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Lesion Formation on Bean Seedling Hypocotyls by *Rhizoctonia solani* as Affected by Size and Nutrition of Propagules

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Severity of diseases caused by *Rhizoctonia solani* is affected by both inoculum density and quality. Thus, only propagules which were larger than 250 μm in size were infective to bean seedlings (Henis and Ben-Yephet, 1970). Direct observations by Scanning Electron Microscopy (SEM) revealed, however, that both infective and non-infective propagules germinate and produce hyphae which spread along the hypocotyls (Lisker, Katan and Henis, 1976). Moreover, lesion production caused by infective propagules occurred always at some distance from the inoculation site (Lisker *et al.*, 1976). In this work observations on some factors involved in lesion formation by *R. solani* are reported.

The isolate of *Rhizoctonia solani* Kuehn was the one used in a previous study (Lisker, Katan and Henis, 1974), and the propagules were prepared essentially as described in that work. A mycelium mat (7-days-old) was blended in tap water for 1 min. The homogenized mycelium was separated on plastic sieves into large infective (400–590 μm) and small non-infective propagules (50–150 μm) by washing them separately with tap water. Bean plants (*Phaseolus vulgaris* L. 'Brittle Wax') were grown at 25 ± 1 °C in closed $20 \times 20 \times 6$ cm plastic boxes which were kept wet ('damp chambers'). Each hypocotyl (1·5–2·0 cm long) was inoculated with a *R. solani* propagule. The propagule was picked up with forceps under a stereoscopic microscope and placed in a 2·5 μl drop of nutrient solution or distilled water previously placed on the hypocotyl surface. Microscopic observations showed no evidence of damage to the propagule or to the hypocotyl. After inoculation, the seedlings were kept in the damp chambers for an additional 2–3 days and then examined for lesion formation. Usually the propagules were placed on the hypocotyl, 1·0–1·5 cm away from the root–stem transition region.

The amino acids used in this study were: Bacto Asparagine (Difco) and DL-Serine (NBC). The composition of the yeast-extract dextrose broth (YE) used was (in g l^{-1}): Bacto yeast extract (Difco), 5·0; Dextrose, 20·0; Bacto peptone (Difco), 5·0; Chloramphenicol (Abic-Israel), 0·25 (Henis and Ben-Yephet, 1970). Bean hypocotyl sap extracts were prepared by homogenizing the tissue and filtering through cheesecloth.

Disease severity was assayed by using a 0–3 scale: 0, no lesion; 1, lesion of 1 mm width; 2, lesion of 1–3 mm width; and 3, lesion > 3 mm width. Disease index was calculated by summing the indices of the lesion formed on each individual plant and calculating the average index of three plants in each of six replicates.

Infective large propagules (400–590 μm) placed on bean hypocotyls produced lesions at a few mm distance from the inoculation site. Various treatments were tried in an attempt to change the location of the lesions. Addition of asparagine (10^{-4} M, 10^{-5} M)

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or serine (10^{-5} M) or the use of larger propagules (800–1000 μm) without amino acid addition, caused an increase in the disease index from 2·0 to 2·9; 3·3 and 4·3, respectively. This increase in virulence had no effect on the location of the lesions.

Infective propagules were placed on the hypocotyl and one drop (2·5 μl) of YE was placed on the hypocotyl at a distance of 3–4 mm from each propagule. Microscopic observations revealed that the propagule germinated and the emerging hyphae extended in all directions as reported elsewhere (Lisker *et al.*, 1976). After reaching the drops, the hyphae grew vigorously but without lesion formation at this site. Later, the developing hyphae formed lesions which were scattered at various sites on the hypocotyl a few mm distant from the propagule. The same pattern regarding lesion site was observed when drops of distilled water were used instead of YE.

Bean hypocotyls were wounded with a sterile needle and inoculated. When large propagules were placed on the tissue immediately after wounding, 90 per cent of the propagules produced lesions at this site. Thorough washing of the damaged tissue with distilled water prior to inoculation with the propagule decreased the number of lesions to 28 per cent. In both cases lesions were not typical, restricted to the border of the wounded tissue, and no infection cushions were formed. Non-inoculated wounded tissue did not form this kind of lesion. The addition of bean hypocotyl extract to infective propagules placed on either intact tissues or wounded ones, 48 h after its injury, did not affect the distance of the lesion from the propagule.

Bean seedlings (70·0 mm long) were inoculated with infective propagules at various distances along the hypocotyl. The infectivity of the propagules was increased by increasing the distance on the hypocotyl between the propagule and the root-stem transition region. The disease index at distances of 2·0, 25·0 and 55·0 mm was 0·9, 1·6 and 2·6, respectively, indicating the presence of areas on the hypocotyls varying in their resistance to the disease (Warren, 1973). Also in these treatments there was no lesion formed underneath the propagule.

In another series of experiments the effect of detached propagules and the remaining hyphae on the hypocotyl was followed. Bean hypocotyls were inoculated with large propagules (one per plant) and at various periods later they were transferred with forceps to new non-inoculated hypocotyls. When the propagules were transferred to a new host after 20 h of growth, lesions were formed on only 10 per cent of the newly inoculated plants as compared to 100 per cent in the control (seedlings inoculated with non-detached propagules). When a 2·5 μl drop of YE was added to inoculation site, infectivity of the transferred propagules was restored; 95 per cent of the plants developing disease symptoms. Extending the nutrients exhaustion period to 40 h resulted in a complete loss of propagule infectivity (disease index = 0). Moreover, when 2·5 μl drops of YE were added to those propagules, lesions were formed on only 10 per cent of the plants. When propagules were transferred 12–15 h after inoculation, 40 per cent of the plants containing the remaining mycelium showed disease symptoms. When a 2·5 μl drop of YE was added to the remaining mycelium (instead of distilled water), 75 per cent of the plants showed lesions. Lesions were formed on all plants by the remaining mycelium if propagules were removed 24 h after inoculation even without the addition of external nutrients. In all cases the pattern of lesion formation remained unchanged.

Bean hypocotyls were rubbed gently over an area of a few square mm with a tooth-pick wrapped with cotton and dipped in chloroform, ether, acetone, alcohol or distilled water, and a propagule was placed on the rubbed site. Ninety per cent of the lesions developing on chloroform-treated hypocotyls were formed underneath the propagule 24 h earlier than the lesions formed with the other treatments. Ether had a similar effect, but the lesions were atypical, a shallow concavity was formed and the necrotic tissue was not uniform. No lesion was formed underneath the propagule with the other

treatments. In non-inoculated, chloroform treated seedlings, a concavity but no discoloration was observed.

In the following experiments, attempts were made to transform small non-infective propagules into infective ones. This was achieved only by addition of YE. The disease index of the inoculated plants increased with increase of YE concentration (Table 1). However, glucose (at concentrations of 10^{-1} M, 10^{-2} M and 10^{-3} M), asparagine (at concentrations of 10^{-4} M and 10^{-5} M), glucose 10^{-1} M + asparagine 10^{-4} M or bean hypocotyl extracts added to small propagules did not affect the infectivity of the small propagules. Microscopic examination revealed that the development of the small propagules on bean hypocotyls after the addition of YE followed the same pattern of extensive germination, cushion formation and lesion location, as described for the large propagules growing on bean hypocotyls without this nutrient (Lisker *et al.*, 1976).

TABLE 1. *Effect of yeast-dextrose broth (YE) on virulence of small non-infective propagules (50–150 μm) of* Rhizoctonia solani*

Concentration of YE (%)	Disease index	Diseased seedlings (%)
0 (water)	0·0	0·0
1	0·1	12·0
10	0·5	37·0
100	3·4	86·0

* 2·5 μl of distilled water or YE solution were added to the propagule at the time of inoculation. The bean hypocotyls were kept in damp chambers at 27 °C for 72 h.

Wounding the hypocotyls with a sterile needle prior to inoculating the wounded site with small non-infective propagules resulted in the formation of lesions at the site of the wounding that were different from the typical ones induced by large infective propagules. They were smaller (up to 1 mm diam.), faintly brown, and confined to the border of the wound. Fifty-five per cent of the wounded inoculated plants showed this type of lesion as compared to 18 per cent in plants in which the wounds were thoroughly washed with distilled water before inoculation. Non-wounded inoculated plants did not show any of these specific symptoms.

Similar to the results presented with the large propagules, lesions were formed underneath the small propagules after the hypocotyls were treated with chloroform and a drop of 2·5 μl YE was added to the propagule. Without YE addition, lesions were indeed formed underneath the propagule but they were atypical, a shallow concavity was formed and the tissue underneath the propagule was not uniformly necrotic.

The consistent formation of the lesions at some distance from the propagule site is indeed puzzling. This pattern could not be changed by using more virulent propagules or by addition of nutrients, and was evident whenever the propagule was placed at a susceptible site on the hypocotyl. This may be explained by assuming the existence of a nutrient gradient from the propagule to the growing hyphae, of substances which enable the formation of infection cushions at specific sites on the hypocotyl surface. Watson and Ford (1972) suggested that some morphogenetic processes in pathogenic fungi depend on their ability to change the existing balance between stimulatory and inhibitory factors. Indeed, the addition of YE to the propagule inhibited the formation of the infection cushion but stimulated hyphal growth on the hypocotyl surface. Changing the location of lesion by chloroform may be due either to the removal of the protective wax layer of the cuticle and/or to the modification of plant nutrients as a result of

cell damage. Ether vapour was found to inhibit the formation of phytoalexins in pea pods (Uehara, 1960).

The present study also shows that alterations in the external supply of nutrients or in the internal reserves of the propagules resulted in pronounced changes in virulence. By supplying suitable nutrients, non-infective small propagules became infective and the virulence of large propagules which was lost upon exhausting, was restored. However, asparagine that increased the virulence of *R. solani* (Weinhold, Bowman and Dodman, 1969; Weinhold, Dodman and Bowman, 1972) had no effect on the virulence of non-infective propagules. Environmental factors which have an effect on the nutritional status of the pathogen and its host at the infection site or in soil may in turn affect the pathogenic capacity of this fungus.

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