Uptake of the Systemic Fungicide Methyl 2-Benzimidazolecarbamate and the Fluorescent Dye PTS by Onion Roots

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ABSTRACT

The Casparian band of the endodermis of the root presents a barrier to the uptake of apoplastic chemicals into plants. A possible pathway for entry of apoplastic chemicals is provided by the gaps in the Casparian band caused by the development of secondary roots. The present study tested whether such gaps are necessary for the fungicide MBC (methyl 2-benzimidazolecarbamate) uptake into the plant from a root treatment. Young onion plants sprouted from bulbs were studied since they showed no secondary root development. The lack of pathways allowing indiscriminate passage of apoplastic substances into the onion plant was confirmed experimentally by showing that plants with intact roots were unable to take up a known apoplastic dye PTS (trisodium, 3-hydroxy-5,8,10-pyrenetrisulfonate). The barrier to PTS penetration was located at the margin of the root, probably in the epidermis. When the outer barrier was mechanically removed, PTS penetrated into the root only as far as the endodermis. In contrast, MBC was readily taken up by onion plants following a treatment of intact roots. Therefore MBC does enter plant roots through the intact endodermis, and also can penetrate the epidermal and hypodermal layers which provide barriers for PTS movement.

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Previous studies on the translocation of the fungicide MBC (methyl 2-benzimidazolecarbamate) indicate that it is transported primarily in the apoplast of the plant (15, 22). At the same time, it is known that MBC can be transported to the foliage following a root treatment and that transport occurs in the xylem of plants (4, 8). Therefore, the chemical must have penetrated to the center of the root, despite the barrier to movement in the apoplast provided by the Casparian strip of the endodermis. The Casparian strip does not remain intact during certain stages of secondary root formation (2, 6, 11) and it has been suggested that these discontinuities provide an entry route into the stele for substances moving in the apoplast (6, 11). In addition, some observations indicating that the main root is more permeable to metal ions at the site of secondary root formation have been mentioned briefly in the plant physiological literature (16, 17). During a previous investigation (15) we used the dye PTS (tri-sodium,3-hydroxy-5,8,10-pyrenetrisulfonate) to investigate the role of secondary root formation in the penetration of an apoplastic chemical into the vascular system of the root. PTS is a nontoxic, fluorescent dye known to be confined to the cell walls when it is applied to plants (7, 25). The dye is also known to be capable of penetrating into the steles of barley roots (13), but the pathway of entry was not elucidated. The barley plants used had numerous secondary roots so it is possible that the dye moved into the roots through the discontinuities in the Casparian band caused by secondary root formation. By applying the dye to specific areas along the lengths of corn roots, we found that dye uptake into the stele was associated with the secondary roots, although uptake did not occur until the secondary roots had penetrated the surface of the primary root (15). The dye was unable to enter secondary root primordia within the main root, probably because of the polysaccharide material secreted over their surfaces described by Karas and McCully (11). Using this information as a basis, we then studied ^{14}C-MBC uptake by the same method. Areas on roots with newly emerged laterals were able to take up ^{14}C-MBC, but treatments to younger areas gave somewhat variable results. Therefore, in order to determine whether MBC could penetrate the endodermal layer when the Casparian strip is intact, another study was initiated using onion plants, which do not have secondary roots. Results of this study are reported here. Preliminary results were presented earlier (15). As in the previous study, PTS was used to determine the response of a truly apoplastic chemical in the test system, and MBC was used because it is relatively stable in plant tissue (14, 22) and would not be likely to break down appreciably in short-term experiments.

MATERIALS AND METHODS.—*Plant growth and treatment.*—Onions (*Allium cepa* L. 'Autumn Spice') were sprouted from bulbs in vermiculite moistened with tap water. Plants were grown in a small (0.45 m^3) controlled-environment chamber for 1 week at 25 C, 12-hour light (1,350 lux), and 50% relative humidity. All bulb scales of the parent bulb were removed and individual plants were separated prior to treatment. All treatments continued for 24 hours at 25 C in continuous light (1,350 lux) in the same chamber. In order to favor transpiration, humidity was lowered by means of absorption of water vapor with anhydrous calcium chloride placed in the chamber 24 hours before the experiment. Treatment solutions were prepared in quarter-strength Knop's solution (12). PTS was used at a concentration of 2×10^{-3} M. Labelled MBC [*ring*-2-(^{14}C) specific activity 6.67 mCi/m mole supplied by International Chemical and Nuclear Corp., Irvine, Calif.] was diluted with unlabelled MBC so that the treatment solution contained 10^{-5} M MBC with a specific activity of 2.6 mCi/m mole. Each plant was placed in a 20-ml vial containing 10 ml of treatment solution which left 3 cm between the surface of the treatment solution and the base of the bulb. Observations with PTS dye showed that the treatment solution did not come in contact with the injured area of the stem. Treatment vials were capped with plastic film (Saran wrap) to prevent evaporation. The amount of water transpired during the experiment was measured by the difference in weight of the vials with plants before and after treatment.

PTS extraction and measurement.—At the end of the treatment time, the onion tops were severed from the roots and viewed under a Chromato-vue Longman ultraviolet (UV) light (maximum emission 365 nm). PTS was extracted from the onion tops by grinding them in a Waring Blendor for 2 minutes in a volume of water 10 times their fresh weight. The homogenate was filtered through Whatman No. 1 filter paper and the amount of PTS in the filtrate was determined with a Turner 430 spectofluorometer, using 404 nm excitation and 509 nm emission. Known amounts of a PTS solution were added to each sample after the initial reading to determine the sensitivity of the instrument in each case.

Microscopic observations of onion roots and PTS.—The structure of the onion root was studied by means of free-hand sections viewed with a Reichert Zetopan microscope employing a high pressure mercury vapor light source (HBO 200), exciter filter BG_{12}, and barrier filter OG. This procedure was not suitable for locating the PTS within the root after treatment, since mounting the cut section in a liquid caused rapid diffusion of the PTS over the surface of the section. Another technique was developed whereby the PTS in the root was detected by means of incident ultraviolet (UV) light without making a wet mount. The submerged root to be observed was removed from the plant and gently blotted. A short segment was then excised with a clean, dry razor blade and mounted in modeling clay in a small, humidified petri dish. The top of the dish was covered with plastic film and the cut end of the segment was viewed under UV light with a dissecting microscope. All photographs were taken with Kodak Tri-X pan film.

Extraction and measurement of ^{14}C-MBC.—Onion tops were removed following treatment, cut into small pieces, frozen and lyophilized. Roots were dipped in acetone for 6 seconds to remove MBC on their surfaces before lyophilization. The dried plant material was combusted in a Packard Tri-Carb Sample Oxidizer 305 and counted in a Nuclear-Chicago Unilux II scintillation counter. Counting efficiency was 30% as determined by the external standard method. This procedure gave total recovery of the label.

RESULTS.—When roots were treated with a solution containing 2×10^{-5} M PTS for a 24-hour period, no dye was visible in the leaves when they were viewed under UV light. In some experiments with large plants, as much as

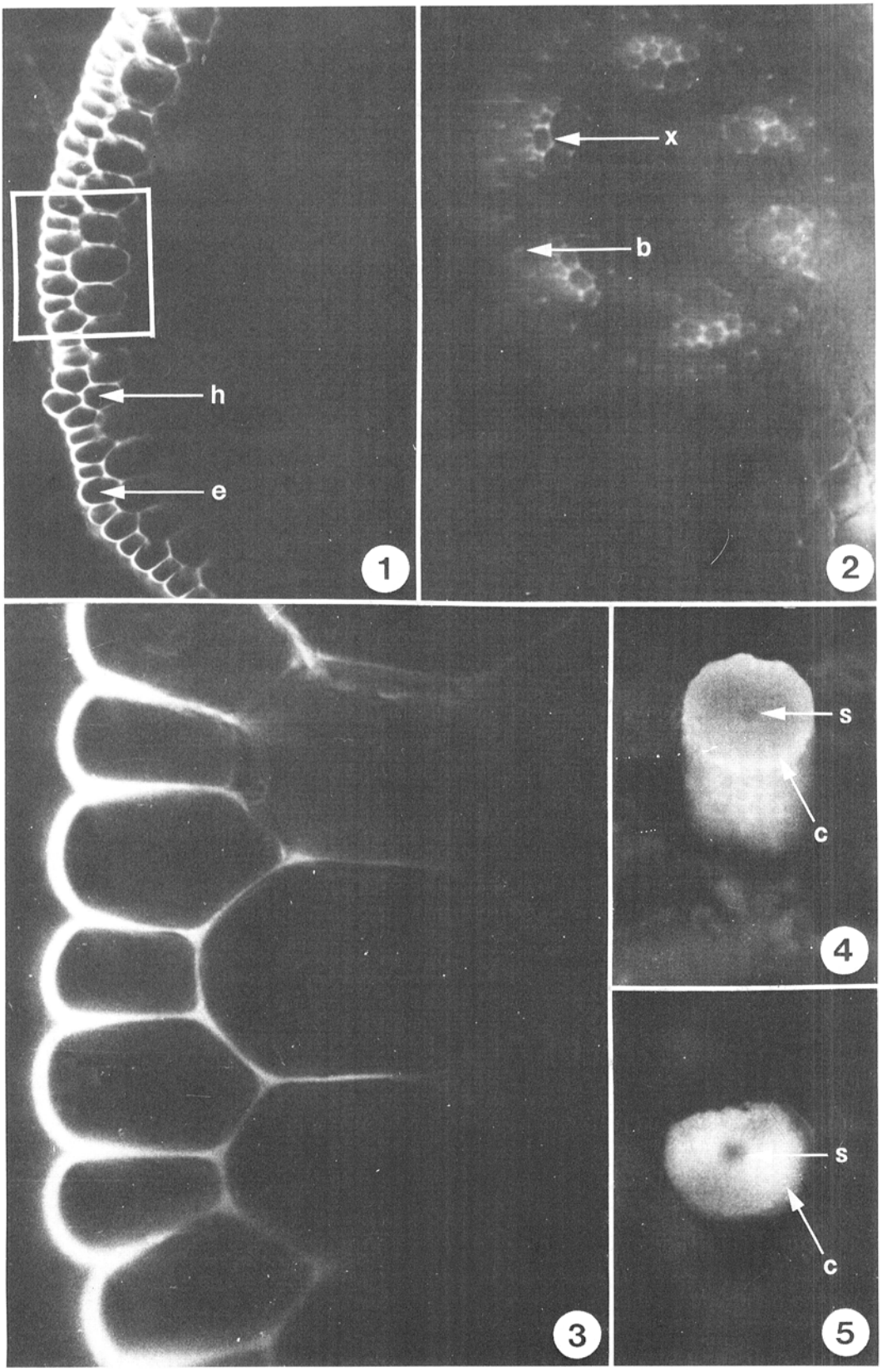
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TABLE 1. Mean values of ^{14}C-MBC (methyl 2-benzimidazolecarbamate) in onion tops and roots following a 24-hour root treatment[a]

	MBC/ml water transpired (nmoles ± std. error)	Ratio of nmoles MBC/ml water transpired:nmoles/ml treatment solution
leaves	3.76 ± 0.31	0.38:1
roots	5.75 ± 0.60	0.57:1
leaves + roots	9.50 ± 0.33	0.95:1

[a]Treatment solutions contained 57,740 dpm/10 nmoles MBC/ml. Results are averages of four replicates.

TABLE 2. Mean amounts of MBC (methyl 2-benzimidazolecarbamate) in onion roots

Av concn (nmoles/g fr wt)[a]	Ratio of concn in root to concn in treatment solution	Ratio of concn in root water[b] to concn in treatment solution
8.41 ± 0.81	0.84:1	0.88:1

[a]Concentration in root = weight MBC ÷ fresh weight tissue.
[b]Concentration in root water = weight MBC ÷ fresh weight − dry weight tissue.

18 ml of water were taken up without any dye being visible in the leaves. The limit of detection of dye in the leaves by this method is in the order of 15 nmoles/g fresh weight of tissue. In order to determine more accurately the amounts of PTS in the leaves, in two experiments the PTS was extracted and measured spectrofluorometrically. No PTS was detected in the leaves despite the high sensitivity of the instrument (0.002 nmoles/g fresh weight onion leaf tissue). Therefore, onion roots very effectively block PTS entry into the plant.

A cross section of the root viewed under UV light showed that the onion root possessed several autofluorescent layers (characteristic of suberized or cutinized material) which could impede free movement across the cortex. These were the walls of the epidermis and hypodermis (Fig. 1) and the Casparian strip in the endodermis (Fig. 2). Fluorescence was especially intense in the walls of the epidermis (Fig. 3). When cut segments of PTS-treated roots were examined under the binocular microscope, the dye was confined to the outer margin of the root (Fig. 4), indicating that an outer layer of the root was acting as the barrier to its entry. When the outer layers were scraped away from a 2-cm length of the root with a razor blade prior to treatment, the dye entered the root as far as the endodermis (Fig. 5).

Having established that very effective barriers to the movement of chemicals in the apoplast exist in intact onion roots, transport experiments were performed with ^{14}C-MBC. In contrast to the dye, the fungicide was taken up following a root treatment and was detected in the leaves (Table 1).

In order to determine whether there is a barrier to MBC movement on the root surface, the amount of MBC in the root was measured after treatment and the concentration in the external treatment solution was measured before and after the experiment. The MBC concentration in the external solution changed less than 5% during the experiment and the MBC initially present in the solution taken up, which was not recovered from the leaves, was found to be present in the roots (Table 1). If MBC movement were hindered at a site inside the root, one would expect an increase in the concentration of chemical in the root over that in the external treatment solution. The data in Table 2 show that the concentration of MBC in the roots at the end of the experiment was less than that in the external solution. If we assume that the MBC is in solution within the root, and express its concentration on the basis of the water content of the root, the concentration of MBC is 88% of that in the treatment solution.

DISCUSSION.—Onion roots proved to be a useful system for study of the uptake of chemicals into the plant. Not only does the Casparian band remain intact due to a lack of secondary root development, but another modified wall layer is present at the margin of the root. Scott et al. (19) have studied the structure of the onion

Fig. 1-5. Cross sections of onion roots viewed with ultraviolet (UV) light. Sections taken 2 cm from the root tip. (1-3) Nontreated roots sectioned and viewed using a microscope with transmitted UV light. **1)** Outer portion of an onion root. Bright areas are autofluorescent. e = epidermis, h = hypodermis (× 230). **2)** Inner portion of an onion root. Bright areas are autofluorescent. b = Casparian band, x = xylem (×250). **3)** Higher magnification of the enclosed area in Fig. 1 (×1,200). (4-5) Segments from roots treated with PTS for 24 hours, cut transversely and viewed using a binocular microscope and incident UV light. Roots were not autofluorescent in the latter viewing conditions. **4)** Slightly oblique view of a cross sectional face of a segment from a root previously treated with PTS. Visible fluorescence indicates dye. s = stele, c = cortex (×31). **5)** Face view of a cross section of a segment from a root treated with PTS. Visible fluorescence indicates dye. Upper edge of root was scraped before dye treatment. s = stele, c = cortex (× 38).

root epidermis in detail, and have reported that a cuticle exists over its surface and that, with age, all epidermal and sub-epidermal walls become cutinized. The same developmental sequence was confirmed in the present study using fluorescent microscopy. It was not possible to distinguish between cutin and suberin since they are both autofluorescent. Hypodermal walls are usually described as suberized (9). The results of the present study showed that PTS applied to onion roots was not transported to the top of the plant and that the primary barrier to its movement was at the margin of the root. De Rufz de Lavison (17) reported that the suberized hypodermis in hyacinth roots constituted a barrier for the movement of iron and sulphocyanide into roots. In the case of onion, the barrier to movement is probably located at the epidermis, since the autofluorescent layer is well-developed there. Regardless of whether the cuticle consists of a separate layer over the epidermis or whether it is made up of materials impregnated in the walls themselves, as argued by von Guttenberg (10), it seems to provide an effective barrier to the entry of substances into the roots via the apoplast.

In contrast to the dye, the fungicide MBC is capable of moving to the tops of onion plants following a root treatment. The roots did not seriously block the uptake and movement of MBC since (i) the concentration of MBC in the external solution did not increase during the experiment, and (ii) MBC did not become concentrated in the roots during the experiment. Thus, we can conclude that MBC traverses the intact endodermis; three possible pathways are apparent. (i) MBC remains in the apoplast and is able to penetrate through the suberized walls of the epidermis, the hypodermis, and endodermis due to the lipid solubility of the chemical. (ii) MBC enters the symplast of the root and moves there until it enters the xylem of the stele. (iii) a combination of i and ii. The first possibility has been suggested to explain the penetration of apoplastic herbicides into the root (1, 3) and MBC is relatively soluble in nonpolar solvents in contrast to PTS which is very water soluble. On the other hand, one could argue that if a chemical were lipid-soluble, it would not likely be confined to the apoplast. The second possibility has also been put forward to explain the movement of herbicides into the stele (3, 5, 20, 21). It would be possible for a chemical to enter the symplast and still display an apoplastic pattern of movement if (i) the chemical entered the symplast of root cells until it reached the xylem, after which it remained in the apoplast, or (ii) the chemical moved freely between the symplast and apoplast. In the latter case, the rapidly moving transpiration stream would tend to remove the chemical from the more slow-moving symplastic transport system, and thus would control its distribution within the plant. The cytokinin-like activity of MBC (18, 23), its transport to cucumber shoot apices following application to a leaf, and penetration into isolated mesophyll cells of apple (24) all indicate its entrance into the symplast, but the amount measured in the symplast in a previous study was small (24). However, the results of the present study show relatively free movement of MBC through the root in comparison with PTS, a chemical known to be apoplastic. The present results are most easily explained by assuming considerable MBC movement within the symplast as well as the apoplast.

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