



Responses of Papaya Plants in a Potting Medium in Containers to Flooding and Solid Oxygen Fertilization

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Papaya (*Carica papaya* L.) production in Florida is concentrated in areas with a high water table that are prone to flooding resulting from storms or hurricanes. This can limit production because the crop is sensitive to flooding stress. Studies were conducted to examine physiological responses of papaya to flooding a portion of or the entire root system and if flooding stress can be reduced by fertilization with solid oxygen compounds. Six-month-old 'Red Lady' papaya plants in Promix® potting medium were divided into 3 flooding treatments: 1) 100% of roots submerged; 2) ~75% of roots submerged; or 3) non-flooded. In separate experiments, plants in each flooding treatment were subdivided into oxygen fertilization treatments by adding different amounts of either magnesium peroxide (MgO₂) or calcium peroxide (CaO₂) or no oxygen fertilization to the potting medium prior to flooding. Redox potential (an indication of oxygen content) of the soil solution, net CO₂ assimilation, stomatal conductance, leaf chlorophyll index, chlorophyll fluorescence, and plant tissue dry weights were assessed. Short-term flooding (2–3 days) of ~75% or 100% of the root system resulted in plant stress as determined by the physiological variables measured. However, plants eventually recovered from flooding after they were unflooded. Application of MgO₂ or CaO₂ to flooded plants did not affect any of the physiological variables measured or dry weights for most chemical fertilization treatments. However, 5 g of MgO₂ increased leaf area and total plant dry weight of flooded plants compared to no oxygen fertilization or fertilization with 10 g of MgO₂. This is in contrast to results of a previous study with 3-week-old papaya seedlings in a gravelly loam soil whereby soil application of CaO₂ reduced the impact of flooding on plant dry weight. Thus, more research is needed with different age plants, different concentrations of solid oxygen fertilizers in different soil types to more thoroughly assess the effects of amending soil with solid oxygen fertilizer on reducing the impact of flooding stress.

Papaya plants are very sensitive to flooding stress (Balerdi et al., 2013; Rodríguez et al., 2014; Thani et al., 2016). For example, net CO₂ assimilation, stomatal conductance and transpiration of papaya plants with 100% of their roots submerged decreased after 1 d of flooding, although these variables recovered to values similar to those of the non-flooded control plants 9 d after plants were unflooded (Rodríguez et al., 2014a, b). However, if 100% of the root system was submerged for more than 2 d, plants never recovered as evidenced by permanent wilting 11 d after plants were unflooded (Rodríguez et al., 2014a, b). Thus, there is a need for identifying methods of reducing the negative impacts of flooding on papaya plants.

Enriching the root zone with O₂ is a method for potentially mitigating the effects of flooding on crops. For example, yield of pepper plants increased by 39% when air was pumped into the soil by increasing oxygen in a subsurface drip irrigation system (Goorahoo et al., 2001). In soybean, cotton, and zucchini fruit production and biomass increased by enriching the root zone with air (Bhattarai et al., 2004). Another method of adding oxygen to the soil is by soil application of "solid oxygen fertilizers." The

redox potential, an indirect indication of oxygen content, of flooded soil can be improved by applying O₂ fertilizers to the soil (Liu and Porterfield, 2014). Solid oxygen fertilizers are available in different formulations such as calcium peroxide (CaO₂) or magnesium peroxide (MgO₂). Addition of MgO₂ to the soil reduced flooding stress of corn (Liu and Porterfield, 2014). For Italian basil, amending the soil with slow release fertilizers (CaO₂ or Ca(OH)₂) increased plant biomass by 15% (Liu et al., 2013). Thani et al. (2016) found that for very young (3-week-old) 'Red Lady' papaya seedlings, application of CaO₂ to a very gravelly loam soil prior to flooding minimized reductions in leaf, stem, root and plant dry weight and increased plant recovery and survival from flooding stress. However, that study was conducted with plants in the very gravelly loam (Krome very gravelly loam) soil found in the fruit crop production area of southern Florida. Reducing flooding stress in papaya by soil application of CaO₂ needs to be tested in different soil types with plants that are older than 3 weeks. Also, in addition to testing CaO₂, the effect of other solid oxygen fertilizers such as MgO₂ on reducing flooding stress of papaya has not been tested.

The objectives of this study were to determine if application of solid oxygen fertilizers (MgO₂ or CaO₂) to the soil could mitigate the negative impacts of flooding a portion or the entire root system

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on the physiology and growth of 6-month-old papaya plants in a potting medium with a high organic matter content.

Materials and Methods

Two experiments were conducted from Mar. 2015 to Aug. 2015 in a greenhouse at the University of Florida/IFAS, Tropical Research and Education Center in Homestead, FL, to test the effects of MgO₂ (Expt. 1) or CaO₂ (Expt. 2) on mitigating responses of papaya plants to partial or total root zone flooding.

For each experiment, papaya (*Carica papaya* L. cv. Red Lady) seeds were soaked in water for 24 h and then sown in flats containing Promix® BX potting medium which has a high organic matter content consisting of 75% to 85% peat moss (Premier Tech, Quebec, Canada). Three weeks after germination, each seedling was transplanted into 11.3-L plastic pots containing Promix® for Expt. 1 and in 13.2-L plastic pots of Promix® for Expt. 2. After plants became established in the pots (after 6 months), they were divided into treatments just prior to flooding.

In each experiment, there were 3 flooding treatments and 3 solid oxygen fertilizer treatments in a 3 × 3 factorial design for a total of 9 treatments. Flooding treatments were: 1) 100% of the roots submerged; 2) 75 % of the roots submerged; or 3) non-flooded, control; 0% of the roots submerged. Plants were flooded by placing each plant pot into a 19-L plastic bucket filled with tap water to: 1) about 5 cm above the soil surface (100% of the roots submerged); 2) cover the lower 75% of the pot (~75% of the roots submerged); or 3) not submerged (non-flooded control). Non-flooded plants were manually irrigated. Tensiometers (Irrometer Company, Riverside, CA) were installed in 6 randomly selected containers for plants in the non-flooded, control treatments to ensure that non-flooded trees were not drought stressed. The tensiometers were maintained between 5 and 7 kPa.

In Expt. 1, the three solid oxygen fertilizer treatments were: 1) 0 g MgO₂ added to the potting medium; 2) 5 g MgO₂ added to the potting medium; or 3) 10 g MgO₂ added to the potting medium. In Expt. 2, the three solid oxygen fertilizer treatments were: 1) 0 g CaO₂ added to the potting medium; 2) 20 g CaO₂ added to the potting medium; or 3) 40 g CaO₂ added to the potting medium. These MgO₂ and CaO₂ concentrations were based on published literature with other crops and adjusting the concentrations based on the soil volume in the present study. All solid oxygen fertilizers were added a few minutes prior to initiating the flooding treatments. In all experiments, treatments were arranged in a randomized complete block design with 5 single-plant replications per treatment combination. In each experiment, plants were unflooded after a statistically significant difference in either net CO₂ assimilation or stomatal conductance was observed between the control (0% of roots submerged) and either root submergence treatment in at any of the oxygen fertilizer concentrations. Plants were unflooded (removed from the buckets of tap water) 72 h after flooding treatments began in Expt. 1 and 48 h after flooding treatments began in Expt. 2. After plants were unflooded, physiological measurements continued for 23 d in Expt. 1 and 21 d in Expt. 2.

Air temperature in the greenhouse was monitored and recorded with a Hobo Pro v2 logger (Onset Computer, Bourne, MA). Temperature of the potting medium was monitored and recorded 3 cm below soil surface with a Hobo Tidbit v2 temperature logger for one plant in each treatment. Air temperature in the greenhouse was monitored and recorded with a Hobo Pro v2 logger (Onset Computer, Bourne, MA).

Redox potential of the potting medium was determined daily for flooded plants until plants were unflooded. Redox potential was determined with metallic ORP indicating electrode (Accumet model 13-620-115, Fisher Scientific, Pittsburgh, PA) connected to a portable voltmeter.

Before plants were flooded, one fully expanded leaf of approximately the same age per plant was tagged for leaf chlorophyll index (leaf greenness), leaf chlorophyll fluorescence, net CO₂ assimilation (photosynthesis), and stomatal conductance measurements. About 10 d after the flooding treatments were initiated (7 d after plants were unflooded), the tagged leaf of several flooded plants (replications) abscised. Therefore, a new fully expanded leaf of approximately the same age was tagged for each plant in each treatment for further measurements. For plants in each treatment, leaf chlorophyll index was measured daily during the flooding period and twice weekly after plants were unflooded with a SPAD-502 meter (Minolta, Inc., Osaka, Japan). The ratio of variable to maximum chlorophyll fluorescence (Fv/Fm), and indication of damage to the photosynthetic apparatus, was determined daily during the flooding period and twice weekly after plants were unflooded in one leaf per plant with an OS-30 hand-held chlorophyll fluorimeter (Opti-Sciences, Inc., Hudson, NH).

Net CO₂ assimilation and stomatal conductance were measured daily during the flooding period and twice weekly after plants were unflooded in one leaf per plant with a CIRAS-3 portable gas exchange system (PP Systems, Inc., Amesbury, MA). Measurements were made at a light saturated photosynthetic photon flux (PPF) of 1200 μmol·m⁻²·s⁻¹, a reference CO₂ concentration of 390 μmol·mol⁻¹ and air flow rate into the leaf cuvette of 200 mL/min.

At the end of the experiment, plants were harvested and roots from each plant were washed in tap water to remove the potting media from the roots. All plant parts were oven-dried at 70 °C to a constant weight and leaf, petiole, stem and root dry weights were determined for each treatment.

Data were analyzed by 2-way analysis of variance (ANOVA) to test for significant statistical interactions between flooding and oxygen fertilizer treatments for any of the dependent variables measured and repeated measures. ANOVA was used to determine the effects of the different root submersion and different solid oxygen fertilizer treatments on any of the dependent variables measured. The SAS statistical software package (SAS Institute, Cary, NC) was used for all data analyses.

Results

EXPERIMENT 1. Mean daily air temperature in the greenhouse was 25.1 °C and temperature in the potting medium ranged from 17.6 °C to 32.4 °C. The redox potential for the ~75% root submergence treatments ranged from 190 mV to 290 mV in the 0 g MgO₂ treatment, 50 mV to 416 mV in the 5 g MgO₂, and 52 mV to 199 mV in the 10 g MgO₂ treatment. The redox potential for the 100% root submergence treatments ranged from 80 mV to 278 mV in the 0 g MgO₂ treatment, 67 mV to 136 mV in the 5 g MgO₂, and 92 mV to 536 mV in the 10 g MgO₂ treatment (data not shown). Anaerobic conditions occur at soil redox potentials ≤ 200 mV (Ponnamperuma, 1972).

The leaf chlorophyll index was significantly lower in the 100 and ~75% root submergence treatments than in the non-flooded control treatment at all MgO₂ concentrations starting 4 d after the flooding treatments began (Fig. 1). However, at all MgO₂

concentrations several days after plants were unflooded, the leaf chlorophyll index of plants with ~75% of the roots submerged returned to levels similar to those of the unflooded control treatment, whereas the leaf chlorophyll index in the 100% root submergence treatments increased to levels higher than those of the 0% or ~75% root submergence treatments (Fig. 1).

The Fv/Fm tended to be lower for plants with ~75% or 100% of submerged roots compared to non-flooded plants 6 d after flooding was initiated for plants receiving 0, 5, or 10 g of MgO₂, although these differences were often not statistically significant at $P \leq 0.05$ (Fig. 2). By the end of the experimental period, Fv/Fm values in both root submergence treatments were similar to those of non-flooded plants regardless of the MgO₂ concentration (Fig. 2).

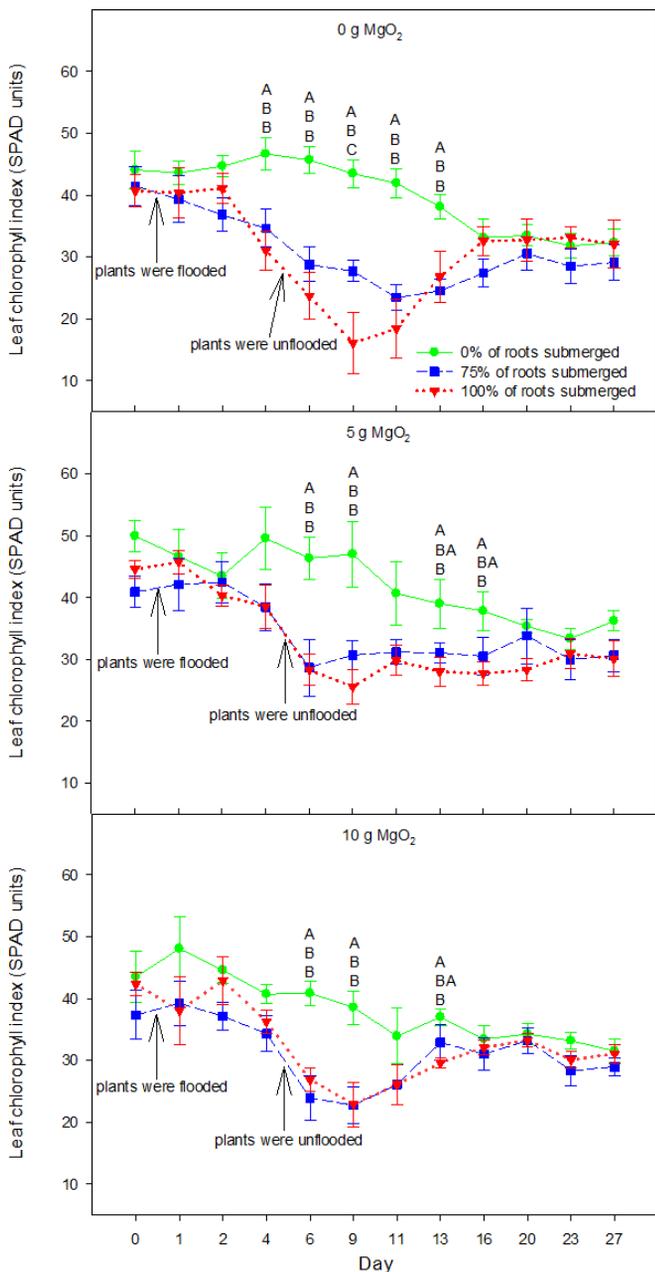


Fig. 1. Effect of flooding on leaf chlorophyll index of papaya grown in Promix® (Expt. 1). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

At all MgO₂ concentrations, net CO₂ assimilation (Fig. 3) and stomatal conductance (Fig. 4) of plants with ~75% or 100% of roots submerged were lower than those of non-flooded plants 1 d after flooding commenced. However, at all MgO₂ concentrations, net CO₂ assimilation (Fig. 3) and stomatal conductance (Fig. 4) levels returned to levels similar to those of the non-flooded controls 3–4 d after plants were unflooded.

Leaf and petiole dry weights were significantly lower in the 100% root submergence treatment compared to the control when either 0 g or 10 g MgO₂ was applied to the soil. Root dry weights were significantly lower in the 100% root submergence treatments than in the control treatment at all the oxygen fertilizer concentrations. There were no significant differences in

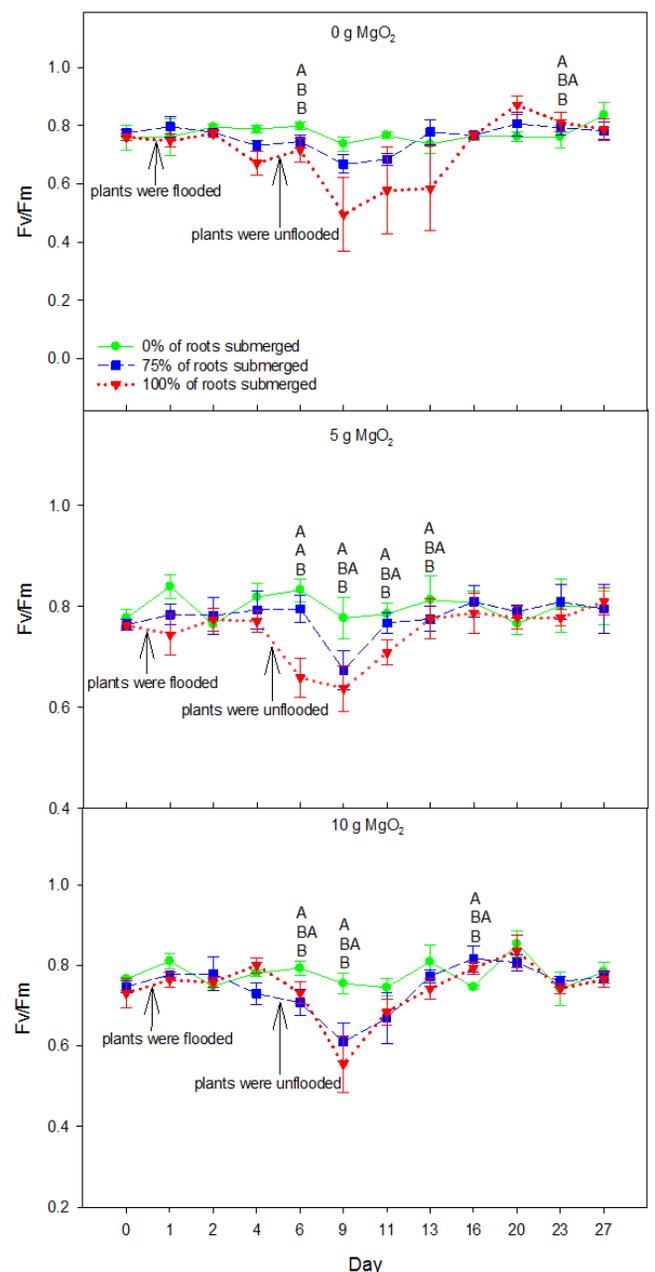


Fig. 2. Effect of flooding on the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) of papaya grown in Promix® (Expt. 1). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

root dry weight between the ~75% submergence treatment and the control in the 5 g and 10 g MgO₂ treatments (Fig. 5). There were no significant differences in the total plant dry weight among the all oxygen fertilizer treatments when 5 g MgO₂ was applied. Total plant dry weight was significantly lower in the 100% root submergence treatment than in the control when 0 g of MgO₂ was applied (Fig. 5).

EXPERIMENT 2. Mean daily air temperature in the greenhouse was 28.7 °C and temperatures in the potting medium ranged from 24.0 °C to 32.8 °C. The redox potential for the ~75% root submergence treatments ranged from 143 mV to 256 mV in the 0 g CaO₂ treatment, -134 mV to 482 mV in the 20 g CaO₂,

and 162 mV to 250 mV in the 40 g CaO₂ treatment. The redox potential for plants in the 100% roots submerged treatments ranged from 326 mV to 497 mV in the 0 g CaO₂ treatment, -40 mV to 310 mV in the 20 g CaO₂, and -85 mV to 267 mV in the 40 g CaO₂ treatment (data not shown).

The leaf chlorophyll index was lower in the ~75 and 100% root submergence treatments than in the non-flooded treatments beginning 3 d after flooding was initiated regardless of CaO₂ concentration (Fig. 6). Several days after plants were unflooded, regardless of the CaO₂ concentration, leaf chlorophyll content of plants in the ~75% and 100% root submergence treatments returned to levels similar to those of the non-flooded plants (Fig. 6).

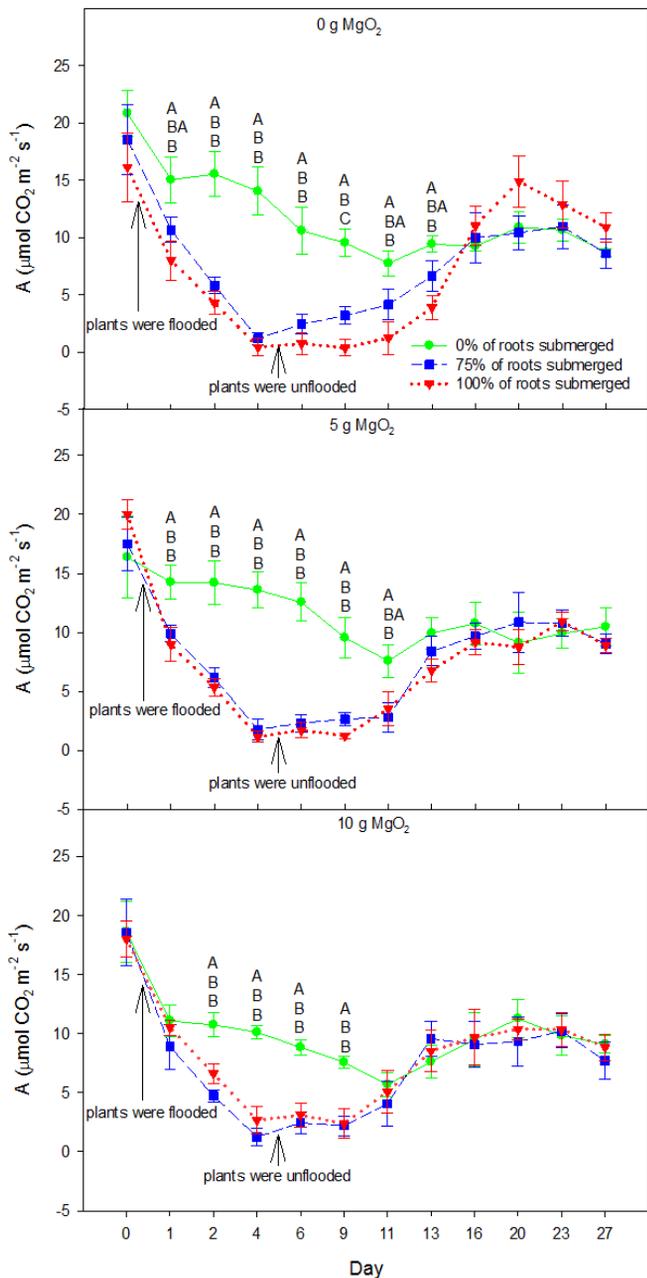


Fig. 3. Effect of flooding on the net CO₂ assimilation (*A*) of papaya grown in Promix® (Expt. 1). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

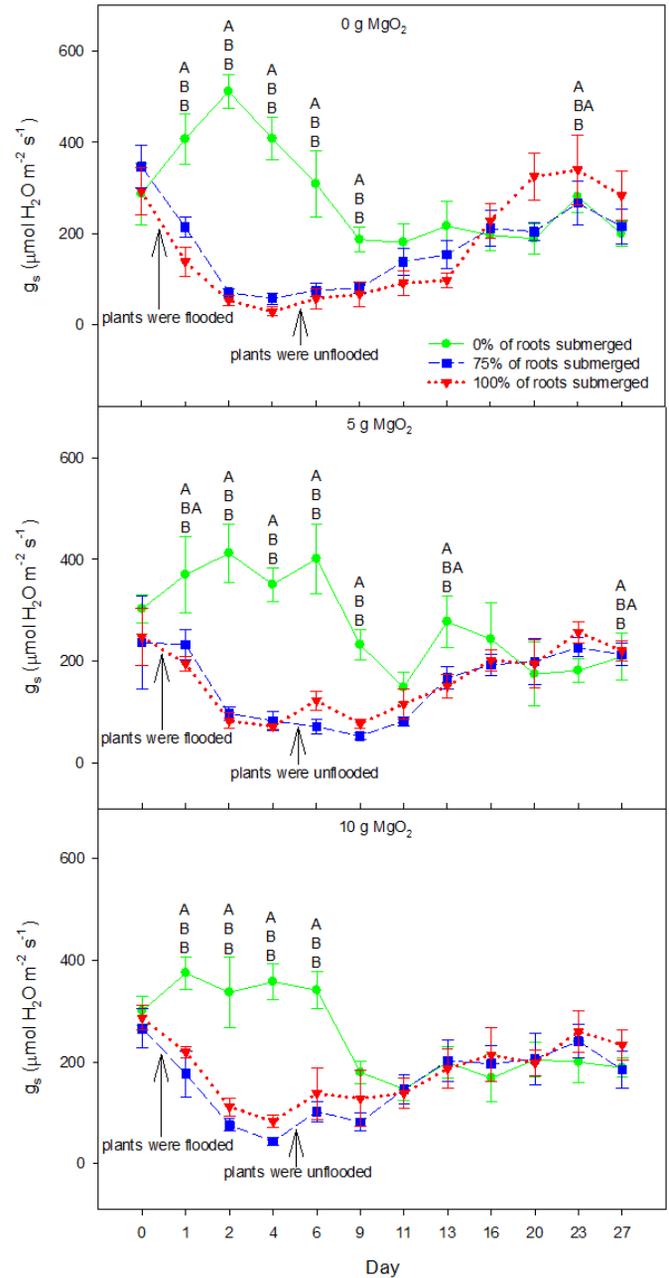


Fig. 4. Effect of flooding on the stomatal conductance (*g_s*) of papaya grown in Promix® (Expt. 1). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

The Fv/Fm of plants in the 100% root submersion treatment was lower than that of plants with 0% or ~75% root submersion at all CaO₂ concentrations (Fig. 7). However, at each CaO₂ concentration Fv/Fm levels of plants with roots submerged returned to levels similar to those of non-flooded plants several days after plants were unflooded (Fig. 7).

Net CO₂ assimilation (Fig. 8) and stomatal conductance (Fig. 9) were lower for plants in the ~75% or 100% root submergence treatment than plants in the non-flooded treatment at all CaO₂ concentrations. However, by the end of the experiment net CO₂ assimilation (Fig. 8) and stomatal conductance (Fig. 9) values of plants in the ~75% root submergence treatment returned to

values similar to those of the non-flooded plants by the end of the study. By the end of the experiment, net CO₂ assimilation (Fig. 8) and stomatal conductance (Fig. 9) values of plants in the 100% root submergence treatment were actually higher than those of the non-flooded plants when 0 or 40 g CaO₂ was applied to the soil.

There were no significant differences in leaf dry weight or petiole dry weight among root submergence treatments regardless of the CaO₂ concentration applied (Fig. 10). Stem dry, root and whole plant dry weights were significantly lower in the 100% root submergence treatment than in the non-flooded control treatment at all CaO₂ concentrations (Fig. 10).

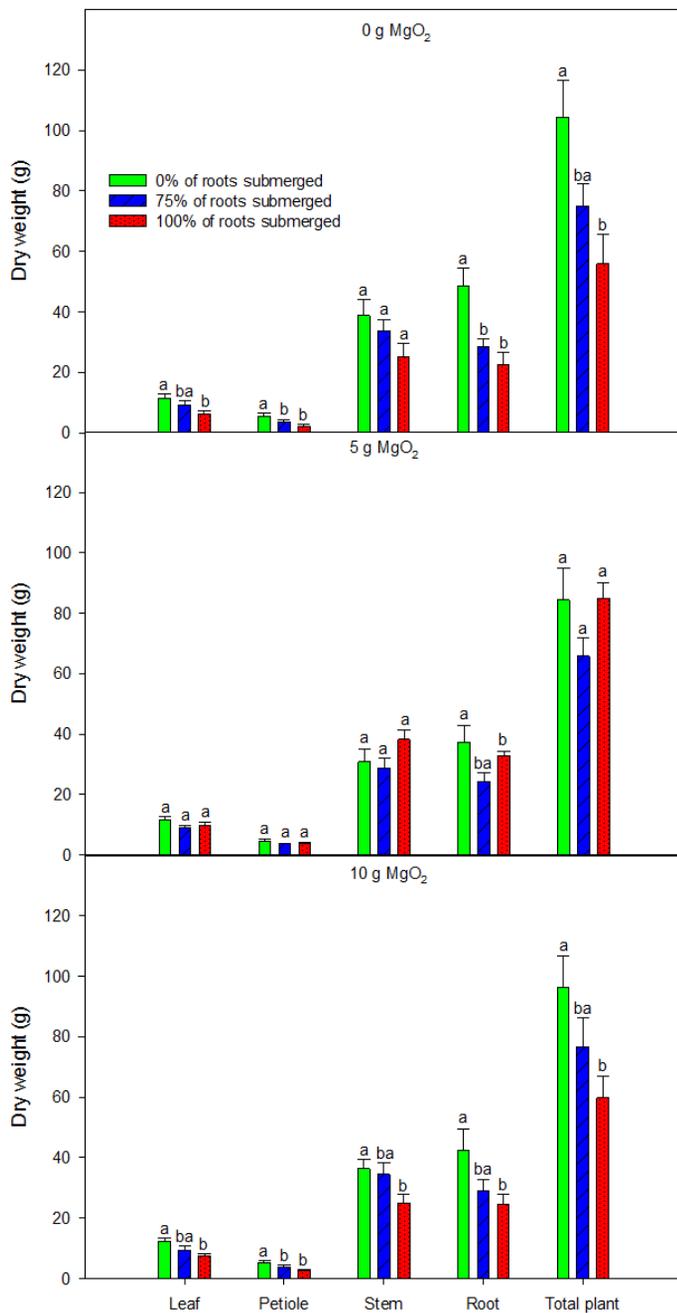


Fig. 5. Effect of flooding on the dry weight of papaya grown in Promix® (Expt. 1). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

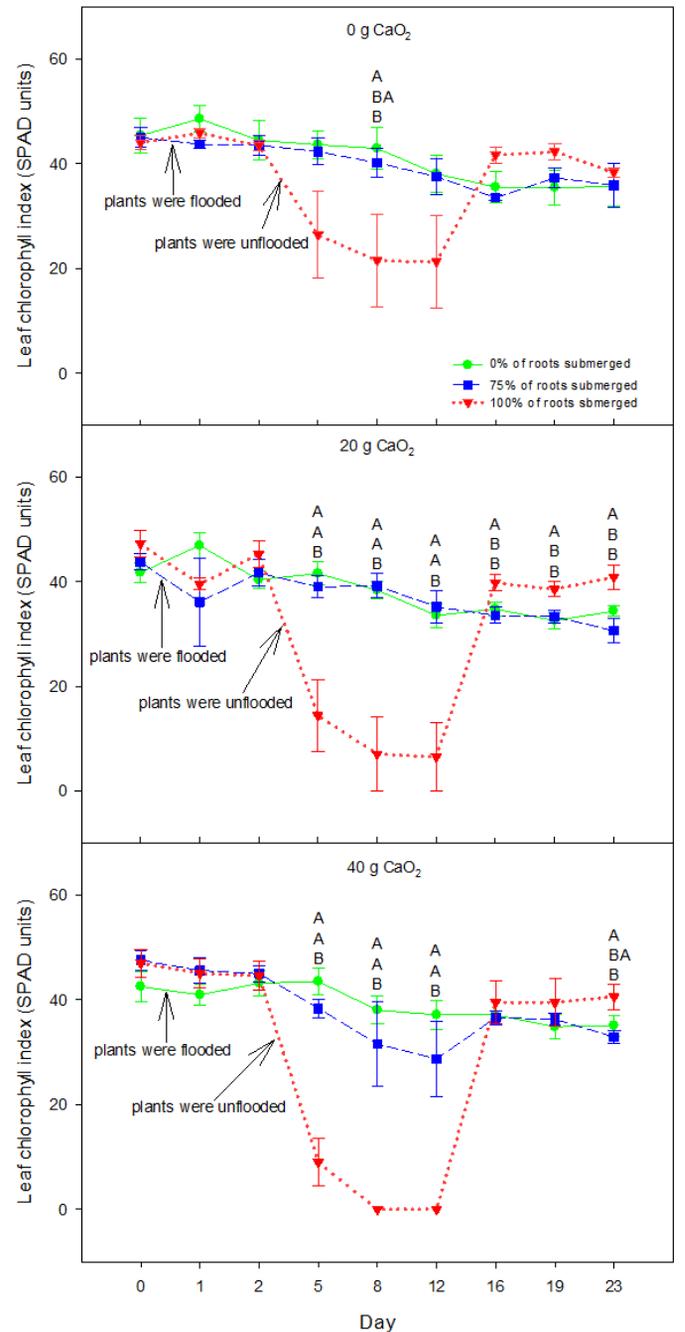


Fig. 6. Effect of flooding on leaf chlorophyll index of papaya grown in Promix® (Expt. 2). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

Discussion

In this study, flooding ~75% or 100% of the root system of 6-month-old 'Red Lady' papaya plants in Promix® potting medium resulted in a decrease in the leaf chlorophyll index, F_v/F_m , net CO_2 assimilation and stomatal conductance compared to non-flooded plants. This early reduction in leaf gas exchange variables was similar to observations by Marler (1995) who found that flooding caused a decline in leaf gas exchange in potted papaya after 1 d of flooding. A reduction in net CO_2 assimilation and stomatal conductance are two of the earliest measurable responses of fruit crops to flooding and are useful indicators of

flooding stress prior to any visible symptoms (Schaffer et al., 1992). In the present study, the negative impact of root submergence on these physiological variables was reversible if plants were removed from flooding within 1–2 d after decreases in net CO_2 assimilation or stomatal conductance were first observed. The transient reductions in the physiological variables measured was generally sufficient to result in a decrease in plant dry weight for plants with 100% of the roots submerged compared to non-flooded plants. In a previous study of the effects flooding on 'Red Lady' papaya plants, Rodríguez et al. (2014) found that net CO_2 assimilation, transpiration and stomatal conductance of flooded plants began to decrease after 1 d of flooding and continued to

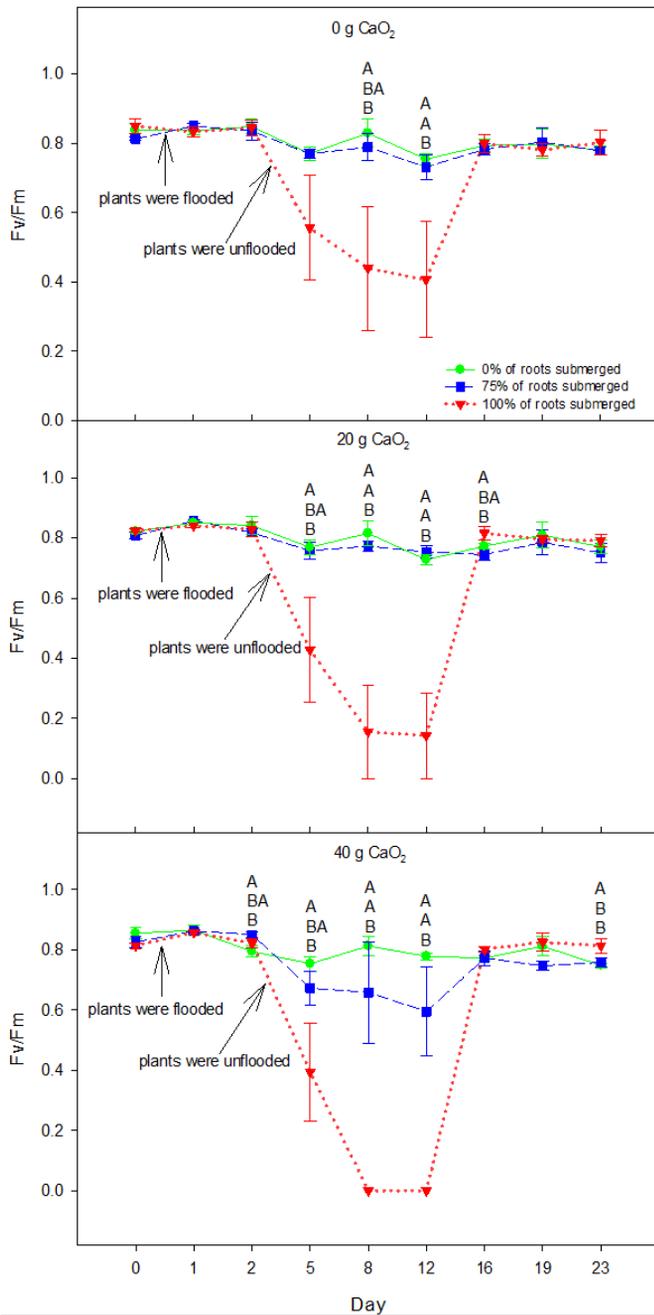


Fig. 7. Effect of flooding on the ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) of papaya grown in Promix® (Expt. 2). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

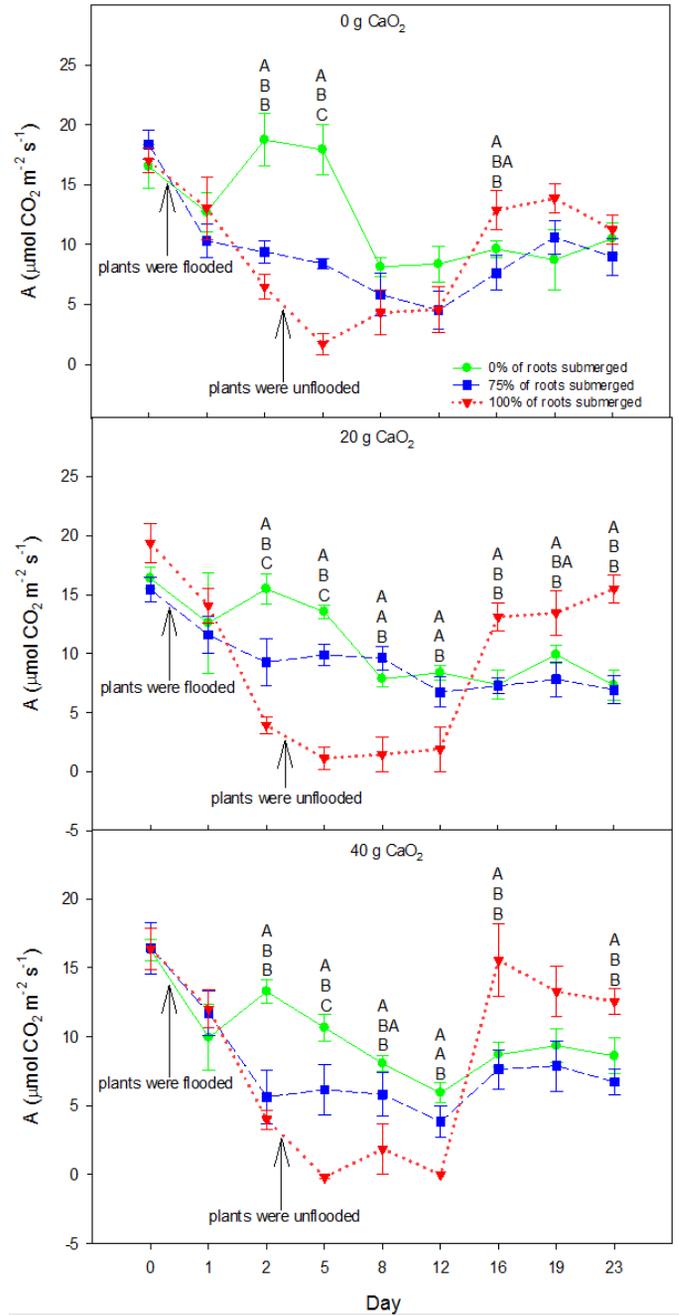


Fig. 8. Effect of flooding on the net CO_2 assimilation (A) of papaya grown in Promix® (Expt. 2). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

decline until plants were unflooded. They also observed that net CO₂ assimilation, stomatal conductance and transpiration of plants with 100% of their roots submerged for 1 d recovered to values similar to those of the non-flooded control plants 9 d after plants were unflooded. However, they also observed that if 100% of the root system was flooded for more than 2 d, plants never recovered as evidenced by permanent wilting 11 days after plants were unflooded. In contrast to the results of Rodríguez et al., we observed that papaya plants flooded for 2–3 ds recovered 7–12 d after plants were unflooded as indicated by an increase in all measured physiological variables in flooded plants to levels

similar to those of the non-flooded plants. The differences in plant recovery between the previous study and the present study may have been due to different temperatures in the greenhouse during the flooding period or differences in plant ages.

In the present study, decreasing the oxygen content in the root zone by application of magnesium peroxide (MgO₂) or calcium peroxide (CaO₂) to the potting medium did not improve net CO₂ assimilation or stomatal conductance of flooded or non-flooded papaya plants. However, application of 5 g of MgO₂ per plant significantly increased the leaf area and leaf, petiole, stem, and total plant dry weights when 100% of the root system was flooded.

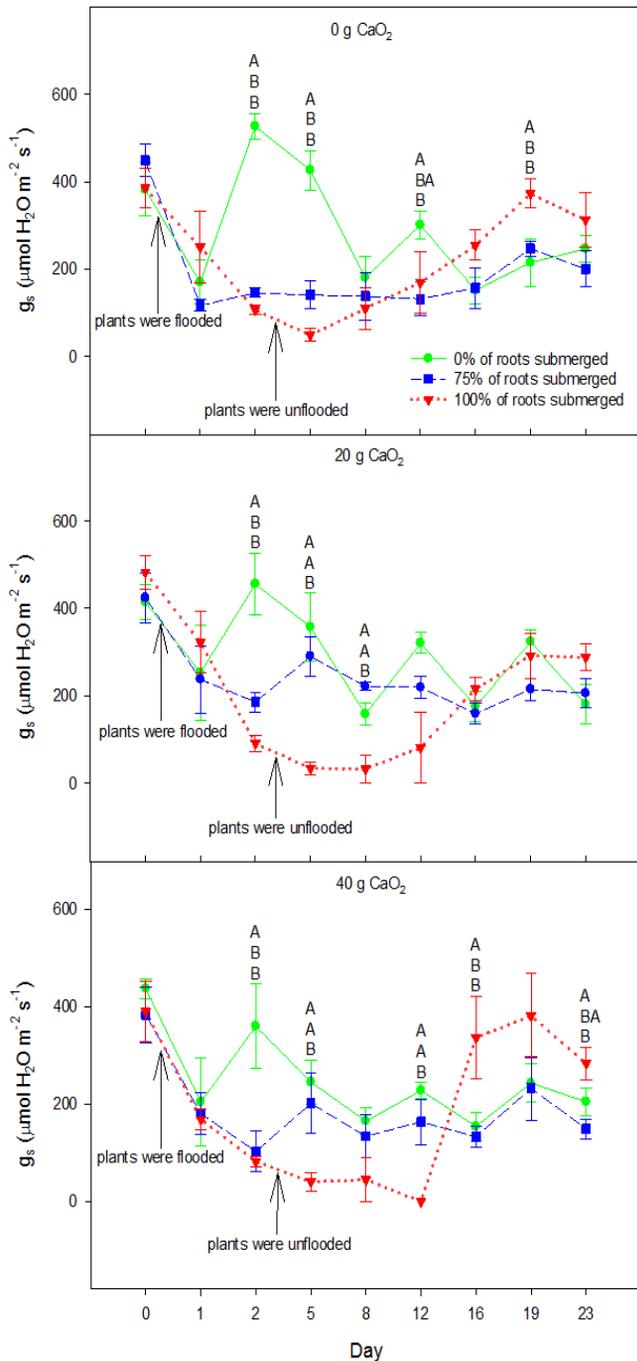


Fig. 9. Effect of flooding on the stomatal conductance (g_s) of papaya grown in Promix® (Expt. 2). Different letters indicate significant difference among treatments ($P \leq 0.05$). Symbols represent means of 5 replicates and bars indicate ± 1 standard error.

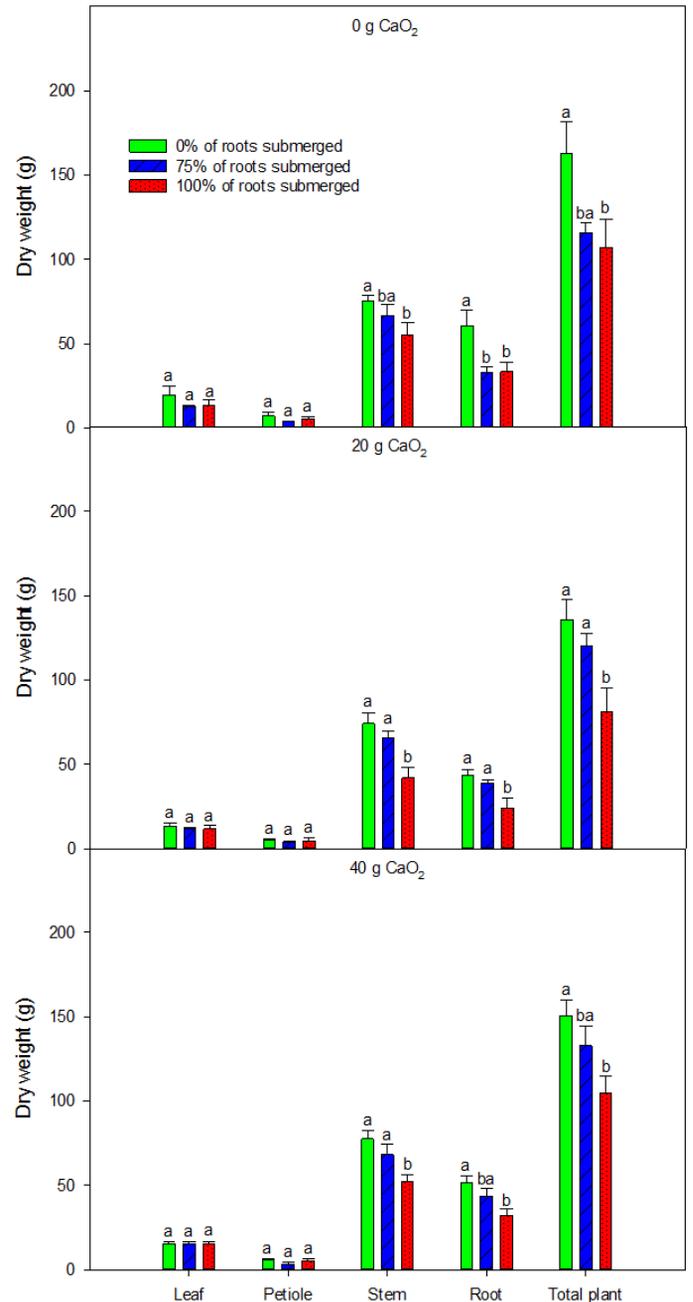


Fig. 10. Effect of flooding on the dry weight of papaya grown in Promix® (Expt. 3). Different letters indicate significant difference among treatments ($P \leq 0.05$). Columns represent means of 5 replicates and bars indicate ± 1 standard error.

Whereas, the application of up to 40 g of CaO₂ per plant did not significantly affect tissue dry weight of plants with ~75% or 100% of the root system was submerged. Gil et al. (2009) observed that addition of hydrogen peroxide (H₂O₂) to the soil significantly improved shoot dry weight of avocado trees but had no significant effects on leaf gas exchange. Similarly, fruit yield of pepper plants increased by 39% when air was pumped into the soil water through subsurface drip irrigation (Goorahoo et al., 2001). In soybean, cotton and zucchini, fruit production and plant dry weight increased by enriching the root zone with air (Bhattarai et al., 2004). Wang and Yeh (2015) observed that application of MgO₂ to the soil prior to flooding increased flood tolerance of chrysanthemum by increasing the net CO₂ assimilation rate, lowering the intercellular CO₂ concentration in the leaves, and increasing the root dry weight. Liu et al. (2013) observed that application of slow-release fertilizers to the soil increased plant dry weight of Italian basil by 15%. The reason why there was no effect of solid oxygen fertilizers on any leaf gas exchange variable for papaya in the present study may have been due the more rapid physiological response of papaya plants to flooding stress than the rate of oxygen release from the solid oxygen fertilizers. Thus, by the time the oxygen was available to the roots, they were presumably not able to absorb or utilize the available oxygen due to root injury or root death. Also, the potting medium used in the study was not sterile. Therefore, it is also possible that a sufficient quantity of additional oxygen from adding MgO₂ or CaO₂ to the soil may have been used by soil microorganisms and thus not readily available to the plants. In contrast to the results of the present study, Thani et al. (2016) found that addition of CaO₂ to Krome gravelly loam soil prior to flooding increased leaf gas exchange, recovery and survival of seedling papaya plants with ~75% or 100% of the roots submerged. The difference between the present results and those of Thani et al., may have been due to the fact that the plants used by Thani et al. were only 3 weeks old and therefore would have a much lower oxygen requirement than the 6-month-old plants in the present study. Also, Krome very gravelly loam soil is extremely porous, and thus O₂ may have been more readily able to reach the roots compared to roots in Promix® with is much less porous with a very high organic matter content.

In conclusion, short-term (2–3 d) of flooding ~75% or 100% of the root system of ‘Red Lady’ papaya in artificial medium with a high organic matter content caused a significant reduction in physiological indicators of plant stress and plant growth, but plants were able to recover from flooding stress within a few weeks after they were unflooded. The addition of MgO₂ or CaO₂ to the potting medium at the concentrations tested did not reduce flooding stress of 6-month-old papaya plants. However, based on results of previous studies where CaO₂ was effective in alleviating

flooding stress of much smaller papaya plants in a more porous soil (Thani et al., 2016), more studies are needed with larger papaya plants in different soils and with higher concentrations of MgO₂ and CaO₂ applied to the soil or potting medium to further test the effects of chemical fertilization on alleviating flooding stress of papaya plants.

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