Microb Ecol (1983) 9:185–187

MICROBIAL ECOLOGY

Detection of Mycoparasitism by Infrared Photomicrography

Yigal Elad, Zili Sadovski, and Ilan Chet

Department of Plant Pathology and Microbiology, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot 76-100, Israel

Abstract. The fungus *Trichoderma harzianum* which parasitizes its host *Rhizoctonia solani* (AG 1-6) was observed under a light microscope and the interaction sites photomicrographed with infrared film. Bright regions indicating infrared irradiation were observed at the interaction sites, apparently due to the high parasitic activity occurring there. The possible use of infrared photomicrography in cell-cell interactions is discussed.

Infrared (IR) photography, used for detecting and localizing diseases in plants and crops [1], has not been applied for detecting sites of cell-cell interaction. In the present work, we have studied a system of *Trichoderma harzianum* Rifai which attacks the soil-borne plant pathogen *Rhizoctonia solani* kühn using IR irradiation as a marker for the interacting sites. Colwell [4] reported that grain rusts cause mesophyll tissues to become compact and semitransparent to IR in the early stages of attack. Infrared photography may also be used for mapping damaged spots on leaves, since their rates of absorbence of IR light vary.

A successful biological control of the pathogens *Sclerotium rolfsii* Sacc. and *R. solani* was achieved by artificial inoculation of the mycoparasitic fungus *T. harzianum* [7, 9, 13].

Fungal hyphal interactions have already been observed by light and electron microscopy [3, 5, 8, 10]. The antagonistic fungus *T. harzianum* coils around its host (Fig. 1) and excretes extracellular lytic enzymes capable of degrading the pathogen's cell walls [6, 12]. This enzymatic activity localized at the interaction sites was sometimes accompanied by the host hyphae being penetrated by the *Trichoderma* spp. [5].

Localizing the interaction sites, between the coiling *T. harzianum* hypha and the host, is essential for the understanding of the mode of parasitism. The 2 fungi, *T. harzianum* and *R. solani,* were grown towards each other in plates on a cellophane membrane placed on water agar (2%) [3]. The interaction sites, observed under a Zeiss light microscope, were photomicrographed either on Eastman Kodak high speed IR film, using Kodak Wratten filter No. 12 or on black and white Pan-X film. Bright regions indicating IR irradiation can be observed at the interacting sites of both hypha only in the IR film but not on the ordinary film (Fig. 2). Similarly, more IR photographs indicated that this phenomenon is constant in these hyphal interactions (Fig. 3). The increased

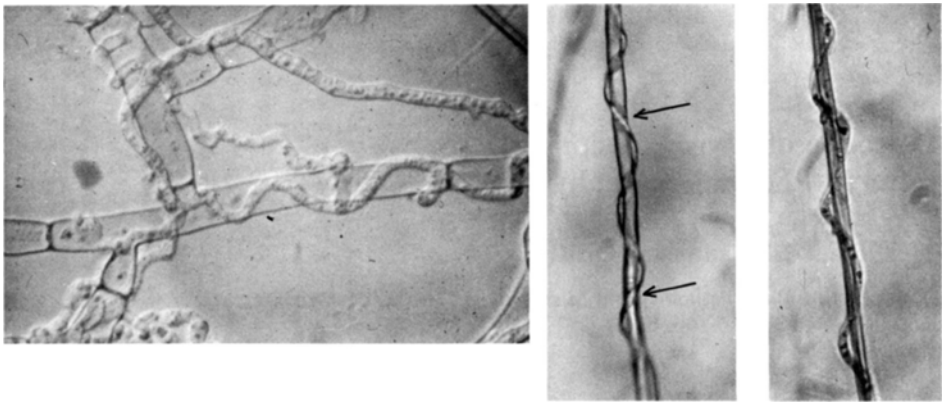

Fig 1. *Trichoderma harzianum* hypha coiling over *Rhizoctonia solani* (×640).
Fig 2. Comparative photographs taken from the same hyphal segment using either IR (left) or conventinal black and white (right) films (×800). *Arrows* indicate the bright regions at the interaction zone.

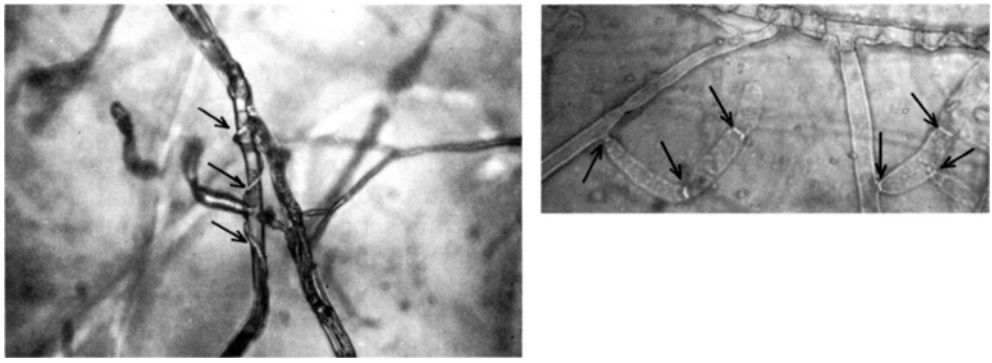

Fig 3. Infrared photomicrographs of *T. harzianum* coiling around *R. solani* hyphae (×640). *Arrows* indicate the bright regions at the interactions sites.
Fig 4. Infrared photomicrograph of *R. solani* hyphae. *Arrows* indicate the bright septa (×640).

irradiation apparently occurs as a result of the high activity of the parasitic fungus which takes place at these specific sites. The differences in reflection may be related either to the active β-(1-3)-glucanase and chitinase which degrade the host's cell walls or to the response of the attacked fungus. Moreover, localized autolytic activity [2] in the hypha itself also can be detected in *R. solani* septa (Fig. 4). This type of photography was performed with 6 different anastomosis groups of *R. solani* (AG 1-6), however, no differences could be observed between them.

This work reveals for the first time that the new technique, applying IR microphotography, can serve as a useful tool for detection of cell-cell interaction as well as on internal activity.

Acknowledgments. This work was supported by a grant from the United States-Israel Binational Agricultural Research and Development Fund (BARD). We gratefully thank Rumia Guvrin, Rina Barak, and Elicheva Avraam.

References

1. Applied Infrared Photography. Eastman Kodak Co Publication No. M-28 (1972)
2. Bartnicki-Garcia S (1968) Cell wall chemistry, morphogenesis and taxonomy in fungi. Ann Rev Microbiol 22:87–108
3. Chet I, Harman GE, Baker R (1956) *Trichoderma hamatum*: its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb Ecol 7:29–38
4. Colwell RN (1956) Determining the prevalence of certain cereal crop diseases by means of aerial photography. Hilgardia 26:223–286
5. Elad Y, Chet I, Boyle P, Henis Y (in press) The parasitism of *Trichoderma* spp. on plant pathogens: ultrastructural studies and detection by FITC lectins. Phytopathology
6. Elad Y, Chet I, Henis Y (1982) Degradation of plant pathogenic fungi by *Trichoderma harzianum.* Can J Microb 28:719–725
7. Elad Y, Hadar Y, Hadar E, Chet I, Henis Y (1981) Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation. Plant Disease 65:675–677
8. Hadar Y, Chet I, Henis Y (1979) Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum.* Phytopathology 69:64–68
9. Henis Y, Chet I (1975) Microbiological control of plant pathogens. Adv Appl Microbiol 19: 85–111
10. Hoch HC, Puller MS (1977) Mycoparasitic relationships: Morphological features of interactions between *Pythium acanthicum* and several fungal hosts. Arch Microbiol 111:207–224
11. Jackson RJ (1964) Detection of plant disease symptoms by infrared. Biol Phot Assoc 32: 45–58
12. Jones D, Gordon AH, Bacon JSD (1974) Co-operative action by endo- and exo-β-(1-3)-glucanase from parasitic fungi in the degradation of cell wall glucans of *Sclerotinia sclerotiorum.* Biochem J 140:47–55
13. Wells HD, Bell DK, Jawarski CA (1972) Efficacy of *Trichoderma harzianum* as a biological control for *Sclerotium rolfsii.* Phytopathology 62:442–447