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Nutrient Uptake in Rust Fungi

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Nutrient uptake in obligate parasitism was recently reviewed by Bushnell and Gay (5) for the powdery mildews. In the rust fungi, the experimental approach to solving this question is quite different, because the fungus mycelium grows only in restricted areas within the leaf. The rust pustules act as foci for the accumulation of many metabolites. The fungus derives nutrients by alteration of the direction of normal phloem transport. Also, since sporulation ruptures the host epidermis, increased water loss through the pustules makes more nutrients available by increased transpiration (23,38).

Many methods have been proposed to study the uptake of nutrients by the rust fungi; Fuchs and Gärtner (11) hoped that the rust pathogen would grow in axenic culture in the correct nutritional environment. Reisener et al (36) fed a leaf with labeled metabolites and analyzed the uredospores to determine which nutrients had been taken up. Shaw and Samborski (41) and Staples and Ledbetter (42) first tried autoradiographic methods to elucidate nutrient uptake. All methods have pitfalls and the three following approaches will be discussed: analysis of uptake during axenic culture, feeding the host with radiolabeled metabolites and subsequent chemical analysis of label distribution, and feeding the host with labeled metabolites and subsequent cytological analysis by autoradiography.

Uptake in axenic culture.

Under natural conditions, biotrophic pathogens derive nutrients from living host cells. Host plants allow optimal development; germ tubes grow as fast as 75 μm/hr and intercellular hyphae about 5–10 μm/hr in a susceptible host. The infection hyphae develop within the host tissue without delay. This is very rapid in contrast to growth on artificial media (39,47), where branching of the fungus generally begins after a delay of 1–4 days, which indicates retarded growth, especially at the intital phases. Later, growth is still much slower than in host parenchyma. However, fungal growth in artificial media can be stimulated, and from such studies we know that rust fungi have some requirements for inorganic salts (6,46). The same effect, although less pronounced, is obvious for carbohydrates (hexoses) (7). The requirements of cultured rust fungi for amino acids are quite specific; most obvious is the need for a sulfur amino acid as a source of nitrogen and sulfur (18,19). The balance of amino acids influences the morphogenic development of *Melampsora lini* (2). For the culture of *Puccinia graminis* f. sp. *tritici*, race 32, a mixture of amino acids and sugars corresponding to what is offered by the host plant resulted in uredospore formation after 10 days and teliospore formation after 28 days (22). The addition of minor constituents such as cholesterol, ergosterol, sitosterol, oleic acid, and ferulic acid (14,17) allowed the culture of different spore forms of *Cronartium fusiforme*. Addition of adenosine triphosphate and ribose to the medium (21) improved axenic culture so that uredospore or teliospore formation can be induced in *P. graminis* as desired. Obviously, the nutrient requirements are specific because the many races of *P. graminis* f. sp. *tritici* each have special nutritional and cultural requirements (4).

Feeding the host radiolabeled metabolites and analysis of label distribution.

Massive increase of respiratory activities (8) and the accumulation of cytokinins (9) in the infected area indicate that the normal patterns of translocation of photosynthetic metabolites in the host cells undergo dramatic alterations. Extensive synthesis of fungal tissue occurs, especially during sporogenesis, and the considerable volume of metabolites required for this synthesis presumably is transported from the host cells into fungal structures. Sugars, mainly sucrose, accumulate in the lesion area (23), and an invertase located on the walls of both host and parasite (25) provides the hexoses used by the fungus. A large pool of different amino acids is provided by the host cell (35,40). Information on which of the amino acids are taken up is important since they serve as sources of nitrogen and sulfur for the rust fungus, and their balance influences fungal growth. To investigate which metabolites provided by the plants are taken up by the fungus, Pfeiffer et al (34) fed wheat leaves with ^{14}C glucose via their hydathodes and analyzed the uredospores of *P. graminis* f. sp. *tritici*. By examining the label distribution within the hexose molecule, they concluded that hexose is taken up and then incorporated into fungal polysaccharides. A study of label distribution in the uredospores also indicated that glucose metabolites are, at least to some extent, incorporated into amino acids. After feeding the host with labeled amino acids, Jäger and Reisener (20) found that lysine and arginine are most probably transported intact from the host to the parasite. Other amino acids (alanine and glycine) appeared to be metabolized to a greater extent before being incorporated into the spore. Although experiments with germinating spores also were performed for comparison, it remained an open question whether at least some amino acids were synthesized by the rust fungus during the parasitic phase or whether all amino acids were derived from the host.

Burrell and Lewis (3) used an "inhibition" technique to determine whether radioactive amino acids were absorbed directly by *Puccinia poarum* or whether they were metabolized in the host cells before their products entered the fungus to be transformed into amino acids. They concluded that serine and alanine may be absorbed apparently directly and more readily than aspartic acid, glutamic acid, or glutamine.

More experiments are needed to investigate why rust fungi in axenic culture exhibit a special need for a sulfur amino acid or glutamic acid as described in the previous sections.

Cytological analysis of nutrient transfer.

Cytological studies do not allow one to trace the pathways followed by a given labeled metabolite on its way to the parasite after it is offered to the host. Metabolism may occur in the host or in the parasite, which will alter it. However, if previous biochemical studies have elucidated the fate of a metabolite, autoradiography can indicate where it accumulates. Then, such studies can demonstrate a relative increase or decrease of the metabolite in the different structures of host and parasite. Furthermore, these experiments have the advantage that the transformations and transpositions being observed have happened under relatively undisturbed conditions.

When ^3H-uridine, ^3H-cytidine, ^3H-leucine (1,44) and ^{14}C-orotic acid (16) were offered to the host, radioactivity accumulated in the infected cell and in the mycelium and uredospores of *Puccinia graminis*. After application of ^{14}C-orotic acid, ^3H-uridine, and ^3H-glycine to infected bean plants, label accumulated in the hyphae and haustoria of the bean rust fungus (*Uromyces phaseoli* var. *typica*) (10,12,42). The initial uptake of nucleosides by a rust fungus appears to coincide with formation of the first haustorium, as shown with *Puccinia coronata* in oat leaves (33).

Differences of label distribution within an infected cell may be recognized. Leucine, cytidine, uridine, and orotic acid or its metabolites accumulate within the nuclei of infected host cells (1,16,45). In bean rust-infected host cells treated with ^3H-glycine, plastids were depleted of label (10). After the similar application of ^3H-lysine, activity accumulated in the plastids (30). The developing haustoria of the bean rust fungus at first had very low activities; a few hours later, however, grain densities were much higher over the mature haustorium (30). This happened at early stages of infection. Later, concentration of label over fungal tissue was much higher when ^3H-glycine or ^3H-leucine was administered (10,26). These dynamics might explain controversial results in some studies of nutrient uptake (1,32,43).

Haustorial function and its role in nutrient transfer may be studied more precisely by comparing nutrient uptake in compatible and incompatible host cells. ^3H-lysine as the transport metabolite appears especially suitable because it is fixed during processing for electron microscopy and is hardly metabolized on its way from host to parasite (30).

In our studies we examined one *compatible* cultivar (Favorit) and two *incompatible* cultivars (Golden Gate Wax and cultivar 017) in combination with our "Hausrasse" of *U. phaseoli*. In cultivar Golden Gate Wax, lysis of cell contents and death of the haustorium occurred not before 6–8 hr after haustorial formation. The intercellular hyphae grew further and tiny pustules within chlorotic spots of the leaf were formed about 10 days later. In plants of cultivar 017, cells collapsed about 20–40 hr after formation of the first haustorium. Subsequently, the rust fungus also died, and a necrotic spot in the leaf indicated the end of the host-parasite interaction (29).

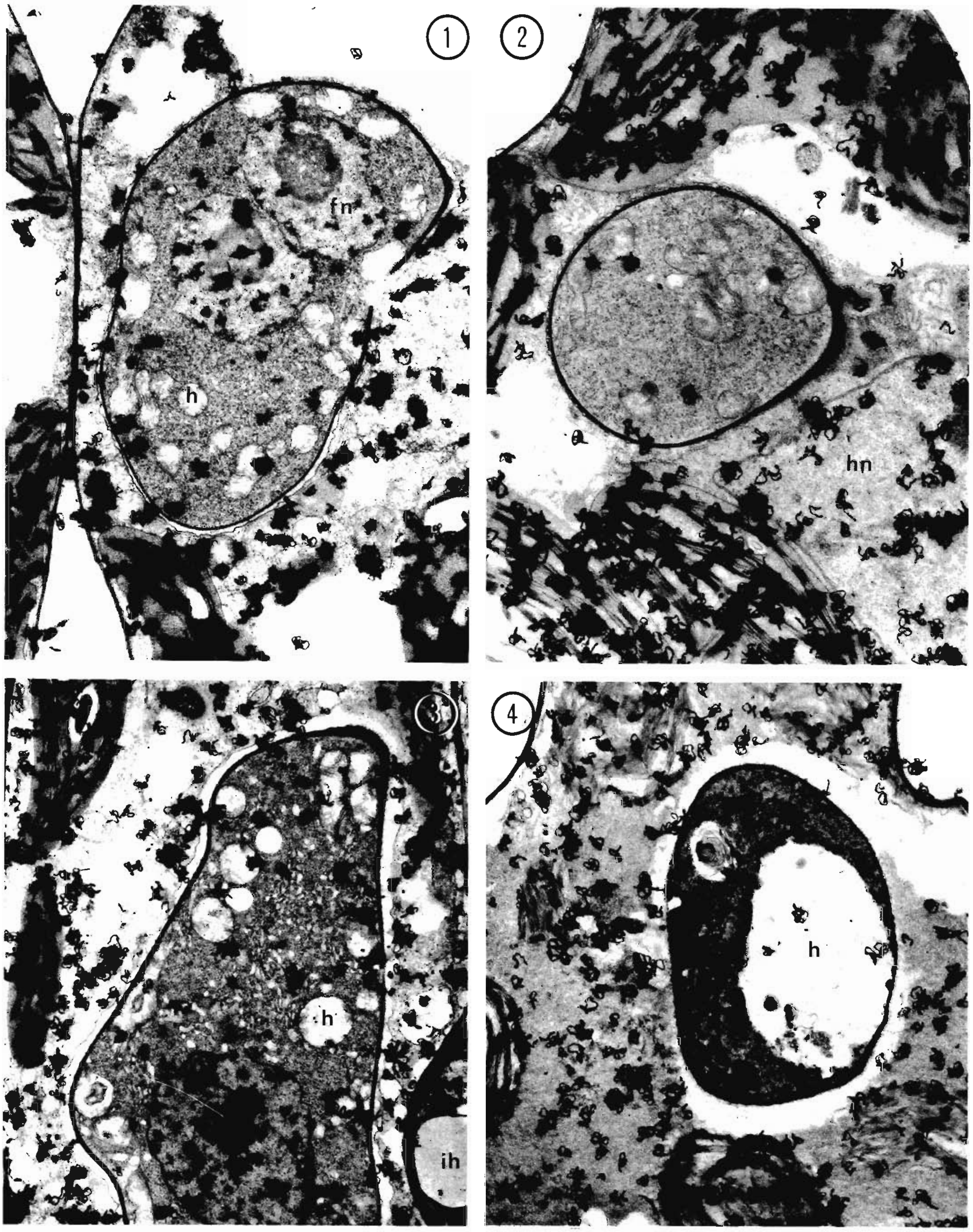
To study nutrient uptake in these combinations, trimmed primary leaves of bean plants were fed with ^3H-lysine as described earlier (30). The uptake of ^3H-lysine or its metabolites into fungal structures was studied by using electron microscopic autoradiography. Analysis of silver grain densities over fungal structures and host cells was performed by using the probability circle method as proposed by Salpeter and McHenry (37). With this method, differentiation between free and bound lysine was not possible. The grain density observed over the surrounding cytoplasm (grains over plastids were not counted, because they were overexposed) corresponded to the pool of free and bound ^3H-lysine offered by the host cell (30).

When haustoria in the cell of the compatible cultivar Favorit (30) and in the incompatible cultivar 017 (Fig. 1) were about 4–8 hr old, silver grains over the haustorium indicated nutrient uptake. The metabolite taken up should be ^3H-lysine. Compared to the surrounding cytoplasm, haustoria exhibited a grain density of 36–38% (Table 1). In the incompatible cultivar Golden Gate Wax

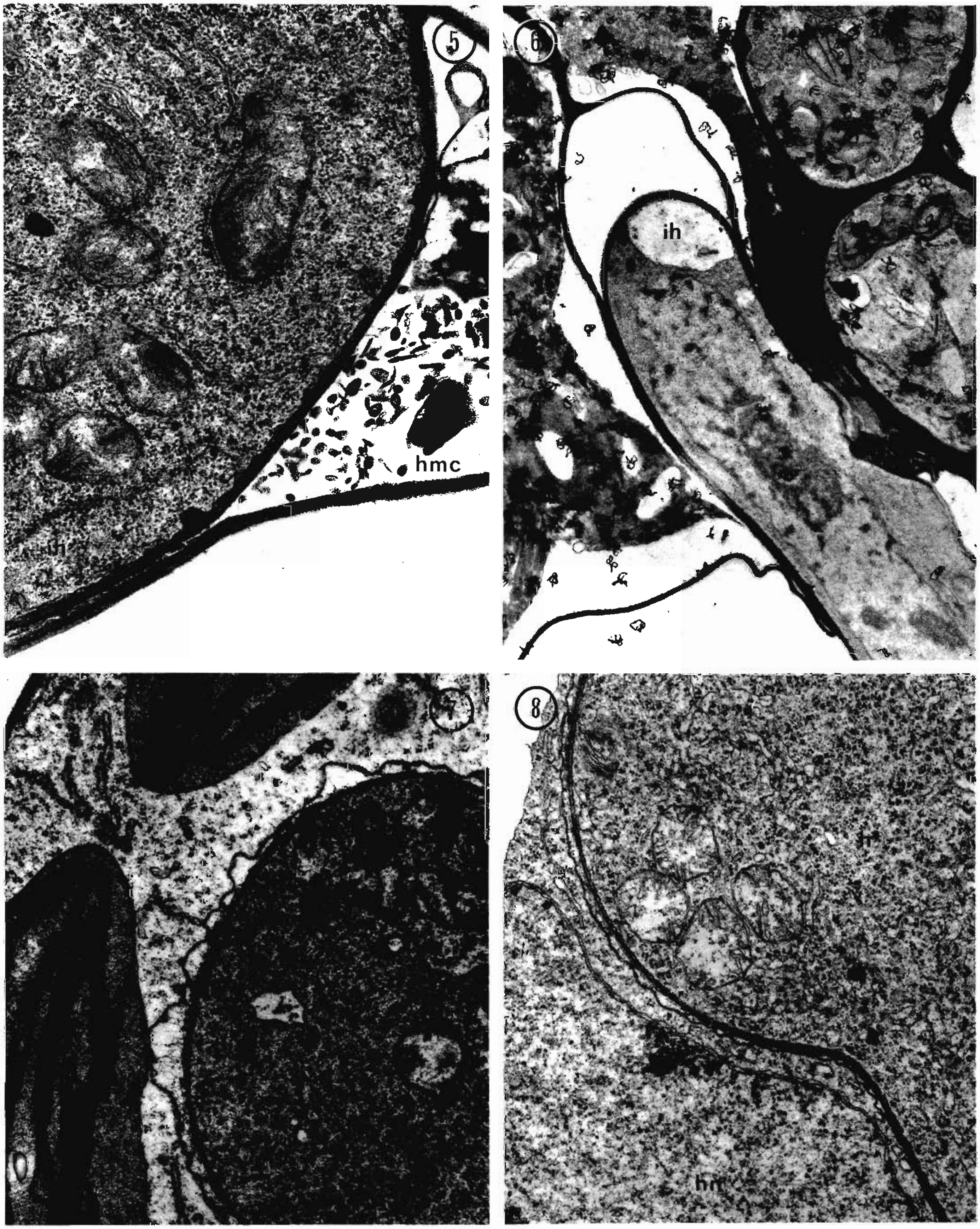
TABLE 1. Relative density of autoradiographically detected silver grains over structures of *Uromyces phaseoli* in comparison to those detected over the surrounding cytoplasm of bean leaf cells after treatment with ^3H-labeled lysine

Structure	Relative density (% difference) of silver grains over fungal structures in cultivars:[a]		
	Favorit[b]	GGW	017
Developing Haustorium	26 ± 5 (350)		
Haustorium			
~4–8 hr old	36 ± 4 (733)	15 ± 2 (538)	38 ± 4 (1,125)
~20–30 hr old	36 ± 3 (1,255)	24 ± 3 (697)	38 ± 3 (1,268)
Hyphae			
~20–30 hr old	27 ± 2 (598)	11 ± 3 (406)	29 ± 2 (1,018)

[a]Grains over at least 10 haustoria in different host cells were counted. The total number of grains counted is indicated in parentheses.
[b]Data from Mendgen (30).



Figs. 1–4. Nutrient uptake by rust haustoria as indicated by the distribution of silver grains after feeding with ^{3}H-lysine. **1,** An approximately 8-hr-old haustorium in the resistant cultivar 017. Silver grains over the haustorium indicate uptake of ^{3}H-lysine or its metabolites (×6,700). **2,** An approximately 8-hr-old haustorium in the resistant cultivar Golden Gate Wax. Compared to Fig. 1, there are few silver grains over the haustorium (×9,800). **3,** An approximately 20- to 30-hr-old haustorium in the resistant cultivar 017. The haustorium has increased in size, but the grain density over the haustorium is similar to that over the younger haustorium in Fig. 1 (×7,000). **4,** In the resistant cultivar Golden Gate Wax, host cell contents are disorganized when the haustoria were about 20–30 hr old. The somewhat increased grain density over the dead haustorium may result from nonspecific diffusion through broken membranes (×8,500).



Figs. 5–8. Differences in fine structure of the haustorial complexes in the two cultivars Golden Gate Wax and 017. **5,** In the incompatible cultivar Golden Gate Wax, the haustoria and their haustorial mother cells are dead. Such a disorganized haustorial mother cell is shown here (hmc). It can be clearly differentiated from the still living mycelium (ih) (×8,000). **6,** The intercellular hyphae in the cultivar Golden Gate Wax are covered by only a few silver grains, although they are growing between disorganized and normal-looking cells covered by many silver grains (×6,700). **7,** An approximately 8-hr-old haustorium in the resistant cultivar Golden Gate Wax. The extrahaustorial membrane appears more undulated than usual and the extrahaustorial matrix is swollen (×20,000). **8,** An approximately 8-hr-old haustorium in the resistant cultivar 017. Host cell organelles and haustorium were not different from those observed in compatible combinations (×10,000).

(Fig. 2) grain density remained much lower compared to that observed in the other combinations.

When the haustoria were about 20–30 hr old, they were larger. Grain density remained the same, indicating a constant uptake of ^{3}H-lysine in cultivars Favorit and 017 (Fig. 3, Table 1.) However, at that stage of infection in cultivar Golden Gate Wax, cell contents of host and parasite had become disorganized and membranes were broken down. However, the haustoria were labeled (Fig. 4). We assume that this labeled material had diffused from the host cell into the haustorium through the damaged membranes. These haustoria and their haustorial mother cells were disorganized and were separated from the rest of the intercellular mycelium by the intervening haustorium mother cell septum (Fig. 5). We assume that the disorganized haustorium and haustorial mother cell can no longer transfer nutrients to the mycelium. The intercellular hyphae that had grown between the disorganized host cells with the dead haustoria exhibited very low grain density (Fig. 6). This observation also may indicate that living intercellular hyphae without live haustoria are less effective in nutrient uptake.

The differences in nutrient uptake by the two incompatible cultivars cannot be readily explained by differences in fine structure (Figs. 7, 8). In plants of cultivar Golden Gate Wax (Fig. 7), the extrahaustorial matrix (sheath) appeared to be swollen in some cases. The extrahaustorial membrane was undulated, but not broken as described recently in another incompatible combination with *P. graminis* f. sp. *tritici* (13). Mitochondria appeared somewhat enlarged, perhaps indicating increased respiration. Compared with the haustorium in cultivar 017 (Fig. 8), no major differences were observed.

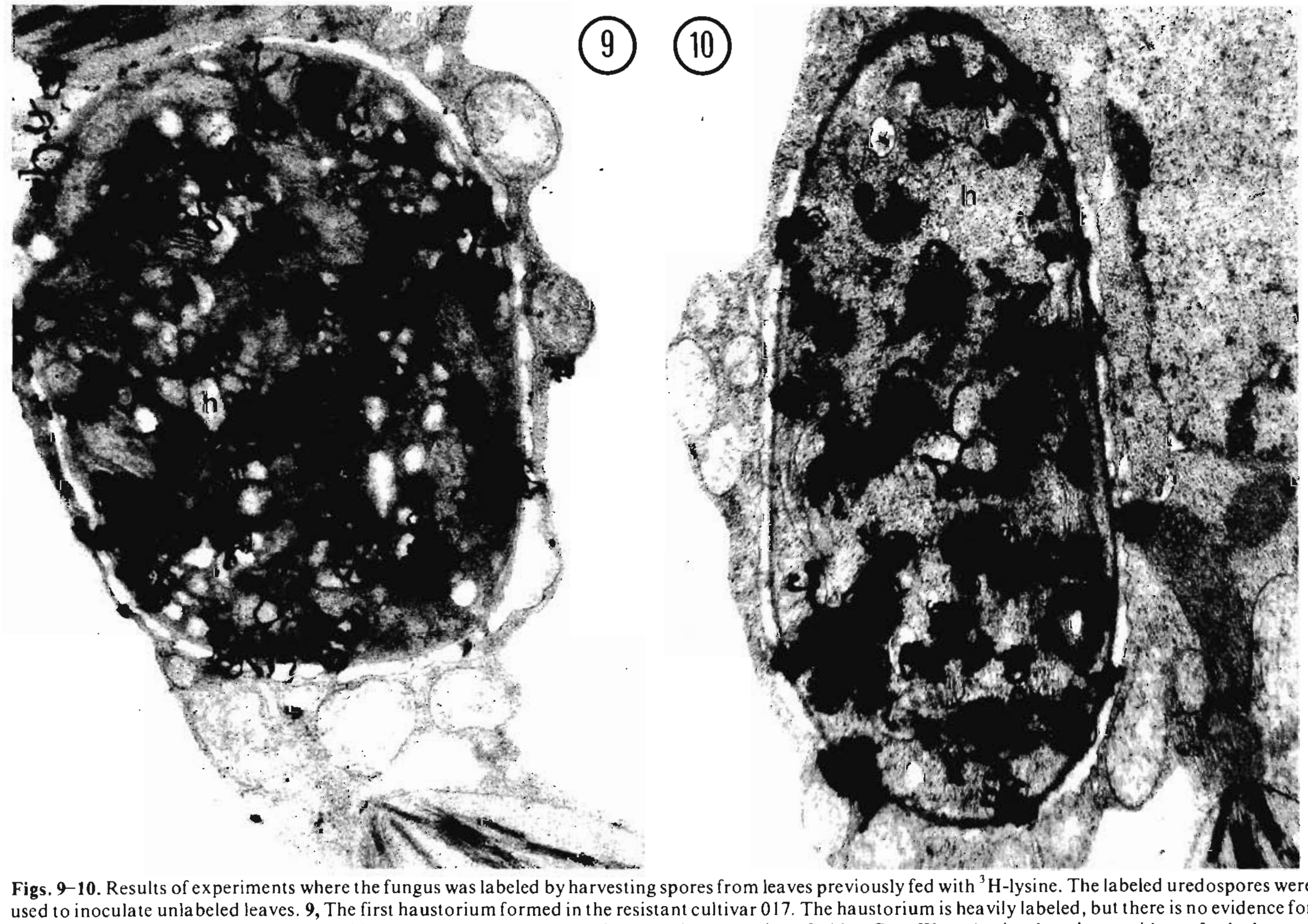
Since any membrane damage of host and parasite could not be detected by direct observation at 4–6 hr after haustorial formation, we tested possible leakage of the fungal plasmalemma by labeling the fungal structures with ^{3}H-lysine as described earlier (30,31). Detection of label in the parasitized cell then would indicate a leakage of ^{3}H-lysine from the parasite to the host. However, in all three cultivars tested at this early stage of host-parasite interaction, the label remained restricted to the structures of the fungus, and no label accumulated over the extrahaustorial matrix (Figs. 9 and 10). This result indicates that the fungal plasmalemma was not damaged in any of the three combinations during this early stage following haustorium formation.

DISCUSSION AND CONCLUSIONS

Nutritional requirements of a rust fungus can be studied starting from rust mycelium in axenic culture. This method has indicated the basic needs of a rust fungus (eg, a balanced mixture of amino acids, a sulfur amino acid, different surgars, inorganic nutrients, and some minor nutrients such as steroids). However, since the fungus grows (39) much better in the host plant by means of haustoria, axenic culture may not reflect the most efficient condition.

Since it is difficult to separate the tissue of host and parasite, biochemical studies of the fungus growing within the plant are difficult to interpret. When a leaf was fed through the hydathode with labeled nutrients, and the spores of *P. graminis* f. sp. *tritici* were subsequently analyzed, it was shown that the fungus took up nutrients in the form of hexoses and amino acids (20,34). Burrell and Lewis (3) consider serine and alanine to be important in the successful establishment and maintenance of the rust fungus in the host.

Cytological studies allow a close look at the host-parasite interface. Many autoradiographic experiments have been



Figs. 9–10. Results of experiments where the fungus was labeled by harvesting spores from leaves previously fed with ^{3}H-lysine. The labeled uredospores were used to inoculate unlabeled leaves. **9,** The first haustorium formed in the resistant cultivar 017. The haustorium is heavily labeled, but there is no evidence for leakage of label into the host cell (×12,700). **10,** A labeled haustorium in the resistant cultivar Golden Gate Wax. Again, there is no evidence for leakage of major amounts of label into the host cell (×9,800).

performed since the first studies of Shaw and Samborski (41) and Staples and Ledbetter (42). A major problem is that we do not know whether the label observed over fungal structures demonstrates the original labeled metabolite that was offered to the host plant. It may even happen that when ^{14}C-metabolites are offered, $^{14}CO_2$ is secreted by the host plant and subsequently refixed by the fungus. Discrimination between free or incorporated labeled metabolites is not yet possible. Therefore, autoradiographic studies are most useful when nutrients are used that are minimally metabolized on their way into the fungal structures and which can be immobilized by chemical fixation to avoid nonspecific diffusion. The results with tritiated lysine have shown that the fungus takes up this metabolite preferentially through haustoria and does not leak major amounts back into the host cell.

Uptake of amino acids may be reduced in incompatible host-parasite combinations (26,28). In the cultivar Golden Gate Wax, grain density over haustoria was reduced to about one third compared to that observed in the compatible cultivar Favorit. It may be speculated that this deficiency is a result of host resistance and occurs before host cell death. A similar phenomenon has been observed in powdery mildew infecting wheat (27). However, this is not a phenomenon always correlated with resistance. In the resistant cultivar 017, uptake of ^3H-lysine was identical to that observed in cultivar Favorit up to 48 hr after inoculation; ie, a few hours before necrotisation of host tissue and fungus occurred. Thus, it appears for this incompatible host-parasite interaction, that nutrient uptake of the haustorium functions normally until other metabolic processes induce the death of host and parasite (29).

The importance of the haustorium for nutrient transfer is underlined by other observations. The haustorial neck wall contains a specialized structure, the "neckband," which is capable of stopping apoplastic flow of materials along the neckwall, a function similar to that of the Casparian strip in vascular plants (15). Thus, the haustorial body is isolated from the hyphae walls and is favored as a structure modified for the uptake of essential substances (15). The haustorial body is surrounded by the extrahaustorial matrix and the invaginated host plasma membrane. The role of the extrahaustorial matrix during nutrient transfer is unclear. Label never accumulated in that area (10,26,30). Therefore, it does not seem to serve as a sink to supply the haustorium with nutrients.

Since in our host-parasite combinations (cultivars Favorit, 017, and Golden Gate Wax) the fungal plasmalemma originated always from the same race of *U. phaseoli*, and since metabolites did not diffuse from the fungus to the host, it appears that the extrahaustorial membrane is possibly the critical barrier governing the success or failure of nutrient transfer between host and parasite. The morphological basis for this could be the specialized structure of this membrane (24). Further studies are needed to determine the structure of membranes in incompatible combinations.

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