Seedcoat Permeability: Uptake and Post-germination Transport of Applied Model Tracer Compounds

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Abstract. The seedcoat permeability, uptake, and transport of model fluorescent tracers were investigated in snapbean (Phaseolus vulgaris), pepper (Capsicum annuum), tomato (Solanum lycopersicum), onion (Allium cepa), cucumber (Cucumis sativus), and lettuce (Lactuca sativa) seeds. Nine fluorescent tracers and one vital stain were selected to represent a diversity of physicochemical properties (lipophilicity, electrical charge, etc.) and to simulate behavior of applied seed treatments. To study seedcoat permeability, tracers were applied to seeds as dry powders, and treated seeds were sown in moistened sand at 20 °C and removed after 18 to 24 h, a time before visible germination. Imbibed seeds were dissected and fluorescence (staining) was observed in embryos with a dissecting microscope under ultraviolet (365 nm) or visible radiation. Seedcoat permeability of species to solutes was grouped into three categories: 1) permeable—snapbeans; 2) selectively permeable—tomato, pepper, and onion; and 3) non-permeable—cucumber and lettuce. Systemic tracers that failed to permeate seedcoats during seed imbition were taken up by roots or hypocotyls after visible germination.

Seed treatments are widely used in agriculture and have desirable properties as pesticides, disinfectants, or plant growth regulators (Taylor, 2003). Many seed treatment active materials have systemic activity providing protection of the aboveground portion of plants for efficient early-season pest protection (Nault et al., 2004). However, movement of a systemic seed treatment from the seed surface to the imbibed or germinated seed is not well understood. Seed treatments may be applied in an aqueous solution as a soak or during priming to infuse active materials into the embryo or eradicating an internal seed pathogen (Taylor et al., 1998). These seed treatments must penetrate the seedcoat or seed-covering tissues to be effective before visible germination. Uptake of compounds applied as seed treatments were examined in different vegetable crop seeds with different pesticides. The herbicide dichlorophenoxyacetic acid was effectively absorbed by pea (Pisum sativum) seeds (Hansen and Buchholtz, 1952) and mustard (Brassica sp.) (Mitchell and Brown, 1947). In contrast, the seedcoat was a barrier for atrazine uptake in turnip (Brassica rapa) (Hocombe, 1968), for the herbicide dipropetryn in cucurbit seeds (Rubin and Demeter, 1986), and for the systemic insecticide phorate in mustard (Sinapis alba) (Bardiner, 1964). These collective results provide evidence that seedcoat permeability differed by crop and by compound. However, the same compound was not tested on several vegetable crop seeds so broader conclusions cannot be drawn from these studies. Therefore, an understanding is needed of the physical–chemical nature of compounds in relation to seedcoat permeability of different vegetable crop seeds.

An understanding of systemic uptake of compounds is based on uptake by plant roots and the major chemical properties are lipophilicity, electrical charge, molecular weight, and H-bonding capacity. Lipophilicity is a measure of the affinity of compounds for the lipid phase of plant tissues (plasma membrane, waxes, cutin, suberin, etc.). Lipophilicity is quantified as log \( K_{ow} \) (where \( K_{ow} \) is the octanol/water partition coefficient). The relationship of log \( K_{ow} \) and plant tissue uptake reveals a Gaussian curve with a range from −1 to 5 log \( K_{ow} \) with a maximum at log \( K_{ow} \) of 2 (Briggs et al., 1982). Moderately lipophilic compounds (log \( K_{ow} \approx 2 \)) are systemic because they permeate the lipid phase of cell membranes in comparison with hydrophilic compounds that are not able to pass through lipid barriers (Briggs et al., 1982). Compounds with high log \( K_{ow} \) (greater than 5) are not systemic because they are strongly retained in the plant lipid constituents (Edgington, 1981) and have limited water solubility. The electrical charge of molecules influences compound diffusion through plasma membranes, and positively charged molecules are often bound by negatively charged cell walls (Edgington and Peterson, 1977). Hydrogen-bond donor and acceptor groups on molecules decrease permeability of molecules through the lipid bilayer of cell membranes. The optimal number of H-donors and acceptors was less than 5 and 10, respectively, based on Lipinski parameters (Tice, 2001). According to Briggs (1997), the “limiting” number of H-donors for agrochemicals is three. Increasing molecular weight impaired molecule penetration across the plasma membrane (Lipinski et al., 1997). Mobile agrochemicals have a molecular weight of ≤300 or less (Briggs, 1997).

The first objective of this article was to study seedcoat permeability of selected species with different morphology and anatomy using fluorescent tracers as model compounds. The research question was to examine the pathway that systemic seed treatments take after being sown in a moist medium: 1) whether seed treatments can diffuse through the seedcoat and then to the embryo; or 2) if seed treatments cannot permeate the seedcoat, so they diffuse in the soil medium and are taken up by roots. The second objective was to study water potential of the soil environment and relative humidity of the air on seedcoat permeability. The third objective was to visualize systemic compound uptake in seedlings.

Materials and Methods

Plant materials. Seeds of six crops were used in the study: lettuce ‘Waldmann’s Green’ (Harris Seeds, Rochester, NY), snapbean ‘Hys- tile’ and pepper ‘Beyton Bell’ (Harris Moran Seed Co., Modesto, CA), tomato ‘Facundo’ (Syngenta Seeds, Golden Valley, MN), cucumber ‘Vlasipik’ (Seminis Inc., Oxnard, CA), and onion ‘Millennium’ (Nunhems, Parma, ID). Seeds used in the experiment did not have physiological or seedcoat-imposed dormancy based on standard germination tests.

Chemicals. Nine fluorescent dyes and one color-forming compound of various chemical classes were selected for their different values of log \( K_{ow} \) and electrical charge (Table 1). Coumarins, coumarin 1 [2H-1-Benzopyran-2-one, 7-(diethylamino)-4-methyl-], and coumarin 151 [2H-1-Benzopyran-2-one, 7-amino-4-(tri-fluoromethyl)-], are both nonionic, lipophilic...
compounds. AMCA (2H-1-Benzopyran-3-acetic acid, 7-amino-4-methyl-2-oxo-) is an anionic tracer. The xanthene, rhodamine B [Xanthium, 9-(2-carboxyphenyl)-3,6-bis(dithiylamino)- chloride (111)] and sulforhodamine B [Xanthium, 3,6-bis(dithiylamino)-9-(2,4-disulfonyphenyl), inner salt, sodium salt (1:1)], are zwitterionic compounds. Fluorescein [Spiro[isobenzofuran-1(3H),9'-(9H)xanthen]-3-one], 5(6)-carboxyfluorescein [Spiro[isobenzofuran-1(3H),9'-(9H)xanthen]-ar-carboxylic acid, 3',6'-dihydroxy-3-oxo], and uranine [Spiro[isobenzofuran-1(3H),9'-(9H)xanthen]-3-one, 3',6'-dihydroxy-, sodium salt (1:2)] are anionic. Acridine (9-aminoacrididine) is cationic. Tetrazole, tetrazolium red (2,3,5-Triphenyl-2H-tetrazolium chloride) is cationic and colorless in water. The tetrazolium salt is a viability stain and is biochemically reduced to the water-insoluble dye, formazan by cellular dehydrogenase activity. In addition, each compound's pKa, molecular weight, and H-donors and acceptors were provided (Table 1). All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Dye application and microscopy. Tracers were applied to seeds as dry powders to avoid exposure to water during the treatment stage. The application rate was 0.5 g tracer per 100 g seeds, and the excess powder was removed though a sieve. Five replications of 20 seeds per replication of each treatment were sown in sand pre-moistened with water at 20 °C and excavated after 18 to 24 h, a time before visible germination. For cucumber seedling uptake studies, treated seeds were sown and seedling parts excised after the first true leaves had developed. The location and intensity of fluorescence in hand-dissected imbibed seeds or seedlings were observed under long ultraviolet (365 nm) radiation with an Olympus SZX12 stereomicroscope (Center Valley, PA) equipped with a SPOT Insight camera and software (Version 4.5; Sterling Heights, MI). Detection of the fluorescent tracers in the embryo was qualitative, and seed tissue autofluorescence, if present, did not interfere with observing the tracers.

**Results**

Seedcoat permeability

Snapbean. Seedcoat permeability of snapbean to applied tracers is shown in Figure 1. Seedcoats were permeable to most fluorescent tracers and the tetrazolium salt. On removal of seedcoats from seed after 18 h of imbibition in sand, strong uniform fluorescence was present on the embryo with coumarins, fluorescein, carboxyfluorescein, uranine, and AMCA showing that seedcoats were highly permeable to these tracers. Time course studies revealed that these tracers diffused uniformly over the seedcoat and not first through the hilum or microyle (data not shown). Tetrazolium red, 9-aminoacrididine, and rhodamine B penetrated the seedcoats; however, fluorescence or red formazan coloration on the surface of the cotyledons was not distributed uniformly. Sulfurhodamine B showed limited staining and only irregularly on the embryo.

**Lettuce and cucumber.** For all tracers, fluorescence or staining was not observed in the embryo tissue after imbibition of lettuce and cucumber seeds (Fig. 3). The tracers were able to diffuse through the tests of cucumber seed but did not permeate the inner seed envelope (perisperm–endo sperm envelope) surrounding the cotyledons.

**Pathways of tracer uptake into seed tissue**

One pathway of movement of nonionic lipophilic tracers (coumarin 1 or coumarin 151) was through the seedcoat to the endosperm and embryo tissues in onion (Fig. 4A). These tracers diffused through the seedcoats before visible germination. In contrast, a charged molecule of rhodamine B was not able to penetrate the onion seedcoat (Fig. 4B) but was absorbed by the radicle after visible germination (Fig. 4C).

**Coumarin uptake by snapbean seeds under different moisture regimes**

Uptake of tracers by snapbean seed at various water potentials and 100% RH are summarized at Table 2. Uptake was rapid in all cases after 18 h imbibition. For all tracers, fluorescence or staining was not observed in the embryo tissue after imbibition of lettuce and cucumber seeds (Fig. 3).

![Fig. 1. Permeability of snapbean seedcoat to applied model tracer compounds of different log $K_{solv}$ and electrical charge.](image-url)
seeds imbibed in sand moistened with water, and treated seeds revealed fluorescence in the embryo after 0.5 d. All seeds had visibly germinated after 2 d when hydrated at 0 MPa. The rate of tracer uptake was slower when seeds were sown in sand moistened with –1 MPa or –2 MPa PEG solutions compared with the 0 MPa control. However, no visible germination was recorded at either water stress by 6 d. Maintaining seeds at 100% RH resulted in limited tracer penetration after 4 d, whereas at 6 d, strong fluorescence was observed in the embryo. Condensation was not observed on the seeds at 100% RH; however, we cannot rule out that liquid water was present on the seed surface.

**Mobility of fluorescent tracers within cucumber seedling tissues**

Mobility of fluorescent tracers within seedling plant tissues was related to the physicochemical properties of the tracer. The nonionic, lipophilic compound, coumarin 151, was found to be xylem-mobile and was detected in roots, shoots, petioles, cotyledons, and first true leaves (Fig. 5). Similar uptake patterns were observed in cucumber for coumarin 1 and rhodamine B (not shown). Fluorescence was not detected in the phloem so downward movement would not occur to the roots. However, detection of the tracer in the roots was attributed to the compound diffusing from the treated seed into the sand and then subsequently taken up by the root in the xylem.

**Discussion**

Snapbean seedcoats were permeable to most applied compounds, but with limited permeability to sulforhodamine B (Fig. 1). The restricted uptake of sulforhodamine B may be attributed to its relatively large molecular weight and large number of H-bond acceptors (Table 1). Similar to snapbeans, soybean (Salanenka and Taylor, 2009), and pea (data not shown) had permeable seedcoats. Collectively, large-seeded legumes have permeable seedcoats to a wide range of applied compounds because they do not possess a semipermeable layer in their seedcoats (Salanenka and Taylor, 2008).

Tomato, pepper, and onion seedcoats were only permeable to the nonionic moderate lipophilic tracers, coumarin 1 and coumarin 151 (Fig. 2). These seeds have barriers in their seedcoats that restrict the diffusion of water-soluble compounds such as amino acids known as semipermeable layers (Taylor et al., 1995). The barrier layers of the seedcoat of these seeds were investigated and composed of suberin in tomato and pepper and cutin in onion (Beresniewicz et al., 1995). Switchgrass (*Panicum virgatum*) had the same permeability characteristics as tomato, pepper, and onion (Salanenka and Taylor, 2009). Similar to these vegetable crop seeds, many grasses have a semipermeable cutinized or suberized membrane in the caryopsis integument that restricts solute transport through the seedcoat (Simpson, 1990).

Lettuce seeds were not permeable to any applied compound (Fig. 3). Lettuce seeds have a semipermeable endosperm envelope surrounding the embryo that restricts solute diffusion (Klein et al., 1971; Speer and Hsiao, 1975). According to Speer and Hsiao (1975), the endosperm of lettuce excludes compounds with molecular weight more than 300 Da. Coumarin 1 and coumarin 151 with molecular weights 229 Da and 203 Da, respectively, did not diffuse into the embryo. This discrepancy

| 0.5 d | 2 d | 4 d | 6 d |
|-------|-----|-----|-----|
| 0 MPa | +   | G   |     |     |
| –1 MPa | –   | +   | +   | +   |
| –2 MPa | –   | +   | +   | +   |
| 100% relative humidity | –   | –   | F/+ | +   |

F = faint; G = seeds germinated.
Amritphale et al. (2010) studied dye transport of differences in seed material preparation. This discrepancy may be the result of the endosperm in the area adjacent to the cotyledons. According to our study, no charged or neutral solutes are retained in the perisperm–endosperm envelope (Yim and Bradford, 1998). Another species with non-permeable seedcoats was castor (Ricinus communis) based on other research in our laboratory (Salanenka et al., 2009). Evidence revealed that wheat seeds took up water vapor from soil (Wuest et al., 1999) and that the uptake rate was not dependent on low water potential after 6 d, revealing that diffusion is independent of germination. Coumarin 1 moved through snapbean seedcoats incubated at 100% RH indicating that water vapor could facilitate diffusion and that liquid water is not necessary. The nonionic moderate lipophilic tracer coumarin 151 (log $K_{ow}$ 1.5) was only able to permeate through seedcoats of large-seeded legumes, whereas the tracer was retained in seedcoats of other crop seeds. Considering that rhodamine B in aqueous solution exists in cationic (HR) or zwitterionic (R-) forms (Mededlov-Petrosyan and Kholin, 2004), limited seedcoat permeability of rhodamine B may be attributed to absorption of the cationic form by seedcoat lignin mediated by hydrogen bonding. Only nonionic coumarin 151 and coumarin 1 were able to pass through seedcoats of the semi-permeable seeds of tomato, pepper, and onion (Fig. 2). Thus, diffusion of compounds into seeds with semipermeable barriers should occur under field conditions.

There are concerns in the seed industry with seed treatment actives and formulations causing phytotoxicity (Taylor et al., 2001). To determine if a systemic a.i. of seed treatment permeates through seedcoat during seed storage that might be a reason of the phytotoxicity, snapbean and soybeans were treated with seed treatment actives and formulations under field conditions.
Results presented in this article have practical implications for seed enhancement technologies (Taylor et al., 1998) such as seed priming or soaking. A long-term objective of many people working with seeds is to incorporate a wide range of solutes into seeds by soaking or other controlled hydration methods to benefit subsequent germination and seedling growth or to disinfest seeds. However, the innate seedcoat permeability can attenuate diffusion of compounds from the soil environment to the embryo. Collectively, seed species with selectively permeable or non-permeable seed-covering tissues may only benefit from water uptake and not the particular chemical compound.

In summary, seedcoat permeability to solutes was grouped into three categories: 1) permeable; 2) selectively permeable; and 3) non-permeable. Snapbean seedcoats were permeable to a wide range of applied chemicals, whereas tomato, pepper, and onion had selective permeability that only allowed penetration of nonionic compounds with moderate lipophilicity. Lettuce and cucumber had non-permeable seedcoats to applied compounds. Therefore, systemic uptake by seeds with a semipermselective or non-permeable layer occurred through the roots.

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