Use of the Electron Microprobe to Measure Exchange of Materials between Host and Pathogen

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ABSTRACT

Relative concentrations of Mg, K, Rb, P, and S in conidia of *Helminthosporium carbonum* and *H. victoriae* on corn leaves (resistant and susceptible to *H. carbonum*) were measured with the electron microprobe X-ray analyzer. All five elements decreased significantly during germination and penetration (0-16 hr after inoculation) of susceptible and resistant tissues. *H. carbonum* regained Mg from susceptible leaves (24-48 hr), but not from resistant leaves. *H. carbonum* conidia on susceptible leaves had 70% and 30% of the original K content at 16 and 36 hr after inoculation. Conidia on resistant leaves had 30% of the original level of K at 16, and almost none at 36 hr. Since Rb, like P and S, was lost from conidia on both resistant and susceptible leaves at equal rates, the results imply an uptake of K from susceptible leaves which is masked by a larger loss. The losses from conidia of *H. victoriae* on both types of corn were comparable to those of *H. carbonum* on resistant corn. Thus, there is an exchange of certain materials between a pathogen and a susceptible host soon after infection, but probably only losses from conidia to the highly resistant host. These observations indicate the potential usefulness of the microprobe for studies of infection and disease development in plants.

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The electron microprobe X-ray analyzer (microprobe) has many potential uses in biological research. It was first used in work with plants in 1966 to determine the distribution of several nutrient elements in corn leaves (9). More recent microprobe studies have shown the K^+ increases in guard cells as stomates open, and decreases as stomates close (7, 14). Another microprobe study was concerned with aluminum uptake and toxicity in corn roots (12). These studies were not possible before the microprobe was available. We are not aware of previous use of the microprobe for studies of plant infections.

The microprobe focuses a beam of electrons on a test sample. Atoms in the sample are thereby excited, causing them to emit X-rays with characteristics peculiar to each element. The X-rays are selected for type and their intensities are measured by an adjustable detector. X-ray intensity, in counts per minute (CPM), gives the relative concentration of each element (13). The instrument can detect and measure all elements above beryllium in the periodic table; as little as 10^{-15} g of some elements can be detected (1).

To explore the possible use of the microprobe in host-pathogen studies, we measured the relative concentrations of several nutrient elements in fungal spores (*Helminthosporium carbonum* Ullstrup) as they germinated on resistant and susceptible corn leaves. The homologous pathogen, *H. victoriae*, was included as a control. The electron beam was easily focused on single conidia and even on individual cells within each conidium. Part of this research has been described in an abstract (3).

MATERIALS AND METHODS.–Conidia of *H. carbonum* race 1 and *H. victoriae* were produced by Lukens' method (11). Cultures were grown on a shaker for 3 days in Fries' medium with the following supplements, in g/liter: yeast extract, 1; KH_2PO_4, 4; and $MgSO_4 \cdot 7H_2O$, 2. Washed mycelium was comminuted briefly in a Waring Blendor; the fragments were washed twice by centrifugation and suspended in 0.02 M phosphate buffer (pH 6.4). A 2-ml aliquot was placed on dry filter paper in a 9-cm petri dish and incubated under continuous light for 3-5 days. Sporulation on the filter paper was abundant.

Leaves of two near-isogenic corn hybrids were used; Pr × K61 is susceptible and Pr1 × K61 is resistant to *H. carbonum* race 1. The second true leaves of plants 10-11 days old were anchored to porcelain plates, with the basal cut ends in water, in a chamber at 100% relative humidity. Conidia were spread on leaves ($10\text{-}15/mm^2$) with a camel hair brush. At various times (0 to 48 hr) after inoculation, spores were removed by gently placing a piece of adhesive tape ("Permacel") on the leaf surface. Spores but not leaf fragments adhered to the tape when it was removed. Tapes were attached to glass slides with spores exposed on the upper surface. The preparations were then frozen, and stored in a desiccator for later analysis with the microprobe.

Concentrations of Mg, K, Rb, P, and S in the conidia were determined with an Applied Research Laboratories microprobe, operating at 16.5 kV and 0.025 μA. To minimize variation in sampling, conidia were removed from pairs of resistant and susceptible leaves at several sampling times. Determinations were

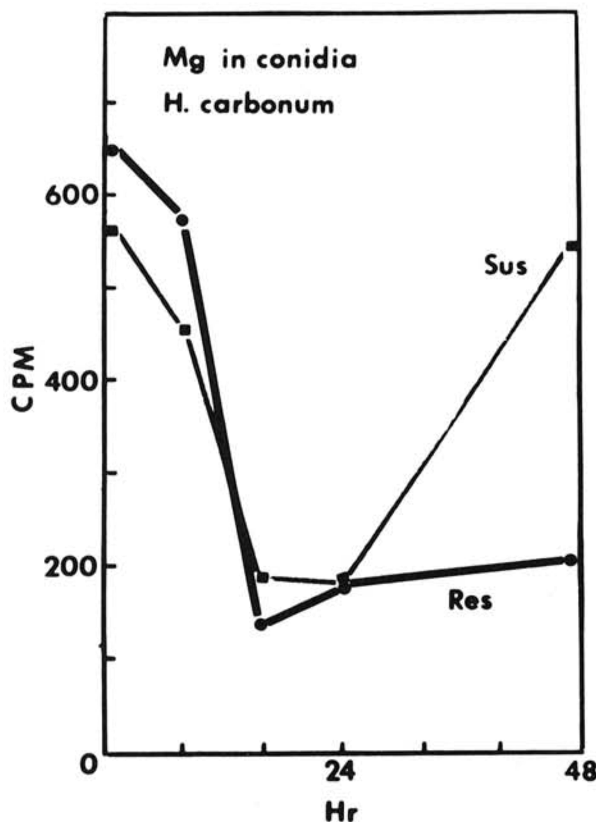

Fig. 1. Relative content of magnesium in conidia of *Helminthosporium carbonum* on resistant (res) and susceptible (sus) corn leaves, at time intervals after inoculation. CPM refers to microprobe counts per min.

made on 15 spores from each leaf for each sampling time, with two determinations (each, 15 seconds) per spore. Background counts for the adhesive tape were subtracted from the average counts for each spore. There was a total of eight experiments on *H. carbonum* with Mg and four with each of the other elements, with comparable results for each element. The data presented are for single representative experiments.

RESULTS.–Conidia of *H. carbonum* lost Mg during germination (0-8 hr) and penetration of leaf cells (8-16 hr) (Fig. 1). The most rapid losses occurred during the penetration phase; by 16 hr, conidia on leaves of both the resistant and the susceptible corn had less than one-third of the original Mg content. Thereafter, the Mg in conidia on the susceptible corn increased to about the original level; Mg return began at about the time that successful colonization was evident (4). On the resistant corn, *H. carbonum* was confined to one or a few cells, and Mg in the conidia remained at a constant low level.

H. carbonum conidia lost K rapidly during germination and penetration (Table 1). At 16 hr after inoculation, the K content of conidia on the resistant corn leaves was 30%, and on the susceptible corn 70%, of the original values. Essentially all K was lost from conidia on the resistant corn leaves by 36 hr after inoculation; conidia on the susceptible corn still contained 30% of the original K content. Both the Mg and K data show differences between spores on the resistant and susceptible leaves at the time resistance was evident.

Conidia of *H. carbonum* were labeled with Rb by growing cultures in Fries' medium supplemented with RbCl (4 g/liter). During germination on the leaf surface (0-8 hr), Rb content of conidia fell from approximately 700 to 550-600 CPM. There were even more drastic losses during the penetration phase (8-16 hr), when the Rb content fell to less than 100 CPM. No further losses were observed at 24 and 48 hr after inoculation. Conidia on the resistant and susceptible corn leaves were comparable in Rb content throughout the experiment. However, Rb was not available for movement from host to pathogen, because leaves contained none at the time of inoculation. This differs from K, which is abundant in plant cells and should be available to a pathogen. Thus, the differences between the losses of Rb and K probably reflect an uptake of K by the successful pathogen.

Phosphorus in *H. carbonum* conidia at 0 time gave 7,500-7,775 CPM, whereas sulfur gave only 250 CPM. Conidia lost P rapidly during germination and penetration of leaves; 50-60% was gone by 16 hr after inoculation, and more than 96% was gone by 36 hr. Approximately 25% of the S was lost by 8 hr. The S count dropped to approximately 100 CPM by 16 hr, and no further decreases were evident at 24 and 48 hr. There were no differences in losses of S and P by conidia on the susceptible and resistant corn leaves, nor were there indications that either P or S were regained by conidia.

H. victoriae germinated and penetrated the epidermal cells of corn, but developed no further (4). The pattern of Mg, Rb, and S losses from *H. victoriae* conidia on corn leaves was comparable to the losses from *H. carbonum* on the resistant corn. For example, Mg was lost during germination and penetration but was not regained by conidia on corn leaves of either type.

DISCUSSION.–These data show that the

TABLE 1. Relative content of potassium in conidia of *Helminthosporium carbonum* on corn leaves, at time intervals after inoculation

Corn type	Hr after inoculation		
	0 hr	16 hr	36 hr
	CPM[a]	CPM	CPM
Susceptible (Pr × K61)	4876	3544	1592
Resistant (Pr1 × K61)	5188	1592	28

[a]CPM = microprobe counts per min.

microprobe has potential in studies of host-pathogen interactions and plant disease development. Detection limits are low enough to determine locations of elements within individual plant (13) or fungal cells. We feel that the microprobe can be even more useful after development of a wider range of techniques for preparing plant samples.

Results of a companion study (4) showed that *H. carbonum* conidia germinated 3 to 4 hr after inoculation, and penetrated cell walls by 12 hr. Hyphal growth was restricted in the resistant (Pr1 X K61) corn leaf by 16 hr after inoculation, as compared to growth in susceptible tissue. The microprobe data indicate a relationship between loss or gain of some nutrient elements in the developing fungus and these events in the colonization process. *H. carbonum* and *H. victoriae* conidia lost significant amounts of Mg, K, Rb, P, and S during germination and penetration of leaf surfaces. The elements presumably are lost to host tissues, or are translocated to the actively growing hyphae. Other spores are known to lose materials to an ambient culture medium before and during germination (5, 10). Such spores may not develop further without exogenous supplies (8). Nevertheless, the extent of P loss was not expected, considering the viability of such spores (6). Losses of P from germinating spores deserves further study.

Helminthosporium conidia appear to continue as functional metabolic units after germination and penetration of plant tissues. This is evident in the fact that such conidia are capable of germinating several times after failure of the first germ tubes (6). Our microprobe data appear to confirm their viability; *H. carbonum* conidia regained Mg from susceptible tissue after germ tube penetration, and appeared to maintain minimal levels of certain other elements. There is probably some movement of K to the fungus, as indicated by the difference between rates of loss of K and Rb from conidia on susceptible leaves [plant cells ordinarily do not discriminate between K and Rb (2) in such movement]. On the resistant leaf, K content in conidia soon falls to very low levels, perhaps below those required for active metabolism. Sulfur is less mobile than Mg or K, although others have shown that ^{35}S is transferred from susceptible wheat leaves to *Erysiphe graminis* f. sp. *tritici* when a functional haustorium is formed (16). Phosphorus in conidia on both susceptible and resistant leaves falls to surprisingly low levels, perhaps also below that required for active metabolism.

The fungal-produced, host-specific toxins of *H. carbonum* and *H. victoriae* could account for the ability of these fungi to obtain nutrients from susceptible but not from resistant tissue (15, 17). Both histological data (4) and our data on Mg content of *H. carbonum* conidia on resistant and susceptible leaves indicate that resistance is evident at about 16 hr after inoculation. Such a time schedule is compatible with the hypothesis that success or failure of the fungus is determined by effectiveness of its toxin (15). Is lack of available nutrients a reason for restricted fungal growth in resistant tissue? Further work with the microprobe may help to answer this question and to clarify other relationships.

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