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14. ABSTRACT

Plants emit volatile mixes characteristic of exposure to both plant and animal (insect) pathogens (bacteria and fungi). The specificity of these emissions was verified in a series of laboratory experiments with tobacco and peanut plants. Plants could provide information about specific exposures if we can interrogate them in open air. Similarly, gene expressions profiles were found to provide fingerprints characteristic of the same exposures. Genes expressed included promoters that could be used to amplify natural responses or develop novel responses for use in "sentinel plants".

15. SUBJECT TERMS

Plants, sentinels, sensors, volatiles.

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FINAL REPORT

GRANT #: N00014-01-1-0848

PRINCIPAL INVESTIGATOR: Jack C. Schultz

INSTITUTION: The Pennsylvania State University

GRANT TITLE: Development of Ground-based Plant Sentinels

AWARD PERIOD: 1 May 2001 - 30 April 2003 (terminated 2002)

OBJECTIVE: To demonstrate that plants can detect and report exposure to a range of potentially pathogenic microbes. The ultimate objective is to develop a plant or plants that can detect and report these selected exposures.

APPROACH: The project had two major thrusts. First, volatile emissions were collected from *Arabidopsis thaliana* and other plant species (e.g., maize, tobacco) exposed to several species and genotypes of plant and animal pathogenic microbes in the laboratory and compared with emissions from ininfected plants to identify volatile "fingerprints" specific to the infecting microbe. Second, suppressive subtractive hybridization was used to compile lists of *Arabidopsis thaliana* genes expressed in response to specific pathogens. Goals of this work included identifying microbe-responsive promoters that could be used to drive reporter constructs, and locating genes responsible for volatile production.

ACCOMPLISHMENTS: The project was terminated early, in its second year. Experiments assessing volatile emissions from tobacco plants exposed to several pathogens were completed. These provided the first evidence that a plant emits a specific mix of volatiles that serves as a fingerprint of the exposure. Volatile fingerprints were found to be highly specific. Volatile emissions allowed us to distinguish between plants treated with two different pathovars of the bacterium *Pseudomonas syringae* (pv. maculicola and pv. tomato), and with wild type and mutant forms of *P. syringae* pv. tomato that differ by only one gene. Key compounds that varied among these volatile mixes were beta ocimene, caryophyllene and methyl salicylate.

Similar experiments were carried out with peanut plants. They were found to emit characteristic volatile

mixtures in response to exposure to white mold. Further experiments indicated that these volatile profiles differ from those elicited by insects, and influence insect growth on infected peanut plants.

To determine whether bacteria other than plant pathogens might elicit similar responses, tobacco plants were exposed to the insect pathogen, *Bacillus thuringiensis* var. kurstaki. Volatile samples collected 48h after leaves were inoculated indicated that this bacterium also elicited a unique mixture. This is the first example of a plant response to an animal pathogen. It is particularly intriguing because B. thuringiensis is thought to be the closest living relative of B. anthracis (anthrax).

Efforts to carry out similar studies of volatiles with *Arabidopsis thaliana* were not completely successful. We found that *A. thaliana* emits few volatiles in very small amounts, making efficient experimentation difficult and reducing the likelihood of their utility in the field. A large number of *A. thaliana* ecotypes were grown from seed, in an attempt to find a better emittor. These trials were underway when the project ended.

The gene expression studies produced independent libraries of *Arabidopsis* genes expressed in plants inoculated with the plant pathogens *P. syringae*, *P. fluorescens*, and *Alternaria brasssicola*. Among these we found several promoter sequences that could be used to drive reporter constructs. These libraries have been added to a microarray comprising a collection of "stress-response genes" from a range of plant exposure circumstances. The project was ended before we could employ this microarray or develop response-reporter constructs from these libraries.

CONCLUSIONS: As proposed, some plants (perhaps all plants) emitted highly distinctive suites of volatiles when exposed to various plant pathogens, and to one animal pathogen. Volatile mixes remain the only phenotypic trait that provides a 'fingerprint' of such exposures. There is considerable variation among plant species (and genotypes) in the volume of volatiles emitted. Gene expression profiles are also distinctive fingerprints of exposure to microbes, as expected. There is preliminary support for the idea that stimulus-specific promoters and reporter constructs could be developed from such profiles.

SIGNIFICANCE: Our studies are the first to show that plants emit specific volatile mixes in response to particular microbial genotypes, even those differing by

only a single gene. This provides evidence for considerably greater environmental sensitivity and responsiveness than anticipated for plants. The molecular resources developed here have been carried forward and are in use in an NSF-sponsored study of *Arabidopsis* responses to pests and in a DARPA-sponsored study of plant receptor/response circuits.

PATENT INFORMATION: No patent applications have been filed as a result of this work.

AWARD INFORMATION: Tumlinson was appointed to the R.O. Mumma Chair in Entomology at Penn State University. Raina was appointed Associate Professor with tenure at Syracuse University.

PUBLICATIONS AND ABSTRACTS (for total period of grant):

Cardoza, Y.J., H.T. Alborn and J.H. Tumlinson. (2002) In vivo volatile emissions of peanut plants induced by fungal infection and insect damage. J. Chem. Ecol. 28: 161-174

Cardoza, Y.J. (2002) Pathogen-induced plant chemical defenses: Effect on insect herbivores and parasitoids. Ph.D. Dissertation, University of Florida, Gainesville, Florida.

Cardoza, Y.J., C.G. Lait, E.A. Schmelz, J. Huang and J.H. Tumlinson. (2003) Fungus-induced biochemical changes in peanut plants and their affect on development of beet armyworm, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae), larvae. Environ. Entomol. 32:220-228

Huang, J., Cardoza, Y.J., Schmelz, E.A., Raina, R., Engelberth, J.E. and Tumlinson, J.H. (2003) Differential volatile emissions and salicylic acid levels from tobacco plants in response to different strains of Pseudomonas syringae. Planta. 217:767-775.

De Moraes CM, Schultz JC, Mescher MC, Tumlinson JH. (2004) Induced plant signaling and its implications for environmental sensing. J Toxicol Environ Health A. 67:819-834.