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Oil emulsions enhance transcuticular movement of captan in apple leaves

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Abstract

The purpose of this study was to determine whether oil emulsions enhanced penetration of captan, a chloroalkyl thio heterocyclic non-systemic fungicide through apple leaf cuticles. Cuticles from apple leaves were isolated with enzymes and treated with 1% soybean oil emulsified with Latron B-1956® and K1, and two commercial soybean oil emulsions, dormant oil and SunSpray on the outer morphological surface. Where captan and oil were applied together, the percentage of captan that penetrated the cuticle never exceeded 3.5% even under a worst-case scenario but this could be sufficient to cause phytotoxicity. When applied on the outer morphological surface of the cuticle, all oil formulations increased captan penetration by more than 50% over the amount penetrated through control cuticles. The greatest penetration of captan was through cuticles treated with dormant oil. A significant amount of captan (51% over the control amount) penetrated through the inner morphological surface of the cuticle. Captan penetration was more than 40% greater through the abaxial than through the adaxial cuticles. Increasing captan concentration increased captan penetration and a linear relationship existed between captan concentration and captan penetration through the cuticle. The study clearly showed that oil emulsions facilitated penetration of significant amount of captan through the cuticle, which may have serious implications for phytotoxicity of leaves.

Keywords: Captan; Cuticle; Dormant oil; Latron B-1956; *Malus domestica*; Soybean oil

1. Introduction

Captan [*N*-(trichloromethylthio)-4cyclohexene-1,2-dicarboximide] is a micro-fine wettable powder for use in water as a spray for the control of certain fungal diseases such as scab, black rot, Botrytis blossom-end rot, sooty blotch, powdery mildew etc. of fruit crops. Captan is not a systemic fungicide, however, problems often arise when captan is mixed with other agrochemical sprays involving adjuvants, which enhance the transport of captan through the plant cuticle and if large amounts enter the leaf, then phytotoxicity is bound to occur (Solel and Edgington, 1973). For instance, in peach, captan in combination with dodine (n-dodecylguanidine acetate), a surfactant, caused phytotoxicity of leaves, but there was no phytotoxicity when captan was applied alone (Lukens, 1970). They further reported that captan normally decomposes on the surface, but when it decomposes inside the leaf, it results in phytotoxicity of leaves. Adjuvants that enhance uptake of captan include spray oils, some spreader-stickers, and other petroleum-based carriers commonly found in products that are formulated as liquids or emulsifiable concentrates.

Soybean oil, an environmentally safe compound is formulated with an emulsifier before it is applied for pest mortality in apple (Myers et al., 1996; Pless et al., 1995; Bondada et al., 2006a). Depending upon disease incidence, the application of captan may follow or precede soybean oil sprays. If absorbed by the leaves, captan will cause phytotoxicity, especially in 'Red Delicious' trees, which are more susceptible to captan injury than most other cultivars. Captan and soybean oil applied together or

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within several days of one another have been shown to cause phytotoxicity of leaves, especially on the lower surface (Sam's personal communication). We hypothesize that such phytotoxicity resulted largely from considerable amount of captan gaining entry into the leaf by penetrating the cuticle chiefly facilitated by the emulsified soybean oil. However, there is no study that examined the influence of soybean oil on the absorption of captan by apple leaves. In order to minimize phytotoxicity and gain an insight into its mechanistic basis, it is important that one elucidate the absorption of captan into the leaf in the presence and absence of soybean oil.

Penetration and absorption of a compound by leaves is determined by the ability of the compound to move through the cuticle, a process known as transcuticular penetration or movement (Solel and Edgington, 1972). Using isolated cuticles, the transcuticular movement of water, nutrients, pollutants, and pesticides has been already investigated in various leaves and fruits (Price, 1982; Chamel, 1986; Schönherr and Riederer, 1988; Bukovac et al., 1990; Chaumat et al., 1992; Bondada et al., 2006b). Captan can penetrate the cuticle and enter the leaf causing toxicity. However, its penetration characteristics in apple leaves under the influence of various oil emulsions are not known. The objective of this study was to examine the transcuticular movement of captan in the presence of various soybean oil emulsions through enzymatically isolated apple leaf cuticles. Enzymatically isolated apple leaf cuticles were chosen as model systems to study captan penetration as this system elucidates the kinetics and mechanism of penetration by providing meaningful quantitative transport parameters (Bukovac and Petracek, 1993). Furthermore, transcuticular movement is one of the major factors determining the performance of fungicides (Solel and Edgington, 1973). For captan transcuticular movement, we used the system developed by Solel and Edgington (1972) as it more closely simulates leaf absorption of fungicides from spray application.

2. Materials and methods

2.1. Isolation of cuticles

Fully expanded apple 'Golden Delicious' apple [*Malus domestica* (Borkh.)] leaves from an apple orchard were collected during mid-summer and used for isolating cuticles. Using a cork borer (3.3 cm diameter), leaf disks were punched out, from which cuticles were isolated by decomposing the pectic layer underneath the cuticle by treating the disks with pectinase enzyme (Orgell, 1955). After 2–3 days, the disks were transferred into water and stirred gently, which separated the cuticles from other tissues. The cuticle disks were washed gently by several changes of the water in which they were floating. The cuticles were then air dried and stored in a jar.

2.2. Measurement of cuticular penetration of captan

Penetration of captan was determined using a finite-dose diffusion system (droplet–cuticle–agar). Cuticle disks were checked under a microscope for imperfections and to determine whether the cuticles were from the adaxial (astomatous) or abaxial (stomatous) side of the leaves. Thereafter, cuticles were supported by circular aluminum foil with an inner diameter of 0.8 cm and an outer diameter of 1.7 cm. Cuticles always were mounted with the external surface facing upward using vein elevation.

The mounted cuticle disks were placed on the surface of 20 ml potato-dextrose agar (PDA) in petri dishes. The edges of aluminum rings were sealed with vacuum grease in order to prevent lateral diffusion of captan. Spores of the fungus, *Penicillium cyclopium*, which are sensitive to captan (91% pure powder form, Crescent Chemical Company, Inc., New York, NY) were sprayed on the medium with a fine atomizer 24 h after the application of captan. Captan was suspended in water to the desired concentration and applied to the cuticle in a 5-μl droplet by a micro-syringe. The plates were then maintained at a temperature (20 °C) optimal for the development of fungus, and when growth was apparent, the zone of inhibition was measured. The amount of chemical which moved through the cuticle during the experimental period was assessed using a standard curve. The standard curve was obtained from a series of known concentrations applied to filter paper (Whatman 541) disks of 1.7 cm in diameter. The diameter of the zone of inhibition was plotted against the concentration of the fungicide which gave a straight line. There were a total of three experiments.

2.3. Experiment 1

This experiment was conducted with abaxial cuticles and included the following treatments: (1) captan spray (control), (2) application of 1% soybean oil emulsified with Latron B-1956 spreader sticker (0.1%, v/v), a non-ionic surfactant (Rohm and Haas, Philadelphia, PA) to dried captan droplets, (3) to fresh wet captan droplets, and (4) application of captan to dried 1% soybean oil droplets.

2.4. Experiment 2

This experiment was also conducted using abaxial cuticles. The treatments consisted of (1) captan spray (Control), (2) captan + 1% soybean oil emulsion (Latron B-1956 as the emulsifier), (3) captan + 1% soybean oil emulsion (K1 as the emulsifier, a non-ionic emulsifier, an experimental emulsifier from Central Soya, Fort Wayne, IN), (4) captan + 1% soybean oil emulsion (Latron as the emulsifier) on the inner morphological surface of the cuticle. The treatments 1, 2 and 3 were applied on the outer morphological surface of the cuticle.