Favorable traits are being introgressed into the genetic background of the most important Spanish melon types, 'Piel de Sapo' and 'Amarillo Canario' (Dias 2003; Fita *et al*. 2005b). This program has reached the 3rd and 4th backcross generations (**Fig. 6**). It should be noted that Pat 81 has unfavorable agronomic features. It develops small and soft fruits, with green flesh which is acid and has a low content of soluble solids (**Fig. 7**). We have studied the agronomic value of the backcross generations obtained. At the third backcross generation we found values for fruit weight, total soluble solid content, flesh thickness, etc. similar to those of the cultivated variety. There was no difference in these traits between the third and the fourth backcross generations, which implies that the third backcross can already be selfed to obtain the first resistant pre-commercial materials (Fita *et al*. 2005b; **Fig. 7**).

Grafting

Another method for enlarging the capacity of soil exploration and enhance resistance to soilborne pathogens has been grafting plants. The popularity of grafting to overcome plant diseases is increasing, and nowadays it is used routinely for watermelon in the Mediterranean basin to avoid Fusarium wilts. Although all cucurbits can be hosts of *M. cannonballus* and *A. cucurbitacearum* (Mertely *et al*. 1993a; Armengol *et al*. 1999), some species have been reported to be tolerant to one or both fungi. The slow disease development and the large root system enable these plants to complete the growing season (García-Jiménez *et al*. 1990; Edelstein *et al*. 1999; Cohen *et al*. 2000). However, the results of grafting melons onto *Cucurbita* rootstocks varies. In general, the use of grafted plants reduces the wilt incidence, but the response in terms of yield or quality depends on many factors as rootstock-scion or the cultivation method. Moreover, in melon-cucurbita graftings, the occurrence of rootstock-scion incompatibility has been reportedly hampering the extensive use of *Cucurbita* rootstocks (**Fig. 8B**) (Edelstein *et al*. 1999; Traka-Mavrona *et al*. 2000; Edelstein *et al*. 2004; Cohen *et al*. 2005). Using melon as rootstock not only avoids incompatibility problems, but also the detrimental effects of some cucurbit rootstocks in fruit quality (Cohen *et al*. 2002; Nisini *et al*. 2002). Accession Pat 81 has been tested as rootstock for melon, and has had excellent results (**Fig. 8A**). Its use as a rootstock avoids the occurrence of wilt and does not have a greater detrimental effect on fruit quality than when grafting on *Cucurbita*. Moreover, no incompatibility has been reported (Fita *et al*. 2007, and unpublished).

ACHIEVEMENTS AND PERSPECTIVES

The significance of vine decline in melon crops has increased throughout the world. As the presence of the disease is a sum of different factors, the only control alternative is that of integrated management. This must combine the use of the available tolerant/resistant germplasm (via grafting or improved lines with resistance to fungus and improved root systems) with the use of techniques that avoid the build-up of the inoculum, and an adequate management of the crop to avoid unnecessary stresses. The efforts of several scientists and the development of new methodologies have contributed to advances in these three directions.

Methodologies, such as real-time PCR and image analysis, are now being used to accurately phenotype segregant populations for resistance and root structure. Genotyping of these populations is also currently being conducted to identify markers linked to the resistance and the root structure traits for a marker-assisted selection. Also, the *Monosporascus cannonballus*-melon system has been used to produce an EST sequence collection under the Spanish Melon Genome initiative (Puigdomènech *et al*. 2007). Several cDNA libraries have been constructed from healthy roots and infected roots with *M. cannonballus* (using roots of both resistant and susceptible genotypes). These sequences have been included in an oligo-microarray of melon (along with other cotyledon, leaves and fruit-derived sequences) that has been used to perform differential expression studies (Puigdomènech *et al*. 2006). The results of these studies will provide candidate genes for further investigation into the response of melon genotypes to this soilborne disease.

ACKNOWLEDGEMENTS

We would like to acknowledge the intensive field work done by Eva Martínez (COMAV), the technical support of Dr. Cristina Roig (COMAV) and the recommendations on the *M. cannonballus* culture by Dr. Roberto Betrán (Instituto Agroforestal Mediterráneo). This research has been supported by the AGL2003-04817/AGR from the Spanish Ministry of Science and Technology.

REFERENCES

Aegerter BJ, Gordon TR, Davis RM (2000) Occurrence and pathogenicity of fungi associated with melon root rot and vine decline in California. *Plant Dise*ase **84**, 224-230

Alfaro-García A, Armengol J, Bruton BD, Gams W, García-Jiménez J, Martínez-Ferrer G (1996) The taxonomic position of the causal agent of acremonium collapse of muskmelon. *Mycologia* **88**, 804-808

Armengol J, Sanz E, Martínez-Ferrer G, Sales R, Bruton BD, García-Jiménez J (1998) Host range of *Acremonium cucurbitacearum*, cause of acremonium collapse of muskmelon. *Plant Pathology* **47**, 29-35

Armengol J, Sales R, García-Jiménez J (1999) Effects of soil moisture and water on survival of *Acremonium cucurbitacearum*. *Journal of Phytopathology* **147**, 737-741

Batten JS, Scholthof KGB, Lovic BR, Miller ME, Martyn RD (2000) Potential for biocontrol of Monosporascus root rot/vine decline under greenhouse conditions using hypovirulent isolates of *Monosparascus cannonballus*. *European Journal of Plant Pathology* **106**, 639-649

Beltrán R, Sales R, García-Jiménez J, Armengol J (2005) Population dynamics of *Monosporascus cannonballus* ascospores in marsh soils in eastern Spain. *European Journal of Plant Pathology* **113**, 357-365

Beltrán R, Vicent A, García-Jiménez J, Armengol J (2007) Quantification of *Monosporascus cannonballus* ascospores in muskmelon fields in eastern Spain. *Journal of Phytopathology* **155**, 248-250

Biernacki M, Bruton BD (2001) Quantitative response of *Cucumis melo* inoculated with root rot pathogens. *Plant Disease* **85**, 65-70

Bruton BD (1995) Optimum CFU concentrations for testing pathogenicity of California cucurbit isolates of *Monosporascus cannonballus*. *Phytopathology* **85**, 1119

Bruton BD, Miller ME (1997a) Ocurrence of vine decline diseases of muskmelon in Guatemala. *Plant Disease* **81**, 694

Bruton BD, Miller ME (1997b) Occurrence of vine decline of melons in Honduras. *Plant Disease* **81**, 696

Bruton BD, Davis RM, Gordon TR (1995) Occurrence of *Acremonium cucurbitacearum* sp. and *Monosporascus cannonballus* in the major cantaloupe and watermelon growing areas of California. *Plant Disease* **79**, 754

Bruton BD, Zhang JX, Howell CR, Miller ME (1998) Evaluation of *Trichoderma virens* as a potential biocontrol agent of *Monosporascus cannonballus* in muskmelon. *Phytopathology* **88**, 12

Bruton BD, García-Jiménez J, Armengol J (1999) Analysis of the relationship between temperature and vine declines caused by *Acremonium cucurbitacearum* and *Monosporascus cannonballus* on muskmelon. *Subtropical Plant Science* **51**, 13-28

Bruton BD, García-Jiménez J, Armengol J, Popham TW (2000) Assessment of virulence of *Acremonium cucurbitacearum* and *Monosporascus cannonballus* on *Cucumis melo*. *Plant Disease* **84**, 907-913

Chen JF, Adelberg J (2000) Interspecific hybridization in *Cucumis* – progress, problems, and perspectives. *HortScience* **35**, 11-15

Clarke JM, McCaig TN (1993) Breeding for efficient root systems In: Hayward MD, Bosemark NO, Romagosa I (Eds) *Plant Breeding: Principles and Prospects*, Chapman and Hall, London, pp 485-499

Cohen R, Schreiber S, Nerson H (1995) Response of melofon lines to powdery mildew, downy mildew, Fusarium wilt, and sudden wilt. *Plant Disease* **79**, 616-619

Cohen R, Elkind Y, Burger Y, Offenbach R, Nerson H (1996) Variation in the response of melon genotypes to sudden wilt. *Euphytica* **87**, 91-95

Cohen R, Pivonia S, Shtienberg D, Edelstein M, Raz D, Gerstl Z, Katan J (1999) Efficacy of Fluazinam in Suppression of *Monosporascus cannonballus*, the causal agent of sudden wilt of Melons. *Plant Disease* **83**, 1137-1141

Cohen R, Pivonia S, Burger Y, Edelstein M, Gamliel A, Katan J (2000) Towards integrated management of Monosporascus wilt of melons in Israel. *Plant Disease* **84**, 496-505

Cohen R, Horev C, Burger Y, Shriber S (2002) Horticultural and pathological aspects of Fusarium wilt management using grafted melons. *HortScience* **37**, 1069-1073

Cohen R, Burger Y, Horev C, Porat A, Edelstein M (2005) Performance of Galia-type melons grafted onto *Cucurbita* rootstock in *Monosporascus cannonballus*-infested and non-infested soils. *Annals of Applied Biology* **146**, 381-387

Crosby K (2000) Narrow-sense heritability estimates for root traits and *Monosporascus cannonballus* tolerance in melon (*Cucumis melo*) by parent-offspring regression. *Acta Horticulturae* **510**, 149-154

Crosby K, Wolff D, Miller M (2000) Comparisons of root morphology in susceptible and tolerant melon cultivars before and after infection by *Monosporascus cannonballus*. *HortScience* **35**, 681-683

Dias R de CS (2003) Mejora de la resistencia al colapso del melón: control genético y desarrollo de cultivares resistentes. PhD Thesis, Universidad Politécnica de Valencia, Spain, 152 pp

Dias R De CS, Picó B, Herraiz J, Espinós A, Nuez F (2002) Modifying root structure of cultivated muskmelon to improve vine decline resistance. *HortScience* **37**, 1092-1097

Dias R De CS, Picó B, Espinós A, Nuez F (2004) Resistance to melon vine decline derived from *Cucumis melo* ssp. *agrestis*: genetic analysis for root structure and root response. *Plant Breeding* **123**, 66-72

Edelstein M, Cohen R, Shriber S, Pivonia S, Shtienberg D (1999) Integrated management of sudden wilt in melons caused by *Monosporascus cannonballus* using grafting and reduced rates of methyl bromide. *Plant Disease* **83**, 1142-1145

Edelstein M, Burger Y, Horev C, Porat A, Meir A, Cohen R (2004) Assessing the effect of genetic and anatomic variation of *Cucurbita* rootstocks on vigour, survival and yield on grafted melons. *Journal of Horticultural Science and Biotechnology* **79**, 370-374

El-Desouky SM, El-Wakil AA (2003) Occurrence of *Monosporascus* root rot and vine decline of cantaloupe and watermelon in Egypt. *Egyptian Journal of Phytopathology* **31**, 141-150

Esteva J, Nuez F (1994) Field resistance to melon dieback in *Cucumis melo* L. *Cucurbit Genetics Cooperative* **17**, 76-77

Fita A, Picó B, Nuez F (2004) Estudio de la genética de parámetros de raíz en melón. *Actas de Horticultura* **41**, 211-213

Fita A, Roig C, Picó B, Nuez F (2005a) Study of natural variation in root structure within *Cucumis melo* L. using *in vitro* culture. *Cucurbit Genetics Cooperative* **28**, in press

Fita A, Dias R de C, Picó B, Nuez F (2005b) Caracterización agronómica de líneas avanzadas de mejora resistentes al colapso del melón. *Actas Portuguesas de Horticultura* **8**, 82-87

Fita A, Picó B, Nuez F (2006a) Análisis de imagen como herramienta en la mejora del sistema radicular de melón. *Actas de Horticultura* **45**, 119-120

Fita A, Picó B, Nuez F (2006b) Implications of the genetics of root structure in melon breeding. *Journal of the American Society for Horticultural Science* **131**, 372-379

Fita A, Picó B, Roig C, Nuez F (2007) Performance of *Cucumis melo* ssp. *agrestis* as a rootstock for melon. *Journal of Horticultural Science and Biotechnology* **82**, 184-190

García-Jiménez J, Velázquez MT, Alfaro A (1989) Secuencia de síntomas en el colapso del melón. *Boletín de Sanidad Vegetal y Plagas* **4**, 333-342

García-Jiménez J, García-Morato M, Velásquez MT, Alfaro A (1990) Ensayos preliminares de control de la muerte súbita del melón mediante la utilización de portainjertos resistentes. *Boletín de Sanidad Vegetal y Plagas* **16**, 709-715

García-Jiménez J, Velásquez MT, Jordá C, Alfaro-García A (1994) *Acremonium* species as the causal agent of muskmelon collapse in Spain. *Plant Disease* **78**, 416-419

García-Jiménez J, Armengol J, Sales R, Jordá C, Bruton BD (2000) Fungal pathogens associated with melon collapse in Spain. *EPPO Bulletin* **30**, 169-173

Gennari S, Mirotti A, Sportelli M (1999) Reperimento di *Monosporascus cannonballus* da piante di cocomero. *Informatore Fitopatologico* **2**, 38-40

Ghignone S, Tamietti G, Girlanda M (2003) Development of specific PCR primers for identification and detection of *Rhizopycnis vagum*. *European Journal of Plant Pathology* **109**, 861-870

Gwynne BJ, Davis RM, Gordon TR (1997) Occurrence adn pathogenicity of fungi associated with melon vine decline in California. *Phytopathology* **87**, S37

Iglesias A (2000) *Resistencia genética al colapso del melón*. Thesis dissertation, Universidad Politécnica de Valencia, Spain, pp 15

Iglesias A, Nuez F (1998) Caracterización de diversas entradas de melón frente al colapso o muerte súbita. *Actas Horticultura* **22**, 139-147

Iglesias A, Picó B, Nuez F (2000a) Artificial inoculation methods and selection criteria for breeding melons against vine decline. *Acta Horticulturae* **510**, 155-162

Iglesias A, Picó B, Nuez F (2000b) Pathogenicity of fungi associated with melon vine decline and selection strategies for breeding resistant cultivars. *Annals of Applied Biology* **137**, 141-151

Iglesias A, Picó B, Nuez F (2000c) A temporal genetic analysis of disease resistance genes: resistance to melon vine decline derived from *Cucumis melo* var *agrestis*. *Plant Breeding* **118**, 1-6

Infantino A, Uccelletti A, Di Stefano G, Ciuffreda G, Frisullo S (2002) First report of *Monosporascus cannonballus* on melon in Italy. *Journal of Plant Pathology* **84**, 139-140

Jackson LE, Koch GW (1997) The ecophysiology of crops and their wild relatives. In: Jackson LE (Ed) *Ecology in Agriculture*, Academic Press, San Diego, pp 3-37

Johnson WC, Jackson LE, Ochoa O, Van Wijk R, Peleman J, St. Clair DA, Michelmore RW (2000) Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild progenitor, differ at QTL determining root architecture and deep soil water exploitation. *Theoretical and Applied Genetics* **101**, 1066-1073

Karlatti RS, Abdeen FM, Al-Fehaid MS (1997) First report of *Monosporascus cannonballus* on melons in Saudi Arabia. *Plant Disease* **81**, 1215

Kim DH, Rasmussen Sl, Stanghellini ME (1995) *Monosporascus cannonballus* root rot of muskmelon: Root infection and symptom development in relation to soil temperature. *Phytopathology* **85**, 1195

Kim DH, Stanghellini ME, Waugh MM, Mayberry KS (2001) Vine decline of melons caused by *Monosporascus cannonballus* II. Postharvest disease management strategies. *Phytopathology* **91**, 48

Koike ST, Subbarao KV, Davis RM, Turini TA (2003) Vegetable diseases caused by soilborne diseases. Pub 8099 UC Davis, 12 pp

Krikun J (1985) Observation on the distribution of the pathogen *Monosporascus eutypoides* as related to soil temperature and fertigation. *Phytoparasitica* **13**, 225-228

Kwon MK, Hong JR, Kim YH, Kim KC (2001) Soil-environmental factors involved in the development of root rot/vine on cucurbits caused by *Monosporascus cannonballus*. *Plant Pathology Journal* **17**, 45-51

Lobo-Ruano M (1990) Colapso del melón producido por hongos del género *Monosporascus*. *Boletín de Sanidad Vegetal y Plagas* **16**, 701-707

Lovic BR, Martyn RD, Millar RD (1993) Pathogenicity of *Monosporascus cannonballus* as related to colony characteristics and geographic origin. *Phytopathology* **83**, 466

Lovic BR, Martyn RD, Miller ME (1994) Association of dsRNA with reduced aggressiveness and phenotypic variability in *Monosporascus cannonballus*.

Phytopathology **86**, 776

Lovic BR, Martyn RD, Lobo M (1996) Agresividad de los aislados españoles de *Monosporascus cannonballus*. *Resúmenes del VIII Congreso Nacional de la Sociedad Española de Fitopatología*, Cordoba, Spain, 139 pp

Lovic BR, Martyn RD, Miller RD (1995a) Sequence analysis of the ITS regions of rDNA in *Monosporascus* spp. to evaluate its potential for PCR-mediated detection. *Phytopathology* **85**, 655-661

Lovic BR, Valadez VA, Martyn RD, Miller ME (1995b) Detection and identification of Monosporacus spp. With genus-specific PCR primers and nonradioactive hybridization probes. *Plant Disease* **79**, 1169-1175

Martínez-Culebras PV, Abad-Campos P, García-Jiménez J (2004) Molecular characterization and PCR detection of the melon pathogen *Acremonium cucurbitacearu*m. *European Journal of Plant Pathology* **110**, 801-809

Martyn RD (2007) Late-season vine declines of melons: pathogical, cultural or both? *Acta Horticulturae* **731**, 345-356

Martyn RD, Miller ME (1996) *Monosporascus* root trot/ vine decline: An emerging disease of melon worldwide. *Plant Disease* **80**, 716-725

Martyn RD, Mertely JC, Miller ME, Bruton BD (1991) The role of a thermophilic fungus in the root rot/vine decline disease of muskmelon in Texas. *HorScience* **26**, 488

Martyn RD, Lovic BR, Maddox DA, Germash A, Miller ME (1994) First report of Monosporascus rot/vine decline of watermelon in Tunisia. *Plant Disease* **78**, 1220

Martyn RD, Battern JS, Park YJ, Miller ME (1996) First report of *Monosporascus* root rot/vine decline of watermelon in Mexico. *Plant Disease* **80**, 1430

Merghany MM (2006) Effect of some agricultural practices as alternatives for methyl bromide against the sudden wilt of melon, Galia type. *Annals of Agricultural Science* **44**, 223-250

Mertely JC, Martyn RD, Miller ME, Bruton BD (1991) Role of *Monosporascus cannonballus* and other fungi in a root rot/vine decline disease of muskmelon. *Plant Disease* **75**, 1133-1137

Mertely JC, Martyn RD, Miller ME, Bruton BD (1993a) An expanded Host range for the muskmelon pathogen *Monosporascus cannonballus*. *Plant Disease* **77**, 667-673

Mertely JC, Martyn RD, Miller ME, Bruton BD (1993b) Quantification of *Monosporascus cannonballus* ascospores in three commercial muskmelon fields in South Texas. *Plant Disease* **77**, 766-771

Miller ME, Martyn RD, Lovic BR, Bruton BD (1995) An overview of vine decline diseases of melons. In: Lester GE, Dunlap JR (Eds) *Cucurbitaceae'94 Evaluation and enhancement of Cucurbit germplasm*, Gateway Printing, Edinburg, TX, USA, pp 31-35

Miller ME, Amador J (2001) Efficacy of drip irrigation applications of fludioxonil for control of Monosporascus root rot and vine decline. *Phytopathology* **91**, 199

Nisini PT, Colla G, Granati E, Temperini O, Crinò P, Saccardo F (2002) Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. *Scientia Horticulturae* **93**, 281-288

Peretz-Alon I, Ucko O (2005) Combined soil fumigants: synergistic performance and improved yield. *Acta Horticulturae* **698**, 135-140

Picó B, Roig C, Fita A, Nuez F (2005) Detección de *Monosporascus cannonballus* en raíces de melón mediante PCR cuantitativa en tiempo real. *Actas Portuguesas de Horticultura* **7**, 169-176

Picó B, Fita A, Dias R, Roig C, Nuez F (2007) Advances in breeding melons for resistance to vine decline. *Acta Horticulturae* **731**, 39-46

Pivonia S, Cohen R, Kafkafi U, Ben Ze'ev IS, Katan J (1997) Sudden wilt of melons in southern Israel: Fungal agents and relationship with plant development. *Plant Disease* **81**, 1264-1268

Pivonia S, Cohen R, Kigel J, Katan J (2002a) Effect of soil temperature on disease development in melon plants infected by *Monosporascus cannonballus. Plant Pathology* **51**, 472-479

Pivonia S, Cohen R, Katan J, Kigel J (2002b) Effect of fruit load on the water balance of melon plants infected with *Monosporascus cannonballus*. *Physiological and Molecular Plant Pathology* **60**, 39-49

Pivonia S, Cohen R, Levita R, Katan J (2002c) Improved solarization of containerized medium for the control of *Monosporascus* collapse in melon. *Crop Protection* **21**, 907-912

Pivonia S, Cohen R, Kigel J, Levita R, Katan J (2004) Effect of irrigation regimes on disease expression in melon plants infected with *Monosporascus cannonballus*. *European Journal of Plant Pathology* **110**, 155-161

Pollack FG, Uecker FA (1974) *Monosporascus cannonballus*, an unusual ascomycete in cantaloupe roots. *Mycologia* **66**, 346-349

Puigdomènech P, Arús P, Garcia-Mas J, González M, Nuez F, Blanca J, Picó B, Roig C, Aranda M, González D (2006) The MELOGEN project: EST sequencing, processing and analysis. Proceedings Cucurbitaceae 2006, Asheville, North Carolina, USA, pp 186-192

Puigdomènech P, Martínez-Izquierdo JA, Arús P, García-Mas J, Monforte AJ, Nuez F, Picó B, Blanca J, Aranda M, Arnau V, Robles A (2007) The Spanish melon genomics initiative. *Acta Horticulturae* **731**, 47-54

Radewald KC, Ferrin DM, Stanghellini ME (2004) Sanitation practices that inhibit reproduction of *Monosporascus cannonballus* in melon roots left in the field after crop termination. *Plant Pathology* **53**, 660-668

Reuveni R, Krikun J (1983) The occurrence and distribution of *Monosporascus eutypoides* under arid zone conditions in Israel. *Transactions of British Mycological Society* **80**, 354-356

Reuveni R, Krikun J, Shani U (1983) The role of *Monosporascus eutypoides* in a collapse of melon plants in an arid area of Israel. *Phytopathology* **73**, 1223-1226

Robinson RW, Decker-Walters DS (1997) *Cucurbits*, CAB International, New York, 226 pp

Sales JR, Becerra do Nascimento IJ, Souza Freitas L, Beltrán R, Armengol J, Vicente A, García-Jiménez J (2004) First report of *Monosporascus cannonballu*s on melon in Brazil. *Plant Disease* **88**, 84

Sales JR, Beltrán R, Michereff SJ, Armengol J, García-Jiménez J, Medeiros EV (2006) Analysis of different types of sugar in the extraction method of ascospores of *Monosporascus cannonballus* in soil. *Fitopatologia Brasileira* **31**, 185-187

Sanz L, Saler R, Armengol J, Monte E, García-Jiménez J, Grondona I (1998) Antagonismo de *Trichoderma* spp. Frente a *Monosporascus* sp. y *Acremonium cucurbitacearum* causantes del colapso del melón. *Resúmenes del IX Congreso Nacional de la Sociedad Española de Fitopatología*, 287 pp

Stanghellini ME, Rasmussen SL (1992) A quantitative method for the recovery of ascospores of *Monosporascus cannonballus* from field soil. (Abstr.) *Phytopathology* **82**, 1115

Stanghellini ME, Kim DH, Rasmussen SL (1996) Ascospores of *Monosporascus cannonballus*: Germination and distribution in cultivated and desert soils in Arizona. *Phytopathology* **86**, 509-514

Stanghellini ME, Kim DH, Waugh M (2000) Microbe-mediated germination of ascospores of *Monosporascus cannonballus*. *Phytopathology* **90**, 243-247

Stanghellini ME, Ferrin DM, Kim DH, Waugh MM, Radewald KC, Sims JJ, Ohr HD, Mayberry KS, Turini T, McCaslin MA (2003) Application of preplant fumigants via drip irrigation systems for the management of root rot of melons caused by *Monosporascus cannonballus*. *Plant Disease* **87**, 1176-1178

Stanghellini ME, Kim DH, Waugh MM, Ferrin DM, Alcantara T, Rasmussen SL (2004a) Infection and colonization of melon roots by *Monosporascus cannonballus* in two cropping seasons in Arizona and California. *Plant Pathology* **53**, 54-57

Stanghellini ME, Waugh MM, Radewald KC, Kim DH, Ferrin DM, Turini T (2004b) Crop residue destruction strategies that enhance rather than inhibit reproduction of *Monosporascus cannonballus*. *Plant Pathology* **53**, 50-53

Stipanovic RD, Zhang J, Bruton BD, Wheeler MH (2004) Isolation and identification of hexaketides from a pigmented *Monosporascus cannonballus* isolate. *Journal of Agricultural and Food Chemistry* **52**, 4109-4112

Traka-Mavrona E, Koutsika-Sotiriou M, Pritsa T (2000) Response of squash (*Cucurbita* spp.) as rootstock for melon (*Cucumis melo* L.). *Scientia Horticulturae* **83**, 353-362

Troutman JL, Matejka JC (1970) Three fungi associated with cantaloupe roots in Arizona. *Phytopthology* **60**, 1317

Tsay JG, Tung BK (1995) The occurrence of Monosporascus root rot/vine decline of muskmelon in Taiwan. *Plant Pathology Bulletin* **4**, 25-29

Uematsu S, Sekiyama K (1990) Comparison of morphological characateristics and pathogenicity of *Monosporascus cannonballus* Pollack *et* Uecker collected in Japan, distribution in melon plants with root rot symptoms, and survival in soils under laboratory conditions. *Soil Microorganism* **35**, 7-12 (in Japanese with English abstract)

Uematsu S, Onogi S, Watanabe T (1985) Pathogenicity of *Monosporascus cannonballus* Pollack *et* Uecker in relation to melon root rot in Japan. *Annals of the Pytopathological Society of Japan* **51**, 272-276

Watanabe T (1979) *Monosporascus cannonballus*, an ascomycete from wilted melon roots undescribed in Japan. *Transects of the Mycological Society of Japan* **20**, 312-316

Waugh MM, Stanghellini ME, Kim DH (2001) Scanning electron microscopy of germinated ascospores of *Monosporacus cannonballus*. *Mycological Research* **105**, 745-748

Waugh MM, Kim DH, Ferrin DM, Stanghellini ME (2003) Reproductive potential of *Monosporascus cannonballus*. *Plant Disease* **87**, 45-50

Wheeler MH, Bruton BD, Puckhaber LS, Zhang J, Stipanovic RD (2004) Identification of 1,8-dihydroxynaphthalene melanin in *Monosporascus cannonballus* and the analysis of hexaketide and pentaketide compounds produced by wild-type and pigmented isolates of the fungus. *Journal of Agricultural and Food Chemistry* **52**, 4113-4120

Wells CE, Eissenstat DM (2003) Beyond the roots of young seedlings: the influence of age and order on fine root physiology. *Journal of Plant Growth Regulation* **21**, 324-334

Wolff DW (1995) Evaluation of melon germplasm for resistance to *Monosporascus* root rot/vine decline. In: Dunlap JR, Lester GE (Eds) *Cucurbitaceae '94, Evaluation and Enhancement of Cucurbit Germplasm*, Gateway Printing, Edinburg, Texas, USA, pp 224-228

Wolff DW (1996) Genotype, fruit load, and temperature affect *Monosporascus* root rot/vine decline symptom expression in melon. Cucurbits Towards 2000. *Proceedings of the VIth Eucarpia Meeting on Cucurbits Genetics and Breeding*. Málaga, Spain, pp 280-284

Wolff D, Miller M (1998) Tolerance to *Monosporascus* root rot and vine decline in melon (*Cucumis melo* L.) germplasm. *Hortscience* **33**, 287-290

Zobel RW (1995) Genetic and environmental aspects of roots and seedlings stress. *HortScience* **30**, 1189-1192