An Analysis of Photosynthesis in Poplar Inoculated with Endophytic Bacteria

Daniel Bornstein
John F. Kennedy High School
Bellmore, NY

PERSONAL SECTION

My high school's Advanced Science Research program afforded me an opportunity that was essentially impossible within the bounds of the high school curriculum: to combine my interests in science and international issues. In the summer prior to my sophomore year, I remember reading a *Wall Street Journal* article titled "Feeding Billions, a Grain at a Time," discussing how both rising food prices and climate change threatened decades of progress on global agriculture. Then, a few months later, *The New York Times* launched an article series called "The Food Chain," highlighting issues in international agriculture. I found it puzzling that while two prominent newspapers were featuring agriculture coverage, very few people in the United States were aware of global food issues.

And that's when I realized an unfortunate reality of the American people: our country is complacent about its food supply. The federal government's subsidies to large farms guarantee a stable food supply, leading Americans to take their food security for granted.

But upon reading those *Wall Street Journal* and *New York Times* articles, I began to formulate the vision that agriculture is the fundamental issue in the developing world. After all, without a stable food supply, how can poor people escape poverty and reach the path to prosperity? Indeed, the world took action on this very question in the 1960s, launching the "Green Revolution," a successful development effort that introduced high-yielding crop varieties and expanded fertilizer and irrigation in Asia and Latin America. Yet today there is a glaring flaw of the Green Revolution: its emphasis on fertilizer and irrigation is making Asian and Latin American farmers dependent on finite resources. The world needs a transition toward sustainable agriculture that relies on nature—not on resources that can be exhausted.

My support for sustainable agriculture led to a project that launched my scientific journey (an experiment I completed prior to completing my Intel Science Talent Search research). I searched the Northeast Organic Farming Association's database and obtained a plot of land at Sophia Garden, a local Community-Supported Agriculture farm. I investigated intercropping soybeans with red clover in an attempt to lower farmers' dependency on fertilizer and irrigation. I hypothesized that delaying the seeding of red clover would avoid competition between the two crops, enabling a balance between economic viability and environmental sustainability. I prepared the soil for seeding, then planted the soybean and red clover seeds manually: each plot was 15 feet by 5 feet, with 10 rows per plot, and 18-inch spacing between rows. Crop biomass and soil nutrient levels tested my hypothesis. Contrary to my hypothesis, these measurements suggested that simultaneously planting soybean and clover soybeans achieves the best balance between economic viability and environmental sustainability.

There was, however, one major limitation to implementing my farming study: the prevalence of biofuels that displace food crops. Competition between food and fuel is a major obstacle to feeding a world whose population, according to the United Nations, is expected to reach 9 billion by 2050. That dynamic encouraged me, for my Intel Science Talent Search project, to research how the use of poplar as a biofuel could avoid displacement of food crops by biofuels (research has shown that the injection of poplar with endophytic bacteria may enable poplar growth on soils unsuitable for food crops). An expert scientist—guidance that was absent from my farming study—would be a key part of my goal. That was why I contacted Dr. Alistair Rogers of Brookhaven National Laboratory.

What distinguishes me from other high school researchers is that I am not only a young scientist but also an activist for global agriculture. I have used my position as a Student Columnist for my local newspaper and as a writer for College News Magazine to counteract indifference on global agriculture. To convince people of this problem's urgency, I have written columns about the work of the World Food Prize (founded by Norman Borlaug, the father of the Green Revolution) and about President Obama's vision for increased attention to agriculture (Obama was the driving force behind a pledge by the G-8 nations last summer to allocate $20 billion for agricultural development in Africa). What's more, I read *Dead Aid* by Zambian economist Dambisa Moyo. Moyo's most powerful argument is her criticism of American food aid policy: the United States' policy of shipping American-grown food to Africa is counterproductive since it prevents African nations from building their own agriculture sectors.

For other high school students interested in science, I encourage you to choose a topic for which you have a passion. Make sure that you stay attuned to the current events related to your topic, ranging from government actions to the work of nonprofit organizations. Having this activist mindset will make you more enthusiastic about your research and give you a sense of fulfillment. My wish is to see more high school students embracing scientific research as a way to contribute to global security and peace.

I hope to be remembered as my high school research program's trailblazer into agriculture science. I was encouraged to learn that Ross Shulman, a student who entered the Advanced Science Research program in the 2009-10 school year, is interested in studying agriculture. When he told me he has a cousin who travels around the world as an organic farmer, I had no doubt that he, too, will focus on agriculture from an international perspective. I am thrilled that Ross will be working with the scientist who mentored me last summer, Dr. Alistair Rogers of Brookhaven National Laboratory. I will stay in touch with Ross and offer him guidance in his agriculture research journey.

RESEARCH SECTION

The use of biofuels such as corn-based ethanol in developed and developing nations offers political, economic, and environmental advantages. Reducing dependence on oil enables a more sustainable energy future. A setback to the use of corn-based ethanol, however, is the competition for land between biofuels and food crops. Given the combination of a growing world population and a decrease in the availability of arable land, displacing food crops with biofuels risks a failure to meet the global food demand.

Focusing on limitations on photosynthesis may identify opportunities to improve crop productivity, given that photosynthesis is the only remaining major trait available for any further increases in yield potential on the scale of the past 50 years (Long et al 2006).

Poplar *(Populus deltoides x Populus nigra* OP-367), a fast-growing plant, may present a more viable alternative to corn-based ethanol. Ways to improve poplar productivity warrants examination for the long-term. The inoculation of endophytic bacteria into poplar may produce growth-promoting effects that enable poplar establishment on marginal soils, effectively avoiding competition between food crops and biofuels (Taghavi et al, 2009).

Several factors need to be investigated in analyzing the viability of poplar as a biofuel. Measuring ratio of intercellular CO_2 to atmospheric CO_2 together (C_i/C_a ratio) with the stomata's degree of openness (called stomatal conductance) and photosynthesis can identify a potential stomatal limitation of photosynthesis (Lawlor & Cornic, 2002). In addition, maximum quantum efficiency (light-use efficiency) has been shown to be an indicator of stress across varying conditions (i.e. drought, freezing, heat, frost) in a wide range of crops (Percival & Sheriffs, 2002; Greaves and Wilson, 1987; Brennan and Jeffries, 1990; Yamada et al, 1996).

METHODOLOGY

80 poplar plants, grown in a greenhouse, were divided into four treatments: poplar without inoculation; inoculation with Enterobactor 638; inoculation with Pseudomonas W619; and inoculation with both bacteria in combination. All measurements were taken with the LI-6400 Portable Photosynthesis System. The advantage of this instrument is that measurements can be recorded in real time: the instrument was brought into the greenhouse and the poplar leaves were placed in the leaf chamber. Conditions in the leaf chamber were set to match ambient conditions. Ambient conditions in the greenhouse

remained constant (air temperature =30°C ± 4 S.D., CO_2 concentration =376 μmol mol^{-1} ± 14 S.D., relative humidity=65% ± 5 S.D.).

In terms of gas exchange measurements, stomatal conductance, C_i/C_a ratio, and photosynthetic rate were measured. In terms of chlorophyll fluorescence, maximum quantum efficiency was measured. A one-way analysis of variance (ANOVA) was performed for all measurements collected over the analysis period.

RESULTS

Maximum quantum efficiency was significantly decreased by 7% in poplar inoculated with Enterobactor (Fig. 1). There was no significant effect of endophytic bacteria inoculation on poplar's photosynthetic rate (Fig. 2), stomatal conductance (Fig. 3), or the Ci/Ca ratio (Fig. 4).

(Results begin on next page)

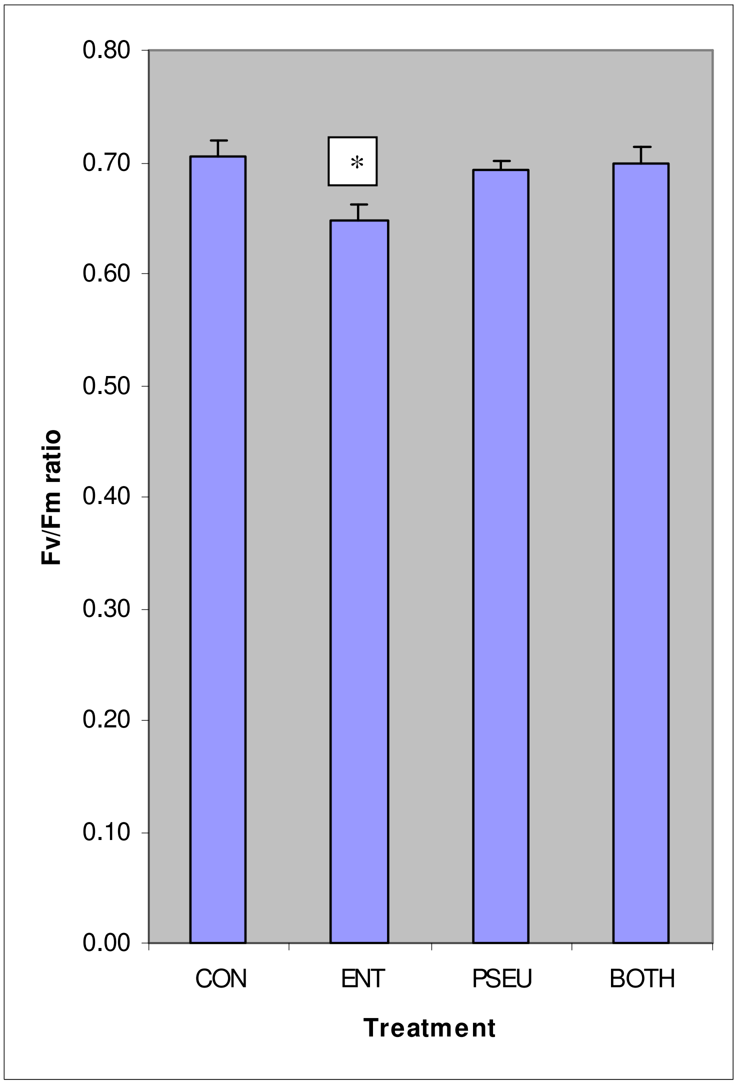

Figure 1. Maximum quantum efficiency measured in poplar (*deltoides x Populus nigra)* four times over the course of one week. Treatments tested were uninoculated control (CON), plants inoculated with the endophytic bacteria *Enterobacter* 638 (ENT) (*p<.05), *Pseudomonas* W619 (PSEU) and with both bacteria in combination (BOTH), bars show mean ± S.E.

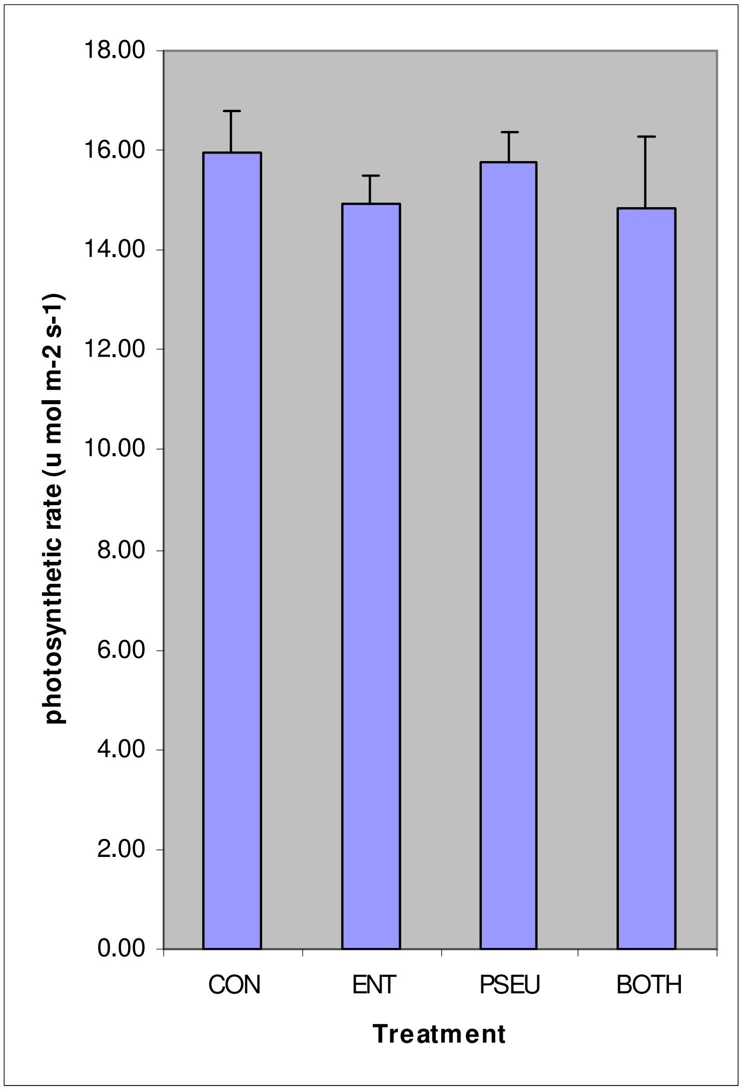

Figure 2. Photosynthetic rate measured in poplar (*deltoides x Populus nigra)* four times over the course of one week. Treatments tested were uninoculated control (CON), plants inoculated with the endophytic bacteria *Enterobacter* 638 (ENT), *Pseudomonas* W619 (PSEU) and with both bacteria in combination (BOTH), bars show mean ± S.E.

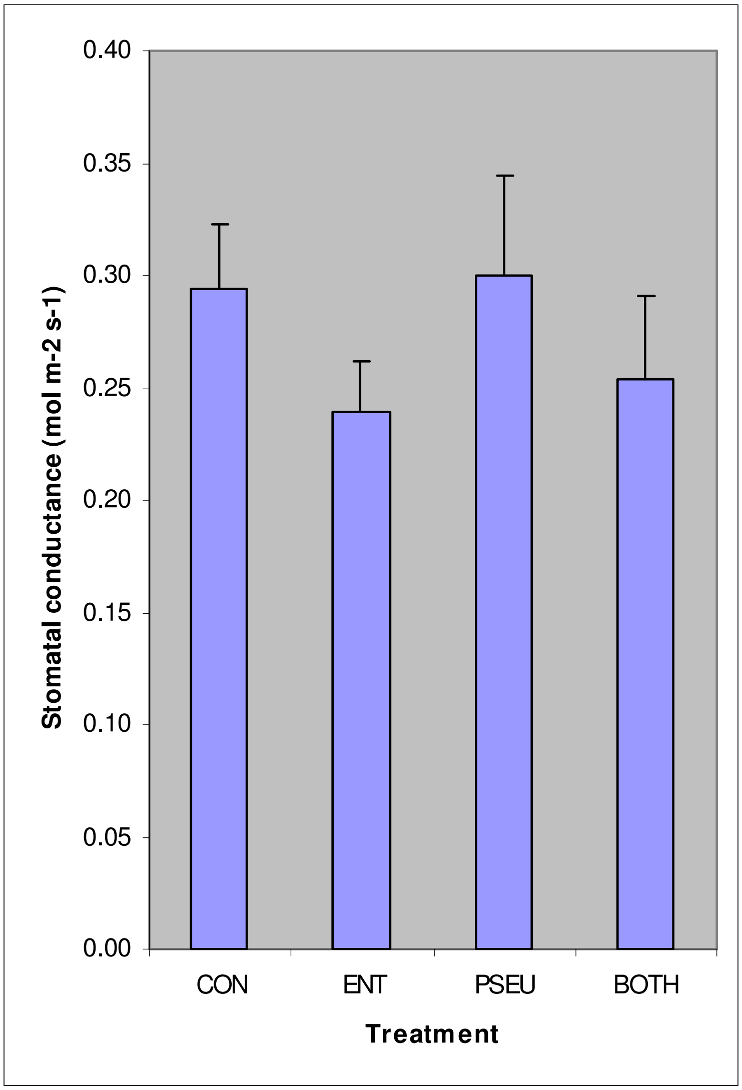

Figure 3. Stomatal conductance measured in poplar (*deltoides x Populus nigra)* four times over the course of one week. Treatments tested were uninoculated control (CON), plants inoculated with the endophytic bacteria *Enterobacter* 638 (ENT), *Pseudomonas* W619 (PSEU) and with both bacteria in combination (BOTH), bars show mean ± S.E.

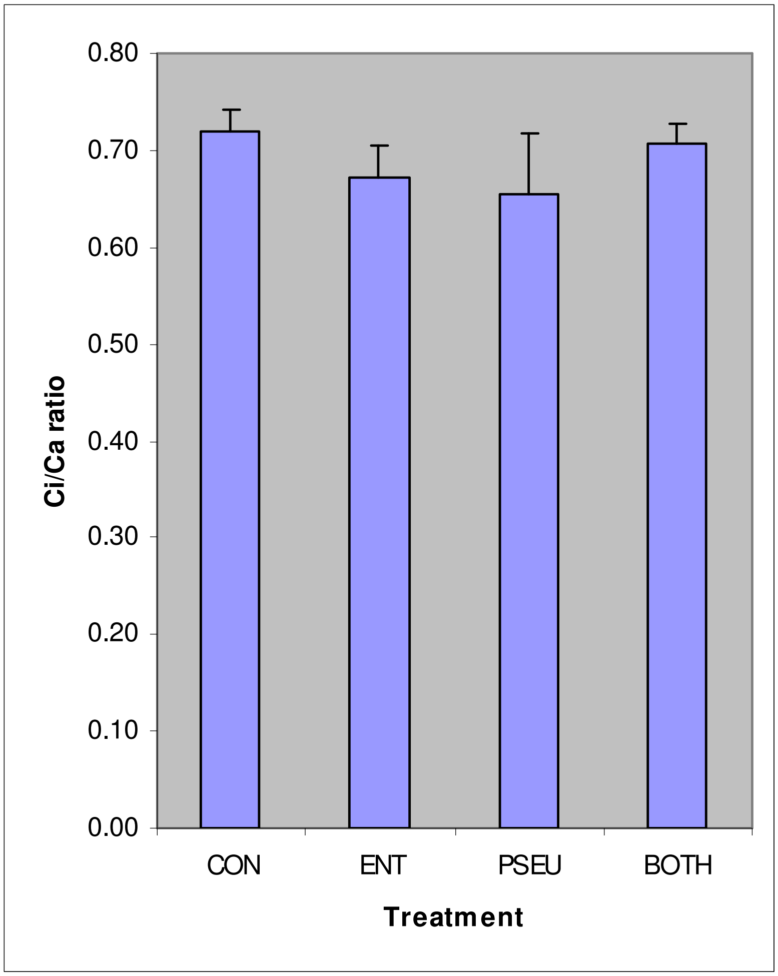

Figure 4. Ratio of intercellular CO_2 to ambient CO_2 measured in poplar (*deltoides x Populus nigra)* four times over the course of one week. Treatments tested were uninoculated control (CON), plants inoculated with the endophytic bacteria *Enterobacter* 638 (ENT), *Pseudomonas* W619 (PSEU) and with both bacteria in combination (BOTH), bars show mean ± S.E.

DISCUSSION

The higher Fv/Fm value of poplar without inoculation compared with Enterobactor inoculation suggests that the Enterobactor treatment experienced a stress. Photoinhibition—the exposure of poplar to excess light energy that reduces photosynthetic capacity—may be the stress present, given that the decrease in Fv/Fm determines the degree of photoinhibition (Kitao et al 2000). However, Similar to Powles and Osmond (1979), it is uncertain whether changes in photochemical properties are attributable to primary damage to reaction centers, interference with the transfer of excitation energy to reaction centers, or inhibition of specific steps in electron transport.

FUTURE RESEARCH

Further studies must more deeply examine photoinhibition to ascertain its causes and to test poplar's ability to recover from photoinhibition. These results indicate that poplar inoculated with endophytic bacteria may be less able to cope with environmental stress; therefore, future investigations of poplar under extreme temperature and precipitation conditions should be undertaken. This study had limited statistical power to detect small but physiologically important changes due to inoculation with endophytic bacteria. Increasing statistical power, by increasing replication and reducing variation, may allow the detection of these important differences.

CONCLUSION

Given the reliability of Fv/Fm as an indicator of stress across various conditions (Percival and Sheriffs, 2002), these data suggest that plants inoculated with Enterobactor may be more prone to photoinhibition under extreme temperature or precipitation conditions. This negative impact contrasts the benefits of endophytic bacteria described by Taghavi et al. (2009). The absence of a decrease in photosynthesis in Enterobactor may be explained by the small decrease in F_v/F_m. If the F_v/F_m decrease were more pronounced, then it is more likely that a reduction in photosynthesis would be observed.

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