Effect of Zein Films on the Growth of Tomato Plants and Evaporative Water Loss

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Abstract. Zein is an alcohol-soluble protein isolated from corn. The effect of ground cover films prepared from zein on the growth of tomato plants and corresponding evaporative water loss was investigated in greenhouse experiments. Results indicated that there was a decrease in water loss from the growth media for pots treated with zein films compared to the control (no film). There was an 11% increase height and 65% increase in dry weight of the treated plants relative to the control. In a second experiment, tomato plants mulched with zein isolates, low in free fatty acids (LFFA), exhibited an 18% increase in height and a 28% increase in dry weight compared to the control. Tomato plants treated with black polyethylene sheathing mulch were the tallest of the plants tested and had the greatest dry weight. Adding corn gluten meal directly to the soil surface resulted in tomato plants that were 26% taller and 29% heavier than those grown in untreated soil. Zein isolate films appear to be a viable ground cover replacement for polyethylene sheathing.

Nonbiodegradable, polymeric materials presently used as ground cover to control weeds and contain or reduce water evaporation are inexpensive to manufacture but expensive to use because of additional costs in collecting the material after the growing season. In addition, polymer materials, which can cover up to 70% of a field, increase surface water runoff (Hapeman, 2003). As a result more of the pesticides applied on such fields are found in the runoff. Photodegradable mulches eliminate the need to remove and dispose of plastic at the end of the growing season, but have not been widely adopted because they tend to degrade prematurely (Greer and Dole, 2003). Films prepared from zein, an alcohol soluble protein extracted from the endosperm of the corn kernel, could be an attractive replacement for synthetic ground cover materials. Zein is a hydrophobic protein principally used by the food industry for the preparation of edible films and coatings. Although zein films are considerably more hydrophilic than synthetic films, such as polyethylene and poly (vinylidene chloride), they are still capable of retarding water vapor transmission (Parris et al., 1995).

Commercially, zein is extracted from corn gluten meal, a byproduct of the wet milling process, using aqueous alcohol (Lawton, 2002). It has been shown that corn gluten meal contains a substance that inhibits root formation in several species, including crabgrass (Christians, 1993). Since commercial zein is extracted from corn gluten meal, films prepared from commercial zein could release these substances, possibly formed by bacterial proteolysis of the film, and inhibit the growth of unwanted grasses. Zein isolated from dry-milled corn contains a mixture of lipids and zein proteins (Dickey et al., 1998). The lipid portion is composed primarily of triacylglycerides and free fatty acids that act as endogenous plasticizers and further enhance the film’s flexibility and water vapor barrier properties. It’s estimated cost is $1.00/lb compared to $0.50/lb for bulk polyethylene. Films would be applied in a similar manner to black polyethylene ground cover but the major difference would be that the films would not be picked up at the end of the growing season but allowed to degrade in the field.

In this study we compared the effect of ground cover films prepared from commercial zein and zein isolated from ground corn on the growth of tomato plants and evaporative water loss.

Materials and Methods

Preparation of Zein films. We obtained corn zein (F-4000) protein from Freeman Industries (Tuckahoe, NY) and poly (ethylene glycol), average MW = 400 (PEG), from Aldrich Chemical Co. Inc. (Milwaukee, Wis.). Black polyethylene sheathing, 4 mil (0.1 mm) thick, was obtained locally and food-grade corn gluten from Extremely Green (Abington, Mass.). Corn zein isolates were prepared by batch extraction with 70% ethanol from dry-milled (preground) yellow dent corn, (Davis Feeds, Perkasie, Pa.) as described by Dickey et al. (1998). Zein isolates, low in free fatty acids (LFFA) were prepared by batch extraction from freshly ground yellow dent corn (Pioneer Hi-Bred International, Inc., Johnston, Iowa). These isolates were LFFA because lipases present in freshly ground corn were not given the opportunity to hydrolyze the triacylglycerides to FFA as in pregrown corn. Corn zein films were prepared by dissolving a total of 2.0 g zein F-4000 + 0.5 g PEG or 2.5 g zein isolate in 75 mL 90% ethanol. The mixture was heated with stirring at 60 °C for 10 min. Ethanol solutions were cast in polystyrene Petri dishes, (Thomas Scientific, Swedesboro, N.J.), 150 mm in diameter. The solutions were dried in a vacuum oven, adjusted to 33.75 kPa at 50 °C.

Fractionation of zein proteins. Zein proteins from ground corn were fractionated and identified on the basis of solubility using a modification of the method described by Esen (1988). Zein isolate (50 g) was stirred with 250 mL of hexane for 30 min and the hexane was decanted. The procedure was repeated and the defatted isolate was allowed to dry under nitrogen. The zein isolate was then dissolved in 200 mL 60% 2-propanol + 0.5% m-cetaptoethanol. 2-Propanol (60 mL) was added to the solution and the β-, γ-, and δ-zeins were precipitated at 4 °C overnight. The purified zeins were isolated by centrifuging at 12,000 g, for 10 min at 4 °C, then lyophilized. The supernatant, containing the α-zeins, was diluted with 1600 mL water and 72 mL 3 sodium acetate (pH 6.0). The solution was stored overnight at 4 °C then centrifuged at 5000 g, for 10 min at 4 °C. The supernatant was discarded and the pellet containing the α-zein was analyzed by gel electrophoresis.

Lipid analysis. To determine the level of free fatty acids (FFA) in the zein isolate, duplicate 0.5-g samples of isolate were homogenized with a mixture of 8 mL chloroform, 16 mL methanol and 4.3 mL water. The homogenate was diluted with 8 mL chloroform and 8 mL water to form two separate layers. The chloroform layer containing triacylglycerides (TAG) and FFA was separated and quantified by normal phase-HPLC according to the method of Moreau et al. (1996).

Experiment 1: Effect of zein films prepared from corn gluten meal or isolated from dry-milled yellow dent corn on the growth of tomato plants and evaporative water loss.

Table 1. Experimental treatments and composition of ground cover.

| Treatment            | Composition           |
|----------------------|-----------------------|
| Control              | No film               |
| Zein F-4000          | 2.0 g Zein + 0.5 g PEG|
| Zein isolate         | 2.5 g Zein            |
| Experiment 2         |                       |
| Control              | No film               |
| Zein F-4000          | 2.0 g Zein + 0.5 g PEG|
| Zein isolate         | 2.5 g Zein            |
| Zein isolate, LFFA   | 2.5 g Zein            |
| Polyethylene         | 4-mil black plastic sheathing |
| Corn gluten          | 2.0 g granular        |

5See Material and Methods section for the preparation of zein films, polyethylene sheathing and application of corn gluten meal to soil surface.

LFFA = low free fatty acids.
Table 2. Effect of zein films on water loss (g/pot) of potted tomato plants.\textsuperscript{a}

| Treatment          | Days 0 | Days 7 | Days 9 | Days 11 | Days 14 | Days 16 | Days 18 | Days 21 | Days 39-40 |
|--------------------|--------|--------|--------|---------|---------|---------|---------|---------|------------|
| Control\textsuperscript{b} | 181 ± 4.4 a | 177 ± 2.7 a | 99 ± 3.1 a | 181 ± 1.6 a | 251 ± 8.5 a | 145 ± 12.3 a | 176 ± 10.2 a | 198 ± 8.7 a | 100 ± 19.0 a |
| Zein F-4000        | 153 ± 1.6 ab | 154 ± 3.2 ab | 88 ± 0.7 a | 161 ± 3.3 ab | 234 ± 9.5 ab | 132 ± 3.4 a | 169 ± 9.0 a | 192 ± 5.3 a | 122 ± 7.2 a |
| Zein Isolate       | 117 ± 12.9 b | 120 ± 9.5 b | 67 ± 0.9 a | 131 ± 11.9 b | 195 ± 5.8 ab | 117 ± 0.3 a | 153 ± 8.0 a | 189 ± 11.3 a | 132 ± 8.3 a |

\textsuperscript{a}Means in the same column with no letter in common are significantly different (\textit{p} < 0.05). The estimate of the Bonferroni LSD (least significant difference) is 47.2; \textit{n} = 3.

\textsuperscript{b}No film.

Table 3. Lipid extracted from zein isolates.

| Samples          | Lipid wt (\%) |
|------------------|---------------|
| Zein isolate     | TAG = 5.75 ± 1.76 | FFA = 35.28 ± 1.00 |
| Zein isolate, LFFA | 6.66 ± 0.11 | 11.93 ± 0.15 |

\textsuperscript{a}TAG = triacyglycerols; FFA = free fatty acids.

Table 4. Effect of various films and corn gluten meal on the water loss (g/pot) of potted tomato plants.\textsuperscript{a}

| Treatment          | Days 0 | Days 7 | Days 9 | Days 11 | Days 14 | Days 16 | Days 18 | Days 21 | Days 39-40 |
|--------------------|--------|--------|--------|---------|---------|---------|---------|---------|------------|
| Control\textsuperscript{b} | 102 ± 1.9 a | 71 ± 1.4 a | 156 ± 3.7 a | 145 ± 1.9 a | 110 ± 2.5 a | 159 ± 4.0 a | 75 ± 4.7 a | 128 ± 8.0 a | 145 ± 7.1 a |
| Zein F-4000        | 75 ± 2.1 bc | 63 ± 1.18 a | 145 ± 3.3 ab | 140 ± 2.2 ab | 101 ± 2.7 a | 155 ± 4.3 ab | 75 ± 2.4 a | 118 ± 2.4 a | 146 ± 5.1 a |
| Zein Isolate       | 56 ± 3.4 bc | 45 ± 1.0 a | 106 ± 9.3 b | 100 ± 3.8 bc | 87 ± 3.3 ab | 132 ± 3.2 a | 65 ± 1.6 a | 108 ± 4.2 ab | 133 ± 3.0 a |
| Zein Isolate LFFA\textsuperscript{a} | 54 ± 1.8 abc | 51 ± 1.8 a | 104 ± 5.9 b | 84 ± 1.7 cd | 75 ± 3.4 ab | 114 ± 3.8 b | 61 ± 2.6 a | 107 ± 5.5 ab | 137 ± 8.0 a |
| Polyethylene       | 35 ± 2.1 c | 31 ± 1.8 a | 59 ± 1.6 c | 45 ± 2.7 d | 45 ± 1.3 b | 68 ± 4.7 c | 39 ± 3.6 a | 75 ± 5.8 b | 104 ± 13.1 a |
| Corn Gluten        | 97 ± 2.7 ab | 67 ± 1.1 a | 154 ± 3.0 a | 118 ± 3.0 abc | 99 ± 1.7 a | 157 ± 5.9 a | 75 ± 2.3 a | 112 ± 6.2 ab | 139 ± 3.6 a |

\textsuperscript{a}n = 5. Data collected for days 26, 28, 31, and 33 are not presented since effects of mulch treatment were negligible. Means in the same column with no letter in common are significantly different (\textit{p} < 0.05). The estimate of the Bonferroni LSD (least significant difference) is 43.0.

\textsuperscript{b}No film.

\textsuperscript{a}LFFA = low free fatty acids.
than the control, while the corn gluten (CG) treatment was not significantly different from the control treatment. For the first 12 d (again excluding day 7), water loss from the zein isolate treatment was signifi cantly less than the control. After 17 d, there was no difference in water loss among the six treatments, except for PE being signifi cantly lower than the control at day 21 and at day 26 (not shown). Examining the effect of day on each treatment led to maximum water loss at 28 d (not shown) for each treatment with a decrease in water loss over the last three periods.

The plants were harvested after 55 d. The height response shows that the PE treatment was signifi cantly taller than the control treatment. There was no evidence of any signifi cant height difference between the other treatments and the control treatment (Table 5). The shoot fresh weight response shows signifi cantly higher values for PE, CG and zein isolate, LFFA than the control treatment. There was no evidence of a signifi cant difference in the shoot fresh weight of zein F-4000 or zein isolate and the control treatment. The shoot dry weight response shows signifi cantly higher values for PE than for the control treatment. The plants treated with zein films tended to have greater dry weights than controls. The number of flowers didn’t differ signifi cantly between treatments.

It appears that difference in water loss after treatment of tomato plants with zein fi lms was due to the presence of lipid in the zein isolates since the water loss of plants treated with zein F-4000 was not statistically different from the control.

Conclusions

Zein isolate fi lms appear to be a viable ground cover replacement for polyethylene sheathing. In contrast to polyethylene sheeting, zein is a potentially inexpensive, degradable product prepared from a renewable resource. Lipids present in the isolate increase water retention in the soil and enhance the growth and dry weight of the plant. Film degradation can be controlled by blending the isolate with commercial zein.

The data presented here suggest a potential valuable contribution of zein fi lms to agriculture in arid climates. The initial deterioration of the fi lms could be timed, through manipulation of the ingredients, to coincide with the closure of the plant canopy when transpiration is the overwhelming contributor to water loss. Subsequent biodegradation of the fi lms would release nutrients to stimulate plant growth and the fi lm need not be disposed of after harvest as with plastic mulches.

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Table 5. Effect of various fi lms and corn gluten meal on plant growth.†

| Treatment                  | Ht (cm)     | Shoot fresh wt (g) | Shoot dry wt (g) | Flowers |
|----------------------------|-------------|--------------------|------------------|---------|
| Control                    | 41.5 ± 2.84 bc | 23.81 ± 1.32 c     | 3.48 ± 0.26 b    | 1.2 ± 0.37 a |
| Zein F-4000                | 40.5 ± 1.18 c  | 25.78 ± 1.59 bc    | 3.74 ± 0.30 ab   | 0.4 ± 0.24 a  |
| Zein Isolate               | 40.7 ± 1.76 bc | 28.96 ± 2.13 bc    | 4.21 ± 0.30 ab   | 1.2 ± 0.37 a  |
| Zein Isolate, LFFA†        | 49.0 ± 2.32 bc | 32.73 ± 1.48 ab    | 4.46 ± 0.24 ab   | 2.0 ± 0.71 a  |
| Polyethylene               | 55.6 ± 3.91 a  | 38.32 ± 1.50 a     | 5.18 ± 0.53 a    | 2.8 ± 0.92 a  |
| Corn Gluten                | 52.4 ± 2.48 ab | 38.28 ± 1.24 a     | 4.47 ± 0.13 ab   | 1.6 ± 0.24 a  |
| Corn Gluten, LFFAx         | 49.0 ± 2.32 bc | 32.73 ± 1.48 ab    | 4.46 ± 0.24 ab   | 2.0 ± 0.71 a  |

†n = 5, numbers in the same column followed by the same letter are not signifi cantly different (p < 0.05).

†No fi lm.

LFFA = low free fatty acids.