Degradation and Nitrogen Release of Zein-based Bioplastic Containers[1]

Matthew S. Helgeson[2], William R. Graves[3], David Grewell[4], and Gowrishankar Srinivasan[5]
Department of Horticulture, Iowa State University, Ames, IA 50011

Abstract

Environmental problems caused by petroleum-based plastics have led to interest in alternatives made from biodegradable polymers (bioplastics), but little effort has been made to evaluate horticultural containers made from these materials. We hypothesized the stability and longevity of containers made from polymers of the hydrophobic corn (*Zea mays* L.) protein, zein, is sufficient to make commercial use of zein-based containers feasible. Our objectives were to fabricate containers from zein, to determine longevity under conditions typical of horticultural production, and to identify limitations of the containers that might be overcome by further research. Zein-based, bioplastic containers of two wall thicknesses were filled with either a peat-based, soilless potting substrate or with coarse perlite and irrigated every 2 or 4 days. After 10 weeks, weight loss of containers was determined as a measure of their degradation. Containers filled previously with soilless substrate lost nearly twice as much weight as containers filled with perlite, and irrigation every 4 days led to greater weight loss than irrigation every 2 days. The containers released nitrogen (N) as they degraded; as much as 208 mg N·kg^{-1} was present in leachate after irrigation with water. In a second experiment, to simulate the potential practice of installing plants in the landscape without container removal, bioplastic containers of two sidewall thicknesses were filled with the soilless potting substrate and planted in either drained or saturated field soil, and the two substrates were either sterilized (autoclaved) or nonsterilized. After 12 weeks, containers in drained soils had greater weight loss than containers in saturated soils regardless of substrate sterilization treatment. Zein-based, bioplastic containers appear suitable for crops having production cycles of < 3 months, and the containers will decompose and release N if installed with plants in the landscape. Further research is needed to increase the longevity of zein-based containers for crops with longer production cycles. In addition, the influence of containers made from zein on plant growth needs to be determined, and potential effects of degrading containers installed in the landscape on the establishment of transplants warrant investigation.

Index words: corn, *Zea mays*, maize, plastic, protein, container, sustainable.

Significance to the Nursery Industry

Rising costs of petroleum and negative consequences of disposing petroleum-based plastic containers in landfills have led to increased interest in bioplastics. Containers made from zein, a protein from corn (*Zea mays*), may serve as a sustainable alternative to petroleum-based containers. Zein is prolamine protein processed from corn gluten and is a byproduct of the wet milling of corn. Zein-based containers are completely biodegradable. Used zein containers removed from root balls should be compostable, or subsequent research may show plants produced in zein containers can be installed into landscapes or transplanted to a larger container without removing the bioplastic container. Composting or planting degrading containers with transplants would circumvent disposal problems inherent to conventional plastic containers, thereby saving commercial horticulturists time and money. Fertilizer costs also may be reduced by the use of zein containers, which release N as they degrade. The limited longevity of the zein containers we studied in this initial evaluation would be suitable for crops with short production cycles, but modifications in container design or composition have the potential to expand the range of crops that can be produced in these containers.

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[2]Graduate Research Assistant.

[3]Professor. graves@iastate.edu

[4]Assistant Professor, Department of Agricultural and Biosystems Engineering. dgrewell@iastate.edu

[5]Graduate Research Assistant, Department of Materials Science and Engineering.

Introduction

Synthetic plastics accounted for 26.8 million metric tons of municipal solid waste in the United States in 2006, only 7% of which was recycled (15). The market for horticultural containers has been dominated by synthetic plastics for several decades. These conventional containers are structurally strong, light in weight, and easy to ship (2). They also have been inexpensive (2), though recent surges in the cost of crude oil have led to price increases. Conventional plastics are not biodegradable, and difficulties associated with disposal of synthetic plastics used in horticulture have raised concerns about environmental sustainability. Recycling of nursery containers is limited because of a lacking infrastructure, poor resin quality, and ultraviolet degradation; therefore, most containers are deposited in landfills (2).

Alternatives to petroleum-based plastic containers have been explored. Pressed peat moss and paper fiber containers have been available for many years as biodegradable alternatives to traditional plastics, but use of peat and paper containers is limited because of unpredictable longevity, high evaporative water loss, and low strength (3). More recently, byproducts of the processing of numerous agricultural commodities have been evaluated for their potential as components in horticultural containers. Biodegradable plastics (bioplastics), which are decomposed by naturally occurring microorganisms (1), have received attention recently for their potential applications in agriculture (9). Replacement of synthetic plastics with bioplastics may reduce dependence on fossil fuels and reduce emissions of greenhouse gases (7). Bioplastics can be derived from abundant agricultural commodities, and because of their biodegradability, plants may be installed into the landscape without removing the container. Discarding containers at composting facilities might be another option.

Research herein focused on zein, a protein from corn, as the primary biorenewable component of bioplastic containers. Zein has been utilized for many industrial products, including fibers, food coatings, adhesives, and pharmaceuticals. Polymers made from zein have been examined for industrial applications since the mid-20th century (10, 13). Zein is hydrophobic, and products made from it are relatively water-insoluble (13). In contrast, proteins of other major agricultural commodities, such as soybean (*Glycine max* [L.] Merr.), are soluble in water, so bioplastic products made from soybean tend to disintegrate rapidly when subjected to moisture (14, 19). In a typical nursery or greenhouse environment, containers are subjected to high moisture. Therefore, we hypothesized the hydrophobic properties of zein would make it suitable as a bioplastic material for horticultural applications.

The work described in this paper represents what appears to be the first attempt to make horticultural containers from zein. To our knowledge, no commercial manufacturer has the capacity to mass-produce zein-based containers. Therefore, the studies we report were conducted with containers made by hand. Our main objective was to determine the effects of substrate type, substrate sterility, and substrate moisture content on biodegradation of containers made from zein. Because we hypothesized the longevity of these containers would vary depending on the thickness of their sidewalls, containers with both relatively thin and thick sidewalls were compared. Additionally, we hypothesized that N from proteins might be released as these containers decompose. Therefore, an additional objective was to quantify N in leachate during our trials.

Materials and Methods

Container preparation. Zein protein (Global Protein Products, Marina, CA) was dissolved in 90% ethyl alcohol at 1 zein:4 ethyl alcohol (by weight) with a magnetic stirrer for 10 min at 100C (212F). The solution was poured into an ice bath, and zein was precipitated to form a dough-like material that was kneaded to remove excess solvent. The bioplastic material, while in the dough form, was molded around the outside of a round container made of conventional plastic with an outer diameter of 10.2 cm (4.0 in) and a height of 8.6 cm (3.4 in) (Kord Products, Brampton, ON, Canada). Before making each container, Quick Silicone mold release agent (Slide Products, Inc., Wheeling, IL) was sprayed on the conventional-container molds to facilitate release from the bioplastic container. Thin- and thick-walled containers of the same shape and size were made from 30 and 40 g (1.06 and 2.41 oz) of dry zein per container, respectively. Bioplastic containers were removed from molds after two weeks of drying in a laboratory at 21C (70F). Thin-walled and thick-walled containers had a mean wall thickness of 1.3 and 2 mm (0.05 and 0.08 in), respectively, and a mean weight of 24 and 34 g (0.85 and 1.2 oz), respectively. Four 6-mm-(0.2-in-) diameter drain holes were drilled in the bottom of each dry container.

Influence of substrate and irrigation on degradation. Zein containers of both thicknesses, as well as round Jiffy-Pots® (Jiffy Products of America Inc., Norwalk, OH) and round paper fiber containers (Kord Products), all with top diameters of ≈10 cm (3.9 in) and heights of 9 cm (3.5 in), were filled with one of two substrates and irrigated by hand with 200 ml tap water at two frequencies. The substrates were a soilless, peat-based substrate (Fafard® 52, Fafard®, Inc., Agawam, MA) and coarse perlite; one-half of the containers of each type were filled with each substrate. Half of the containers within each combination of container type and substrate were irrigated every 2 days, the others every 4 days. There were 48 containers in this four (container type) × two (substrate) × two (irrigation) factorial combination of treatments, with three containers (experimental units) per treatment combination. Containers were arranged in a completely randomized design in a growth chamber in which 16-hr photoperiods were provided by cool-white fluorescent and incandescent lamps. Photosynthetically active radiation, measured with a quantum sensor (LI-COR, Lincoln, NE) at container height at five locations, averaged 211 ± 37 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Day/night air temperatures were 26 ± 2/15 ± 2C (79 ± 4/59 ± 4F), respectively, and corresponded to the photoperiod schedule.

Leachate was collected from each container after irrigation on day 72 of treatment. Total inorganic N (NO_3^--N + NH_4^+-N) in the leachate was determined with Lachat® flow injection analysis (Lachat Instruments, Milwaukee, WI). Treatments ended on day 73. After substrates were removed, containers were dried in a laboratory at 21C (70F) for two weeks and then weighed. Empty containers were weighed before and after treatments so biodegradation could be expressed as relative (percentage) weight loss.

Influence of substrate sterilization and aeration on degradation of planted containers. Thick- and thin-walled zein containers were made as described previously and filled with Fafard® 52. Each filled container was planted individually into a larger conventional plastic container [13.8 cm (5.4 in) top diameter, 15 cm (5.9 in) height] filled with Hayden Storden loam soil to simulate installing a plant in the bioplastic container in which it was produced into a landscape with mineral soil. Bioplastic containers were buried up to the top 1 cm (0.4 in) of the sidewall, which remained above the top of the soil in the larger container. Larger containers either were allowed to drain after irrigations or were kept saturated. For half of these two-container experimental units, both the Fafard® 52 and soil were sterilized (autoclaved) immediately before use, whereas nonsterilized Fafard® substrate and soil were used for the other half of the units. Moisture content of the upper 6 cm (2.4 in) of Fafard® 52 in drained larger containers was measured every 2 days with a Theta Probe (model HH1, model ML 1 sensor; Delta-T Services, Cambridge, England). When the moisture content of the Fafard® 52 was ≤ 0.2 $m^3 \cdot m^{-3}$, inner and outer containers were irrigated simultaneously with a total of 500 ml deionized water. Soil was kept inundated with water in the saturated containers. There were 24 experimental units, three in each of the eight factorial treatment combinations [two (bioplastic container thickness) × two (sterile vs. nonsterile) × two (moisture conditions)]. Containers were arranged in a completely randomized design on a bench in a glass-glazed greenhouse in which no supplemental irradiance was provided and night and daytime air temperature was 22 ± 2.5C (72 ± 4F).

After 12 weeks, bioplastic containers were removed from the larger containers, separated from the substrate surrounding them, and weighed after drying in a laboratory at 21C (70F) for 2 weeks. Differences between final and initial weights of empty containers were used to quantify biodegradation as relative weight loss.

Table 1. **Weight loss and total nitrogen (N) release in leachate from containers after 73 days. There were three treatment factors: container type, substrate, and irrigation frequency. All four container types were filled with either Fafard® 52 medium or perlite, and containers were irrigated with 200 ml of tap water every 2 or 4 days. Treatments were arranged in a complete factorial combination resulting in a total of 16 treatments with three replicates per treatment. Containers were arranged in a completely randomized design in a growth chamber.**

Treatment	Weight loss (% of initial wt.)[z]	Total N ($mg \cdot kg^{-1}$)[z]
Container type		
Fiber	—	3.2c[y]
Peat	—	8.1c
Thin bioplastic	33.7a[y]	82.6b
Thick bioplastic	20.9b	120.5a
Substrate		
Perlite	18.8b	29.7b
Fafard®	35.8a	165.8a
Irrigation frequency (days)		
2	23.3b	85.7b
4	31.3a	114.5a

[z]Weight loss means calculated only from bioplastic containers, and total N means for substrate and irrigation frequency calculated only with data for bioplastic containers.

[y]Mean separation within each column by treatment category (container type, substrate, or irrigation frequency) at $P \leq 0.05$ by Fisher's least significant difference. Mean separation statistics were assessed separately for container type (n = 11 or 12), substrate (n = 11 or 12), and irrigation frequency (n = 11 or 12).

Data analysis. Data for container degradation (weight loss of each container) and total N content in leachate were analyzed for main effects and interactions by using the general linear model (GLM) procedure of SAS/STAT®, version 9.1.3 (Cary, NC). Because weight loss of each container was expressed as a percentage of its initial weight, the data were square-root transformed before analysis but are reported as nontransformed data to ease interpretation. Means associated with effects that showed significance in the GLM analyses were separated using Fisher's least significant difference at $P \leq 0.05$.

Results and Discussion

During the first experiment, degradation of zein-based bioplastic containers, which was assessed as weight loss, was influenced by sidewall thickness, substrate, and irrigation frequency (Table 1). Thin- and thick-walled containers lost > 30 and 20% of their initial weight, respectively (Table 1). Because degradation is dependent on microorganisms that colonize substrate, zein-based bioplastic containers used in greenhouses and nurseries probably will degrade primarily from the inside out, and container longevity likely will increase with increasing sidewall thickness. The sidewall thicknesses of the containers we used might make them suitable for crops with short production cycles, such as annual bedding plants, herbaceous perennials to be sold bare-root, vegetable seedlings, or other crops that can be finished or grown to a transplant stage within about 3 months. Further research is needed to assess how the degradation and longevity of zein-based containers in greenhouses or in outdoor production systems are influenced by conditions of the growth environment, such as irrigation or precipitation, humidity, and ultraviolet radiation. In addition, the amount of time to move plants to retail markets and into landscapes and gardens must be considered as research and development of biopastic containers for horticulture continue. Additional effort should focus on strategies for increasing longevity of zein-based bioplastic containers to increase the feasibility of using them to produce crops with production cycles exceeding three months. The bioplastic containers we studied were made by hand and therefore had sidewalls that were not as uniform as would be expected for sidewalls of machine-molded containers from a commercial manufacturer. Slight variations in thickness of the walls of our containers may have lessened their structural properties and longevity, and commercially fabricated containers might have increased longevity simply due to uniformity of the sidewalls. Future research could be designed to examine how addition of chemical cross-linking agents (8, 18), plasticizers (10), and organic fibers affects the mechanical properties of zein-based bioplastics, as well as container longevity and cost.

Weight loss was greater for the 4-day irrigation treatment than the 2-day irrigation treatment, and weight loss was greater when containers were filled with Fafard® 52 than with perlite (Table 1), which we used because of its high porosity and lack of organic matter. Effects of moisture and aeration on microbial activity may explain the heightened degradation of zein containers irrigated less frequently; we speculate microorganisms populated the substrate adjacent to the walls of our containers better when aeration in the substrate was enhanced due to relatively infrequent irrigation.

More of the surface area of inner sidewalls of bioplastic containers filled with Fafard® 52 appeared to have degraded compared with bioplastic containers filled with perlite (Fig. 1). Sidewalls of containers with Fafard® 52 appeared to have degraded, whereas sidewalls of containers with perlite seemed to retain most of their original thickness, but they cracked extensively (Fig. 1). The appearance of bioplastic containers filled with the two substrates reflected the differences between them in mean weight loss and suggest the mode of degradation of zein-based containers will differ depending on the substrate they contain. These observations suggest a new hypothesis that warrants testing; we suspect microorganisms responsible for degrading zein colonized the

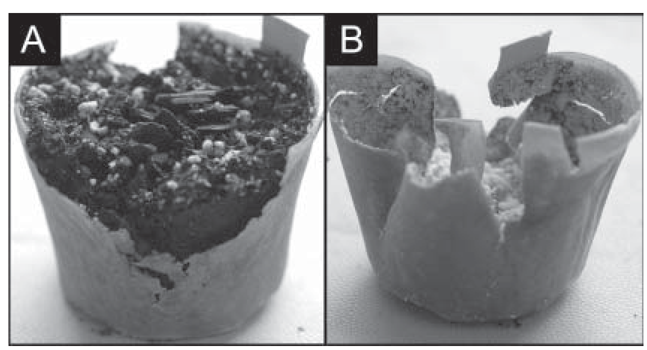

Fig. 1. **Representative zein-based bioplastic containers filled with (A) Fafard® 52 medium or (B) perlite and held under treatment conditions in the first experiment for 73 days. Sidewalls of containers filled with Fafard® 52 degraded extensively, whereas containers filled with perlite cracked extensively, but were less degraded.**

Table 2. **Weight loss of bioplastic containers after 12 weeks. There were three treatment factors: container type, moisture, and sterilization. Bioplastic containers were manufactured to two different thicknesses, thin and thick. Containers were filled with Fafard® 52 medium and placed in a larger container of either drained or saturated field soil. The field soil and potting substrate was either sterilized or nonsterilized. Treatments were arranged in a complete factorial combination resulting in a total of eight treatments with three replicates per treatment. Containers were arranged in a completely randomized design on a greenhouse bench (n = 12).**

Treatment	Weight loss (% of initial wt.)
Container type	
Thin bioplastic	58.0a[z]
Thick bioplastic	48.7b
Moisture	
Drained	68.0a
Saturated	38.7b
Sterilization	
Nonsterilized	55.9a
Sterilized	50.7a

[z]Mean separation within each column by treatment category (container type, moisture, or sterilization) at $P \leq 0.05$ by Fisher's least significant difference.

interface of the organic Fafard® 52 and the inner container sidewall, but not the interface of the sidewall and perlite, which is inorganic and a poor source of nutrients. Peat and fiber containers showed few signs of degradation. Peat containers had mold on the outer walls, and were prone to breakage upon handling, particularly soon after they were saturated with irrigation water. Fiber containers remained structurally stable, and no mold was evident on their outer sidewalls.

Total N in leachate from both thin- and thick-walled bioplastic containers was greater than N in leachate from fiber and peat containers (Table 1). Leachate from bioplastic containers filled with Fafard® 52 contained more than five times the total N than leachate from containers filled with perlite, and irrigation every 4 days led to 34% more N in leachate compared with irrigation every 2 days (Table 1). An interaction existed between irrigation frequency and substrate. Containers with Fafard® 52 had 126 and 205 mg $N \cdot kg^{-1}$ (ppm) in leachate when irrigated every 2 and 4 days, respectively. In contrast, containers filled with perlite had means of 37 and 24 mg $N \cdot kg^{-1}$ (ppm) in leachate when irrigated every 2 and 4 days, respectively, illustrating the interaction was due to enhanced N release when Fafard® 52 was used and irrigated every 4 days. We speculate that N accumulated in the substrate over a longer period in the 4-day irrigation treatment than in the 2-day treatment and then leached in greater concentration during irrigation (Table 1). The presence of N in leachate from these bioplastic containers is consistent with the fact that zein protein contains ≈15% N (4, 11). Containers filled with Fafard® 52, which degraded more than containers filled with perlite, contained comparably high concentrations of N in leachate, presumably because enhanced microbial activity fostered breakdown of zein and a consequential release of N into the substrate (Table 1). Fafard® 52 substrate contains a starter fertilizer (N–P–K) that likely influenced N in leachate. However, N in the starter fertilizer is water-soluble and probably leached after a few irrigations. Because peat and fiber containers containing Fafard® 52 had low concentrations of N at day 72, while zein containers had greater concentrations of N, we surmise that the N originates from mineralization of the protein. The release of N from zein containers should be explored further to determine whether it enhances plant growth and reduces the need for supplemental fertilization.

During the second experiment, when averaged over moisture and sterilization treatments (no interaction existed), thin-walled bioplastic containers lost more of their initial weight than thick-walled bioplastic containers (Table 2). Placement of bioplastic containers into soil, which was intended to simulate the possible practice of transplanting or installing without container removal, led to more weight loss when the soil was drained than when the soil was saturated (Table 2). In drained soils, the bioplastic degraded extensively and lost structure and shape, whereas containers in saturated soil, though misshapen, remained intact (Fig. 2). Use of sterilized substrate did not influence weight loss of the containers (Table 2). Microorganisms can quickly repopulate previously sterilized soils (17), which we presume occurred during this experiment. Containers placed in saturated conditions probably were exposed to hypoxia, which may have suppressed colonization by zein-degrading microorganisms. Our results are consistent with previous observations of other bioplastics, which degrade under aerobic conditions but not under

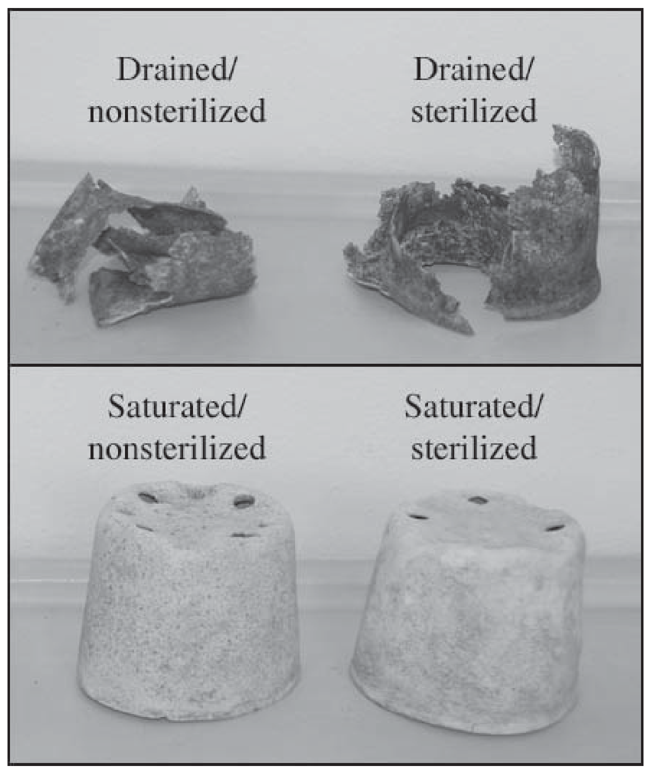

Fig. 2. **Representative bioplastic containers placed in saturated soil (bottom) were intact and, though misshapen, retained their original form at the end of the second experiment. In contrast, containers placed in drained soil (top) degraded extensively and lost structural integrity. Prior sterilization of media in the containers and of the soil in which the containers were planted did not influence weight loss of the containers, which was used to quantify degradation.**

anaerobic conditions (12). Because hypoxic conditions develop in some managed landscapes, particularly after heavy precipitation or soil compaction, further research should explore biodegradability of containers made from zein in soils that differ in moisture content and physical properties. If transplants are installed without removing the containers in which they were produced, the rate of container degradation could be critical to plant establishment. Because of problems resulting from roots circling within conventional containers, installers are encouraged to disrupt the root mass mechanically to improve establishment (5, 6, 16). Delays in container degradation might increase the extent of root circling, and corrective measures after plant installation would be challenging. A thorough understanding of factors influencing container degradation should be developed before recommending that transplants grown in containers made from zein be installed without removing the container.

This investigation provides the first insight concerning the horticultural potential of containers made from zein. Although we consider the containers studied prototypes rather than products ready for commercial use, our results suggest the concept of using zein to fabricate containers for horticulture is worthy of additional attention. Further research is warranted because bioplastics made from zein, a byproduct of a major agronomic crop, would be completely degradable and compostable without the need for special facilities or processes. Currently, commercially produced zein is relatively expensive when compared to feedstocks for petroleum-based plastics, but new technologies promise to make zein extraction and recovery easier and cheaper (11). The possibility for competitively priced biodegradable containers in the future provides incentive for continued evaluation of zein-based bioplastic containers. Strategies to increase container longevity for crops that typically are kept in a container of a given size for more than about 3 months should be explored, as should the feasibility of producing zein-based bioplastic containers commercially by standard molding techniques. Finally, our findings that containers made from zein release N justifies further studies to explore how N from these containers may benefit plant growth during production, may reduce the need to apply N fertilizers, and may aid establishment of crops transplanted or installed with their containers.

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