Horticultural evaluation of zein-based bioplastic containers

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Horticultural evaluation of zein-based bioplastic containers

by

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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. Containers were fabricated from polymers of the corn \((Zea~mays~L.)\) protein, zein. My first objective was to determine the longevity of zein-based containers under conditions typical of horticultural production. Zein containers of two wall thicknesses were filled with either a peat-based, soilless potting substrate or coarse perlite, and they were irrigated every 2 or 4 days. After 10 weeks, weight loss of containers was determined as a measure of their degradation. Containers filled with the peat-based 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. In another 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. My second objective was to test the hypothesis that biodegradation of zein-based containers provides nitrogen (N) that promotes growth of geranium \((Pelargonium \times hortorum\) L.H. Bail.). Zein containers provided root zones up to 298 and 277 mg·kg\(^{-1}\) \(\text{NH}_4^+\)-N and \(\text{NO}_3^-\)-N, respectively. Unlike plants in conventional plastic containers, leaves of geraniums in zein containers remained dark green when produced without fertilization. Electrical conductivity (EC) and pH of substrate in zein containers increased above ranges recommended for many horticultural crops, and \(\text{NO}_2^-\), which can be toxic to plants, was
present in the substrate of zein containers. These chemical changes seem responsible for reduced canopy height and width, leaf area, root system length, and shoot dry weights of geraniums in zein containers compared with geraniums in conventional plastic containers. My third objective was to determine if geraniums grown in zein containers could be transplanted and reestablished successfully with the container intact, thus eliminating the need for disposal of containers. When transplanted with zein containers intact, root and shoot growth of geraniums were reduced until after six weeks, when biodegradation of containers was nearly complete. Migration of roots through the zone of the degraded container and into surrounding substrate was observed approximately three months after transplanting. Our data show the zein-based bioplastic containers we made are suitable for crops having production cycles < 3 months. Geraniums can be produced and transplanted in zein-based containers, but additional research is needed to solve problems that can result from chemical changes to the root zone during production, and from chemical and physical effects on reestablishment after transplant.
CHAPTER 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

Introduction

Because of concerns of global warming, our nation’s dependence on fossil fuels, and increasing solid wastes, there is interest in replacing petroleum-based products with sustainable alternatives. Plastic containers manufactured from petroleum are widely used in the nursery and greenhouse industry for crop production. Production of these plastic containers is energy intensive, and most of the containers are deposited in landfills after a single use or they accumulate as waste near plant nurseries. Bioplastics, which are plastics manufactured from renewable sources that are biodegradable, may help reduce our dependence on fossil fuels and reduce the amount of plastic waste sent to landfills each year. Bioplastics are already used for many single-use items such as disposable tableware and trash bags, but few attempts have been made to apply bioplastic technology to nursery and greenhouse containers. Biodegradable containers would provide many advantages over petroleum-based containers. Biodegradable containers could be composted rather than deposited in landfills, or landscapers could simply install plants with their containers intact if the containers degrade quickly and allow for plant establishment.

Zein is a protein from corn (Zea mays L.) extracted during the corn wet-milling process (Shukla and Cheryan, 2001). It is a by-product of ethanol production. Zein has many properties that make it an appealing bioplastic feedstock. Its hydrophobic properties help it resist water, it is 100% biodegradable, and it consists of amino acids that contain nitrogen that could support plant growth.

Zein was processed into a bioplastic, formed into plant containers, and evaluated for use in horticultural applications. I assessed the biodegradability and longevity of bioplastic
containers and made specific recommendations for cropping systems. Container performance was evaluated and growth of plants in zein-based containers was compared to growth of plants in conventional containers. Attributes of bioplastic containers made from zein were identified, as were problems that must be overcome before containers like those I used can be broadly recommended for horticultural applications.

**Thesis Organization**

This thesis contains two manuscripts. The first manuscript, chapter 2, has been published in the *Journal of Environmental Horticulture* and is formatted for that journal. It provides information about the biodegradability of zein-based containers under conditions typical of settings where plants are produced. The second manuscript, chapter 3, is formatted for submission to the *Journal of Environmental Horticulture*. It provides information about effects of biodegrading zein containers on substrate and plant growth. Chapter 4 provides general conclusions of the research. Following the conclusions are two appendixes with information relevant to the topic not presented in the manuscripts.

**Literature Review**

In the greenhouse and nursery industry, the use of containers for plant production has been extensive for over 50 years. It is estimated that during 2007, nearly 750 million potted floricultural plants were sold (U.S. Dept. of Agriculture, 2008). The total number of nursery crops sold during 2006 was nearly 510 million, which includes bare root, balled and burlaped, and containerized plants (U.S. Dept. of Agriculture, 2007). The vast majority of containers are made from petroleum-based plastics, and over 250 million kg of plastic are
used to manufacture them (Amidon Recycling, 1994; Lawrence, 2005). High-density polyethylene, polypropylene, and polystyrene are the main resins used for plastic containers because of their light weight, high strength, ease of shipping, and high moisture tolerance (Amidon Recycling, 1994).

The environmental impact of producing petroleum-based plastic is large, and there is great interest in plastics derived from agricultural feedstocks (bioplastics) as sustainable alternatives (Gross and Kalra, 2002; Mohanty et al., 2002). Petroleum-based plastics represent a large solid waste problem. Plastics account for 28 million metric tons (31 million tons) or 12% of all municipal solid waste generated in the United States (U.S. Environmental Protection Agency, 2008). Nationally, landfill space is becoming limited and there is a strong need to reduce the amount of plastic waste generated. Bioplastics degrade by the action of naturally occurring microorganisms such as bacteria, fungi, and algae, and therefore, bioplastics could be composted rather than deposited in landfills. Although conventional plastic containers can be recycled, this practice is uncommon because of a lack of infrastructure to collect and recycle containers. Other limiting factors include the low-quality resins used for containers, degradation from ultraviolet radiation, fears of contamination from herbicides and pesticides, and spread of pathogens (Amidon Recycling, 1994). Municipal recycling programs, nurseries, garden centers, and other organizations are beginning to accept containers for recycling, but the percentage of plastics recovered remains low (Missouri Botanical Garden, 2009).

Many sources claim that manufacturing of bioplastics requires less fossil fuel and contributes less to global warming when compared with production of petroleum-based plastics. A life cycle assessment (LCA) examines and quantifies environmental impacts of
products or processes. Polylactic acid (PLA), poly-\(\beta\)-hydroxybutyric acid (PHB), and polyhydroxyalkanoate (PHA) are three main bioplastics produced from agricultural by-products, primarily from corn and soybean \([\textit{Glycine max} \text{ (L.) Merr.}]\). LCAs of PLA, PHB, and PHA indicate a reduction in energy use (fossil fuels) for manufacturing, fewer greenhouse gas emissions, and less eutrophication (Harding et al., 2007; Vink et al., 2003; Yu and Chen, 2008). More comprehensive studies, which closely examine factors such as fertilizer, pesticide, and limestone use during production of corn and soybean, indicate less of an advantage of bioplastics over petroleum-based plastics (Landis et al., 2007). Conflicting conclusions and the lack of LCAs for many bioplastic materials prevents us from understanding the true environmental impact of bioplastics. However, it is important to continue research and development of other alternatives that may prove to be environmentally sustainable.

\textit{Nonplastic Alternatives to Petroleum-based Containers}

In horticulture, alternatives to petroleum-based products have been developed, such as paper and peat containers. These containers are considered more environmentally “green” because they are made from compressed paper and peat-moss and can be composted rather than deposited in landfills. These containers have an advantage over plastic containers because they allow air to move through the container walls, allowing evaporative cooling and fewer problems related to high root-zone temperature. Several disadvantages have led to limited use of peat and paper pots. The cost remains comparatively high compared with plastic containers of the same size. Longevity of paper and peat pots is limited because of a loss in structural integrity when subjected to high moisture, freezing and thawing, and high
temperature (Beattie and Berghage, 1998). The bottoms of paper pots will often break loose when lifted off the ground. Peat and paper container manufacturers market the containers to be directly planted into the ground where the roots of plants will migrate through the container wall; however, root establishment can be impeded by the container, so most gardeners remove the container before planting (Lahde and Kinnunen, 1974). Regardless of these problems, herbs and vegetables are typically grown in peat containers, and some nursery stock is grown in paper containers.

Coconut coir containers are made from the outer husk of a coconut (Cocos nucifera L.). These containers reportedly lead to better growth than peat containers, maintain moisture in the substrate and can be transplanted into the ground. These containers are new on the market, and there are few records of their performance.

Containers made from processed poultry feathers have been investigated but are not commercially available. In greenhouse studies, plants performed as well in feather containers as peat and plastic containers and could be directly planted into the ground without impeding growth (Evans and Hensley, 2004).

Bioplastic Containers

Although bioplastics are used for many single-use items such as disposable tableware, food packaging, shopping and trash bags, and agricultural films, there have been few efforts to examine the many different types of bioplastics for horticultural containers. Several companies produce and sell biocomposite plastics containers. A biocomposite is a product consisting of a binding material, which may or not be petroleum-based, and a particulate or fibrous material (John and Thomas, 2008). Many of the biocomposite containers on the
market utilize rice hulls as the particulate fill of the composite; however, the binding materials used are not disclosed. The biodegradability of these biocomposites is in question because they may contain significant quantities of nondegradable petroleum-based polymers. Plant growth in these containers has not been well documented in the scientific literature or by commercial growers. PLA, PHB, and PHA are bioplastics derived from the fermentation of plant starches and sugar. These bioplastic materials are beginning to be produced commercially on a large scale and may prove useful for manufacturing horticultural containers. High cost and uncertain performance probably have limited their development. There has also been considerable interest in plant proteins, such as those from soybean and corn, for bioplastics that could be formed into containers for plant production.

Zein-based Bioplastic Containers

Zein is a protein from corn and is a byproduct of the corn wet-milling process and corn gluten (Lawton, 2002). Historically, zein has been used in many industries, including the manufacture of fibers, adhesives, coatings, binders, and plastics. Zein was produced on a large scale until the mid-20th century. Because zein was not cost-competitive with petroleum-based material developed at that time, interest in zein diminished (Lawton, 2002). The only current commercial use of zein is for coatings on pills and food products, such as fruits and vegetables. There is renewed interest in zein because of concerns over the continued use of petroleum-based products and their impact on climate change and problems associated with waste disposal. Zein is being explored for applications such as lubrication sticks, temporary protective coatings, nonfood packaging, identification cards, chewing gum, coatings for hay bales, and biodegradable plastics.
Several properties of zein make it promising as a bioplastic for horticultural uses. Zein is renewable and 100% biodegradable. Zein is hydrophobic, which makes it relatively resistant to water and high humidity, conditions typical of a greenhouse or nursery setting. Comparatively, protein from soybean is hydrophilic and quickly loses structural integrity when subject to moisture. Furthermore, zein contains $\approx 15\%$ nitrogen (Cohn et al., 1924). If containers are made from zein, nitrogen may be available for plant uptake as degradation of the container progresses. Use of zein is limited by its high cost, which in 2009, is as much as $10$ to $40$/kg. Alternative extraction methods and new markets promise to decrease the cost considerably, however (Dickey et al., 1999; Lawton, 2002; Shukla and Cheryan, 2001). Most feedstocks for petroleum-based plastics sell for $0.30$-$0.90$/kg. The research herein focuses on the use of zein as the main constituent of bioplastic containers.
Literature Cited


CHAPTER 2. DEGRADATION AND NITROGEN RELEASE OF ZEIN-BASED BIOPLASTIC CONTAINERS

A paper accepted for publication in the Journal of Environmental Horticulture

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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 < 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 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.

**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 21°C (70°F). 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 (3.5 in) cm, 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 µmol·m⁻²·s⁻¹. Day/night air temperatures were 26 ± 2/15 ± 2°C (79 ± 4/59 ± 4°F), 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 21°C (70°F) 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$^3$/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) \times two (sterile vs. nonsterile) \times 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.

**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 upon 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 bioplastic 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 affect 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 seemed 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 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·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·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, whereas 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 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 with 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.
**Literature Cited**


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 replications per treatment. Containers were arranged in a completely randomized design in a growth chamber.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight loss (% of initial wt.)&lt;sup&gt;z&lt;/sup&gt;</th>
<th>Total N (mg·kg&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;z&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Container type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiber</td>
<td>_ _ _</td>
<td>3.2 c&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peat</td>
<td>_ _ _</td>
<td>8.1 c</td>
</tr>
<tr>
<td>Thin bioplastic</td>
<td>33.7 a&lt;sup&gt;y&lt;/sup&gt;</td>
<td>82.6 b</td>
</tr>
<tr>
<td>Thick bioplastic</td>
<td>20.9 b</td>
<td>120.5 a</td>
</tr>
<tr>
<td>Substrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perlite</td>
<td>18.8 b</td>
<td>29.7 b</td>
</tr>
<tr>
<td>Fafard®</td>
<td>35.8 a</td>
<td>165.8 a</td>
</tr>
<tr>
<td>Irrigation frequency (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23.3 b</td>
<td>85.7 b</td>
</tr>
<tr>
<td>4</td>
<td>31.3 a</td>
<td>114.5 a</td>
</tr>
</tbody>
</table>

<sup>z</sup>Weight loss means calculated only from bioplastic containers, and total N means for substrate and irrigation frequency calculated only with data for bioplastic containers.

<sup>y</sup>Mean separation within each column by treatment category (container type, substrate, or irrigation frequency) at P ≤ 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).
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 were either sterilized or nonsterilized. Treatments were arranged in a complete factorial combination resulting in a total of eight treatments with three replications per treatment. Containers were arranged in a completely randomized design on a greenhouse bench (n = 12).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight loss (% of initial wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Container type</strong></td>
<td></td>
</tr>
<tr>
<td>Thin bioplastic</td>
<td>58.0 a</td>
</tr>
<tr>
<td>Thick bioplastic</td>
<td>48.7 b</td>
</tr>
<tr>
<td><strong>Moisture</strong></td>
<td></td>
</tr>
<tr>
<td>Drained</td>
<td>68.0 a</td>
</tr>
<tr>
<td>Saturated</td>
<td>38.7 b</td>
</tr>
<tr>
<td><strong>Sterilization</strong></td>
<td></td>
</tr>
<tr>
<td>Nonsterilized</td>
<td>55.9 a</td>
</tr>
<tr>
<td>Sterilized</td>
<td>50.7 a</td>
</tr>
</tbody>
</table>

*Mean separation within each column by treatment category (container type, moisture, or sterilization) at P ≤ 0.05 by Fisher’s least significant difference.*
Figure Captions

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.

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.
(Fig. 1)
(Fig. 2)
CHAPTER 3. ZEIN-BASED BIOPLASTIC CONTAINERS ALTER ROOT-ZONE CHEMISTRY AND GROWTH OF GERANIUM

A paper to be submitted to the *Journal of Environmental Horticulture*

Matthew S. Helgeson¹, William R. Graves², David Grewell³, and Gowrishankar Srinivasan⁴

Abstract

Bioplastic containers made from the corn (*Zea mays* L.) protein zein may offer alternatives to conventional, petroleum-based plastics. We tested the hypothesis that biodegradation of zein-based containers provides nitrogen (N) that promotes growth of geranium (*Pelargonium ×hortorum* L.H. Bail.) and to determine if geraniums grown in zein containers could be transplanted and reestablished successfully with the container intact, thus eliminating the need for disposal of containers. Zein containers provided root zones up to 298 and 277 mg·kg⁻¹ NH₄⁺-N and NO₃⁻-N, respectively, and unlike plants in conventional plastic containers, leaves of geraniums in zein containers remained dark green when produced without fertilization. Electrical conductivity (EC) and pH of the substrate in zein containers increased above ranges recommended for many horticultural crops, and NO₂⁻, which can be

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toxic to plants, was present in the substrate of zein containers. These chemical changes may be responsible for reduced canopy height and width, leaf area, root system length, and shoot dry weights of geraniums in zein containers compared with geraniums in conventional plastic containers. In a second experiment, when transplanted with zein containers intact, root and shoot growth of geraniums were reduced until after six weeks, when biodegradation of containers was nearly complete. Migration of roots through the zone of the degraded container and into surrounding substrate was documented approximately three months after transplanting. Our data show that geraniums can be produced and transplanted in zein-based containers, but additional research is needed to solve problems that can result from chemical changes to the root zone during production, and from chemical and physical effects on reestablishment after transplant.

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

**Significance to the Nursery Industry**

Bioplastic containers made from zein, a protein from corn, biodegrade and therefore might be installed with transplants or composted rather than discarded in landfills. Through this research, which was the first test of producing plants in zein-based containers, we documented influxes of various forms of N, and increases in EC and pH in the substrate. These chemical changes seem to inhibit geranium growth. When geraniums are transplanted with zein containers intact, establishment of roots into the surrounding substrate is delayed until degradation of the container is nearly complete. If zein-based bioplastics are to be used to produce horticultural crops, additional research will be needed to identify ways to control
the release of N from containers and the changes in substrate EC and pH. Researchers also should define the roles of substrate chemistry and physical inhibition in the posttransplant delay in establishment we documented. Subsequently, strategies can be identified to overcome the problem and allow rapid establishment of transplants in the zein-based containers in which they were produced.

Introduction

Bioplastic containers manufactured from renewable materials may offer alternatives to horticultural containers made from conventional plastics. Conventional containers are manufactured from petroleum-based polypropylene and polyethylene, which are not renewable and typically discarded in landfills after a single use, contributing to restricted space for solid wastes (13). Nationally, no infrastructure exists to collect and recycle containers used in nurseries and greenhouses, although some municipalities are beginning to accept containers for recycling (19). Bioplastics manufactured from agricultural materials such as corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) are biodegradable and can be composted rather than deposited in landfills (3, 4, 28). Biocomposites, which are composed of matrices of various natural polymers and reinforcement materials such as cellulosic fibers from wood, coir, rice and soybean hulls, and corn stover, are also biodegradable (14). Many of these materials are underutilized byproducts of ethanol and biodiesel production, and utilizing these materials for bioplastics and biocomposites would increase their value, enhance profitability for producers, and expand local economies. Replacing petroleum-based plastics with bioplastics may reduce our nation’s dependence on diminishing fossil fuels and reduce emissions of greenhouse gases (7, 32).
Bioplastic containers are not common in horticulture, due in part to high cost and uncertain performance. Consumer awareness of the benefits of bioplastics has grown, and there is increasing demand for container manufacturers to produce economically viable, biorenewable products so growers can offer biodegradable containers to consumers (10). Alternatives to petroleum-based plastics, such as containers made from peat, paper, and coir have been developed, but there are several disadvantages to these containers. Peat containers are more expensive than plastic containers, they lose structural integrity when moist, and they wick water from the substrate, leading to the need for growers to increase the frequency and quantity of irrigation. Because substrates dry quickly in peat containers, roots often are damaged. Paper containers biodegrade slowly when transplanted into the landscape, which often leads to them being removed before transplant and needing to be disposed. These limitations explain the limited use of peat and paper containers in the nursery and greenhouse industries. Research and development of alternatives may facilitate widespread use of bioplastic containers in the horticulture trade.

Zein, a protein from corn, can be processed into bioplastic containers. Zein has many industrial uses, is a byproduct of ethanol production, and is extracted from corn gluten meal (26). The hydrophobic properties of zein, compared to properties of other proteins like those from soybean, and its biodegradability, suggest that zein may be suited for horticultural containers. Although zein is currently expensive when compared with feedstocks for conventional plastics, new markets and new extraction methods promise to lower its price (26).

Helgeson et al. (9) reported that containers made from zein are biodegradable and have longevity suitable for crops with short production schedules, including annuals,
vegetables, herbs, and some herbaceous perennial species. As microorganisms degrade containers made from zein protein, inorganic nitrogen (N) that may be available to plants is generated (9). Breakdown of protein into plant-available \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) starts with mineralization. Enzymes produced by microorganisms break down organic N (proteins) into \( \text{NH}_4^+ \). \( \text{NO}_3^- \) is subsequently produced by the process of nitrification, which is the conversion of \( \text{NH}_4^+ \) to \( \text{NO}_2^- \) by the bacteria \textit{Nitrosomonas}, quickly followed by the conversion of \( \text{NO}_2^- \) to \( \text{NO}_3^- \) by the bacteria \textit{Nitrobacter} (29). Containers that provide plant-available N may allow growers to reduce inputs of N fertilizer.

Our objectives were to test the hypothesis that the biodegradation of zein-based containers provides suitable forms and quantities of N to support growth of geranium (\textit{Pelargonium \times hortorum}), which we selected as a model crop. We also tested the hypothesis that a geranium grown in a zein container could be transplanted with the container intact without adversely affecting reestablishment of the transplant. We speculated that roots would migrate through the degrading container and establish into the surrounding substrate, thus eliminating the need for disposal of containers.

Materials and Methods

\textit{Container preparation.} Zein protein (Global Protein Products, Marina, CA), the plasticizer glycerol, and the solvent 90% ethanol (Fisher Scientific, Fair Lawn, NJ) were blended in a ratio of 20:4:1 (by weight). The formulation was extruded with a PL 2000 series single-screw extruder (76 cm length and 3.18 cm diameter; C.W. Brabender Instruments, Inc., South Hackensack, NJ). Barrel temperature varied linearly from 70\( ^\circ \text{C} \) (158\( ^\circ \text{F} \)) in the feed zone to 105\( ^\circ \text{C} \) (221\( ^\circ \text{F} \)) in the die. The extrudate was pelletized for compression molding (C.W.
Brabender Instruments, Inc.), which was carried out with an aluminum container mold and 136 t Wabash Press (Wabash MPI, Wabash, IN). Molding was completed at 105°C (221°F) with a force of 13.6-t for 5 min. Height of the containers was 88 mm (3.5 in), and bottom and top diameters were 75 mm (3.0 in) and 105 mm (4.1 in), respectively. Molded bioplastic containers had a sidewall thickness and bottom thickness of 1.7 mm (0.07 in) and 1.9 mm (0.09 in), respectively. Four 9-mm-diameter drainage holes were drilled in the bottom of each container.

Experiment 1. Our goal was to model the release of plant-available N from zein containers during seven weeks of growth of geraniums. The factorial treatment design included two container types (zein bioplastic or conventional plastic) and two types of fertilizer [Hoagland solution no. 1 with nitrogen (+N; 210 mg NO₃⁻·N·kg⁻¹) or without nitrogen (-N)] (11), for a total of four treatment combinations (Zein +N, Zein –N, Plastic +N, Plastic –N), each of which was replicated 10 times. The 40 containers were arranged in a completely randomized design in a glass-glazed greenhouse with 16-h photoperiods provided by high-pressure sodium lamps. Mean daily maximum photosynthetically active radiation was 631 ± 31 µmol·m⁻²·s⁻¹. Air temperature ranged from 21 to 32°C (70 to 89°F), with a mean of 24 ± 1.2 (75°F ± 2.2°F).

Conventional plastic containers (Kord Products, Brampton, ON, Canada) that had a height of 8.6 cm (3.4 in) and bottom and top diameter of 6.8 cm (2.6 in) and 10 cm (4.0 in), respectively, were compared to zein containers. Each container was filled with a soilless, peat moss-based substrate (Sun Gro® Sunshine® LC1 mix, Sun Gro Horticulture Distribution Inc., Bellevue, WA). One rooted cutting of *P. ×hortorum* ‘Rocky Mountain
Salmon Rose’ was planted in each container. Each container/plant constituted an experimental unit.

To establish plants, 200 ml of 50% Hoagland solution with N was applied to all experimental units in all treatment combinations for the first two weeks. For the remaining five weeks, one-half of bioplastic containers and conventional containers (Zein +N, Plastic +N) received 100% Hoagland solution with N, and the remaining one-half (Zein –N, Plastic –N) received 100% Hoagland solution without N. All containers were irrigated to container capacity with 200 mL twice weekly. After each irrigation, leachate was collected from five randomly selected containers from each treatment combination, using the Pour-Thru method (2). An HI 9811 meter (Hanna Instruments, Woonsocket, RI) was used to determine EC and pH. Lachat flow injection analysis (Lachat Instruments, Milwaukee, WI) was used to analyze leachate for NO$_3^-$-N, NH$_4^+$-N. NO$_2^-$-N also was quantified during the final 17 d.

Plants were disbudded until three weeks before harvest.

Plants were harvested after 47 d. Canopy height and width were measured. Relative greenness of the three youngest fully expanded leaves on each plant was determined by using a SPAD-502 Chlorophyll Meter (Minolta Camera Co., LTD., Tokyo, Japan). Surface area of the same three leaves was measured with a LI-COR 3100 leaf area meter (LI-COR, Lincoln, NE). Leaves from three experimental units of each treatment combination were combined for analysis of NH$_4^+$-N and NO$_3^-$-N by using Lachat flow injection analysis. The shoot of each plant was harvested by severing the primary stem just above where the callus had formed on the cutting during propagation. Substrate was washed from roots, and root-system development was quantified by measuring its length as the distance from the origin of roots on the stem cutting to the tip of the most distal root as root systems were suspended in the
atmosphere. Weights of roots and shoots were recorded after drying them at 67°C (153°F) for
72 h.

Experiment 2. We tested the hypothesis that a plant grown in a zein container could
be transplanted with the container intact and reestablished successfully because roots would
migrate through the degrading container into surround substrate. Our protocol was intended
to simulate planting a bioplastic container into the landscape or into a larger container with
soilless substrate. We used zein and conventional containers like those described for
experiment 1. Plants also were grown in round Jiffy-Pots® (Jiffy Products of America Inc.,
Norwalk, OH) with a height of 9 cm (3.5 in) and bottom and top diameter of 6.5 cm (2.6 in)
and 10 cm (3.9 in), respectively. Twenty containers of each type were filled with the soilless
substrate used in experiment 1. A rooted cutting of *P. ×hortorum* ‘Rocky Mountain Salmon
Rose’ was planted in each container. Plants were irrigated with 100% Hoagland solution no.
1 twice weekly for seven weeks except for the first two weeks, when 50% solution was used.

All plants were transplanted into larger azalea containers (Kord Products) filled with
the soilless substrate 51 d after planting. The containers were made of conventional plastic
and were 15 cm (5.9 in) tall, with bottom and top diameters of 15 cm (5.9 in) and 20 cm (7.9
in), respectively. Ten of the plants grown in zein, conventional plastic, and peat containers
were removed from the production container before transplanting. The remaining 10 of each
container type were transplanted with the container intact, such that the top of the original
root ball was level with the substrate in the larger container. Ten experimental units per
treatment combination consisted of the geranium (production container either removed or
intact) transplanted into a larger azalea container (N = 60). Containers were arranged in a
completely randomized design in a glass-glazed greenhouse with 16-h photoperiods provided
by high-pressure sodium lamps. Mean daily maximum photosynthetically active radiation was $631 \pm 31 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Air temperature ranged from 21 to 32°C (70 to 89°F), with a mean of $24^\circ\text{C} \pm 1.2^\circ\text{C} (75^\circ\text{F} \pm 2.2^\circ\text{F})$. Each plant received 1 L of 50% Hoagland solution no. 1 every 3 to 7 d, when the surface of the medium appeared dry. EC, pH, NO$_3$-N, NH$_4$+-N, and NO$_2$-N of leachate were measured after each irrigation as during experiment 1, except that NO$_2$-N was measured throughout treatments. At 73 d after transplant, 2 L of distilled water was applied to each container to reduce EC of the substrate. Plants were disbudded until three weeks before harvest.

Five replications per treatment combination were harvested on each of two dates, 42 and 84 d after transplant. Height and width of plants, relative leaf greenness, and leaf area were determined as in experiment 1. The shoot of each plant was harvested by severing the primary stem just above where the callus had formed on the cutting during propagation. Roots that had migrated through the original container or beyond the original root ball were washed free of substrate and removed. Roots remaining within the volume occupied by the original production container were then washed free of substrate. Shoots and both sets of roots from each plant were dried at 67°C (153°F) for 72 h and weighed.

Data analysis. Data from both experiments were analyzed for main effects and interactions by using the general linear models (GLM) procedure of SAS/STAT®, version 9.1.3 (SAS Institute Inc., Cary, NC). Data were transformed when necessary to equalize variances. Treatment means for foliar N, relative greenness, height, width, leaf area, root length, and dry weights were separated using Tukey’s honestly significant difference (HSD)
test. Means for pH, EC, NO$_3^-$-N, NH$_4^+$-N, and NO$_2^-$-N at each date of leachate collection was separated using Tukey’s HSD test.

**Results and Discussion**

*Experiment 1.* Initial degradation of zein containers during production of geraniums led to various concentrations of the three forms of plant-available N we measured in the leachate. NH$_4^+$-N in leachate from zein containers increased during the first few weeks and peaked at 241 and 298 mg·kg$^{-1}$ 27 d after transplant for containers in the Zein +N and Zein – N treatments, respectively (Fig. 1A). In contrast, little NH$_4^+$-N was detected in leachate from plastic containers. Some NH$_4^+$-N detected soon after planting likely was from starter fertilizer in the substrate, which would have rapidly leached or been used by the plant. Because the fertilizer we applied contained no NH$_4^+$-N, the presence of NH$_4^+$-N in substrate of zein containers suggests that ammonification, the conversion of organic N in the zein protein to NH$_4^+$-N, occurred as these containers began to degrade. NO$_2^-$-N was in leachate from zein containers when we first tested for it at day 30, and concentrations were higher thereafter (Fig. 1B). NO$_2^-$-N was not in leachate from plastic containers. The presence of NO$_2^-$-N in zein containers suggests that oxidation of NH$_4^+$-N to NO$_2^-$-N occurred in the substrate. NO$_2^-$-N, generally considered toxic to plants, is scarce in soils and soilless substrate because the rate of oxidation of NO$_2^-$-N usually exceeds the rate of oxidation of NH$_4^+$-N (8). However, when applied to neutral or alkaline soils, NH$_4^+$-N may cause accumulation of NO$_2^-$-N by inhibiting *Nitrobacter*, which is sensitive to NH$_4^+$ and pH (21, 25). The NH$_4^+$-N in zein containers, along with elevated pH (Fig. 2A) may explain the accumulation of NO$_2^-$-N. NO$_3^-$-N began to increase in leachate from containers in the Zein –
N treatment by day 37 and rose to 277 mg·kg⁻¹ over time (Fig. 1C). Among all treatments, NO₃⁻-N detected before day 15 can be attributed to the application of Hoagland solution with NO₃⁻-N for plant establishment (Fig. 1C). NO₃⁻-N concentrations then declined in the substrate of containers in the Zein –N and Plastic –N treatments due to leaching (Fig. 1C). The resurgence of NO₃⁻-N in the substrate of containers in the Zein –N treatment suggests the oxidation of NO₂⁻-N to NO₃⁻-N occurred. High pH and concentrations of NH₄⁺-N may have inhibited *Nitrobacter* temporarily and thus may account for the lack of NO₃⁻-N until day 37 (21, 25). It is also possible that *Nitrobacter* populations needed to increase before considerable amounts of NO₂⁻ were oxidized.

The pH and EC of leachate from zein containers changed over time in ways not observed for leachate from plastic containers. pH of leachate from containers in the Zein +N and Zein –N treatments increased similarly for the first 30 d, approached eight, and then declined (Fig 2A). In contrast, pH of leachate from Plastic +N and Plastic –N remained within an acceptable range for geraniums, which has been defined as 6.0 to 6.6 (Fig. 2A) (31). The increase in pH of leachate from zein containers may be attributed to the ammonification of protein. The subsequent decrease in pH may be attributed to nitrification (23). EC of leachate from containers in the Zein +N and Zein –N treatments increased over time to 4.7 and 5.1 dS·m⁻¹, respectively (Fig 2B). In contrast, the EC of leachate from containers in the Plastic +N and Plastic –N treatments remained in the acceptable range for geranium of 2.0 to 3.5 dS·m⁻¹ (Fig. 2B) (31). Increased soluble salts of N in the substrate of zein containers probably contributed to increased EC of leachate.

Although N released from containers prevented chlorosis of geraniums in the Zein –N treatment, these and other chemical changes to the root zone reduced growth of roots and
shoots. Leaf greenness of geraniums grown in the Zein –N treatment was 30% greater than that of leaves in the Plastic –N treatment (Table 1). Greenness data from SPAD meters often are correlated with chlorophyll content and can indicate N deficiency (16). Leaves of geraniums in the Zein –N treatment showed elevated NO₃⁻-N and nearly seven times as much NH₄⁺-N as leaves of geraniums in the Plastic –N treatment (Table 1), effects that we attribute to the N released during initial degradation of zein containers. Our results are consistent with a previous study of containers made from a different high-protein material, processed poultry-fibers, which provided N to substrate as they biodegraded (5).

Compared with geraniums in the Plastic +N treatment, geraniums in the Zein +N treatment had reduced shoot height, and geraniums in the Zein +N and Zein –N treatments had reduced shoot width (Table 2). Surface area of selected leaves of geraniums in the Zein +N and Zein –N treatments was 55 and 66% less, respectively, than that of geraniums in the Plastic +N treatment (Table 2). Dry weight of shoots of geraniums grown in the Zein +N and Zein –N treatments was less than that of plants in the Plastic +N treatment (Table 2). Although mean root weight of geraniums grown in zein containers was not different than that of geraniums grown in the Plastic +N treatment, mean length of root systems of geraniums grown in the Plastic +N treatment was four times greater than that of geraniums grown in zein containers (Table 2). Roots in zein containers were similar to a root system subjected to extensive pruning. Minimal extension was apparent, tips were necrotic where they approached the inside container wall, and there was extensive branching. Growers may welcome the root-pruning effect of zein containers, which seems to reduce the potential for problematic circling roots (30). Subsequent research should examine whether this effect is taxon-specific, how differences in container size, and the ratio of substrate volume and area
of container sidewalls alter the effect, and whether chemical inhibitors of root growth in corn gluten meal (17, 18), elevated EC, or other chemical changes to the root zone are responsible.

Reduced growth of geraniums in zein containers may be due to the influx of various forms of N in the containers, the changes in root-zone EC and pH, or both (Figs. 1 and 2). Populations of organisms capable of nitrification may have been low when the release of \( \text{NH}_4^+ \)-N began, allowing an accumulation of \( \text{NH}_4^+ \)-N (20) that curtailed plant growth (1, 6). \( \text{NO}_2^- \)-N also inhibits the growth of plants (12, 22, 24). The sum of \( \text{NH}_4^+ \)-N and \( \text{NO}_3^- \)-N concentrations in leachate from zein containers frequently exceeded the 200 to 300 mg N·kg\(^{-1}\) applied to most greenhouse crops, and the \( \text{NH}_4^+ : \text{NO}_3^- \) ratio was unstable over time, so the zein containers we used pose challenges to the production of crops requiring specific forms and quantities of N (20). Furthermore, EC in the substrate of zein containers increased to values known to reduce growth, kill root tips, and promote formation of leaves that are small and unusually dark green (31, 15), which are symptoms we observed. Changes in substrate pH in zein containers also are potentially detrimental due to effects on nutrient availability (20, 27). This research, which is the first to examine plant growth in containers made from zein, has demonstrated dynamic and important effects of the containers on substrates and plants. Although strategies are needed to restrict the rate of release of N, the potential for modest N release and the highly branched root systems we observed are potentially beneficial for production of horticultural crops. The dynamic changes in the EC and pH of substrate we have documented, however, must be targeted as problems with zein containers to overcome through additional research and product development.
Experiment 2. Concentration of ${\text{NH}}_4^{+}$-N in leachate from experimental units with the zein container intact was greater 7 and 13 d after transplant than that of units with a plastic container intact (Fig. 3A). Leachate from experimental units with plastic containers removed consistently had $< 5$ mg ${\text{NH}}_4^{+}$-N · kg$^{-1}$ after transplant, whereas leachate from units with the zein container removed had 10 mg ${\text{NH}}_4^{+}$-N · kg$^{-1}$ 7 d after transplant (Fig. 3A). The rapid decline of ${\text{NH}}_4^{+}$-N concentrations in leachate from experimental units with the zein container intact suggests that bacteria capable of nitrification were established in the zein containers. Alternatively, ${\text{NH}}_4^{+}$-N concentrations may have been diluted by the volume of fertilizer solution applied after transplant, which was greater than that applied in experiment 1. In addition to high ${\text{NH}}_4^{+}$-N, NO$_2^-$-N was elevated at 13 d after transplant in leachate of experimental units with zein containers intact ($P < 0.0001$). Leachate from these units contained 5.3 mg NO$_2^-$-N · kg$^{-1}$, whereas $< 1$ mg·kg$^{-1}$ was present in leachate from other units, and all concentrations decreased to $< 1$ mg NO$_2^-$-N · kg$^{-1}$ thereafter. Concentrations of NO$_3^-$-N in leachate from experimental units with the zein container intact increased to 362 mg·kg$^{-1}$ by 25 d after transplant and declined to 153 mg·kg$^{-1}$ at the end of the experiment (Fig. 3B). EC increased and decreased similarly, suggesting NO$_3^-$-N was a primary determinant of EC (Fig. 4B). pH of leachate from experimental units with the zein container intact usually was lower than the pH of leachate from units in other treatments (Fig. 4A). This contrasts with the elevated pH of leachate documented from zein containers during experiment 1 (Fig. 2A). This difference may be explained by the ammonification of protein in experiment 1, followed by extensive oxidation of ${\text{NH}}_4^{+}$-N to NO$_3^-$-N in experiment 2.

Establishment of plants transplanted with zein containers intact was delayed for more than six weeks, when degradation of containers was nearly complete and roots began to
extend beyond the original root zone. At the harvest conducted 42 d after transplant, canopy dimensions, surface area of selected leaves, shoot dry weights, and dry weights of roots migrated beyond the original container of geraniums transplanted with zein containers intact were less than those of plants in the other treatments (Table 3). In contrast, transplant method (container intact vs. removed) led to no differences among plants grown in peat containers. Roots penetrated through the walls and drainage holes of peat containers and through the drainage holes of plastic containers. There was comparatively little growth of roots through the walls or drain holes of containers made from zein (Table 3). Collectively, these results suggest the walls of containers made from zein acted as a barrier to root penetration and suppressed plant growth. The failure of roots to move through even the drain holes of containers made from zein suggests chemicals inhibiting plant growth were present in substrate near the container walls. In contrast, extension of roots through the sidewall of containers made from poultry feathers was not impeded after transplanting under simulated field conditions, though the sidewall thickness of those containers was not specified (5).

At the harvest 84 d after transplant, geraniums transplanted with zein containers intact no longer had reduced canopy height and width, root and shoot dry weight, and leaf area when compared to geraniums transplanted with peat containers intact or with plastic containers removed (Table 3). Roots of plants transplanted with zein containers intact had migrated through fissures in sidewalls, which we estimated were > 50% disintegrated. Dry weight of roots that had extended beyond the production container was not different for plants produced in zein and peat containers (Table 3). The improved growth measures at 84 d compared to 42 d after transplanting geraniums with zein containers intact can be explained by physical and chemical changes of degrading containers. As degradation progressed, EC,
pH, and concentrations of NO$_3^-$-N and NH$_4^+$-N decreased to within ranges recommended for geranium (Fig. 3) (31). It is unclear why canopy height and width and shoot dry weights of plants transplanted with zein containers removed exceeded that of plants in other treatments by 84 d after transplant.

This is the first evaluation of plant growth in zein containers and the first examination of the possibility of transplanting plants with zein containers intact. Several challenges have been identified, including large influxes of substrate nitrogen levels, pH and EC, inhibition of roots, and reduced shoot growth. The cause of root inhibition should be identified before potential advantages such as N supply and transplantability are further explored. Moreover, adjustments to container sidewall thickness, use of larger containers with a greater substrate : zein ratio, and changes to container composition including the use of other materials, fillers, and coatings are all possible strategies to overcome root inhibition. These strategies may also help to provide a modest release of N and an appropriate rate of degradation for plant production and establishment into the landscape.
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    and pH of a peat-based substrate affect growth, nutrient uptake, and chlorosis of

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Table 1. Relative greenness (SPAD) and mean foliar NH$_4$$^+$-N and NO$_3$$^-$$^-$-N concentrations of leaves of geraniums after seven weeks of growth in two container types (zein and plastic) with two types of fertilizer [with nitrogen (+N) and without nitrogen (-N)]. Means of SPAD are from 10 replications, and means of N concentrations are from three replications for each combination of container type and fertilizer.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative greenness (SPAD)</th>
<th>Concentration (mg·kg$^{-1}$)</th>
<th>NH$_4$$^+$-N</th>
<th>NO$_3$$^-$$^-$-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zein +N</td>
<td>65.3 a</td>
<td>1374 b$^z$</td>
<td>760 a</td>
<td></td>
</tr>
<tr>
<td>Zein –N</td>
<td>62.0 ab</td>
<td>1704 a</td>
<td>476 b</td>
<td></td>
</tr>
<tr>
<td>Plastic +N</td>
<td>59.2 b</td>
<td>393 c</td>
<td>70 c</td>
<td></td>
</tr>
<tr>
<td>Plastic –N</td>
<td>43.6 c</td>
<td>253 d</td>
<td>0 d</td>
<td></td>
</tr>
</tbody>
</table>

$^z$Means within each column followed by the same letter are not different at $P \leq 0.05$ using Tukey’s HSD test.
Table 2. Mean shoot height, shoot width, leaf area, leaf greenness, root system length, root dry weight, and shoot dry weight of geranium after seven weeks of growth in two container types (zein and plastic) with two types of fertilizer [with nitrogen (+N) and without nitrogen (-N)]. Leaf area and root length are measures of the three youngest fully expanded leaves on each plant and the length from the origin of roots to the most distal root, respectively. Means are of 10 replications for each combination of container type and fertilizer.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Leaf area (cm²)</th>
<th>Root system length (cm)</th>
<th>Dry weight (g) Root</th>
<th>Dry weight (g) Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zein +N</td>
<td>7.9 b</td>
<td>18.3 b</td>
<td>38.3 b</td>
<td>5.0 b</td>
<td>0.69 b</td>
<td>3.21 b</td>
</tr>
<tr>
<td>Zein –N</td>
<td>9.5 ab</td>
<td>17.6 b</td>
<td>46.1 b</td>
<td>6.3 b</td>
<td>0.77 b</td>
<td>3.37 b</td>
</tr>
<tr>
<td>Plastic +N</td>
<td>10.1 a</td>
<td>20.9 a</td>
<td>69.9 a</td>
<td>21.0 a</td>
<td>0.92 b</td>
<td>5.37 a</td>
</tr>
<tr>
<td>Plastic –N</td>
<td>8.3 b</td>
<td>17.0 b</td>
<td>43.3 b</td>
<td>24.5 a</td>
<td>1.23 a</td>
<td>4.57 a</td>
</tr>
</tbody>
</table>

²Means within each column followed by the same letter are not different at $P \leq 0.05$ using Tukey’s HSD test.
Table 3. Shoot height, shoot width, inside root dry weight, outside root dry weight, shoot dry weight, leaf greenness (SPAD), and leaf area of geraniums grown in peat, plastic, and zein containers for 51 d and transplanted into larger azalea containers. One-half of the plants were removed from their original container (peat, plastic, zein) before transplanting, and the remaining one-half were transplanted with the containers intact (container removed or container intact). There were two harvests, 42 and 84 d after transplant. Inside root weight is the dry weight of the root system contained within the volume occupied by the original container. Outside root weight is dry weight of roots that had migrated through the original container or beyond the original root ball. Means are of five replications for each combination of pot type, transplant method, and harvest date.

<table>
<thead>
<tr>
<th></th>
<th>Height (cm) Days after planting</th>
<th>Width (cm) Days after planting</th>
<th>Root dry weight (g) Inside Days after planting</th>
<th>Outside Days after planting</th>
<th>Shoot dry weight (g) Days after planting</th>
<th>SPAD Days after planting</th>
<th>Leaf area (cm²) Days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>42</td>
<td>84</td>
<td>42</td>
<td>84</td>
<td>42</td>
<td>84</td>
<td>42</td>
</tr>
<tr>
<td>Peat container intact</td>
<td>15.5 b</td>
<td>21.7 ab</td>
<td>36.5 ab</td>
<td>41.9 b</td>
<td>1.09 a</td>
<td>1.4 ab</td>
<td>0.41 a</td>
</tr>
<tr>
<td>Peat container removed</td>
<td>16.8 ab</td>
<td>21.8 ab</td>
<td>36.5 ab</td>
<td>45.1 b</td>
<td>1.07 a</td>
<td>1.6 ab</td>
<td>0.40 a</td>
</tr>
<tr>
<td>Plastic container intact</td>
<td>16.5 ab</td>
<td>20.3 ab</td>
<td>33.3 b</td>
<td>43.2 b</td>
<td>1.34 a</td>
<td>1.9 ab</td>
<td>0.13 b</td>
</tr>
<tr>
<td>Plastic container removed</td>
<td>16.4 ab</td>
<td>22.4 ab</td>
<td>38.1 ab</td>
<td>45.1 b</td>
<td>1.31 a</td>
<td>1.5 ab</td>
<td>0.43 a</td>
</tr>
<tr>
<td>Zein container intact</td>
<td>10.2 c</td>
<td>19.4 b</td>
<td>24.9 c</td>
<td>39.6 b</td>
<td>0.90 a</td>
<td>1.3 b</td>
<td>0.01 c</td>
</tr>
<tr>
<td>Zein container removed</td>
<td>18.3 a</td>
<td>27.1 a</td>
<td>41.4 a</td>
<td>52.3 a</td>
<td>1.03 a</td>
<td>1.9 a</td>
<td>0.74 a</td>
</tr>
</tbody>
</table>

*Means within each column followed by the same letter are not different at P ≤ 0.05 using Tukey’s HSD test.

*Means are of 10 replications.
Figure Captions

Fig. 1. Change in substrate NH$_4^+$-N (A), NO$_2^-$-N (B), and NO$_3^-$-N (C) during growth of geranium for seven weeks. Treatments included two container types (zein and plastic) and two types of fertilizer [with nitrogen (+N) and without nitrogen (-N)]. Means within each date followed by the same letter are not different at $P \leq 0.05$ using Tukey’s HSD test (n = 5). An asterisk following a letter indicates unmarked means within that date share the same letter.

Fig. 2. Change in substrate pH (A) and EC (B) during growth of geranium for seven weeks. Treatments included two container types (zein and plastic) and two types of fertilizer [with nitrogen (+N) and without nitrogen (-N)]. Means within each date followed by the same letter are not different at $P \leq 0.05$ using Tukey’s HSD test (n = 5). An asterisk following a letter indicates unmarked means within that date share the same letter.

Fig. 3. Change in substrate NH$_4^+$-N (A) and NO$_3^-$-N (B) of azalea containers into which geraniums, previously grown for 51 d, were transplanted. One-half of the plants were removed from the original containers (peat, plastic, or zein) before transplanting and the remaining one-half were transplanted with the containers intact (container removed or container intact). Means for peat containers were similar to plastic containers and were not presented to ease interpretation. Means within each date followed by the same letter are not different at $P \leq 0.05$ using Tukey’s HSD test (n = 5). An asterisk following a letter indicates unmarked means within that date share the same letter. Letters are not provided for graph A after 13 d after transplant because the values are low, and the differences are not meaningful.
Fig. 4. Change in substrate pH (A) and EC (B) of azalea containers into which geraniums, previously grown for 51 d, were transplanted. One-half of the plants were removed from the original containers (peat, plastic, zein) before transplanting and the remaining one-half were transplanted with the containers intact (container removed or container intact). Means for peat containers were similar to plastic containers and were not presented to ease interpretation. Means within each date followed by the same letter are not different at $P \leq 0.05$ using Tukey’s HSD test ($n = 5$). An asterisk following a letter indicates that unmarked means within that date share the same letter.
(Fig. 1)
(Fig. 2)
(Fig. 3)
(Fig. 4)
CHAPTER 4. GENERAL CONCLUSIONS

General Discussion

Bioplastics hold potential to have many advantages over conventional petroleum-based plastics for the greenhouse and nursery industries. First, the reduction or elimination of use of petroleum-based plastics in horticulture is one step toward reducing our nation’s demand for petroleum. Biorenewable materials, especially byproducts of other goods and processes, may be sustainable feedstocks for plastic. The commercial production of petroleum-based plastics has large environmental impacts, such as high energy use and greenhouse-gas emission. Bioplastic may be a more sustainable, environmentally friendly alternative. Additionally, bioplastic containers can be composted rather than add to the nationwide shortage of landfill space.

My research has been the first attempt to evaluate zein-based bioplastics for horticulture. Although several questions have been answered, many new questions have evolved, and several challenges have been identified that must be addressed. We have determined that zein-based containers are biodegradable, and that degradation begins as soon as containers are filled with a moist substrate. Under the conditions we used, containers begin to lose structural integrity after about three months, so they should be restricted to crops with short production cycles such as annuals, vegetables, and some perennials. Microorganisms that degrade zein require a nutrient-rich substrate, such as a peat-based substrate, and aerobic conditions.

Degradation of zein-based containers provides various forms of plant-available nitrogen (N) to the substrate. Although the nitrogen provided by containers prevented chlorosis of geranium, chemical changes to the substrate reduced plant growth. Degradation
of zein seems to have led to adverse concentrations of $\text{NH}_4^+$ and $\text{NO}_2^-$, and to elevated electrical conductivity (EC) and pH, which were documented in the substrate of zein containers and may have inhibited growth of roots and shoots. Some essential micronutrients become unavailable at elevated pH, and root damage, stunting, and death can result from elevated EC.

When transplanted with zein containers intact, root and shoot growth of geraniums were reduced until after six weeks, when biodegradation of containers was nearly complete. Migration of roots through the zone of the degraded container and into surrounding substrate was documented approximately three months after transplanting. These results suggest that the containers, as we made and used them, are not suitable for directly planting into the ground along with a plant. Although containers eventually degrade and roots eventually establish into the surrounding soil, the initial reduction of growth after transplant is unacceptable. For installing or transplanting plants with zein containers intact to succeed, chemical changes to the substrate must not cause root inhibition, and containers must degrade quickly after transplant to allow roots to migrate through the remnants of the container and establish into the surrounding root zone.

**Recommendation for Future Research**

More effort must be focused on container performance. Zein has many advantages as a bioplastic for plant containers, such as biodegradability, N content, and water resistance, yet there are many shortcomings of the containers I produced. Containers must be improved structurally to have the durability of conventional plastic containers. They must not be brittle or crack easily. Containers must have increased longevity to be suitable for crops with
various production cycles. Changes to the root zone as containers degrade must be minimal or be harmless to plants, and N concentrations, pH, and EC must be maintained within acceptable ranges for the duration of crop production and beyond.

There are several possibilities for improved container performance. Container size may be affecting changes to the root zone. It is likely that changes to the substrate would be less severe in a larger container with a greater substrate : zein ratio. Future research should compare the chemical changes to the substrate with various container sizes. Slowing the degradation of zein containers is another avenue to prevent drastic changes to the substrate. Applying a coating on inner container walls may be one strategy for reducing the rate of container degradation. If containers could be coated with a material that degrades slowly, the degradation of zein might be delayed, lessening impacts on the substrate. Ideally, the coating would exert its effects temporarily and break down quickly after crop production.

Reducing the thickness of sidewalls of zein containers would decrease the total amount of zein present in a container, which could reduce the chemical changes to the substrate. However, reducing sidewall thickness may compromise container longevity, so changes to the chemical and physical composition of the polymers and container should also be examined as methods to decrease the rate of degradation.

Physical changes to the container composition could include the use of fillers to create a composite material. Dry distillers grain (DDG’s), spent germ, lignin, and cellulosic fibers, such as corn stover, soybean and rice hulls, coir, and wood fiber are among the numerous possibilities to incorporate into zein containers to reduce the overall content of zein and increase structural properties. All these materials are underutilized byproducts of other processes, so their use might partially offset the high price of zein protein.
Directly transplanting containers in soil along with a plant is a promising method for container disposal. Although chemical root inhibition is a major challenge to overcome, another challenge is to design containers that physically break down quickly once planted, ideally in one to two weeks, allowing roots to establish into the surrounding soil. Containers begin to break down during crop production, and because crops have different production times and are in retail markets for different periods of time, a rate of degradation suitable for all horticultural crops in containers will be a great challenge. Containers cannot degrade too extensively and fail structurally before they are sold to consumers, but they cannot require several months to degrade in the soil, preventing roots from establishing. Various container sidewall thicknesses, time-released coatings, and other chemistries should be examined for this practice to be practical.

Although the root-pruning effect of zein-based containers can be detrimental, this effect also may be advantageous because it prevents circling or girdling roots, which are problems associated with containerized nursery stock. It is likely that the root-pruning effect I observed was severe because of the small size of the container and the low ratio of substrate to zein. Further research should examine the root-pruning effect in a larger container and determine if root pruning is limited to the areas in close proximity to the container wall and if plants can be produced successfully without adverse effects on plant growth. The root-pruning seemed similar to air root-pruning and could replace and eliminate the use of copper-based root-pruning chemicals used as coatings on plastic and fiber containers to prevent circling roots.
APPENDIX A. GROWTH OF SALVIA IN ZEIN CONTAINERS

Shoot and root growth of salvia (*Salvia splendens* Sellow ex Roem. & Schult) grown in zein containers was compared to shoot and root growth of salvia grown in plastic containers. Seedlings were transplanted into zein containers, which were manufactured as described in chapter 3. Plastic containers were used for comparison. One-half of plants of each container type received Hoagland solution no. 1 with nitrogen (+N) and the remaining one-half of plants received Hoagland solution no. 1 without nitrogen (-N). Hoagland solution with N supplied 210 mg NO₃⁻·kg⁻¹. Plants received 200 ml of fertilizer solution once weekly and were watered as needed between fertilizer applications. Plants were placed in the greenhouse and grew for eight weeks. Substrate leachate was collected from containers after each fertilizer application and measured for pH and EC. Leachate also was analyzed for NO₃⁻, NO₂⁻, and NH₄⁺. Plants were harvested after eight weeks. Canopy height and width were measured. Relative leaf greenness was measured on the three most recent fully expanded leaves on each plant by using a SPAD-502 Chlorophyll Meter (Minolta Camera Co., LTD., Tokyo, Japan). Total leaf area was measured using a LI-COR 3100 leaf area meter (LI-COR, Lincoln, NE). Substrate was washed from roots, and dry weights of roots and shoots were recorded after drying them at 67C (153F) for 72 h.

**Results and Discussion**

Dry weights of roots of plants grown in zein containers were not different than those of plants in plastic containers. However, root structure in zein containers differed from structure of roots in plastic containers (Table 1). Roots of plants grown in zein containers were short and heavily branched. Roots of plants grown in plastic containers were longer.
and less branched, and more typical of roots of potted plants. These observations are similar to those made of the roots of geranium grown in zein containers as discussed in chapter 3. Substrate EC, pH, and nitrogen (N) concentrations were similar to the results found in chapter 3, so the data are not presented. High substrate EC, pH, and N may explain the rooting response of plants grown in zein containers.

Even though root structure of saliva grown in zein containers was different than root structure of the control, shoot growth was unaffected. Plants grown in Zein +N and Zein -N treatments had equal or better growth than plants in the control treatment (Plastic +N) (Table 1). Plants grew poorly in Plastic –N probably because they did not receive any N in the fertilizer. The increased growth of plants grown in Zein-N compared with plants grown in Plastic –N can be attributed to the nitrogen released from zein containers during container degradation. There was a lack of chlorosis (as measured by SPAD) of leaves of plants in the Zein-N treatment compared with plants in the Plastic –N treatment, which also can be attributed the nitrogen released from the zein containers. Plant width, leaf area, and shoot dry weight were greatest for Zein +N. We suspect the additional N provided from the degradation of containers led to the greatest growth of plants in the Zein +N treatment.

These results for shoot growth are different than what was observed among geraniums grown in zein containers as discussed in chapter 3. Geraniums had reduced shoot growth when grown in zein containers compared with growth in plastic containers. We suspect geraniums may be more susceptible than salvia to high substrate EC and high concentrations of NO₃⁻-N, NO₂⁻-N, and NH₄⁺-N.

Although plants grown in zein containers sequester N released from containers during degradation, the N may be in excess, causing root tips to become necrotic. Shoot growth of
some crops appears to be more affected by the change in root zone chemistry of zein containers.
Table 1. Mean height, width, total leaf area, root dry weight, and shoot dry weight of saliva after eight weeks of growth in two container types (zein and plastic) with two fertilizer regimes [with nitrogen (+N) and without nitrogen (-N)]. Means are of 10 replications for each combination of container type and fertilizer.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Total leaf area (cm²)</th>
<th>Dry weight (g)</th>
<th>Roots</th>
<th>Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zein +N</td>
<td>17 a²</td>
<td>30.6 a</td>
<td>1519 a</td>
<td>2.9 a</td>
<td>9.5 a</td>
<td></td>
</tr>
<tr>
<td>Zein –N</td>
<td>14.2 b</td>
<td>25.2 b</td>
<td>762 b</td>
<td>2.3 a</td>
<td>4.5 c</td>
<td></td>
</tr>
<tr>
<td>Plastic +N</td>
<td>15.3 ab</td>
<td>24.0 b</td>
<td>860 b</td>
<td>2.6 a</td>
<td>6.3 b</td>
<td></td>
</tr>
<tr>
<td>Plastic –N</td>
<td>6.4 c</td>
<td>6.9c</td>
<td>46 c</td>
<td>0.6 b</td>
<td>.3 d</td>
<td></td>
</tr>
</tbody>
</table>

²Means within each column followed by the same letter are not different at $P \leq 0.05$ using Fisher’s LSD test.
APPENDIX B. SUPPLEMENTARY FIGURES

Fig. 1. The appearance of zein-based containers as they were manufactured for experiments in chapter 3.
Fig. 2. The root-pruning effect caused by zein-based containers. Roots of *Pelargonium × hortorum* were necrotic where they approached the sidewalls. Death of root tips seemed to cause increased root branching (A). In another experiment, a conventional plastic container was cut in half vertically and placed inside a zein-based container. *Dendranthemum × grandifolium* (synonym *Chrysanthemum × morifolium*) was grown in this container for eight weeks. Roots that approached the sidewall of the container that was not blocked by conventional plastic (left side of root ball) were few and necrotic, whereas roots that approached the sidewall where conventional plastic lined the inside wall (right side of root ball) were numerous and appeared healthy (B).
Fig. 3. *Dendranthemum ×grandifolium* grown in zein containers developed symptoms typical of iron and manganese deficiency after five weeks. Increased substrate pH, which was present in substrate of zein containers, may be the main cause for iron and manganese unavailability to plants.
ACKNOWLEDGMENTS

I would like to thank the Department of Horticulture at Iowa State University for providing financial support for my graduate work. I would like to thank Dr. William Graves, my major professor, for your advice and guidance throughout the years. Without your inspiration as an undergraduate to continue my education I would not be where I am today. Because of you I will never forget how to indentify slender deutzia, how to pronounce Pseudotsuga menziesii, or how to spot a leatherwood from a hundred feet away.

I thank my committee member, Dr. Richard Gladon, for your constructive criticism and eye for detail. You are rich with wisdom and my time asking you questions was well spent! I also thank my committee member, Dr. David Grewell, and his graduate student Gowrishankar Srinivasan. Your bioplastic knowledge and time spent manufacturing containers were critical to my research.

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I thank my family, especially my parents, for your support and encouragement from the beginning. To Julie, thank you for everything. You are my best friend and we have a great future ahead of us.