Coating Technologies for Seed Treatment Applications

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Abstract

Seed coating technologies serve as delivery systems for biological and chemical seed and/or seedling performance enhancing treatments. Seed treatments must be applied uniformly over the seed surface for maximum efficacy. Here, application uniformity was studied using a fluorescent tracer mixed with *Rhizobium* inoculant and applied to pea seeds. A digital camera with UV illumination was used to examine application uniformity. Imaging software discriminated between non-treated, properly treated and over-treated portions of seeds. Seeds treated with an 'on-farm' auger had better uniformity of coverage compared to hand mixing. Doubling the amount of liquid improved the coverage for both methods. New chemistry seed treatments with systemic activity have the ability to control foliar pests. Bean seeds were treated with imidacloprid or thiamethoxam at 63 and 30 mg ai / 100 grams of seeds, respectively. These systemic insecticidal seed treatments were analyzed from both true leaf and trifoliate leaf tissue 28 days after sowing. Both systemic seed treatments were detected in leaves, with imidacloprid having a higher concentration than thiamethoxam. Thiamethoxam concentration was similar between first leaves and trifoliates, whereas imidacloprid was detected at over 4x higher concentration in the true leaves than the trifoliates.

INTRODUCTION

Seed Coating Technologies

Seed coating technologies used to apply chemical and biological seed treatments have been well described (Taylor et al., 1998; Taylor et al., 2001; Taylor, 2003). They range from 'on farm' applications to sophisticated methods that are conducted with specialized equipment by the seed industry. For example, liquid *Rhizobium* formulations may be mixed with seed by hand or with an auger just prior to sowing. In contrast, film coating, encrusting and pelleting are performed by the seed industry on high-value horticultural crop seeds. Formulations and equipment to coat seeds have been reviewed by Halmer (2000). Coating formulations can be both cosmetic or functional. This paper will illustrate functional aspects of coatings as delivery systems for biological or chemical compounds.

Rhizobium Seed Treatments

Specific biological agents may be applied as seed treatments that can colonize roots and enhance nutrient availability. The bacterium *Rhizobium* develops a symbiotic relationship with the crop to fix nitrogen and it is the most widely used biological seed treatment in agriculture. *Rhizobium* seed treatments can reduce production costs by decreasing the use of chemical nitrogen fertilizers and have the potential to decrease soil and water pollution. Coating systems were described to provide a conducive environment for *Rhizobium* establishment (Scott, 1989).

Rhizobia may have short longevity after application, and commercial formulations of the inoculant, *Rhizobium leguminosarum* biovar. *viceae*, must be applied to pea (*Pisum sativum*) seeds 'on farm' and then sown the same day. The *Rhizobium* may be applied to seeds by hand mixing or can be treated in bulk with an auger (Cell-Tech C, Application guide). A major concern with these methods is achieving application uniformity.

Systemic Chemical Seed Treatments

Seed treatments may have contact or systemic activity which is determined by the physical/chemical properties of the active ingredient (Peterson, 1989). Systemic plant protectants have broad utility to control insects and diseases in the seedling and early vegetative phases of plant growth. Seed treatments with systemic activity challenge the need for in-furrow and other soil applications of protectant pesticides. Moreover, a reduction in pesticide usage per hectare may exceed 85% when comparing a seed treatment with an in-furrow treatment (Taylor et al., 2001).

New chemistry pesticides in the chloronicotinyl insecticide class (CNI's or neonicotinoids) have been developed as systemic seed treatments (Brandl, 2001). The two compounds of interest are imidacloprid and thiamethoxam. Both compounds exhibited good efficacy to control flea beetles (*Chaetocnema pulicaria*), the vector for the disease Stewart's Wilt caused by the pathogen (*Erwinia stewartii*) on sweet corn (*Zea mays*) (Kuhar et al., 2002). However, pesticide persistence in the plants was not determined in those studies. Thus, there is a need to quantify and verify the presence of pesticide concentration in leaves.

In this paper, seed treatments included *Rhizobium* (a beneficial microbe to establish a symbiotic relationship with legumes to fix nitrogen) and chemical plant protectants to control pests. Application uniformity was examined with fluorescence photography and imaging software. Pesticide residue time course studies were illustrated for selected seed treatments and leaf age.

MATERIALS AND METHODS

Seed Coating Uniformity

A fluorescent tracer ('Saturn Yellow EPX-17', Day-Glo Color Corp., Cleveland, OH) was added to the *Rhizobium* inoculant (Cell-Tech C, LiphaTech, Milwaukee, WI) at 1 part tracer to 3 parts inoculant. This mixture was applied to pea (*Pisum sativum*) cultivar 'Bolero' seeds using two methods and at two dilutions. The first method was to hand mix the inoculant with 1 kg of seeds in a container at the recommended rate (2.78 ml/kg). Hand mixing was continued for 60 seconds before allowing seeds to dry on a screen. The second method involved a controlled drip at the feed end of an auger with bristle brushes used to load seeds into a planter's hopper. In the first of the two dilutions, only the liquid from the treatment bag provided by Cell-Tech C plus fluorescent tracer was used (1x dilution). The 2x dilution was prepared by adding one volume of water to the liquid prior to coating.

Material coverage on seed surfaces was evaluated by examining the levels of fluorescent expressed under UV excitation. Fluorescent images of each treatment were recorded using a digital camera (Coolpix995, Nikon Corp., Tokyo, Japan). Images were then processed using 'ImageJ ver. 1.27' software (public domain, National Institutes of Health, USA) to provide data for uniformity evaluations.

Photographs were taken in a dark room in which samples were excited using a hand-held UV source (Model UVG-54, 254 nm hand held, Ultra Violet Products, Inc. San Gabriel, CA) clamped at a fixed distance above seed samples. The camera was also mounted above seed samples. Seed samples of 50 g were packed flat into a 10 x 10 cm tray and arranged so that one image included all four treatment combinations. In this way, UV excitation and camera settings were identical for all treatments evaluated. The image data of equal area extracted from each of the four treatment sections (fig. 1) contained approximately 632k 8-bit picture elements (pixels). An 8-bit pixel has 0-255 possible levels of light intensity. Only the 'green' data set from this RGB image was used to discriminate between shadows between seeds, and non-treated, uniformly treated and over-treated areas on seed surfaces.

Systemic Chemical Seed Treatments

Snap bean (*Phaseolus vulgaris*) cultivar 'Hystyle' seeds (Harris Moran, Modesto, CA) were treated with imidacloprid (Gaucho 480, Gustafson LLC, Plano, TX) at 63 mg ai/100 g seeds, or thiamethoxam (Cruiser 5FS, formerly known as Adage, Syngenta Crop Corp., Greensboro, NC) at 30 mg ai/100 g seeds. Seed treatments were performed by Syngenta. Field studies were conducted at Cornell's New York State Agricultural Experiment Station in Geneva, NY, and seeds were sown on July 2, 2002 in replicated plots. Leaf samples were collected by selecting old (first true) leaves or new (trifoliate) leaves from 20 random plants, freeze drying and then grinding the dry samples. Samples were collected 28 days after sowing. Imidacloprid and thiamethoxam was extracted with 50% methanol or water, respectively, and the extracts homogenized and centrifuged. ELISA kits were used to analyze imidacloprid (EnviroLogix Inc., Portland, ME) and thiamethoxam (Beacon Analytical Systems, Inc. Portland, ME).

RESULTS AND DISCUSSION

Seed Coating Uniformity

Pea seeds were treated with 'Saturn Yellow', a fluorescent tracer, and a liquid *Rhizobium* inoculant by hand or by mechanical auger mixing (Fig. 1). The application rate for each method was 1x or 2x. This was achieved by adding one volume of water to the suspension, thus providing a 2x rate. Since the concentration of both inoculant + fluorescent tracer was held constant for all treatments, the fluorescence distribution could be fairly compared between each treatment. In this study, all four treatments were illuminated with UV light and photographed at the same time.

Our goal was to provide objective data to characterize application uniformity on the seed surface, which are not biased by the background area between seeds. This was achieved by sampling specific regions on these images, and ranges of light levels were determined for the following types of seed surfaces. Pixels having light levels less than 15 did not represent any actual seed surface and were thus factored out of the analysis. Pixels having levels from 16 to 100 were those of non-treated seed surfaces (no observable fluorescent tracer). Levels from 101 to 200 were areas determined to have a relatively uniform deposit of tracer, and areas with levels of 201 to 255 were considered 'hot spots' with an excessive amount of tracer present.

Three categories of seed surface application were quantified from the images: uniform deposition, no treatment and over-treatment (hot spots). The uniformity of distribution of the fluorescent tracer in each category differed by application method (hand vs. auger) and application rate (1x vs. 2x) (Fig. 2). The recommended hand mix at 1x resulted in over 60% non-treated surface as the fluorescent indicator did not cover the concave surfaces of the pea seed. In contrast, the auger method had less than 40% non-treated surface. Doubling the amount of liquid decreased the proportion of non-treated surface by more than 10 percentage points within each method. Only the auger at 2x revealed an increase in the percentage of over-treatment (10% vs. 2%) in comparison with the other treatments.

In field studies, nodulation from pea seeds inoculated with the 2x hand mix was compared with the 2x auger method. The auger method resulted in a greater level of nodulation than the hand mix (3.0 vs. 2.3 ($P=0.02$) on a 4 point rating scale for nodulation).

Systemic Chemical Seed Treatments

Imidacloprid and thiamethoxam were analyzed in the bean leaf tissue 28 days after sowing (Fig. 3). In the trifoliate leaves, imidacloprid had a concentration 5.2 times greater than thiamethoxam. Imidacloprid was 4.7 times more concentrated in the true leaves than in the trifoliates, while thiamethoxam concentration was not different between trifoliate and true leaves. Concentration differences of each systemic seed treatment and leaf tissue may be attributed to several factors including uptake rate, ability to translocate in the plant, and metabolism of the active compound with time. Moreover, systemic compounds may be lost due to leaching and adsorption to the soil particles.

The commercial ELISA kits had good sensitivity and specificity for each systemic compound. Procedures were developed to extract each compound from leaf tissue as described in the methods. Thiamethoxam is more water soluble than imidacloprid so different solvents were employed (water and 50% methanol/water, respectively). The imidacloprid kit is available commercially, while the thiamethoxam kit was obtained with a Materials Transfer Agreement between Cornell and Syngenta.

CONCLUSIONS

A fluorescent tracer with the liquid inoculant facilitated the visualization and assessment of application uniformity. Overall, hand mixing resulted in poorer uniformity than the auger technique, while doubling the amount of liquid improved uniformity for each method. The method was effective even with treated seeds, as the seed lot used in this study was commercially treated with a fungicide that contained a red dye. The digital camera with imaging software provided a method to quantify application uniformity, and this system could be used for many other purposes.

Both imidacloprid and thiamethoxam were detected in leaf tissue. These treatments provided protection against potato leaf hopper in field studies (Nault and Taylor, unpublished). It should be noted that imidacloprid (Gaucho 480) is labeled as a seed treatment on edible legumes including bean seeds, but that leafhoppers are not on the label (cited on the World Wide Web 7, Aug. 2002, http://www.gustafson.com/). Thiamethoxam is labeled on bean seeds to control leaf hoppers (cited on the World Wide Web 19, Feb. 2004). In summary, there is tremendous potential for systemic seed treatments to control early season pests.

ACKNOWLEDGMENTS

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Figures

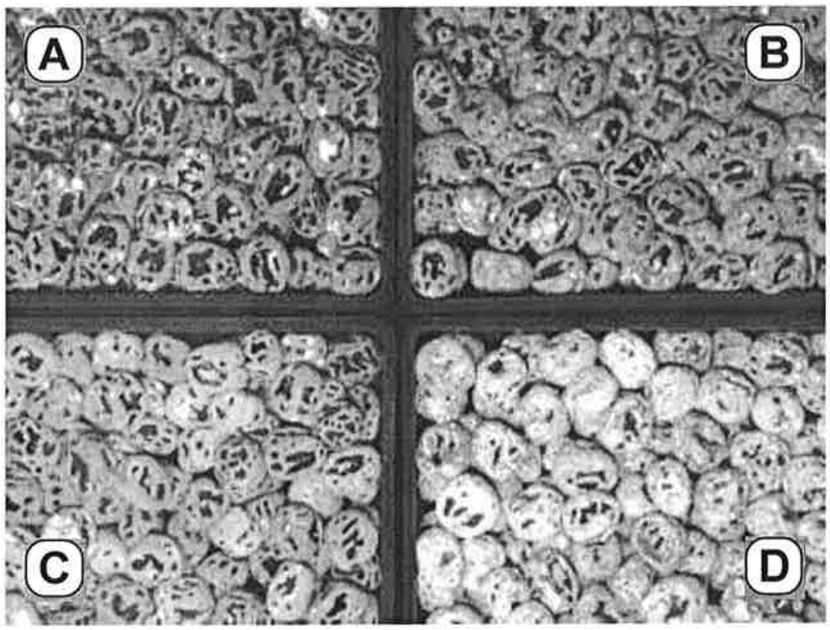

Fig. 1. Pea seeds treated with *Rhizobium* inoculant with fluorescent tracer: A) hand-mixed with one volume liquid (1x), B) hand-mixed with two volumes liquid (2x), C) auger with bristle brushes mixing (1x) and D) auger with bristle brushes mixing (2x). The concentration of both inoculant + fluorescent tracer was held constant for all treatments, only the volume of liquid changed (1x vs. 2x).

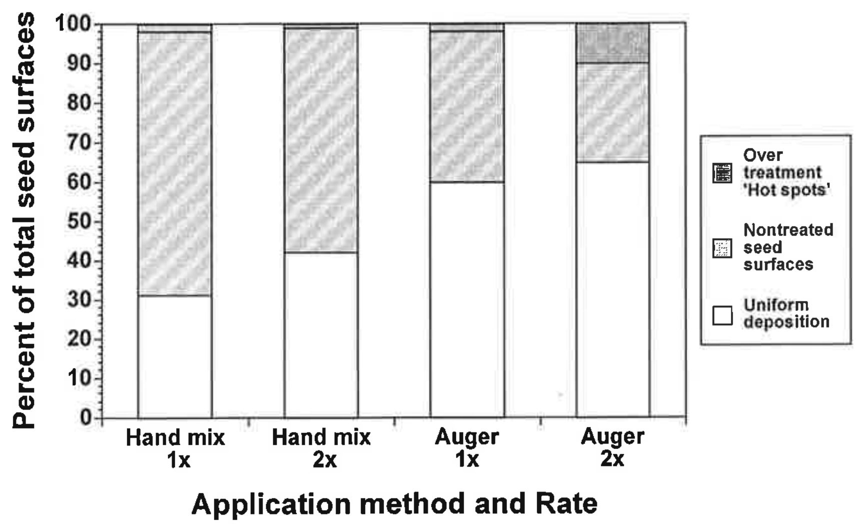

Fig. 2. Fluorescence distribution of non-treated, properly treated and over-treated pea seeds from Figure 1.

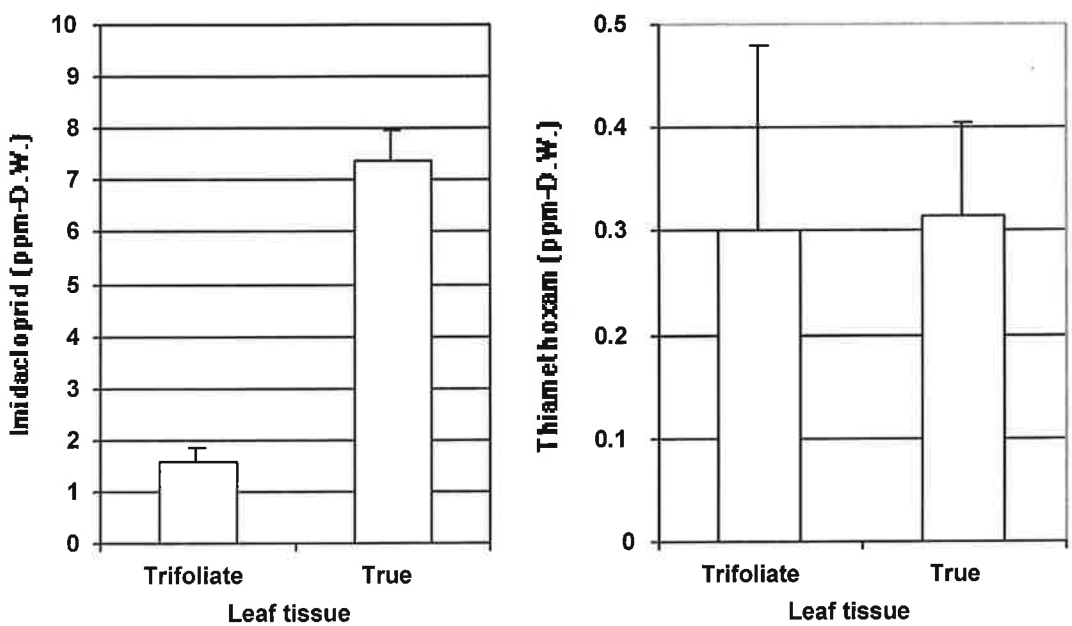

Fig. 3. Imidacloprid and thiamethoxam concentration in the first true leaves and trifoliates 28 days after sowing. Concentration expressed as ppm on dry weight basis.