KAPSOL™

KLONDIKE™
AGRICULTURAL
PRODUCTS

Use of Gelatin Capsules as a Form of Seed Enhancement in Tomato *(Lycopersicon esculentum)*.

Studies conducted by Dr. Brant Touchette
at Elon University.

brant@klondikeag.com
daniel@klondikeag.com

klondikeag.com

Use of Gelatin Capsules as a Form of Seed Enhancement in Tomato (*Lycopersicon esculentum*).

By

Brant W. Touchette[1]*, and Daniel S. Cox[2]

1. Department of Biology, 2015 Campus Box, Elon University, Elon NC, 27244, USA. (btouchette@elon.edu)
2. Klondike Agriculture Products, 580 Kennedy Rd., Akron OH, 44305, USA. (daniel@klondikeagriculturalproducts.com)

*Corresponding author

Running heading: Tomato Seed Encapsulation

Abstract

Seed enhancements involve post-harvest modifications of seeds intended to improve germination and plant performance. This includes seed modifications that facilitates the delivery of other plant-benefiting components (e.g., nutrients or plant protectants). This study considers the use of tomato-seed encapsulation as a possible extension of seed coatings. Placing seeds within gelatin capsules offers potential benefits including space for greater volumes of additives, separation between protectant chemicals and seeds, diminished human exposure to agrochemicals, and improved uniformity for mechanical planters. Therefore, the objectives of this study were to determine to what degree seed encapsulation alters plant emergence, affects plant performance, and serves as a possible delivery-system for controlled-release fertilizers. The results suggest that seed encapsulation may delay initial plant emergence by one day, and between one and two days for fertilizer treatments. Gelatin capsules alone improved early root development, promoted plant growth, and increased fruit production; indicative of gelatin's biostimulant properties. The addition of controlled-release fertilizers (especially Florikan) appeared to provide greater aboveground, belowground, and total plant mass, and higher fruit yield. The results of this study support the notion that seed encapsulation can improve tomato performance, and that other component(s) can be successfully delivered to provide additional plant benefits.

Keywords: Biostimulant, Capsule, Fertilizer, Gelatin, Seed Encapsulation, Tomato.

Introduction

Post-harvest modifications of seeds used to improve germination and/or plant performance are often referred to as 'seed enhancement' (Taylor *et al*., 1998). This characterization also applies to any seed modification that facilitates the delivery of seeds along with other beneficial components employed during planting. While broadly defined, seed enhancement can be categorized into three general techniques (*i*.) pre-sowing hydration or liquid priming, (*ii*.) seed conditioning, and (*iii.*) seed coatings (Taylor *et al*., 1998; Jamieson, 2006). For the most part, these techniques are not mutually exclusive, and can be combined in different ways to provide cumulative benefits that improve seed quality, germination, and/ or growth.

Seed coat technologies typically include pelleting, encrusting, or film coating. Pelleting involves the layered deposition of materials that can alter the shape and size of the original seed. This change in conformation can improve plantability, especially for seeds that are small or irregularly shaped (Barut, 2008; Sidhu *et al*., 2019). In most cases, seeds are coated with both an adhesive binder and a filler agent (or bulking agent). Pelleted materials can also contain plant protectants such as fungicides and insecticides, thereby providing additional benefits to seeds and emerging plants (Heijbroek and Huijbregts, 1995; Taylor *et al*., 2001). Seed coatings, which were adapted from the pharmaceutical industry, involve the uniform deposition of polymers, plasticizers, and colorants forming a film that acts as a physical barrier (Taylor *et al*., 1998). The reduced friction among coated seeds, partially attributed to its improved uniformity, has been shown to enhance flow characteristics in mechanical planters (Hill, 1999; Barut, 2008). As with pelleting, plant protectants and other beneficial material can be applied to seeds through film coating (Scott, 1998; Rocha *et al*., 2019). The spatial separation between the seed surface and the chemical protectant, however, is not as great as those achieved through pelleting and some film-

coatings may be toxic or inhibitory to seeds of some crops (Taylor *et al*., 1998; Hill, 1999). As such, film coatings are often preferred as a means to reduce overall exposure of chemicals (used as seed treatments) to agricultural workers.

One possible extension of seed coatings is the use of pharmaceutical capsules in which seeds and other beneficial components (or plant protectant chemicals) can be placed inside a single unit. This seed encapsulation approach may combine the benefits of both pelleting and coating. That is, seed encapsulation may provide precise uniformity with reduced friction allowing for the use of mechanical planters, may offer needed separation between protectant chemicals and seeds (perhaps extending seed viability), and minimize exposure of agrochemical chemicals to workers. Pharmaceutical capsules also come in different sizes, and depending on the size and shape of the seed, capsules may provide sufficient space to deliver greater volumes of beneficial additives at the time of sowing. Moreover, pharmaceutical capsules are relatively inexpensive (1,000 capsules for 10 US Dollars; at the time of this writing), and do not require equipment such as side-vented-drum coating machines that provide needed ventilation for drying seed coat formulations (Yehia, 2008). Finally, pharmaceutical capsules can be made from different compounds, including gelatin (collagen-based material from animal bone or hide) or plant-based hydroxypropyl methyl cellulose (HPMC). Gelatin, protein hydrolysates, and other amino acid-based products may also behave as effective plant biostimulants, with enhanced plant growth and/or yields observed a variety of corps (Morales-Payan and Stall, 2003; Parrado *et al*., 2008; Ertani *et al*., 2009; Koukounararas *et al*., 2013; Amirkhani *et al*., 2016; Wilson *et al*., 2018). In cucumber, for example, there was a positive correlation between the amount of gelatin provided to the seed and both plant growth and total tissue nitrogen content (Wilson *et al*., 2018).

Due to the potential advantages associated with seed encapsulation within gelatin capsules, we sought to evaluate the efficacity of this technology and to explore its potential as a possible delivery system for materials that may enhance plant performance in tomato (*Lycopersicon esculentum* Mill.). More specifically, the objectives of this research were to (*i*.) determine if and to what degree seedling emergence was altered when placed within gelatin capsules, (*ii*.) characterize any differences in tomato performance following emergence of encapsulated seeds, (*iii.*) evaluate the use of seed encapsulation as a possible vehicle for the delivery of controlled-release fertilizers (a surrogate for other beneficial agrichemicals), and (*iv.*) owing to the plant-biostimulant properties of gelatin, consider if there are any long-term influences on flower and fruit production in tomatoes.

Materials and Methods

Experimental Design

Seeds of tomato plants (*Lycopersicon esculentum* Mill. Cv. Early Girl) were encapsulated in pharmaceutical gelatin capsules (bovine gelatin extract from hide, size 00; Capsuline Inc., Dania Beach, FL) with or without controlled-released fertilizers. Fertilizer treatments involved three different manufactures including Coor's (13:13:13, N:P:K; Coor Farm Supply, Smithfield, NC), Florikan with nutricote (18:6:8; Florikan, Sarasota, FL), and Osmocote (14:14:14; ICL Specialty Fertilizers, Dublin, OH). Two fertilizer pellets were placed in each capsule, which accounted for approximately 45, 100, and 55 mg fertilizer per capsule for Coor's, Florikan, and Osmocote, respectively. For all encapsulated treatments (with and without fertilizer), the remaining void space within the capsule were loosely filled with a dried mixture consisting of compost (60%) and peat (40%).

To evaluate seedling emergence, five seeds (control) or five encapsulated seeds (treatments) were planted equidistantly in 3.4 L polypropylene pots with sandy-loam soils in mid-March. A total of 50 pots were employed in this study (n=10) and were placed in a randomized complete block design that accounted for the north-south orientation of the glasshouse benches (Hartung *et al.*, 2019). The climate-controlled glasshouse maintained temperatures between 25 and 30°C, with relative humidity fluctuating between 34 and 89% throughout the study. Plants were watered daily with approximately 400 mL of water.

Plant Measurements

Emergence (and survival) was monitored daily for the first 16 days, and then twice-a-week through the remaining 24 weeks. In this study, successful seedling emergence was characterized by the presence of aerial cotyledons, and was reported as percent emergence from each experimental unit (i.e., pot). One plant from each pot was selected and evaluated weekly (over 26 weeks) for changes in plant height (growth). Flower and fruit production was also monitored within each pot from 7- to 26-weeks. Plants were harvested at 3-, 7-, and 10-weeks, and evaluated for biomass. At the end of 26 weeks, a forth plant was harvested for biomass estimates. For biomass, plants were carefully removed from the pots, separated between above- and below-ground structures, dried in a laboratory oven at 60°C until constant weight, and massed.

As mentioned, fruit production was recorded throughout the study. This included cumulative number of fruit produced (both unripened and ripened), as well as the number of ripened fruit. Once the fruit had fully ripened (based on deep-red coloration) it was harvested, and fresh weight was immediately recorded.

Data Analyses

Harvested-ripen fruit data was used to calculate the mean individual fruit mass, mean number of fruit per plant, and total fruit mass produced per plant, and was statistically analyzed to compare controls against encapsulated treatments (with and without fertilizers) using generalized linear models (GLM). Wald chi square tests for pairwise evaluations were conducted when significant treatment responses were identified by GLMs. Similarly, seedling emergence data, including number of days until first seedling emergence, third seedling emergence, and the fifth seedling emergence (for pots with 100% germination) were statistically analyzed using GLMs followed by Wald chi square tests when treatment differences were detected.

For longitudinal data including changes in plant height, seedling emergence/and survival over time, cumulative flower and fruit production, and biomass (recorded over 4 different intervals), we employed generalized estimating equations (GEE), which is an extension of GLMs designed for repeated-measures analyses (Zeger and Liang, 1986; Ballinger, 2004), to compare controls against encapsulated treatments. GEEs were selected because of the model's ability to evaluate non-normal longitudinal data that is often characteristic of count data. Wald chi square tests were performed on parameters identified by GEE to have significant treatment responses. All statistical analyses were conducted using SPSS software version 26 (IBM Corp. 2019), where comparisons were considered significant at an $\alpha = 0.05$.

Results

When considered over time, there were significant differences in seedling emergence among the control and encapsulated treatments ($p < 0.001$). That is, seedling emergence appeared earlier for controls in comparison to treatments, and the Coor's treatment, particularly, seemed to lag other treatments in both timing of germination and total emergence (figure 1). As such, the number of days (after sowing) until first emergence was also different among the control and experimental treatments. In this case, there was a one-day delay in emergence for Capsule, Florikan, and Osmocote treatments, compared to the control ($p = 0.021$, 0.002, and $<$ 0.001, respectively), and a two-day delay in emergence for the Coor's treatment (table 1; $p <$ 0.001). The number of days until complete (100%) seedling emergence also reviled significant treatment delays ($p < 0.001$). While there were no differences in the number of days for complete emergence between control and Capsule treatments (both around nine days; $p = 0.789$), the encapsulated fertilizer treatments were delayed by 2-, 3-, and 8-days for Florican, Osmocote, and Coor's treatments, respectively (table 1; $p \leq 0.046$). Nevertheless, when you consider the total seedling emergence after 30 days, although there was a trend of lower emergence in the Coor's treatments (only $86 \pm 5.2\%$ germinated), there were no statistical differences among the control and encapsuled treatments (table 1; $p = 0.190$).

Plant growth over time, as indicated by a change in overall height, was significantly different among the control and encapsulated treatments ($p = 0.005$). Although there were no differences in plant height over time between the control and Osmocote treatment ($p = 0.162$), the remaining treatments (Capsule, Coor's, and Florikan) revealed taller plants throughout most of the study (figure 2; $p \leq 0.018$). Differences among plant height appeared to be most pronounced between 7- and 14-weeks, where there was a clear separation between controls and

184 experimental treatments. By 14-weeks, the height of the control plants begins to match those of
185 some encapsulated treatments, and appears to be associated with a lag in plant growth for
186 encapsulated plants around the time of flower induction (~ 8 to 9 weeks).

187 There were significant differences in plant mass among control and encapsulated
188 treatments. By the third week, aboveground biomass in control plants were larger than both
189 Coor's and Osmocote treatments (table 2; $p \leq 0.034$). During this time, however, the Capsule
190 treatment had twice as much belowground biomass compared to the control ($p = 0.008$), and all
191 encapsulated treatments (with and without fertilizers) had more than two-times the root/shoot
192 ratios observed in the controls (ratio of 0.32 ± 0.03 in the controls, compared to values at or
193 above 0.68 for encapsulated treatments; table 2; $p \leq 0.014$). For week-7 and beyond, these
194 biomass characteristics began to change. At that point, aboveground biomass among control and
195 encapsulated treatments were no longer statistically different, except for the Florikan, which was
196 statistically larger than the controls ($p \leq 0.007$). Similarly, belowground biomass was greater in
197 Florikan treatments relative to the control for week-7, -10, and -26 ($p < 0.001$), and periodic
198 increases in belowground biomass were also observed in Capsule (week-26; $p = 0.045$), Coor's
199 (week-26; $p = 0.033$), and Osmocote (week-7; $p = 0.004$; table 2). Total plant biomass closely
200 mirrored aboveground biomass, with lower mass in the Coor's treatments compared to the
201 control on week-3 ($p = 0.002$), followed by significant increases in total biomass for Florikan
202 (week-7 through week-26; $p < 0.001$) and Osmocote (week-7; $p = 0.018$) treatments.

203 As with other growth metrics, there were notable enhancements in the total number of
204 flowers produced per plant for fertilized treatments (figure 3a). That is, while there were no
205 statistically significant differences in flowers produced per plant over time between control and
206 Capsule ($p = 0.167$), Coor's, Florikan, and Osmocote treatments had greater number of flowers

produced over the 26-week period ($p = 0.019$, 0.005, and < 0.001, respectively). By the end of the study, control and Capsule treatments produced 8.3 ± 0.9 and 11.5 ± 1.9 flowers per plant (respectively), compared to 12.2 ± 1.6, 13.1 ± 1.4, and 16.5 ± 2.86 flowers per plant for Coor's, Florikan, and Osmocote treatments (figure 3a). The elevated flower production in fertilized treatments, however, did not translate into a significantly higher number of fruit (both unripen and ripen) produced per plant over the same 26-week period; although a trend of more fruit per plant is noted in encapsulated treatments (figure 3b; $p = 0.069$). Interestingly, when only ripen-harvested fruit is considered (i.e., not including green immature fruit that remained on plants), there were significantly more fruit produced by Capsule and Florikan treatments compared to the control ($p = 0.032$, and 0.018, respectively; figure 4a). Similarly, when you consider total ripen fruit mass produced per individual plant (g plant^{-1}), both Capsule and Florikan produced more fruit by mass than the control ($p = 0.011$, and 0.007, respectively; figure 4b). For all encapsulated treatments, the fresh weight of ripen fruit was greater than those observed in the control ($p \leq 0.014$; figure 4c).

Discussion

In this study, we investigated the use of gelatin seed encapsulation as a possible technique for seed enhancement in tomatoes. The use of pharmaceutical gelatin capsules alone provided some plant benefits including better root development within the first three weeks, higher plant growth within the first 12 weeks (as indicated by changes in height), and improved fruit production. These results are consistent with other studies involving protein hydrolysates as a possible plant biostimulant (Taylor *et al*., 1998; Calvo *et al*., 2014; Skwarek *et al*., 2020). In a study by Colla *et al*. (2014), for example, tomato cuttings that were exposed to plant-derived

protein hydrolysate had significantly greater shoot and root dry weights, along with greater root length, diameter, and surface areas in as little as eight days after treatment. In a study by Parrado *et al*. (2008), there were significant improvements in plant height, number of flowers per plant, and number of fruit per plant after 18 weeks in tomatoes treated with a similar plant-derived hydrolysate extract. In a study more comparable to ours, involving pharmaceutical capsules as a biostimulant, there were significant increases in both leaf area and plant mass after 28 days for tomatoes planted with as little as one-half of a gelatin capsule (Wilson *et al*., 2018). These reported growth and performance benefits, however, are not restricted to tomatoes, as other studies have shown beneficial biostimulant-like responses involving animal- or plant-derived protein hydrolysates in arugula, broccoli, cucumber, kiwifruit, maize, papaya, passionfruit, pea, pepper, and snapdragon (Quartieri *et al*., 2002; Morales-Pajan and Stall, 2004; Ertani *et al*., 2009; Colla *et al*., 2014; Cristiano *et al*., 2018; and Wilson *et al*., 2018).

As mentioned, the findings from this study were comparable to those observed by Wilson *et al*. (2018), wherein tomato seeds planted along with gelatin capsules appeared to have beneficial growth responses. The difference between our study and Wilson *et al*. (2018), however, is that we placed seeds within gelatin capsules to function as both a biostimulant and as a potential delivery system for both seed and other plant-benefiting components, rather than placing seeds adjacent to capsules where it can serve primarily as a biostimulant. To test whether capsules can effectively deliver other beneficial components, we added controlled-release fertilizers. The results from this study suggest that the addition of fertilizers can delay total seed emergence (i.e., 100% germination); from one to two days for Florikan and Osmocote, to as much as eight days for Coor's. Although some of the delay may be attributed to the time necessary to allow water to dissolve the capsule and initiate seed germination, nutrient pulses

253 may also inhibit germination and early seedling growth (Bremner and Krogmeier, 1989;
254 Bremner, 1995). That is, under certain circumstances, elevated pulses of N, and to a lesser degree
255 P, can adversely affect the growth and development of tomatoes (Magalhas and Wilcox, 1984;
256 Jones, 1998; Barreto *et al*., 2016). This notion is supported, in part, by the responses observed in
257 the Coor's treatment including seedling emergence delays, lower aboveground mass within the
258 first three weeks, and comparatively lower ripened fruit production. While the Coor's fertilizer is
259 approximately 13% nitrogen, only 8.4% is considered controlled-release and remaining nitrogen
260 is in the form of conventional ammonia and urea. Ammoniacal nitrogen, when applied directly or
261 as a hydrolytic biproduct of urea, has been shown to adversely affect seed germination
262 (Openshaw, 1970; Bremner and Krogmeier, 1989). Perhaps this blend of conventional and
263 controlled-release fertilizers produced an elevated pulse of nutrients that initially influenced
264 germination and early growth. Nevertheless, aside from delays in emergence, the addition of
265 small amounts of controlled-release fertilizers in gelatin capsules appeared to provide some
266 additional benefits to tomatoes. This is especially true for Florikan treated tomatoes, which
267 consistently maintained higher aboveground, belowground, and total plant dry mass, as well as
268 higher ripen-fruit production with larger fruit mass.
269 Although animal-derived protein hydrolysates have been shown to improve plant growth
270 (Calvo *et al*., 2014), it is unclear how these biostimulants would affect long-term flower and fruit
271 production in tomato. The results from this study suggest that tomatoes, with seeds initially
272 encapsulated with controlled-release fertilizers, would produce 50 to 100% more flowers,
273 depending on the fertilizer used. While the total number of fruit produced per plant (both ripen
274 and unripen) was not significantly different among the treatments, both Capsule and Florikan
275 treatments did produce more mature/harvested fruit. This discrepancy can be explained, in part,

by earlier fruit development in treated plants, and a disproportionally higher number of unripened-green tomatoes remaining on control plants by the end of the study. Interestingly, all encapsulated treatments produced between 16.9 and 19.6% larger fruit by weight. Larger fruit size is constant with other tomato studies that employed controlled-release fertilizers either solely or as mixed blends with conventional fertilizers (Cole *et al*., 2016; Incrocci *et al*., 2020; Qu *et al*., 2020). It is also possible that the larger fruit observed in the Capsule treatment, without fertilizer, was attributed to the biostimulant properties of gelatin and/or degradation products from the capsule serving as plant nutrients.

Seed coatings can be described as any enhancement that directly applies plant-benefiting material to seeds. This includes pelleting, film coating, and seed encrusting, and may involve slurries and dry powders (Jolayemi, 2019; Qiu *et al*., 2020). Unlike film coatings, however, dry powders generally do not adhere well to seed surfaces resulting in poor dousing, loss of uniformity, and formation of dust, while thick seed coatings may break or disintegrate before sowing (Halecky *et al*., 2016; Jolayemi, 2019; Qiu *et al*., 2020). Furthermore, externally applied seed coatings may not be able to provide enough dosage of beneficial material to be effective (Qiu *et al*., 2020). This study considered an alternative approach using pharmaceutical gelatin capsules to encase seeds along with other plant-benefiting components. Depending on seed size, capsules can provide sufficient void space to add greater volumes and/or multiple types of plant-benefitting components. Gelatin alone has been shown to ack as a biostimulant (Calvo *et al*., 2014; Wilson *et al*., 2018), and the addition of other agrichemicals may provide additive benefits without the concern of material loss or human exposure as observed in other forms of coating. Moreover, gelatin capsules promote uniformity in the seeds and can be easily sowed using mechanical planters. Finally, animal-protein hydrolysates have been found to be an efficient,

safe, and sustainable biostimulant or fertilizer, with no harmful or toxic effects on soil microbiota the environment (Corte *et al*., 2014; Jolayemi, 2019). Therefore, seed encapsulation using gelatin capsule may provide unique advantages that are not offered by other forms of seed enhancement. The results of this study support the notion that seed encapsulation can improve tomato performance under certain circumstances, and that other component(s) can be successfully delivered within the capsule to provide additional benefits to the plant. Further research is necessary to determine the best ways to utilize this technology, especially its roles in seed enhancement and as a delivery system of plant-benefitting materials.

Acknowledgements

Funding for this research was provided by Klondike Agriculture, and the Departments of Biology and Environmental Studies at Elon University.

References:

Amirkhani, M., Netravali, A.N., Huang, W., and Taylor, A. G. (2016). Investigation of soy protein based biostimulant seed coating for broccoli seedling and plant growth enhancement. *HortScience*, **51**, 1121–1126. https://doi.org/10.21273/HORTSCI10913-16

Ballinger, G.A. (2004). Using generalized estimating equations for longitudinal data analysis. *Organizational Research Methods*, **7**, 127-150. https//doi.org/10.1177/1094428104263672

Barreto, R.F., Prado, R.M., Leal, A.J.F., Troleis, M.J.B., Silva Junior, G.B., Monteiro, C.C., Santos, L.C.N., and Carvalho, R. F. (2016). Mitigation of ammonium toxicity by silicon in tomato depends on the ammonium concentration. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*, **66**, 483-488. https://doi.org/10.1080/09064710.2016.1178324

Barut, Z.B. (2008). Seed coating and tillage effects on sesame stand establishment and planter performance for single seed sowing. *Applied Engineering in Agriculture*, **24**, 565-571. doi:10.13031/2013.25268

Bremner J.M. (1995). Recent research on problems in the use of urea as a nitrogen fertilizer. In *Nitrogen Economy in Tropical Soils. Developments in Plant and Soil Sciences* (ed. N. Ahmad), vol. 69, pp. 321-329, Springer, Dordrecht. https://doi.org/10.1007/978-94-009-1706-4_30

Bremner, J.M., and Krogmeier, M.J. (1989). Evidence that the adverse effect of urea fertilizer on seed germination in soil is due to ammonia formed through hydrolysis of urea by soil urease. *Proceedings of the National Academy of Sciences*, **86**, 8185-8188. https://doi.org/10.1073/pnas.86.21.8185

Calvo, P., Nelson, L., and Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. *Plant and soil*, **383**, 3-41. https://doi.org/10.1007/s11104-014-2131-8

Cole, J.C., Smith, M.W., Penn, C.J., Cheary, B.S., and Conaghan, K.J. (2016). Nitrogen, phosphorus, calcium, and magnesium applied individually or as a slow release or controlled release fertilizer increase growth and yield and affect macronutrient and micronutrient concentration and content of field-grown tomato plants. *Scientia Horticulturae*, **211**, 420-430. https//doi.org/10.1016/j.scienta.2016.09.028

Colla, G., Rouphael, Y., Canaguier, R., Svecova, E., and Cardarelli, M. (2014). Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. *Frontiers in Plant Science*, **5**, 448. https://doi.org/10.3389/fpls.2014.00448

Corte, L., Dell'Abate, M.T., Magini, A., Migliore, M., Felici, B., Roscini, L., Sardella, R., Tancini, B., Emiliani, C., Cardinali, G. and Benedetti, A. (2014). Assessment of safety and

efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates—a laboratory multidisciplinary approach. *Journal of the Science of Food and Agriculture*, **94**, 235-245. http//doi.org/10.1002/jsfa.6239

Cristiano, G., Pallozzi, E., Conversa, G., Tufarelli, V., and De Lucia, B. (2018). Effects of an animal-derived biostimulant on the growth and physiological parameters of potted snapdragon (*Antirrhinum majus* L.). *Frontiers in plant science*, **9**, 861. https://doi.org/10.3389/fpls.2018.00861

Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C., and Nardi, S. (2009). Biostimulant activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *Journal of Plant Nutrition and Soil Science,* **172**, 237–244. https://doi.org/10.1002/jpln.200800174

Halecky, A., Ren, N., Lu, J., Wang, J., and Lockwood, F. (2016). Correlation of the mechanical properties of seed coating films and dust-off, flowability, and plantability tests. In *Pesticide Formulation and Delivery Systems: 36th Volume, Emerging Trends Building on a Solid Foundation*. ASTM International. doi: 10.1520/STP159520160082

Hartung, J., Wagener, J., Ruser, R., and Piepho, H.-P. (2019). Blocking and re-arrangement of pots in greenhouse experiments: which approach is more effective? *Plant Methods*, **15**, 143. https://doi.org/10.1186/s13007-019-0527-4

Heijbroek, W., and Huijbregts, A.W.M. (1995). Fungicides and insecticides applied to pelleted sugar-beet seeds-III. Control of insects in soil. *Crop Protection*, **14**, 367-373. https://doi.org/10.1016/0261-2194(94)00015-Z

Hill, H.J. (1999). Recent developments in seed technology. *Journal of New Seeds*, **1**, 105-112. https://doi.org/10.1300/J153v01n01_09

IBM Corp. (2019). IBM SPSS Statistics for Mac OS, Version 26.0. Armonk, NY.

Incrocci, L., Maggini, R., Cei, T., Carmassi, G., Botrini, L., Filippi, F., Clemens, R., Terrones, C., and Pardossi, A. (2020). Innovative controlled-release polyurethane-coated urea could reduce N leaching in tomato crop in comparison to conventional and stabilized fertilizers. *Agronomy*, **10**, 1827. https://doi.org?10.3390/agronomy10111827

Jamieson, G. (2006). New perspectives on seed enhancement. *Acta Horticulturae*, **782**, 143-150. https://doi.org/10.17660/ActaHortic.2008.782.15

Jolayemi O.L. (2019). *Enhancing Sugar Beet's Early Growth and Establishment by Using Protein-Based Biostimulants*. Alnarp: Sveriges lantbruksuniversitet. Introductory Paper at the Faculty of Landscape Architecture, Horticulture and Crop Production Science, 2019:2

Jones Jr, J.B. (1998). Phosphorus toxicity in tomato plants: when and how does it occur? *Communications in Soil Science and Plant Analysis*, **29**, 1779-1784. https://doi.org/10.1080/00103629809370068

Koukounararas, A., Tsouvaltzis, P., and Siomos, A.S. (2013). Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization levels. *Journal of Food, Agriculture and Environment,* **11**, 644–648.

Magalhaes, J.R., and Wilcox, G.E. (1984). Ammonium toxicity development in tomato plants relative to nitrogen form and light intensity. *Journal of Plant Nutrition*, **7**, 1477-1496. https://doi.org/10.1080/01904168409363295

Morales-Payan, J.P., and Stall, W.M. (2003). Papaya (*Carica papaya*) response to foliar treatments with organic complexes of peptides and amino acids. *Proceedings of the Florida State Horticultural Society,* **116**, 30–31.

Openshaw, M.D. (1970). *The Effect of Ammonia on Germination and Development of Seedlings in Soil*. Ph.D. Dissertation in Agronomy, Iowa State University, Ames, Iowa.

Parrado, J., Bautista, J., Romero, E.J., Garciìa-Martiìnez A.M., Friaza, V., and Tejada, M. (2008). Production of a carob enzymatic extract: potential use as a biofertilizer. *Bioresource Technology*, **99**, 2312–2318. https://doi.org/10.1016/j.biortech.2007.05.029

Qiu, Y., Amirkhani, M., Mayton, H., Chen, Z., and Taylor, A.G. (2020). Biostimulant seed coating treatments to improve cover crop germination and seedling growth. *Agronomy*, **10**, 154. https//doi.org/10.3390/agronomy10020154

Qu, Z., Qi, X., Shi, R., Zhao, Y., Hu, Z., Chen, Q., and Li, C. (2020). Reduced N fertilizer application with optimal blend of controlled-release urea and urea improves tomato yield and quality in greenhouse production system. *Journal of Soil Science and Plant Nutrition*, **20**, 1741-1750. https://doi.org/10.1007/s42729-020-00244-8

Quartieri, M., Lucchi, A., Marangoni, B., Tagliavini, M., Cavani, L. (2002). Effects of the rate of protein hydrolysis and spray concentration on growth of potted kiwifruit (*Actinidia deliciosa*) plants. *Acta Horticulture*, **594**, 341-347. https://doi.org/10.17660/ActaHortic.2002.594.42

Rocha, I., Ma, Y., Souza-Alonso, P., Vosátka, M., Freitas, H., and Oliveira, R.S. (2019). Seed coating: a tool for delivering beneficial microbes to agricultural crops. *Frontiers in Plant Science*, **10**, 1357. https://doi.org/10.3389/fpls.2019.01357

Scott, J.M. (1998) Delivering fertilizers through seed coatings. *Journal of Crop Production*, **1:2**, 197-220. https://doi.org/10.1300/J144v01n02_08

Sidhu, H., Wiesenborn, D., Johnson, B., Monono, E., and Eriksmoen, E. (2019). Coating of hulled seeds improved field plantability and grain yield of extra-large confectionary

412 sunflower achenes. *Crop Science*, **59**, 1182-1190.
413 https://doi.org/10.2135/cropsci2018.06.0400
414 Skwarek, M., Nawrocka, J., Lasoń-Rydel, M., and Ławińska, K. (2020). Diversity of plant
415 biostimulants in plant growth promotion and stress protection in crop and fibrous
416 plants. *Fibres and Textiles in Eastern Europe*, 28, 34-41. DOI: 10.5604/01.3001.0014.0931
417 Taylor, A.G., Allen, P.S., Bennett, M.A., Bradford, K.J., Burris, J.S., and Misra, M.K. (1998).
418 Seed enhancements. *Seed Science Research*, **8**, 245-256.
419 https://doi.org/10.1017/S0960258500004141
420 Taylor, A.G., Eckenrode, C.J., and Straub, R.W. (2001). Seed coating technologies and
421 treatments for onion: challenges and progress. *HortScience*, **36**, 199-205.
422 https://doi.org/10.21273/HORTSCI.36.2.199
423 Wilson, H.T., Amirkhani, M., and Taylor, A.G. (2018). Evaluation of gelatin as a biostimulant
424 seed treatment to improve plant performance. *Frontiers in Plant Science*, **9**,
425 1006. https://doi.org/10.3389/fpls.2018.01006
426 Yehia, I. (2008). Factors affecting the design of coating machine for crop seeds. *Misr Journal of*
427 *Agricultural Engineering*, **25**, 147-159.
428 Zeger S.I., and Liang K.-Y. (1986). Longitudinal data analysis for discrete and continuous
429 outcomes. *Biometrics*, **42**,121-130.

Figure Captions

Figure 1. Seedling emergence over time for controls and encapsulated treatments including Capsule only, and capsules with controlled-release fertilizers (Coor's, Florikan, and Osmocote). Data are presented as means ± 1 SE. Significant differences among treatments, based on GEEs following a repeated-measures design, are identified by letters following the name of the treatment listed in the legend, wherein different letters identify significant differences among the treatments (n = 10).

Figure 2. Plant height over time for controls and encapsulated treatments including Capsule only, and capsules with controlled-release fertilizers (Coor's, Florikan, and Osmocote). Data are presented as means ± 1 SE. Significant differences among treatments, are identified by letters following the name of the treatment listed in the legend, wherein different letters identify significant differences among the treatments (n = 10).

Figure 3. Cumulative flower production per plant (panel-A), and cumulative fruit production (both ripen and unripen) per plant (panel-B) for controls and encapsulated treatments including Capsule only, and capsules with controlled-release fertilizers (Coor's, Florikan, and Osmocote). Data are presented as means ± 1 SE. Significant differences among treatments, are identified by letters following the name of the treatment listed in the legend for panel-A, wherein different letters identify significant differences among the treatments (n = 10).

Figure 4. Number of ripen-fruit harvested per plant (panel-A), total ripen-fruit mass (fw) harvested per plant (panel-B), and average fruit mass (fw) for controls and encapsulated treatments including Capsule only, and capsules with controlled-release fertilizers (Coor's, Florikan, and Osmocote). Data are presented as means ± 1 SE. Significant differences among treatments, are identified by letters above the bars, wherein different letters identify significant differences among the treatments (n = 10).

Figure 1

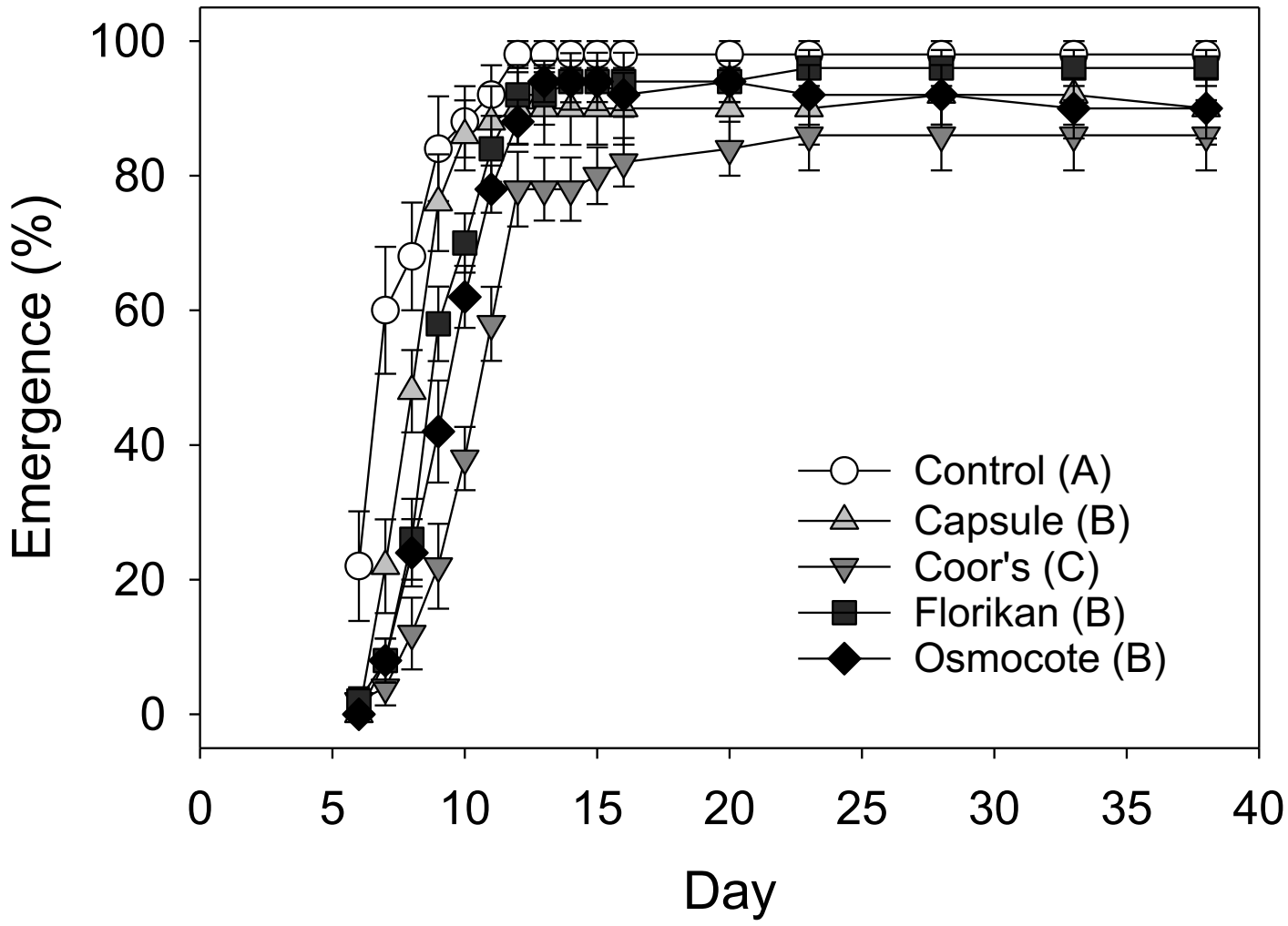
Emergence (%)
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Day
Control (A)
Capsule (B)
Coor's (C)
Florikan (B)
Osmocote (B)

Figure 2

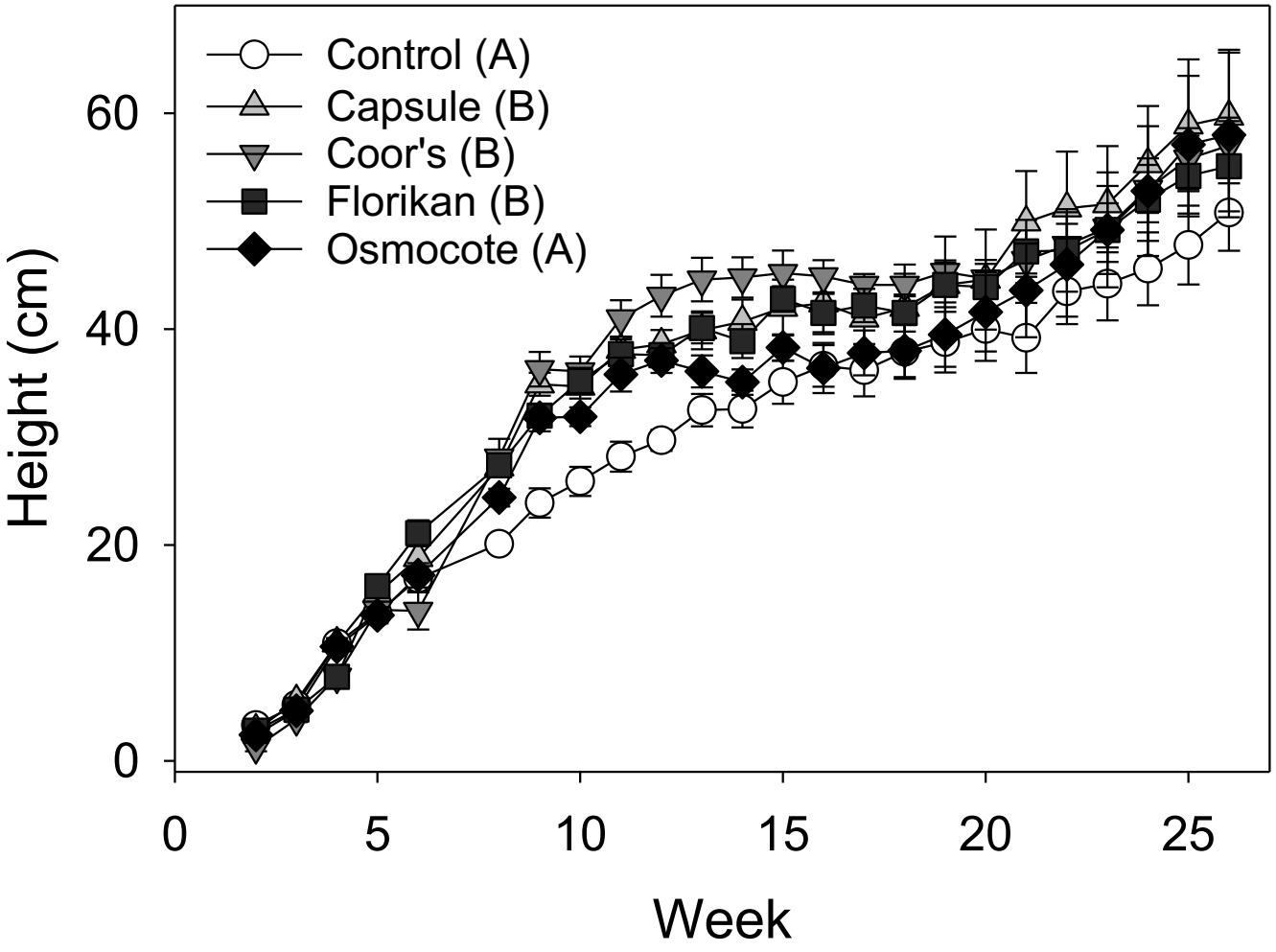
Control (A)
Capsule (B)
Coor's (B)
Florikan (B)
Osmocote (A)
Height (cm)
0
20
40
60
0
5
10
15
20
25
Week

Figure 3

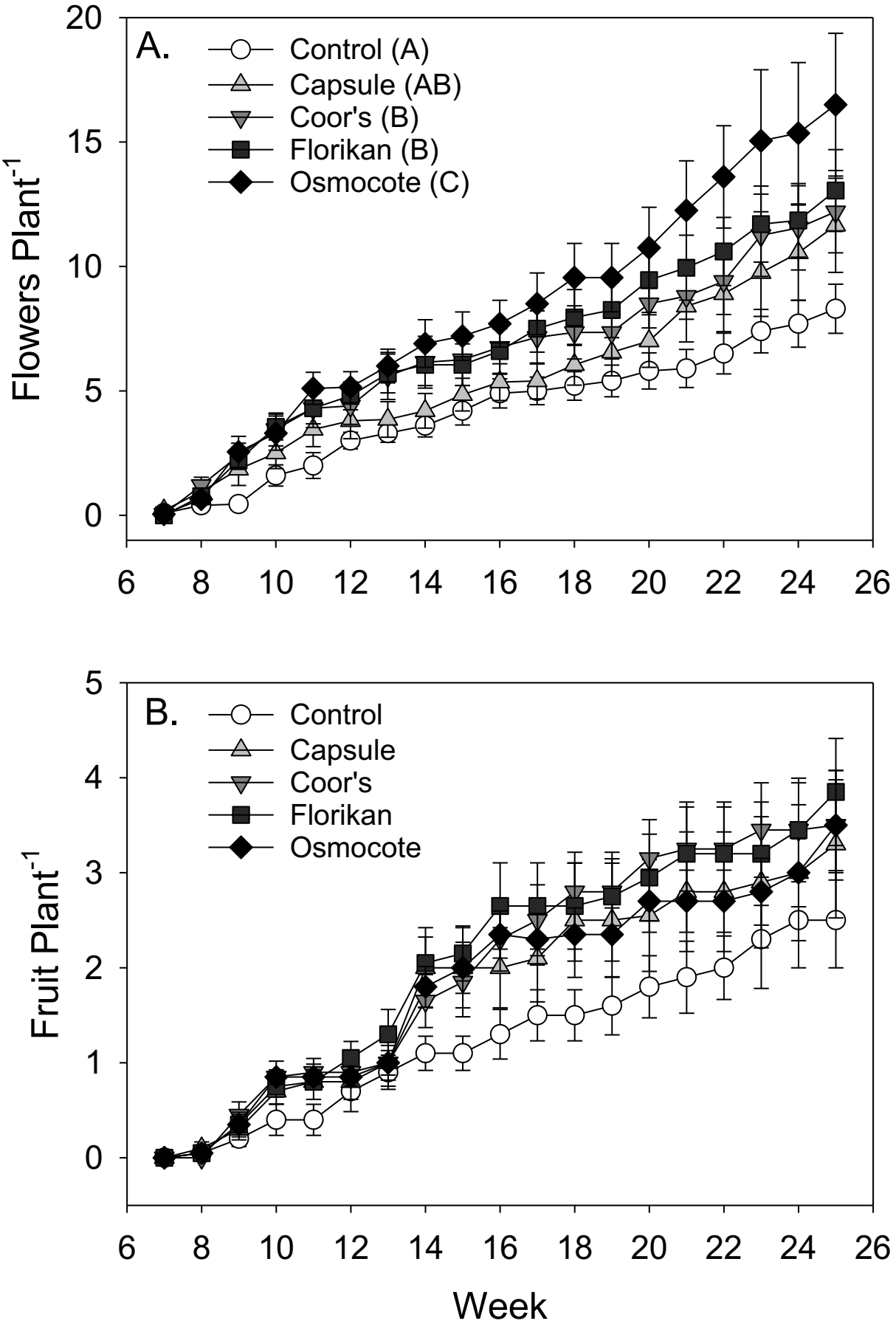
A.
Control (A)
Capsule (AB)
Coor's (B)
Florikan (B)
Osmocote (C)
Flowers Plant-1
0
5
10
15
20
6
8
10
12
14
16
18
20
22
24
26
B.
Control
Capsule
Coor's
Florikan
Osmocote
Fruit Plant-1
0
1
2
3
4
5
Week

Figure 4

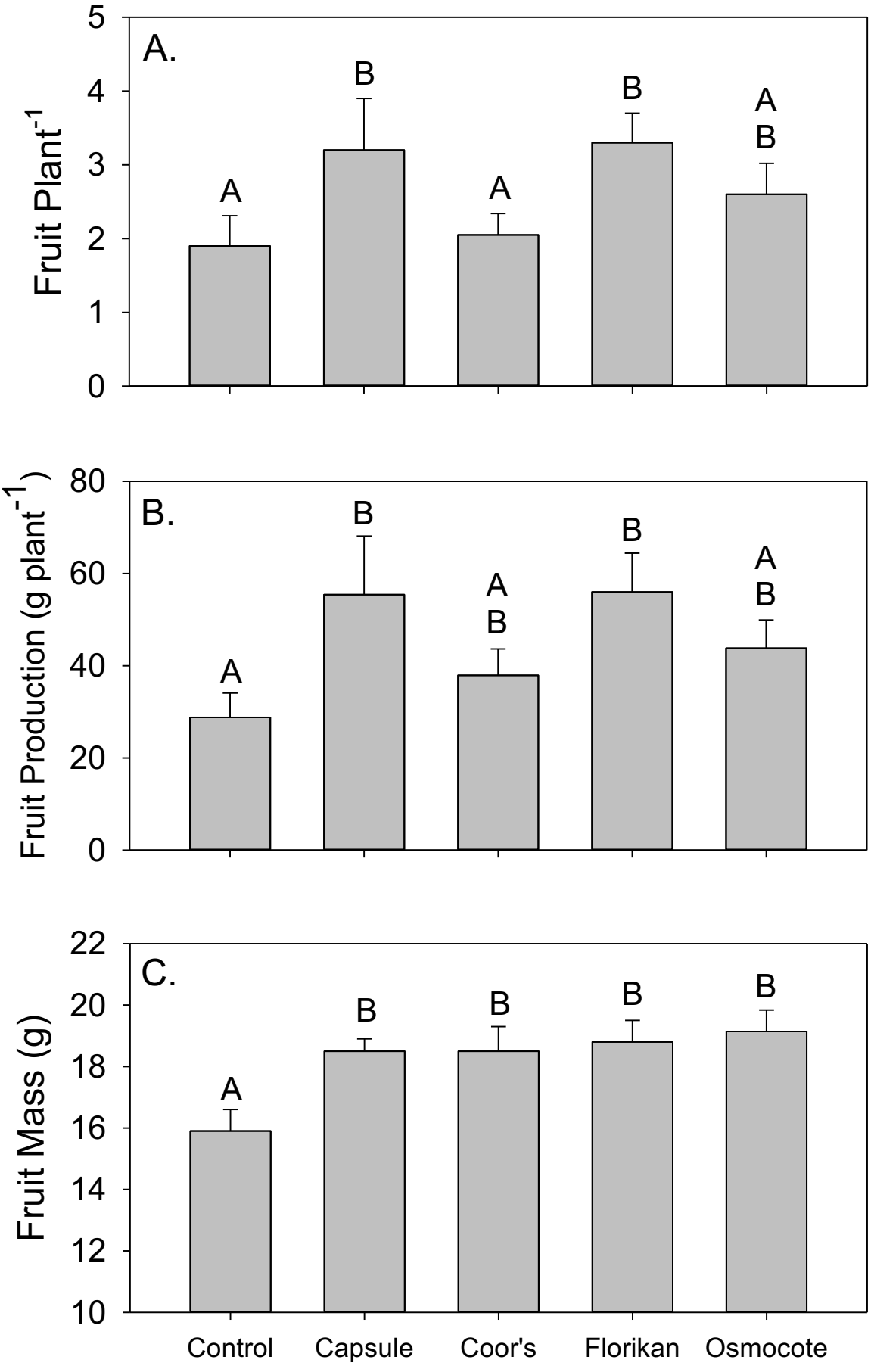
A.
Fruit Plant-1
5
4
3
2
1
0
A
B
A
B
A
B
B.
Fruit Production (g plant-1)
80
60
40
20
0
A
B
A
B
B
A
B
C.
Fruit Mass (g)
22
20
18
16
14
12
10
A
B
B
B
B
Control
Capsule
Coor's
Florikan
Osmocote

Table 1. Seedling emergence including number of days until first observed (First Emerge), 3 out of 5 Emerged (60%; Third Emerge), 5 out of 5 emerged (100%; Fifth Emerge), and total percent emergence after 30 days. Treatments included control, encapsulated seeds (Capsule), and seeds encapsulated with controlled-release fertilizers (Coor's, Florikan, and Osmocote). Data are presented as means ± 1 SE. Significant differences from the controls are identified by asterisks ($\alpha = 0.05$). Note, 10 replicates for all parameters, except Fifth Emerge where $N \geq 6$.

Parameter	Control	Capsule	Coor's	Florikan	Osmocote
First Emerge (d)	6.5 ± 0.17	7.4 ± 0.16*	8.6 ± 0.43*	7.7 ± 0.30*	7.9 ± 0.31*
Third Emerge (d)	7.7 ± 0.47	8.9 ± 0.35*	11.1 ± 0.31*	9.4 ± 0.27*	9.8 ± 0.25*
Fifth Emerge (d)	9.3 ± 0.66	9.7 ± 0.31	17.0 ± 2.49*	11.6 ± 0.26*	12.2 ± 0.31*
Emergence (%)	98 ± 2.0	90 ± 5.4	86 ± 5.2	96 ± 2.7	90 ± 4.5

Table 2. Dry mass measured on plants including aboveground-, belowground-, and total- dry mass, and root/shoot mass ratios for control and treated plants (capsule, Coor's, Florikan, and Osmocote). Plants were harvested on 3-, 7-, 10-, and 26- weeks after planting. Data are presented as means ± 1 SE. Significant differences from the controls are identified by asterisks ($\alpha = 0.05$).

Parameter (wk)	Control	Capsule	Coor's	Florikan	Osmocote
Aboveground Mass (g)					
Wk-3	0.11 ± 0.02	0.11 ± 0.01	0.04 ± 0.01*	0.08 ± 0.01	0.07 ± 0.01*
Wk-7	0.65 ± 0.11	0.74 ± 0.07	0.54 ± 0.12	1.03 ± 0.01*	0.90 ± 0.15
Wk-10	0.74 ± 0.10	1.02 ± 016	0.66 ± 0.19	1.46 ± 0.16*	0.95 ± 0.34
Wk-26	3.09 ± 0.31	3.69 ± 0.52	4.02 ± 0.49	4.82 ± 0.41*	3.27 ± 0.53
Belowground Mass (g)					
Wk-3	0.03 ± 0.01	0.06 ± 0.01*	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Wk-7	0.23 ± 0.03	0.27 ± 0.03	0.25 ± 0.05	0.56 ± 0.08*	0.43 ± 0.05*
Wk-10	0.22 ± 0.04	0.34 ± 0.07	0.23 ± 0.03	0.73 ± 0.12*	0.34 ± 0.12
Wk-26	1.26 ± 0.14	1.90 ± 0.29*	1.94 ± 0.30*	2.59 ± 0.30*	1.63 ± 0.13
Total Mass (g)					
Wk-3	0.14 ± 0.03	0.17 ± 0.02	0.07 ± 0.01*	0.14 ± 0.02	0.11 ± 0.01
Wk-7	0.88 ± 0.13	1.01 ± 0.09	0.80 ± 0.15	1.59 ± 0.14*	1.32 ± 0.18*
Wk-10	0.95 ± 0.13	1.36 ± 0.22	0.98 ± 0.22	2.18 ± 0.23*	1.21 ± 0.46
Wk-26	4.35 ± 0.42	5.59 ± 0.77	5.96 ± 0.69	7.41 ± 0.67*	4.90 ± 0.59
Root/Shoot					
Wk-3	0.32 ± 0.03	0.68 ± 0.14*	0.89 ± 0.11*	0.69 ± 0.11*	0.68 ± 0.11*
Wk-7	0.38 ± 0.05	0.38 ± 0.04	0.53 ± 0.08	0.55 ± 0.07	0.56 ± 0.09
Wk-10	0.29 ± 0.03	0.34 ± 0.05	0.37 ± 0.07	0.51 ± 0.07*	0.40 ± 0.06
Wk-26	0.42 ± 0.03	0.52 ± 0.05	0.50 ± 0.05	0.54 ± 0.06	0.58 ± 0.08