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Title of Proposal: Analysis of wound regeneration in vascular tissue regions for transgenic corn and cotton expressing variation in lignin concentration and composition.

Narrative:

A)

-Investigate developmental differences between transgenic and parent plant types with respect to lignin concentration, structure, and function.

-Successfully photograph and analyze differences in wound response between transgenic and parent plant types.

-Theorize the potential impact of any differences that arise in terms of fitness, biodiversity, and conservation concerns.

B)

The first investigations into the abnormal lignin content of Bt transgenic plants were in 2001 by Stotzky and Saxena. My project will borrow many of the methods from their paper published in the American Journal of Botany. They planted seeds of Bt hybrids and the non-Bt isolines in a uniform soil mixture and carefully controlled variables such as water, soil content, and light exposure. They also had field-grown groups to use as comparison to the laboratory controlled groups. They grew their specimens for 90-97 days, which is longer than is necessary to get results for the purpose of this experiment; 60 days should be adequate. Their methods for calculating lignin content will be especially useful. They used 0.01% toluidine blue for staining and then observed microscope cross-sections from cut stems. They also derived the lignin content by chemical analysis methods, which involve drying portions of stem, grinding them up, and processing through an 80-mesh sieve before applying acetyl

bromide. Their final results showed significantly increased lignin levels in the transgenic plants when compared to parent type. The next phase of their research involved answering some of the concerns from these results.

Another paper by these authors, published in 2001, answered the question of potential for soil contamination of Bt toxin leached out and how it might effect common soil invertebrates such as the earth worm. They concluded that there was little concern that Bt transgenic plants will harm non-target soil invertebrates. More recently in 2005, they had a paper documenting their findings that transgenic Bt plants decompose less in soil then non-Bt plants. This finding was significant because it showed that Bt plants interact differently with their environment. Specifically they pushed the notion that more work must be done because the ecological and environmental relevance of their conclusions was not known.

Other researchers have also tried to raise awareness of the abnormal phenotypes that Bt transgenic plants display. In 2002, Buxton and Casler had a paper published in which they showed that the genetic modification of lignin whether intended or unintended had an effect of the fitness of the plants they investigated. Taken together these experiments should be a significant cause of concern for the companies selling transgenic plants, the farmers using them, and people concerned about the environment to which these plants are being introduced.

The research that is specifically inspiring this project comes from a paper by Breitler et al. in 2001. What this research looked at was a region of maize

genome that directs high level, wound-inducible expression of the cry1B gene which protects transgenic rice plants from stemborer attack. The cry1B gene is the specific gene taken from *Bacillus thuringiensis* that gives Bt-transgenic plants their insecticide abilities. The significance of this is that this paper demonstrated the first evidence that plant responses to wounding are different in Bt plants by providing Bt rice plants a new level of defense from stemborer attacks. My paper is going to investigate similar ideas but in a different part of the plant, stem and root as opposed to leaf tissue, and in two different types of plants. This is the most recent work done directly related to my topic and this project design will be a logical step from the works by these authors in investigating differences between transgenic and parent plants.

C)

This project presents an exciting challenge that will allow me to apply the research, laboratory, and writing methods I have been honing during the past three years as an undergraduate to a project of my own choosing and my own design. In particular, the subject of genetic modification is controversial and very relevant to the direction that the biological sciences are heading. My proposed experiment borrows from the results and conclusions of recently published work by other scientists that were also curious, confused, or even concerned about the unexpected phenotypic changes in commercially marketed transgenic plants. My goal is to take what they did a step further and test whether those observed differences translate into different and unique environmental responses. To my

knowledge, the subject of this research has not been investigated in this way before and I have every intention for my work to contribute to the every-growing body of literature concerning transgenic plants.

D)

For the purpose of investigating any differences between transgenic plants and non-transgenic plants, cotton and corn were chosen because both have been modified with the Bt gene and both have significant differences in vascular structure and should make for an interesting comparison. Approximately 20 plants from both species their varieties (parent and Bt) will be germinated, planted in a mixture of 33% sand, 33% gardening soil, and 33% vermiculite, and allowed to grow under optimal conditions for several weeks. At that time small horizontal slices will be made to all the plants in the regions under investigation with the point of a very fine scalpel. The plants will be allowed to continue to grow and heal for one week and then microscope slides of the areas of interest for each plant will be made. After pictures are taken histo-chemical tests will be conducted to determine chemical composition. Finally, the best examples will be set aside, stained, and photographed under fluorescence microscopy.

E)

The growing phase of this project will be started during the fall 2006 semester and continued during the winter semester break. Microscope slides of healed areas will be made and assed with histo-chemical tests or photographed

with a compound and fluorescence microscope during the beginning of Spring 2007.

F)

The results of this project will be presented at Quest day 2007, as well as submitted to an appropriate biological journal for review.

Equipment Purchase: Chemical Kits, Digital Camera, Film/Developing, Grow setup
Equipment amount: $300
Equipment Rental:
Equipment Rental amount:
Photocopying: 400+ pages of printing and photocopying
Photocopying amount: $60
Postage:
Postage amount:
Professional Assistance:
Professional Assistance amount:
Publication Costs:
Publication Costs amount:
Software Purchase:
Software Purchase amount:
Supplies: Razors, pots, soil, sand, and vermiculite
Supplies amount: $75
Telephone:
Telephone amount:
Travel: Travel to and from ESF to do fluorescence microscopies
Travel amount: $50
Misc Costs: Food for 5 days during traveling
Miscellaneous Costs Amount: $50
Miscellaneous Costs 2:
Miscellaneous Cost 2 Amount:
Total Funds Requested: Cost of growing set-up is TBD based on available resources
Total Funds Requested amount: $535 appx.

Budget Justification: 80+ plants will be grown requiring the appropriate size set up (pots, soil, trays, lighting); then after intentional wounding and subsequent healing microscope slides of plant tissue will be made and pictures taken of the areas of interest. 160 or more photos will likely be taken requiring a camera and microscope apparatus. Then chemical analysis (chemical kit) will quantify the lignin differences that were qualified in the photos. Other expenses include photocopy/printing of references and paper and travel expenses of going to another university to use their fluorescence microscopy.

Additional Funds no
Final Report no
Submit Final Report na

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