Zein-based Bioplastic Containers Alter Root-zone Chemistry and Growth of Geranium[1]

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Abstract

Bioplastic containers made from zein, a protein from corn (*Zea mays* L.), have been developed but not evaluated as alternatives to conventional, petroleum-based plastics. We tested the hypothesis that biodegradation of zein containers provides nitrogen (N) that promotes growth of plants and examined whether plants grown in zein containers could be transplanted successfully without removing the container, thus eliminating the need to dispose of containers. Zein containers provided root zones of geranium (*Pelargonium ×hortorum* L.H. Bailey) with up to 298 and 277 mg·kg^{-1} of NH_4^+-N and NO_3^--N, respectively, and unlike geraniums in conventional plastic containers, leaves of plants in zein containers remained dark green when produced without fertilization. Electrical conductivity and pH of the substrate in zein containers increased above ranges recommended for many horticultural crops, and NO_2^-, which can be toxic to plants, was present in the substrate. These chemical changes may have been responsible for reduced canopy height and width, surface area of selected leaves, length of root systems, and dry weight of shoots of geraniums in zein containers compared with geraniums in conventional plastic containers. In a second experiment, when geraniums were transplanted without removing zein containers, growth of roots and shoots was reduced until after six weeks, when biodegradation of containers was nearly complete, and extension of roots past the zone of the degraded container was documented approximately 12 weeks after transplanting. Geraniums can be produced and transplanted in containers made from zein, but additional research must solve problems that result from altered root-zone chemistry during production and from chemical and physical impediments that delay transplant success.

Index words: corn, *Zea mays*, *Pelargonium ×hortorum*, maize, nitrogen, plastic, protein, sustainable.

Significance to the Nursery Industry

Zein containers biodegrade and therefore might be installed with transplants or composted rather than discarded in landfills. This research, the first test of producing plants in zein containers, showed the early stages of container degradation during plant production lead to excessive concentrations of nitrogen (N), high electrical conductivity (EC), and elevated pH of the container substrate. These chemical changes were associated with reduced growth of geranium. 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, and if the containers are not removed at transplant, additional research is needed to identify ways to slow N release, to maintain desirable EC and pH of substrate, and to permit unimpeded extension of roots through degrading walls of transplanted containers.

Introduction

Bioplastic containers manufactured from renewable materials may offer alternatives to horticultural containers made from conventional plastics. Conventional containers made from petroleum-based polypropylene and polyethylene can be recycled but usually are not (18); typically, they are discarded in landfills after a single use (13). Bioplastics manufactured from renewable agricultural materials such as corn (*Zea mays*) and soybean [*Glycine max* (L.) Merrill] are biodegradable and can be composted rather than deposited in landfills (3, 4, 27). Replacing petroleum-based plastics with bioplastics may reduce our dependence on diminishing fossil fuels and reduce emissions of greenhouse gases (7, 31).

Bioplastic containers are not common in horticulture, due in part to high cost and uncertain performance. As consumer awareness of benefits of bioplastics has grown, demand is increasing for container manufacturers to produce economically viable, biorenewable products (10). Peat, clay, and paper containers have provided growers alternatives to petroleum-based plastics but have several disadvantages. Peat and clay containers are more expensive than conventional plastic containers, and both wick water from substrate, leading to the need for increased irrigation. In addition, peat containers lose structural integrity when moist, while clay containers are heavy and fragile. Paper containers biodegrade slowly when transplanted into the landscape, so they often are removed before transplant and need to be disposed. Research and development of bioplastics made from agricultural byproducts might lead to competitively priced horticultural containers with desirable structural integrity and the capacity to degrade quickly when a crop produced in them is transplanted without the container removed.

Zein, a hydrophobic protein from corn (25), can be processed into bioplastic containers that are biodegradable. The longevity of prototypes of zein containers makes them suitable for crops with short production schedules, including annuals, vegetables, herbs, and some herbaceous perennials (9). Zein is expensive compared to feedstocks for conventional plastics, but new markets and new extraction methods promise to lower its price (25). Producing plants in zein containers might lower production cost because N applications can be reduced. As microorganisms degrade the protein in zein containers, inorganic N that may be available to plants is generated (9). The process begins with mineralization, which leads to NH_4^+. Nitrate subsequently is

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produced via nitrification, which is the conversion of NH_4^+ to NO_2^- by the bacteria *Nitrosomonas*, quickly followed by the conversion of NO_2^- to NO_3^- by the bacteria *Nitrobacter* (28). Although degrading zein is known to release N into container substrates (9), effects of N from the degradation of zein on plants in the containers have not been determined. The N may either enhance or curtail plant growth depending on its quantity, its chemical form, and its effects on root-zone pH and electrical conductivity (EC).

Objectives of this research were to test the hypothesis that the biodegradation of zein containers provides suitable quantities and forms of N to support growth of geranium, 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

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 70C (158F) in the feed zone to 105C (221F) in the die. The extrudate was pelletized for compression molding (C.W. Brabender Instruments, Inc.) with an aluminum mold at 105C (221F) and a 136-t Wabash Press (Wabash MPI, Wabash, IN) that provided 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. The 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 (11) with nitrogen (+N; 210 mg NO_3^--N·kg^{-1}) or without nitrogen (–N)], for a total of four treatment combinations (Zein +N, Zein –N, Plastic +N, Plastic –N), each of which was replicated 10 times. The conventional plastic containers (Kord Products, Brampton, ON, Canada) had a height of 8.6 cm (3.4 in), four drainage holes, and bottom and top diameter of 6.8 cm (2.6 in) and 10 cm (4.0 in), respectively. One rooted stem cutting of 'Rocky Mountain Salmon Rose' geranium was planted in each container by using a soilless, peat moss-based substrate (Sun Gro® Sunshine® LC1 mix, Sun Gro Horticulture Distribution Inc., Bellevue, WA). The 40 containers/plants, each of which constituted an experimental unit, 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 (*PAR*) was 631 ± 140 μmol·m^{-2}·s^{-1}. Air temperature ranged from 21 to 32C (70 to 89F), with a mean of 24 ± 1.2C (75 ± 2.2F). Plants were disbudded for the first 26 d of treatments.

To establish plants, 200 ml of 50% Hoagland solution with N was applied to all plants twice weekly during the first two weeks. Thereafter, one-half of bioplastic containers and conventional containers (Zein +N, Plastic +N) received 100% Hoagland solution with N twice weekly, and the remaining one-half (Zein –N, Plastic –N) received 100% Hoagland solution without N. An HI 9811 meter (Hanna Instruments, Woonsocket, RI) was used to determine EC and pH of leachate collected after each irrigation (2) from five randomly selected containers from each treatment combination. Lachat flow injection analysis (Lachat Instruments, Milwaukee, WI) was used to analyze leachate for NO_3^--N and NH_4^+-N. Nitrite also was quantified in leachate collected during the final five irrigations.

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 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 air. Weights of roots and shoots were recorded after drying them at 67C (153F) 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 surrounding substrate. The protocol was intended to simulate planting a bioplastic container into the landscape or transplanting into a larger container. Zein and conventional containers like those described for experiment 1 were used. 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 stem cutting of 'Rocky Mountain Salmon Rose' geranium was planted in each container. Plants were irrigated with 100% Hoagland solution no. 1 twice weekly for 51 d except for the first two weeks, when 50% solution was used. Containers were arranged in a completely randomized design in the greenhouse where plants in experiment 1 were treated simultaneously.

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 *PAR* was 349 ± 156 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air temperature ranged from 18 to 30C (64 to 86F), with a mean of 22 ± 1.5C (72 ± 2.8 F). Each plant received 1 liter of 50% Hoagland solution no. 1 every 3 to 7 d, when the surface of the medium appeared dry. Electrical conductivity, 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 liters of distilled water was applied to each container to reduce EC of the substrate.

Five replications per treatment combination were randomly assigned to each of two harvest dates, 42 and 84 d after transplant, and plants were disbudded until three weeks before their assigned harvest date. Height and width of plants, relative leaf greenness, surface area of selected leaves, and shoot dry weight were determined at harvest as in experiment 1. 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. Both fractions of the root system from each plant were dried at 67C (153F) 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, surface area of selected leaves, root length, and dry weights were separated with Tukey's honestly significant difference (HSD) test. The same test was used to separate means for pH, EC, NO_3^--N, NH_4^+-N, and NO_2^--N at each date of leachate collection.

Results and Discussion

Experiment 1. Initial degradation of zein containers during production of geraniums led to various concentrations of the three forms of N measured. During the first few weeks, NH_4^+-N increased and peaked at 241 and 298 $mg \cdot kg^{-1}$ 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 conventional 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 applied contained no NH_4^+-N, the long-term 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. There was NO_2^--N in leachate from zein containers when we first tested for it at day 30, and concentrations were higher thereafter (Fig. 1B). No NO_2^--N was detected 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. Generally considered toxic to plants, NO_2^--N 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* (20, 24). The NH_4^+-N in zein containers, along with elevated pH (Fig. 2A), may explain the accumulation of NO_2^--N. Nitrate began to increase in leachate from containers in the Zein –N treatment by day 37 and rose to 277 $mg \cdot kg^{-1}$ over time (Fig. 1C). Among all treatments, NO_3^--N detected before day 15 can be attributed to the application of Hoagland solution with NO_3^--N for plant establishment (Fig. 1C). Nitrate concentrations then declined in the substrate of containers in the Zein –N and Plastic –N treatments due to leaching (Fig. 1C). The resurgence of NO_3^--N in the substrate of containers in the Zein –N treatment suggests oxidation of NO_2^--N to NO_3^--N occurred. High pH and concentrations of

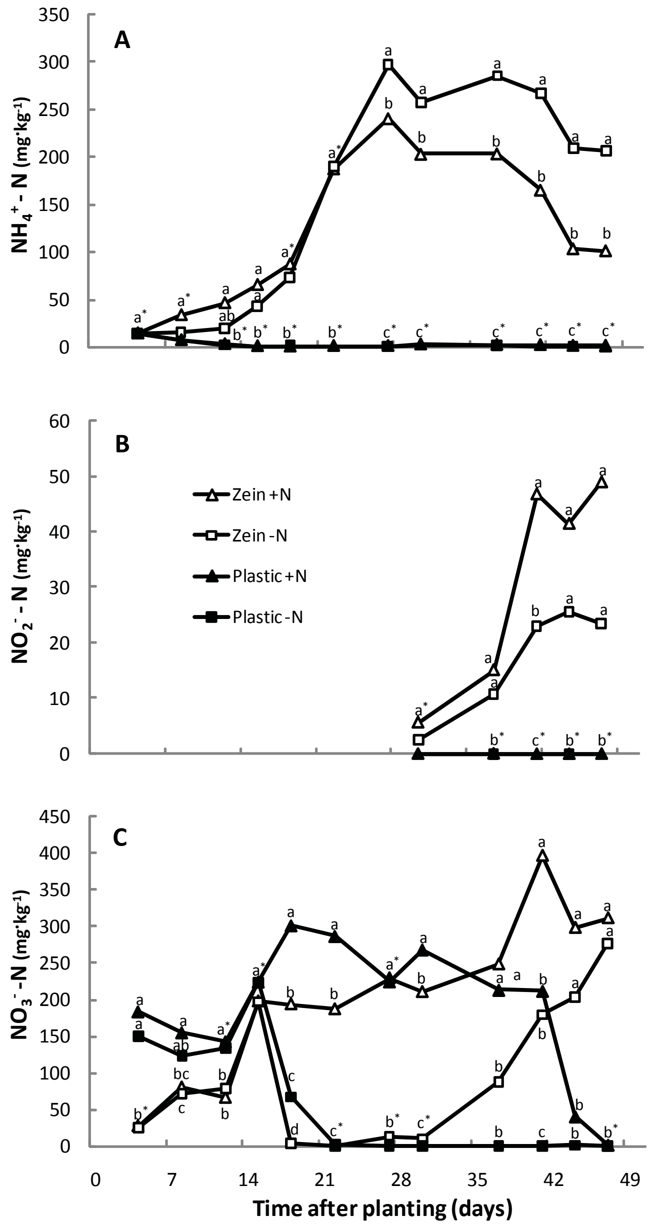

Fig. 1. Change in substrate NH_4^+-N (A), NO_2^--N (B), and NO_3^--N (C) during growth of geranium for seven weeks. Treatments included two container types (zein and plastic) and two types of fertilizer [with nitrogen (+N) and without nitrogen (–N)]. Means within each date followed by the same letter 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.

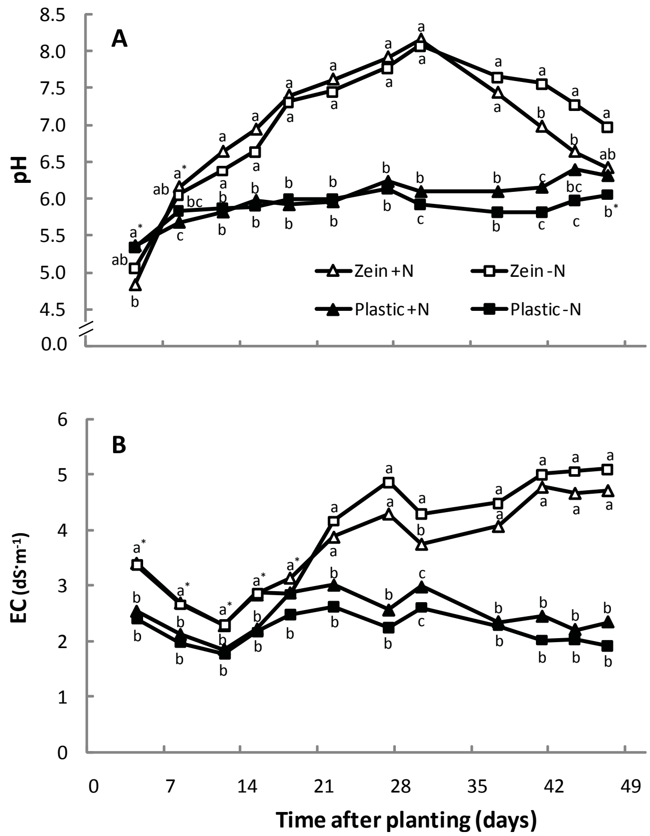

Fig. 2. Change in substrate pH (A) and EC (B) during growth of geranium for seven weeks. Treatments included two container types (zein and plastic) and two types of fertilizer [with nitrogen (+N) and without nitrogen (–N)]. Means within each date followed by the same letter are not different at $P \leq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

NH_4^+-N may have inhibited *Nitrobacter* temporarily and thus may account for the lack of NO_3^--N until day 37 (20, 24). It is also possible that *Nitrobacter* populations needed to increase before considerable amounts of NO_2^- were oxidized.

The pH and EC of leachate from zein containers changed over time in ways not observed for leachate from plastic containers. The 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 containers in the Plastic +N and Plastic –N treatments remained within an acceptable range for geraniums, which has been defined as 6.0 to 6.6 (Fig. 2A) (30). The increase in pH of leachate from zein containers may have been due to ammonification of protein, and the subsequent decrease in pH may be attributed to nitrification (22). Electrical conductivity 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, 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) (30). Increased soluble salts of N in the substrate of zein containers probably contributed to increased EC of leachate.

Although N released from containers probably explains the lack of chlorosis observed among geraniums in the Zein –N treatment, chemical changes to the root zone likely were responsible for reduced growth of roots and shoots. Leaf greenness of geraniums in the Zein –N treatment was 30% greater than that of leaves of plants in the Plastic –N treatment (Table 1). Greenness data from SPAD meters often are correlated with chlorophyll content and can indicate N deficiency (15). Leaves of geraniums in the Zein –N treatment showed elevated NO_3^--N and nearly seven times as much NH_4^+-N as leaves of geraniums in the Plastic –N treatment (Table 1), effects that we attribute to the N released as zein containers began to degrade. 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 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 in zein containers was not different than that of geraniums in the Plastic +N treatment, mean length of root systems of geraniums in the Plastic +N treatment was four times greater than that of geraniums in zein containers (Table 2). Roots in zein containers appeared 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 (29). 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 inhibitors of root growth previously found in corn gluten meal (16, 17), 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 substrate, changes in root-zone EC and pH, or both (Figs. 1 and 2). Populations of organisms capable of nitrification may have been low when release of NH_4^+-N began, allowing an accumulation

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.

Treatment	Relative greenness (SPAD)	Concentration (mg·kg^{-1}) NH_4^+-N	Concentration (mg·kg^{-1}) NO_3^--N
Zein +N	65.3a	1374b	760a
Zein –N	62.0ab	1704a	476b
Plastic +N	59.2b	393c	70c
Plastic –N	43.6c	253d	0d

[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, surface area of selected leaves, root dry weight, shoot dry weight, and root system length 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 distance from where roots initially formed on stem cuttings to the tip of the most distal root, respectively. Means are of 10 replications for each combination of container type and fertilizer.**

Treatment	Shoot height (cm)	Shoot width (cm)	Surface area of selected leaves (cm^2)	Dry weight (g)		Root system length (cm)
				Root	Shoot	
Zein +N	7.9b[z]	18.3b	38.3b	0.69b	3.21b	5.0b
Zein –N	9.5ab	17.6b	46.1b	0.77b	3.37b	6.3b
Plastic +N	10.1a	20.9a	69.9a	0.92b	5.37a	21.0a
Plastic –N	8.3b	17.0b	43.3b	1.23a	4.57a	24.5a

[z]Means within each column followed by the same letter are not different at $P \leq 0.05$ using Tukey's HSD test.

of NH_4^+-N (19) that curtailed plant growth (1, 6). Nitrite also inhibits the growth of plants (12, 21, 23). The sum of NH_4^+-N and NO_3^--N in leachate from zein containers frequently exceeded the 200 to 300 mg N·kg^{-1} applied to many greenhouse crops, and the NH_4^+:NO_3^- ratio was unstable over time, so zein containers pose challenges to the production of crops, especially those that require specific forms and quantities of N (19). Furthermore, EC in the substrate of zein containers increased sufficiently to reduce growth, kill root tips, and promote formation of unusually small, dark green leaves (14, 30), which are symptoms we observed. Changes in substrate pH in zein containers also are potentially detrimental due to effects on nutrient availability (19, 26).

Experiment 2. Concentration of 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 conventional plastic container intact (Fig. 3A). Leachate from experimental units with plastic containers removed consistently had < 5 mg NH_4^+-N·kg^{-1} after transplant, whereas leachate from units with the zein container removed had 10 mg NH_4^+-N·kg^{-1} 7 d after transplant (Fig. 3A). The rapid decline of NH_4^+-N concentrations in leachate from experimental units with the zein container intact suggests bacteria capable of nitrification were established in the zein containers. Alternatively, 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 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 NO_2^--N at 5.3 mg·kg^{-1}, whereas < 1 mg·kg^{-1} was present in leachate from other units, and all concentrations decreased to < 1 mg·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). Electrical conductivity increased and decreased similarly, suggesting NO_3^--N was a primary determinant of EC (Fig. 4B). The 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), which we attribute to ammonification. It appears that the extended duration of treatments in experiment 2 compared with experiment 1 permitted extensive oxidation of NH_4^+-N to NO_3^--N and a consequential decreased in pH.

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, dry weight of roots that

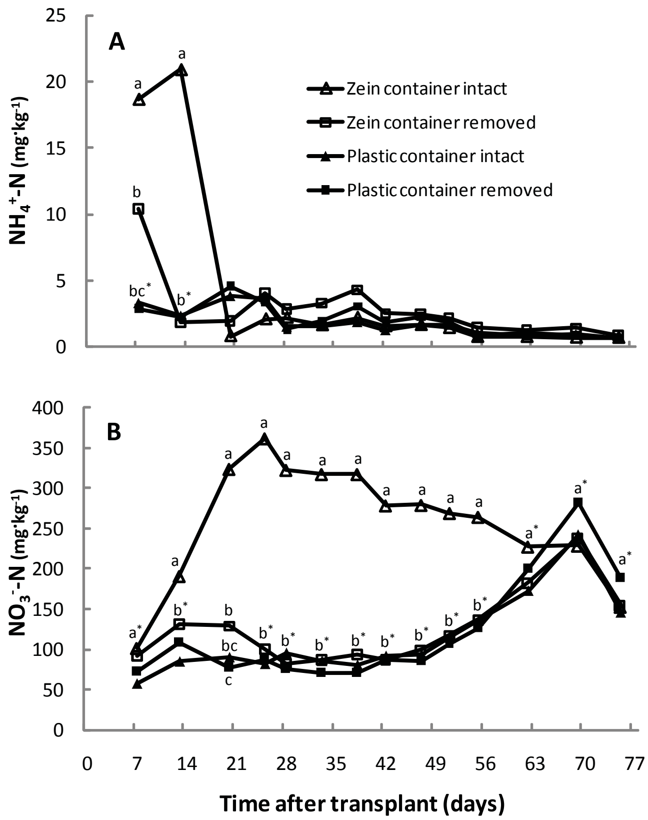

Fig. 3. **Change in substrate NH_4^+-N (A) and NO_3^--N (B) of azalea containers into which geraniums, previously grown for 51 d, were transplanted. One-half of the plants were removed from the original containers (peat, plastic, or zein) before transplanting and the remaining one-half were transplanted with the containers intact (container removed or container intact). Means for peat containers were similar to those for 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.**

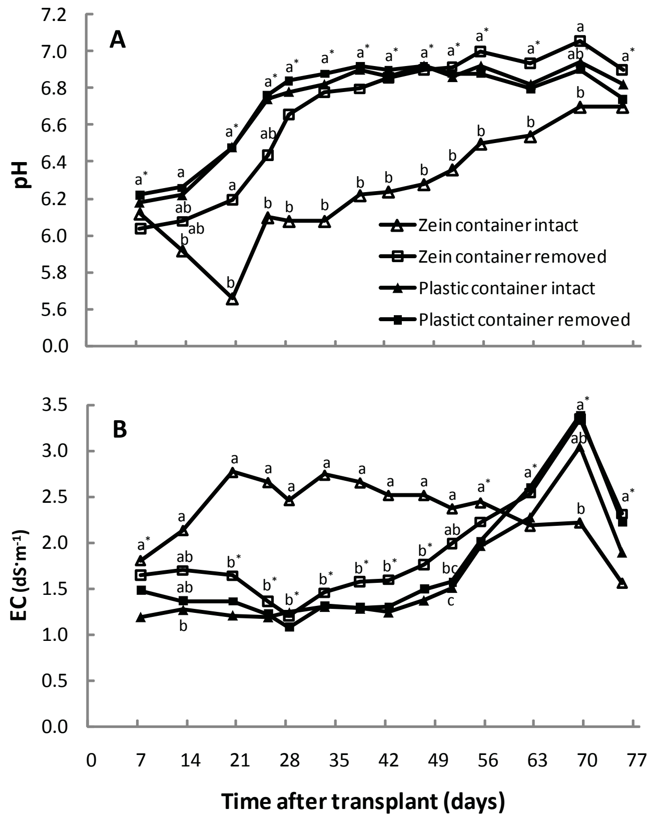

Fig. 4. Change in substrate pH (A) and EC (B) of azalea containers into which geraniums, previously grown for 51 d, were transplanted. One-half of the plants were removed from the original containers (peat, plastic, zein) before transplanting and the remaining one-half were transplanted with the containers intact (container removed or container intact). Means for peat containers were similar to those for those for 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.

had migrated beyond the original container, shoot dry weight, and surface area of selected leaves were less for geraniums transplanted with zein containers intact than for 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 conventional 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 show the walls of zein containers were a barrier to root penetration and suppressed plant growth. 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 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 appeared > 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 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, pH increased (Fig. 4A), EC decreased (Fig. 4B), and concentrations of NH_4^+-N (Fig. 3A) and NO_3^--N (Fig. 3B) decreased to within ranges recommended for geranium (30). 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 84 d after transplant.

Table 3. Shoot height, shoot width, inside root dry weight, outside root dry weight, shoot dry weight, and surface area of selected leaves 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. Means are of five replications for each combination of pot type, transplant method, and harvest date.

	Shoot height (cm)		Shoot width (cm)		Root dry weight (g) Inside[z]		Root dry weight (g) Outside[y]		Shoot dry weight (g)		Surface area of selected leaves[x] (cm²)	
Treatment	42 DAP[w]	84 DAP	42 DAP	84 DAP	42 DAP	84 DAP	42 DAP	84 DAP	42 DAP	84 DAP	42 DAP	84 DAP
Peat container intact	15.5b[vu]	21.7ab	36.5ab[u]	41.9b	1.09a	1.4ab	0.41a	0.7b	14.5a	24.8bc	166abc	182bc
Peat container removed	16.8ab	21.8ab	36.5ab	45.1b	1.07a	1.6ab	0.40a	0.9ab	15.1a	28.7b	180ab	158c
Plastic container intact	16.5ab	20.3ab	33.3b	43.2b	1.34a	1.9ab	0.13b	0.6b	14.2a	28.6b	119cd	185abc
Plastic container removed	16.4ab	22.4ab	38.1ab	45.1b	1.31a	1.5ab	0.43a	0.8ab	17.9a	26.8bc	150abc	172bc
Zein container intact	10.2c	19.4b	24.9c	39.6b	0.90a	1.3b	0.01c	0.5b	8.2b	16.2c	81d	229a
Zein container removed	18.3a	27.1a	41.4a	52.3a	1.03a	1.9a	0.74a	1.3a	17.9a	39.7a	212a	213ab

[z]Inside root weight is the dry weight of the root system contained within the volume of the original container.
[y]Outside root weight is dry weight of roots that had migrated through the original container or beyond the original root ball.
[x]Leaf area is a measure of the three youngest fully expanded leaves on each plant.
[w]Days after planting.
[v]Means within each column followed by the same letter are not different at $P \leq 0.05$ using Tukey's HSD test.
[u]Means are of 10 replications.

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. Dynamic and important effects of the containers on substrate, including large influxes of N and altered pH and EC are associated with inhibition of root and shoot growth. The cause of root inhibition should be identified, and the influx of various forms of N and rapid changes in substrate EC and pH should be targeted as problems with zein containers to be overcome through additional research and product development. Adjustments to container sidewall thickness, use of larger containers with a greater substrate:zein ratio, blending zein with other feedstocks, and use of coatings on sidewalls 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 container degradation for plant production and establishment into the landscape. Preserving the capacity of zein containers to promote highly branched root systems that do not circle around the inner container wall should be a goal of continued research.

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