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Oxygen Amendment on Growth and Nitrogen Use Efficiency of Flooded Italian Basil

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Flooding is a frequent, and often unavoidable cause of stress, in vegetable production in Florida. Flooding results in hypoxia; that is, oxygen deficiency. This study was conducted with traditional Italian basil (*Ocimum basilicum* L.), cv. Genovese OG, treated with either a fast- or slow-release solid oxygen compound under flooding in a plastic house in Homestead, Florida. Application of fast- and slow-release oxygen compounds to flooded basil seedlings increased leaf chlorophyll content by 53% and 58%, respectively, compared to controls. Oxygen amendment increased redox potential of flooded soil 20-fold. Application of oxygen-containing compounds is a potentially effective method to alleviate hypoxic conditions in soil, which, in turn, enhances N use efficiency and improves crop productivity.

Keywords *Ocimum basilicum*, Oxygen bioavailability, Soil redox potential, Biomass

Florida weather is characterized by frequent hurricanes and heavy rains, which often results in hypoxic conditions (deficiency in the amount of oxygen

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reaching root tissues) affecting crops. Hurricane Irene (October 1999) resulted in approximately \$77 million losses in vegetable crop production. Economic loss of the vegetable industry caused by floods as a result of 35.3-mm rainfall in December 2000 was estimated at \$13 million (Li et al., 2009). Muñoz-Carpena et al. (2009) reported that approximately 67% of Florida's vegetable production was impacted by floods. Although most Florida soils are normally well drained, low-lying areas are prone to flooding during periods of high rainfall. These climatic and hydrologic circumstances are further complicated by the nature by which vegetable crops obtain oxygen. Root systems of vegetable crops such as basil (*Ocimum basilicum* L.) must obtain bioavailable oxygen from the soil. Although air is a mixture with 21% oxygen, the diffusion rate of oxygen in water is only 1/10,000 that in air and is slow to move into flooded soil (Holbrook and Zwieniecki, 2003). Based on oxygen bioavailability, soil undergoes the following changes over the duration of flooding: before flooding, normoxia (normal levels of oxygen); one or 2 days after flooding, hypoxia; and more than 2 days after flooding, anoxia (absence of oxygen). For vegetable production, soils need to be normoxic during the growing season.

Basil has potent antioxidant, antiviral, and antimicrobial properties and has the potential for use in treating cancer (Manosroi et al., 2006). Extracts of this vegetable are toxic to mosquitoes (*Culiseta longiareolata*; Maurya et al., 2009) and have antifungal and insect-repelling properties (Dube et al., 1989). Basil was selected for conducting oxygen amendment tests because it is sensitive to hypoxia. The project investigated the effects of fast- and slow-release oxygen compounds on basil growth, nitrogen use efficiency, and redox potential of flooded soil.

MATERIALS AND METHODS

Traditional Italian basil, cv. Genovese, seed (Johnny Selected Seeds, Winslow, ME) was used.

Soil Preparation and Plant Culture

One hundred and forty-four 7-cm diameter pots (internal volume: 353 cm³) were filled with Pro-Mix (A.F.E.C. Fertilizer Company, Homestead, FL) potting medium and each pot was planted with about 20 seeds spaced 1.5 cm apart. Pots were irrigated with tap water once daily. Seed emerged 3 days after planting. On day 7, seedlings in pots were fertilized with a solution a 20N–20P–20K fertilizer, 100 g/20 L. The N was from urea (10.85%), nitrate nitrogen (5.50%), and ammoniacal nitrogen (3.85%); P₂O₅ was from phosphoric anhydride; and K₂O was from muriate of potash, boron (B; 0.02%), water-soluble copper (Cu; 0.005%) from EDTA chelation, iron (Fe; 0.10%) from EDTA chelation, magnesium (Mg; 0.05%) from magnesium sulfate, manganese (Mn; 0.05%) from EDTA chelation, and zinc (Zn; 0.05%) from EDTA chelation.

Oxygen Fertilizers

The main ingredients of the slow-release solid oxygen-containing compounds were calcium peroxide and calcium hydroxide (Solvay Chemical and Pharmaceutical Group, Houston, TX). The major component of fast-release solid oxygen-containing compound was carbamide peroxide (Catalog No. AC24102-0010, Fisher Scientific, Hampton, NH).

Chemicals and Materials

The chemicals used for this study were from Fisher Scientific: ammonium acetate ($C_2H_7NO_2$; Catalog No. MA-X12201), α, α' -dipyridyl ($C_{10}H_8N_2O_2$; Catalog No. AC11750-0100), calcium hydroxide (Catalog No. AC21918-0010), ferric chloride ($FeCl_3$; Catalog No. S71935), urea (CH_4N_2O ; Catalog No. BP169-500). The N-P-K fertilizer and 7- and 12.5-cm Pacific Nursery Pots were from A.F.E.C. Fertilizer Company.

Oxygen Amendment Treatments

Four-week-old seedlings were transferred to 48 12.5-cm pots (internal volume: 1349 cm^3) containing Pro-Mix medium. Specific amounts of oxygen-containing compounds were incorporated into soil to produce the treatments: 1) 650 mg urea as control 1 (CK1); 2) 1000 mg carbamide peroxide as a fast-release oxygen-containing amendment (OF1) containing 650 mg urea and 350 mg hydrogen peroxide; 3) 1000 mg calcium hydroxide [$Ca(OH)_2$] as control 2 (CK2); and 4) 1000 mg slow-release oxygen-containing amendment (OF2) that contains 400 mg $Ca(OH)_2$ and can form approximately 600 mg $Ca(OH)_2$ after releasing bioavailable oxygen. Each treatment consisted of 12 pots randomly grouped for the four treatments. Six uniform seedlings were maintained in each pot. One day after repotting, all 48 pots with plants were fertilized at the same concentration as before repotting. On the third day after repotting, six pots from each treatment were flooded with tap water for 4 days; the remaining six pots were not flooded. The experimental design was a split plot, with flooding being the main plot and oxygen amendment treatment being the split with six replications.

Height, Chlorophyll, and Biomass Measurements

Plant heights were measured on days 1 and 5 after flooding. Leaf chlorophyll content index was determined with a SPAD meter (SPAD-502, Minolta Camera Co., Ltd., Osaka, Japan) on day 5. On day 6, plants were harvested by cutting shoots at the soil surface and samples were dried to constant weight in a forced air oven at 75°C . Dry matter weights were recorded.

Nitrogen Use Efficiency

Nitrogen use efficiency (NUE ($\text{kg} \cdot \text{kg}^{-1}$)) was determined and defined as the biomass production per unit of nitrogen supplied as follows:

$$\text{NUE}(\text{kg} \cdot \text{kg}^{-1}) = \frac{\text{Biomass}}{\text{N}_{\text{applied}}}$$

For convenient comparison, relative N use efficiency (RNUE) was determined and defined as:

$$\text{RNUE}(\%) = \frac{\text{NUE}_{\text{flooded}}}{\text{NUE}_{\text{corresponding-non-flooded}}} \times 100$$

Pro-Mix Potting Medium Redox Potential (Ferrous Iron Detection)

Six 3.8-L pots filled with Pro-Mix potting medium were individually flooded in six 19-L buckets filled with 7.6 L of tap water: three were oxygen amended with 10 g OF2 (41.7 mM bioavailable oxygen) incorporated into the medium; the other three were not oxygen amended. There were three replicates. To ensure that ferric ions were available in the Pro-Mix potting medium, 100 mL of 0.18 μM FeCl_3 (10 $\text{mg} \cdot \text{L}^{-1}$ Fe^{3+}) solution was added to each pot 1 h prior to flooding. After 10 days, 10 g of soil was sampled from each pot. The sampled soil was immediately treated with 10 mL of 1.28 mM α, α' -dipyridyl dissolved in 1 M ammonium acetate solution. Absorption of the $[\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3]^{2+}$ complex was colorimetrically determined with a spectrophotometer (DU 640, Beckman Coulter, Brea, CA, USA) at 520 nm with an extinction coefficient (ϵ_{520}) of 5.2 $\text{mM}^{-1} \cdot \text{cm}^{-1}$ (Anderson and Howard, 1984; Ding and Demple, 1996).

Soil Redox Potential (Indicator of Reduction in Soil)

White PVC tubes were sanded with 100-grit sandpaper and used to study soil reduction (Jenkinson and Franzmeier, 2006). Tubes were 15 cm in length and 2.1 o.d. and 1.5 cm i.d. Tubes were coated with Fe^{3+} ions by submerging in a saturated FeCl_3 solution (500 mL deionized water plus 505 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) for 24 h. Coated tubes were dried at room temperature for 3 days. The air-dried tubes were further dried in a forced air oven at 75 °C for 24 h to stabilize the coatings on the tubes. After stabilization, 12 cm of the 15-cm tubes was inserted into flooded sandy soils with or without the slow-release oxygen compound (83.4 mM bioavailable oxygen in slow-release oxygen-containing amendment incorporated into soil per bucket). The remaining 3 cm of the tube was submerged in water above the flooded soil. The tubes were for measurements and photographic record after 2 weeks. White (ferric iron was reduced to ferrous iron and then dissolved in soil solution without oxygen-releasing

compound) and brown (ferric iron was not reduced and remained on the PVC tube with oxygen-releasing compound) areas of treated tubes were measured for redox potential quantification. The average value was calculated from nine measurements for the control and treatment.

Statistical Analysis

The data were subjected to one- and two-way analyses of variance (ANOVAs) using SAS (ver. 9.1, SAS Institute, Inc., Cary, NC). If interactions were significant the critical ranges ($LSD_{2,0.05}$) of Duncan's multiple range test were used to separate means (Hubbard, 2001).

RESULTS AND DISCUSSION

The main effects of flooding and treatment affected biomass, chlorophyll content, plant height, and N use, and the interaction affected chlorophyll content (Table 1). Physiologically, this interaction might be related to compensatory responses of photosynthesis for plants under stress. This result indirectly supports defoliation findings that nonstructure carbohydrate content did not differ between intact and defoliated plants because of the compensatory effects of plants (Vanderklein and Reich, 1999). Chlorophyll content was affected by the interaction because chlorophyll biosynthesis, a complicated biochemical process, needs active chemical energy supplied from adenosine triphosphate (ATP). With an ATP shortage, biochemical processes will be retarded. Leaf chlorophyll content may be the most sensitive indicator of basil plants to the shortage of soil oxygen bioavailability.

The Oxygen amendment \times Flooding interaction affected chlorophyll content (Figure 1). Nonflooded plants had higher chlorophyll than flooded plants but there was no difference between controls without flooding. Oxygen-treated plants produced more chlorophyll than controls, and there was no difference between treatment type or whether plants were flooded. Increased chlorophyll

Table 1: Results of ANOVA for biomass, chlorophyll content, plant height, and N use in basil due to flooding and oxygen amendment treatment.

Source	Biomass	Chlorophyll content	Height	Relative N use efficiency
Flood (F)	**	*	*	*
Treatment (T)	**	*	*	*
Interaction T \times F	NS	**	NS	NS

NS, *, **Nonsignificant and significant at $P < 0.05$ or $P < 0.01$, ANOVA.

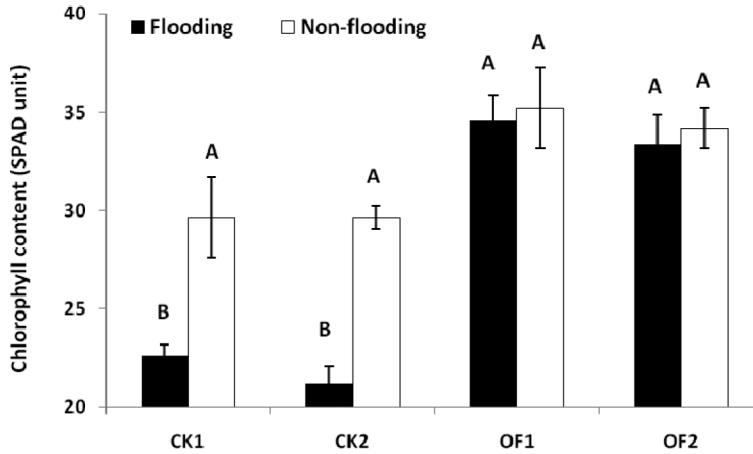


Figure 1: Difference in chlorophyll content of flooded basil plants without (CK1 and CK2) or with fast-release (OF1) or slow-release (OF2) oxygen compound. Histograms with different letters are significantly different, $P < 0.05$; $LSD_{2,0.05} = 6.4$ SPAD units.

might be attributed to enhanced oxygen bioavailability provided by amendment with compounds supplying bioavailable oxygen so that plants can maintain aerobic respiration under flooding. In plants, chlorophyll is probably synthesized from succinyl-CoA and glycine through the immediate precursor, protochlorophyllide, to chlorophyll a and b (Meskauskiene et al., 2001). This biosynthesis process consumes active chemical energy, ATP. Flooded basil plants were in anaerobic conditions and might not be able to produce enough ATP for chlorophyll biosynthesis due to lack of the terminal electron acceptors in the respiratory chain because of hypoxia. This study did not analyze ATP contents of plants with or without oxygen amendment. One mole of glucose can produce 36 to 38 moles of ATP in normoxia but only 2 moles of ATP in hypoxia (Rich, 2003) because there is not enough bioavailable oxygen serving as the final electron acceptor to convert all of the reducing power formed from respiration into ATP (Figure 2). The difference in chlorophyll content between basil plants with and without oxygen amendment indirectly supports Rich's (2003) findings. The sufficient available energy of oxygen-fertilized plants was able to ensure all of the biochemical and physiological processes in metabolism.

Flooding caused plants to have significantly lower biomass, a lower growth increment, and lower relative N use efficiency (Table 2). The highest biomass was produced by the fast-release oxygen treatment. The slow-release oxygen amendment had a biomass value 15% better than controls. There was a significant height increment of flooded basil plants with fast-release oxygen amendment treatment. The slow-release oxygen amendment was significantly better than control 1 but not significantly better than control 2. Both controls were similar in height increment. Oxygen amendments produced similar relative N use efficiency and both were significantly better than controls (Table 2).

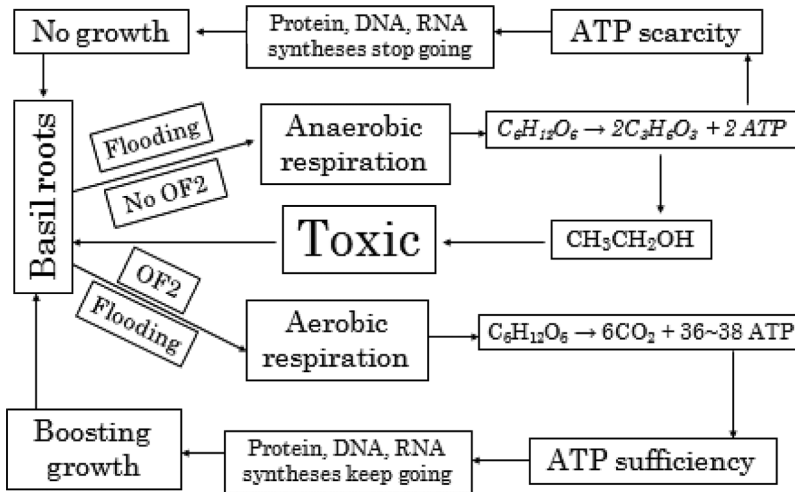


Figure 2: Possible mechanisms for using oxygen amendment to increase yield of basil affected by flooding.

Table 2: Effect of flooding or oxygen-containing compound treatment on plant biomass, plant height, and N use efficiency.

Source	Biomass (mg/pot)	Height increment (cm)	Relative N use efficiency (%)
Flooding			
Yes	3400.8b ^a	2.63b	78.0b
No	4306.6a	4.94a	100.0a
Treatment			
Control 1 ^b	3300.0b	2.75c	83.3b
Control 2	3378.0b	3.29bc	84.3b
Fast release			
Oxy. amendment	4931.1a	4.71a	96.0a
Slow release			
Oxy. amendment	3805.8b	4.38ab	92.3a

^aValues in a column followed by the same letter are not significantly different, $P < 0.05$, Duncan's multiple range test.

^bControl 1: 650 mg urea per pot; control 2: 1000 mg calcium hydroxide. Fast-release oxygen amendment: 1000 mg urea hydrogen peroxide containing 650 mg urea and 350 hydrogen peroxide; slow-release oxygen amendment containing 400 mg calcium hydroxide and forming approximately 600 mg calcium hydroxide after releasing bioavailable oxygen.

Basil plant biomass was lower when plants were flooded (Table 2) because of oxygen deficiency and energy shortage. Under the same flooding condition, oxygen amendment increased biomass production of flooded basil plants compared to plants without oxygen amendment. The plants with the fast- and slow-release solid oxygen compounds increased biomass by 79% and 27% compared to those without oxygen amendment (Table 2). Poor growth due to lack of oxygen indicates sensitivity of plants to hypoxic stress resulting

from flooding. The plants might also be suffering from toxicity due to ethanol accumulation and other toxicants usually formed under anaerobic metabolism (Blokhina and Fagerstedt, 2010).

The fast-release oxygen compound is water soluble, releasing bioavailable oxygen quickly for a short period of time. Fast-release oxygen compounds can provide flooded plants with more bioavailable oxygen than slow-release oxygen compounds for a few days. If the flooding period is longer than 5 days, more than one application of the fast-release compounds may be needed. The slow-release oxygen compound is not water soluble and releases bioavailable oxygen gradually for up to 6 months. The slow-release oxygen compound would likely be preferred to alleviate long-term flooding problems. A combination between fast- and slow-release oxygen compounds may be more advantageous than the use of one type of oxygen amendment, but more research is needed.

Nitrogen use efficiency is defined as basil biomass per unit of N supplied. Because it is positively associated with biomass and negatively related with N application rate, every factor influencing basil growth and N bioavailability impacts N use efficiency (Sowers et al., 1994). Nitrogen use efficiency of flooded plants was reduced due to their inability to obtain oxygen needed to produce active chemical energy. Nitrogen influx is an active process needing metabolically supported transport across membranes and the flow of nutrient ions down the resultant electrochemical gradient (Cernusak et al., 2009; Fiscus and Kramer, 1975). Under flooded conditions, the bioavailability of N decreases due to dilution and leaching. Oxygen amendment may overcome some limitations and improve N use efficiency. Oxygen amendment may alleviate hypoxic problems caused by flooding or overirrigation in basil production.

Redox potential analysis of flooded soil with or without oxygen amendment supported the results. The slow-release solid oxygen compound had fewer (11.7 ± 2.1 nM) ferrous ions than the control (194.9 ± 23.6 nM ferrous ions). Analysis of ferric-coated PVC tubes, an indicator of reduction in soil, also showed that the oxygen amendment held 93.6% ferric iron on the tube inserted in flooded soil for 2 weeks (Figure 3). This indicates that oxygen amendment maintained approximately 94.0% of the applied ferric iron during the 10- to 14-day period. This implied that oxygen amendment improved soil redox potential. Qualls et al. (2001) reported that the ferrous iron level in 10- to 15-cm-deep soil ranged from 1.4 to 17.7 nmol·g⁻¹ soil. Patrick (1964) found that a Crowley silt loam soil had concentrations of exchangeable ferrous iron of up to 82.4 μmol·g⁻¹ soil at redox potentials above the 100 to 200 mV range. Oxygen amendment in this study produced a healthy redox status for flooded soil because the solid oxygen amendment is a strong oxidizing agent and can supply bioavailable oxygen. This may explain why oxygen amendment was able to improve flooded basil growth and biomass.

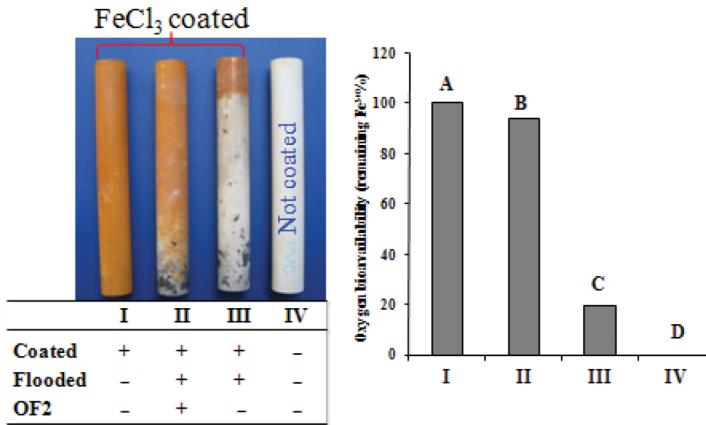


Figure 3: Difference in redox potential of soil flooded for 2 weeks. $P < 0.05$; $LSD_{2,0.05} = 4.5\%$ (color figure available online).

Oxygen-producing compounds occur in nature. In theory, every electron acceptor can supply bioavailable oxygen. Scott and Renaud (2007) listed a group of redox pairs. Among these, oxygen is the first choice for plants to use, followed by nitrate, manganese oxide, ferric iron, and carbon dioxide. Nitrate can be considered an oxygen amendment because its oxygen can serve as a respiratory electron acceptor in plants when soil is flooded and gaseous or dissolved oxygen is not available. Nitrate N fertilizers were able to alleviate flooding problems and reduce damage to vegetable crops (Li et al., 2009; Rao et al., 2002). However, the oxygen capacity of all electron acceptors on that list is too low to meet oxygen requirements for normal plant growth and development. Without oxygen amendment, flooded basil plants suffer from toxicants formed under hypoxia.

In flooded soil, there are a series of chemical reactions. Due to oxygen deficiency at the beginning of flooding, a colorless gas, nitrous oxide (N_2O), which is 310-fold more powerful than carbon dioxide (CO_2) with respect to the greenhouse effect (Finlayson-Pitts and Pitts, 2000; Houghton et al., 1996), may be emitted. Flooded plants may use nitrate as their oxygen source to accept electrons from the respiration chain. This might explain why foliar application of nitrate solutions alleviated growth-limiting factors associated with flooding (Li et al., 2009; Rao et al., 2002). If the flooding situation is not improved, another colorless, extremely poisonous gas, hydrogen sulfide (H_2S), may be released in addition to release of methane (CH_4). Methane is 25-fold more powerful with regard to the greenhouse effect (Finlayson-Pitts and Pitts, 2000; Houghton et al., 1996) than CO_2 . This release of methane is a typical anoxic condition that can kill plants. Oxygen amendment may alleviate anoxia or hypoxia from occurring and minimize emission of greenhouse gases.

Oxygen amendment may be a potential technique for the Florida vegetable industry to reduce economic loss caused by flooding. More research is needed to determine when and how to apply this novel material to vegetable crops grown under different soil and climatic environments.

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