Effects of wounding, humidity, temperature, and inoculum concentrations on the severity of corky dry rot caused by *Fusarium semitectum* in melon fruits

Michelle Jardelina de Oliveira¹, Delson Laranjeira¹, Marcos Paz Saraiva Câmara¹, Francisco Ferraz Laranjeira², Josep Armengol³ and Sami Jorge Michereff¹

¹Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, 52171-900, Recife, Pernambuco, Brazil. ²Laboratório de Fitopatologia, Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil. ³Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Valencia, Spain. *Author for correspondence. E-mail: sami@depa.ufrpe.br

ABSTRACT. Corky dry rot, caused by *Fusarium semitectum*, is the main postharvest disease of melons in Brazil. This study investigated the effects of wounding, humidity, temperature, and inoculum concentration on the severity of corky dry rot under controlled conditions. Cantaloupe and honeydew melon types were inoculated by spraying conidial suspensions of three *F. semitectum* isolates. In all experiments, the tested *F. semitectum* isolates did not differ in relation to disease severity, but, the cantaloupe melon showed higher levels of severity. No lesions appeared on fruits that lacked wounds, and increasing wound age reduced lesion severity. Melons that were inoculated with *F. semitectum* developed symptoms regardless of the presence or absence of a moist chamber at the post-inoculation stage, but the lesions were larger under moist chamber conditions. There were no symptoms at 10°C, but a temperature increase from 15 to 25°C resulted in a disease severity increase. The largest lesions were observed when both melon types were inoculated with a concentration of 10⁶ conidia mL⁻¹, but even the lowest concentration (10¹ conidia mL⁻¹) was sufficient for causing lesions. Injury reduction and/or the acceleration of melon healing, as well as environmental variable control and a reduction of inoculum sources, are essential to reducing corky dry rot severity.

Keywords: *Cucumis melo*, fruit rot, postharvest pathology, epidemiology.

Efeito de ferimento, umidade, temperatura e concentração de inóculo na severidade da podridão seca causada por *Fusarium semitectum* em frutos de melão

RESUMO. A podridão seca causada por *Fusarium semitectum* é a principal doença pós-colheita do melão no Brasil. Este estudo investigou o efeito de ferimento, umidade, temperatura e concentração de inóculo na severidade da podridão seca em condições controladas. Frutos de melão dos tipos cantaloupe e honeydew foram inoculados por meio da pulverização de suspensão de conídios de três isolados de *F. semitectum*, inoculados separadamente. Em todos os experimentos os isolados de *F. semitectum* testados não diferiram em relação à severidade da doença, porém, a variedade cantaloupe apresentou maiores níveis de severidade da doença. Não houve lesões em frutos sem ferimento e o incremento na idade do ferimento reduziu a severidade. Melões inoculados com *F. semitectum* desenvolveram sintomas independentemente de câmara úmida pós-inoculação, mas as lesões foram maiores em condições de câmara úmida. Não houve sintomas a 10°C, mas o aumento da temperatura de 15 para 25°C resultou num incremento da severidade da doença. As maiores lesões foram observadas na concentração de inóculo de 10⁶ conídios mL⁻¹ nos dois tipos de melão, mas a menor concentração (10¹ conídios mL⁻¹) foi suficiente para causar lesões. A diminuição de injúrias nos frutos de melão e/ou a aceleração da cicatrização, bem como o controle das variáveis ambientais e a redução das fontes de inóculo são essenciais para redução da severidade da podridão seca.


Introduction

Melon (*Cucumis melo* L.) is an economically important crop in Brazil, where it represents the second largest Brazilian fresh fruit export, and places the country among the top ten melon exporters in the world. The northeastern region of Brazil accounts for 85% of the country’s melon production and covers approximately 14,900 ha. The main producing areas are located in the States of Rio Grande do Norte, with 7,582 ha resulting in an annual production of 201,250 ton, and Ceará, with 4,304 ha resulting in an annual production of 124,157 ton (AGRIANUAL, 2012).

Postharvest rots cause severe losses in Brazilian melon production. It is estimated that between 10 to...
30% of postharvest losses are due to corky dry rot, which is caused by *Fusarium semitectum* Berk. & Ravenel. (Synonym: *Fusarium incarnatum* (Roberge) Sacc., *Fusarium pallidoroseum* (Cooke) Sacc.). This disease was detected in 1999 in melons from Rio Grande do Norte State, and its incidence has progressively increased (TERAO et al., 2008).

Corky dry rot is a major cause of cantaloupe melon losses (*C. melo* var. *cantaloupenis* Naud.) (BRUTON, 1995; 1996; CARTER, 1979; MCGOVERN, 1994), but the rot also affects honeydews (*C. melo* var. *inodorus* H. Jacq.) and several other melon varieties (BRUTON, 1995; 1996; DUTHIE, 1996; BRUTON et al., 1998; BRUTON; DUTHIE, 1996; DIAS; TERAO, 2006).

Disease symptoms may appear in the field (at the preharvest phase), close to fruit maturity, or in the postharvest phase. Lesions are variable in length, from 2 mm to 8 cm, and they also vary in depth, from 2 mm to 3 cm. They are especially prevalent on the stems and the blossom ends of the fruit, but they can occur on any area of the fruit. Evidence of this disease consists of a thickening of the net and/or development of corky tissue along the vein tracts. Cracks are often found in large lesions. Under high humidity, a light pink to beige mycelium appears on the rind surface immediately above the corky lesion. Affected zones will first appear as small white spongy areas beneath the rind. In time, these areas will turn dark brown, dry, and mealy. In large lesions, the brown tissue is surrounded by a white, moist, spongy tissue that extends into the flesh of the fruit. A distinct margin separates the diseased and healthy tissues, and the lesion can easily be removed intact from the flesh (BRUTON; DUTHIE, 1996; CARTER, 1979; MCGOVERN, 1994).

Controlling corky dry rot is very difficult. Therefore, it is essential to adopt integrated management strategies. There is no known resistance to corky dry rot (MCGRATH, 2004). Preharvest fungicide applications have been somewhat ineffective because of the difficulty of reaching sufficient fruit coverage. Attempts at postharvest control have also yielded erratic results. Using fungicides in combination with a hot-water treatment (57°C for 1 min.) has provided some success in controlling the disease, but study results on these combined treatments are inconclusive. Wounding avoidance during harvest and packing, proper storage and transit temperatures, and prompt handling of melons upon arrival at the market can provide some protection against postharvest decay (BRUTON, 1995; 1996; DUTHIE, 1996). More recently, the control of corky dry rot through the activation of systemic resistance (BI et al., 2010; GE et al., 2008; GONDIM et al., 2008) and the use of ethylene inhibitors (ALVES et al., 2005; TERAO et al., 2009) have been investigated, but with highly variable results.

Brazilian melons that are exported, particularly those that are destined for the European Union countries, are required to be of high quality, undamaged, and phytopathogen-free. Corky dry rot represents a serious obstacle to the commercialization of melons, particularly as foreign commodities (GONDIM et al., 2008). Melon fruits that are affected by corky dry rot are not marketable. Although a working knowledge about the conditions that favor disease development is essential to improve disease management strategies, little information is available on the epidemiology of corky dry rot in melon (BRUTON; DUTHIE, 1996).

The present study was undertaken to evaluate the effects of wounding, humidity, temperature, and inoculum concentrations of *F. semitectum* on the severity of corky dry rot in melons under controlled conditions.

**Material and methods**

**Fungal isolates**

Three single-spore *F. semitectum* isolates (CMM-589, CMM-685 and CMM-687) were used for all experiments. The isolates were obtained from melon fruits that were exhibiting symptoms of corky dry rot in three growing areas of northeastern Brazil, which are located in the agricultural area of Mossoró (Rio Grande do Norte State). The isolates were morphologically identified as *F. semitectum* after being grown on potato dextrose agar (PDA) (Acumedia Co., Lansing, MI, USA) and Spezieller Nährstoffärmer agar (SNA) culture media (LESLIE; SUMMERELL, 2006) for 10 days at 25°C with a 12-h photoperiod. Additionally, part of the translation elongation factor 1-alpha (EF1-α) gene was used to confirm the identity of this *Fusarium* species through a BLAST DNA sequence database search (http://isolate.fusariumdb.org). A representative sequence of the DNA region of study from isolate CMM-687 was deposited at GenBank (Q004800). This sequence had a 99.06% similarity with the sequence of *F. semitectum* (= *F. pallidoroseum*) (NRRL 31160).

All experiments were conducted twice. Prior to the experiments, the isolates were cultivated in Petri dishes containing PDA and incubated at 25 ± 2°C with a 12-h photoperiod using fluorescent light (40 μmol m−2 s−1) for 10 days.
Fungal inoculum was prepared by gently rinsing the agar surface with sterile water and, then, filtering the resulting mycelium and conidia suspensions through four layers of cheesecloth. The conidia suspensions were counted with a hemocytometer and adjusted by adding sterile tap water. Before inoculation, Tween 20 (0.05%) was added to the inoculum. The germination of each batch of inoculum was measured prior to inoculation to ensure conidia viability. Suspensions with less than 90% conidia germination were discarded.

Fruits

Melon fruits from cantaloupe (cv. Torreon) and honeydew (cv. Orange Flesh) types were harvested from a commercial plantation at the ripening stage and selected for uniformity of size, ripeness, and absence of injuries. These fruits were used in all the experiments. Before inoculation, the fruits were carefully washed, surface-disinfested by immersion in a 1.5% NaOCl solution in sterile water for 5 min., rinsed twice with sterile distilled water, and allowed to air dry on a laboratory bench.

Wounding effects

Wounded and non-wounded fruits were used in this experiment. To make wounded fruits, the epidermis was punctured at four equidistant points. Five wounds (3 mm deep and 0.8 mm wide) were made at each point with a sterile needle prior to inoculation. Immediately after wounding, fruits were sprayed with a conidial suspension (2 mL per fruit; 10^6 conidia mL^{-1} in sterile water plus Tween 20 (0.05%)) of each *F. semitectum* isolate. Control fruits were inoculated with sterile water plus Tween 20 (0.05%). The inoculated and control fruits were placed on plastic moist chambers (50 x 30 x 20 cm). To encourage high humidity, the bottom of each moist chamber was lined with a distilled water-soaked paper towels. Trays were kept in growth chambers at 25 ± 2°C under fluorescent light (12-h photoperiod, 40 μmol m^{-2} s^{-1}). Fruits were removed from the moist chambers after 48h of incubation and were kept at the same temperature for an additional 3-day period. The lesion size (mm²) at each inoculated point was measured 5 days after inoculation by measuring its length and width in two perpendicular directions. The full factorial combination of the treatments (3 x 2 x 2), which are represented by three *F. semitectum* isolates, two melon varieties and wounded and non-wounded fruits, was arranged in a completely randomized design with four replicates per treatment and five fruits per replicate.

Wound age effects

Wounded fruits were sprayed with a conidial suspension (2 mL per fruit; 10^6 conidia mL^{-1} in sterile water plus Tween 20 (0.05%)) of each *F. semitectum* isolate. The inoculations were carried out immediately after wounding, and 12 and 24h after. The control fruits were inoculated with sterile water and Tween 20 (0.05%). The incubation conditions and disease evaluation were carried out the same way as described in the experiment on wounding effects. The full factorial combination of treatments (3 x 2 x 3), which represented three *F. semitectum* isolates, two melon varieties and three wound ages, was arranged in a completely randomized design with four replicates per treatment and five fruits per replicate.

Humidity effects

Wounded fruits were inoculated with *F. semitectum* isolates by spraying (2 mL per fruit; 10^6 conidia mL^{-1} in sterile water plus Tween 20 (0.05%)) the fruits immediately after wounding. Control fruits were inoculated with sterile water and Tween 20 (0.05%). The inoculated and control fruits were subjected to two different conditions: incubation in moist chambers or incubation without moist chambers, both for 48h. The incubation conditions, experimental design and disease evaluation were the same as described for the wounding effects experiment.

Temperature effects

Fruits were wounded and inoculated with *F. semitectum* isolates by spraying (2 mL per fruit; 10^6 conidia mL^{-1} in sterile water and Tween 20 (0.05%)) the fruits immediately after wounding. The control fruits were inoculated with sterile water and Tween 20 (0.05%). The inoculated and control fruits were placed in moist chambers and, then, incubated in growth chambers at 5, 10, 15, 20, 25, 30 and 35°C under fluorescent light (12-h photoperiod, 40 μmol m^{-2} s^{-1}). After 48h of incubation, the fruits were removed from the moist chambers and left for an additional 3-day period at the same temperatures. The disease evaluation was performed as described for the wounding effect experiment. The full factorial combination of treatments (3 x 2 x 7), which represented three *F. semitectum* isolates, two melon varieties and seven temperatures, was arranged in a completely randomized design with four replicates per treatment and five fruits per replicate.

Inoculum concentration effects

Fruits were inoculated by spraying them with a conidial suspension (2 mL per fruit) of each *F. semitectum* isolate immediately after wounding,
at six different concentrations ($10^1, 10^2, 10^3, 10^4, 10^5, 10^6$ conidia mL$^{-1}$ in sterile water and Tween 20 (0.05%)). Control fruits were inoculated with sterile water and Tween 20 (0.05%). The incubation conditions and disease evaluation were the same as those described in the experiment on wounding effects. The full factorial combination of treatments ($3 \times 2 \times 6$), which represented three $F. \text{semitectum}$ isolates, two melon varieties and six inoculum concentrations, was arranged in a completely randomized design with four replicates per treatment and five fruits per replicate.

**Statistical analysis**

Analyses of variance (ANOVARs) were conducted with data that were obtained from the experiments on the effects of wounding, wound age, humidity, temperature, and inoculum concentration. In all cases, ANOVA analyses indicated that the data between the two repetitions were similar ($p > 0.05$), and thus the data from all variables in both experiments were combined. First, the data from each experiment were subjected to three-way ANOVA analyses. In all experiments, the $F. \text{semitectum}$ isolates did not differ significantly ($p > 0.05$) among one another in relation to the levels of disease severity that they could induce, and significant interactions with melon types were not found. On the other hand, significant differences in disease levels between the different melon types were found in all experiments. Therefore, only the isolates' averages within each melon type were considered across all statistical analyses. The wounding and humidity treatments were compared using Student's t-test ($p \leq 0.05$), whereas the wound age treatments were compared using Fisher's LSD test ($p \leq 0.05$). These statistical analyses were performed with Statistix v. 9.0 software (Analytical Software, Tallahassee, FL, USA). Data from the temperature and inoculum concentration experiments were analyzed by fitting linear and nonlinear regression models with the aid of TableCurve 2D v. 5.01 software (SYSTAT Software Inc., Chicago, IL, USA). The best-fit curves showing corky dry rot severity (lesion size) were selected on the basis of the coefficient of determination ($R^2$) and the mean squared residue. The significance of the regressions and their parameters were verified by F test ($p \leq 0.05$).

**Results and discussion**

This is the first study in which the effects of wounding, humidity, temperature, and $F. \text{semitectum}$ inoculum concentration have been determined with relation to the severity of corky dry rot in melon fruits.

The three $F. \text{semitectum}$ isolates did not differ significantly ($p > 0.05$) among themselves in relation to the levels of induced disease severity in any of the experiments. High levels of intraspecific variability exist within $F. \text{semitectum}$ isolates that are obtained from a single host, such as cotton (ABD-ELSALAM et al., 2003), alfalfa (ZACCARDELLI et al., 2006) and red-fleshed dragon fruit (HAVA et al., 2010). In our case, the occurrence $F. \text{semitectum}$ isolates with similar virulence levels in melon fruits may be due to the low number of isolates because they all came from the same agricultural region.

Regarding the effects of different melon varieties, the cantaloupe showed significantly ($p \leq 0.05$) higher levels of corky dry rot severity in all experiments than those of the honeydew (Figures 1A, B, C, 2 and 3). The susceptibility difference between these melon varieties may be associated with the structural characteristics of the fruit epidermis. Honeydew melons have a considerably thicker epidermis and no net in comparison to cantaloupes. The membrane integrity of the hypodermal tissue in melon fruits appears to be highly correlated to their shelf life (LESTER, 1988).

**Wounding effects**

There was no appearance of symptoms in melon fruits when inoculations were performed without wounding (Figure 1A), indicating that the pathogen is unable to penetrate the fruit directly. Fruit injuries were essential to initiating the infection process, which facilitated pathogen penetration. In addition, injured tissue increases the metabolic activity of injured cells (GUZMÁN et al., 1999), leading to an elevated respiration rate, induction of ethylene synthesis, and increased water loss, resulting in accelerated fruit deterioration (ADASKAVEG et al., 2002; JACOMINO et al., 2004). In the field, melon fruits can suffer various forms of injuries during crop management, such as when fruits are dragged on the ground, through interactions with cutting instruments or small stones, or by the activity of insects, which tend to scrape the surface, leave pits, or penetrate the fruits in their search for food (VIANA et al., 2001). It is also worth noting that the penetration of melon fruits by $F. \text{semitectum}$ can also occur through natural openings, including lenticels or cracks in the epidermis that are associated with net development (BRUTON et al., 1998).
Wound age effects

Fruit wound ages significantly influenced the severity of corky dry rot. This factor was also found to reduce the lesion size with increasing wound age (Figure 1B). In both melon varieties, the lesions were larger in fruits that were immediately inoculated after wounding than in the other treatments. This may be a consequence of increased water availability in the form of exudates that were released soon after the injury. The healing process may also have influenced the disease severity because the cantaloupe produces lignin and suberin during tissue healing, depositing these compounds on the walls of injured cells to form a lesion periderm (JACOMINO et al., 2004), which may provide a structural resistance mechanism against infection. Another aspect to consider is that fresh wounds generally accelerate the rate of respiration and ethylene synthesis because of the ensuing high metabolic activity of cell wounds that occur within minutes after cutting (GUZMÁN et al., 1999). The lower severity that was observed in older wounds can also be attributed to an accumulation of phytoalexins and pathogenesis-related (PR) proteins in response to mechanical injury, which can inhibit pathogen actions. Phytoalexins are produced by healthy cells near the injury site in response to substances that are produced in damaged cells, which accumulate and can provide resistance to the corky dry rot fungus (ADASKAVEG et al., 2002).

Humidity effects

The melon fruits that were inoculated with *F. semitectum* developed symptoms of corky dry rot regardless of the presence or absence of a moist chamber (Figure 1C), indicating that free water was not needed, at least not in the form of condensation on the fruit surface, for spore germination and host penetration. This result contrasts with the observation that the presence of a film of water is essential for *F. semitectum* spore germination (PEREZ; VIDAL, 2002). The occurrence of infections by this pathogen in the absence of a moist chamber indicates that the mere moisture that is associated with exudates that were released by the fruit after wounding was sufficient to start the processes that were involved in pathogenesis, as also found in yellow melon fruits inoculated with the bacterium *Acidovorax avenae subsp. citrulli* (Schaad et al.) Willems et al. (SILVEIRA et al., 2004). Moreover, it is important to consider that *F. semitectum* is resistant to low humidity conditions and can remain in the field for long periods under conditions of extreme drought (BADGER, 1965).

Corky dry rot lesions were higher in fruits that were subjected to a moist chamber (Figure 1C), indicating that high moisture stimulates the infection process by *F. semitectum* and/or increases host susceptibility, influencing the rate of disease progression and demonstrating the greatest severity, as has been observed previously in banana fruits.
after harvest (PEREZ; VIDAL, 2002). The moisture loss of fresh produce is determined by relative humidity. High levels of humidity help to maintain fruit turgidity and to reduce water loss but may also be conducive to disease development (ADASKAVEG et al., 2002). In the melon-producing areas of northeastern Brazilian, there is no high humidity occurrence during the day. However, there are several short periods of wetness at night. This dampness may be sufficient to stimulate the *F. semitectum* infection process, as also happens in other pathosystems in semi-arid regions (ROTEM, 1978). The dew formation that occurs as a result of differences between day and night temperatures also provides periods of wetness in the early hours of the day. Furthermore, interruptions in periods of wetness by dry days may be offset by the moisture that is added by some cultural practices that are used by northeastern Brazilian melon producers, such as drip irrigation, plastic mulch, and row covers made from agrotextiles.

### Temperature effects

The temperature significantly influenced the severity of corky dry rot in melon fruits. In general, the disease severity increased as temperature increased from 15 to 25°C, whereas lesions were smaller when the temperature was higher than 25°C (Figure 2). There were no symptoms at 10°C (Figure 2), confirming observations about the importance of refrigerating fruits at that temperature to control corky dry rot. However, refrigeration is not lethal to *F. semitectum* because the disease begins to develop normally again after the fruits are removed from the cold (TERAO et al., 2006). In addition to influencing fruit metabolism, the temperatures may also possibly affect pathogen metabolism and the kinetics of enzymes that are involved in the infection process, which increase or reduce activity depending on the prevailing conditions, which, in turn, affect disease development (ADASKAVEG et al., 2002).

The logistic model with an asymmetric power peak (Figure 2) provided excellent fit curves to describe the progress and severity of corky dry rot with respect to the temperature, with coefficients of determination (R²) of 99.9 and 98.6% for cantaloupe and honeydew melon, respectively. The chosen model had four parameters (a, b, c, d), and the first two parameters are of great biological interest because they reflect the maximum estimated lesion size (a) and the temperature at which the maximum lesion size occurred (b). The cantaloupe displayed a maximum estimated lesion size (421.6 mm²) that was significantly (p ≤ 0.05) larger than in the honeydew (254.9 mm²), when the regression parameter was compared by t-test using the confidence interval. On the other hand, the two melon varieties did not differ significantly (p > 0.05) in view of the optimum temperature for the disease, for which the estimated values were 23.0 and 23.9°C for the cantaloupe and honeydew, respectively (Figure 2).

![Figure 2](image.png)

**Figure 2.** Effect of temperature on corky dry rot severity (lesion size) in fruits of cantaloupe (cv. Toreon) and honeydew (cv. Orange Flesh) melon types inoculated with *Fusarium semitectum*. Values are presented as the means from two independent experiments. Temperatures and disease severity were related by the nonlinear regression analysis using the logistic power peak model, shown at the base of the graphs.

The optimum temperatures that were estimated for the development of corky dry rot in this study were lower than 30°C, which was previously considered to be more favorable for disease development in melon fruits (DIAS; TERAO, 2006). In banana fruits, higher crown rot severity caused by *F. semitectum* was observed to occur at temperatures between 25 and 27°C, whereas the disease development was strongly inhibited below 15°C, and no symptoms were observed at 38°C (PEREZ; VIDAL, 2002). Melon fruits undergo pre-cooling before export to lower their temperature to 10-15°C, and this action could be considered to be an efficient control of corky dry rot at postharvest. However, the transport trucks that serve the domestic Brazilian market lack refrigeration, as do the supermarkets and/or outdoor markets, which favor the incidence of corky dry rot.

### Inoculum concentration effects

The inoculum concentration of *F. semitectum* significantly influenced the severity of corky dry rot in melon fruits, and lesion sizes increased with
increasing inoculum concentrations. Larger lesions were observed at a concentration of $10^6$ conidia mL$^{-1}$ in both varieties of melons, but even the lowest concentration of inoculum ($10^1$ conidia mL$^{-1}$) (Figure 3) was sufficient for causing lesions. The incidence of corky dry rot, even with very low inoculum concentrations, indicates the high infection potential of *F. semitectum* spores.

![Figure 3. Effect of *Fusarium semitectum* inoculum concentrations on corky dry rot severity (lesion size) in fruits of cantaloupe (cv. Toreon) and honeydew (cv. Orange Flesh) melon types. Values are presented as means from two independent experiments.](image)

The single linear model ($y = a + bx$) provided excellent curve fits to describe the severity and progress of corky dry rot with respect to the inoculum concentrations of *F. semitectum*, with coefficients of determination ($R^2$) at 99.9 and 98.6% for cantaloupe and honeydew melons, respectively (Figure 3). The cantaloupe experienced an estimated rate of lesion size progress (64.19 mm$^2$ per spore concentration unit) that was significantly ($p \leq 0.05$) higher than the honeydew (34.32 mm$^2$ per spore concentration unit), when the regression parameter was compared by $t$-test using the confidence interval, once again demonstrating the high sensitivity of the cantaloupe to corky dry rot in comparison to the honeydew.

The increased severity of corky dry rot in melons with increasing concentrations of *F. semitectum* inoculum highlights the importance of reducing the inoculum to minimize the risk of epidemics during the postharvest period, which may be achieved by measures that reduce the level of inoculum in the field, packing house and in storage (DIAS; TERAO, 2006). Considering that melon fruits that are left in the field can be a source of *F. semitectum* inoculum, the elimination of infected fruits during and after each crop season is a very important measure for preventing the spread of disease.

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**Conclusion**

Management practices involving the reduction of injury to fruits and/or the acceleration of wound healing are essential for reducing the severity of corky dry rot in the postharvest treatment of melon fruits.

The control of environmental variables, such as humidity and temperature, as well as the reduction of inoculum sources can help to reduce the severity of corky dry rot in melons.

**References**


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