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# TRACER TECHNIQUES FOR THE STUDY OF HOST-PARASITE RELATIONS

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**Abstract**

TRACER TECHNIQUES FOR THE STUDY OF HOST-PARASITE RELATIONS.

Autoradiographic techniques have been used to study the interaction of many facultative and obligate parasites, including viruses. After feeding the host plant with labelled substrates, labelled material accumulates in the infected cells and seems to penetrate into structures of the parasite. After labelling the parasite, its influence on the host may be studied. We use this technique to study the interaction of host (bean) and parasite (bean rust) during the infection process. After infection with uredospores labelled with tritiated orotic acid, the radioactivity is retained almost completely within the young haustorium at 22 h after inoculation. This may indicate a very small influence of the parasite on its compatible host. In incompatible host-parasite combinations, the infection process proceeds in a different way. The use of autoradiographic techniques to compare combinations of varying compatibilities will be discussed.

Many autoradiographic studies indicate that the transport and the distribution of solutes in plants can be significantly altered by infection [1]. This has been shown in a number of obligate and facultative parasites, including viruses [2]. Generally, radioactive material accumulates in a zone around infection sites. Accumulation of labelled material seems to be caused by increased metabolic activity in and transport of labelled substrates to the infection sites [1, 3]. Autoradiographs of thin sections through rust-infected tissues have demonstrated an accumulation of labelled substances in different organelles of the infected cell (e.g. nuclei), and a passage of the label from the host through the sheath of the haustorium into the haustorium and into the mycelium of the fungus [4-7]. This has been confirmed by biochemical studies [8].

Onoe, Tani and Naito [9] have labelled the host with $^{3}$H-uridine, $^{3}$H-cytidine, $^{3}$H-adenosine, $^{3}$H-guanosine and $^{14}$C-orotic acid, and allowed unlabelled uredospores to infect the plant. During formation of the infection structures of the fungus (prehaustorial stage) there was no uptake of nucleosides. But when the haustorium was formed, uptake of nucleosides began.

Mount and Ellingboe [10] quantitatively measured the transfer of $^{35}$S and $^{32}$P isotopes from the host plant to the extracellular mycelium of E. graminis f. sp. tritici. The transfer of radioactivity detected in the surface mycelium seemed to be correlated with the morphological development of the intracellular haustoria. Inhibition of penetration or haustorial development in incompatible combinations resulted in altered patterns of transfer of radioactivity from plant to parasite [11].

Autoradiography also allows a study of the parasite's influence on its host. $^{35}$S- and $^{14}$C-labelled uredospores were used to infect their hosts, and some days later labelled substances were found in different parts of

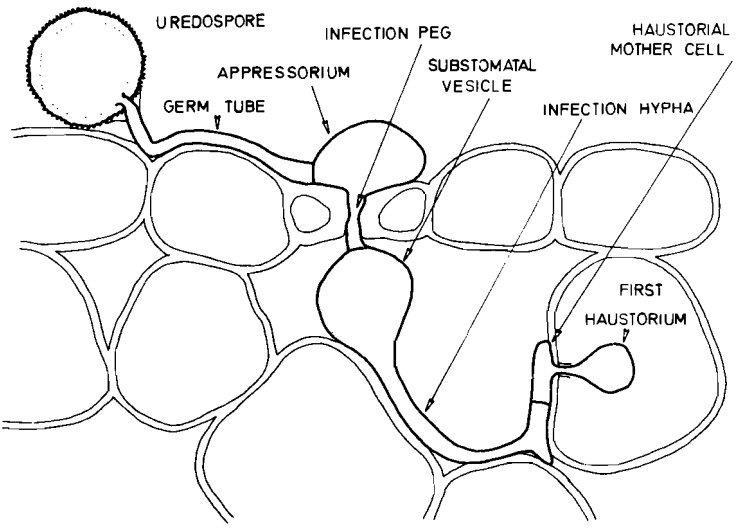


FIG.1. The bean rust infection process in a compatible combination of Uromyces phaseoli and Phaseolus vulgaris ('Favorit') (schematic).

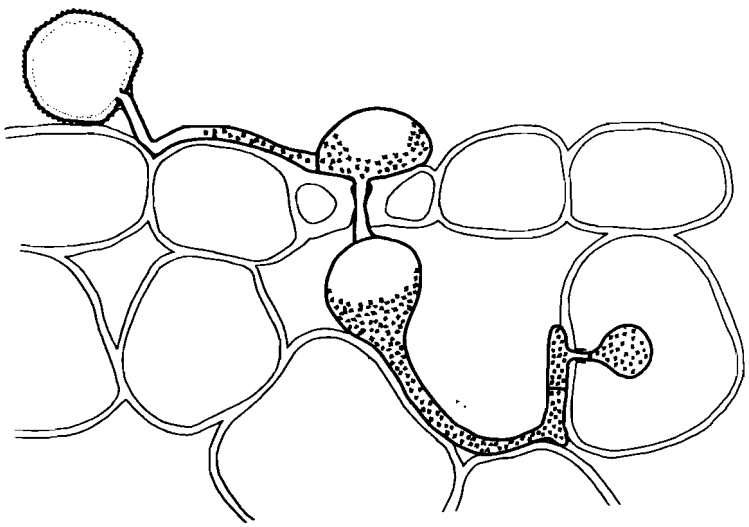

FIG.2. The infection process of tritium-labelled uredospores 22 h after inoculation (schematic).

the host plant [12, 13]. These studies were performed some days after inoculation. During this long incubation period, the unspecific exchange of labelled material could not be excluded. Such exchange may happen when $^{14}CO_2$ secreted by the fungus may be refixed by the plant during photosynthesis and when $^{35}SO_4$ is formed which may diffuse into adjacent cells. Isotope exchange also contributes to unspecific diffusion. These difficulties might be reduced by exposing host and parasite during short periods only to the labelled substrate.

In order to examine a possible substrate flow and the influence of the parasite on the host during infection, labelled bean rust uredospores were produced by feeding infected plants with $^{3}$H-orotic acid [14]. We used such spores to infect non-labelled bean plants [14]. The normal infection process is shown in Fig.1 and the distribution of silver grains 22 hours after inoculation in Fig.2. Obviously, young haustoria of the rust are able to take up substrates from the host, but the release of labelled material from the parasite to the host is, at least during the very early stage after inoculation, rather low. This may indicate that in the compatible combination employed, the bean rust fungus exerts a relatively small influence on its host during penetration into the cell.

Electronmicroscopical studies of the infection process [15] also show that in comparison with older stages of infection, no changes of organelles can be observed that might indicate severe damage to the infected cell during penetration of the fungus. Some days after infection, however, the cell organelles of the host begin to degenerate [16]. In autoradiographic studies of such stages of infection [12, 13], the label of the fungal spores could be detected in the parasite and in the host [13]. This may indicate that the relations between host and parasite change during the course of infection.

For further information, it would be important to know whether the relation between host and parasite differs during the infection process or is disturbed in incompatible host-parasite combinations. To find appropriate combinations, we shall study the infection process of bean varieties that do not allow a normal sporulation of the rust fungus. For comparative studies of compatible and incompatible host-parasite combinations, information on the early steps of the infection process is needed, since defence reactions of the plant mostly start during formation of the first haustorium [17, 18]. Even varieties that similarly react macroscopically may show a different infection process [19].

Based on observations with the light and the electron-microscope, we intend to use the following varieties for studies with labelled spores. Variety 'Favorit' represents the compatible reaction, variety 057 shows no macroscopical symptoms after infection (sometimes a very faint chlorosis) and variety 017 shows necrotic spots three days after inoculation. The infection process differs in the three varieties.

In comparison with observations in the cultivar 'Favorit' [15], haustoria in variety 057 develop with some delay, remain rather tiny and the host cell collapses at once without an increase of oxidative enzymes as cytochemical studies have shown. At this stage of infection, fungal hyphal die.

Haustoria in variety 017 seem to develop normally at first, but increased peroxidative activity is observed around the haustorial neck and later around the haustorial body. Necrosis of cells occurs and fungal growth stops at about 28 h after infection.

These studies in connection with a cytochemical characterization of the host parasite interactions [19, 20] will be performed to elucidate more precisely different mechanisms of resistance in incompatible combinations.

## CONCLUSIONS

We do not propose that the plant breeder should apply these methods to study the interaction of host and parasite in varieties that show a different

compatibility towards a pathogen. This is too difficult and the success may be questionable.

But we hope to characterize in the resistant varieties different types of defence reactions of the bean or different mechanisms of incompatibility in this host-parasite (bean-bean rust) system. The autoradiographic studies to detect the flow of substrates and the cytochemical localization of oxidative enzymes during infection are only one important aspect of this characterization, by which we hope to give the breeder new information on the behaviour of the bean rust in his resistant varieties. This might be useful for further selection experiments.

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## DISCUSSION

R. ANTOSZEWSKI: I imagine you could more effectively label your material by keeping your plant in tritiated water of high specific radioactivity.

K. MENDGEN: This would give highly labelled spores indeed. But I fear that the diffusion of tritiated water and the high rate of isotope exchange migth give place to unspecific diffusion.

A. MICKE: Rohringer and his colleagues at Winnipeg have found a case where RNA is passed from the pathogen to the host. Is this not in contrast to your finding that no labelled compound was transmitted from the fungus to the bean tissue?

K. MENDGEN: I do not think that my results contradict Rohringer's findings. At the moment, autoradiography is not sensitive enough to detect single molecules that might function as a messenger.

F.G.H. LUPTON: I congratulate you on your very elegant technique and on the very clear-cut results, but do you know whether you are considering resistance reactions determined by major genes, such as breeders consider too unstable for safe use?

K. MENDGEN: I hope to attack this problem by using bean varieties that are known to be resistant against different races of bean rust and different bacteria at the same time.

A. MICKE: I think that monogenic and oligogenic inherited resistance mechanisms could be very valuable in composing stable resistance. If we would have selection methods for such components of resistance, we could combine several of them into one variety, and the resulting resistance might be unspecific and long lasting.

K. MENDGEN: This aim seems to be very promising and we plan indeed to combine several 'components of resistance', as you call it. But first of all we have to find the appropriate bean varieties with the methods described.

S.W. ZAGAJA: Mr. Mendgen, you told us in your oral presentation that the technique developed by you is too laborious to be used in screening the seedlings. Would it also be too time-consuming to be applied in parent screening?

K. MENDGEN: I do not think that it is too laborious for parent screening and we shall in fact do this. Having found enough promising varieties, we shall proceed as Mr. Micke proposed.