WASHINGTON SEA GRANT
PROJECT COMPLETION SUMMARY REPORT

INSTRUCTIONS: Please provide a lay summary for your completed project that includes the following project elements:

- objectives
- methodology
- rationale
- major findings
- significance of results

If relevant, also include:

- students supported (number and degree level)
- partnerships
- outreach activities

Please note that this summary will be submitted in the Washington Sea Grant annual report to the National Sea Grant Office and will be available to the public via the NIMS database and the Washington Sea Grant website.

WSG Project Number:	**R/B-44**
Project Title:	Crab Shell Chitosan as a Pesticide Sticker and Immune Response Activator
Project period:	2/1/2004 – 1/31/2008

Principal Investigator(s) and Affiliation:

Lee Hadwiger Plant Pathology, Washington State University

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*(Please provide your summary here. Character limit: **5,000 characters**, including spaces):*

The first objective was to work with a manufacturer of chitosan and various pesticide industries currently manufacturing agriculture chemicals to define additional uses of chitosan as a pesticide "sticker". The Vanson/Halo Source company was cooperative with testing, and developed a second compound called SeaKlear that contained the copper sulfate pentahydrate (antifungal) ingredient and thus became the source of both of the ingredients, having previously produced chitosan lactate serving as the sticker ingredient. A publication in Plant Health Progress relayed our research data to those growers likely to adapt this treatment in the control of potato late blight, designed and approved for the organic grower. Two additional field tests run by an independent testing company again indicated this treatment offered potatoes protection, however not as effectively as the organically synthesized fungicides, however the latter chemicals have

not been approved for the organic grower. The second objective to develop patents was unattainable, because the ingredients were not chemically unique.

Research directed to the third objective, to understand the mode by which chitosan activates defense genes in plants, was very productive. Chitosan effectively reduces the presence of HMG A (previously HMG-I/Y) in the vicinity of the DNA promoter region of one of the defense genes (termed PR genes). Its action in inducing PR genes is less destructive to the phosphorylation content of the nucleosome where gene transcription occurs than the other signal Fsph DNase released by the invading fungus. Chitosan when extensively tested in combination with key metabolic inhibiting or enhancing compounds was found to have an additive effect in the production of the plant's natural antifungal compound, pisatin (published in Plant Science). These tests directed research toward phosphatase and kinase inhibitors and subsequently to the ubiquitination of nuclear proteins, e.g. HMG A and histones. Taken together our results were the basis of a review paper in Phytopathology. This review systematically describes a scenario of how external signals from the pathogen, such as chitosan, can activate the PR set of genes in developing plant immunity. Thus the chitosan tool has provided us with an explanation for how the defense genes that constitute the bases for immunity can become activated in the presence of plant pathogenic organisms, called inappropriate pathogens. The resistance exerted is called non-host resistance, an area in which my laboratory has played a major role in defining. A simple explanation is that chitosan enters the plant cytoplasm, then on to the nucleus, where, as a polycationic polymer, it competes for sites on the DNA molecule with other basic polymers (histones). The displacement of histones allows the transcription complex to progress through gene's open reading frames activating the defense genes. The defense genes are selectively enhanced, because they are in regions with lower barriers to the transcription complex.

The agricultural application of chitosan is on two levels. A high level application induces natural plant immunity. At a much lower level chitosan as a sticker holds onto active ingredients that in turn afford protection. More recently we found that an intermediate level, holding maximum copper sulfate pentahydrate, is highly effective as a herbicide removing Milfoil weeds from lakes, while limiting the copper's diffusion into open water. Finally, this combination removes Fairy Rings from golf courses and lawns.

As a result of Sea Grant funding, chitosan's potential for application has become of world wide interest. It remains one of the most available, economically viable, marine products known to man. This known value increases its worth of chitosan sufficiently so that the source material, crab shell wastes, is seldom allowed to pollute because of the inherent value of the chitosan within.

Although formal funding from The Washington Sea Grant Program has ended, the research outlined in the objectives will continue albeit at a less vigorous rate. There is a continuing effort by my laboratory to understand more about the chitosan action and how its commercial value can be further enhanced. The acreage of land converted to organic farming continues its exponential growth and potatoes can not be grown successfully in most areas without fungicidal protection. The chitosan/copper sulfate pentahydrate treatment remains one of the very few options the organic grower has to protect potatoes and other vegetables from disease.