



Effects of Added Carbon Dioxide on Iodine Staining of Soybean Leaves: A Pilot Study Toward Early Detection of Magnesium Deficiency

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Abstract

Magnesium (Mg) deficiency lowers crop yield and the nutritional value of food crops, but early-stage deficiency produces few visible symptoms and is difficult to diagnose. One of the earliest changes in Mg-deficient leaves is the accumulation of carbohydrates, including starch, which can be detected with iodine staining. Rising atmospheric carbon dioxide (CO₂) has also been associated with lower mineral concentrations in plants. In this pilot study, we tested whether iodine staining and simple image analysis could detect changes in soybean leaves exposed to added CO₂. Three groups of 20 soybean plants were grown in enclosed containers: a control group and two experimental groups that received 7 g or 14 g of gaseous CO₂ each week for four weeks. Each week, leaves were collected from the lower nodes, cross sections were stained with Lugol's iodine solution, and microscope images were analyzed in MATLAB by separating each image into its red, green, and blue channels and recording the peak count of each channel's intensity histogram. Stained sections from both experimental groups appeared darker than those of the control group, and their peak blue-channel counts rose to values five to six times higher than any control value, generally reaching a maximum in week 3. Because the most common blue value in these images was near zero, this pattern reflects darker staining rather than more blue color. These results are consistent with greater starch accumulation in CO₂-treated leaves, but Mg was not measured, and elevated CO₂ can increase leaf starch directly through enhanced photosynthesis. Future studies should measure leaf Mg directly and include Mg-deficient controls to test whether iodine staining can serve as a low-cost early indicator of Mg deficiency.

Keywords: *magnesium deficiency; carbon dioxide; soybean; starch; iodine staining; image analysis*

1. Introduction

Magnesium (Mg) is an essential nutrient for humans, supporting protein synthesis, vitamin D activation, bone health, and kidney function (Schwalfenberg & Genuis, 2017). Because people obtain much of their Mg from plant foods, Mg deficiencies in crops can reduce both crop yield and the nutritional value of food. In plants, Mg deficiency can result from Mg-poor parent rock, long-term unbalanced fertilization, removal of Mg through harvests, leaching by excessive rainfall or irrigation, acidic soil, and high temperatures (Yan & Hou, 2018). Rising atmospheric carbon dioxide (CO₂) is another concern: plants grown under elevated CO₂ tend to have lower concentrations of many minerals, including Mg (Loladze, 2014).

Visible symptoms of Mg deficiency include interveinal chlorosis, in which the leaf veins stay green while the tissue between them turns yellow, brown, or reddish, as well as early leaf drop (Ye et al., 2019; Kobayashi & Tanoi, 2015). However, many of these symptoms overlap with those of other nutrient deficiencies, so visual cues alone cannot reliably identify Mg deficiency. Moreover, the earliest stage of Mg deficiency produces little or no visible change. Before chlorosis or any decline in photosynthesis appears, Mg-deficient leaves begin to accumulate sugars (Hermans & Verbruggen, 2005) and, in particular, sucrose and starch, because Mg is required to load sucrose into the phloem for export from the leaf (Cakmak & Kirkby, 2008). Starch accumulation is therefore a potential early indicator of Mg deficiency.

Mg is mobile within the plant. When the supply is insufficient, plants move Mg from older leaves to younger, actively growing leaves, so deficiency typically appears first in the older, lower leaves (Kobayashi & Tanoi, 2015; Cakmak & Kirkby, 2008). Starch can be visualized with iodine: iodine forms a dark blue-black complex with starch, whereas tissue without starch takes on the yellow-brown color of the iodine solution. Iodine staining is inexpensive and requires only basic equipment, which makes it attractive for use by farmers and growers.

The objective of this pilot study was to explore whether iodine staining combined with simple image analysis could detect early changes in the leaves of soybean plants exposed to added CO₂, as a step toward a low-cost method for detecting early Mg deficiency. Soybean was chosen because it is an important staple crop and a fast-growing dicot that produces many leaves for sampling. We hypothesized that short-term exposure to added CO₂ would increase starch staining in soybean leaves, consistent with an early-stage Mg deficiency.

2. Materials and Methods

2.1 Plant Growth and CO₂ Treatment

Soybean seeds (heterozygous yellow soybean; Carolina Biological Supply Company) were grown in three trays of 20 seeds each in Miracle-Gro Vegetable & Herbs Garden Soil. One tray served as the control group and received no added CO₂; the other two trays served as experimental groups. The plants were kept in enclosed 64-L containers at room temperature to limit the loss of added CO₂ and received natural sunlight during late winter in California (approximately 11–13 hours of daylight). Plants were watered weekly with a spray bottle before CO₂ was added.

After all seeds had germinated and developed leaves on their first node, gaseous CO₂ from commercial canisters (approximately 40 g each) was sprayed onto the leaves. To measure the dose, each canister was weighed before spraying, and CO₂ was released until the canister mass had decreased by 7 g (experimental group 1) or 14 g (experimental group 2). CO₂ was added once a week for four weeks. If fully retained in a 64-L container, 7 g of CO₂ corresponds to a concentration of roughly 6% (about 60,000 ppm) and 14 g to roughly 12%, far above the current atmospheric average of about 410 ppm (Phys.org, 2019). The actual CO₂ concentration inside the containers was not monitored.

2.2 Leaf Sampling and Iodine Staining

Each week, leaves approximately 1 cm long and 1 cm wide were collected from each node of the plants. Each leaf was cut thinly through the middle to expose the internal cells and

observed with a Celestron TetraView LCD digital microscope. Enough Lugol's iodine solution was then added to cover the exposed cells. After five minutes, the leaves were patted dry and observed again under the microscope. The microscope light was adjusted so that individual cells were clearly visible, and images were captured with the microscope's 5-megapixel camera.

2.3 Image Analysis

Images were analyzed with a custom MATLAB program (see Acknowledgments). Each image was manually cropped to the region of interest and separated into its red, green, and blue channels. For each channel, the program computed a 256-bin intensity histogram and recorded its peak count, that is, the number of pixels sharing the most common intensity value. These peak counts were plotted as grouped bar graphs for each node and week (Figures 1–5). Because the stained sections were predominantly red-orange to dark brown, the most common blue value was near zero; a higher peak blue count therefore indicates more pixels with almost no blue, corresponding to darker, more intensely stained tissue, rather than more blue or purple color. Only nodes 1 and 2 were analyzed because nodes 3 and 4 developed later in the experiment and lacked data from earlier weeks. No statistical tests were performed.

3. Results

After the first week, standing water inside the trays caused a fungal infection that spread throughout the plants; fungus was most abundant on the lower (node 1) leaves and had to be partially wiped off before staining.

In the control group, the peak blue count for node 1 leaves stayed between about 1.2×10^5 and 2.8×10^5 throughout the four weeks, and in week 2 the green peak (about 2.6×10^5) exceeded the blue peak (Figure 1). In experimental group 1, the peak blue count for node 1 remained near 2.2×10^5 in weeks 1 and 2, rose above 1.4×10^6 in week 3, and fell to about 7.1×10^5 in week 4 (Figure 2). For node 2 of the same group, the peak blue count increased every week, from about 0.8×10^5 in week 1 to more than 1.4×10^6 in week 4 (Figure 3). In experimental group 2, the peak blue count for both nodes rose to about 1.75×10^6 in week 3; in week 4 it dropped to about 2.9×10^5 for node 1 but remained high, at about 1.4×10^6 , for node 2 (Figures 4 and 5). Peak red counts remained below about 1×10^5 in all groups, and peak green counts rose modestly in week 3 in experimental group 2. Overall, peak blue counts in the experimental groups reached values five to six times higher than any control value, generally peaking in week 3.

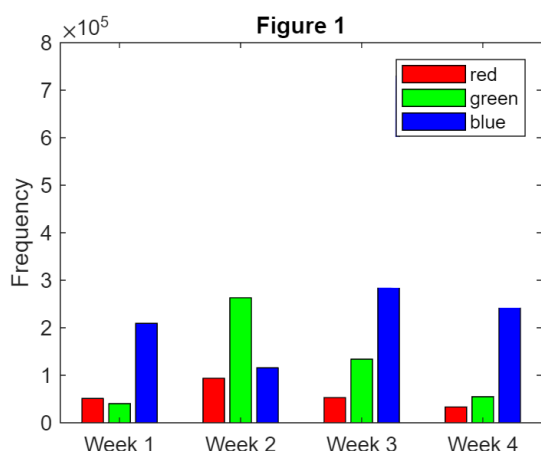


Figure 1. Peak histogram counts for the control group, node 1, over four weeks. Each bar shows the number of pixels at the most common intensity value of the red, green, or blue channel of stained node 1 leaf images. Note that the y-axis scales differ among Figures 1–5.

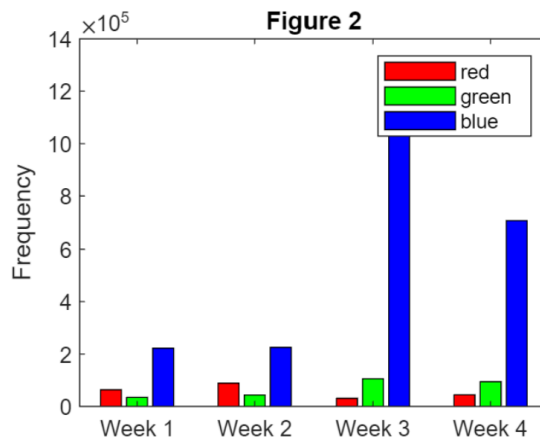


Figure 2. Peak histogram counts for experimental group 1 (7 g CO₂), node 1, over four weeks. The week 3 blue bar exceeds the upper limit of the axis.

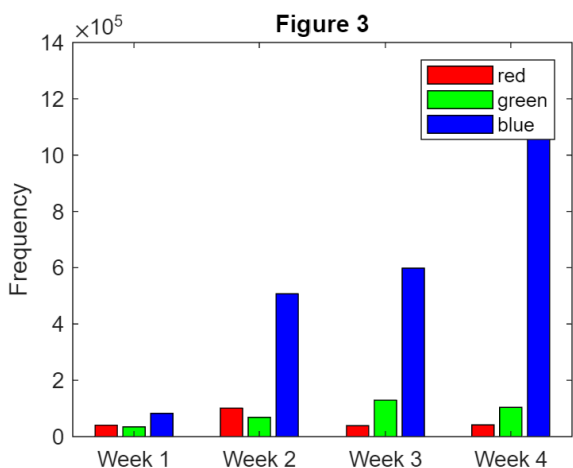


Figure 3. Peak histogram counts for experimental group 1 (7 g CO₂), node 2, over four weeks. The week 4 blue bar exceeds the upper limit of the axis.

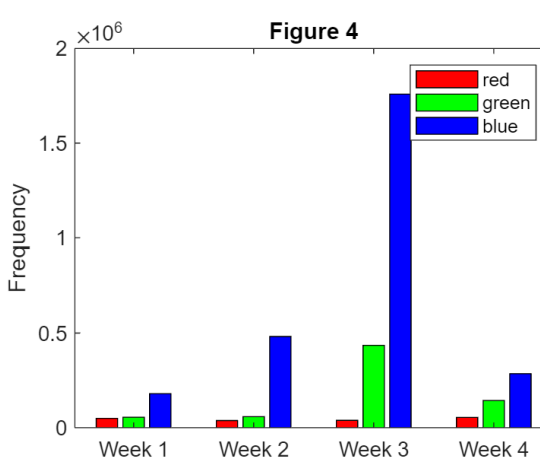


Figure 4. Peak histogram counts for experimental group 2 (14 g CO₂), node 1, over four weeks.

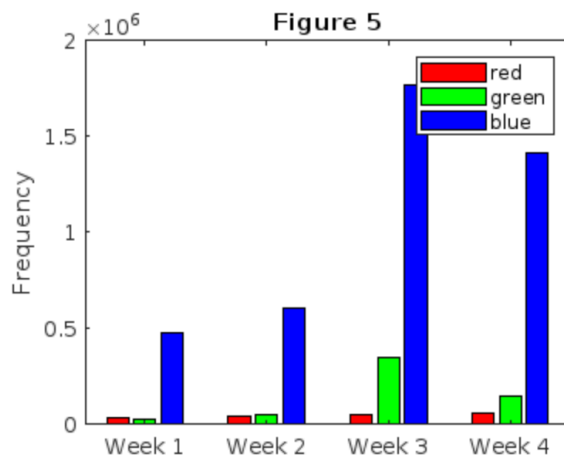


Figure 5. Peak histogram counts for experimental group 2 (14 g CO₂), node 2, over four weeks.

Representative stained sections from week 3 are shown in Figure 6. Sections from both experimental groups appeared darker and more intensely stained than those from the control group, with experimental group 2 appearing darkest.

Figure 6

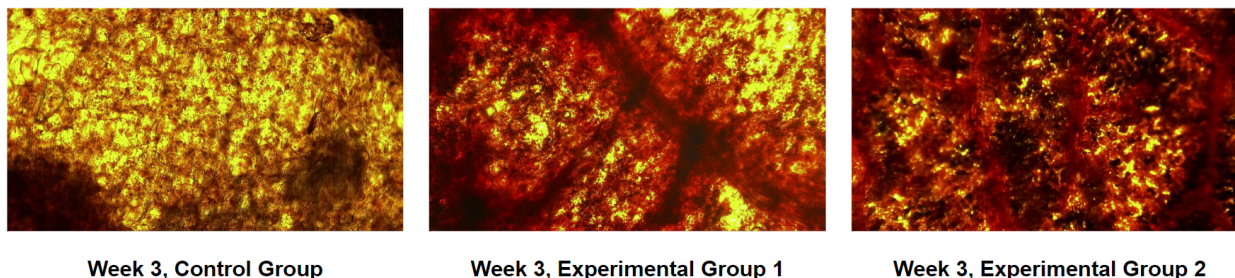


Figure 6. Representative iodine-stained leaf sections from node 1 in week 3. Control group (left), experimental group 1 (center), and experimental group 2 (right).

4. Discussion

This pilot study explored whether iodine staining combined with simple image analysis could detect changes in soybean leaves exposed to added CO₂. Leaves from both CO₂-treated groups stained darker than control leaves, and their peak blue counts, which reflect the number of very dark, low-blue pixels, rose well above control values, generally peaking in week 3. Because iodine forms a dark complex with starch, these results are consistent with greater starch accumulation in the CO₂-treated leaves, which supports our hypothesis.

However, two explanations could account for this increase. First, as hypothesized, added CO₂ may have contributed to an early Mg deficiency; Mg-deficient leaves accumulate starch because sucrose export is impaired (Cakmak & Kirkby, 2008), and elevated CO₂ is associated with lower Mg concentrations in plants (Loladze, 2014). Second, elevated CO₂ increases photosynthetic carbon uptake (Ainsworth & Long, 2005), so the extra CO₂ could have raised leaf starch directly, regardless of Mg status. Because Mg was not measured in this study, these two explanations cannot be distinguished, and the results do not show that a Mg deficiency developed.

The pattern among nodes also deserves comment. The peak blue count for node 2 of experimental group 1 increased steadily over four weeks, whereas the other experimental series peaked in week 3. The decline in week 4 for node 1 leaves may reflect the fungal infection, which was heaviest on the lower leaves; wiping fungus off these leaves before staining may also have removed some tissue or starch. Green-channel values are not directly related to iodine staining and mainly reflect less-stained leaf tissue in each image.

If validated, iodine staining could offer farmers an inexpensive way to check crops for early signs of Mg deficiency and apply Mg-containing fertilizers, such as Epsom salt (magnesium sulfate), before yield or nutritional quality is affected. Because rising atmospheric CO₂ may lower the mineral content of crops (Loladze, 2014), low-cost monitoring tools of this kind may become increasingly useful.

5. Limitations and Future Directions

This study has several important limitations. First, Mg concentrations in the leaves were not measured, so the link between staining and Mg status is inferred rather than demonstrated. Second, the CO₂ doses were far higher than current or projected atmospheric levels and the actual concentrations in the containers were not monitored. Third, the fungal infection introduced an uncontrolled variable that affected the lower leaves most. Fourth, the image metric (peak histogram count) depends on image size and cropping and does not directly measure stain color or intensity; each bar is derived from a single image, and no replicate measurements or statistical tests were performed. Finally, the figures use different y-axis scales, which makes direct visual comparison among groups difficult.

Future studies should measure leaf Mg directly, include a group grown under Mg-deficient conditions as a positive control, monitor CO₂ concentration continuously, and analyze multiple images per node with a metric such as mean stain intensity, using statistical tests to compare groups. Sampling leaves at a consistent time of day would also be important, because leaf starch content changes over the day–night cycle.

6. Conclusion

In this pilot study, soybean leaves exposed to added CO₂ stained darker with iodine than control leaves, suggesting greater starch accumulation within three weeks of treatment. This is consistent with, but does not demonstrate, an early Mg deficiency, because elevated CO₂ can also increase leaf starch directly. With direct Mg measurement and improved image analysis, iodine staining may be developed into an inexpensive tool for detecting early Mg deficiency in crops.

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