TRICOTHECENES AND THEIR POSSIBLE INVOLVEMENT IN PATHOGENICITY OF
MYROTHECIUM RORIDUM.

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ABSTRACT: Macrocytic trichothecene roridin E, produced in culture or in plant host tissue by a strain of Myrothecium roridum pathogenic to muskmelon (Cucumis melo L), and roridin A and myrotoxin A+B, trichothecenes from M. roridum strains nonpathogenic to muskmelon were investigated for their role in pathogenicity of M. roridum. Roridin E did not affect the growth of the pathogen *in vitro* but significantly increased sporulation and lesion diameter on inoculated muskmelon leaves whereas roridin A and myrotoxin A+B did not affect sporulation and lesion size. All trichothecenes induced phytotoxic reactions in muskmelon and increased electrolyte leakage from muskmelon leaf tissue. Muskmelon cultivar rankings with respect to the phytotoxic responses and level of electrolyte leakage induced by roridin E were similar to cultivar rankings after inoculation with M. roridum. Roridin A and myrotoxin A+B did not elicit cultivar-specific responses. Characteristic lesions caused by the pathogen were similar to lesions caused by roridin E. These results suggest that roridin E is involved in the establishment of pathogenicity by M. roridum in muskmelon tissue.

INTRODUCTION:

The trichothecene mycotoxins are a family of related sesquiterpenoids produced by fungal genera such as Fusarium, Trichothecium, Trichoderma, Cephalosporium, Myrothecium, and Stachybotrys (ref. 1). The trichothecenes are divided into two groups: simple and macrocytic. The simple trichothecenes (e.g. T-2 toxin, deoxynivalenol, diacetoxyscripenol) are among the commonly recognized mycotoxins and pose serious hazards to animals and humans (ref. 1). The macrocytic trichothecenes (e.g. roridins, verrucarins, myrotoxins) are encountered less often but are typically more toxic than the simple trichothecenes (ref. 1). Trichothecenes, especially the macrocytic ones are also highly phytotoxic (ref. 2,3).

Since a wide array of macrocytic trichothecenes can be produced by various pathogenic and nonpathogenic strains of M. roridum, a study of their role(s) in disease development on host plants is pertinent and

could be useful in selection of resistant plant cultivars. In this report, we examine effects of roridin E and related macrocyclic trichothecenes on the pathogenicity of M. roridum to muskmelon.

MATERIALS AND METHODS:

Isolation and characterization of the macrocyclic trichothecenes used in this study have been described elsewhere (ref. 4,5). Partially purified crystalline roridin E from M. roridum isolated from muskmelon (ref. 4), myrotoxins from M. roridum isolated from tomato (ref. 5), and roridin A, from a M. roridum soil isolate, were used. Aqueous 1% methanol was used as a solvent to prepare the toxin solutions because of its low toxicity to plant tissue and fungal cells.

Plant material used in this study included fully expanded leaves of five muskmelon genotypes. When inoculated with M. roridum, 'Amarelo' and 'Iroquois' are relatively susceptible, 'Hales Best' and 'PMR 45' are relatively resistant and 'Perlita' has intermediate resistance (ref. 6). Fully expanded leaves were inoculated either with 25 μ l of a solution of 5 μ g ml⁻¹ roridin A, roridin E and myrotoxin A+B, or toxins plus 25 μ l of a spore suspension containing 10⁶ spores ml⁻¹. After 7 days in petri dishes in the dark, lesion diameters, including both necrotic and chlorotic areas, were measured.

To assess the effects of the trichothecenes on muskmelon leaf tissue cellular membranes, leaf discs (18 mm diam) were excised from uninoculated leaves of three muskmelon genotypes ('Hales Best', 'Iroquois' and 'Perlita'), agitated in 50 ml of deionized water for 30 min to remove adhering electrolytes, and floated on 25 ml of a solution containing 0.5 μ g ml⁻¹ of the various trichothecenes. The leaf discs were incubated on an orbital shaker (150 rpm) for 2 hr after which 5 ml aliquots were collected and tested for electrolytic conductivity with a conductivity bridge (YSI model 31).

To examine the effects of trichothecenes on growth and sporulation of M. roridum, droplets of a spore suspension containing 10⁶ spores ml⁻¹ plus various concentrations of trichothecenes were transferred into polystyrene culture plates containing Bacto-dextrose agar and spore germination recorded after 18 hr. To assess the role of roridin E on growth of M. roridum on muskmelon leaves, 25 μ l droplets of a spore suspension, with or without 5 μ g ml⁻¹ trichothecene, were placed on detached leaves and after 7 days, lesion diameters were measured and the level of sporulation estimated.

RESULTS AND DISCUSSION:

Trichothecenes produced necrotic lesions on all muskmelon genotypes. Necrosis first appeared 24 hr after inoculation. The rate of chlorotic and necrotic spot development differed with each trichothecene. Roridin A caused larger lesions than roridin E while myrotoxin A+B induced the smallest lesion. All muskmelon genotypes responded the same to roridin

A and myrotoxin A+B but had differential responses to roridin E. The responses to roridin E were also highly correlated with genotype responses following inoculation with M. roridum whereas responses to roridin A and myrotoxin A+B were not. These results are important in that roridin E, produced by the muskmelon pathogen M. roridum, appears to possess selectivity for identifying genotype resistance in muskmelon varieties.

All 3 trichothecenes induced cellular leakage in muskmelon leaf tissue. The amount of leakage increased with increasing trichothecene concentration. When the muskmelon cultivars were treated with roridin A and myrotoxin A+B from nonpathogenic isolates (to muskmelon), the level of leakage was the same. With roridin E, however, 'Iroquois', the most susceptible genotype had the highest amount of electrolyte leakage while 'Hales Best', the most resistant had the lowest amount of electrolyte leakage. The differences in the effect of roridin E on the different genotype membranes may be due to the presence of toxin receptors or binding sites in susceptible genotype membranes and absence of such receptors in resistant genotype membranes as reported by others (ref. 7).

Roridin E and myrotoxin A+B produced by muskmelon and tomato isolates respectively inhibited germination of other M. roridum isolates but not themselves. However, roridin A produced by the soil isolate of M. roridum reduced spore germination of all isolates including the isolate that produce it. The tolerance shown by the pathogenic strains of M. roridum to the trichothecenes they produce, may also indicate the existence of a recognition phenomenon between the fungus and its metabolites that allows the fungus to grow even though toxic metabolites are present.

Addition of roridin E to the three M. roridum isolates at inoculation caused a significant increase in lesion size and sporulation on both resistant and susceptible genotypes. The amount of sporulation was greatest on the susceptible genotype. Addition of roridin E to the other two M. roridum isolates did not affect either lesion size or amount of sporulation. The increased growth of M. roridum on muskmelon tissues in the presence of roridin E could not be attributed solely to an increasing leakage of nutrients from the plant tissues which theoretically would become available to the pathogen since roridin A and myrotoxin A+B are also capable of causing electrolyte leakage but did not influence growth of M. roridum. In addition to roridin E causing electrolyte leakage in the host tissue, it may also be eliciting a virulence factor in the fungus.

In conclusion, the results obtained in this study suggest that although macrocyclic trichothecenes produced by *Myrothecium* species are structurally similar, their biological effects on plant tissue, fungal cells and plant-fungal interactions are significantly different. Although the production of macrocyclic trichothecenes by M. roridum is

probably not necessary for its pathogenicity, trichothecenes such as roridin E, produced by plant pathogens, exhibit genotype-specific phytotoxicity that may act to increase virulence of the pathogen.

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