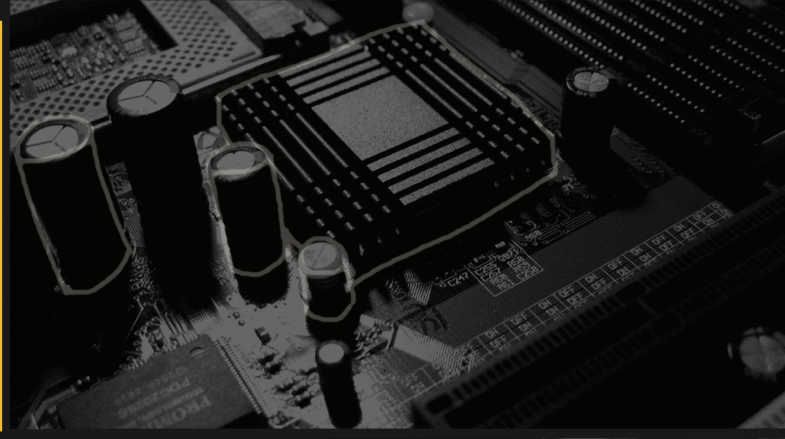
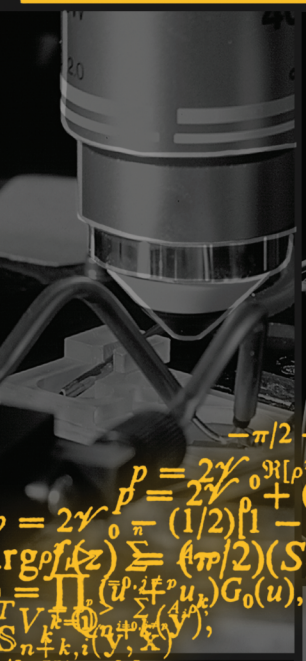
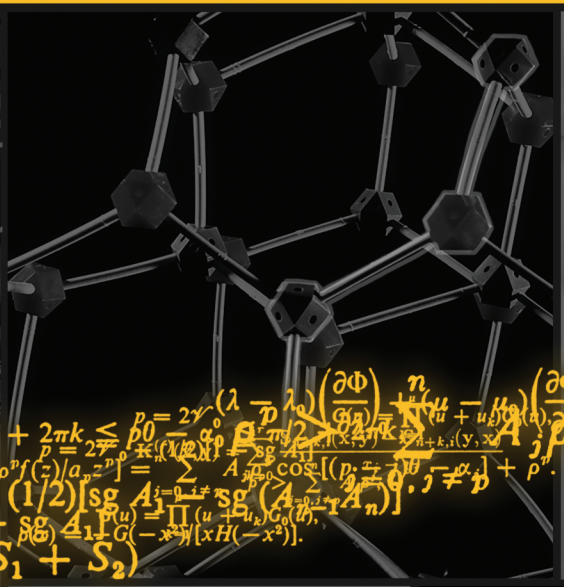
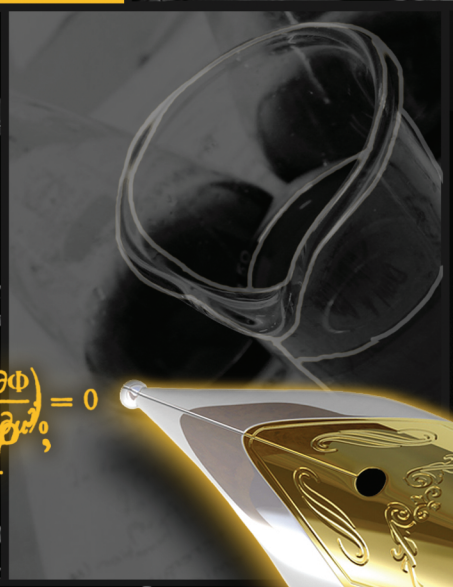
The TOWER

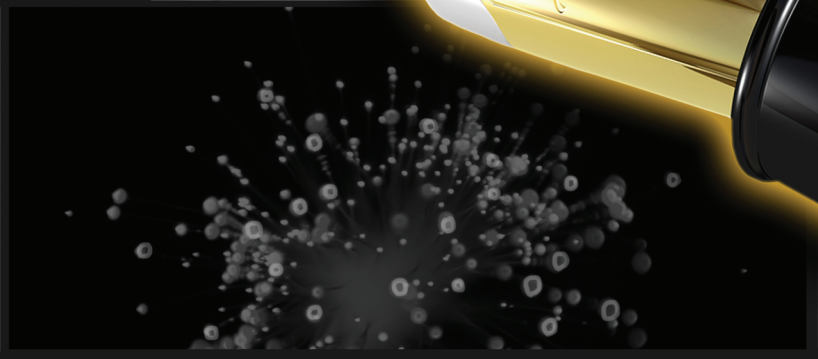
Undergraduate Research Journal
at the Georgia Institute of Technology











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The Tower is an interdisciplinary research journal for undergraduate students at the Georgia Institute of Technology. The goals of our publication are to:

- showcase undergraduate achievements in research;
- inspire academic inquiry;
- and promote Georgia Tech's commitment to undergraduate research endeavors.

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The Tower would also like to thank the Georgia Tech Board of Student Publications, the Undergraduate Research Opportunities Program, the Georgia Tech Library and Information Center, Auxiliary Services, and the Student Government Association for their support and contributions.

Letter from the Editor

The Tower has come a long way since our founding 4 years ago. *The Tower* was founded by a group of students dedicated to showcasing the world class research that undergraduate researchers perform here at Georgia Tech. With the third volume of *The Tower* printed, I would like to proudly announce that we are in the process of moving to a biannual publication from the annual publication we once were. A biannual publication will allow the many students at Tech who participate in undergraduate research twice the opportunity to publish their research in our journal.

While full research articles will remain the core of our publication, the increased diversity of content present in volume three is a trend I hope to continue in future publications. Showcasing undergraduate research does not just mean filling up a journal with technical content. The five interviews in this volume allow us to capture a more personal aspect to undergraduate research. Our interview of Professor Ada Yonath, a Nobel Prize Winner, provides a faculty perspective towards research. When I asked her how a student should decide to pursue a career in research, she responded by saying, "only if he or she has curiosity." Since most scientists find their curiosity through undergraduate research, the most important aspect of *The Tower* is to stress the importance of undergraduate research to the technological advancement of human society.

For this reason, I would like to urge all potential undergraduate researchers to explore the various submission types that we accept. In addition to research articles, we hope to see submissions in the category of dispatches, perspectives, and synopsis articles. These three formats are shorter, and emphasize the importance of the type of research the undergraduate researcher is performing.

Starting with this volume, we will cover fellowship winners, such as Alice Wang—a Fulbright Scholar in Cyprus—in every print publication. I believe such coverage helps readers learn about the various fellowships that can help them advance their careers. We also featured the work of three undergraduates, Natasha Barbely, Michelle Delcourt, and Liam Rattray. The loss of Liam Rattray to our community was tragic, and we at *The Tower* felt it was important to include a piece on the various issues Liam was passionate about.

In this volume, I would also like to introduce our new undergraduate research director and *The Tower*'s faculty advisor, Dr. Chris Reaves. He comes to us from the University of Alabama Birmingham, and we hope to continue working with him into the foreseeable future.

Special thanks to our authors for providing the content that makes our journal possible. I would also like to thank all of our staff for putting together the journal. With each volume, our staff creates a journal that is better than the last. Finally, thanks to our Student Media Director, Mr. Mac Pitts, and our accountant, Ms. Marlene Beard-Smith. Without the work of these two wonderful people, *The Tower* could not exist.

Check out our website at gttower.org for digital copies of our journals, video interviews, and other announcements. Be sure to keep on the lookout for our next call for papers for our journal as well as the call for abstracts for the Undergraduate Research Kaleidoscope.

Cheers,

Michael Chen

Editor-In-Chief, 2010-2012

Letter from the Director

It's a great honor to be able to join Georgia Tech as its first Director of Undergraduate Research and Student Innovation. These first 5 months have flown by and I have been amazed at the work of our undergraduates and the faculty and graduate students that mentor them. We have had a very busy semester and the staff and I at UROP would like to take this opportunity to share with you some of the undergraduate research activities that occurred this spring.

In March, UROP hosted a reception for 100 of the highest sought after high school students in the Atlanta area and their guests prior to the final round of The InVenture Prize Competition. Faculty and student members of some 20 different labs demonstrated their various works, which included robots, software, medical devices, etc. Our guests then joined the packed audience to watch the InVenture Prize Competition.

On April 5th, UROP hosted its 6th Annual Undergraduate Research Spring Symposium. Kudos to all the students participating and their mentors! We had over 130 students present their research and gave out some 22 awards to those that were judged as exemplary in their research endeavors (enclosed we recognize the winners as well as interview one of our Outstanding Undergraduate Researchers). Also, we owe a big thanks to many in the Tech community that volunteered to serve as judges, specifically our faculty, graduate students and post-docs, as well as those that served as moderators, scorers, greeters, set up personnel and much more. Thanks also to the many student groups that were key to the success of the symposium and associated research awards, specifically the Student Activities Board for Undergraduate Research (SABUR), The Tower Undergraduate Research Journal, and the Biomedical Research and Opportunities Society (BROS). This event would not be possible without the members of the Georgia Tech community contributing their time to make this event the success it has become.

Also, this April Georgia Tech sent 7 undergraduate students to represent Georgia Tech at the ACC Meeting of the Minds Conference held in Miami, FL. The students certainly stood out and were stellar in their presentations. Enclosed you will see the perspective of one our student representatives who attended the conference and discusses the benefits of attending.

We encourage each of you to get involved in undergraduate research either as a student researcher or as a mentor. We are reminded on a daily basis of the great rewards students earn from their research experience. Please contact our office for additional information on getting involved and the support we offer for research. You can also visit our website at www.undergradresearch.gatech.edu or friend us on Facebook.

Many thanks to those who have welcomed me so warmly to Tech! I have thoroughly enjoyed my first few months and look forward to many more with you.

Best regards,

Chris Reaves
Director of Undergraduate Research

Director's Biography

CHRIS REAVES

Director of Undergraduate Research and Student Innovation

The New Year brought a new director to the Undergraduate Research Opportunities Program with the announcement of Dr. Christopher W. Reaves to the position of Director of Undergraduate Research and Student Innovation at Georgia Tech. A specialist in community and local economic development, public policy, and program evaluation, Dr. Reaves' research has covered topics as diverse as public transit compliance of disability standards, effectiveness of domestic violence prevention initiatives, educational delivery systems and economic development programs. For the last fourteen years, he has been actively engaged in environmental and sustainable development research and practice, specifically assisting local governments in developing their capacity to redevelop contaminated properties.

Prior to coming to Tech, he was director of the Office for Undergraduate Research at the University of Alabama at Birmingham, where he was charged with planning and implementing programs for undergraduate researchers, promoting and informing students about the benefits and opportunities for undergraduate research, designing new support programs, coordinating with departments and central administration, and monitoring and reporting program statistics and learning outcomes.

Dr. Reaves received his Ph.D. in Urban and Public Affairs from the University of Louisville and an M.A. in Public Administration and B.S. in Education from Jacksonville State University. While at Louisville, Kentucky, he was actively involved in policy research pertaining to brownfield (contaminated property) redevelopment. Dr. Reaves' professional interests include, student research/innovation/creative activities, program evaluation, public policy in sustainable development and urban planning, among others. He reports being excited about the new position at Georgia Tech and is eager to lead the institute's existing emphasis on undergraduate research and burgeoning focus on undergraduate and graduate student innovation.

Getting Involved

CALL FOR PAPERS

The Tower seeks submissions on a rolling basis. Volume deadlines are posted every semester at gttower.org. Papers may be submitted in the following categories:

***Article*:** the culmination point of an undergraduate research project; the author addresses a clearly defined research problem

***Dispatch*:** reports recent progress on a research challenge; narrower in scope

***Perspective*:** provides personal viewpoints and invites further discussions through literature synthesis and/or logical analysis

Synopsis: summary of already published articles highlighting the work of involved undergraduates

If you have questions, please e-mail review@gttower.org. For more information, including detailed submission guidelines and samples, visit gttower.org.

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ADA YONATH

Professor, Wiezmann Institute of Science
Nobel Prize Laureate in Chemistry, 2009

by Tyler Kaplan

This year the Tower Undergraduate Research Journal had the pleasure of interviewing Dr. Ada Yonath, an Israeli crystallographer who is well known for her work in the discovery of the structure of the ribosome, for which she received the 2009 Nobel Prize in Chemistry. After receiving the Nobel Prize in 2009, she became the first Israeli woman to win a Nobel Prize, out of nine Israeli Nobel laureates, and was the first woman in 45 years to win the prize in Chemistry.

While growing up in the Geula quarter of Jerusalem, her childhood was plagued by difficulty, as her father passed away when she was very young and she was required to care for the rest of the family and raise her younger sister. Despite these challenges, she was sent to a school in Tel Aviv which she paid for by giving math lessons to other students. Yonath told us that she became inspired to study science by both the books that she read as a child, and by one of her early school teachers who encouraged her to pursue her interest in science.

When asked about the role of undergraduate research in the scientific community, Yonath told us that it is important that we encourage people at a young age to begin a passion for science, and cultivate that throughout their years in school. She believes that undergraduate research

is a great way to do that, and says that some of her most devoted students in the lab have been undergraduates. However, she also believes that one should not pursue a career in science if you do not have a strong passion for research and discovery. The career path is difficult, and can be extremely discouraging for someone who only has a weak passion or a small desire to follow it.

Yonath's research focuses mainly on protein biosynthesis through ribosomal crystallography, a form of research that she developed many years ago, despite the scientific community's uncertainty about its validity. Through the research she has done in this area, she was able to discover the complete high-resolution structures of ribosomal subunits and the asymmetric ribosome, which provides the framework for the process of polypeptide polymerization. Through this pioneering work that she completed, she was able to introduce cryo biocrystallography, which is now considered a common technique in structural biology that allows for the completion of projects that were previously considered to be impossible.

Yonath currently serves at the Weizmann Institute as the Martin S. and Helen Kimmel Professional Chair, and is the recipient of a number of other awards in the scientific community, including the Wolf Prize in Chemistry (2009) and the L'Oréal-UNESCO Award for Women in Science (2008).

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NATASHA BARBELY

School of Aerospace Engineering

by Azeem Bande-Ali

Natasha Barbely is an Aerospace Engineer and the recipient of the 2011 College of Engineering Outstanding Undergraduate Researcher award for her research at NASA Ames and GTRI.

Q Why did you choose your major?

A I chose the field because I find it to be both fascinating and exciting. Throughout my childhood I was exposed to space themed movies like Star Wars, which made me even more interested in the field of aerospace.

Q What do you study in Aerospace Engineering?

A I work in aeroacoustics, which is the study of the sounds that aircraft make. My research attempts to reduce the sound that aircraft generate by figuring out what is happening and why it is happening.

Q How did you get involved with this research?

A I got interested in research while interning at NASA Ames which is in California, right in Silicon Valley. While there I became very interested in the work, and subsequently came back to Georgia Tech to perform the research I am doing now.

Q You have done work at NASA Ames and GTRI. How does your work at these two places fit together?

A At NASA Ames, I worked on rotorcraft acoustics which carries over to Georgia Tech where I work with Prof. Sankar on the PSU WoP/BoP code. At NASA Ames I do a lot of experimental work but at Georgia Tech I am able to carry out more experiments by myself with the help of Prof. Ahuja.

Q What do you exactly do in your research?

A At NASA I was able to work with a 40 feet wind tunnel where large scale experiments are conducted. Back at Georgia Tech I worked on the PSU WoP/BoP code which is a code that helps us predict the noise helicopters make. And here at GTRI I was able to conduct experiments on my own with the help of Dr. Ahuja.

Q What is the important of Aeroacoustics?

A First of all, it is important for military aircraft to be quiet because noise increases thier chances of being detected in a covert situation. Additionally, many animals are frightened by loud noises, and can thus be scared out of their own ecosystem by loud aircraft. By studying aeroacoustics and reducing the noise created by aircraft, we can help our soldiers and we can protect the environment.

Q What did you get out of your undergraduate research?

A Through my undergraduate research I was able to become well-versed in my field, and also discover a passion that I want to pursue for the rest of my life.

Q It is a common belief that undergraduate students do not get to do much in research. Tell us if you have found that to be true or not?

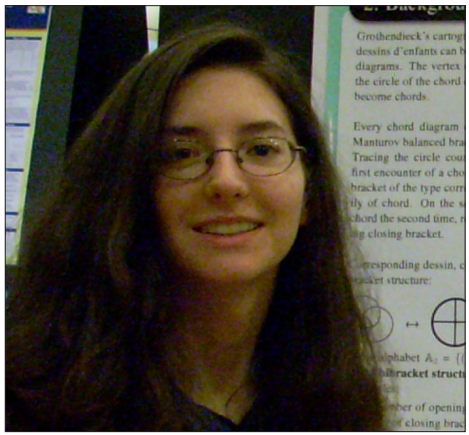
A I really feel like Georgia Tech teaches you to produce quality work as well as understand the fundamentals of your field. Your professors try to develop you into a great engineer. I find it very untrue that undergraduates cannot do much research. Undergraduates can find a number of opportunities if they look hard enough for them. For instance, I am conducting my own experiments here at GTRI as an undergraduate.

Q Have you published any of your works?

A I have published two papers. In the first one, I helped find study acoustic reflections in the wind tunnel in NASA Ames and the other one was on the Boeing-SMART rotor, also at NASA Ames.

Q You recently won an all-college award for your undergraduate research. Tell us how that felt.

A It felt wonderful, but I owe my award to Dr. Ahuja, Dr. Sankar, and all my mentors at NASA, because they always kept on pushing me and stood by me even when my days were tough.



MICHELLE DELCOURT

School of Mathematics

by Shereka Banton

Michelle Delcourt is a mathmatician and the recipient of the 2011 College of Sciences Outstanding Undergraduate Researcher award for her research at various REUs and at Georgia Tech.

Q How and when did you get involved in undergraduate research at Georgia Tech and the other NSF research experience for undergraduate (REU) programs you participated in?

A One of my most influential experiences was my first introduction to undergraduate research the summer after my freshman year. I was part of a team of three undergraduate students at Clemson University working on a problem from functional analysis at the Clemson REU in Computational Number Theory and Combinatorics.

Q Having worked at different schools, would you say there are advantages to participating in REUs as opposed to working in one lab at your home institution?

A Working at different institutions gives one different perspectives from advisors on how to approach research and what problems are important. REU programs in particular also provide opportunities to interact with students with similar interests from other institutions.

Q What did you do on a day-to-day basis during your different REUs? Did you have indepen- dent projects?

A Day-to-day activities varied by program. The Clemson program was structured so that students worked in groups of three. LSU and Georgia Tech had individual research. Generally I worked individually and met frequently with my advisor.

Q What is the coolest thing you've done in your research?

A One of the coolest skills that I learned during research was using a parallel computing system. My team wrote a parallelizable algorithm and then ran tens of thousands of jobs over the course of a summer.

Q What has been the value of your research learning- and career-wise (such as receiving the NSF Fellowship, attending graduate school, and publishing)?

A My participation in three REU programs in mathematics has shown me the importance of having curiosity, determination, and the ability to collaborate with others. During each project, I learned to formulate stimulating questions, write my results in a rigorous way, and present my findings to a variety of audiences. The skills that I have cultivated during my previous research experiences will assist me in the completion of my doctoral dissertation.

As a result of my research, I have published a paper in the Proceedings of the American Mathematical Society, an honor that surely helped me to get accepted to graduate schools as well as win an NSF Graduate Research Fellowship.

Q What graduate school are you attending, and in what program will you be a Ph. D candidate?

A I will be attending the University of Illinois at Urbana-Champaign in the fall as a PhD candidate in Mathematics. I will be participating in the Research Experience for Graduate Students program there this summer.

Q Are you considering different research areas for graduate school?

A My current interests are in combinatorics and graph theory. I will also be taking courses in other areas of mathematics as well as theoretical computer science. I am considering applying algorithmic techniques to graph theory or combinatorial approaches to proofs in other branches of mathematics.

Q What are your current career plans (academia, industry, government)?

A Ultimately I aspire to become a professor at a research university, and completing my PhD is the first step towards that goal. I intend to continue to learn and broaden my horizons afterwards through a post-doctoral position. After I have prepared myself, I will pursue a tenured position and continue to spread excitement and knowledge. As a future academician I understand there are four crucial components to being a well-rounded professor: research, teaching, service, and interacting with the mathematical community.

Q If you had any advice for current MATH undergraduates, or undergraduates in general, at Georgia Tech who are interested in conducting research what would that be?

A My two main pieces of advice concerning undergraduate research are to start as soon as possible and not to be intimidated. Apply for as many opportunities as you can and start early. The applications for most REU programs require a curriculum vitae, letters of recommendation, a personal statement, and a research statement. Writing these is good practice if you plan on applying for fellowships or graduate school. Getting a research position is competitive. If you are rejected, do not become discouraged; think about how to improve your application and apply elsewhere. In my experience obtaining my first position was the hardest. After you have participated in research, a letter from that adviser is invaluable to obtaining another position.

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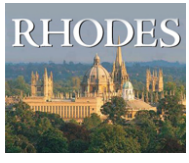

THE HARRY S. TRUMAN SCHOLARSHIP

THE GEORGE J. MITCHELL SCHOLARSHIP

ALICE WANG

School of Physics, School of Electrical and Computer Engineering
Fulbright Scholar, 2010

by Dr. Karen Adams

As fall of 2009 and her senior year approached, Alice Wang knew she wanted to apply for a Fulbright but was not sure where she wanted to go nor what she wanted to do. She discussed her interests with Dr. Karen Adams in the Fellowship Office, and they discussed several possible countries. Alice needed some time to think so she went to the library to read about countries of interest.

When she returned to the Fellowships Office, she had chosen the country for her Fulbright application—Cyprus. The year before there had been nine applications for two awards in Cyprus, so she knew what her chances of appointment might be. Language requirements for Cyprus state that English is generally used but that projects that use source materials (i.e., old documents) may require knowledge of Greek or Turkish. She would not be using archival materials and knew she would be fine in terms of language.

Alice decided her project should involve developing something for computers, but she was not sure what would be appropriate. She immediately began asking advice from people on the Georgia Tech campus to see if they knew people working on Cyprus-related projects or if they

had contacts in Cyprus. She found several helpful people, and ultimately wrote two universities in Cyprus, one Greek and one Turkish, in the attempt to obtain a letter of invitation from each.

The project Alice developed was suggested to her by her hosts and focused on developing an interactive Cyprus-like island that would allow young people in both the Greek and Turkish communities to deal virtually with some of the tension-causing problems on the island. The hope was that young people could learn how to deal with problems and that there would be less conflict between the two communities in the future. Alice learned in the spring of 2010 that she was selected for a Fulbright award to Cyprus.

Alice spent her first three Fulbright months travelling throughout the island and interviewing non-governmental and international organizations, conflict resolution scholars, and individuals involved in bi-communal peace building efforts. She wanted to create a relevant product that would be useful to Cyprus' problems and that Cypriots would use, so she worked to gauge the needs of potential users. Through interviews she found there was a need to develop a narration software to bring the two communities closer.

Alice originally proposed using the social software "Second Life" to provide a forum to help young Greeks and Turks in Cyprus interact. However, the Second Life idea in her proposal would not have been useful since Second Life is not widely used in Cyprus, so Alice is currently working to develop software in a Facebook application. She has made a photo gallery on where users can rearrange uploaded pictures on a virtual desktop and write about what is happening in

the pictures. Her hope is that users learn that pictures can be interpreted differently and do not present absolute truths. She is conducting user experience testing on the software.

Alice has also become interested in the conditions of foreign migrant workers in Cyprus since in recent years Cyprus has become a target destination for cheaply paid laborers from Eastern Europe and Asia. She submitted a proposal to Fulbright for a second project that was approved to study the working and living conditions of female Asian migrant workers in Cyprus. She is conducting interviews with migrant women in Limassol and Nicosia.

Aside from her projects, Alice has joined a Cypriot community choir and traveled to Prague to compete in an international vocal competition with them. She is taking Greek lessons and filming a publicity video for the Limassol Hospice. She states that "Cyprus is the most naturally beautiful place I've been, and I have fun every weekend hiking and traveling around the island."

A Fulbright award can be to any of 140 countries and can be a life-changing event. Students can do a one-year master's degree, a research project, or serve as an English Teaching Assistant during the Fulbright year. Alice is doing one year of research and is using knowledge learned at Georgia Tech to help others and will bring back in-depth knowledge of another culture.

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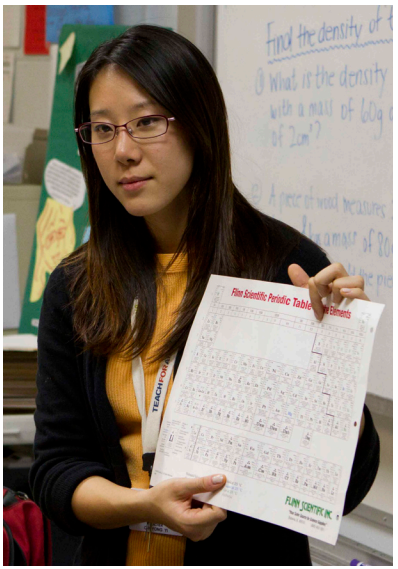
CHU YI

School of Chemistry and Biochemistry
Teach for America Corps Member, 2011

by Michael Chen



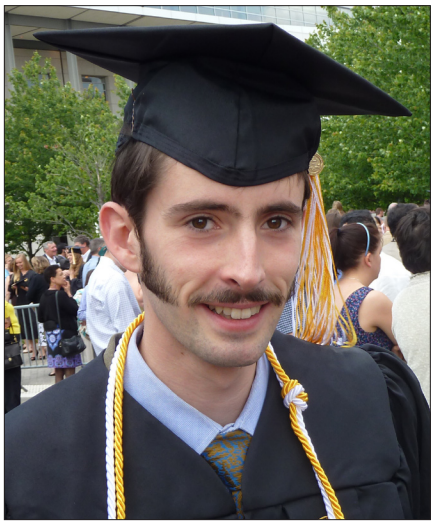
Graduate school or industry? Every year, the ritual of applying for jobs or graduate schools preoccupies the minds of most graduating seniors who are involved in undergraduate research. Yet, for one Georgia Tech alumna, neither of the options above fit her passions. Chu Yi, a member of the class of 2011, decided to spend her first two years out of college in school, but not a graduate school. Chu is a 2011 Teach for America corps member, teaching chemistry classes to high school students at Westlake High School in Atlanta, GA. An undergraduate researcher during her time at Georgia Tech, Chu believes that many of the same skills and motivations present at the benchtop apply to the classroom as well. "I get to experiment...to figure out the best learning method[s] within a class," said Yi.

According to Yi, teaching is even more rewarding than her undergraduate research experience because she sees the results of her work every single day in the form of her students' growth. During her childhood, Chu attended multiple high schools and saw the discrepancies between schools that have and schools that have not. "I wanted to do something to close the achievement gap that I experienced firsthand," said Yi. She joined Teach for America to try to

address these discrepancies by providing the knowledge she gained from her years at Tech to the high school classroom.

A commonly held belief at Georgia Tech is that Teach for America is only valuable for those who plan to pursue a career in K-12 education. Yi disagrees, saying "Teach for America offers a great starting point for any type of career...because teaching challenges you in so many different ways, and helps you grow as a person, as a teacher, as an organizer, [and] as an orator." In fact, Yi believes that Teach for America prepares college students for any job out there. "Working with close to one-hundred kids every day...and convey[ing] messages in an efficient, yet simple manner...will prepare anyone for anything they want to do in their life" said Yi.

Why should Georgia Tech students join Teach for America? "Because it is important to give back, using whatever we get from Georgia Tech to help the community," said Yi. She adds that Georgia Tech trains people to be innovative, which benefits the classroom because she believes "science must be taught in a non-traditional way" in order to pique the interest of high school students. Yi challenges Georgia Tech students to bring what they learn at Tech into the K-12 classroom because she believes that there is no cause nobler than inspiring high school students to pursue a career in science.



IN MEMORIAM: LIAM RATTRAY (1989-2011)

School of Public Policy

by Tyler Kaplan

Liam Rattray, a 2011 B.S. Public Policy Graduate from the Georgia Institute of Technology was scheduled to have a feature in this year's issue of *The Tower Undergraduate Research Journal*, but regrettably passed away the week before the interview was scheduled to take place. Rattray, who was known around campus for his dedication to his research, was to be interviewed regarding his work on sustainable food initiatives and urban farming in Atlanta.

He was involved in a project called ArkFab, which is dedicated to vertical farming capacity building. The project—a finalist at Georgia Tech's 2011 Ideas to Serve Competition—uses a multistage bioconversion process to grow foods such as fruits and vegetables by upcycling organic waste streams from businesses within the immediate area. The food that is produced within this system is grown within 10 or less miles of the market it will eventually reach, and is grown free of chemicals, hormones or irrigation. Additionally, the project will allow for training centers to operate in the immediate area to continue the education process and promote use of the system.

Rattray, whose research was advised by Associate Professor Douglas Noonan in the School of Public Policy, received a number of awards throughout his time at Tech, and was greatly

celebrated for the quality of his research. In 2011, he was named the School of Public Policy Outstanding Student, and also received a PEER Fellowship, through which he worked on open source software policy indicators. Throughout his undergraduate career, he was lauded for his infectious enthusiasm and undying dedication to his work. He will be greatly missed by both the Public Policy Department and the Georgia Tech community as a whole, and will forever be remembered as one of the hardest working, passionate individuals to grace the research community.

The Georgia Tech Honors Program is dedicating part of its Student Challenge Fund as the Liam Rattray Grant, providing funding opportunities for students undertaking projects in sustainability. Additionally, Liam Rattray's family has set up a donation program to three groups close to Liam's heart: the AMPS-UGRO garden, Truly Living Well, and the permaculture course at Koinonia. Donations are accepted at rememberliam.com.

MATHEMATICAL MODELING OF THE CHEMICAL REACTION NETWORK THAT PROTECTS MITOCHONDRIA IN HUMAN NEURAL CELLS FOLLOWING TRAUMATIC BRAIN INJURY

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"Following traumatic brain injury, human neural cells experience an increase in reactivity between peroxynitrite and superoxide dismutase. This reaction prevents superoxide dismutase from performing its essential role in the cell, which is to act as a catalyst in a reaction which protects mitochondria in the cell from damage (Bayir, 2007). Since mitochondria are vital to a cell's survival, it is desirable to understand the mechanism that a cell uses to protect itself from harm when these reactions occur. The goal of this research is the mathematical development of these processes using the techniques of chemical kinetics, so that we may gain understanding of the complicated system of chemical reactions governing this mechanism. This mathematical development includes analyzing the concentration versus time of all reactants, discovering the time scales when they react, and analyzing which reactions in particular influence the tendency of the concentration of a particular reactant to reach equilibrium. This analysis and the interpretation of the results will provide mathematical support for the proposed protection mechanism, furthering our understanding of how the brain cell behaves under the stress of traumatic head injuries. We find that this system can indeed be modeled by a system of ordinary differential equations, whose solution can be interpreted to accurately describe the system. The oxidation percentages and ratios of the different enzymes involved can be plotted and interpreted, and the amount that each reaction forces the concentration of each reagent to change at turning points can be determined."

I. MATHEMATICAL MODELING OF MITOCHONDRIAL PROTECTION

A major topic in the field of chemical kinetics is studying the rate at which reactions occur and how the concentrations of reactants in a chemical reaction change with time. A basic result from this field states that the rate at which a reaction occurs is directly proportional to the concentration of the reactants (de Mol, 2010). This law, known as the rate law, makes it possible to model, given a system of chemical reactions, the rate of change of each reactant's concentration with ordinary differential equations. This concept can be applied to very complex systems of reactions relatively easily.

Techniques from the field of chemical kinetics can be applied directly to computational biology, where the systems of chemical reactions studied occur inside human cells. Most human cells, including the brain cells studied in this paper, contain mitochondria. Mitochondria are organelles responsible for, among other things, producing adenosine triphosphate, which the cell uses as a source of chemical energy. Therefore, the healthy function of the cell is entirely dependent on the mitochondria being healthy (Mcbride et al., 2006). Damage to mitochondria is responsible for cardiac dysfunction (Lesnefsky, 2001) and other mitochondrial disorders (Gardner, 2005). Even the aging process and eventual death of cellular organisms is related to the function of mitochondria (Terman, 2010). Since the mitochondria play such a vital role to the cell, it would be beneficial to the cell to minimize any chemical having a detrimental effect on the mitochondria. Therefore, it is of vital importance to the advancement of cellular biology and healthcare applications that reactions damaging or inhibiting the function of mitochondria be understood, and in particular, what the cell does to defend against such reactions.

A medical application based on the study of how mitochondria defend themselves is related to brain cells (Bayir, 2007). Essentially, the following reactions tend to protect the mitochondria from damage in neural cells following traumatic brain injury. Superoxide ($O_2^{\bullet-}$) is produced in mitochondria. The enzyme superoxide dismutase (SOD) prevents nitric oxide from reacting with the superoxide, a reaction that would form peroxynitrite, which is harmful to enzymes. So, there must be a sufficient amount of SOD present to prevent these harmful reactions from occurring and thus to prevent damage to the mitochondria. These processes are described in greater detail by Rafikov et al. (2007). The goal of this paper is the mathematical development of these processes using the techniques of chemical kinetics.

In the given chemical network, pictured in Figure 1 (K. Hosking, pers. comm., August 11, 2009), a mechanism is proposed to "protect" the enzyme catalysts nitric oxide synthase (NOS), SOD, and catalase (CAT). An enzyme is protected when it reacts with hydrogen peroxide (H_2O_2). This is called oxidation and is denoted by a plus sign following the symbol for the enzyme catalyst. An enzyme is "infected" when it reacts with peroxynitrite ($ONOO^-$), and it cannot perform its function (Bayir, 2007). This is called nitration and is denoted by a minus sign following the symbol for the enzyme catalyst. When an enzyme reacts with hydrogen peroxide, it can perform its normal function while being "blocked" from reacting with peroxynitrite. An enzyme is said to be free if it has not been oxidized or nitrated and a free enzyme is denoted by placing a zero after its symbol. If a certain proportion of the free enzymes are nitrated by peroxynitrite before they perform their function, or before they are oxidized by hydrogen peroxide, the cell can no longer sustain its mitochondrial function and dies. Within the brain cell, there is an initial concentration of CAT0, SOD0, and NOS0 of approximately 10-3 moles per liter, as well as a large concentration of molecular hydrogen (large compared with the concentrations of the other reagents). As mentioned above, mitochondria produce superoxide under such stresses as traumatic injury. At time zero, a disturbance of the cell causes the mitochondria to inject superoxide at a constant rate, k1, into the cell, and an external reaction to the system shown in Figure 1, catalyzed by NOS0, injects nitric oxide ($NO^{\bullet}$) into the region near mitochondria. At early times, the superoxide can react in three ways: 1) with nitric oxide to produce peroxynitrite, 2) with molecular hydrogen to produce hydrogen peroxide, and 3) with molecular hydrogen catalyzed by SOD to produce hydrogen peroxide. Once all of the ingredients are present, the following reactions occur: 4) hydrogen peroxide reacts with CAT0, SOD0,

and NOS0, 5) peroxynitrite reacts with CAT0, SOD0, and NOS0, 6) an external reaction is catalyzed by the NOS+ and injects nitric oxide into the environment, 7) hydrogen peroxide decays into water, 8) hydrogen peroxide leaves the environment and 9) nitric oxide leaves the environment. All of the results assume that reactions 7 through 9 happen at slow enough rates to be neglected. This assumption has a negligible effect on the concentrations on the timescale considered in this study.

At very long times (approximately 60 seconds), the following trends are predicted based on Rafikov's physical intuition about the system: the superoxide, hydrogen peroxide, and the nitric oxide concentrations will reach a stable terminal value, while the peroxynitrite will increase linearly (pers. comm. June, 2009). The

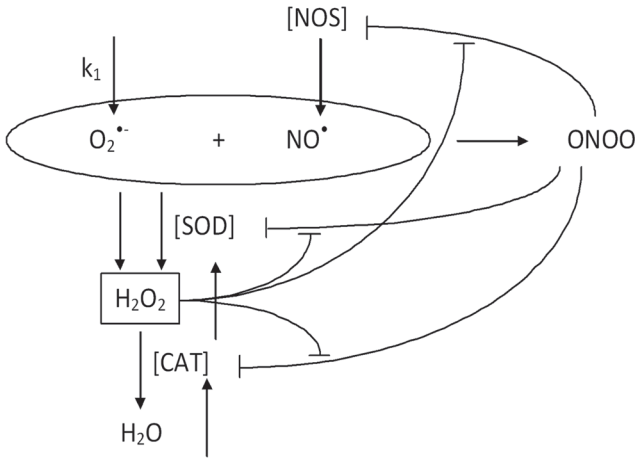

Figure 1. The chemical network describing the mechanism by which mitochondria defend themselves inside human neural cells following traumatic brain injury. Enzymes are contained in brackets, the lines with bars at one end show the enzyme nitration and oxidation reactions, and the arrows show all other reactions.

free enzymes will decay to zero on the same timescale (about 60 seconds) as they either get oxidized or nitrated, and the oxidized and nitrated enzymes will reach a terminal value as the free enzyme runs out.

The purpose of this study is to, for the first time, mathematically quantify the system of reactions describing the process by which mitochondria in human brain cells defend themselves following traumatic brain injury. This mathematical quantification includes calculating the concentration in time of all reactants involved, determining which reactions influence the tendency of the concentrations to stabilize, discovering the timescale during which these processes occur, and calculating the oxidation ratio and percent as a function of the physical parameters of the system of reactions. All of these results are obtained, but more knowledge, which is currently unknown, such as the exact initial concentrations of the reagents and the rate at which they react with each other, is required for an exact mathematical description.

II. METHOD

The system of equations given in Table 1 was solved using Matlab R2008a. The function ode23tb was employed, a solver recommended by Mathworks for solving stiff systems of differential equations (Mathworks, 2009). Stiffness can be interpreted as resulting from the difference in scale of the reaction rates. For the calculations of various derivatives below, the symbolic manipulation shell in Matlab R2008a was used.

The variable associations are given in Table 2.

The following vector is used for the reaction constants (k_i is the i^{th} entry):

$k=(10^{-3},10^{5},7\times10^{9}, 8\times10^{6},10^{3.15},0,10^{10},2\times10^{-1},0,10^{3},10^{3},10^{3})$.

The equations in Table 1 give the rate of change of the concentration of each reactant. The estimated value for each k_i gives the rate at which the two reactants it multiplies react, or in some cases, the probability that the one ingredient it multiplies will react. The k_i are determined by Rafikov in part by previous experimental data (pers. comm.). The units of concentration in these equations are moles per liter. The unit of time is seconds. Since the overall units must balance, the reaction rate constants have varying units, depending on whether the term they are in is linear or quadratic in concentration. The construction of the

equations can be understood by comparing the terms on the right hand sides to Figure 1. For example, the right hand side of the equation giving the rate of change of superoxide concentration, equation (1a), has four terms. The first term gives the rate at which superoxide is initially injected into the system. The second term denotes the decrease in superoxide due to its reacting with molecular hydrogen in the environment and producing hydrogen peroxide. The third term denotes the same reaction, but catalyzed by the free and oxidized SOD. The last term denotes the decrease in superoxide due to its reacting with nitric oxide and producing peroxynitrite. All of the equations can be constructed in this way. Note that some of the reaction constants have been set to zero. These constants are associated with reactions (8) and (9), hydrogen peroxide and nitric oxide leaving the environment, which are assumed to be negligible on the timescales analyzed in this study.

Reaction modeling equations	
$\dot{x}_1 = k_1 - k_2 x_1 - k_3 x_1 (x_8 + x_{10}) - k_9 x_1 x_3$	(1a)
$\dot{x}_2 = k_2 x_1 + k_3 x_1 (x_8 + x_{10}) - k_4 x_2 (x_{11} + x_{13})$ $-k_5 x_2 x_5 - k_6 x_2 x_8 - k_7 x_2 x_{11} - k_8 x_2$	(1b)
$\dot{x}_3 = k_{10}(x_5 + x_7) - k_9 x_1 x_3 - k_{11} x_3$	(1c)
$\dot{x}_4 = k_9 x_1 x_3 - k_{12} x_4 x_5 - k_{13} x_4 x_8 - k_{14} x_4 x_{11}$	(1d)
$\dot{x}_5 = -k_5 x_2 x_5 - k_{12} x_4 x_5$	(1e)
$\dot{x}_6 = k_{12} x_4 x_5$	(1f)
$\dot{x}_7 = k_5 x_2 x_5$	(1g)
$\dot{x}_8 = -k_6 x_2 x_8 - k_{13} x_4 x_8$	(1h)
$\dot{x}_9 = k_{13} x_4 x_8$	(1i)
$\dot{x}_{10} = k_6 x_2 x_8$	(1j)
$\dot{x}_{11} = -k_7 x_2 x_{11} - k_{14} x_4 x_{11}$	(1k)
$\dot{x}_{12} = k_{14} x_4 x_{11}$	(1l)
$\dot{x}_{13} = k_7 x_2 x_{11}$	(1m)

Table 1. The differential equations modeling the system of reactions in Figure 1.

Most of the solutions to this system of equations have the general feature where they increase or decrease at a nearly constant rate and then reach equilibrium. It is useful to know which reaction in the network is most responsible for driving the concentration towards equilibrium. This happens twice in the most well behaved set of solutions (a set of solutions is considered well behaved if it is continuous, non-oscillatory, and finite), at two different time

Reactant	*Variable*
$O_2^{\bullet -}$	x_1
H_2O_2	x_2
$NO^{\bullet}$	x_3
$ONOO$	x_4
NOS^0	x_5
NOS^-	x_6
NOS^+	x_7
SOD^0	x_8
SOD^-	x_9
SOD^+	x_{10}
CAT^0	x_{11}
CAT^-	x_{12}
CAT^+	x_{13}

Table 2. The variable associations that are made between the chemical reactants and the variables of the differential equations

scales. The dependence of the turning points on the different reactions at the first temporal stabilization point is calculated. This first stabilization happens almost immediately.

It is a result of elementary differential calculus that when a graph reaches a local maximum or minimum, the first derivative equals zero, and the second derivative measures the magnitude and tendency for the graph to curve up or down. It is useful to proceed by making a few approximations which are valid at very

short times, solving for the stabilization values of a few of the reactants, and constructing formulae for the second derivatives of each reactant. Then the dependence of each of these equations on the various reactions can be measured by taking the partial derivative of each equation with respect to each reaction rate k. Now, plots can be constructed showing the dependence of the second derivative concentration formulae on each reaction rate. This is done by taking the magnitude of the partial derivative of each second derivative formula with respect to each reaction rate k_i, and plotting this versus the subscripts of the k_i. These plots have the subscript of each reaction constant along the x axis simply for visual clarity.

The graphs of the concentrations of superoxide, nitric oxide, and hydrogen peroxide increase very rapidly when the reaction starts. At this timescale, almost all of the enzymes are free. So, take

$\dot{x}_1=\dot{x}_2=\dot{x}_3=0$. When the graphs level off, $x_6=x_7=x_9=x_{10}=x_{12}=x_{13}=0$.

When these simplifications are made, the first temporal equilibrium concentration values of superoxide and hydrogen peroxide can be solved for, shown in Table 3.

Now, the second derivative of the concentration of each reactant in terms of these stabilization values can be calculated, taking the same approximations into account (Table 4). Where

$$X = x_{1,stable}(k_2+k_3x_8) - x_{2,stable}(k_4x_{11}+k_5x_5+k_6x_6+k_7x_{11}). \qquad (4)$$

The partial derivatives of equation (3a) with respect to each reaction constant are shown in Table 5 as an example.

III. RESULTS

The graphical solutions to the system of equations modeling the concentrations of the reactants are shown in Table 6. The unit of concentration in all of the graphs is moles per liter and the unit of time is seconds. The graphs for x_8 and x_{11} are identical to the graph for x_5, the graphs for x_9 and x_{12} are identical to the graph for x_6, and the graphs for x_{10} and x_{13} are identical to the graph for x_7. These solutions are interpreted in the discussion section.

The example of a graphical representation of the magnitudes of the partial derivatives of the second derivative concentration formula of superoxide shown in Figure 3 has been rescaled by dividing each magnitude by the corresponding k value, effectively putting each value on the same order of magnitude so the graph may be read easily. The positive points in this graph reflect the tendency for the associated reaction rate to cause positive curvature in the concentration graph at the first stabilization point, and the negative points give the concentration graph negative curvature. The magnitude of the point gives the relative influence of each reaction rate on the curvature of the concentration graph.

The quantity called oxidation ratio is defined to be the ratio of the terminal values of the oxidized enzyme to the nitrated enzyme. To measure how variations in k_1 and k_{10} affect the oxidation ratios of the different enzymes, a program was written which solves equations (1a)-(1m) for a matrix of k_1 and k_{10} values, where each ranges between two input values, records the oxidation ratio for each enzyme each time the system is solved, triangulates over the range of k_1 and k_{10} values and plots a surface showing the value of the oxidation ratio versus variation in k_1 and k_{10} (Figure 4).

Various other graphs can be produced to determine how variations in certain parameters will influence the percent of the initial amount of a certain enzyme that will be oxidized. These graphs could be useful if, for example, one needed to determine a value for k_1 or k_{10} for a desired oxidation percent without interpolating in three dimensions, shown in Figure 5. Another useful graph shows the first temporal stabilization value of superoxide, nitric oxide, or hydrogen peroxide (Figure 6) versus the oxidation percentage.

Stable concentration formulae

$$x_{1,stable} = \frac{k_1}{k_2 + k_3x_8} \qquad (2a)$$

$$x_{2,stable} = \frac{x_{1,stable}(k_2 + k_3x_8)}{k_4x_{11} + k_5x_5 + k_6x_8 + k_7x_{11}}$$

$$= \frac{k_1}{k_4x_{11} + k_5x_5 + k_6x_8 + k_7x_{11}} \qquad (2b)$$

Table 3. The stable values of superoxide and hydrogen peroxide at the first temporal stabilization point.

These could be used to predict the stabilization values of either of these reactants given the desired oxidation percentage.

IV. DISCUSSION

Despite being the first attempt to model this system of reactions mathematically, all of the goals stated in the introduction are accomplished. Namely, the concentration in time of all the reactants have been computed (Figures 2a-g). Reactions that

Second derivative formulae	
$\ddot{x}_1 = -k_9 k_{10} x_5 x_{1,stable}$	(3a)
$\ddot{x}_2 = x_{2,stable}^2 (x_5 k_5^2 + x_8 k_6^2 + x_{11} k_7^2)$	(3b)
$\ddot{x}_3 = k_{10} x_5 (-k_9 x_{1,stable} - k_{11})$	(3c)
$\ddot{x}_4 = k_9 k_{10} x_5 x_{1,stable}$	(3d)
$\ddot{x}_5 = x_5 (k_5 x_{2,stable})^2 - k_5 x_5 X$	(3e)
$\ddot{x}_7 = -k_5 x_{2,stable}^2 x_5 + k_5 x_5 X$	(3f)
$\ddot{x}_8 = x_8 (k_6 x_{2,stable})^2 - k_6 x_8 X$	(3g)
$\ddot{x}_{10} = -k_6 x_{2,stable}^2 x_8 + k_6 x_8 X$	(3h)
$\ddot{x}_{11} = x_{11} (k_7 x_{2,stable})^2 - k_7 x_{11} X$	(3i)
$\ddot{x}_{13} = -k_7 x_{2,stable}^2 x_{11} + k_7 x_{11} X$	(3j)
$\ddot{x}_6 = \ddot{x}_9 = \ddot{x}_{12} = 0$	(3k)

Table 4. The second derivative formulae for the concentrations of the reactants in terms of the first temporal stabilization values and the reaction rates.

influence the tendency of the concentrations to reach equilibrium can be determined through the second derivative dependence graphs (Figure 3), the timescale during which the reactions occur can be read off the plots in Figures 2a-g, and the oxidation ratio and percent can be calculated easily (Figures 4-5).

Partial derivative of second derivative formulae for $O_2^{\cdot-}$	
$\frac{\partial}{\partial k_1}\ddot{x}_1 = -\frac{k_9 k_{10} x_5}{k_2 + k_3 x_8}$	(5a)
$\frac{\partial}{\partial k_2}\ddot{x}_1 = -\frac{k_9 k_{10} k_1 x_5}{(k_2 + k_3 x_8)^2}$	(5b)
$\frac{\partial}{\partial k_3}\ddot{x}_1 = -\frac{k_9 k_{10} k_1 x_5 x_8}{(k_2 + k_3 x_8)^2}$	(5c)
$\frac{\partial}{\partial k_9}\ddot{x}_1 = -\frac{k_{10} k_1 x_5}{k_2 + k_3 x_8}$	(5d)
$\frac{\partial}{\partial k_{10}}\ddot{x}_1 = -\frac{k_9 k_1 x_5}{k_2 + k_3 x_8}$	(5e)
$\frac{\partial}{\partial k_4}\ddot{x}_1 = \frac{\partial}{\partial k_5}\ddot{x}_1 = \frac{\partial}{\partial k_6}\ddot{x}_1 = \frac{\partial}{\partial k_7}\ddot{x}_1 = \frac{\partial}{\partial k_8}\ddot{x}_1 = \frac{\partial}{\partial k_{11}}\ddot{x}_1 = \frac{\partial}{\partial k_{12}}\ddot{x}_1 = \frac{\partial}{\partial k_{13}}\ddot{x}_1 = \frac{\partial}{\partial k_{14}}\ddot{x}_1 = 0$	(5f)

Table 5. The partial derivatives with respect to the reaction rates of the second derivative of the superoxide concentration formula.

In what follows, when referring to a region inside the stressed human brain cell where mitochondria are present, the terms "the system" or "the environment of the system" will be used.

Superoxide, hydrogen peroxide, and nitric oxide all have two different temporal equilibrium points. The first temporal point of interest is reached very quickly, on the order of milliseconds, and the second point is reached after about 60 seconds (Figures 2a-g). Initially, the superoxide that has been injected into the system by the mitochondria builds up very rapidly and reacts on the same timescale with the large amount of molecular hydrogen in the environment to produce a lot of hydrogen peroxide very quickly. As this is happening, the nitric oxide is building up from the external reaction. When the first stability point is reached, the superoxide is split between reacting with the molecular hydrogen and the nitric oxide. Until the next and final stable point is reached, the superoxide continues to increase slightly as more is injected, the hydrogen peroxide continues to increase resulting from the increase in superoxide, and the nitric oxide decreases because the rate at which it reacts with superoxide to form peroxynitrite dominates the rate at which it is injected into the environment.

There is a slight delay in the production of peroxynitrite because all of the initial superoxide goes into producing hydrogen peroxide;

Graphical solutions to equations (1a)-(1m)

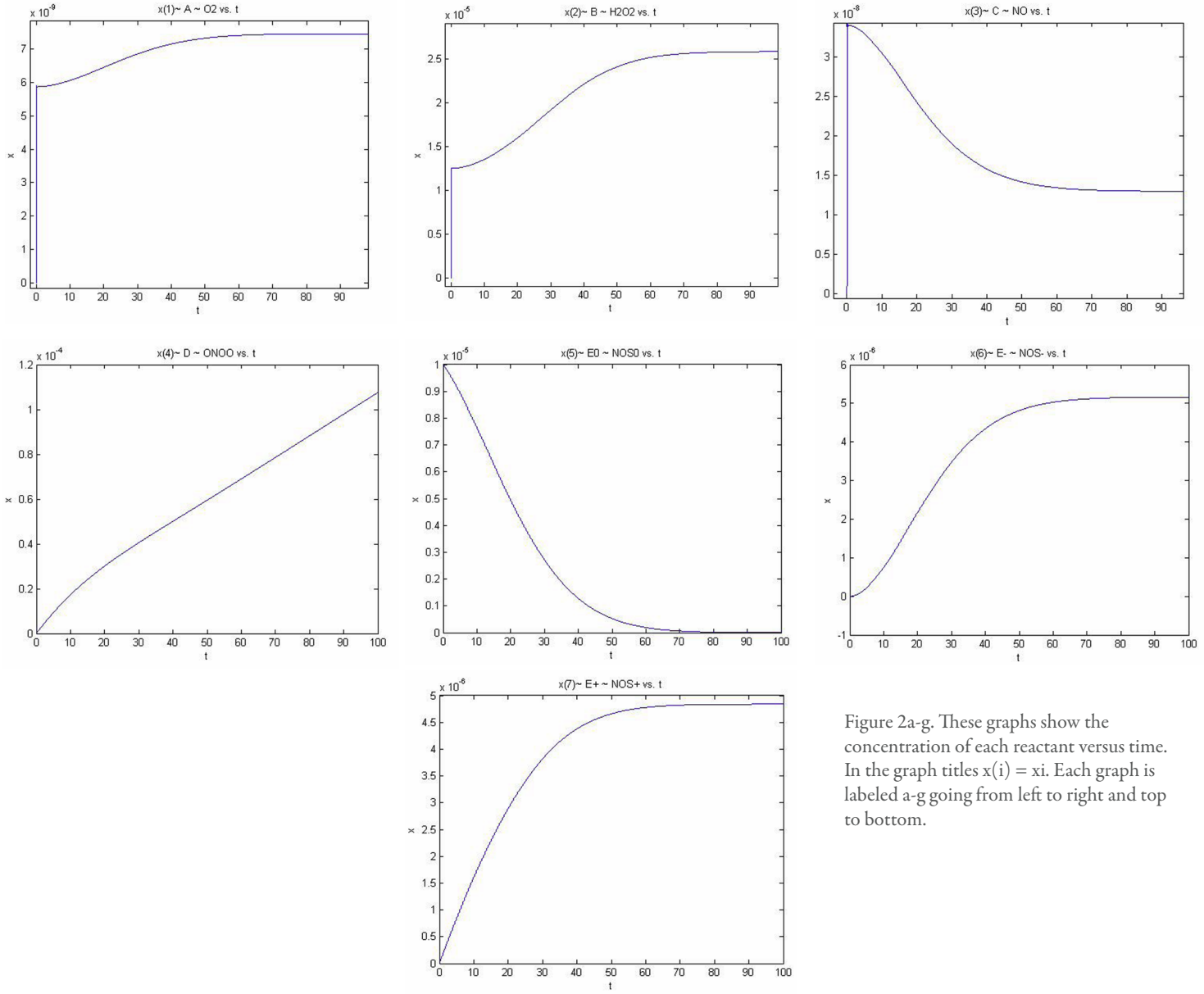

Figure 2a-g. These graphs show the concentration of each reactant versus time. In the graph titles x(i) = xi. Each graph is labeled a-g going from left to right and top to bottom.

it takes a short time for the reactants superoxide and nitric oxide to be devoted to producing peroxynitrite. After this, peroxynitrite increases linearly. If it is assumed that all the superoxide and nitric oxide goes into producing peroxynitrite and approximately none of the peroxynitrite goes into infecting the enzymes, the equation giving the rate of change of peroxynitrite reduces to an equation in which the growth is linear, namely $\dot{x}_4=k_9x_{1,stable}x_{3,stable}$, where superoxide and nitric oxide have reached their constant, stable values.

CAT0, whose initial concentration is 10-5 moles per liter, decays to zero in about 60 seconds. It splits into oxidized and nitrated CAT based on whether it bonds with hydrogen peroxide or peroxynitrite. After the same time-period, the CAT- and CAT+ level off to a terminal value because the entire amount of CAT0 has reacted. For this choice of parameters, the terminal values of CAT- and CAT+ are the same order of magnitude, showing that half the enzyme is protected, so the protection mechanism is highly pronounced. At the very beginning times, there is a slight delay in the production of CAT-, whereas the CAT+ increases rapidly. This is because there is a slight delay in the production of peroxynitrite whereas hydrogen peroxide gets produced very rapidly at the start. The analysis of SOD and NOS is similar to the analysis of CAT.

It is very difficult to give a direct interpretation of the second derivative dependence graphs (e.g. Figure 3) because the dependence is so complex. However, some general comments about the usefulness of these graphs can be made. These graphs show directly which reactions are responsible for causing the concentration to stabilize. In an experiment, one has certain control over the reaction rates, especially over the rate at which superoxide and nitric oxide are injected into the environment. Therefore, one of these graphs could be used to decrease the negative curvature at one of the turning points in a concentration graph by observing how varying reaction rates in a controlled experiment effects the magnitude of the negative points. This could be useful if one wished to delay the stabilization of NOS, allowing more time for the free NOS to prevent the formation of peroxynitrite, which, as was discussed before, is harmful to the mitochondria.

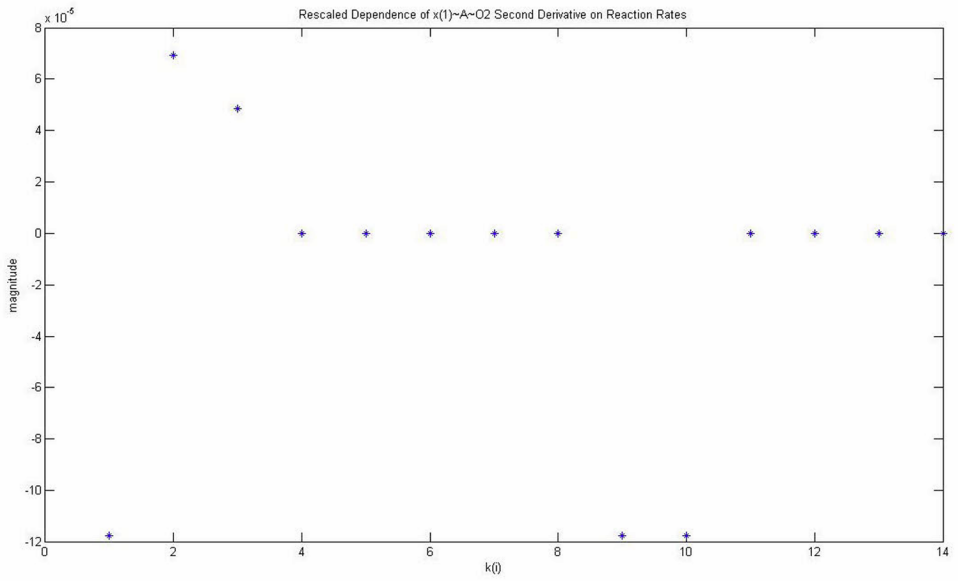

Figure 3. Dependence of x1 second derivative on reaction rates.

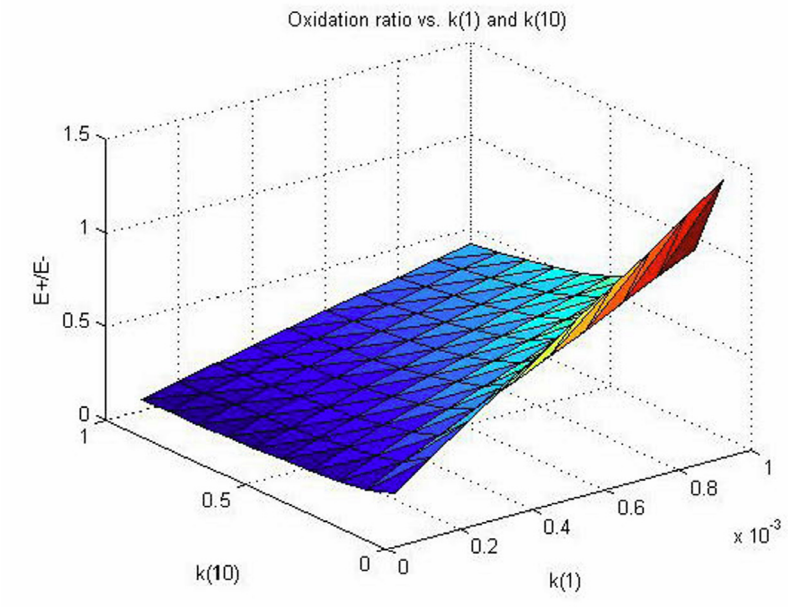

Figure 4. Oxidation ratio versus variation in k1 and k10.

The same analysis for the second temporal stabilization points is not so straightforward. It is very difficult to solve for the stabilization values of superoxide or hydrogen peroxide explicitly as before because of the dependence on x3 ,x4, and the oxidized and nitrated enzymes. So, the formulas for the second derivatives would depend on more than just the constants and initial enzyme values. The approximations which led to the formulae for the first temporal stabilization point forced the second derivatives of some of the reactants to equal zero. If similar substitutions are made for the second temporal stabilization point, nearly all of the second derivative formulae equal zero, and any further analysis would be trivial. One way around this would be to take the values of x3 and x4 at the turning points into account when evaluating the derivatives, but this would skew the dependencies. Unfortunately, this is the most biologically relevant information; showing that an enzyme's stabilization is highly dependent on the reaction between the enzyme and superoxide would show that the protection mechanism detailed in the introduction is highly pronounced in the system. Another method to analyze the dependence of the turning points on each reaction should be developed.

Fortunately, there is another way to analyze how pronounced the proposed protection mechanism is in the system: the oxidation ratio (Figure 4). The oxidation ratio measures directly how prominent the protection mechanism is; a ratio of approximately one means that an equal amount of enzyme is being oxidized as nitrated, meaning that about half the enzyme concentration can still perform its proper function. An experimenter has a certain amount of control over the reaction rates, particularly k1 and k10, the rates at which superoxide and nitric oxide are injected into the environment. These graphs could be useful to, for example, extrapolate the required k1 and k10 for a desired oxidation ratio.

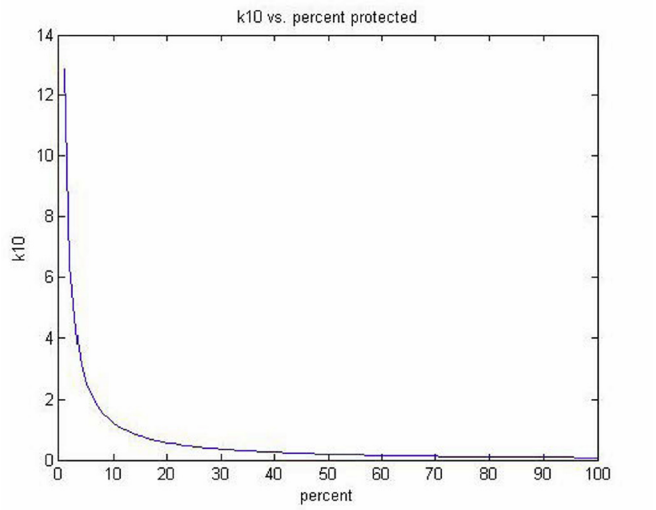

Figure 5. k10 versus oxidation percentage.

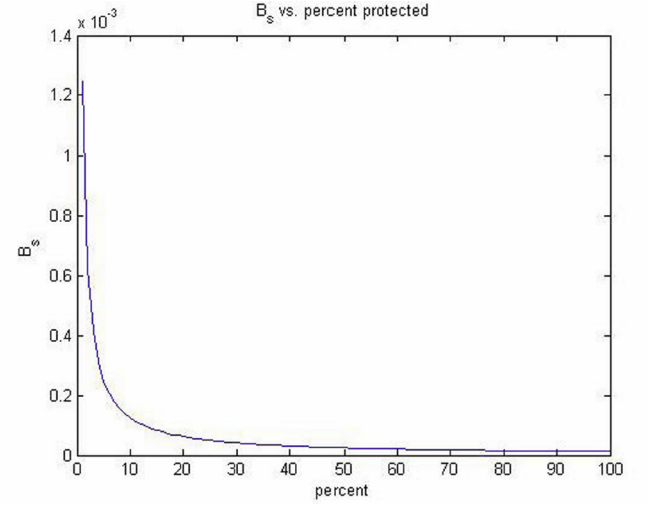

Figure 6. First stabilization value of hydrogen peroxide versus oxidation percentage.

V. CONCLUSION

The major goal of this study was to understand how the given chemical system behaves and to determine whether a mathematical model of the proposed neurocellular protection mechanism was feasible. By modeling the chemical system with a system of differential equations, the actual concentration of each reactant can be graphed versus time and interpreted to confirm physical intuition about the system of reactions. Furthermore, the timescale during which these reactions occur can be deduced. Although there is slight uncertainty in the reaction rates and initial concentrations of the enzymes, a qualitative description of the system is definitely possible. Through the analysis of various graphs, one can determine the dependence of the reactants' second derivative formulae on the reaction rates and also determine the oxidation ratio or percent given very little information about the system. In order to make a model such as the one developed in this paper more quantitative, more information must be known about the cell, including a better approximation of the initial conditions of the reactants' concentrations and a better knowledge of the reaction rates. For example, it would be desirable to know the exact threshold at which enough of the enzyme is nitrated to damage the mitochondria in the cell enough to kill the cell. With this information, treatments of patients who have sustained brain damage could be targeted at preventing the cell from crossing this threshold.

The same analysis could be effectively applied to an even larger subsystem of the cell, stripping off various simplifications and approximations, and furthering our understanding of the chemical processes that occur within stressed cells and in cells in general.

VI. ACKNOWLEDGMENTS

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PROSPECTS OF IMPLEMENTING A VHAND GLOVE AS A ROBOTIC CONTROLLER

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“There are numerous approaches and systems for implementing a robot controller. This project investigates the potential of using the VHand Motion Capturing Glove, developed by DGTech, as a means of controlling a programmable robot. A GUI-based application was utilized to identify and subsequently reflect the extended or closed state of each finger on the glove hand. A calibration algorithm was implemented on the existing application source code in order to increase the precision of the recognition of extended or closed finger positions as well as enhance the efficiency of the hand signal interpretation. Furthermore, manipulations were made to the scan rate and sample size of the bit signal coming from the glove to improve the accuracy of recognizing dynamic hand signals or defined signals containing sequential finger positions. An attempt was made to sync the VHand glove signals to a Scribbler robot by writing the recognized hand signals to a text file which were simultaneously read by a Python-based application. The Python application subsequently transmitted commands to the Scribbler robot via a Bluetooth serial link. However, there was difficulty in achieving real-time communication between the VHand glove and the Scribbler robot, most likely due to unidentified runtime errors in the VHand signal interpretation code.”

I. INTRODUCTION

Robots are a pivotal aspect of modern technology because machines are created for the simple goal of making life easier. Through machines, humans are able to automate redundant processes; eliminate human error from a production system; explore or excavate hazardous environments; and accomplish many other mundane or superhuman activities. As research in artificial intelligence develops, robotic autonomy becomes ever more feasible. The methods of controlling or communicating with robots transcend beyond employing a remote control or transmitting encoded commands through a communication link. The capabilities of artificial intelligence motivate various research endeavors that focus on communication with robots through physical interaction or visual and verbal cues.

A potential communication mechanism currently under investigation involves the visual recognition of hand signals. Humans have already established a code of sign language used for communication between people who have hearing or vocal deficiencies. Robots could be programmed to recognize these signals by using a camera to visually record hand signal images from a human, and through image processing algorithms, interpret these recorded hand signals. If robots can distinguish between varying hand signals, then programmable logic could be used to interpret the hand signal and execute a functional command.
The building blocks to such research endeavors involve manipulating the DGTech VHand Motion Capturing Glove, in order to communicate with a programmable robot. By utilizing a binary search tree populated with signal definitions, it was possible to alter the C++ based GUI hand signal application, provided by DGTech, to associate various finger positions to a calibrated / predetermined hand signal definition [1]. The ultimate goal of hand signal recognition research would be to implement similar algorithms investigated in this study in order to design robots capable of recognizing sign language through simulated machine vision by means of cameras and other visual sensors . This mode of robotic interfacing would expand the realm of artificial intelligence, making it ever more feasible for human to robot interaction in daily life.

II. BACKGROUND AND RELEVANCE OF DIGITAL GLOVE RESEARCH

Before delving into the details of the VHand research project, it is important to address the related research which preceded the VHand glove and provided a technological basis from which to expand upon. Common uses of glove-based input devices include virtual reality applications, human to computer gesture interfacing, and teleoperative (remote) controls [2]. Most of the teleoperative control applications, in which glove-based input is used to remotely manipulate or control a particular device, relate to the aim of VHand research which seeks to control the Scribbler robot teleoperatively using the VHand glove. Researchers from the Universidad Autónoma del Estado de México investigated the potential of teleoperation through glove-based input in a project that utilized a digital glove to control a robotic arm [3]. The capabilities of the digital glove implemented in this project exceed that of the VHand glove; as the glove, developed 5th Dimension Technology, is able to measure the pitch and roll of a user's hand as well as the finger positions. The researchers mapped the signal output from the digital glove to a control scheme that manipulated the rotation of the shoulder, elbow, and wrist of the robotic arm and further indicated whether the gripper claw should open or close. The results of this project yielded support for the teleoperative control of robots using glove-based input, and in addition demonstrated that computer data networks can serve as a communication channel between digital gloves and robots.

Human to computer gesture interfacing is another aspect of glove-based input research explored in a study by The Robotics Institute at Carnegie Mellon University [4]. The researchers developed a hand signal recognition system which could acknowledge sign language executed by a user wearing a Virtual Technologies 'CyberGlove'. The CyberGlove is comparable to the VHand as they use similar sensor technology to record the finger positions of the user [5]. The hand signal recognition system developed at Carnegie Mellon was based on a statistical model known as the Hidden Markov Model (HMM) which essentially incorporates probability distributions to identify sequences. In this case the sequences, or gestures, were comprised of individual hand signals

referred to as states of the sequence. The system would associate a series of states as a predefined gesture and could also learn new gestures by receiving user input on the definition of the new gesture and the states it consists of. Though the recognition system was able effectively identify gestures and learn new ones, the complexity of the system's algorithm made it difficult to implement. For testing purposes, the researchers treated the glove hand as a whole; meaning the system could only recognize open hand and closed fist positions, and not individual finger positions.

The research with the VHand glove attempts to integrate the teleoperative and gesture interfacing aspects of glove-based input to develop a system where a robot can recognize gestures executed by a human and consequently operate based off of the given hand signals. The VHand signal interpretation system implements a node-based model, in the form of a binary search tree, to recognize hand signals from the glove. The binary search tree is an algorithm that allows the computer to search through a hierarchical model of nodes, which are defined as individual hand signal positions . As the computer receives input from the glove indicating a particular hand signal, the computer will transition through the model until it reaches a node signifying the completion of a gesture. This article will expound more on the algorithm developed for hand signal recognition and shed light on the possibilities of teleoperative robotic control using sign language.

III. DETAILS ON HARDWARE

A. VHand Glove Specifications

The specifications on the VHand Glove, Figure 1, are as follows [6]:

The glove communicates with a PC using a port connection with a DB9 serial cable. The capturing application, from which the research project is based, is a C++ application compiled with Microsoft Visual Studio.

IV. APPROACH FOR VHAND SIGNAL INTERPRETATION

A. Original Source Code

The original hand signal interpretation application, provided by the DGTech developers, contained the initial source code for the GUI motion capturing application. The application, shown in Figure 2, primarily consisted of a visual display which represented the glove hand of the user.

There are five bars representing the five fingers of a hand. The bars graphically indicate how far each finger can extend or contract as detected by the sensors. When a finger is contracted the signal reading from the sensor will be close to 0, which is reflected on the display as a white or empty bar. As the user begins to extend

Table 1: VHand Glove Specifications

Sensors:	5 resistance bend sensors, one per finger
Resolution:	10 bit (1024 positions)
Transmission:	RS-232 38400 BAUD full duplex
Frequency:	100 Hz
Power Supply:	12 V DC
OS:	Windows 95, 98, 2000, XP

Table 1: VHand Glove Specifications

the finger, a blue filler rises along the bar indicating the percentage of contraction detected by the sensor. The signal coming from the sensors are 10 bits in length which correspond to a minimum value of 0 and a maximum value of 1023 .

B. Prior Source Code Modifications

Numerous modifications were made to the developer's source code in order to enable the glove hand capturing application to recognize and interpret specific sign language hand signals, including the addition of two classes: Interpreter.cpp and Tree.cpp. These classes are responsible for defining the finger configurations for each hand signal and subsequently matching the hand signals to it respective robot signal command.

Figure 1. Two images of the DGTech VHand glove. The picture above shows the complete DGTech glove package including the serial link, com port, and power adapter.

A five bit signal was created in the Interpreter class and is composed of one bit for each finger on the glove hand. The most significant bit represented the thumb and each subsequent bit represented the next finger on the hand with the least significant bit representing the pinky finger. A threshold value was calculated by averaging the maximum and minimum sensor values in a series of manual calibration tests to determine a reliable threshold by which the Interpreter could consistently distinguish between an extended or contracted finger. This value was established as ~28% of the maximum signal range, so that if the 10 bit signal from the finger sensors exceeded the 28% threshold, a value of 1 was given to the corresponding bit in the 5 bit signal. For instance, if the user were to extend only their index finger past the sensor value of 288 then the corresponding 5 bit signal created by the Interpreter class would be 01000. From this 5 bit signal, the interpreter class could identify the hand signal executed by the user and iterate through the binary search tree to find specific definition which represents the signal 01000. The following is a list of all predefined hand signals of capturing application [1].

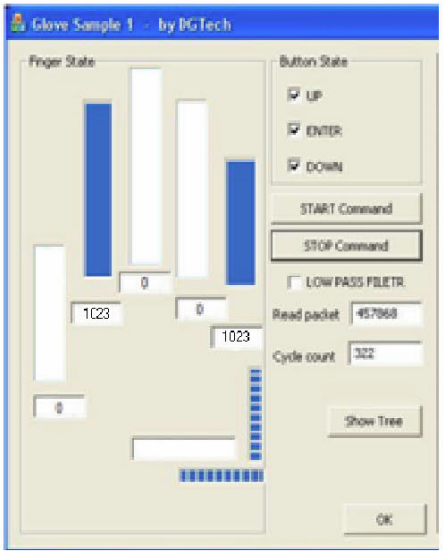

Figure 2. A screenshot of the original GUI interface of the interpreter application provided by DGTech.

C. Recent Contributions and Code Modifications

Following the source code modifications, in Section B, there were several problems remaining that hindered the effectiveness of the application. Foremost, the finger sensors were quite volatile and the current formula used to calculate the threshold was not robust enough to account for the unpredictable nature of the sensors. The sensor output of the maximum and minimum finger position values were never close to 1024 or 0, respectively, in operating conditions. The recorded values of extended and closed finger positions would intermittently fluctuate during each execution of the application. Moreover, the size of the user's hand would also impact the signal readings. These flaws had the potential to cause the application to not recognize the correct finger positions of the glove user. Furthermore, it was difficult to perform a dynamic hand signal that could be recognized by the application because the application could not effectively transition between nodal states due to scanning and timing issues.

To correct these issues, an algorithm was implemented into Interpreter.cpp, which replaced the old threshold calculation

formula. The user was given an option after running the application to initialize a calibration sequence. The calibration sequence prompted the user to fully extend all fingers for five scan rate cycles and then fully close all fingers for another five cycles. The recorded maximum and minimum finger position values were registered and used in a new formula which takes the difference between these values and defines the threshold as a variable percentage of the difference.

Table 2: Static Hand Signals

Signal Definition:	Binary Representation:
“Zero”	00000
“One”	01000
“Two”	01100
“Three”	11100
“Four”	01111
“Five”	11111
“Six”	01110
“Seven”	01101
“Eight”	01011
“Nine”	00111
“Distress”	00100
“Good Job”	10000
“Fly”	10001

Table 2: Static Hand Signals

In addition, manipulations were made to the sample size and window size variables in order to increase the scan rate of the application, since the original scan rate was too slow to be effective. Originally the sample size was 20 packets, meaning that the Interpreter.cpp class would register and average 20 consecutive 10 bit packets from the glove sensors to generate one sample to be interpreted by the class. Furthermore, the window size variable dictates how many samples are averaged together to identify one set of finger positions or hand signal. The window size value was originally set to 30. Noting that the default scan rate of the glove sensors is 100 Hz as indicated in the hardware specifications, a sample size of 20 and a window size of 30 would correlate to a functional scan rate of 1/6 Hz or the recognition of one finger position every 6 seconds. Testing proved that this rate was too slow to effectively detect dynamic hand signals; therefore, the sample size was reduced to 5 packets and the window size reduced to 20 samples which resulted in a functional scan rate of 1 Hz or one hand signal per second. A scan rate of one hand signal per second represents a more realistic rate at which sign language signals are executed, resulting in an increase in the effectiveness of the interpreter application.

Table 3: Dynamic Hand Signals

Signal Definition:	Binary Representation:
"Come here"	{01000, 00000, 01000, 00000}
"Go”	{01000, 00000, 00100, 00000, 01000, 00000, 00100}
“Bye bye”	{01111, 00000, 01111, 00000}
“Wait / Pause”	{11111, 11110, 11100, 11000, 10000}
"Hold / Get"	{11111, 00000}
“Open”	{00000, 00001, 00011, 00111, 01111, 11111}
“Shoot / Fire”	{11000, 01000, 11000, 01000}
“Stop / Hault”	{00000, 11111}

Table 3: Dynamic Hand Signals

V. COMMUNICATION BETWEEN THE VHAND GLOVE AND SCRIBBLER ROBOT

To test the practicality of the VHand glove serving as a robotic controller, an attempt was made to employ the VHand as a controller for a Scribbler robot, shown in Figure 3. However, due to the lack of a Bluetooth serial API for C++ applications, the VHand application could not directly communicate with the Scribbler robot, which is reliant on Bluetooth communications.

A work-around communication medium was conceived which involved writing the hand signal output commands to a text file. Concurrently a Python-based application would read the text file, associate the command with an applicable Scribbler function, and transmit the command to the robot. Given the complexity of the interpreter application's source code, this approach provided a simple and feasible means for porting the signal commands from the C++ based signal interpreter application to the Scribbler robot.

For the sake of simplicity, only basic commands were used for the purpose of testing the communication link with the scribbler. The commands were Forward (FRWD), Backward (BACK), Turn Left (LEFT), and Turn Right (RGHT). The practice of using 4 letters to represent each command made it easier to read in the commands using the Python application.

To establish a Bluetooth serial communication link between the Python application and the Scribbler robot, several key steps found in the Institute of Personal Robots in Education (IPRE) Robotic Services website must be performed [7]. Primarily, BASIC Stamp Editor v2.4.2 was utilized to program the Stamp microcontroller on the robot and download the IPRE services which enable use of the Fluke device, Figure 4.

The IPRE Fluke facilitates a Bluetooth serial link for wireless communication to the Scribbler robot. After reprogramming the Scribbler robot's microcontroller using the IPRE services, the robot is capable of recognizing various functions and commands executed in Python.

Figure 3. The Scribbler robot used to test the teleoperative capabilities of the VHand glove. The Scribbler's API has built-in functions to control the movement of the robot, play sound bytes, and activate the onboard LEDs.

The Python application contained a simple script which read in the command currently stored in the output text file and consequently sent the appropriate Scribbler function command to the robot. The program would loop repeatedly using a While() loop and a time delay of 1 second was implemented to slow the rate at which the application would send commands to the robot. If the recognized command from file had not changed between scan cycles, the application would not send another command.

Although the concept seemed sound in theory, the computer could not handle both the C++ and Python applications running simultaneously. However, the Scribbler robot could execute a list of hand signal commands that were saved to a text file after the glove application recorded a sequence of hand signal commands.

VI. RESULTS AND DISCUSSION

Currently, by utilizing the calibration sequence, the glove capturing application is capable of recognizing all of the hand signals performed by any user no matter the size of their hand or the condition of the glove sensors. The glove was tested by both a male and female researcher. Despite the fact that the glove did not fit properly on the male's hand, both researchers were able to successfully execute all of the defined hand signals including the

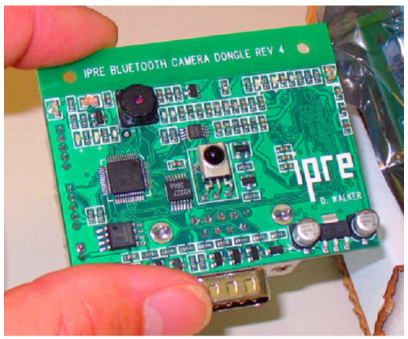

Figure 4. The IPRE Fluke device facilitates Bluetooth serial communication to the Scribbler robot. The Fluke is attached to the serial port located on the front of the robot.

dynamic signals that involved 5 - 6 sequential finger positions. This testing indicated that the signal recognition problems which remained after the initial code modifications have been corrected. Figure 5 displays a screenshot of the signal interpretation GUI after successfully executing a dynamic hand signal command.

In the execution of the "Shoot / Fire" command shown in Figure 5, the user must execute the following sequence of hand signals involving the thumb and index finger: {11000 01000 11000 01000}. The completion of this sequence is indicated in the binary value tracking field on the right side of the GUI.

Note that although the sensor values were not exactly zero for the contracted fingers, the program was still able to recognize these sensors as being in the closed position due to the threshold values set from the calibration algorithm.

Moreover, the current state of the project is able to provide support for the fact that sign language, through glove-based input, can be used to control a robot. Even though the VHand glove was unable to communicate with the Scribbler robot in a real-time environment, it is feasible that a researcher can develop the necessary run-time environment using a different programming language to achieve real-time communication.

In concomitance to the real-time communication issue is the fact that the glove application is prone to arbitrarily crashing during runtime. This runtime error is inherent to the developer's

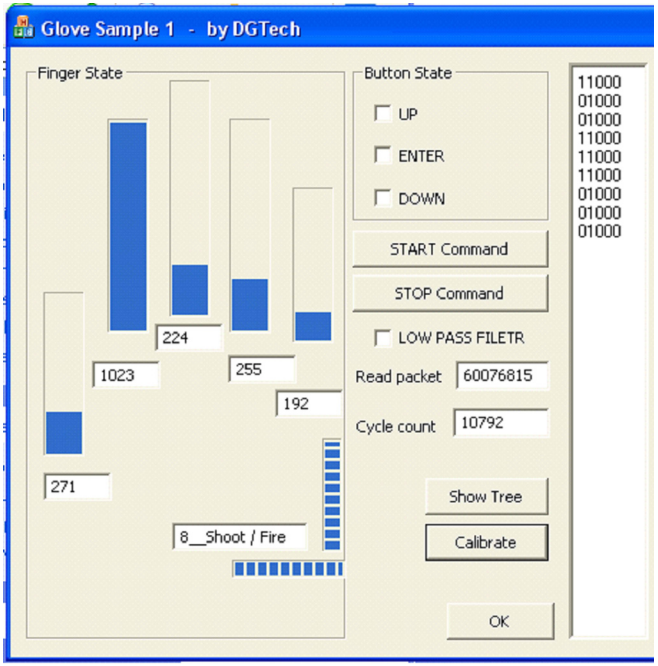

Figure 5. Screenshot of the hand signal interpreter application showing the successful execution of the "Shoot / Fire" dynamic hand signal. The text field on the right side of the GUI tracks the binary signals received from the glove.

source code and was documented in an earlier research report [7] detailing the preceding code modifications mentioned in Section 4B. Efforts were spent debugging the program in order to isolate the cause of this runtime error, yet the cause remains unknown. A probable speculation is a memory leak error in the interpreter application, which causes the computer to crash when the application exceeds the available RAM. Another supposition is that the VHand drivers provided by DGTech are defective.

Regardless of the issues involving communication between the VHand and the Scribbler, there are several lessons to be learned from this research endeavor. Primarily the algorithm for signal interpretation in conjunction with the method for calibrating the glove to a user's hand is an applicable technique for future endeavors involving glove-based input. The VHand signal interpretation system boasts a simple recognition algorithm that

is robust enough to identify hand signals involving each of the 5 fingers in the hand. Pending advancements in robotic image processing, concepts of this algorithm can be applied to a robot; enabling it to recognize human sign language visually using a camera as the sensory input.

VII. FUTURE WORK

There are many possibilities for advancement which remain with regards to the development of the hand signal recognition application. One immediate change would be to improve the means of implementing the calibration algorithm. As is, the calibration sequence is optional, and can be activated by pressing its respective pushbutton in the GUI application. However, the configuration settings established after calibration are lost when the program is closed. A useful feature to include would be to have the option of saving the finger position configuration of the user so that they would not have to repeat the calibration sequence on every execution of the program application.

If the reliability of the finger sensors improves, there would be an opportunity to allow for more finger states than simply extended or closed. This could be implemented by increasing the hand signal size from 5 bits to 10 bits which would enable up to four recognized positions for each finger. Another option would be to forego using binary signals for finger position recognition and, instead, use the 0 to 1024 integer value utilized in the developer's source code. Variables could be created to store each integer value representing the fingers of the glove hand. This integer value can be converted into a percent value of finger contraction. This implementation would enable greater possibilities when controlling a robot by allowing enhanced signal recognition capabilities. Glove users would be able to execute forms of sign language, such as the letter C, which require the computer to acknowledge a bend in the fingers as a defined finger position and not simply extended or contracted.

One other focus of improvement for the VHand glove capturing application is to identify the cause of the runtime errors. These errors pose a hindrance to the application's viability and restrict potential utilization of the application. One consideration is to recode the program in a different programming language such as C# or Python, which may eliminate any potential memory leaks existing in the current source code. Moreover, these programming languages are supported by the IPRE robotic services and provide Bluetooth serial capabilities which allow for wireless communication between the VHand glove and a robot.

Refining the programming of the VHand signal interpretation algorithm would pave the way for various captivating applications of the VHand glove. Using a Bluetooth serial link, it would be possible to facilitate teleoperative communication of the VHand glove with an autonomous humanoid such as the Robosapien. The Robosapien is a commercially available, toy robot programmed with artificial intelligence and personality attributes. The robot comes with a remote control to activate any of the various pre-programmed functions; however, the Robosapien can also interact with its environment without human control. Future research could explore the plausibility of reprogramming the Robosapien to receive Bluetooth signals from the VHand glove. Employing the VHand signal interpretation algorithm, a nearby computer could interpret the hand signals coming from the VHand glove and wirelessly relay the appropriate commands to the Robosapien. This would effectively replace the controller that comes with the robot allowing contemporary sign language to dominate the behavior of the robot.

VIII. ACKNOWLEDGMENT

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THE GMO CONTROVERSY AND ITS EFFECTS ON BRAZIL'S AGRICULTURAL SECTOR: FOCUSING ON SOYBEAN TRADE WITH THE EUROPEAN UNION

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"The investigation of genetically modified organisms (GMOs) is a recently popularized issue within the realm of international science and technology research. It has been a mere 18 years since the United States' FDA declared that genetically engineered foods are not inherently dangerous and do not require special regulation. In the midst of the publicized controversy surrounding the idea of genetically engineered food items, Brazil has been caught between two differing forces in the agricultural realm: commercial farmers, researchers, and agribusiness vs. environmentalists and consumer advocates. The history of GMO production in Brazil encompasses heated battles due to both internal and external disagreements. In addition to a general concern about the risks of growing and consuming bioengineered agriculture, the Brazilian government has struggled to integrate GMOs into the farming sector because Brazil's largest agricultural importer, the European Union, has remained hostile to GMOs, placing strict rules on the importation, labeling, and distribution of these foods within their markets."

I. INTRODUCTION

Because of this controversy, I have chosen to examine the implications of Brazil's decisions regarding GMO production and trade within the past twelve years, focusing on the Brazil-EU trade partnership and the effects of GMO suppression on the Brazilian agricultural community. In order to closely look at the changes that have occurred, I will focus on the soybean sector for two reasons: Brazil is the second largest producer of soybeans in the world and their first controversial GM legislation, proposed in 1998, concerns the use of Monsanto's Roundup Ready (RR®) Soybeans. Upon the introduction of this first piece of legislation, Brazil was hesitant to integrate biotechnology into its commercial farms, pressed by the European Union to remain GM-free because a large majority of European consumers were anti-GM, fearful of the unknown effects of bioengineering. But, as the GM conflict has persisted in Brazil, their federal government has repeatedly supported the dissemination of biotech crops and subsequent research and development in the field, while the EU continues to resist GM foods. So, why did Brazil make the change?

I will discuss the interactions between Brazil and the EU during Brazil's tense period of GM controversy, noting the nature of their relationship as trading partners and how trade patterns have changed since Brazil has stepped into the international GMO market between the years of 1998-2008. I suggest that Brazil's executive body and agricultural sector has been unable to resist the influence of large corporations, such as Monsanto, therefore they have seen an enormous increase in the percentage of GM soy crops produced and exported. But, rather than harming Brazil's economy, this has increased the EU's participation in trade with Brazilian soy growers. Since Brazilian regulations on GM crops are still in the process of being solidified, Brazil has the largest percentage of non-GMO soybean acreage in the world, with almost 30% of their soybean production classified as conventional. The EU has not shifted its trading focus away from Brazil, instead it has become even more interested in utilizing both the GM (on a limited scale) and the non-GM soy crops available, further distancing its market from the worlds' other important soy producers, including the United States and Argentina, who both have over 90% of their soy crop as GM.

II. THE DEVELOPMENT OF THE GMO CONTROVERSY IN BRAZIL

In September 1998, Brazil's National Biosafety Technical Commission (CTNBio) announced the Commercial Release of the Genetically Modified Soybean (Roundup Ready® Soybean), concluding that "there is no evidence of environmental risk or to the human or animal health from the use of the genetically modified soybean in question." This was the first attempt at GMO acceptance in Brazil, coming only two years after the manufacturer, Monsanto, introduced this herbicide tolerant bean on the international market. The resolution would allow Brazilian soybean farmers to purchase transgenic seeds from Monsanto as a five-year study was conducted to validate the new crops as harmless. Just weeks after this approval, backlash arose in the form a class-action lawsuit filed by environmentalists and consumer nongovernmental organizations (NGOs) within the 6th Civil Law Circuit of Brasilia. They claimed that CTNBio "didn't know enough about the safety of genetically modified crops when it cleared Monsanto." As a result, the lower court granted a preliminary order rescinding Monsanto's permission to distribute the RR® seeds. After this decision, Monsanto and the federal government appealed to the regional federal court.

The judges of this higher court denied the appeal in 2000, overruling CTNBio's decree, immediately placing an outright ban on GMOs in the region indefinitely, requiring that an environmental impact study (EIS) be conducted and labeling requirements be established before any other GMOs were taken into consideration. Quoted in the Wall Street Journal, Marilena Lazzarini, the executive coordinator of the Brazilian Institute for Consumer Defense (IDEC), one of the organizations that filed the suit against Monsanto, praised the court's decision: "We hope that now they [Monsanto] will stop their irresponsible goal of liberating bioengineered seeds in the country without the necessary evaluation of risks to the environment and human beings." In addition to IDEC's opposition to GMOs, the international conservationist organization Greenpeace vehemently opposed Brazil's new decree on soy planting. Mariana Paoli, the Campaign Coordinator of Greenpeace's Genetic Engineering Brazil depart-

ment stated: "We do not yet know the consequences of genetically modified organisms into the environment and public health. Therefore, we consider the application of the precautionary principle and the implementation of an EIS." Under the precautionary principle, the burden of proof that GMOs are NOT harmful falls on those who are supporting their use. So, therefore, in order for the genetically-modified foods to be considered acceptable, it would be the responsibility of an informed, ruling body to determine any risks and make that information available to the public.

After the GMO ban in 2000, some planters were stuck with Roundup Ready® seeds that they had already acquired from Monsanto, and some were anxious to use the product, so they began to smuggle the seeds from Argentina, who at the time was the world's second largest GMO producer. Argentina began using RR® seeds in 1996, and by 2003 almost 100% of their soy farms produced GM crops. The majority of these illegal crops were brought from Argentina into Brazil's state of Rio Grande do Sul, located right across the border in the southernmost part of the country. The federal government under President Fernando Henrique Cardoso did not support the court's ban on GM crops and, therefore, has been accused of "indirectly encouraging the growing of GM soya, [because] it lacked a clear policy on GM crops and failed to adequately monitor crops." Although Cardoso did not take a strong stance against Monsanto's seed, the state government took preventative measures, passing laws explicitly banning the cultivation of GMOs. They also tried to take advantage of the European Union's concern about GM foods, urging farmers to stick with the non-GM crops and protest the relentless push toward GMOs from companies like Monsanto. One pamphlet they published even said that science should be "under public control to benefit life, not under private control to [benefit] profit."

In the end, the state government was unable to implement these preventative measures, and it is estimated that around six million tons of transgenic soybeans (80% of the region total) were ready to be harvested after the 2003 season. This vast act of piracy had not gone unnoticed on the international soybean market; corporations and farmers across the globe who were legally growing the RR® soybeans spoke out against the ineffectiveness of Brazil's government to curb the theft of Monsanto's intellectual property. So, in March 2003, the federal government under newly elected President Luiz Inácio Lula da Silva, issued Provisional Measure (PM) 113, allowing the commercial use of the illegal crops that had already been grown using pirated seed. Following the publication of this measure in the United States, producers' concerns continued to escalate: Brazil's illegal exportation of GM soybeans gave them a distinct competitive advantage over the U.S., who had to pay both high taxes on their goods and royalties to the corporation. This emergency measure did not permit Monsanto to bring more RR® seeds in, but in an effort to save the Rio Grande do Sul farmers from losing millions of dollars of crop that would otherwise be destroyed, the Brazilian government deemed this a "conduct adjustment," only if the farmers would agree to not plant GMOs again. Monsanto tried to fight back against the Intellectual Property Rights violation by "requiring exporters in Brazil to sign license agreements" in order to export the RR® Soy that had been temporarily allowed in 2003 by issue of Presidential Decree, but since the crops were allegedly being placed back on the "ban list" the following year, such agreements were ignored and the illegal crops were distributed at the government's command.

At this point, PM 113 had set place very few, weak requirements such as the labeling of products in which GMOs consisted of 1% or more of the total volume. The labeling constraints were never adopted, and the Ministry of Agriculture even admitted that there "were not enough accredited laboratories available to certify GM and non-GM soybeans. President Lula, who passed this measure in 2003, was eager to make some changes regarding biotechnology, feeling pressure from GM corporations such as Monsanto, who were ready to see compensation for the harvesting of their RR® product. In an attempt to make sure the federal courts could not issue another moratorium on GMOs by disregarding all other entities who should be consulted on such an assessment, a new Brazilian Biosafety Law (No 11,105) was passed in March 2005. This law created the National Biosafety Council (CNBS), and re-established CTNBio as a group of 27 members from all facets of

agriculture, from a consumer rights specialist to a representative from the Ministry of Defense. Also, it provided safety norms and inspection mechanisms that all sectors of GM production and trade would be required to follow, including parameters within the construction, storage, research, and marketing sectors.

The most stirring portion of this law is that the responsibility of determining the safety GM products was completely handed to the newly formed CNBS. With this law in place, CTNBio was now allowed to provide the final word regarding the accepted technical opinion of GMOs. They were now in the position to implement much needed policies: monitoring research, authorizing new species of GM plants, and regulating the registration and farming of accepted crops. In Brazil, in order for a law to enter into effect, a regulatory decree must also be signed by the President, much like an Executive Order in the U.S. This decree cannot change the verbiage or provisions of the law, but it can create bureaucratic obstacles that could change the overall effectiveness of the law. Knowing that the Decree was needed, IDEC struck again, and the Federal Public Prosecutor filed a lawsuit in Brazil's Supreme Court called a Direct Action of Unconstitutionality (AIDN), claiming that the law was unconstitutional and, therefore, could be challenged in the highest court of law.

After eight months of rigorous debate among governmental officials, President Lula signed Decree No 5,591, implementing the provisions of Law No 11,105 and allowing CTNBio to finally get on track, regulating the GM trade in Brazil and expanding the use of GM crops throughout the nation. The law required that two-thirds vote was necessary within the CNBS to approve a new biotech agricultural product. Since the anti-GMO presence in Brazil was so fanatical and (even) militant, they were able to gain membership within CTNBio and block the passage of new GM regulations, inciting many scientists to ultimately leave the commission as no progress had been made and there were 500 pending new product requests. So, in 2007, President Lula signed Law No 11,460, changing the previous law, calling for a majority vote within the CNBS, rather than a two-thirds vote.

III. HOW HAS THE EUROPEAN UNION AGRICULTURAL SECTOR DEVELOPED WITHIN THE CONTEXT OF THIS GMO MOVEMENT?

In 1999, multiple countries in the EU started urging the European Commission (EC) to place a de facto moratorium on any new GMO approvals and in July 2000, EU ministers accepted the proposal, agreeing that no new GMOs would be accepted into the European market until further labeling and tracing methods were researched, tested, and implemented. Europeans have exhibited a growing concern about food health and safety since the late 1990s, prompted by the disturbing emergence of mad cow disease and instances of AIDS-contaminated HIV blood. Today, the EU still has very hard guidelines for GM crops, allowing very few GM foods into the country and almost no cultivation within the borders. The EU's opposition to the GM revolution has been intensified in recent years due to the steady growth of the anti-GMO movement across the continent, supported by the EC's regulatory approach and embedded in a general sense of apprehension. Europeans were susceptible to far-fetched information about GM effects, hearing stories about dangerous additives, unhealthy processing methods, and the risk of cross-contamination between GM crops and GM-free crops, because it was very difficult to contain the two varieties in their designated areas once seeds were cross-pollinated and multiple harvests had been conducted. In order to gain access to the EU soybean market, Monsanto was granted a reportedly vague patent two years before the GM controversy began in Brazil: 1996. This patent encompasses genetically modified plants that have been made resistant to glyphosate, Monsanto's own herbicide, also known as Roundup®. In agreement with the patent, the EC allowed Monsanto to introduce RR® soy into the EU market, but it was not (and to this day, is not) allowed to be cultivated on EU land.

The EC stresses the need to prevent "contamination" within the natural crop harvest, issuing a Commission Recommendation in July 2003 that outlines specific procedures to "ensure the co-existence of genetically modified crops with conventional and organic farming." Although the first point of this document states that no form of agriculture will be excluded from the EU, the reality

is that the guidelines that have been put in place in order for GM growers to gain access into the European market are so stringent that even the most experienced and preeminent corporations cannot break-through in a reasonable amount of time. As Brazil's government grapples with the idea of GMOs, some farmers have expressed their interest in remaining GM-free, seeing this as a way to remain competitive in the EU market is to produce the soybeans that they want to buy. Farmers believe that the price for natural soy will increase as the availability on the international market is contained primarily in Brazil, since it is the last major non-GE soy exporter in the world. As of 2006, the Federation of Rural Workers and Family Farmers in South Brazil (FETRAF-Sul/CUT) committed to selling more than 50,000 metric tons of GM-free soybeans to the European market with the help of the Dutch Soy Coalition. A report published by the EC's Directorate General for Agriculture and Rural Development published a study in 2007 urging the world's major soybean producers to consider the EU's harsh regulations and realize that even if the European Food and Safety Agency (the EU-wide regulatory agency for GM-related issues) gives clearance to a certain food or feed, that does not mean that all Member States have accepted the GMO, as well. In fact, there has never been a majority agreement among Member Nations concerning any GM product.

There have been many studies conducted investigating the EU's weariness of biotech foods, and many scholars have suggested that negative consumer perception lies in a widespread lack of knowledge about the effects of GM crops, causing fear and rejection that will continue to grow as long as the anti-GM movement advertises exaggerated defects in GM cultures and the EC implements different submission hurdles and labeling policies to which biotech companies must conform. In her paper, "Governing GMOs in the EU: A Deviant Case of Environmental Policy-Making?," G. Kristin Rosendal suggests the apparent lack of support is due to the ineffectiveness of "environmental policy in the face of influential corporate interests." She presents four theses to explain the EU's resistance to the biotech industry: a lack of internal unity, limited access to decision making, the strength of counterbalancing forces, and industry interests for protectionist reasons. The GM backlash, she says, has been fueled by counterbalancing forces within the internal sector, including the work of Environmental NGOs (ENGOs) such as Greenpeace/Friends of the Earth. An interesting element of the public opinion, she claims, is that "people [put] more trust in information from ENGOs compared to industry as well as regulatory authorities." Her conclusion looks to the future of GM policy in the EU, deeming the effect of external activities (such as WTO disputes) as a "dark horse" that will increase opposition to GM foods in the beginning, but in the long run these events will help strengthen the GM movement in countries surrounding and interacting with the EU, hopefully obstructing their harsh policies and facilitating a change in the EC's standards.

Another scholar, Sylvie Bonny, also discusses the influence of NGOs on the anti-GM movement, but rather than focusing on their work within the policy sector, she focuses on their ability to exploit the fear that many Europeans already possess concerning biotech foods by creating media hype and promoting non-GM products in all sectors. In her paper, "Why are most Europeans opposed to GMOs?", Bonny conducts a case study, comparing the anti-GM movement in France to that in the EU as a whole. She focuses on the development of this overwhelmingly negative response, linking the GM conflict to a strong distrust of firms and public authorities as food safety issues were widely publicized and the problems of industrial pollution came to the forefront around the same time that GM products were gaining ground in the international market. She also attributes the negativity to the strong influence of NGOs and other associations that focus only on the risks, representing a segment of the population that began as a small circle of environmentalists, but has evolved into an enormous movement including economic interest groups, human rights activists, and agribusiness firms. By incorporating many different media outlets into their publicity schemes, Bonny claims that this dynamic sector of the public has been able to provide inescapable sources of suspicious information to the public, criticizing the GM movement on all levels and encouraging public support.

In contrast to focusing on the transformation of public opinion with regards to policy support or media exploitation, Joyce Tait "analyzes

the risk-related problems that have arisen over the introduction of GM crops and food products in the context of the adoption of the Precautionary Principle (PP)" in her paper titled "More Faust than Frankenstein." This standard, established as a guideline at the 1992 United Nations Conference on Environment and Development (UNCED) through the Rio Declaration on Environment and Development, states that in order to protect the environment, each nation needs to interpret the safety of new technologies according to their ability, without disregarding potential damages due to a lack of certainty or scientific evidence. Ironically, in the EU's circumstance, a precautionary stance was originally taken as an attempt to draw support from the public, avoiding the problems that arose while under a preventive regulatory system. Tait proposes the idea that the PP should have helped to "smooth the path" for new products, acting as a mechanism for confidence as the community could rest assured that through this method, GM foods would go through a stringent admissions process with "effective oversight of the industry's activities." She describes the "overall trait trajectory," attributing the seamless rise of the anti-GM movement to a "perfect timing" sort of event, involving three important actors in the GM market; just as agri-business began arguing against regulation of the industry (in an attempt to gain further access into the market), GM promotional advertisements were attracting public attention and environmental NGOs (and others) were realizing the influence they had on public opinion due to the recent effects of the BSE Crisis (Mad Cow Disease). As Tait suggests, the Precautionary Principle was an important measure effecting the public impression of GM foods in the EU and the grade of confidence consumers had concerning the safety/reliability of testing procedures. In addition to the effect that the PP had on the EU's public view of GMOs, this strategy of acceptance played a large role in the creation of biosafety policy by the Brazilian government in the late 1990s, which will be illustrated in the following analysis of the EU-Brazil relationship during the GM controversy.

IV. WHAT ARE THE OUTCOMES OF BRAZIL-EU INTERACTIONS DURING THIS PERIOD?

After reviewing the evolution of GM agriculture and its social, economic, and political implications within both the EU and Brazil, the soybean-trade relationship between these two countries and how it has transformed since the introduction Monsanto's RR® Soybean in Brazil will now be considered. Due to a widespread feeling of dissent in the EU concerning the introduction of GM products into their economy, it seems natural for their trade relations with Brazil to go sour after their GM policy is broadened, allowing multiple new products by using their own process of admission and regulatio¬¬n. The EU is Brazil's largest agricultural export market, but even though it is assumed that Brazil is losing out because it has become a GM-soya producer and the EU is primarily interested in non-GM crops for human consumption, I suggest that Brazil has actually benefitted as an actor on the international market from the change. Since the other major soy producing countries (mainly Argentina and the US) have shifted towards primarily growing and exporting GM soy rather than non-GM soy, Brazil has taken a hold of the non-GM soy market as the only remaining producer. In the EU, therefore, they have a monopoly on the non-GM soy product market, which greatly improves the economic outlook of non-GM soy farmers in Brazil and fortifies their position in the international soy market.

Because of Brazil's historical background concerning the implementation of GM policy, including a distinctive transitional period, internal controversy, moratoriums, set-backs, and sometimes militant opposition, they saw stunted GM growth from the outset. Unlike the other two largest soybean producers in the world, Brazil's GM soya acreage as a percentage of their total soya acreage is hovering around 70% and conventional soy crops hold the other 30%. Neither Argentina (99% GM) nor the United States (90% GM) produce a significant non-GM soya crop that could be exported to the EU. Even though the United States is the world's largest international soybean exporter, when solely looking at the EU's soybean imports, we can see that they receive a much bigger portion of their soybean products from Brazil, and the difference between the quantity of crops imported has grown

every year since 2002, when United States' participation began to decline (see Figure 1). Another interesting indicator is the fact that even though the quantity of Brazil's exports have not experienced constant, progressive growth, the value of all soy brought into the EU has increased by 50% since 2006. Promoting this positive trade growth, the EU now allows the importation of hybrid-GM soybeans that contain a small percentage of Monsanto's RR® product.

This concession increases the amount of soybean product that Brazil exports across the Atlantic and strengthens the EU-Brazil trade partnership, which has become especially important in the face of recent events that have heightened skepticism over importing from countries that mostly produce GM crops. For example, in 2009, EC scientists discovered traces of RR maize® residue in several bulk shipments of soy being imported into the EU from the U.S, causing the EC to reject over 180,000 tons of GM soy. This incident could be duplicated in the near future; Argentina currently harvests multiple GM varieties that are not allowed in the EU and are not even in the assessment process. According to the EU's Zero-Tolerance Policy, "any shipment of food or feed must be completely free from even trace amounts of GM crops that have not been approved." Fortunately, Brazil does not currently allow any GM varieties that are not also allowed in the EU (at least in some portion). Because of this connection, the EU will focus their attention on Brazil's agricultural sector since a GM/non-GM mix-up is much less likely to occur. In that regard, Brazil will be poised to take over the EU's import market, boosting Brazil's soybean price and giving them a monopoly on the entire soy sector, both GM and non-GM.

In addition to the possibility of taking over the U.S's importation of soy to the EU because of legal restrictions and bans, Farm Chemicals International published an article in 2006 describing the changes in the EU soybean import market, claiming that U.S. imports had declined (previously confirmed in Figure 1) and the EU was shifting its focus to Brazil. This adjustment, they say, can be attributed to the fact that "Brazilian soybeans generally have a higher protein and oil content, and because European crushers prefer non-GM soybeans." Also, the article indicates that the Brazilian soybean shipping season lasts longer that in the United States, which they claim is generally competitive only between October-December.

Generally, the relationship between the EU and Brazil as trading partners has been very strong, faltering slightly during the GM controversy. After Brazil's decision to integrate GM soybeans, there was an uproar from the EU community as they struggled to convince Brazil's government to retain a precautionary stance on biotech foods. The EU sought to continue the importation of non-GM soybeans (and other vegetable products) as the European anti-GM faction grew to an overwhelming majority, including both retailers and consumers. In 2005, for example, the British Retail Consortium (BRC) called on Brazilian farmers to "resist further growth of GM planting because it will be enormously difficult to maintain trust in the food chain should Brazil's supply of non-GM soybeans dry up." The BRC implored UK food companies to "place firm orders for non-GM soya for animal feed because they "feared the availability of non-GM soya products would continue to decrease if they did not express their need for them. In addition to this fear of losing non-GM products in the EU, Brazilian food manufacturers feared that they would lose their partnerships with the European consumer market.

In 2006, the Brazilian Institute for Consumer Protection (IDEC, the same organization that filed a lawsuit against the 1998 Commercial Release of the GM Soybean) published an article titled, "Food Companies Have Adopted Policies Against GMOs." IDEC points out ten different food manufacturers in Brazil who have adopted policies against GM food products "as a way to meet the European consumer market." The article cites a 2005 Greenpeace study on consumer acceptance in the EU, claiming that 90% of all large retailers and 73% of all food and drink manufactures have a GM-free policy. An important aspect of the publication is a focus on environmental preservation, stating that with conventional soybean planting, a farmer can easily respect the environment around his crops. In the end, César Borges de Souza, the Vice President of Caramuru Alimentos (a grain processor and exporter in Brazil), said that the decision to adopt a non-transgenic policy was a "consequence of European

politics to trace the origin and processing of the product they consume." These two perspectives on the status of the non-GM soybean market between Brazil-EU are the two central positions of the non-transgenic movement, providing insight and analysis of each side's reactions.

After discussing the implications of the non-GM movement among separate economic entities in the market, the value of GM soybeans shall be shown in comparison to the average value of GM-free soybeans that are exported to Europe (see Figure 2). It is clear that the EU values non-GM crops much more than GM crops: in 2008, the value of 1,000 KG of non-GM soya was almost € 800; for GM soya it was barely € 400. This data provides a compelling argument for the proliferation of non-GM crops in Brazil. But, I am not suggesting that GM crops should be replaced – they should be supplemented with additional non-GM cropland.

Brazil has an enormous amount of arable land that has not been cultivated yet, and if non-GM producers utilize these resources to expand the non-GM market, the potential economic gains are astonishing. It is estimated that there are between 124-247 million acres of unused land that could be transformed into non-GM soy farms. By looking at Table 1 and Figure 3, we can see that between 1998 and 2009, the area of soybean crop harvested in Brazil climbed from about 30 million acres to almost 55 million acres, as production increased by 2/3. At 55 million acres, Brazil was producing almost 57 million metric tons of soy as a whole (GM and non-GM). Non-GM soy production is estimated to have equaled 14.34% of the total soybean production at that time, so about 8.17 million metric tons of non-GM soy were harvested in 2009. If the Brazilian agricultural sector develops unused arable land and the area of soybean cropland is increased to the conservative value of 100 million acres, then there is the potential for a harvest of 104 million metric tons of soybeans. If non-GM soy rises to just 30% of production, there could be 31+ million metric tons of non-GM soy harvested in Brazil. In this event, EU retailers and soy-processors would be able to provide many more non-GM food items to European consumers, who are still desperately seeking non-GM alternatives. The economic benefits of this non-GM market expansion would have an extensive impact on Brazil's lucrative soy sector: the country could potentially become the world-leader in exporting both GM-soy and non-GM soy products.

Through this analysis, I have concluded that non-GM production is still an essential part of the Brazilian economy. Brazil would face harmful economic repercussions if they, as the world's last large-scale provider of non-GM crops, stopped harvesting conventional (non-GM) soybeans. Brazil's current success as an international soybean exporter can be attributed to the influence of widespread GM-discontent in the EU on the expansion of the Brazilian agricultural sector. Brazil was in a unique position at the emergence of the GM movement in the late 1990s because they, unlike Argentina and the United States, did not latch on to the GM "bandwagon." Their current GM production level is nowhere near the levels of other world leaders in the international agricultural market. As Brazilian consumer defense organizations and environmentalists rallied against the harvesting of transgenic soybeans, the European Union urged Brazil's anti-GM movement to push harder, knowing that if Brazil's soy crops became 90%+ genetically modified, they would have no market for importing the non-GM soy products that their consumers were demanding. So, with the EU's support, Brazil has slowly transitioned to an international GM-soy producer while maintaining a large sector of conventional soy crop. Therefore, Brazil is able to trade soy products in two distinct markets, reaping the benefits of both GM and non-GM consumer bases, rather than solely profiting from GM-soy materials.

As a result of my analysis of the trading relationship between the EU and Brazil, I conclude that without the EU's support of their non-GM soy sector, Brazil would not have been able to reach its current level of soy production. Since GM-soy production in Brazil was stunted early-on, the soy-export market would have been quickly surpassed by other GM-soy providers; the proliferation of non-GM soy farming has secured Brazil's place in the international market and brought far-reaching economic prosperity.

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ASSESSING THE GENOTOXIC EFFECTS OF MICROPARTICULATE EXPOSURE IN DROSOPHILA MELANOGASTER

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"Exposure to microparticulate matter and endocrine disruptors has been linked to severe pathological and disruptive effects on human health. Airborne microparticles are confirmed vectors for various pulmonary and cardiovascular conditions as well as adverse genotoxic and cytotoxic effects. Endocrine disruptors are especially detrimental since they selectively interfere with the sex hormone functions of the host organism and can potentially disrupt ecosystems by hindering reproduction in affected species. Despite the fact that there are numerous studies assessing the cytotoxic effects of airborne microparticulate matter, there is a clear deficiency of conclusive data and topical research assessing the genotoxic effects of microparticles on organisms. The aim of this study was to evaluate the significance of microparticulate exposure in an urbanized setting in order to assess whether anthropogenic causes are producing detrimentally quantifiable genotoxic effects and possibly endocrine disruption. Drosophila melanogaster was used as a model test subject to analyze for survivorship, induced genotoxicity, and distorted sex ratios across generations. Samples of microparticulate matter were collected from four locations of varying degrees of urbanization and incorporated into the parental generation and observed over two generations. Microparticulate exposure did in fact have an observable generational selection effect on D. melanogaster. We also observed distorted sex ratios in the F1 generation; however, endocrine disruption was not attributable to exposure. Based on a comet assay, we found clear indications that genotoxic damage was linked to the extent of microparticulate exposure."

I. INTRODUCTION

Microparticles are defined as particles ranging in size from 0.1-100 μm. Most microparticulate matter is released by anthropogenic processes that are prominent in industrial and developing areas and contains harmful elements such as sulphates, strong acids, and trace metals (Buschini et al., 2001). Major contributors of microparticulate matter include incineration wastes (Cormier et al., 2006), vehicle exhaust (Slapsyte et al., 2006), and industrial manufacturing and processing plants. An adverse effect of microparticulate exposure on microorganisms is genotoxic damage, which is defined as any toxic effect that interferes with the transmission, maintenance, or replication of genetic material (Buschini et al., 2001). Genotoxic damage, which carries serious ramifications for normal genetic transmission and generational succession on the prokaryotic and eukaryotic level, can be a valuable tool for screening of pollution and environmental harm (Boettcher et al., 2010). The biological and ecological consequences of transmitted genotoxicity can have a pronounced effect on the population dynamics, organismal viability, and survivability of susceptible species, especially due to its known connection between mutagenesis, carcinogenesis, ageing and other pathologies (Wessel et al., 2007). For example, one study (Mondal et al., 2010) found that premenopausal women exposed to high levels of indoor air pollution from biomass fuel reported increased frequencies of micronucleated cells leading to chromosomal damage that can lead to respiratory diseases such as COPD and lung cancer. These factors may have far-reaching ecological effects contingent upon the ecological significance of the affected organisms and the extent of microparticulate and exposure (Aras et al., 2010)

There is little available data concerning the genotoxic effects of airborne microparticles in multicellular organisms because most research has focused on microorganisms or cell cultures. The results of such existing studies, including two assays by the University of Parma which determined that mutagenesis correlated with concentration of microparticulates in both Salmonella typhimurium (mutant strains TA98 and 100) and Saccharomyces cerevisiae (D7 strain) (Buschini et al., 2001), warrant further research at the multicellular organismal level to determine if the impact of microparticulate pollution carries broader health implications. One such study did in fact find correlations between ambient air microparticle concentrations and various cardiovascular, pulmonary, and immunological complications in humans (Cormier et al., 2006). The results of this study reveal a strong connection between microparticulate exposure and harmful health effects and illustrate the necessity for focused and quantitative follow-up research.

Another cause for concern that may be attributed to microparticulate exposure is the possibility for endocrine disruption in the organisms. Endocrine disrupters are exogenous substances that can disrupt the physiological functions of endogenous hormones by imitating the physical structures of natural hormones. They act as false substrates to receptors and cause erroneous activations of functional pathways and cascades that can have consequences on sexual and functional development. Endocrine disruptors can affect an organism's development in utero (Wise et al., 2007); the adverse effects carry over to adult life and possibly to future progeny following genetic transmission, as shown by the emergence of male black bass which are now producing oocytes in the testes and vitellogenin (a protein normally produced only by female fish to form egg yolks) in the bloodstream (Hinck et al., 2009). The results of endocrine disruption are not exclusive to aquatic species and the effects have been shown to cause pregnancy and intrauterine growth complications in humans (Casals-Casas et al., 2008). Since endocrine disrupters are fat soluble, a major source of exposure in humans comes from our food sources, which are vectors of endocrine disrupters due to the host organisms' exposure to anthropogenic chemicals and microparticulates in their natural settings. Further research into the sources of endocrine disrupters, such as microparticles, will ensure that we can address and remedy the cause and spread of these pathogenic agents.

The primary goal of our study is to analyze the genotoxicity from microparticulate exposure in D. melanogaster. We have chosen D. melanogaster as a model organism because its genome has been extensively studied and completely mapped, showing that D. melanogaster has very similarly preserved genomic analogues to mammals and their functional genetic pathways are very comparable to that of humans (Bier, 2005). D. melanogaster is a fitting candidate for inferential studies of genotoxic effects on other eukaryotic organisms, and has been extensively used to test for several human diseases such as Parkinson's Disease and Huntington's Disease. In order to test for these genotoxic effects,

samples of airborne particulate matter collected on Teflon filters from four different locations with varying degrees of urbanization were taken to determine if levels of urbanization induced a correlating selective strain on D. melanogaster. After observing survivorship, genotoxicity from microparticle exposure was assessed by performing a comet assay on selected groups which would be indicative of the extent of any induced genotoxicity. The second objective of our study was to assess possible endocrine disruption caused by airborne microparticulates. We analyzed the sex ratios of D. melanogaster across two generations in order to assess any abnormalities that may be attributable to endocrine disruption. We formed the hypothesis that exposure to microparticles will result in observable and quantifiable effects in genotoxicity, skewed sex ratios, and survivability, though these effects will not be carried over to the F2 generation.

II. METHODS

We evaluated survivorship over two generations of D. melanogaster by exposing the parental generation to microparticulate extracts obtained from previously set up Teflon filters from the sites. The first site studied was Yargo National Park, a protected reserve located northeast to metropolitan Atlanta. In contrast, we also collected samples from the abandoned Atlanta Fire Station 58, which is located in a heavily industrialized area and in the proximity of a railroad station. Lastly, we acquired filters from Fort McPherson and a heavily traffic-congested location in South DeKalb in order to provide a range of microparticle sources of differing levels of urbanization and, theoretically, corresponding levels of microparticulate effects. Figure 1 illustrates two of these sample filters. The microparticulates from these samples were extracted by water and acetone in order to extract the hydrophilic and hydrophobic compounds. The filter was subjected to an extraction with 8 mL of water for 30 minutes, followed by an extraction with an acetone solution for another 30 minutes. These extracts were used in the culture media of D. melanogaster in order to simulate exposure to the microparticulates in the parental generation. The growth media for the control setup was prepared according to instructions in the supplied Carolina Drosophila Manual (Flagg et al., 2005) which instructed the addition of 16 mL water to 16 mL growth media. For the experimental water setups, we used the same ratio but changed the measure to 8 mL of water to 8 mL of growth media in order to maximize the efficacy of the extract on the D. melanogaster. We prepared the acetone experimental setup with 0.5% acetone, which was found to be most feasible for viability. This resulted in a setup that used of 0.04 mL acetone along with 7.96 mL of water in 8 mL of growth media for the experimental acetone treatments.

Figure 1. Photograph showing Teflon filters containing microparticles. The filter on the left was collected from YG site and the filter on the right was collected at SD site.

Each parental setup was created using the above method. However, for the F1 generation we selected two males and two females from the parental groups and allowed them to produce larvae for 9 days, at which time the four parental flies were cleared in order to control for inter-generational mixing. After 16 days from initial culture, the number of females and males as well as sex ratios calculated from the F1 generation. Additionally, the F2 generation was set up on this day (16 days from F1 setup) using the same procedure as the F1 generation and followed the standardized schedule for clearing and counting.

Genotoxicity was assessed by performing a comet assay, which determines the amount of DNA damage as a result of microparticulate exposure by the appearance of “comets”. Comets indicate the amount of DNA damage sustained by the length of the observable “tail” which signifies the migration of damaged DNA fragments during electrophoresis. All reagents used were prepared beforehand in accordance with the Trevigen kit (#4205-050-K) instructions. We removed hindgut tissue from

four females from each treatment group, added it to a mixture of 50 µL of cold 1x PBS and 20 mL EDTA, ground it with a tissue probe and placed it on ice. 20 µL of the resulting cell suspension was transferred into the 200 µl of 37°C LMA. The solution was mixed and 50 µL was pipetted and spread evenly on a sample area of a CometSlide. Each slide was refrigerated in darkness at 4°C for 30 minutes. These slides were then bathed in a lysis solution for 60 minutes at 4°C. They were then immersed in an alkaline solution for 60 minutes at room temperature in the dark. Next, electrophoresis was conducted at 1 volt/cm for 40 minutes at 4°C. Excess electrophoresis solution was drained, and the slides were immersed in deionized water twice for 10 minutes each. Immersion in 70% ethanol for 5 minutes followed, and the slides were dried at 37°C for 15 minutes. The slides were stained by placing 100 µL diluted SYBR Green 1 on the agarose circles of the slides, which were then refrigerated for 5 minutes. Slides were tapped to remove excess stain, and the slides were then allowed to dry in the dark for 10 minutes. We viewed slides with epifluorescence microscopy at 521 nm and photographed them.

III. RESULTS

Treatment (F1)	P Values
FS Acetone	0.010
FT Acetone	0.031
FT Water	0.003
SD Acetone	0.015
SD Water	0.005

Table 1. Significant p values for observed sex ratio data compared to expected 1:1 sex ratio from F1 and F2 generations using Fisher's Exact Test.

Treatment	P Values
FS Acetone	0.048
FT Acetone	0.001
FT Water	0.001
SD Acetone	0.001
SD Water	0.001

Table 2. Significant p values for percentage of observed comets from the F1 generation using Fisher's Exact Test.

Treatment (F1)	P Values
SD Acetone	0.001
SD Water	0.098
FS Water	0.001
FT Water	0.012

Table 3. Significant p values when comparing experimental groups to control groups for survivorship data from F1 and F2 generations using Fisher's Exact Test.

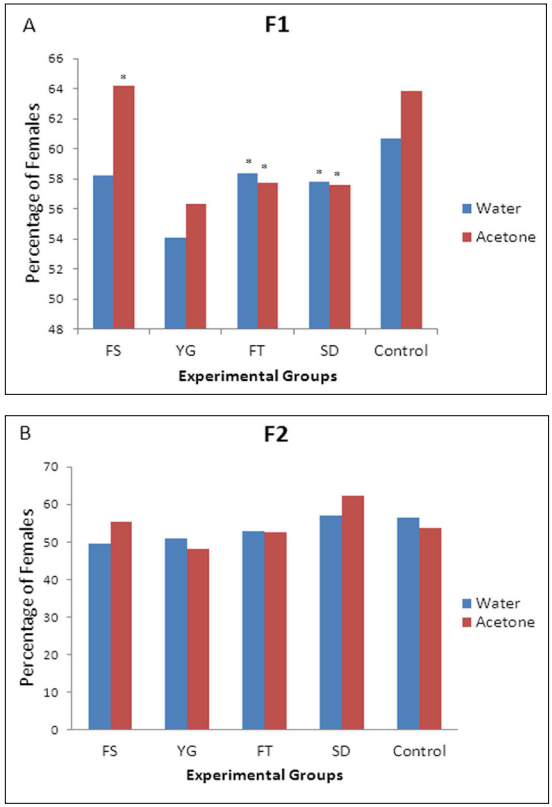


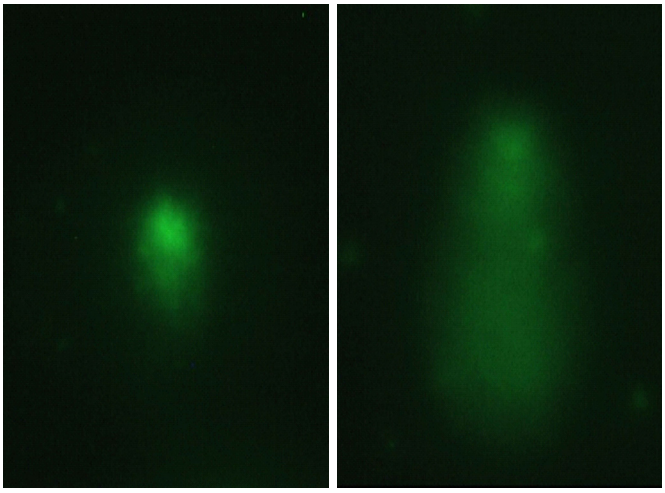
Figure 2. Percentage of female D. melanogaster in the F1 generation [A] and the F2 generation [B] within all treatment and control groups at Fire Station 58 (FS), Yargo National Park (YG), Fort McPherson (FT) and the South Dekalb traffic site (SD).

* Significant deviation in comparison of actual sex ratio to expected sex ratio of 1:1, Fisher's Exact Test, ($p < 0.05$).

Microparticulates from the urbanized locations significantly affected the F1 sex ratio from the expected 1:1 ratio (Figure 2, {Fisher's Exact Test}, $P < 0.05$). These p values are listed in Table 1. No significance was found in the F2 generation. The F1 generation had a higher ratio of females than males, but the F2 generation normalized towards the normal 1:1 ratio.

Genotoxic damage was observed through the comet assay for the urban sites in comparison to the undamaged DNA in the control sites (Figure 3). All tested sites showed significant deviation (Figure 4, {Fisher's Exact Test}, $P < 0.05$ for the FS acetone extraction and $P < 0.001$ for all extractions at the SD and FT sites). These p values are listed in Table 2.

The exposure to microparticles from all urban sites showed a significant effect on survivability (Figure 5, {Fisher's Exact Test}, $P < 0.05$). These p values are listed in Table 3. The treatment groups generally showed a trend of enhanced survivability in the F1 generation for both water and acetone treatments with the exception of the FS site. The F2 generation showed no significant difference in survivability.



A B

Figure 3. Photographs taken using fluorescence microscope at 400x magnification of a non-comet (undamaged DNA) from the control acetone treatment [A], and a visible comet from the SD acetone treatment [B].

IV. DISCUSSION

Our data infers that concentrated microparticulate exposure from urban locations leads to significant and observable effects on D. melanogaster in terms of genotoxicity, altered sex ratios, and survivability. The heavily urbanized settings (South DeKalb, Atlanta Fire Station 58, and Fort McPherson) showed significant deviations from their respective controls, in contrast to the protected Yargo National Park. Assessing genotoxicity, we found concrete indications of genotoxic damage by observing significant amounts of comets found in both the water and acetone extractions for the SD and FT sites. Figure 3 shows fluorescence microscopic images that illustrate a comet formation in contrast to a non-comet formation. These sites showed substantial statistical significance with p values lower than 0.001 and clear indications of induced genotoxic damage due to microparticle exposure (Figure 4). This reinforces our projection that both hydrophobic and hydrophilic microparticulate matter are directly connected to inducing genotoxicity in D. melanogaster.

In order to assess whether these effects were attributable to endocrine disruption and would have a cross-generational effect, we analyzed the sex ratios for each treatment and compared these ratios to an expected sex ratio of 1:1. We found significant variation using the Fisher's Exact Test for both the water and acetone extraction at the SD and the FT sites (Figure 2). As before, these sites showed expressed effects due to microparticle exposure in comparison to YG. Additionally, the FS acetone extraction also showed significance whereas YG failed to show any discernable significant deviation from the expected 1:1 sex ratio. We were also able to confirm that these effects are only applicable to the generations that were directly exposed to microparticulate matter and the effects did not carry over to the F2 generation for all treatments and extractions (Figure 2 B), as was observed by other studies that monitored the generational effect of toxic exposure on D. melanogaster (Hurst et al., 2001). This indicates that endocrine disruption did not occur, which is reassuring since it could have had significant ramifications on the population dynamics of D. melanogaster and its ecologically coupled species.

We assessed the survivability of all treatments in order to address any direct population perturbations attributed to microparticulate

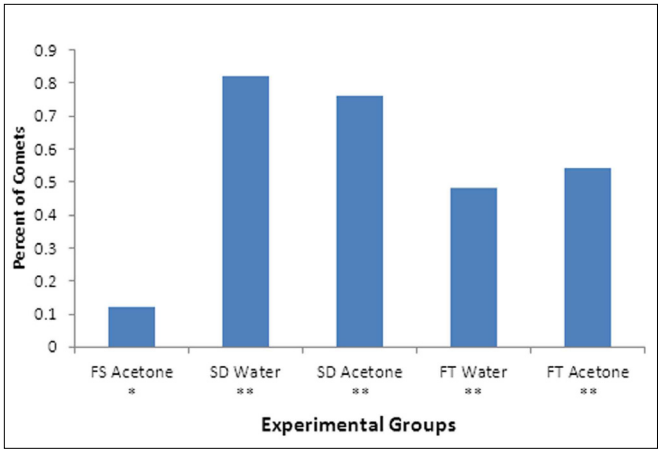


Figure 4. Percentage of comets visible in samples of D. melanogaster after comet assay was performed. Cells from the hindguts of D. melanogaster females were lysed and ran via gel electrophoresis. The cells were then stained with SYBR-green fluorescent dye and photographed using fluorescence microscopy. Comets were identified as cells with observed DNA damage in the form of dimerization or strand breakage. Extractions at Fire Station 58 (FS), South Dekalb traffic site (SD), and Fort McPherson (FT) from the F1 generation were examined in comparison to the water and acetone control groups which exhibited no visible comets.

* Significant deviation for percentage of observed comets in comparison to control group, Fisher's Exact Test, ($p < 0.05$).
** Greater significant deviation for percentage of observed comets in comparison to control group, Fisher's Exact Text, ($p < 0.001$).

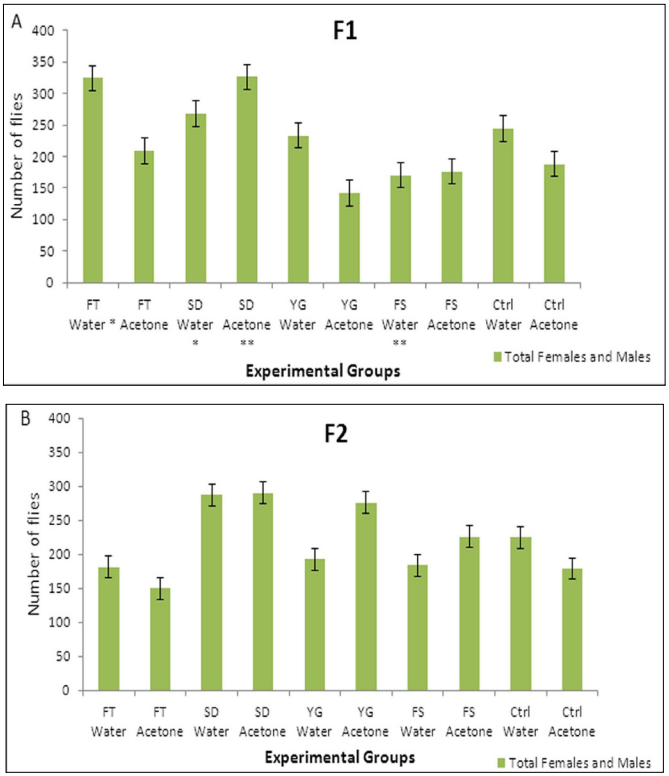


Figure 5. Survivorship of D. melanogaster in the F1 generation [A] and the F2 generation [B] within all treatment and control groups at Fire Station 58 (FS), Yargo National Park (YG), Fort McPherson (FT) and the South Dekalb traffic site (SD). Standard error bars are displayed.

* Significant deviation in comparison to control group, Fisher's Exact Test, ($p < 0.05$)
** Greater significant deviation in comparison to control group, Fisher's Exact Test, ($p < 0.001$).

exposure. Unexpectedly, we found an enhancement effect on the survivorship of the F1 generation in the experimental treatments (Figure 5A). In areas with heavy exposure, such as FT and SD, there was a marked variation from the control and an increase of survivability in comparison to non-urban sites such as YG, indicating the occurrence of hormesis. Further toxicity tests should be conducted in order to observe the degree to which microparticulate exposure elicits positive selection.

Though the results of this study show a strong correlation between microparticulate exposure and its expressed effects on D. melanogaster survivability, genotoxicity and altered sex ratios, we cannot confirm direct causation due to the possible influence of localized, extraneous variables. Therefore, this study illustrates the need for appropriate chemical analysis and more focused research to conclusively determine the specific cause of the aforementioned effects.

V. CONCLUSION

Our tests infer that there was a trend of increased observed effects of microparticle exposure to D. melanogaster, which was especially evident when comparing results from the rural YG treatment

to more heavily urbanized regions as FT and SD. We found statistically significant evidence of genotoxicity in the SD and FT locations which suggests that heavily urbanized and industrial locations contribute significantly to microparticulate exposure on local organisms which consequently results in genotoxic damage. These results warrant follow-up research in order to clearly define the specific compounds causing genotoxic effects and the exact vectors and mechanisms responsible for inducing such effects. Fortunately, we found that endocrine disruption was not attributed to microparticle exposure and that both the genotoxic effects and distorted sex ratios were only experienced by the generations directly exposed to microparticulate matter and not inherited by their progeny. As mentioned before, although this experiment used D. melanogaster as our test model, the results are directly applicable to eukaryotes. Further research should be conducted to distinguish what concentrations of microparticles are responsible for the observed effects and whether these effects are consistent with varying population densities.

VI. ACKNOWLEDGEMENTS

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EFFECTS OF SALINITY CONCENTRATIONS ON ARABIDOPSIS FUNCTIONAL TRAITS

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“Excessive soluble salts in the soil are harmful to most plants. In fact, no toxic substance restricts plant growth more than salt does on a world scale. Understanding the mechanisms of plant salt tolerance will lead to effective means to breed or genetically engineer salt tolerant crops. Salt tolerance research also represents an important part of basic plant biology, contributing to our understanding of subjects ranging from gene regulation to ion transport, osmoregulation and mineral nutrition. Using a survey of published literature, we asked whether salinity mitigates a functional response, examined the form of responses, and surveyed the evidence for saline effect on functional traits. Our primary goal was to seek general patterns of saline effects on Arabidopsis for broad categories of functional traits. This research review is aimed at answering these general questions: 1) Does salinity affect phenotypic expression? 2) How does salinity affect reproductive fitness? 3) Can salt inhibit germination? 4) Whether saline disrupts ion transport through root structures? 5) Is there variation in salinity tolerance among the ecotypes? We surveyed nine peer-reviewed journals from 1999 to 2010 and organized the articles we found (12) into categories that best answered one of our five questions. Our overall findings suggest that saline does have a pertinent effect on Arabidopsis and we go into further detail, in regards to the answers to our questions. Ultimately, this review is significant because we show that Arabidopsis is adaptive to increase its salt tolerance. Also, these results can beneficially impact the agricultural sector in increasing crop yields or vegetative output.”

I. INTRODUCTION

Plants need essential mineral nutrients to grow and develop. However, excessive soluble salts in the soil are harmful to most plants. In fact, no toxic substance restricts plant growth more than salt does on a world scale (Xiong et al. 2002). It is estimated that salinity affects at least 20% of world's arable land and more than 40% of irrigated land to various degrees (Rhoades and Loveday 1990). In extreme cases, productive agricultural land could no longer sustain agricultural production and had to be abandoned. This may have contributed to the decline of some human civilizations in history. Soil salinization is also one of the driving forces of land degradation throughout the world and has a formidable impact on the agricultural industry (Zhang et al. 2010).

Based on their capacity to grow on high salt mediums, plants are traditionally classified as glycophytes or halophytes (Flowers et al. 1977). Halophytes are tolerant to high concentrations of sodium chloride; some can withstand salts that are more than twice the concentration of seawater. Most plants, including the majority of crop species, are glycophytes and cannot tolerate high salinity. For glycophytes, salinity imposes ionic stress, osmotic stress, and secondary stresses such as nutritional disorders and oxidative stress (Zhu 2001a). Sodium toxicity represents the major ionic stress associated with high salinity. Additionally, some plant species are also sensitive to chloride, the major anion found in saline soils. The low osmotic potential of saline solutions hampers plant water uptake, resulting in "physiological drought." For halophytic plants that are tolerant of sodium toxicity, osmotic stress may be the main cause of growth inhibition.

Understanding the mechanisms of plant salt tolerance will lead to effective means to breed or genetically engineer salt tolerant crops. Salt tolerance research also represents an important part of basic plant biology, contributing to our understanding of subjects ranging from gene regulation and ion transport, to osmoregulation and mineral nutrition. Additionally, some aspects of salt stress responses are intimately related to drought and cold stress responses (Zhu 2001b). Using a survey of published literature, we asked whether salinity mitigates a functional response, examined the form of responses, and surveyed the evidence for saline effect on functional traits.

Salt Stress and Arabidopsis

Our review focused primarily on the glycophytic plant Arabidopsis thaliana because of its ubiquity, use as a model organism, and ecotypic variation in salinity tolerance. Although salt sensitivity among genetically different geographic varieties (ecotypes) of Arabidopsis exists, a systematic comparison among different ecotypes has not been reported. This comparison between ecotypes may localize salt tolerance functional traits if relatively large differences in salt tolerance exist between ecotypes. For this research review, we did not limit our definition of salinity to purely sodium chloride.

Research with other glycophytic plant species has shown that upon exposure to high salinity, plants may exhibit a reduced growth rate, accelerated development or death if the stress is severe or prolonged (reviewed by Lazof and Bernstein 1999). Like many other glycophytes, the sensitivity of Arabidopsis to salt stress is exhibited at all stages of development. Thus, functional traits are pertinent in characterizing specific Arabidopsis developmental characters, affected under saline conditions.

Definition of Functional Traits

In reviewing current scientific literature, derivatives for the definition of a functional trait were ubiquitous. In the broadest sense, a functional trait can be defined as any phenotypic character that influences organismal fitness through biochemical, physiological, morphological, develop-mental, or behavioral mechanisms (Geber and Griffen 2003). Most often, a functional trait affects fitness through performance measures, such as growth rate, competitive ability, herbivore resistance or tolerance, attractiveness to pollinators, and so on. Table 1.0 synthesizes common derivations of traits definitions used in this review.

In practice, the distinctions between functional and performance traits and between performance and fitness components are difficult and subjective because the designations will often differ between researchers investigating different levels of function. For example, leaf size is often considered a functional trait by ecologists, whereas a developmental biologist might view it as a performance measure because it is the outcome of leaf meristem size and rates of cell division and expansion. Leaf photosynthetic rate is also considered a functional trait by ecologists, but as a measure of net carbon gain it can also be viewed as a performance trait that is

		Definition	Application
Functional Trait	(1) Physiological processes: photosynthesis, respiration (Calow 1987)	Individual	Individual
	(2) Life history processes: germination, growth production (Weiher et al 1999)	Individual	Individual/ population
	(3) Individual Fitness (Reich et al 2003)	Individual	Individual
	(4) Performance measures (Geber and Griffen 2003)	Individual	Individual
Performance Trait	Fitness components: growth, reproduction, survival (Geber and Griffen 2003).	Individual	Individual
Response Trait	Response of a plant to environmental changes (Keddy 1992)	Individual	Individual
Effect Trait	Effect of a plant on ecosystem functioning (Diaz and Cabido 2001)	Individual	Ecosystem
Functional Marker	Function s.l. at any organization level (more easily measurable than the function itself) (Garnier et al. 2004)	Individual	Any organization level

Table 1. Examples of plant functional traits found in literature, while the function, component or process they are supposed to capture the levels of definition and application.

determined by biochemical and transpiration properties of leaves (Geber and Dawson 1997).

In our review, we focus on traits related to vegetative function that affect survival, vegetative growth and size, and fertility. We do not address sexually selected traits.

Review Goals

Our primary goal was to seek general patterns of saline effects on Arabidopsis for broad categories of functional traits (i.e. physiology, morphology) and fitness measures (i.e. vegetative fitness, fertility). We addressed five questions of general significance to studies of Arabidopsis salt tolerance. This research review is aimed at answering these general questions:

1. Does salinity affect phenotypic expression?

2. How does salinity affect reproductive fitness?

3. Can salt inhibit germination?

4. Whether saline disrupts ion transport through root structures?

5. Is there variation in salinity tolerance among the ecotypes?

Our article provides a comprehensive review of saline effects on Arabidopsis functional traits and is one of the few papers that formally investigate saline variations among ecotypes.

II. METHODS

Literature Survey

We conducted a broad literature review of three online databases (ISI Web of Science, MEDLINE, and PubMed) accessible through the Georgia Tech Library. The search yielded twelve peer-reviewed journal articles from nine journals ranging in date from 1999 to 2010. These the journals include: Journal of Plant Ecology, The American Society of Plant Biologists, Plant Molecular Biology, Journal of Experimental Botany, Journal of Plant Nutrition, Plant Cell and Environment, Journal of Plant Physiology, Plant and Soil, and Acta Physiologiae Plantarum.

The articles were sorted into categories that best answered one of our five questions. Note: a particular article could have been sorted into two of the question categories if we felt that it was applicable and offering an alternative/supplementing viewpoint.

III. RESULTS

Our overall findings suggest that saline does have a profound effect on Arabidopsis and we go into further detail, in regards to the answers to our inquiries.

Question 1: Does salinity affect phenotypic expression?

The literature reviewed indicated across the board that salinity does affect phenotypic expression. In comparison to 11 other Brassicaceae species, Orsini et al. 2010 presented the variability that Arabidopsis phenotypic expression takes. A first comparison between different species was aimed at assessing their performance in saline environments in terms of both general growth and survival. Leaf traits and height were common functional traits that varied. A. thaliana and T. salsuginea, the latter known to tolerate very high NaCl concentrations (Inan et al. 2004), were used as controls. Under the imposed experimental conditions at 150 mM NaCl, leaf area was significantly reduced by the stress in A. thaliana (Figure 1). Similarly, Arabidopsis root growth was significantly inhibited at 150 mM NaCl, while others were less affected. Significantly tolerant root systems were found for M. triloba and T. parvula (Figure 1).

The NaCl lethal dose of 50% to the population (LD50NaCl) was used to assess plant survival to salt stress. Most species revealed their halophytic nature since their survival threshold was between 200 and 400 mM NaCl. This was much higher than Arabidopsis, whose LD50NaCl was 150 mM (see Appendix IA). Knowledge of the dose-response relationship establishes causality that the NaCl has in fact induced the observed phenotypic effects.

After germination, seedling growth is also very sensitive to NaCl (Xiong 2002). Two-week-old Columbia ecotype seedlings growing in the soil were irrigated with 0, 50, 75, and 100 mM NaCl, respectively. Xiong et al. (2002) observed the bleaching of leaves, retarded growth, and delayed development of seedlings treated with higher concentrations of the salt. Specifically, at concentrations higher than 50 mM, NaCl statistically correlated ($p < 0.01$) to the degradation and death of Arabidopsis.

The manner in which NaCl affects performance traits in Arabidopsis is striking. Why is leaf area more profoundly affected than root length? (Figure 1) Counterintuitive? Actually, no.

The imino acid, proline, has been reported to accumulate in plants subjected to salt stress (Mansour 2000). Proline, which accumulates in larger amounts than any other amino acid (Abraham et al. 2003), contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Mansour 1998). Total free amino acids in the leaves have been reported to be higher in salt tolerant than in salt sensitive ecotypes of Arabidopsis (Ashraf and

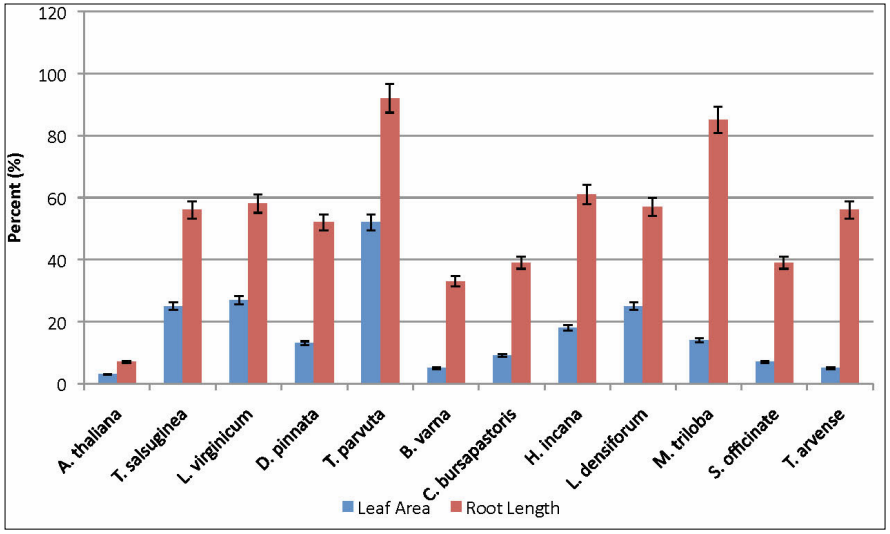


Figure 1. Effect of 150 mM NaCl stress on leaf area and root length of different Arabidopsis species synthesized from several articles. Salt treatments were administered when plants were 4-6 cm tall. Leaf area and root length were measured using a scanner and image processing software. Values are expressed as a percentage (%) of leaf area and root length in non-salinized plant controls.

Species	Response to salinity	References
Oryza sativa	decrease	Alamgir and Ali (1999)
Vicia faba	decrease	Gadallah (1999)
Amaranthus tricolor	decrease	Wang and Nil (2000)
Bruguiera parviflora	decrease	Parida et al. (2002)
Pancratium maritimum	increases at low salinity; decrease at high salinity	Khedr et al. (2003)
Arabidopsis thaliana	increase	Quintero et al. (1996)
Fragaria ananassa	increase	El-Baz et al. (2003)

Table 2. Changes in proline in response to salinity

Tufail 1995). Petrusa and Winicov (1997) demonstrated that salt tolerant Arabidopsis ecotypes rapidly doubled their proline content in roots, whereas in most Arabidopsis ecotypes the increase was slow in leaves.

There are two alternative routes in proline biosynthesis in higher plants: the L-ornithine and the L-glutamate pathways. It is also known that, as in plants, both ornithine and glutamate are precursors of proline biosynthesis in microorganisms and mammals. Delauney et al. (1993) showed that two enzymes: pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), play major roles in proline biosynthetic pathway. Plants over-expressing P5CS have shown increased concentration of proline and resistance to both drought and salinity stresses (Kishor et al. 1995). However, whether proline accumulation in these transgenic plants resulted in increased stress tolerance through osmotic adjustment or other mechanisms is unknown (Sharp et al. 1996). Table 2 presents the physiological reaction of several model plants to salt stress, in regards to their proline formation.

Transgenic approach to improve plant stress tolerance has appreciable results. Overproduction of proline by genetically manipulated Arabidopsis ecotypes showed tolerance to NaCl (Hong et al. 2000). Nanjo et al. (2003) demonstrated that introduction of antisense proline dehydrogenase cDNA in Arabidopsis over expresses proline and showed tolerance to salinity (600 mmol NaCl).

Question 2: How does salinity affect reproductive fitness?

Salt accumulation has detrimental effects on seed maturation (Labidi et al. 2005). For their study, the diversity of some effects of salinity on rosette growth, seed production, and seed viability in Arabidopsis thaliana was explored.

There was a large inter-ecotype variability of the effect of salt on all factors contribution to reproduction (number of flower per axis, flower fertility, and seed viability). Among the five ecotypes, those with highest levels of Na^+ and Cl^- ions in the silique valves produced the less viable seeds. The increased presence of sodium ions up-taken by Arabidopsis limited potassium nutrition; thus, limiting size and quantity of seeds produced (Xiong et al. 2002). Under increased saline stress and lower potassium absorption, reproductive functional seed traits were impaired (Figure 2).

The potassium ion is the preferred inorganic cation of living cells, and Arabidopsis is no exception to this rule; yet almost invariably the concentration of K^+ in the soil solution is lower than the cytosolic K^+ concentration (100-200 mM), meaning that Arabidopsis must actively take up and concentrate K^+ using various types of ion transporters (Rodriguez et al. 2000). Because Na^+ is similar to K^+, and many K^+ transporters do not discriminate sufficiently between these cations, excess external Na^+ cannot only impair K^+ acquisition, but also lead to accumulation of Na^+ in Arabidopsis cells. In order to avert Na^+ toxicity most glycophytes rely on restricting Na^+ intake, but because the cell's interior is electronegative relative to the extracellular space, and because cation transporters in cell membranes are somewhat permeable to Na^+, there is constant influx of Na^+ down this electrochemical gradient that cannot be completely prevented (Amtmann et al. 1999, Hasegawa et al. 2000). Moreover, the outcome of long-

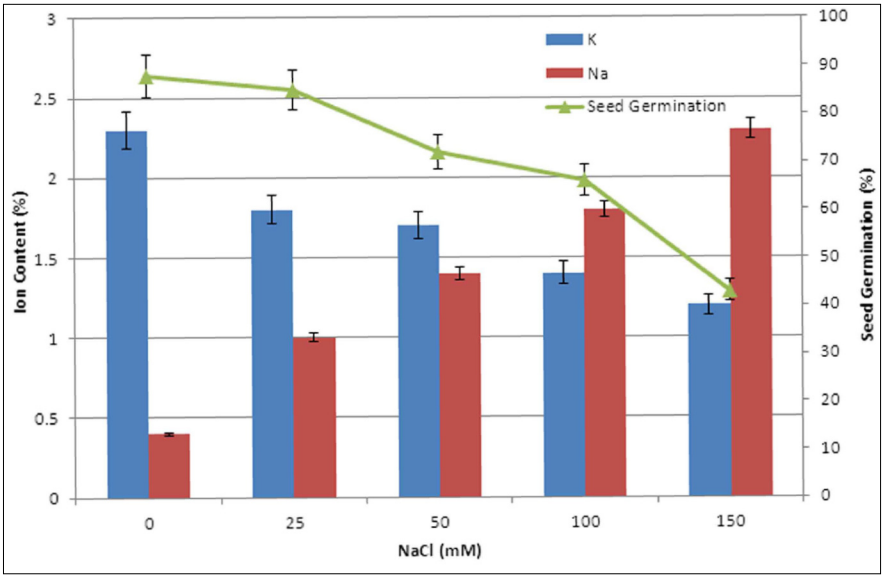


Figure 2. Salt stress impairs K nutrition in Arabidopsis. With increased concentration of NaCl in the culture medium, Na^+ content in plants increases whereas K content decreases. Arabidopsis seedlings (ecotype Columbia) growing for two weeks were treated with NaCl. Seedlings were allowed to grow for 14 days before harvesting and analyzing ion contents (dry weight basis). Source: Xiong et al. 2002. The reproductive success of the seedlings can be represented by the percent of germinated seedlings that the Columbia ecotypes produced. The general trend is that as the salt concentrations increase, seedling germination decreases and consequently the ecotype's reproductive fitness. Source: Berthomieu et al. 2003.

term inhibition of K^+ acquisition by competing Na^+ is chronic K^+ deficiency. Such deficiencies are the biochemical explanations to the decreasing percent seed germination along a salt gradient, reflected in Figure 2, and consequently decreased reproductive fitness.

Question 3: Can salt inhibit germination?

Seed germination behavior of Arabidopsis ecotypes were closely related to the salinity level of the habitats over which they were distributed. Ecotypes from the habitats with higher salinity had generally higher final germination proportions, but shorter mean times to germination than those from the habitats with lower salinity (Zhang et al. 2010). The inhibition of germination could be due either to NaCl-induced seed mortality or to unfavorable external conditions (Debez et al. 2004). Figure 2 underscores that inherent correlation between salt concentrations and the seed's ability to successfully germinate. As explained previously, sodium ions biochemically inhibit potassium ion uptake. Chronic potassium deficiency arrest cell growth and germination processes from undergoing. Particularly, Debez et al. (2004) observed that saline inhibited germination without damaging the seeds, which could recover their high capacity to germinate, when transferred to pure water. Hence, the main factor involved in the salt induced dormancy of Arabidopsis seems to be the low water potential of the saline medium.

Question 4: Whether saline disrupts ion transport through root structures?

Saline inhibition of germination stems from the realization that NaCl inhibits certain minerals from absorption by the roots. Hence, salinity tolerance can be attributed to three different mechanisms: Na^+ exclusion from the shoot, Na^+ tissue tolerance and osmotic tolerance (Jha et al. 2010). Jha et al. found that not only are genes differentially regulated between ecotypes, the expression levels of the genes can also be linked to the concentration of Na^+ in the plant. An inverse relationship was found between a particular gene expression in the roots and total plant Na^+ accumulation, supporting a role for that particular root gene in Na^+ efflux from the plant. Similarly, ecotypes with high expression levels of this gene in the roots had lower root Na^+ concentrations, due to the hypothesized role of the gene.

This new evidence supplements older theories that excessive sodium ions at the root surface may disrupt plant potassium nutrition that is vital for the maintenance of cell turgor, membrane potential, and the activities of many enzymes (reviewed by Lazof and Bernstein 1999). Because this particular gene expression

inhibits the majority of Na^+ uptake, the sodium ions then act as competitive inhibitors of potassium ion uptake. Sodium ions are not required for the growth of most land plants. Land plants do not seem to have transport systems specifically for Na^+ uptake. However, Na^+ can still enter plant cells via several routes. Since the concentration of Na^+ in the soil solution is usually much higher than that in the cytosol of root cells, Na^+ movement into root cells is passive. Current evidence suggests that Na^+ enters root cells mainly through various cation channels (Xiong 2002). These channels could be voltage dependent cation channels and due to the similarity between Na^+ and K^+, Na^+ could enter the roots in place of K^+ causing differential expression of functional traits (reviewed in Blumwald et al. 2000).

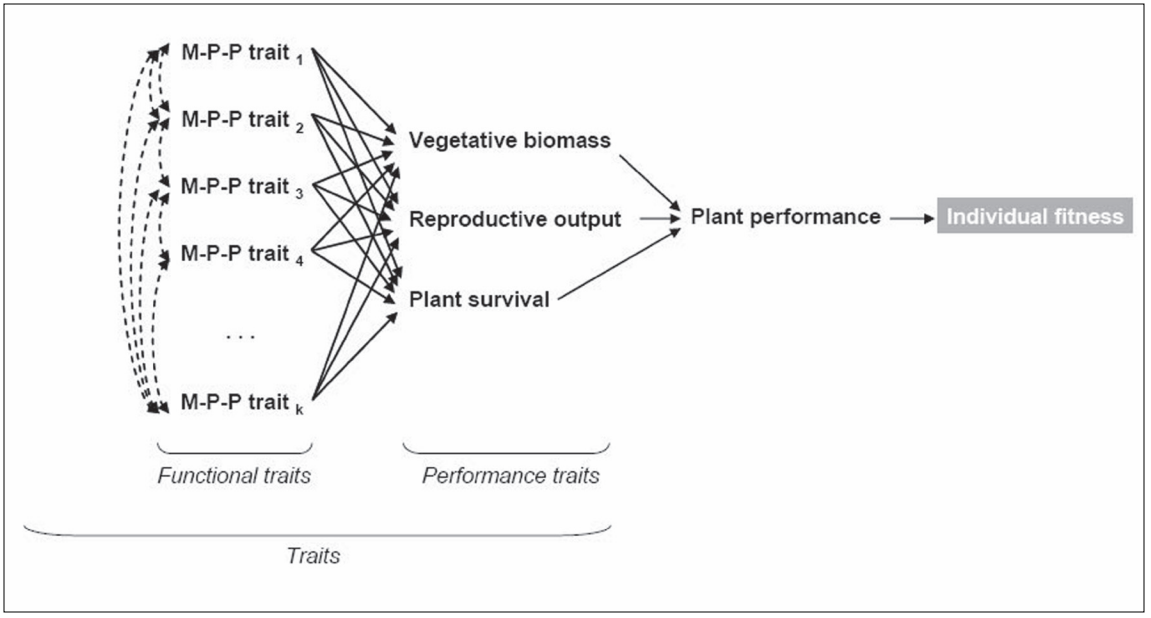
Question 5: Is there variation in salinity tolerance among the ecotypes?

All the literature we reviewed contained experimental designs that compared Arabidopsis ecotypes or genetically similar species. As well, in all cases there was statistically significant variation in functional traits between the ecotypes. Yet, underscoring this mantra was Katori et al.'s (2010) experiment where 350 Arabidopsis thaliana accessions large-scale soil pot experiments were performed to understand the natural variability in salt tolerance. The evaluation revealed a wide variation in the salt tolerance among accessions on the 35th day of 500 mM NaCl treatment. Salinity treatments on agar plates renders immediate salinity shock to plants, whereas the treatment using soil pots exposes plants to a gradual increase of NaCl stress because the water contained in soil is gradually substituted with saline water. Thus, treatments in soil pots stratified Arabidopsis responses, with gradual accumulation to high saline concentrations.

IV. DISCUSSION

Our primary goal was to seek general patterns of saline effects on Arabidopsis for broad categories of functional traits and

Figure 3.0 The current paradigm of trait, performance and fitness interconnectivity. This figure reflects current, widely accepted theories augmented with Arnold's (1983) framework of plant ecology. Morpho-physio-phenological (M-P-P) traits (from 1 to k) modulate one or all three performance traits (vegetative biomass, reproductive output and plant survival) that determine plant performance and individual fitness. M-P-P traits may be inter-related (dashed double-arrows).



demonstrate that this literature is pertinent in identifying and synthesizing knowledge for agricultural applications.

As Zhang et al. (2010) studied, soil salinization is a leading cause of land degradation, an urgent problem in the agricultural industry, where soil salinities can be high enough to impede optimal growing conditions. Although data on functional trait fitness are well-appreciated by community ecologists, stressors on these traits have largely been overlooked by population biologists focusing on the demography of a single species. Similarly, this review provides in depth research on inter-special variation. Solutions to the ever mounting battle against salinity in the agriculture industry can be achieved through differentiating glycophytes or halophytes plants. Genetic engineering and gene manipulation also provides an avenue for phenotypic plant alteration and adaption to high saline environments.

Additionally, we have shown that saline stress influences phenotypic expression, and more specifically Arabidopsis' reproductive trait expressions. Germination, the resultant of these trait expressions, was also shown to be inhibited, along with ion transport—the metabolic ignition for germination. Yet, more significantly, saline is a stressor of functional traits. These traits then assume particularly important roles as determinations for survival and, more long term, an individual's fitness (Figure 3). Thus, this research review becomes of significant importance because it has critical implications for real world applications. Scientists and geneticists are now enabled to create better fit individuals, grown in a specific habitat, to increase crop yields or vegetative output.

Because Arabidopsis is a glycophyte and is very sensitive to salt, one might assume that this plant is not suitable for studying the mechanisms of salt tolerance. However, previous studies with cultured glycophytic plant cells indicated that these cells could be adapted to tolerate high concentrations of salt that would kill un-adapted cells (Xiong et al 2002). Additionally, this review is necessary in the field of botany because our results for the model organism, Arabidopsis, can be applied to the general realm of glycophytic plants. The fact that adaptation can increase plant salt tolerance suggests that glycophytes do have salt tolerance machinery that may not be operating effectively in un-adapted conditions. Thus, further research is needed to invent adaptive machinery in glycophytes to allow optimal growth in salt saturated sectors of the planet. Therefore, the difference in salt tolerance between glycophytes and halophytes appears to be quantitative rather than qualitative, and basic salt tolerance mechanisms are probably conserved in all plant species. Our research review on Arabidopsis salt tolerance has provided the preliminary and

APPENDIX IA: Arabidopsis Ecotypic Response to Salinity Stressors

	Leaf Area Reduction (%)	Root Length Reduction (%)	Lethal Dose NaCl Response (mM)	K Ion Content % (@ 100 mM NaCl)	Na Ion Content % (@ 100 mM NaCl)
A. thaliana	3	7	145	1.7	1.5
T. salsuginea	25	56	591	5.7	5.3
L. virginicum	27	58	500	4.9	4.7
D. pinnata	13	52	223	3.7	4.5
T. parvuta	52	92	600	8.4	9
B. varna	5	33	263	3.3	4.3
C. bursapastoris	9	39	343	4.1	4.4
H. incana	18	61	307	4.5	4.6
L. densiforum	25	57	498	5.2	2.3
M. triloba	14	85	600	8.9	7.6
S. officinate	7	39	339	3.4	3.9
T. arvense	5	56	296	1.9	1.6

confirmatory evidence for other specialized fields, such as genetic agricultural engineering, to apply our results and create adaptive mechanisms to optimize growth. Further research is needed in this field to determine the specific differences in salt sensitivity/ tolerance and genetic markers for predisposition towards salt tolerance.t.

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