WORKING TITLE:

MECHANICAL STIMULATION CONTROLS CANOPY ARCHITECTURE AND IMPROVES VOLUME UTILIZATION EFFICIENCY IN BIOREGENERATIVE LIFE-SUPPORT CANDIDATE CROPS

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ABSTRACT

Key Words

Thigmomorphogenesis, Plant Dwarfing, *Capsicum annum* ‘California Wonder’, Pepper, Bioregenerative Life-Support

ABBREVIATIONS

VUE – Volume Utilization Efficiency
ALS – Advanced Life Support
MS – Mechanical Stimulation

1. INTRODUCTION

It has long been recognized that mechanical stimulation (MS; stress), such as wind action, rubbing, constriction, shaking, and encounters with physical barriers can have a dramatic influence on plant morphological development[1-5]. Jaffe (1973) demonstrated that daily MS to partially mature internode tissue, applied by rubbing stem tissue between two fingers, could induce dramatic reductions in internode length resulting dwarf phenotypes in a range of crop species. Jaffe (1973) coined the term thigmomorphogenesis, (*thigma* being the Greek word for *touch*) to describe these long term morphological responses to touch. Over the ensuing 40 years many others have followed up on Jaffe's work, most notably Cary Mitchell's group at Purdue (West Lafayette, IN, USA) and Janet Braam at Rice University (Houston, TX, USA). It is now known that thigmomorphogenesis includes a wide range of responses including, but are not limited to, shortening of internodes, stem thickening, reduced leaf expansion, changes in chlorophyll content, and alterations in plant hormone levels[5-11].

A significant amount of thigmomorphogenesis research conducted during the 1980s-90s was sponsored by the National Aeronautics and Space Administration (NASA)[1,4,10,12,13]. Given the physical rigours of spaceflight it is important to understand how mechanical and vibrational stimuli affected plant growth, and importantly, how MS could be used to counter the absence of a gravity vector that may otherwise result in plants with leggy growth or being susceptible to breakage[14]. The research findings were fairly consistent, at least in terms of the effects of MS on plant architecture; MS results in shorter more compact plants[3,11,13,15]. These findings were significant in that mass and volume are major limiting factors in the design and development of bioregenerative life-support systems. Taking advantage of thigmomorphogenesis to produce dwarf plant architectures, thereby reducing mass and volume requirements, is of interest.

Crops selected for use in bioregenerative systems need to conform to the many constraints of spaceflight. As noted, a major constraint are the extremely limited real estate available for plant production. Unlike most terrestrial agricultural applications that strive to optimize the use of a given *area* of arable land, spaceflight agriculture requires researchers to maximize *volume* use efficiency (VUE). This can be achieved through genetic manipulations, chemical interventions (e.g., exogenous growth regulators), the selection of dwarf cultivars, and through specific horticultural management practices utilizing standard crop species[16-20]. These dwarfing mechanisms are now being combined with advances in light emitting diode (LED) systems which, due to their cool operating temperature, allows for close proximity of the crop and light source, enabling significant improvements in VUE. Volume use efficiencies have improved to the point that viable stacked or vertical agricultural production industries have emerged, in addition to other applications such as molecular farming that often employ multi-layered or vertical production architectures (Goto, 2012).

Public and private efforts are rapidly advancing both the notion and the technology required to send humans to the Moon and Mars for extended periods. Bio-regenerative or advanced life-support (ALS) systems utilizing plants and other biological machinery to sustain human life have long been considered critical for such extended missions beyond low Earth orbit[21]. The plants and associated microbial communities in these bio-regenerative systems provide, in whole or in part, critical life-support services including food production, air revitalization (oxygen production and carbon dioxide removal), and wastewater recycling[22,23]. Modifying the architecture of any given crop, through such responses as thigmomorphogenesis, could help reduce the equivalent system mass (ESM) of bio-regenerative systems ultimately leading to viable 'agriculture in space' (Drysdale et al, 2003).

The objective of the presented study was to re-examine thigmomorphogenesis as a tool for improving VUE in bioregenerative life-support and vertical agriculture system designs, while adding to the knowledge base in this domain. The data obtained from the fruiting study were also used to generate a rudimentary VUE model for vertical agriculture applications, given recent advances (lighting) in controlled environment system technology.

2. MATERIALS & METHODS

Two experiments were conducted to determine the vegetative response, and fruiting response of *Capsicum annum* 'California Wonder' to mechanical stimulation (MS). Response data were used as a baseline for volume utilization efficiency calculations for hypothetical spaceflight and vertical agriculture applications.

2.1 Vegetative Response Study

***2.1.1 Plant Material Preparation and Growth Conditions**:* Four seeds of *Capsicum annum* 'California Wonder' (Lake Valley Seed Company Inc., Boulder, CO) were sown in each of 18, 1.67L pots containing a standard potting media (Fafard ProMix 2B, Sun Gro Horticulture Distribution Inc., Agawam, MA), in which 8.3 g/L of 18-6-8 slow release fertilizer (Nutricote Total, Florikan E.S.A. Corp., Sarasoda, FL) was incorporated. Plants were hand watered daily with deionized water and supplemented with a half strength Hoagland's solution twice per week for the duration of the trial. The chamber was maintained at a 23/20°C day/night temperature profile, a 16-h photoperiod, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, constant relative humidity of 65%, and 800 ppm CO_2.

2.1.2 Layout: Pots were randomly assigned to positions in a 3 x 6 grid within the growth chamber. Treatment levels (1-control; 2-mechanically stimulated) were randomly assigned to each grid position in a completely randomized design structure. The experiment was replicated to validate the results.

2.1.3 Treatment Application: Mechanical stimulation (MS) was initiated after a 1-week acclimation period following transplanting into pots. Tightly wrapped cotton-tipped inoculation sticks (InoculatorZ™, Biolog Inc., Hayward, CA) were used to apply gentle but firm strokes along each side of the most recently developed internode at an application rate of 10 stroke per side (total of 40). The internode was supported during the treatment by the placing two fingers on the internode opposite to the point of MS. The MS was applied twice daily on weekdays and once daily on weekends. Treatments were applied in the morning between 09:00-10:00 (2-3 h after lights came on) and in the evening (16:00-17:00), for the duration of the experiment. In order to avoid confounding the MS dwarfing effect with the amount of incident light between the treatments, the MS plants were placed on vertical risers, as needed, to ensure that the top of each of the 18 plants was at the same height. The treatments were applied for seven weeks after which the plants began to flower and the experiment was ended.

2.1.4 Measurements: After the seven-week treatment period plants were destructively harvested. Shoot fresh mass (SFM), shoot dry mass (SDM), root dry mass (RDM), leaf area (LA), number of leaves, height to first bifurcation, number of nodes to first bifurcation, total height, stem diameter at the cotyledon and sixth nodes, relative chlorophyll levels (SPAD), and number of flower initials were all measured the day of harvest.

2.2 Fruit Production Study

***2.2.1 Plant Material Preparation**:* Seeds of *Capsicum annum* 'California Wonder' (Lake Valley Seed Company Inc., Boulder, CO) were sown in mineral wool starter plugs (Grodan AO, Rockwool BV, Roermond, NL), placed in a germination tray, covered with a humidity dome, and placed in a controlled environment growth chamber (Environmental Growth Chambers, Chagrin Falls, OH. USA). The chamber was maintained at a constant 26°C during the germination period, with a 16-h photoperiod, relative humidity of 65%, and ambient CO_2 (chamber had CO_2 monitoring but no active control; levels ranged between 380-420 ppm). After two weeks, 10 seedlings were selected for uniformity and transplanted into 1.67L pots with media prepared as described in section 2.1.1. After 8 weeks' growth in the pots, all plants were pruned to open up the center of the plant to allow proper air movement. The leaf area, fresh mass, dry mass, and flowers on the removed tissue were measured and included in the final tally for each plant.

2.2.2 Layout: The light distribution in the chamber used for this study varied significantly along the long axis of the chamber, ranging from 240 -350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) at canopy height; blocking on light intensity was implemented to accommodate the lack of uniformity. Pots were randomly assigned to one of five block positions within the growth chamber. Treatment levels (1-control; 2-mechanically stimulated) were randomly assigned to one of the two positions within each block in a randomized complete block design structure.

***2.2.3 Treatment Application*:** Mechanical stimulation (MS) was initiated after a 1-week acclimation period following transplanting into 1.67 L pots. The MS was applied once daily in the morning within 2-3 hours of the lights coming on for the duration of the experiment (11-weeks). Once the plants had bifurcated, each of the most recently matured internodes on each branch received MS, with the application per internode reduced to 10 strokes. In order to avoid confounding the MS dwarfing effect with the amount of incident light between the treatments, the MS plants were placed on scissor lifts (Fisherbrand Lab Jacks, Fischer Scientific) to ensure that the top of the MS plant was at the same canopy height as the control plant in each block. As the plants began to branch, the uppermost internode on each primary branch received the MS. The MS was only applied once daily during this trial, with treatments applied between 09:00-10:00 for the 11-week duration (12-weeks total in pots) of the trial.

2.2.4 Measurements: After 12 weeks the plants were destructively harvested. Similar to the previous experiment SDM, LA, height to first bifurcation, number of nodes to first bifurcation, total height, diameter at first true leaf node, and SPAD readings were recorded. Additional fruit number, total fruit fresh mass, total fruit volume, and total fruit dry mass data was recorded for each plant. Resources were insufficient to allow leaf counts or root measurements during this study. Plants from each block were placed on a black drop cloth and photographed from above. A 100-cm ruler was included in the frame to allow post-harvest measurement of shoot diameters (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA).

3. RESULTS

3.1 VEGETATIVE STUDY

Significant reductions were observed for all growth metric, although the shoot to root dry mass ration did not differ (Fig. 1 A-H). The relative chlorophyll levels (SPAD) were significantly greater in MS leaves (Fig. 2). The stem thickness of MS plants increased at the first node, relative to the control plants, but the difference was reversed at the sixth node (Fig. 3)

3.2 FRUITING STUDY

The reductions in plant metrics observed in the vegetative study were not observed at the time of harvest of mature, fruit bearing plants, with the notable exception of total plant height (Fig. 4). The reduced number of flowers observed in the vegetative study was also noted in the fruiting study with significantly fewer fruit being produced by the MS plants (Fig.5 A). Although there were fewer flowers and resulting fruit produced, the total fruit volume, fresh mass and dry mass were not significantly different between the control and MS groups (Fig. 5 B-D)

4. CALCULATIONS

Data from the fruiting trial were used as the basis for volume use efficiency (VUE) calculations. The calculations focus on vertical components of VUE as crown diameter (area utilization), reductions, although statistically significant, were not considered practically significant except on extremely large scales; scales not likely to be realized in any practical spaceflight application. It is assumed that the vertical use improvements are additive with respect to VUE. It is accepted that the following calculations are relatively simplistic in that they assume static interactions between plants in terms of light competition and other environmental factors. In the growth study from which the data were gathered, care was taken to ensure a uniform access to light although neighbour shading did occur. In less regulated systems some plants height heterogeneity will increase resulting in further variation through shading and other competitive effects. Regardless, the exercise is valuable for highlighting the potential for using MS as a tool for improving VUE in controlled environment agricultural systems.

4.1 Vertical Use Calculations

The mean shoot heights for the control and MS plants were 59.0 ± SD 1.4 cm and 47.2 ± SD 1.5 cm respectively (Figure 4C), which translates to a 20% plant height reduction. For the purposes of this calculation the mean plus the standard deviation will be used in order to buffer the crop variance. Assuming that in a stacked plant production system there is a total fixed height requirement for lighting and rooting hardware of 30 cm, then the total vertical distance required for control and MS plants is approximately 90.4 cm (60.4 + 30 cm) and 78.7 cm (48.7 + 30 cm) respectively. This represents an overall reduction in system height of 12.9% under MS. Carrying this calculation forward, in the total height required to accommodate six stacked trays of control plants (6 x 90.4 cm = 542.4 cm; round to 555 cm), one additional layer could conceivably be included, if MS were employed (7 x 78.7 cm = 550.9 cm; Figure 6). Clearly this example is not feasible in current spaceflight scenarios given the nearly 6 m vertical distance required to realize the additional layer of plants; however, it is relevant to vertical farming in terrestrial settings where significant production increases could be achieved. Recognizing this spaceflight limitation, it still may be possible to grow plants otherwise unsuited for spaceflight production systems (e.g., Lada, Veggie, or proposed “salad machine” concepts) based on their crown architecture under conditions where MS is absent[24]. Applying MS to these plants may prevent them from outgrowing the plant production hardware, making them viable test species.

5. DISCUSSION

Mechanical stimulation of *Capsicum annum* (cv California Wonder) resulted in significant reductions in overall plant height in both the vegetative and fruiting study (Fig. 1H, 4C). The reductions were sufficient enough to realize improved VUE potential in life-support and other vertically integrated production systems (Fig. 6), although the mode of that improvement differs between terrestrial and spaceflight applications. The potential for improving VUE is greatest in terrestrial settings where large volumes (e.g., warehouse scale production facilities) can be exploited, such as the scales modelled in Fig. 6. Long term space applications, such as a growth chamber system on the Lunar or Martian surface, could also realize these VUE improvements. In the near-term, spaceflight cropping system applications will be tightly constrained in the vertical dimension, as well as the horizontal. This said, MS in concert with other interventions such as root restriction[18] or on its own, could be used to expand the species options for existing plant production hardware (e.g., Veggie) by reducing the vertical space requirements for typically taller crops such as *Capsicum spp.*

Crown diameter was also significantly reduced in the presented fruiting study, but unlike other horticultural interventions (e.g., root restriction) examined by the authors[18], the reductions were not sufficient in terms of area utilization to justify an increased planting density under conditions of MS. The observed crown diameter reductions would only result in improved plant densities on scales currently impractical for both terrestrial and spaceflight applications (e.g., 12 m wide production benches; calculations not shown).

Other vegetative production metrics were reduced under the MS treatment during the vegetative experiment (Figure 1-3); however, those vegetative differences did not persist or become evident in the fruiting trial (Fig. 4), with the notable exceptions of total height and stem thickness (Fig. 4H and E). Some of the discrepancy between the vegetative and fruiting studies may lie in an increase in light competition/shading effects. During the fruiting study, there was insufficient room at maturity to exclude all incidence of shading between neighbouring plants. This increased light competition may have dampened some of the thigmomorphogenic effects through shade adaptation responses which tend to elongate plants and increase leaf area[25,26].

In addition to being able to squeeze crop plants into a smaller volume and still maintain productivity, MS could also be used as a countermeasure to ensure crop plants develop structurally sound support tissue under microgravity conditions. Humans require significant countermeasure interventions to reduce the negative impacts of microgravity of bone and muscle tissue [27]; it stands to reason that crop plants making up part of a bioregenerative life-support system may also benefit from microgravity countermeasures. In the absence of a significant gravity vector plant cell walls and, by elaboration, supporting tissues (e.g., branches supporting fruit) can be modified, although consensus on the degree and direction of the modifications is elusive[14,28-32]. Very little (if any) research has examined the effects of direct mechanical stimuli on crop plants in a microgravity setting. Having said this, it should be noted that the vibrational environment of space research platforms, such as the International Space Station (ISS), do impose a certain baseline level of mechanical stimulation to all plant experiments, but it is low—typically

< 0.001 g acceleration. Cleary the approach taken for this study required human intervention for each plant, which would translate into “crew time” in space. But automated systems for applying thigmo- or seismic- stimuli might be envisioned. For example, allowing canopies to growth through a grid and mechanically shaking the grid each day to stimulate all the plants at once But such approaches would require validation for efficacy.

Given the importance that crop plants will play in the future of human exploration, it is imperative that attention be directed to all the various spaceflight environment parameters that will influences the ability of the crops to deliver their life-support functions. Concurrently, potential interventions, such as MS, that could contribute to improvements in VUE as well as providing countermeasures to the rigours of the spaceflight environment should be considered.

6. CONCLUSIONS

Thigmomorphogenesis can be utilized to improve volume utilization efficiency in peppers (*Capsicum annum* cv. California Wonder), a candidate crop for fresh food production in space. The effect occurred primarily through a reduction in average plant height. Reductions in vegetative growth metrics during the juvenile growth phase (growth leading up to and including early anthesis) were not observed during the mature or fruiting phase, with the notable exception of reduced plant height. Early flower production and fruit set was reduced under MS; however, the total edible biomass was not reduced, with MS plants producing fewer but larger fruits. The overall reduction in plant height due to MS was sufficient to realize theoretical improvements in VUE for large vertical farming systems. The reduced heights observed could improve VUE in single tier spaceflight hardware (e.g., Veggie; Massa 2016 (this issue)) in that crops that would not normally fit in these spaceflight systems may be accommodated if MS can be applied. Although the potential for using MS to induce thigmomorphogenic phenotypes has long been appreciated, it is only recently that the growth systems themselves could take advantage of the modified crop architecture associated with MS. It is with this in mind that renewed attention should be given to developing procedures for environmentally modifying crops for spaceflight applications.

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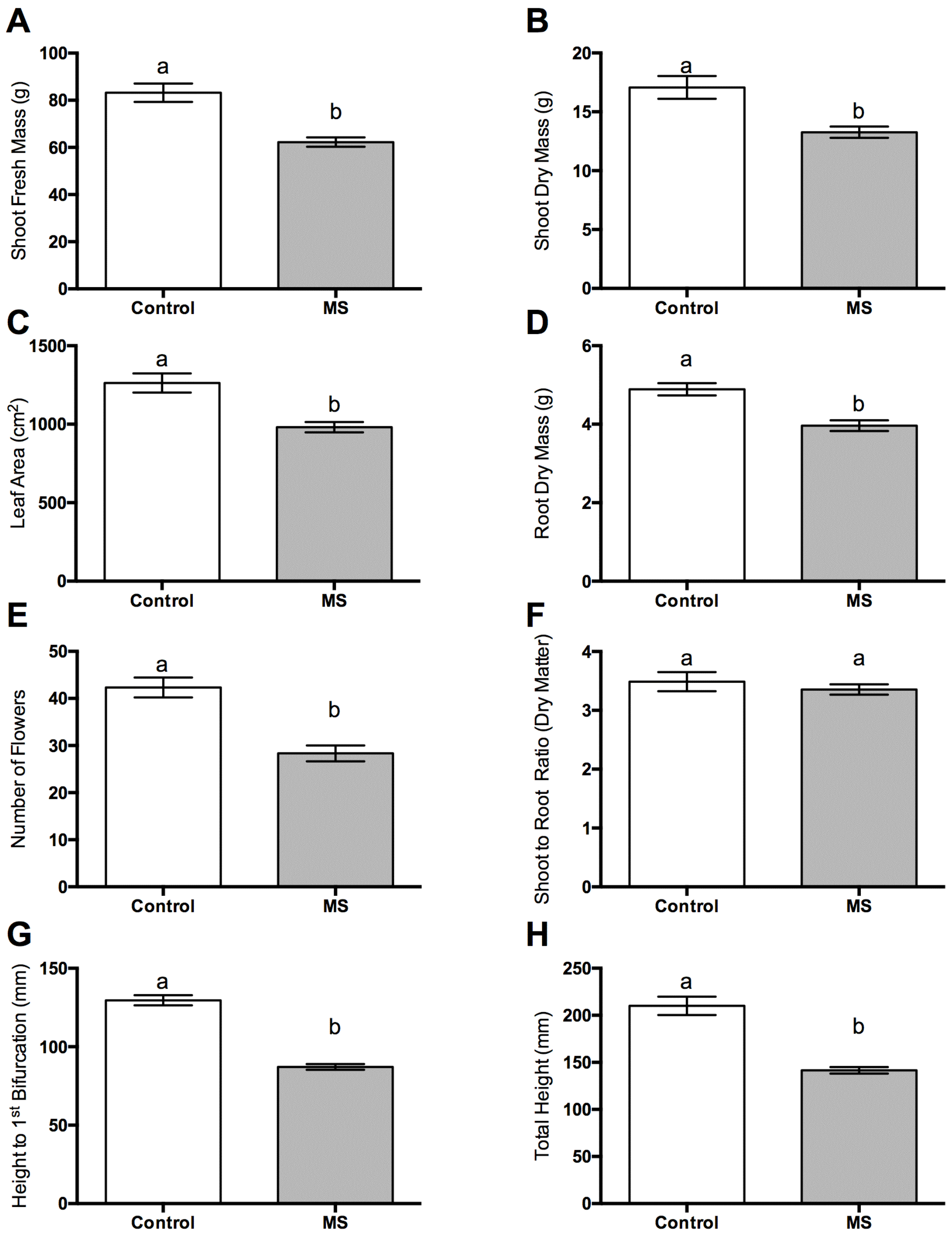

Figure 1: Plant growth response to mechanical stimulation during juvenile and early anthesis growth stages: (A) Shoot fresh mass; (B) Shoot dry mass; (C) Leaf area; (D) Root dry mass; (E) Flower production; (F) Shoot to root dry matter ratio; (G) Height at the first stem bifurcation; (H) Total height. Columns with the same letter appearing above do not differ at p≤0.05. Error bars are the SEM.

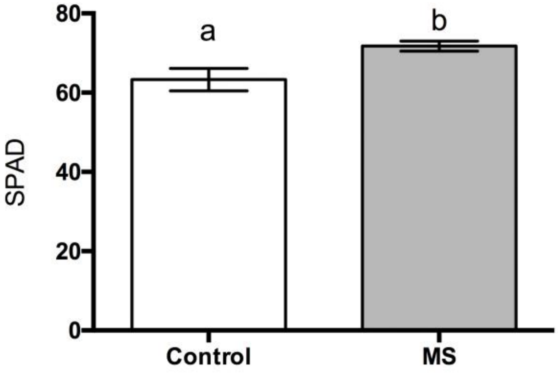

Figure 2: Relative chlorophyll levels (SPAD) in the last fully expanded leaf under control and mechanical stimulation treatments. Columns with the same letter appearing above do not differ at p≤0.05. Error bars are the SEM.

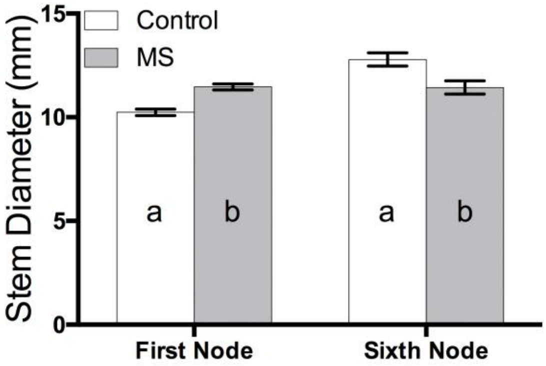

Figure 3: Stem diameter at the first and sixth node for control and mechanically stimulated pepper plants. Bars within each grouping (e.g., first node) with the same letter do not differ at p≤0.05. Error bars are SEM.

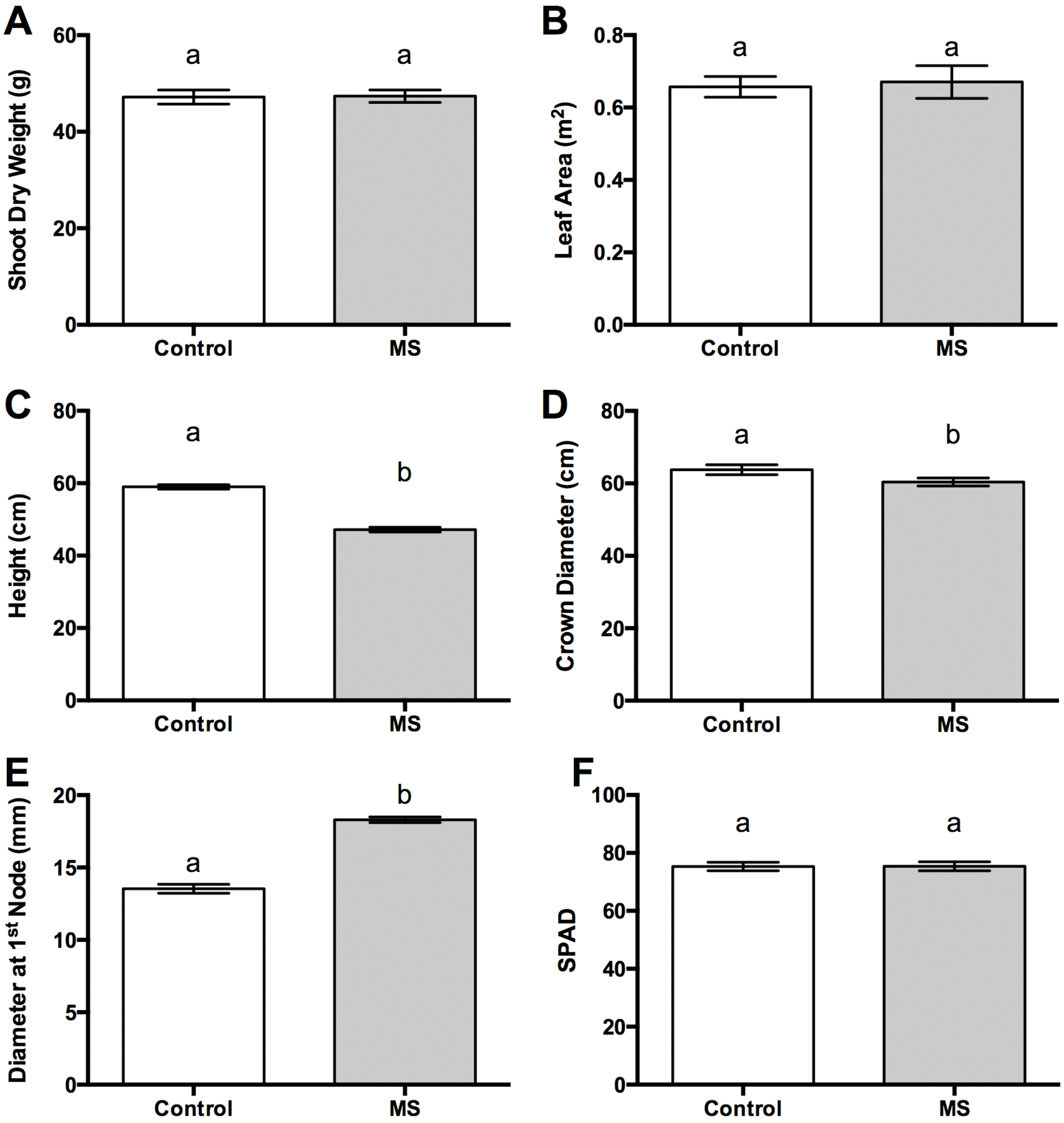

Figure 4: Vegetative shoot metrics for control and mechanically stimulated pepper plants during fruit set and maturation: (A) Shoot dry mass; (B) Leaf area; (C) Total height; (D) Crown diameter; (E) Stem diameter at the first node; (F) Relative chlorophyll level (SPAD). Columns with the same letter appearing above do not differ at $p \leq 0.05$. Error bars are the SEM.

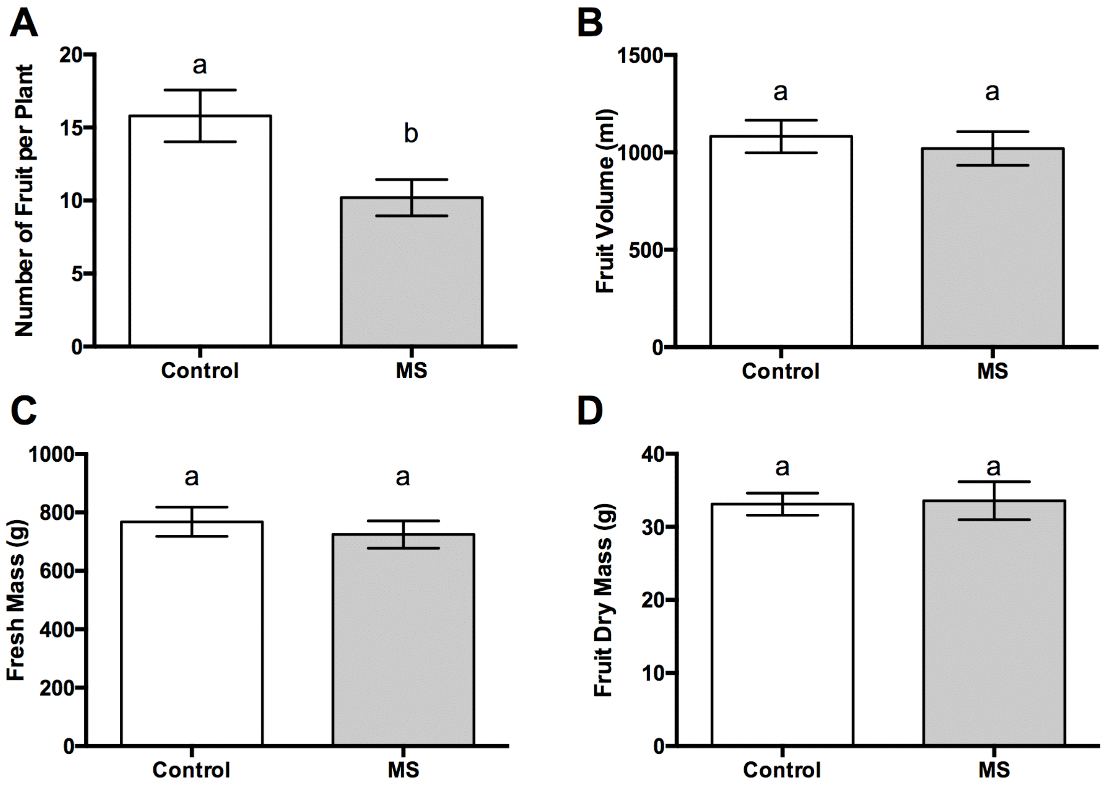

Figure 5: Fruit production metrics for control and mechanically stimulated pepper plants: (A) Average number of mature fruit per plant; (B) Mean total fruit volume per plant; (C) Mean total fruit mass per plant; (D) Mean total fruit dry mass per plant. Columns with the same letter appearing above do not differ at p≤0.05. Error bars are the SEM.

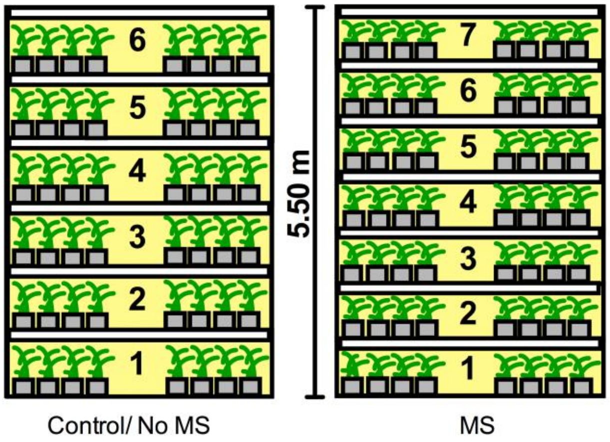

Figure 6: Theoretical Volume Utilization Efficiency improvement potential in stacked crop production system based on the mean height reductions observed in the fruiting experiment presented.