



Gelatin capsules as a delivery system for tomato (*Lycopersicon esculentum*) seed enhancements

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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 examined 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, minimised human exposure to agrochemicals, and improved uniformity for mechanical planters. The objectives of this study were to determine if seed encapsulation alters seedling emergence, plant performance and serves as a delivery-system for controlled-release fertilizers. The results demonstrate that seed encapsulation delayed initial plant emergence by one day, and between one and two days for encapsulation with fertilizer treatments. Gelatin capsules alone in comparison with the control 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, 18:6:8) provided greater aboveground, belowground and total plant mass. The results of this study support the concept that seed encapsulation can improve tomato performance, and that other component(s) can be successfully delivered to provide additional plant benefits.

Keywords: biostimulant, fertilizer, plant growth, protein hydrolysates, seed coating

Introduction

Post-harvest modifications of seeds used to improve germination and/or plant performance are often referred to as 'seed enhancements' (Taylor *et al.*, 1998; Afzal *et al.*, 2020). This characterisation also applies to any seed modification that facilitates the delivery of seeds along with other beneficial components employed during planting. While broadly defined, seed enhancements can be categorised into three general techniques (i) pre-sowing hydration or liquid priming; (ii) seed conditioning; and (iii) seed coatings (Taylor *et al.*,

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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 plant 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). Film coating was adapted from the pharmaceutical industry and involves the uniform deposition of polymers, plasticisers and colourants forming a film that acts as a physical barrier and often weighs less than 10% of the total seed mass (Taylor *et al.*, 1998; Pedrini *et al.*, 2017). The reduced friction among film 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 in film coatings, however, is not as great as those achieved through seed encrusting and pelleting. Consequently, depending on the nature of the applied chemical(s), film-coatings may be more toxic or inhibitory to seeds of some crops (Taylor *et al.*, 1998; Hill, 1999). Nevertheless, 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 and planted as a single unit (Cox, 2014). This seed encapsulation approach may combine the benefits of both pelleting and film 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 (thus reducing potential phytotoxicity); and minimize exposure of workers to agrochemicals. Pharmaceutical capsules are commercially available 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 writing), and do not require specialised equipment such as fluidised bed, rotary coater or rotating pan (Yehia, 2008; Pedrini *et al.*, 2017). 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 in a variety of crops (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 efficacy 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) characterise differences in tomato performance following emergence of encapsulated seeds; (iii) evaluate the use of seed encapsulation as a means to deliver 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 with a 0.90 mL capacity; Capsuline Inc., Dania Beach, FL) with or without controlled-released fertilizers (figure 1). Capsules, seeds, fillers and fertilizers were assembled using a semi-automatic capsule filling machine (CN-100M; iPharmachine, Zhejiang, China). Fertilizer treatments involved three different manufactures including Coor (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; table 1). Two fertilizer prills were placed in each capsule, which accounted for approximately 45, 100 and 55 mg fertilizer per capsule for Coor (C.COR), Florikan (C.FLR) and Osmocote (C.OSM), respectively. For all encapsulated treatments (including without fertilizer; C.NO), the remaining void space within the capsule was loosely filled with a dried mixture consisting of compost (60%) and peat (40%).

To evaluate seedling emergence, five seeds (CTRL) or five encapsulated seeds (C.NO; C.COR, C.FLR and C.OSM) 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 (10 pots per treatment) and were placed in a randomised 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

Seedling 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 characterised 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 fourth 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 then weighed.

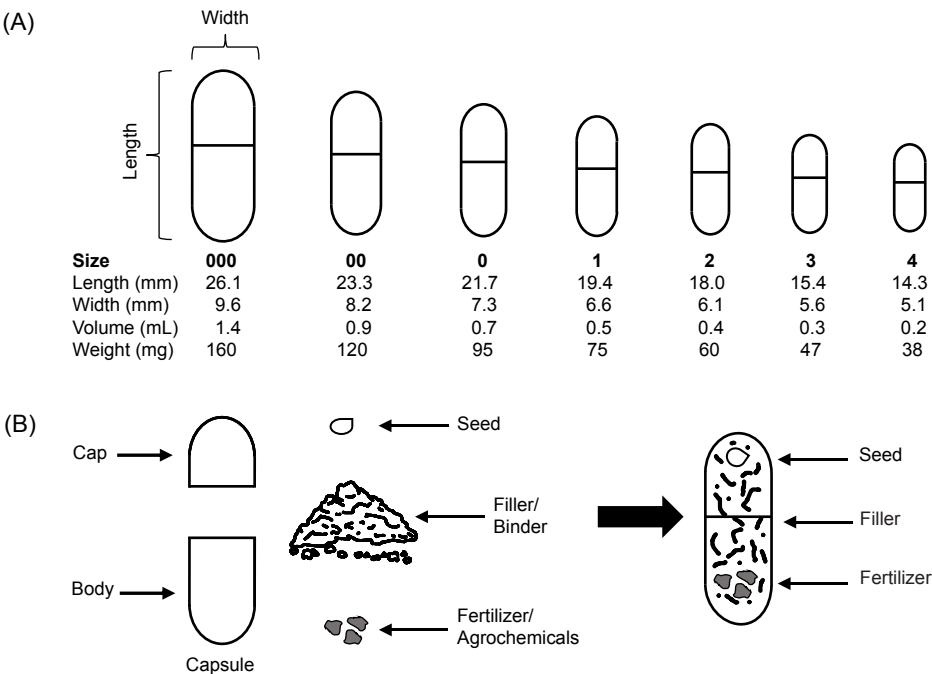


Figure 1. (A) Characteristics of different capsules including overall length, width, volume and weight. Note size 00 gelatin capsules were employed in this study. (B) Assembly features for seed encapsulation using tomato seeds, filler and fertilizer (agrochemicals) used in this study. A notable advantage of seed encapsulation is the separation between seed and fertilizers / agrochemicals provided by the filler/binder material.

Table 1. Characteristics of the controlled-release fertilizers and gelatin capsules applied to tomato cv. ‘Early Girl’ seeds, including N-P-K composition, additional elements ($\leq 5\%$ composition), release time, coating material and quantity applied for each treatment.

Fertilizer/ capsule	N-P-K	Additional elements	Release time	Coating material	Applied (mg)
Capsule	16-0-0	Ca, K, Na	Immediate	Gelatin	120
Coor	13-13-13	Fe, S, Zn	6-month	Sulphur-coated urea	45
Florican	18-6-8	S, Mg, Fe, Mn, Cu, B, Mo	12-month at 25°C	Polyolefin resin	100
Osmocoat	14-14-14	Ca, S	3-4 month at 21°C	Dicyclopentadiene, glycerol ester	55

Fruit production was recorded throughout the study as 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 colouration) it was harvested, and fresh weight was immediately recorded.

Data analyses

Seedling emergence data, including number of days until 1st, 3rd and 5th seedling emergence (for pots with 100% germination) were statistically analysed using generalised linear models (GLM). Wald χ^2 tests for pairwise evaluations were conducted when significant treatment responses were identified by GLMs.

For longitudinal data including seedling emergence/and survival over time, changes in plant height, cumulative flower and fruit production, and biomass (recorded over 4 different intervals), we employed generalised estimating equations (GEE), which is an extension of GLMs designed for repeated-measures analyses (Zeger and Liang, 1986; Ballinger, 2004), to compare CTRL 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 χ^2 tests were performed on parameters identified by GEE to have significant treatment responses.

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 CTRL against encapsulated treatments (C.NO; C.COR, C.FLR and C.OSM) using GLMs, followed by Wald χ^2 tests when treatment differences were detected. All statistical analyses were conducted using SPSS software version 26 (IBM Corp.), where comparisons were considered significant at an $\alpha = 0.05$.

Results

Seedling emergence

When considered over time, there were significant differences in seedling emergence among the CTRL and encapsulated treatments ($P < 0.001$). Seedling emergence was earlier for CTRL in comparison to other treatments. C.COR treated seeds, particularly, lagged behind other treatments in both timing of germination and total emergence (figure 2). The number of days (after sowing) until first emergence was also different among the CTRL and experimental treatments. In this case, there was a one-day delay in emergence for C.NO, C.FLR and C.OSM treatments, compared to the CTRL ($P = 0.021$, 0.002 and < 0.001 , respectively), and a two-day delay in emergence for C.COR ($P < 0.001$).

The number of days until complete (100%) seedling emergence also revealed significant treatment delays ($P < 0.001$). While there were no differences in the number of days for complete emergence between CTRL and C.NO (both approximately nine days; $P = 0.789$), the encapsulated fertilizer treatments were delayed by 2-, 3- and 8-days for C.FLR, C.OSM and C.COR treatments, respectively (figure 2; $P \leq 0.046$). Nevertheless, when the total seedling emergence after 30 days was considered, although there was a trend of lower emergence in C.COR (86% germinated), there were no statistical differences among the CTRL and encapsulated treatments (figure 2; $P = 0.190$).

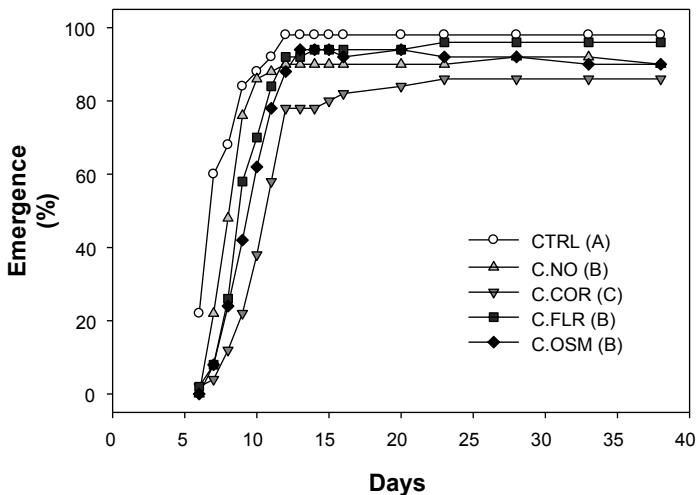


Figure 2. Emergence of tomato cv. 'Early Girl' seedlings over time for control treatment (CTRL; seeds not encapsulated) and encapsulated treatments including C.NO (encapsulated seeds, no fertilizer) and C.COR, C.FLR and C.OSM (encapsulated seeds with controlled-release fertilizers, Coor, Florikan and Osmocote). 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 ($n=10$ pots per treatment, each with five seeds or encapsulated seeds).

Plant height

Plant growth over time, as indicated by a change in overall height, was different among the CTRL and encapsulated treatments ($P=0.005$). Although there were no differences in plant height between the CTRL and C.OSM ($P=0.162$), the remaining treatments (C.NO, C.COR, and C.FLR) had taller plants throughout most of the study (figure 3; $P\leq 0.018$). Differences in plant height were most pronounced between 7- and 14-weeks, where there was a clear separation between CTRL and experimental treatments. By 14-weeks, the height of the CTRL plants began to match those of some encapsulated treatments and appeared to be associated with a lag in plant growth for encapsulated plants around the time of flower induction (~ 8 to 9 weeks).

Flowers and fruit production

There were notable enhancements in the total number of flowers produced per plant for fertilized treatments (figure 4A). While there were no statistically significant differences in flowers produced per plant over time between CTRL and C.NO ($P=0.167$), C.COR, C.FLR and C.OSM treatments had a 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, CTRL and C.NO 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 C.COR, C.FLR and C.OSM treatments, respectively (figure 4A).

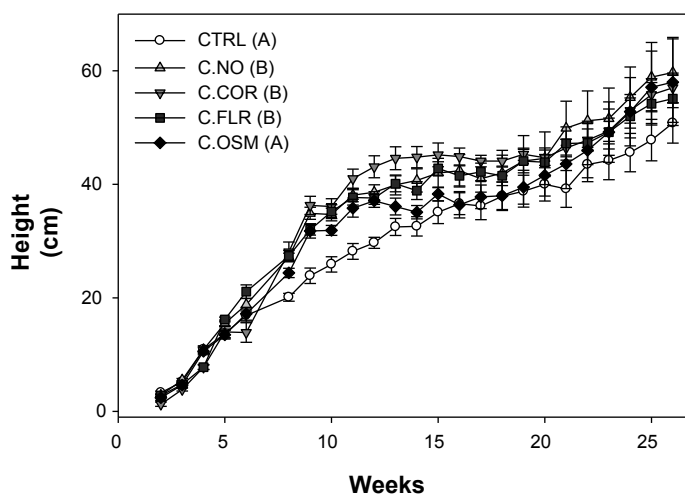


Figure 3. Change in height of tomato cv. 'Early Girl' plants over time for control treatment (CTRL; seeds not encapsulated) and encapsulated treatments including C.NO (encapsulated seeds, no fertilizer) and C.COR, C.FLR and C.OSM (encapsulated seeds with controlled-release fertilizers, Coor, Florikan and Osmocote). Data are presented as means \pm 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 ($n=10$ pots per treatment, each with five seeds or encapsulated seeds).

The elevated flower production in fertilized treatments, however, did not translate into a significantly higher number of fruit (both unripe and ripe) produced per plant over the same 26-week period; although a trend of more fruit per plant was recorded in encapsulated treatments (figure 4B; $P=0.069$). Interestingly, when only ripe-harvested fruit was considered (i.e., not including green immature fruit that remained on plants), there were significantly more fruit produced by C.NO and C.FLR treatments compared to the CTRL ($P=0.032$ and 0.018 , respectively; figure 5A). Similarly, when considering total ripe fruit mass produced per individual plant (g plant^{-1}), both C.NO and C.FLR produced more fruit by mass than the CTRL ($P=0.011$ and 0.007 , respectively; figure 5B). For all encapsulated treatments, the fresh weight of ripe fruit was greater than those measured in the CTRL ($P\leq 0.014$; figure 5C).

Biomass

There were differences in plant mass among CTRL and encapsulated treatments. By the third week, aboveground dry mass in CTRL plants was greater than that of both C.COR and C.OSM treatments (table 2; $P\leq 0.034$). During this time, however, the C.NO treatment had twice as much belowground biomass compared with CTRL ($P=0.008$), and all encapsulated treatments (with and without fertilizers) had more than two-times the root/shoot ratios observed in the CTRL (ratio of 0.32 ± 0.03 in the CTRL, compared to values at or above 0.68 for encapsulated treatments; table 2; $P\leq 0.014$). At week-7 and beyond, these biomass characteristics began to change. At that point, aboveground

biomass among CTRL and encapsulated treatments were no longer statistically different, except for the C.FLR, which was statistically greater than the CTRL ($P \leq 0.007$). Similarly, belowground biomass was greater in C.FLR treatments relative to the CTRL for week-7, -10, and -26 ($P < 0.001$), and periodic increases in belowground biomass were also observed in C.NO (week-26; $P = 0.045$), C.COR (week-26; $P = 0.033$) and C.SOM (week-7; $P = 0.004$; table 2). Total plant biomass closely mirrored aboveground biomass, with lower mass in the C.COR treatment compared with CTRL at week-3 ($P = 0.002$), followed by significant increases in total biomass for C.FLR (week-7 through week-26; $P < 0.001$) and C.OSM (week-7; $P = 0.018$) treatments.

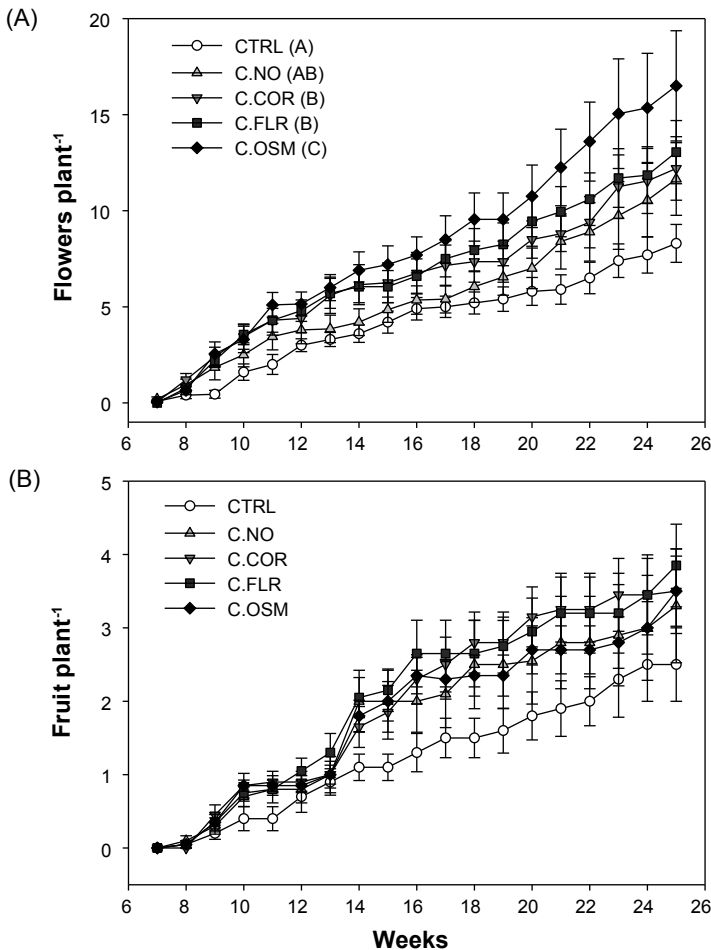


Figure 4. (A) Cumulative flower and (B) fruit (ripe and unripe production per tomato cv. 'Early Girl' plant for control treatment (CTRL; seeds not encapsulated) and encapsulated treatments including C.NO (encapsulated seeds, no fertilizer) and C.COR, C.FLR and C.OSM (encapsulated seeds with controlled-release fertilizers, Coor, Florikan and Osmocote). Data are presented as means \pm 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 ($n = 10$ pots per treatment, each with five seeds or encapsulated seeds).

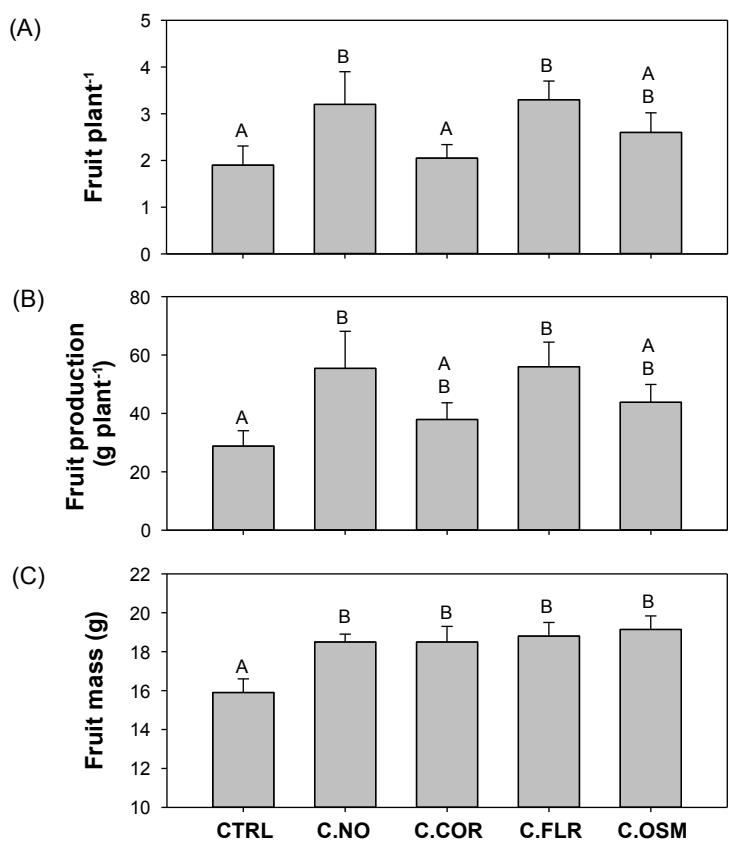


Figure 5. (A) Number of ripe fruits harvested per tomato cv. ‘Early Girl’ plant, (B) total ripe-fruit mass (fresh weight) harvested per plant and (C) average fruit mass (fresh weight) for control treatment (CTRL; seeds not encapsulated) and encapsulated treatments including C.NO (encapsulated seeds, no fertilizer) and C.COR, C.FLR and C.OSM (encapsulated seeds with controlled-release fertilizers, Coor, Florikan and Osmocote). Data are presented as means \pm 1 SE. Significant differences among treatments, are identified by letters above the bars, wherein different letters identify significant differences among the treatments (n = 10 pots per treatment, each with five seeds or encapsulated seeds).

Discussion

Gelatin seed encapsulation was investigated as a technique for seed enhancement in tomatoes. The use of pharmaceutical gelatin capsules alone (C.NO) provided some plant benefits including better root development within the first three weeks (table 2), higher plant growth within the first 12 weeks (as indicated by changes in height; figure 3) and improved fruit production (figure 5). 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). Interestingly, increased expression of amino acid and nitrogen transporter genes was observed in gelatin treated cucumber seedlings, suggesting a possible mechanism for gelatin-induced growth enhancement (Wilson *et al.* 2015). These reported growth and performance benefits, however, are not restricted to tomatoes and cucumbers, as other studies have shown beneficial biostimulant-like responses involving animal- or plant-derived protein hydrolysates in arugula, broccoli, 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; Wilson *et al.*, 2018).

Table 2. Dry mass of tomato cv. ‘Early Girl’ plants including aboveground-, belowground- and total- dry mass, and root/shoot mass ratios for control (CTRL; seeds not encapsulated) plants and plants from encapsulated seeds (C.NO, C.COR, C.FLR and C.OSM). Plants were harvested at 3, 10 and 26 weeks after planting. Data are presented as means \pm 1 SE. Significant differences from CTRL are identified by asterisks ($\alpha = 0.05$).

Time from planting (weeks)	CTRL	C.NO	C.COR	C.FLR	C.OSM
Aboveground mass (g)					
3	0.11 \pm 0.02	0.11 \pm 0.01	0.04 \pm 0.01*	0.08 \pm 0.01	0.07 \pm 0.01*
7	0.65 \pm 0.11	0.74 \pm 0.07	0.54 \pm 0.12	1.03 \pm 0.01*	0.90 \pm 0.15
10	0.74 \pm 0.10	1.02 \pm 0.16	0.66 \pm 0.19	1.46 \pm 0.16*	0.95 \pm 0.34
26	3.09 \pm 0.31	3.69 \pm 0.52	4.02 \pm 0.49	4.82 \pm 0.41*	3.27 \pm 0.53
Belowground mass (g)					
3	0.03 \pm 0.01	0.06 \pm 0.01*	0.03 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.01
7	0.23 \pm 0.03	0.27 \pm 0.03	0.25 \pm 0.05	0.56 \pm 0.08*	0.43 \pm 0.05*
10	0.22 \pm 0.04	0.34 \pm 0.07	0.23 \pm 0.03	0.73 \pm 0.12*	0.34 \pm 0.12
26	1.26 \pm 0.14	1.90 \pm 0.29*	1.94 \pm 0.30*	2.59 \pm 0.30*	1.63 \pm 0.13
Total mass (g)					
3	0.14 \pm 0.03	0.17 \pm 0.02	0.07 \pm 0.01*	0.14 \pm 0.02	0.11 \pm 0.01
7	0.88 \pm 0.13	1.01 \pm 0.09	0.80 \pm 0.15	1.59 \pm 0.14*	1.32 \pm 0.18*
10	0.95 \pm 0.13	1.36 \pm 0.22	0.98 \pm 0.22	2.18 \pm 0.23*	1.21 \pm 0.46
26	4.35 \pm 0.42	5.59 \pm 0.77	5.96 \pm 0.69	7.41 \pm 0.67*	4.90 \pm 0.59
Root / Shoot					
3	0.32 \pm 0.03	0.68 \pm 0.14*	0.89 \pm 0.11*	0.69 \pm 0.11*	0.68 \pm 0.11*
7	0.38 \pm 0.05	0.38 \pm 0.04	0.53 \pm 0.08	0.55 \pm 0.07	0.56 \pm 0.09
10	0.29 \pm 0.03	0.34 \pm 0.05	0.37 \pm 0.07	0.51 \pm 0.07*	0.40 \pm 0.06
26	0.42 \pm 0.03	0.52 \pm 0.05	0.50 \pm 0.05	0.54 \pm 0.06	0.58 \pm 0.08

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 this 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 C.FLR and C.OSM, to as much as eight days for C.COR (figure 2). 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 may also inhibit germination and early seedling growth (Bremner and Krogmeier, 1989; Bremner, 1995). That is, under certain circumstances, elevated pulses of N, and to a lesser degree P, can adversely affect the growth and development of tomatoes (Magalhas and Wilcox, 1984; Jones, 1998; Barreto *et al.*, 2016). This idea was supported, in part, by the responses observed in the C.COR treatment including seedling emergence delays, lower aboveground mass within the first three weeks and comparatively lower ripened fruit production (table 2; figures 2 and 5). While the Coor fertilizer is approximately 13% nitrogen, only 8.4% is considered controlled-release and remaining nitrogen is in the form of conventional ammonia and urea. Ammonium nitrogen, when applied directly or as a hydrolytic biproduct of urea, has been shown to adversely affect seed germination (Openshaw, 1970; Bremner and Krogmeier, 1989). Perhaps this blend of conventional and controlled-release fertilizers produced an elevated pulse of nutrients that initially influenced germination and early growth. Nevertheless, aside from delays in emergence, the addition of small amounts of controlled-release fertilizers in gelatin capsules provided some additional benefits to tomatoes. This is especially true for C.FLR treatments, which consistently maintained higher aboveground, belowground and total plant dry mass (table 2).

Although animal-derived protein hydrolysates were shown to improve plant growth (Calvo *et al.*, 2014), it is unclear how these biostimulants would affect long-term flower and fruit production in tomato. In this study, flower and fruit numbers per plant are markedly low and is likely attributed to the use of a nutrient-poor sandy-loam substratum. Nevertheless, the results suggest that tomatoes, with seeds initially encapsulated with controlled-release fertilizers, could produce more flowers, depending on the fertilizer used (figure 4). While the total number of fruit produced per plant (both ripen and unripen) was not significantly different among the treatments, both C.NO and C.FLR treatments did produce more mature/harvested fruit (figure 5). 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 CTRL 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 C.NO 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). This study considered an alternative approach using pharmaceutical gelatin capsules to encase seeds along with other plant-benefiting components. Manual seed encapsulation is easily performed using semi-automated filling machines where 300 capsules can be processed in 10 to 15 minutes (basic units cost less than 300 US dollars; at the time of writing). For larger scale seed encapsulation, 25,000 seeds per hour can be encapsulated with control-released fertilizers and/or other plant-benefiting agrichemicals using modified commercially available (e.g., Bosch Inc., Gerlingen-Schillerhöhe, Germany) fully-automated capsule filling machines (Cox, patent pending). Depending on seed size, capsules can provide sufficient void space to include greater volumes and/or multiple types of plant-benefitting components. Gelatin alone has been shown to act 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. 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 idea 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 best ways to utilize this technology, especially its roles in seed enhancement and as a delivery system of plant-benefitting materials.

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References

- Afzal, I., Javed, T., Amirkhani, M. and Taylor, A.G. (2020). Modern seed technology: Seed coating delivery systems for enhancing seed and crop performance. *Agriculture*, **10**, 526. <<https://doi.org/10.3390/agriculture10110526>>
- Amirkhani, M., Netravali, A.N., Huang, W. and Taylor, A.G. (2016). Investigation of soy protein based bio-stimulant 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. <<https://doi.org/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), volume 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., Roupheal, 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. <<https://doi.org/10.1002/jsfa.6239>>
- Cox, D. (2014). *Inventor, Klondike Agricultural Products, Agricultural System, United States Patent US 8,683,742 B1*.
- Cox, D. (patent pending). *Methods and Device for Placing Seeds in Capsules*, Submitted to the United States Patent and Trademark Office.
- 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 hydrolysates 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*, (eds. C. Poffenberger and J. Heuser), ASTM International. <<https://doi.org/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](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>
- Incrocci, L., Maggini, R., Ceì, 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*, Introductory Paper at the Faculty of Landscape Architecture, Horticulture and Crop Production Science, Sveriges Lantbruksuniversitet, Alnarp, 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., García-Martínez A.M., Friaiza, 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>>
- Pedrini, S., Merritt, D.J., Stevens, J. and Dixon, K. (2017). Seed coating: Science or marketing spin? *Trends in Plant Science*, **22**, 106-116.
- 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. and 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**, 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 sunflower achenes. *Crop Science*, **59**, 1182-1190. <<https://doi.org/10.2135/cropsci2018.06.0400>>
- Skwarek, M., Nawrocka, J., Lasoń-Rydel, M. and Ławińska, K. (2020). Diversity of plant biostimulants in plant growth promotion and stress protection in crop and fibrous plants. *Fibres and Textiles in Eastern Europe*, **28**, 34-41. <<https://doi.org/10.5604/01.3001.0014.0931>>
- Taylor, A.G., Allen, P.S., Bennett, M.A., Bradford, K.J., Burris, J.S. and Misra, M.K. (1998). Seed enhancements. *Seed Science Research*, **8**, 245-256. <<https://doi.org/10.1017/S0960258500004141>>
- Taylor, A.G., Eckenrode, C.J. and Straub, R.W. (2001). Seed coating technologies and treatments for onion: challenges and progress. *HortScience*, **36**, 199-205. <<https://doi.org/10.21273/HORTSCI.36.2.199>>
- Wilson, H.T., Xu, K. and Taylor, A.G. (2015). Transcriptome analysis of gelatin seed treatment as a biostimulant of cucumber plant growth. *The Scientific World Journal*, **2015**, 391234. <<https://doi.org/10.1155/2015/391234>>
- Wilson, H.T., Amirkhani, M. and Taylor, A.G. (2018). Evaluation of gelatin as a biostimulant seed treatment to improve plant performance. *Frontiers in Plant Science*, **9**, 1006. <<https://doi.org/10.3389/fpls.2018.01006>>
- Yehia, I. (2008). Factors affecting the design of coating machine for crop seeds. *Misr Journal of Agricultural Engineering*, **25**, 147-159.
- Zeger, S.I. and Liang, K.-Y. (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*, **42**, 121-130.