Influence of Powdery Mildew Infection on ^{35}S and ^{45}Ca Accumulations in Leaves of Apple Seedlings

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ABSTRACT

The influence of powdery mildew on translocation of root-absorbed ^{35}S and ^{45}Ca into leaves and areas of infection of young apple seedlings grown in nutrient solution was investigated by radioautography and counts of mildew-infected and adjacent tissues.

On leaves with young colonies of mildew prior to labeling, sulfur accumulated at the infection site, but there was no accumulation in old colonies. Accumulation of ^{35}S could not be detected 3 days after inoculation in young leaves labeled prior to inoculation. However, accumulation was demonstrated 10 days after inoculation.

There was no detectable accumulation of ^{45}Ca at the infection sites, although the spores became radioactive. The transport of ^{45}Ca to diseased leaves was markedly less than that to healthy leaves of the same plant. Phytopathology 61:1099-1103.

Additional key words: Podosphaera leucotricha, obligate parasitism.

The use of radiotracers to study movement and accumulation of compounds in diseased tissue has contributed valuable information on host-parasite interactions (6, 7, 8, 10, 12). Most of this work refers to rusts and powdery mildews of beans, wheat, or barley. Little is known about the host-parasite relationships of obligate parasites infecting leaves of a perennial plant, e.g., *Podosphaera leucotricha* (Ell. & Ev.), apple powdery mildew.

Yarwood & Jacobson (14) tested the ability of diseased tissues of a variety of plants to selectively accumulate labeled substances. In their studies, mildew-infected tissue of *Pyrus malus* fumigated with sulfur-labeled H_2S contained less ^{35}S than did healthy tissue. However, ^{35}S was not introduced via the translocation stream in their experiments.

Sulfur-containing compounds are well known to control powdery mildews. Recent work has demonstrated that L-methionine inhibits the development of powdery mildew on cucumber leaf discs (3) and apple powdery mildew (4). An amino acid analysis of *Erysiphe graminis* DC. *hordei* E. Marchal spores revealed no sulfur containing amino acids (11). However, sulfur containing amino acid has been found in apple powdery mildew spores (*unpublished data*).

The following investigations were conducted to obtain information on the influence of mildew infections on the accumulation of labeled $SO_4^=$ in apple leaves. In addition, ^{45}Ca was used since calcium is only slightly remobilized in plants once it is deposited (7).

MATERIALS AND METHODS.—Apple seeds germinated in moistened perlite were planted in a greenhouse in peat moss irrigated with 50% Hoagland's (9) solution. When the seedlings were ca. 7 cm tall, they were transferred to a growth room with continuous light of about 500 ft-c, temperature, 24 ± 2 C, and relative humidity, 45-55%. After a few days, radiotracers were added to 0.5% strength Hoagland's solution as $H_2{}^{35}SO_4$ or $^{45}CaCl_2$ (generally 160 μc ^{35}S, 160 μc ^{45}Ca/liter).

Apple seedlings were sulfur-labeled in separate experiments both prior to and after visible mildew was present on the leaves. The labeling period was generally 4 days. At the end of the tracer uptake period, diseased leaves were detached and prepared for radioautography.

In one experiment, the relationship between age of mildew infection and the accumulation of solutes was determined. Healthy seedlings were sulfur-labeled for 2 days before inoculation. At the time of inoculation, activity was determined in the leaves with a Geiger counter. Radioautographs were made of infected leaves 3 and 10 days after inoculations.

Radioautographs were made in light-tight cassettes using Kodak industrial Type M X-ray film. The leaves were placed on foam rubber and separated from the film with Saran Wrap. During the exposure period of 3 to 10 days, the cassettes were kept at 0 F. To locate the areas of mildew infections, the leaves were photographed before radioautographs were made, using panchromatic process film, polarized light, and Wratten G filter.

To determine the influence of mildew infection on the influx of ^{45}Ca into the apple leaves, the entire surface of a young, fully expanded leaf was inoculated with fresh mildew spores using the leaf at the next lower position of the plant as a control. The lower leaf was kept free from mildew infection by painting it several times (over a 7-day period) with a 0.3% Karathane solution. Additional pairs of leaves on each seedling were treated similarly as they reached full expansion. When two or three such treated leaf pairs had developed, six plants were set in a ^{45}Ca-labeled nutrient solution for 30 hr. The corresponding leaves were then harvested, weighed, and wet-digested in con-

centrated HNO_3 on stainless steel planchets, and their radioactivity was determined with a gas flow proportional counter and calculated as counts per minute per unit fresh weight and per leaf.

RESULTS.—The effect of apple mildew on the accumulation of sulfate into leaves with visible mild infections prior to labeling is illustrated in Fig. 1. The radioautographs show that ^{35}S accumulated in some, but not all, areas of infection. The oldest leaf (Fig. 2-a, d), the second of nine developed leaves, was mildew-infected several weeks before the labeling, whereas the mildew spots of the youngest leaf (Fig. 1-c, f) developed 4 days after introduction of the tracer (6 days after inoculation). Matching the photograph (Fig. 1-a) and the radioautograph (Fig. 1-d) of the old leaf showed that not all mildew infections induced an accumulation of ^{35}S. The youngest leaf (Fig. 1-c, f) had an infection on each side of the midrib at the base of the leaf, but spots occurred on the X-ray film only on the left side of the midrib. This

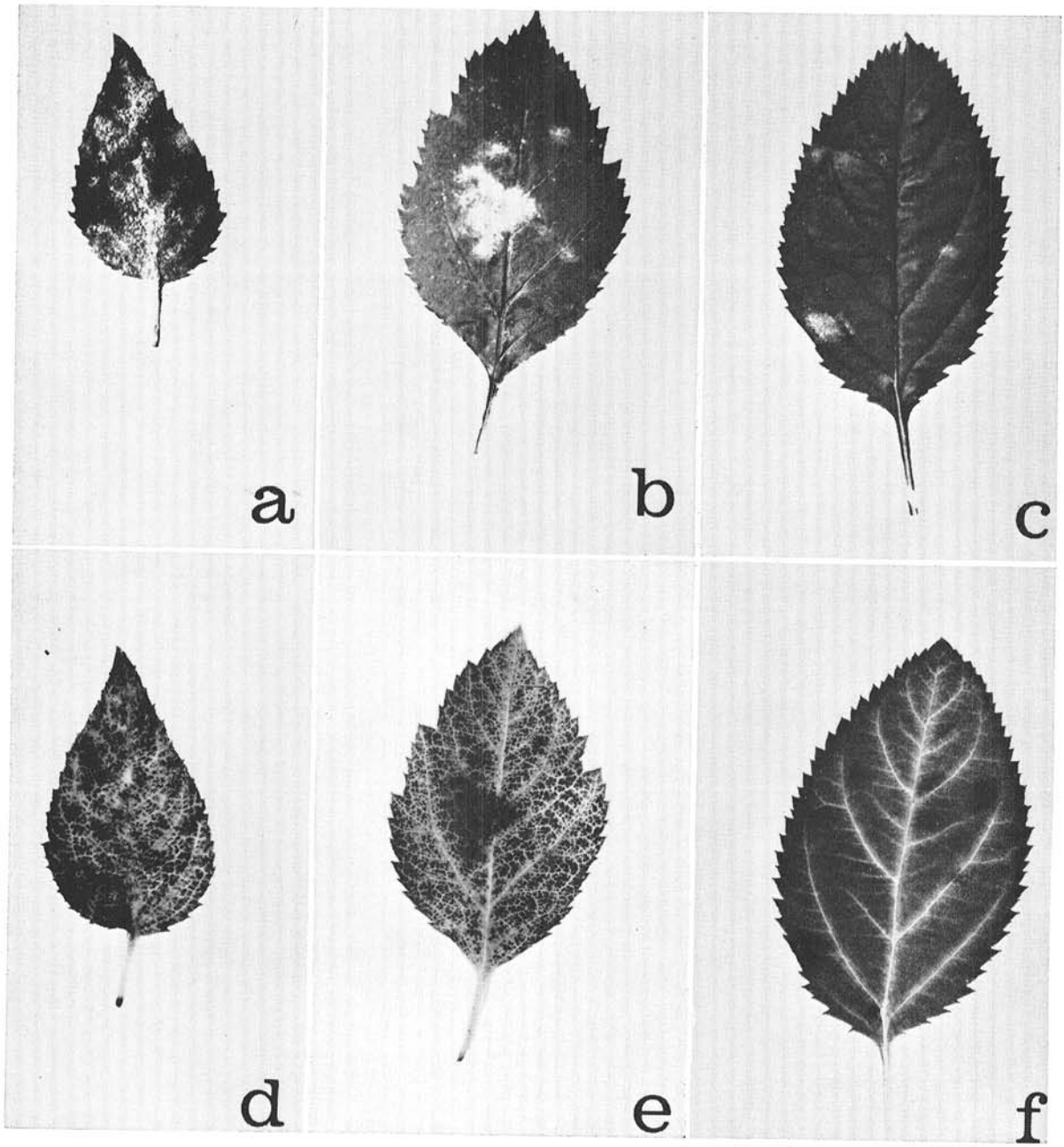

Fig. 1. Accumulation of ^{35}S in leaves of apple seedlings infected with mildew prior to labeling. Illustrations a, b, c are photographs; d, e, f are radioautograms. Leaves illustrated by (a, d), (b, e), and (c, f) are old, intermediate, and young leaves, respectively.

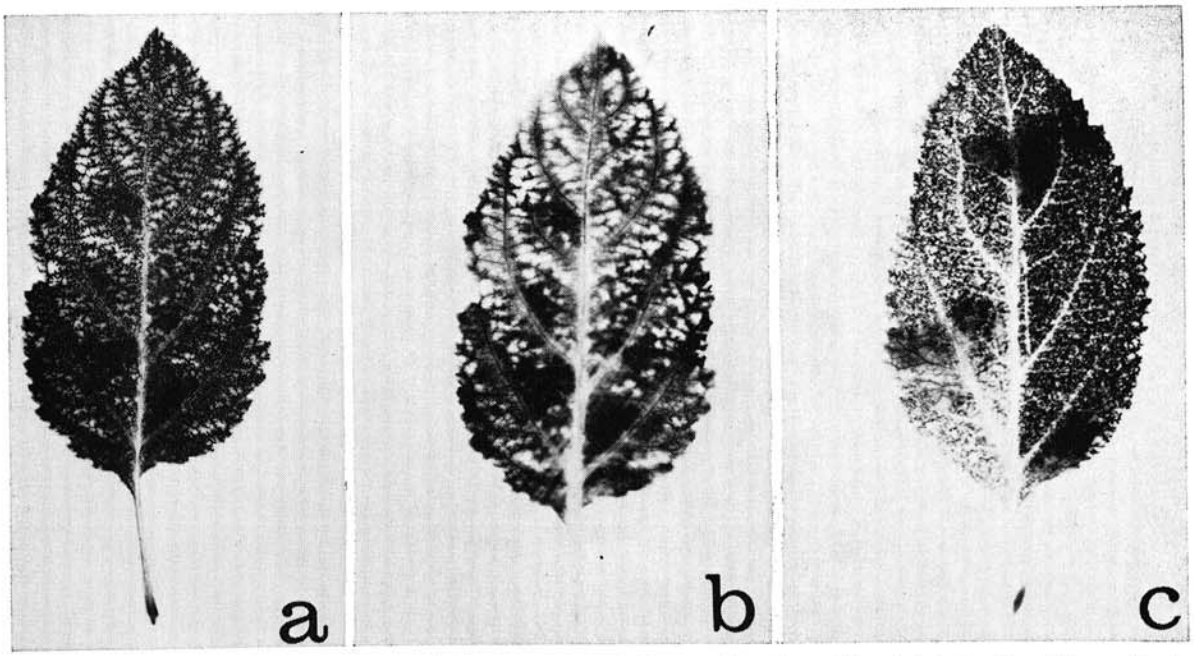

Fig. 2. Radioautograms demonstrating ^{35}S accumulation in leaves of apple seedling infected with mildew prior to labeling. **a)** Spores not removed; **b)** leaf (a) with spores removed; **c)** another leaf with spores removed.

more or less sporadic appearance of spots was typical, even though 30 of 34 radioautographs of diseased leaves of varying age showed at least one accumulation spot corresponding to a mildew infection site. There were no accumulation spots which were not coincident with a mildew infection site.

Mildew spores removed from the leaves with Scotch tape were radioactive. Therefore, the possibility exists that accumulation spots on the radioautograph were not real, but represent less self absorption due to the activity being closer to the film. The radioautograph in Fig. 2 shows a leaf after removal of the spores, illustrating accumulation of translocated ^{35}S in the leaf tissue at the site of a mildew infection, and showing that spots on the radioautograph were not caused artificially because the activity in the spores was closer to the film than to the leaf tissue.

When plants were labeled prior to infection, ^{35}S again generally accumulated at the site of an infection. The darkened areas on the radioautograph were more scattered and had less contrast than when the leaves had infections prior to labeling. Three days following inoculation, no mildew spots were visible on the leaves and no dark areas could be detected on the radioautographs at the inoculation sites even though the plants were labeled 2 days before inoculation. However, 10 days after inoculation, numerous mildew spots had developed which caused definite dark areas on the X-ray film.

Since ^{35}S accumulation did not occur at all of the mildew infections, a more quantative approach was taken to attempt to explain the discrepancies. Apple seedlings with several infected leaves were allowed to take up ^{35}S for 2 days. The infected area of leaves with only one colony/leaf was cut out with a 6-mm cork bore. An adjacent area of healthy tissue was sampled in the same manner. Colonies of varying ages were selected. The radioactivity was determined with a gas flow proportional counter. The data from this experiment is summarized in Table 1.

Sulfur was accumulated by young and intermediate-age colonies but not by old colonies (chi-square analysis shows statistical significance at $P = .05$). The young and intermediate-age colonies had 9 and 13% more activity, respectively. The old colonies had less sulfur than did adjacent tissue, on the average; but the variability was larger on the old leaves, and the difference was not statistically significant.

When apple seedlings absorbed ^{45}Ca, the distribution pattern (Fig. 3) was typical (13). The tracer was well distributed after 4 days in the young leaves, but had barely moved out of the midrib and veins in the older leaves. Three days after inoculation, no accumulation spots were evident. However, a few leaves produced dark areas on the radioautographs 10 days

TABLE 1. Accumulation of ^{35}S in mildewed and adjacent health leaf tissue

Age of colony[a]	Count difference[b]	Per cent[c]
Young (2-4)	+477 *[d]	91
Intermediate (5-8)	+205 *	75
Old (9-14)	−542 NS	33

[a] Days when colonies were visible before counting.
[b] Counts per min mildewed tissue—counts per min healthy tissue.
[c] Per cent of mildew infections having higher counts than adjacent tissue.
[d] * = Statistical significance at 5%; NS = not significant.

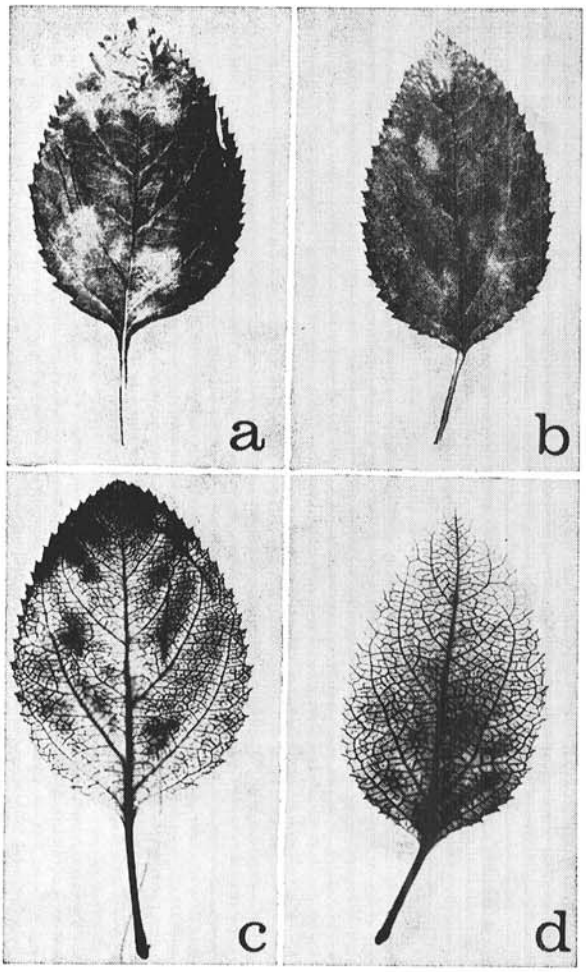

Fig. 3. ^{45}Ca translocation into apple leaves 10 days after inoculation with mildew spores. (Above) Photographs; (Below) radioautographs. (Left) Young leaves; (Right) old leaves.

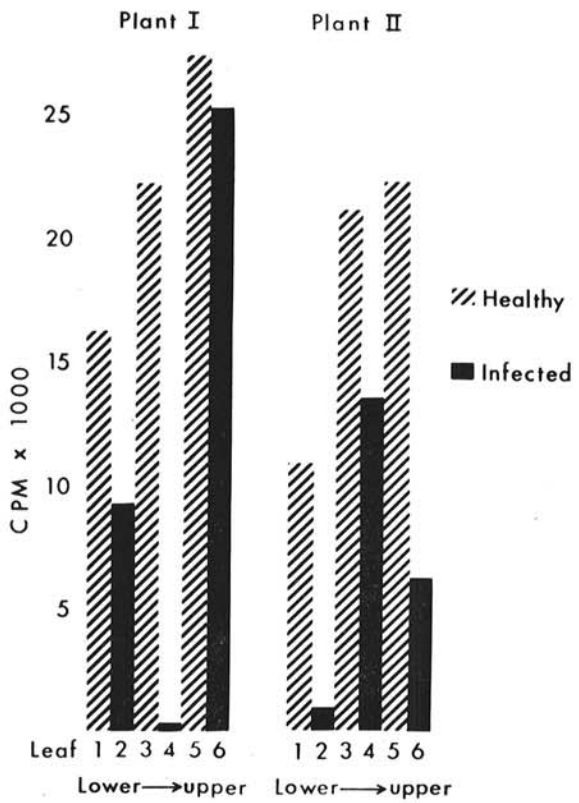

Fig. 4. Distribution of ^{45}Ca in healthy and mildew-infected apple leaves following a 30-hr absorption period by seedling roots. Leaves 2, 4, and 6 were inoculated 10, 7, and 5 days prior to harvest, respectively.

after inoculation (Fig. 3-c). If the spots were carefully compared with those on the photographs of mildew-infected leaves, some matched but most did not. Additional experiments utilizing different techniques, such as pulse-activation of the plant (5) or spot application of the tracer to the leaf base were tried, but true accumulation spots coinciding with mildew occurrence could not be demonstrated. Therefore, accumulation of calcium in association with the mildewed tissue was not demonstrated. Spores collected from the ^{45}Ca experiments were radioactive.

The influence of mildew infection on distribution of ^{45}Ca in apple seedlings after a 30-hr absorption period is presented in Fig. 4. In the healthy apple leaves, the calcium influx increased toward the apex. In the alternate leaves which were mildew-infected, this distribution pattern was broken and the calcium influx into diseased leaves was erratic and severely reduced.

Discussion.—The accumulation of ^{35}S in most areas of mildew infections on apple leaves was demonstrated. Part of the lack of accumulation was found to be associated with the age of the infection. While older apple leaves are not susceptible to new infections, the authors have noted that a small per cent of the colonies on older leaves remain quite active, as measured by their increase in size. This could account for the accumulation of ^{35}S associated with a few of the older colonies; i.e., the active colonies accumulate ^{35}S, whereas the less active ones do not.

Yarwood & Jacobson (14) found that when mildewed leaves were fumigated with H_2S, there was less ^{35}S accumulated in the diseased tissue than in the surrounding healthy tissue. Whether the differences between our results and the previous findings are due to the methods of application or the use of the sulfate versus the sulfide form of sulfur is not known.

Calcium was not found to accumulate in areas of infection. In addition, leaves with mildew infection were found to have less calcium than expected when compared to the adjacent healthy leaves (1, 2). The decrease of total calcium in bean leaves infected with rust had been reported by Durbin (6); however, Ca accumulated in the area of infection.

Where an inorganic metabolite has been found to accumulate at the site of infection, accumulation is generally noted prior to symptom expression. This was not noted with the combination of ^{35}S and apple powdery mildew. However, only one time sequence,

3 days after inoculation, was tested. This might not have been long enough for a field of dominance to be established. Therefore, future time studies are warranted.

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