Connecticut Field stock #9, Jack-O-Lantern) and a common pathogen of the pumpkin, the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, were the organisms selected. Magnesium and manganese were chosen since both ions are important in plant metabolism [4], especially photosynthesis [5], and are also interchangeable as co-factors for many biochemical reactions. The pumpkin seedlings were germinated (98% germination after 5 days) on paper towels and grown in trays of acid-washed quartz sand in a growth chamber adjusted for 16-h days at 30 °C and 21 °C nights. Plants were grown under polyethylene humidity-control chambers which were made by cutting the bottoms from 5-l polyethylene reagent bottles. They maintained high humidity around the plants and also prevented cross-contamination between treatments—an important consideration for working with an infectious disease. The polyethylene was translucent and allowed good temperature equilibration as well. Four pumpkin seedlings were put under each polyethylene chamber. Four chambers were set in each of six trays. Thus for each treatment (complete, minus Mg, or minus Mn) there were 4 plants not inoculated and 28 plants inoculated with the pathogen. The pumpkin seedlings were watered daily with Hoagland's solution, either complete or minus magnesium or minus manganese. Sclerotia of the fungus were supplied by Duane Letourneau (University of Idaho, USA). Cultures of the fungus were obtained by growing sclerotia, which were surface-sterilized in sodium hypochlorite diluted 1:5 for 3–5 min. Cultures were maintained on potato dextrose agar slants. When the pumpkin seedlings were 7 days old (time since seeds imbibed), the two first foliage leaves of each seedling were inoculated with cultures of the fungus which were actively growing. Leaves of the control plants were scratched but not inoculated with the fungus. Daily observations on the progress of the disease were recorded, and records were kept of characteristic necrosis and dieback of infected plants. The experiment was terminated 21 days after plants were inoculated.

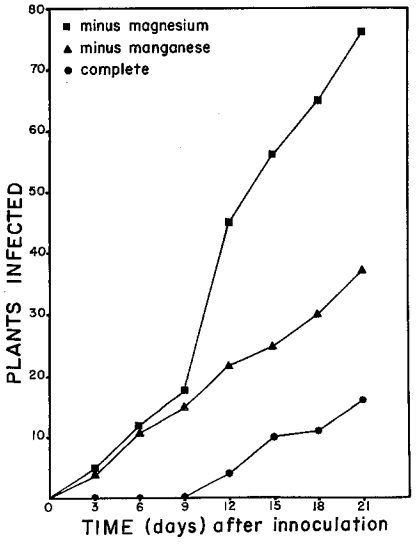

Fig. 1. Cumulative number of pumpkin seedlings infected with *Sclerotinia sclerotiorum* on days following inoculation

Seedlings grown in the absence of Mg or Mn showed disease symptoms within 3 days after inoculations (Fig. 1). Plants on complete Hoagland's solution showed no symptoms for 9 days after inoculation. Plants which were not inoculated (−Mg, −Mn, complete) did not exhibit symptoms during the duration of the experiments. Seedlings low in magnesium were more susceptible to fungal attack than were manganese-deficient plants. However, both were more readily infected than plants watered with complete Hoagland's solution (Fig. 1).

In summary, deficiency of magnesium or manganese greatly increased both time of infection and rapidity of progress of fungal disease for 7-day-old pumpkin seedlings inoculated with *Sclerotinia sclerotiorum*. It would seem advisable to determine the mineral nutrition status of most plants in treatment and prevention of plant disease. Research on the possible subcellular role of magnesium in preventing fungal infection is now under way.

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1. Bottrill, D.E., Possingham, J.V., Kriedemann, P.E.: Plant Soil *32*, 424 (1970)
2. Arny, D.C., Lindow, S.E., Upper, C.D.: Nature *262*, 282 (1976)
3. Interface *5*, 37 (1976)
4. Larkum, A.W.D.: Nature *218*, 447 (1968)
5. Heath, R.L., Hind, G.: Biochim. Biophys. Acta *189*, 222 (1969)

Reduced Lysine Uptake by Bean Rust Haustoria in a Resistant Reaction

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Host-cell necrosis, which commonly occurs when pathogens infect incompatible host varieties, has suggested a causal relationship between abnormal, rapid cell death and resistance (see [1]). It is not known, however, whether the 'hypersensitive' [2] cell death inhibits fungal growth by formation of substances toxic to the pathogen and by depriving the biotrophic pathogen of its food base, the living cell; or whether the hypersensitive reaction is only a consequence of an unsuccessful interaction between the host and pathogen and thus, is not primarily responsible for resistance. Our results are consistent with the latter possibility.

The nutrient uptake (^{3}H-lysine) by *Uromyces phaseoli*, bean rust, was studied by autoradiographic electron microscopy in compatible and incompatible cultivars of bean, *Phaseolus vulgaris*. The cultivar 'Favorit' is compatible, i.e., about 18 h after inoculation with the uredospores, the infection hypha forms the first haustorium in the leaf mesophyll. Within 3 to 5 h later, the haustorium is fully developed and running hyphae differentiate haustoria every 8 h. Eight days later, uredia appear. The bean cultivar 'Golden Gate Wax' is incompatible, i.e., the formation of the first haustorium also begins 18 h after inoculation, but 3–5 h later, disintegration of the plant cell content begins and extends to most infected cells. Haustoria also deteriorate and fungal growth is retarded. Only very small uredia form two weeks later. To study the function of the haustoria, the leaf was fed with lysine by dipping the petiole in 1 ml water with 330-μCi ^{3}H-lysine. One day later, unlabeled uredospores were allowed to infect the labeled leaf under high-humidity conditions. After 24 h, samples were fixed for electron microscopy. In the compatible combination, normal haustoria had developed [3]. In the incompatible host, haustoria of some infection hyphae were in cells with normal fine structure, others were in cells whose membranes were disrupted and whose mitochondria and plastids appeared swollen or disintegrated. After application of an autoradiographic emulsion (Ilford L 4 Gel), it could be seen that haustoria in the compatible cultiver had taken up the labeled

Fig. 1. (a) Haustorium of the bean rust in a cell of the compatible cultivar Favorit. Silver grains over the haustorium indicate transport of ^{3}H-lysine or its metabolites from host to parasite. (b) Haustorium in the incompatible cultivar Golden Gate Wax. It is only slightly labeled (*ha* haustorium, *p* plastid, ×8800)

material (Fig. 1a). In the incompatible bean, all haustoria in cells with intact fine structure were covered with only a few silver grains (Fig. 1b). This indicates that the uptake of lysine or its metabolites by haustoria in the incompatible cultivar is reduced. Obviously, a reduction in nutrient uptake by the haustorium occurs before the hypersensitive death of the host cell begins.

These results cannot yet be generalized. Previous morphologic studies have shown that, as in other host-parasite combinations (e.g. [4]), every combination of host and rust seems to be unique [5]. Different mechanisms might induce hypersensitive cell death [6]. This may explain why there is still much controversy about the significance of premature host cell necrosis in disease resistance [7]. Therefore, a study of other incompatible bean rust-bean combinations is in progress to see whether starvation of haustoria occurs generally.

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1. Müller, K.O., in: Plant Pathology I (eds. Horsfall, J.G., Dimond, A.E.). New York: Academic Press 1959
2. Stakman, E.C.: J. Agric. Res. *10*, 427 (1915)
3. Mendgen, K.: Phytopath. Z. *78*, 109 (1973)
4. Lupton, F.G.H.: Trans. Br. mycol. Soc. *39*, 51 (1956); Slesinski, R.S., Ellingboe, A.H.: Can. J. Bot. *49*, 303 (1971)
5. Heath, M.C.: Physiol. Pl. Path. *4*, 403 (1974)
6. Littlefield, L.J.: ibid. *3*, 241 (1973)
7. Mayama, S., et al.: ibid. *7*, 35 (1975); Tani, T., et al.: ibid. *7*, 231 (1975); Ogle, H.J., Brown, J.F.: Ann. Appl. Biol. *67*, 309 (1971); Heath, M.C.: Phytopathology *66*, 935 (1976)

Somatic Embryogenesis from Tobacco Protoplasts

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Nicotina tabacum has long been classified as the model plant for in vitro culture, but the important step of plant regeneration via embryogenesis from callus or suspension cultures has not been demonstrated. We now report upon embryo-like structure formation from calluses and cell suspensions of protoplast origin of both amphihaploid and amphidiploid *Nicotiana tabacum*, var. Samsun. Mesophyll protoplasts were isolated from greenhouse-grown haploid and diploid tobacco plants by the method of Nagata and Takebe [1]. They were then cultured by a new hanging-drop technique [2] that permits the testing of large numbers of media combinations. Over 2000 hormone combinations were tested in combination with Nagata-Takebe basal medium. The hormones, tested over the range 10^{-4}–10^{-8} *M*, included 2,4-dichlorophenoxyacetic acid, indoleacetic acid, o-naphthaleneacetic acid, parachlorophenoxyacetic acid, and Dicamba as auxins and kinetin, 6-benzylaminopurine and zeatin as cytokinins. In some of these treatments (especially 6×10^{-6} *M* p-chlorophenoxyacetic acid in combination with 2.5×10^{-5} *M* kinetin or 1×10^{-5} *M* Dicamba in combination with 5×10^{-5} *M* 6-benzylaminopurine) structures that were proembryo-like were observed (Fig. 1A). These, if allowed to remain in the same culture medium, gave rise to calluses. The formation of large numbers of embryo-like structures (Fig. 1B), bearing a remarkable resemblance to sexually produced embryos, occurred when cell cultures 3–4 weeks after protoplast isolation were transferred to the medium of Wood and Braun [3] as used by Thomas and Street [4] for inducing embryogenesis from suspensions of *Atropa belladonna*. Recently,

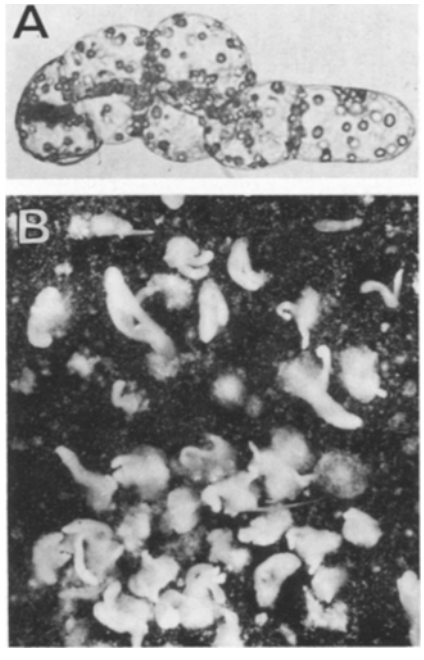

Fig. 1. (A) Proembryo-like structure of *Nicotiana tabacum* formed in liquid medium 24 days after protoplast isolation. (B) Large numbers of embryo-like structures after transfer of protoplast-derived cell cultures to medium of elevated ammonium content

