Myrothecium roridum, a potential pathogen of rapeseed and mustard in Alberta

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Myrotheciurn roridum was isolated from the seed of yellow marsh cress (Rorippa islandica) collected from near Legal, Alberta. It was inoculated on some cultivars of rapeseed and mustard commercially grown in Alberta and found to be pathogenic. Differences in susceptibility between various cultivars were noted. Many cruciferous weeds found in central Alberta are also susceptible. Although, the disease has so far not been found occurring naturally in the fields, it is a potential' pathogen of rapeseed and mustard in Alberta. Some hitherto unreported features of M. roridum, as revealed by light and scanning electron microscopy, are reported.

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On a isolé Myrotheciurn roridurn de la graine du cresson des marais (Rorippa islandica) récolté près de Legal (Alberta). Le champignon a ete inoculé a certains cultivars de colza et de moutarde cultivés commercialement dans la province et s'est révélé pathogene. On a observe une 'difference de sensibilité entre les divers cultivars. Beaucoup de mauvaises herbes de la famille des Cruciferes repandues dans le centre de la province sont egalement sensibles. Bien que la maladie n'ait pas encore ete observée à l'état naturel dans les champs, c'est un agent pathogene potentiel du colza et de la moutarde en Alberta. Lauteur mentionne certaines caracteristiques jusqu'ici non signalées de M. roridurn, révélées par la microscopie optique et électronique.

Myrothecium roridum Tode ex Fr. is plurivorous and is widespread in temperate and tropical regions of the world (9). This fungus is strongly cellulolytic and produces mycotoxins called trichothecenes, which are capable of causing disease and death in animals (7,9).

In 1975, we isolated ***M. roridum*** from the seeds of marsh yellow cress, ***Rorippa islandica*** (Oeder) Borbas. This fungus has a wide host range including some crucifers (2,4,8,9). Since ***M. roridum*** is present in the environment in Alberta, a study was undertaken to determine if it could parasitize cultivars of rapeseed and mustard grown in this area, and also some cruciferous weeds common in central Alberta.

Materials and methods

Myrothecium roridum was isolated from the seeds of yellow marsh cress collected on 20, August 1975 from near Legal, Alberta. It was grown on potato dextrose agar (Difco) at 25°C. Subcultures have been deposited at the Commonwealth Mycological Institute, Kew, England (I.M.I.No. 204824) and at the National Mycological Herbarium, Agriculture Canada, Ottawa (DAOM 164769). Yellow marsh cress is a cruciferous weed native to Canada and occurs in all provinces usually in damp sites **(3).**

Plants were inoculated with ***M. roridum*** in two different ways, a) Detached leaves of Torch **(*Brassica campestris*** L., Polish rapeseed), Midas ***(B. napus*** L., Argentine rape), and Accession No. 311726 ***(B. juncea*** (L.) Coss, oriental mustard, Regional Plant Introduction Station, Ames, Iowa, from Poland, henceforth called the Iowa line) were spot inoculated with a conidial suspension (approx. 100,000 conidia/ml) and kept in moist chambers in petri dishes at room temperature; b) The spore suspension was sprayed on the plants in the greenhouse and in the field at the University of Alberta Farm. The cultivars screened were Midas, Tower (B. ***napus),*** Lethbridge (LB) 22A ***(B. juncea),*** Torch, R-500 ***(B. campestris),*** and Yellow 2 ***(B. hirta*** Moench). The cruciferous weeds were inoculated only in the greenhouse. At first, difficulty was encountered in obtaining good germination of the seeds of some of the weeds. Consequently, based on the work of Corns (5) on dormancy of the seeds in wild mustard and stinkweed, seeds of the weeds collected from different areas in central Alberta were routinely soaked for 24 h in 1000 p.p.m. aqueous solution of the potassium salt of gibberellic acid before sowing. The cruciferous weeds screened were common peppergrass ***(Lepidium densiflorum*** Schrad.), flixweed ***(Descurainia sophia*** (L.) Webb), Indian mustard ***(B. juncea),*** shepherds' purse ***(Capsella bursa - pastoris*** (L.) Medic.), stinkweed **(*Thlaspi arvense*** L.), wormseed mustard **(*Erysimum cheiranthoides*** L.) and yellow marsh cress ***(Rorippa islandica).***

Morphology of the fungus on the leaves of Torch was investigated by light microscopy using a Leitz Wetzlar microscope equipped with an Ultropak incident light illuminator, and by scanning electron microscopy (SEM). The sporulating material was fixed for SEM in three different ways: (a) The material was fixed overnight with osmium tetroxide vapour without any disturbance to the

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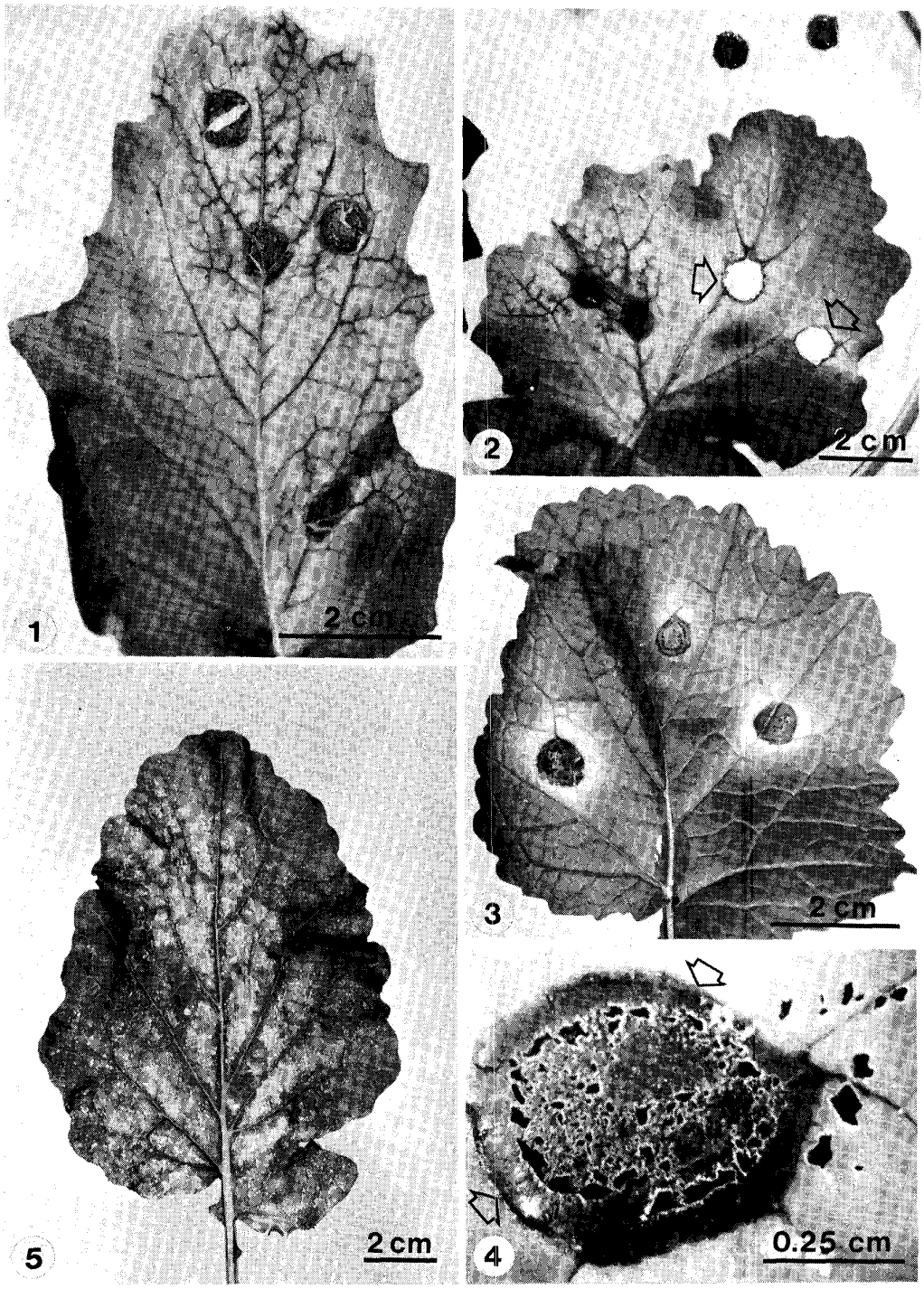

Figures 1-5. Symptoms of ***Myrothecium roridum*** infection on leaves of rapeseed and mustard. 1,2) Detached leaves of Torch rapeseed, 4 days after inoculation. Note the development of spots, often resulting in shot-holes (arrows) and extensive chlorosis. 3) Detached leaf of Iowa line mustard, 4 days after inoculation. Note limited chlorosis around the spot. 4) Close-up of a spot on a detached leaf of Iowa line mustard, 6 days after inoculation. Note the discrete margin around the spot (arrows) and formation of sporodochia inside and outside the spot. 5) Leaf of Midas rapeseed, 2 weeks after inoculation in the greenhouse. Note the bleached areas.

fruiting bodies; (b) The material was gently flushed once with a stream of distilled water and then vapour-fixed with osmium tetroxide; (c) The conidia were fixed overnight in 3% glutaraldehyde in **0.1** M phosphate buffer (pH **7.0),** post-fixed with osmium tetroxide in the same buffer for **4** h, deposited on a Millipore filter **(0.45**μ m), and washed five times with distilled water. Pieces of Millipore filter with the conidia adhering to them were used in further preparations. All the materials for SEM were frozen in liquid Freon 12, briefly stored in liquid nitrogen and dried at **-70°C** in an Edwards-Pearse Tissue Dryer (Model EPD2). After drying, the material was mounted on stubs with conductive glue, coated with gold and examined in a Cambridge Stereoscan S4 scanning electron microscope at the Department of Entomology, University of Alberta.

Results

Symptoms on detached leaves

On Torch a brownish-green, water-soaked spot with a chlorotic halo is evident 24 h after inoculation. Sporodochia of the fungus develop in the spot in 3 to 4 days (Figure 1), and in 4 to **6** days shot holes form (Figure 2) in many leaves. Subsequently the sporodochia form in the chlorotic part of the leaf (specimens deposited DAOM **164768).** Leaves of Torch show extensive chlorosis as a result of infection. In the Iowa line progression of the disease is slower (Figure 3), followed by that in Midas. In both these cases the spot has a discrete brownish margin (Figure **4),** which is absent in Torch.

Symptoms in the greenhouse and field

The leaves develop bleached areas **4** to **5** days after inoculation (Figure 5). Younger leaves are less susceptible than older ones. Sporodochia develop in 2 to 3 weeks.

Based on a visual rating scale of 0 to **5,** where 5 is highly susceptible, **R-500** and Torch were rated as **5,** LB 22A as 3, Midas and Tower as 2, and Yellow 2 as **1.**

All cruciferous weeds except common pepper grass showed infection.

Light microscopy and scanning electron microscopy

Light microscopy revealed that the sporodochia are formed at sites where a clear exudate droplet was initially located. Also, in reflected light a sac-like envelope is seen covering the sporodochium.

Material that was vapour-fixed without washing shows the sporodochia which are irregular in shape and surrounded by a fringe of marginal hyphae (Figures **6,7).** The sac-like envelope covering the sporodochium appears wrinkled in some SEM preparations (Figure 8). Faint outlines of the conidia are seen through this covering due to the shallow transmission effect in the SEM.

Phialides and conidia in various stages of development are seen in material that was vapour fixed after brief washing (Figure 9). Some phialides show cracking of the cell wall at the apex. These were interpreted as stages in formation of the first conidium from the phialide. Later stages show conidia in different stages of extrusion from the phialides. A prominent collar is present (Figure **10).** During later stages of development, usually five or six knob-like appendages are seen on the distal end of the conidium (Figure 9). These appendages are readily washed off since they are not seen in conidia processed through a series of liquid solutions (Figure **11).**

Discussion

Myrothecium roridum is a serious pathogen of some economically important plants (9). Its host spectrum also includes some wild, ornamental, and oleiferous crucifers (2,4,8,9). In Canada, serious outbreaks of ***M. roridum*** have occurred on cultivated pansy ***(Viola tricolor*** var. ***hortensis),*** resulting in considerable damage to the seed crops in B.C. (2). Our results indicate that ***M. roridum*** is capable of parasitizing cultivars of rapeseed and mustard commercially grown in Alberta. However, natural infection of these crops by the pathogen has so far not been found in Alberta and conditions that could lead to this situation are not known at present.

Light and scanning electron microscopy have revealed some hitherto unreported features of ***M. roridum.*** The sporodochia develop in places where a clear exudate droplet was initially present. Macroconidia in ***Fusarium culmorum*** also develop in a similar way **(6).** The sporodochia in ***M. roridum*** are covered with sac-like envelopes. This covering morphologically resembles the envelopes on the exudate droplets on the sclerotia of ***Sclerotinia sclerotiorum*** (1). The phialides have a prominent collar. The developing conidium has **5-6** knob-like appendages on the distal end. These are washed out on passage through liquids. It is of interest that a fantailed appendage is also present on the conidium in ***M. verrucaria*** (9).

Acknowledgments

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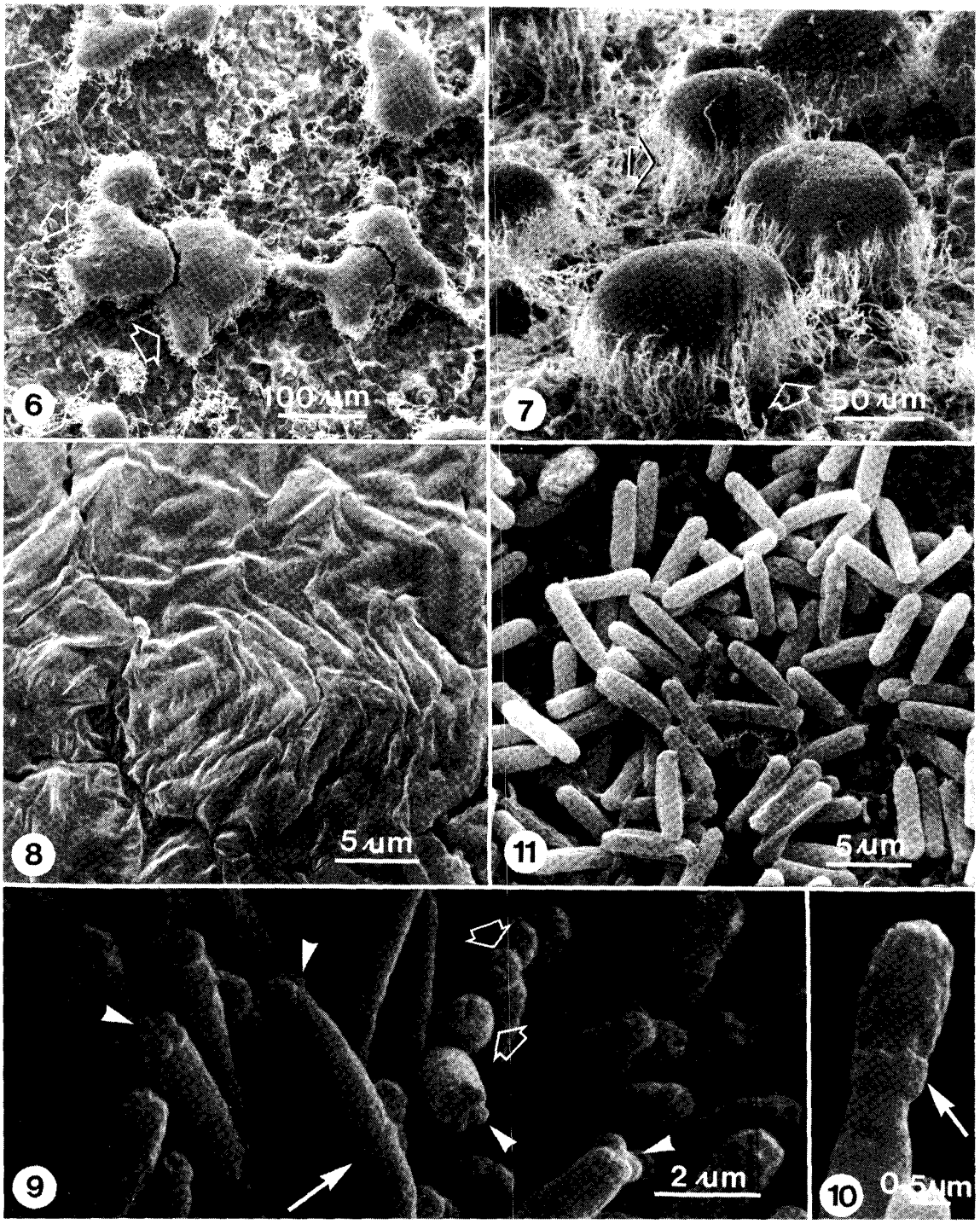

Figures 6-11. Scanning electron microscopy of *Myrothecium roridum* on the leaves of Torch. 6.7) Vapour fixed material as seen from the top (Figure 6) and side (Figure 7). Note the fringes of marginal hyphae (arrows) around the irregularly shaped sporodochia. 8) Surface view of a sporodochium showing the slightly wrinkled sac-like envelope and profiles of conidia. 9,10) Material flushed with distilled water and then vapour fixed showing phialides. Note the collar (small arrows) and knob-like appendages (arrowheads) on distal end of the developing conidium. Phialides with cracked apices represent stages in formation of first conidia (large arrows). 11) Conidia fixed in liquid solutions. Note that the knob-like appendages are not present. The cobweb-like material in the background is the Millipore filter.

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