

Cucurbit Host Range of *Myrothecium roridum* Isolated from Watermelon

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ABSTRACT

In 2010, a foliar and stem-lesion disease that produced moderate to severe defoliation of watermelon was observed in the southern Great Plains. The disease was ultimately determined to be caused by *Myrothecium roridum*. The objective of this study was to compare the susceptibility of the vegetation and fruit of a broad range of commercially important cucurbits to three isolates obtained from these foliar lesions on watermelon. In greenhouse foliar inoculation experiments, cantaloupe, honeydew, cucumber, squash, and watermelon were susceptible to the fungus with cantaloupe and honeydew being the most susceptible and watermelon the most resistant. Furthermore, greenhouse inoculations supported earlier field observations as differential resistance was exhibited among the watermelon cultivars as well as the cucurbit types. All tested cucurbit fruit exhibited interior lesions when inoculated sub-epidermally with *M. roridum* isolates. However, natural infection of watermelon and pumpkin fruit has never been reported.

Keywords: *Myrothecium roridum*; Watermelon; Leaf and Stem Blight; Cucurbits

1. Introduction

Myrothecium roridum Tode ex Fr. is a common soil-inhabiting fungus with a relatively wide host range that includes such agronomic crops as cotton, tomato, cacao, coffee, potato, soybean, and cucurbits, as well as various ornamental plants [1-3]. *M. roridum* has been demonstrated to be seed-transmitted in numerous cases, including cucurbits [4-7], and has been evaluated for biocontrol of water hyacinth [3,8]. Diseases caused by *M. roridum* are generally thought to be associated most frequently with warmer environments during wet conditions [1,9, 10]. In contrast, *M. roridum* was recently reported as an endophyte of the gymnosperm, *Pinus albicaulis*, at high elevation in Oregon [11].

The first known record of *M. roridum* on cucurbits appears to be from Mexican cantaloupe (*Cucumis melo*) intercepted at the Texas border in 1950 and later in the Rio Grande Valley of Texas in 1961 [10]. Although watermelon (*Citrullus lanatus*) has been reported as a host for *M. roridum* in greenhouse testing [3,6,12], the first report of *M. roridum* causing a leaf spot in watermelon under field conditions was from Korea in 2003 [13]. The disease was subsequently reported in Georgia (USA) in 2005 [14] and in Oklahoma (USA) in 2012 [15].

Greenhouse studies have demonstrated that isolates of *M. roridum* from diseased field plants of cucumber (*Cu-*

cumis sativus), gherkin (*Cucumis anguria*), and squash (*C. moschata*) caused disease symptoms on watermelon, pumpkin, and cantaloupe as well as the original host plants [12]. Cabral *et al.* [12] noted that cucurbit isolates demonstrated differential aggressiveness when inoculated onto cucumber, squash, watermelon, and cantaloupe. They further stated that watermelon was the least susceptible of the cucurbits tested. Bean *et al.* [16] reported that a *M. roridum* isolated from cantaloupe fruit produced the trichothecene, roridin E, and later demonstrated that the presence of this mycotoxin was related to lesion size [17]. A correlation between sensitivity to roridin E produced by some isolates of *M. roridum* and level of resistance exhibited by cucurbits has been reported [17,18].

Three distinct phases of disease caused by *M. roridum* have been observed in cucurbits: leaf spot, crown and stem canker, and fruit rot, also known as crater rot [1]. In 2010 at the Wes Watkins Agricultural Research Laboratory, Lane, OK, an outbreak of leaf spot and stem canker occurred in a 0.5 hectare experimental field in which were growing twenty different cultivars of watermelon. This disease was ultimately determined to be caused by *Myrothecium roridum* [15]. The objective of this study was to determine the susceptibility of selected cucurbits to the fungus, specifically *M. roridum* isolates from these diseased watermelon plants.

2. Materials and Methods

2.1. Isolation and Determination of *M. roridum*

Isolations were performed from leaf lesions of three watermelon plants that exhibited moderate to severe disease symptoms in the field [15]. Diseased tissue was plated out on potato dextrose agar (PDA) and incubated for 5 days under laboratory conditions. Mycelia from colonies that emerged from the plated leaf tissue were hyphal-tipped and transferred to PDA. The *M. roridum* isolates from watermelon vegetation were designated 46-100117, 46-100137, and 46-100138.

2.2. Host Range of Commercially Important Cucurbits

Pathogenicity tests, using *M. roridum* isolates 46-100117, 46-100137, and 46-100138 from watermelon vegetation were carried out on healthy seedlings of various cucurbits. Watermelon cultivars tested were "AC 7177", "Dixie Lee", "Sangria", and "Sugar Baby". Cantaloupe cultivars tested were "Bella Toscana", "Caravelle", and "Magnum 45". Cucumber cultivars tested were "Dasher II" and "Poinsett 76". The squash (*Cucurbita pepo*) cultivar tested was "Lemon Drop". The honeydew cultivar tested was "TamDew Improved". Seedlings were planted, grown, and tested in Speedling trays (Speedling Inc., Sun City, FL) (8 rows of 16 cells). Every other 16 cell row in the tray was left empty so that a flat contained up to 64 seedlings at the two true-leaf stage. Rows of seedlings of each cultivar were randomly grown among the flats so that a total of 34 - 40 plants (samples) of each of the eleven cucurbit cultivars were tested. A suspension of 1×10^6 conidia per ml was applied to leaves and stems with a Nalgene aerosol spray bottle (Thermo Scientific, Rochester, NY). Control plants (17 - 23 plants of each of the eleven cultivars) were sprayed with sterile distilled water. After treatment, flats of plants were sealed inside a plastic dew chamber at 21°C - 29°C and 100% humidity for 17 hr. Flats were then removed from the chamber and placed in the greenhouse. PDA plates were sprayed with the same suspension and placed in the plastic dew chambers along with the inoculated plants to make sure the environmental conditions and 17 hr time-frame were conducive to conidial germination. To this end, more than 95% of the spores had germinated and a germ-tube at least 3 times longer than the spore diameter had formed after 17 hr. Temperatures in the greenhouse ranged between 30°C ± 6°C during the day and 21°C ± 2°C at night. Plants were watered twice daily. The experiment was conducted twice for each of the three *M. roridum* isolates.

2.3. Disease Ratings and Statistical Analyses

Disease ratings were made on treated plants and their

controls 7 days after inoculation. The disease rating system employed was an interval scale of 0 to 4 with 0 being healthy; 1 = 1% to 25% of the leaf or cotyledon exhibiting leaf spot, 2 = 26% to 50%, 3 = 51% to 75%, and 4 = 76% to 100%. Separate disease ratings were made on the cotyledons and the first two true-leaves. The experimental design was a factorial with two replications of each treatment combination of fungal isolate and cucurbit host and 34 - 40 samples (plants) for each treatment combination. Analyses of variance were performed to determine significance of main effects and interaction for ratings of cotyledons and true-leaves using the Kenward-Roger method to compute denominator degrees of freedom. Least squares means were computed and compared at $P \leq 0.05$ using Tukey's adjustment. Normally, more than 2 replications are desirable. The interaction of isolates with cultivars was significant at $P \leq 0.05$ with just 2 replications in an experiment designed to determine if there were effects of isolate, cultivar or the interaction of isolates with cultivars.

2.4. Inoculation of Various Cucurbit Fruit with the Fungal Isolates from Watermelon

The host range of various cucurbit fruit to *Myrothecium roridum* was examined by inoculation with the three fungal isolates. Watermelon ("Jubilee"), cantaloupe ("Caravelle"), and pumpkin ("Fall Splendor") (*Cucurbita pepo*), grown at the Lane Research Center, were used for fruit inoculations. Cucumber fruit (cv. unknown) were purchased from a local retail grocery. The fruit were washed using warm water and dish soap and then allowed to dry. Each of 4 to 6 fruit representing the various cucurbits was inoculated at multiple sites (3 to 5) with one of the three fungal isolates by a procedure previously described [19]. Fruit were inoculated by surface disinfecting with 80% ethanol and removing a cylinder of tissue aseptically (1 cm deep) with a cork borer (0.7 cm diameter). The isolates were grown on PDA for 7 days at 25°C prior to inoculation. A PDA disc (0.5 cm diameter) colonized by the fungus was placed into each inoculation site, covered with a small autoclaved cotton ball, and sealed with Kwik Seal Caulk (DAP Inc., Dayton, Ohio). Fruit inoculated with PDA discs without the fungus served as controls. The inoculated fruit were maintained on the laboratory bench at 25°C ± 2°C. After 6 days, the fruit were cut perpendicular to the inoculation sites and the resulting lesions were traced onto transparent film. The area of fungal decay was calculated using an area meter (Li-Cor, Lincoln, NE, USA) and analyzed as a completely randomized factorial experiment, with factors being type of cucurbit and pathogen isolate.

3. Results

Fungal colonies obtained from the diseased watermelon

leaf lesions reached 40 - 60 mm in diameter after 14 days at 25°C. The fungal colonies were white, floccose, wrinkled, and somewhat raised in the center. Sporulation occurred throughout the colony in concentric greenish-black zones. These zones consisted of groups of conidiophores forming sporodochia. Conidia were rod-shaped (1.5 - 2 µm × 5 - 10 µm). The characteristics of the fungus were consistent with those reported for *M. roridum* [9,20]. When sections of symptomatic tissue from test seedlings were plated out (see below), they yielded only *M. roridum* colonies, thereby fulfilling Koch's Postulates.

Healthy seedlings of several cucurbits were subjected to the *M. roridum* isolates to examine the vegetative host range of the fungus. The progress of the disease after 7 days consisted of small tan lesions 1 - 3 mm across on cotyledons and leaves; all five types of cucurbits exhibited some disease symptoms. For cotyledons, there was a significant interaction ($P \leq 0.05$) between cultivars and fungal isolates (**Table 1**). However, when grouping the cultivars by cucurbit type, the interaction of fungal isolate and cucurbit type were not significant. Isolates 46-100137 and 46-100138 were significantly ($P \leq 0.05$) more aggressive on cotyledons as compared to 46-100117. "Bella Toscana" cantaloupe was the most highly susceptible cucurbit tested; it ranked in the top 10 of all treatment combinations (**Table 1**). On the other hand, "Sangria" watermelon was the least susceptible cucurbit tested; it ranked in the bottom 5 of all treatment combinations. The average cotyledon disease ratings ranged from 0.18 to 1.51 over the five cucurbit types with cantaloupe having the highest rank and watermelon the lowest.

Analysis of the disease rating on true leaves showed a significant ($P \leq 0.05$) interaction for cucurbit type, isolate, and cucurbit type by isolate. The average true-leaf disease ratings ranged from 0.11 to 1.67 (**Table 2**). Cantaloupe and squash occupied the first 7 ranks of the average disease rating on true leaves. Fungal isolate 46-100137 tended to be the most aggressive on all cucurbit types. Although the leaf ratings were slightly different, they followed a pattern similar to that observed on the cotyledons. Again, "Bella Toscana" exhibited some of the highest levels of disease and "Sangria" watermelon exhibited some of the lowest (**Table 2**).

Table 3 presents the host range of a selection of cucurbit fruit inoculated with *M. roridum*. In contrast to foliar inoculations, watermelon fruit were more susceptible than cantaloupe fruit. All fruit tested exhibited lesions; the average lesion size ranged from 1.19 cm² in cantaloupe to 3.23 cm² in watermelon. There was a significant interaction between isolate and cucurbit type. There was no significant ($P \leq 0.05$) isolate effect in the fruit.

4. Discussion

The recent plethora of first reports of *M. roridum* causing disease on plants here-to-fore not observed to be susceptible [11,13,14,21-23] suggests that either regional change in weather resulting in local growing conditions more conducive to *M. roridum* infection has occurred or an evolution of *M. roridum* sub-species or races has occurred that facilitates a broader host range.

Even though there are numerous reports of *M. roridum* as a pathogen of cucurbits, little is known about the environmental conditions most conducive to disease development. Several reports [1,9,10] suggest that relatively high temperatures and frequent rain events are prerequisite. In contrast, Chase and Poole [24] noted that 21°C to 27°C was optimum for disease development in *Dieffenbachia maculate* and temperatures of 30°C or higher inhibited lesion formation. Although most inoculation studies have used temperatures in the range of 25°C, Fitton and Holliday [9] reported the optimum temperature for conidial germination was 28°C.

Duration of leaf wetness is another relatively unknown requirement for infection and disease development. Incubation periods of 100% RH following inoculation of cucurbits have ranged between 18 - 72 hr [3,6,12,14]. In the present study, a 17 hr incubation period in the dew chamber was maintained before placing the flats in the greenhouse. Within 2 to 3 days, small leaf spots about 1 to 2 mm in diameter could be observed. Once the plants were placed in the greenhouse at about 30°C and $\leq 50\%$ RH, lesions ceased to enlarge. (The average relative humidity recorded in the month during the field disease outbreak was 76% ± 9%) If previously inoculated plants were re-introduced into the dew chamber (100% RH), misted, and allowed to stay an additional 17 hr, the diameter of the lesions increased to 2 - 5 mm (data not presented).

Foliar disease, caused by *M. roridum*, is a relatively easy disease to control with broad spectrum fungicides used at a frequency dictated by the weather [25]. Many of the fungicides normally used in watermelon production should have good activity against *M. roridum* which may be why it has been reported only twice on watermelon in the US [14,15].

All of the cucurbit fruit, including watermelon, that were inoculated in the present study exhibited decay. However, the inoculum was introduced into the fruit following the removal of the epidermal layer. In the Rio Grande Valley of Texas, up to 30% of cantaloupe fruit in the field exhibited the crater rot symptom in one report [26]. Seebold *et al.* [14] did not observe lesions on watermelon fruit in a mildly affected field in Georgia. Although the disease severity in Oklahoma was more severe, fruit lesions also were not observed [15]. Fruit le-

Table 1. Disease ratings of cotyledons of selected cucurbit seedlings inoculated with *M. roridum* isolates from watermelon leaf lesions.

| <i>M. roridum</i> Isolate | Cucurbit Cultivar ² | Disease Score ¹ Least Squares Mean | Disease Score Standard Error | Mean Separation ³ |
|---------------------------|--------------------------------|---|------------------------------|------------------------------|
| 46-100138 | Bella Tuscana (c) | 1.51 | 0.19 | a |
| 46-100137 | Magnum 45 (c) | 1.38 | 0.16 | ab |
| 46-100138 | Poinsett 76 (cu) | 1.26 | 0.19 | abc |
| 46-100138 | TamDew Imp (hd) | 1.19 | 0.15 | abc |
| 46-100138 | Magnum 45 (c) | 1.19 | 0.15 | abcd |
| 46-100137 | AC 7177 (w) | 1.15 | 0.15 | abcd |
| 46-100138 | Caravelle (c) | 1.14 | 0.15 | abcd |
| 46-100137 | Bella Tuscana (c) | 1.08 | 0.15 | abcd |
| 46-100117 | Bella Tuscana (c) | 1.08 | 0.15 | abcd |
| 46-100137 | Dasher II (cu) | 1.03 | 0.15 | abcde |
| 46-100138 | Lemon Drop (s) | 1.00 | 0.16 | abcde |
| 46-100137 | Sugar Baby (w) | 1.00 | 0.15 | abcde |
| 46-100137 | TamDew Imp (hd) | 1.00 | 0.16 | abcde |
| 46-100137 | Caravelle (c) | 0.92 | 0.16 | abcde |
| 46-100137 | Lemon Drop (s) | 0.91 | 0.16 | abcde |
| 46-100117 | Magnum 45 (c) | 0.88 | 0.16 | abcde |
| 46-100117 | Caravelle (c) | 0.82 | 0.16 | abcde |
| 46-100137 | Dixie Lee (w) | 0.82 | 0.16 | abcde |
| 46-100117 | TamDew Imp (hd) | 0.78 | 0.18 | abcde |
| 46-100117 | Dixie Lee (w) | 0.76 | 0.18 | abcde |
| 46-100117 | Poinsett 76 (cu) | 0.75 | 0.16 | abcde |
| 46-100117 | Lemon Drop (s) | 0.72 | 0.15 | abcde |
| 46-100138 | Sugar Baby (w) | 0.71 | 0.15 | abcde |
| 46-100117 | Dasher II (cu) | 0.68 | 0.16 | bcde |
| 46-100138 | Dixie Lee (w) | 0.66 | 0.16 | bcde |
| 46-100137 | Poinsett 76 (cu) | 0.61 | 0.16 | bcde |
| 46-100138 | AC 7177 (w) | 0.57 | 0.16 | bcde |
| 46-100138 | Dasher II (cu) | 0.56 | 0.16 | bcde |
| 46-100138 | Sangria (w) | 0.53 | 0.15 | bcde |
| 46-100117 | AC 7177 (w) | 0.48 | 0.16 | bcde |
| 46-100117 | Sugar Baby (w) | 0.41 | 0.15 | cde |
| 46-100137 | Sangria (w) | 0.33 | 0.16 | de |
| 46-100117 | Sangria (w) | 0.18 | 0.15 | e |

¹The disease rating system employed was an interval scale of 0 to 4 with 0 being healthy; 1 = 1% to 25% of the leaf or cotyledon exhibiting leaf spot, 2 = 26% to 50%, 3 = 51% to 75%, and 4 = 76% to 100%; ²Letters in parentheses stand for the various cucurbits tested: (c) = cantaloupe, (cu) = cucumber, (hd) = honeydew, (s) = squash, (w) = watermelon; ³Least squares means in Means Separation column were compared using Tukey's adjustment. Means not followed by the same letters are significantly different at $P \leq 0.05$.

Table 2. Disease ratings of first two leaves of selected cucurbit seedlings inoculated with *M. roridum* isolates from watermelon leaf lesions.

| <i>M. roridum</i> Isolate | Cucurbit Cultivar ² | Disease Score ¹ Least Squares Mean | Disease Score Standard Error | Mean Separation ³ |
|---------------------------|--------------------------------|---|------------------------------|------------------------------|
| 46-100138 | Lemon Drop (s) | 1.67 | 0.29 | ab |
| 46-100137 | Magnum 45 (c) | 1.45 | 0.12 | acd |
| 46-100137 | Lemon Drop (s) | 1.35 | 0.12 | acde |
| 46-100137 | Bella Tuscana (c) | 1.14 | 0.11 | abcdefgh |
| 46-100138 | Bella Tuscana (c) | 1.14 | 0.29 | cefij |
| 46-100117 | Lemon Drop (s) | 0.88 | 0.19 | cdefghijk |
| 46-100137 | Caravelle (c) | 0.86 | 0.11 | bfg hijklm |
| 46-100137 | Sugar Baby (w) | 0.81 | 0.11 | bfg hijklmn |
| 46-100138 | Caravelle (c) | 0.76 | 0.29 | efghijklno |
| 46-100117 | Bella Tuscana (c) | 0.76 | 0.19 | efghijkl |
| 46-100137 | Dasher II (cu) | 0.75 | 0.12 | fghijklmn |
| 46-100137 | AC 7177 (w) | 0.74 | 0.12 | fghijklmn |
| 46-100138 | Magnum 45 (c) | 0.70 | 0.29 | cdefghijklnop |
| 46-100137 | Dixie Lee (w) | 0.70 | 0.12 | fghijklmnp |
| 46-100117 | Dasher II (cu) | 0.62 | 0.19 | fghijklmno |
| 46-100138 | Sugar Baby (w) | 0.60 | 0.29 | dghklmnopq |
| 46-100138 | Dixie Lee (w) | 0.58 | 0.29 | dghklmnopq |
| 46-100117 | Magnum 45 (c) | 0.57 | 0.20 | fghijklmnop |
| 46-100117 | Caravelle (c) | 0.53 | 0.19 | klmnopqr |
| 46-100137 | TamDew Imp (hd) | 0.50 | 0.12 | ijklmnoprs |
| 46-100117 | Poinsett 76 (cu) | 0.47 | 0.19 | klmnopqr |
| 46-100117 | Sugar Baby (w) | 0.44 | 0.19 | klmnopqr |
| 46-100137 | Poinsett 76 (cu) | 0.39 | 0.11 | jnopqt |
| 46-100117 | AC 7177 (w) | 0.37 | 0.20 | klmnopqr |
| 46-100138 | AC 7177 (w) | 0.33 | 0.29 | ghklmnopqs |
| 46-100117 | Dixie Lee (w) | 0.31 | 0.20 | imnopqr |
| 46-100138 | Dasher II (cu) | 0.30 | 0.29 | ghklmnopqs |
| 46-100138 | Sangria (w) | 0.30 | 0.29 | ghklmnopqs |
| 46-100138 | Poinsett 76 (cu) | 0.28 | 0.29 | hmpqr |
| 46-100117 | Sangria (w) | 0.22 | 0.19 | nptu |
| 46-100137 | Sangria (w) | 0.18 | 0.12 | ortu |
| 46-100117 | TamDew Imp (hd) | 0.13 | 0.19 | ptu |
| 46-100138 | TamDew Imp (hd) | 0.11 | 0.29 | mt |

¹The disease rating system employed was an interval scale of 0 to 4 with 0 being healthy; 1 = 1% to 25% of the leaf or cotyledon exhibiting leaf spot, 2 = 26% to 50%, 3 = 51% to 75%, and 4 = 76% to 100%; ²Letters in parentheses stand for the various cucurbits tested: (c) = cantaloupe, (cu) = cucumber, (hd) = honeydew, (s) = squash, (w) = watermelon; ³Least squares means in Means Separation column were compared using Tukey's adjustment. Means not followed by the same letters are significantly different at $P \leq 0.05$.

Table 3. Area of fruit lesion cross sections on selected cucurbit fruit inoculated with *M. roridum* isolates from watermelon leaf lesions.

| <i>M. roridum</i> Isolate | Cucurbit | Lesion Area Least Squares Mean (cm ²) | Lesion Area Standard Error | Mean Separation ¹ |
|---------------------------|------------|---|----------------------------|------------------------------|
| 46-100137 | watermelon | 3.67 | 0.27 | a |
| 46-100138 | watermelon | 3.64 | 0.27 | a |
| 46-100117 | cucumber | 2.44 | 0.25 | ab |
| 46-100117 | watermelon | 2.39 | 0.27 | ab |
| 46-100137 | cucumber | 2.15 | 0.25 | b |
| 46-100138 | pumpkin | 1.94 | 0.30 | b |
| 46-100138 | cucumber | 1.87 | 0.25 | b |
| 46-100117 | pumpkin | 1.80 | 0.35 | b |
| 46-100137 | cantaloupe | 1.52 | 0.27 | b |
| 46-100137 | pumpkin | 1.43 | 0.30 | b |
| 46-100117 | cantaloupe | 1.38 | 0.27 | b |
| 46-100138 | cantaloupe | 1.19 | 0.28 | b |

¹Least squares means in Means Separation column were compared using Tukey's adjustment. Means not followed by the same letters are significantly different at $P \leq 0.05$.

sions have been reported on cantaloupe, honeydew, and cucumber [1], but there are no reports of *M. roridum* causing fruit lesions on watermelon or pumpkin. The thick wax layer on watermelon fruit may impede the fungus' ability to cause infection.

Based on the research from a sizeable number of laboratories around the world, it seems likely that all the optimal conditions must come together for an outbreak of *M. roridum* disease to occur on watermelon. An apparently wide range of susceptibility among watermelon cultivars in the field has been observed [15] which suggests a moderately high level of resistance to *Myrothecium* leaf spot in some cultivars. Greenhouse inoculations support field observations by showing differential resistance within cucurbit types. Extensive epidemiological work will be required if *Myrothecium* leaf spot and stem canker becomes more prevalent in major watermelon production areas.

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