Environment and Natural Resources Trust Fund 2015 Request for Proposals (RFP)

| Project Title: | ENRTF ID: 093-D |
|--|---|
| Treatment of Emerald Ash Borer-infested Ash Trees | |
| Category: D. Aquatic and Terrestrial Invasive Species | |
| Total Project Budget: \$ 120,000 | |
| Proposed Project Time Period for the Funding Requested: | <u>1 year, January 1, 2016 - March 31, 20</u> |
| Summary: | |
| Emerald ash borer is a threat to Minnesotas ash trees and treath boreGONE! provides an environmentally-sound and economic al | |
| Name: Thomas Matthews | |
| Sponsoring Organization: Phyllom BioProducts Corporation | |
| Address: 484 Lake Park Ave, #23 | |
| Oakland CA 94610 | - |
| Telephone Number: (858) 349-2251 | |
| Email davematthews@phyllom.com | |
| Web Address http://phyllombioproducts.com | |
| Location | |
| Region: Metro | |
| County Name: Hennepin, Ramsey | |
| | |
| | |
| City / Township: | |

Alternate Text for Visual:

Map of EAB infestations in the Metro Region of Minnesota.

| Funding Priorities Multiple Benefit | s Outcomes Knowledge Base |
|-------------------------------------|---------------------------------|
| Extent of Impact Innovation | _ Scientific/Tech Basis Urgency |
| Capacity ReadinessLeverage | TOTAL |



PROJECT TITLE: Treatment of Emerald Ash Borer-infested Ash Trees

I. PROJECT STATEMENT

The effect of aerial spraying ash trees infested with the emerald ash borer (EAB), *Agrilus planipennis*, with a beetle-specific biological control agent on reducing the population of the subsequent generation will be determined. EAB is native to northeastern China, Korea, Mongolia, and Japan, but was identified in 2002 as the causal agent of ash tree mortality in southern Michigan and Ontario. EAB is now established throughout Lower Michigan, and infestations have also been found throughout the Midwest and Northeastern Unites States, including Minnesota. Ash is an important timber species and landscape tree, and EAB places ash trees throughout North America at risk. The costs to communities and landowners for removal and replacement of ash trees killed by EAB are high. A 2011 economic impact study estimated EAB annual losses at \$1.2 billion in local government and household expenditures, \$380 million in lost residential property values and \$60 million in landowner timber losses. Treatment of infested ash trees is difficult and expensive. EAB infestations are not usually identified until symptoms appear, and by that time the prospects of successfully treating the tree are poor. Current treatment options are limited to systemic injections of chemical insecticides directly into an infested tree, using parasitoid wasps, or tree removal. However, these options are neither economic nor practical; other treatment options are needed to fully protect ash trees from EAB.

The most widely used and successful biological control agents for insects are the crystal (Cry) proteins produced by the bacterium *Bacillus thuringiensis* (*Bt*). The Cry proteins provide the specificity, potency and safety needed to implement a pest control program in environmentally-sensitive areas such as riparian and urban forest ecosystems. *Bt*-based aerial spray products have a long history of successful utilization in the field. Forested areas have been sprayed with products based on *Bt kurstaki* strains that are active against Lepidopteran species, and used in USDA Forest Service and APHIS programs to control the invasive gypsy moth. In Minnesota, the Metropolitan Mosquito Control District has used *Bt israelensis* for several years to control mosquito populations. The Cry8Da toxin produced by *Bt galleriae* SDS-502 has been extensively tested in numerous types of adult EAB bioassays, and possesses very high specific activity against this pest.

The goals of this study are to suppress the subsequent generation of EAB by targeting EAB adults shortly after emergence, and to measure any effects on non-target species. The ash foliage will be treated with boreGONE!TM, a formulated product based on *Bt galleriae* SDS-502, by aerial application. It is expected that ingestion of treated ash foliage will result in cessation of feeding and death of adult EAB. This will in turn suppress populations of both male and female adults before mating, and ultimately reduce the numbers of females laying eggs. The efficacy of the applications to reduce the amount of progeny produced will be measured by comparing branch samples from ash trees before and after treatment with branches from untreated trees for the presence of larvae and galleries. Drop clothes around treated and untreated trees will also be used to collect and quantitate dead adult EAB as well as any dead non-target insects.

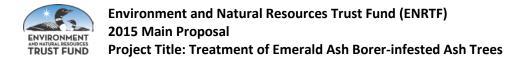
II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Pre-trial Assessment

Budget: \$15,000

A pre-trial assessment of EAB infestation before adult emergence will be performed at each trial site.

| Outcome | Completion Date | |
|--|------------------------|--|
| 1. Two branches per ash tree will be collected from 25 trees within the designated | April 30, 2016 | |
| treated and untreated blocks for each trial site. | | |
| 2. Two 25 cm sections per branch will be whittled back to determine extent of EAB | May 31, 2016 | |
| infestation by counting larvae and galleries. | | |



Activity 2: Aerial Spraying

Budget: \$75,000

Budget: \$10,000

Spraying will be performed based on day degree models of adult EAB emergence and peak flight, most likely in June and July. At least two applications will be performed per site.

| Outcome | Completion Date |
|--|-----------------|
| 1. Data will be collected to measure droplet size and density, as well as foliar coverage of | August 31, 2016 |
| the product. | |
| 2. Ground tarps will be placed around 25 ash trees in each treated and untreated block | August 31, 2016 |
| to collect dead adult EAB as well as any dead non-target insects. | |

Activity 3: Post-trial Assessment

A post-trial assessment of EAB infestation will be performed at each trial site.

| Outcome | Completion Date |
|--|-------------------|
| 1. Two branches per ash tree will be collected from 25 trees within the designated | October 31, 2016 |
| treated and untreated blocks for each trial site. | |
| 2. Two 25 cm sections per branch will be whittled back to determine extent of EAB | November 30, 2016 |
| infestation by counting larvae and galleries. | |

III. PROJECT STRATEGY

A. Project Team/Partners

Thomas D. Matthews, Ph. D., Scientific Advisory Board, Phyllom BioProducts Corp. will act as the primary investigator, will manage the trial, supervise personnel, analyze the data, and prepare the final report (will receive funding from this proposal).

Temporary contract workers (5) will assist in field work and data collection (will receive funding from this proposal).

Contracted aerial spraying service to apply the boreGONE![™] biopesticide (will receive funding from this proposal).

Phyllom BioProducts Corp. will supply the boreGONE![™] biopesticide (will not receive funding from this proposal).

B. Project Impact and Long-Term Strategy

Phyllom BioProducts' boreGONE![™] currently has an Experimental Use Permit from the US Environmental Protection Agency. To obtain full registration of this product, efficacy in the field needs to be demonstrated, and data from this project will help support this effort. The addition of an environmentally and economically sound pest control agent such as boreGONE![™] as another option to suppress populations of EAB and prevent further spread of this pest will help protect the 998 million ash trees in Minnesota.

C. Timeline Requirements: January 1, 2016-March 31, 2017:

March 31, 2016: Assemble field teams and materials for the pre-trial assessment; identify spraying sites; design blocks of ash trees; identify aerial spraying service.

April 30, 2016: Completion of the pre-trial assessment.

May 31, 2016: boreGONE![™] delivered to aerial spraying service; aerial spraying preparations completed. August 31, 2016: Completion of aerial spraying of boreGONE![™].

November 30, 2016: Completion of post-trial assessment.

March 31, 2017: Analysis of data and completion of the final report.

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2015 Detailed Project Budget

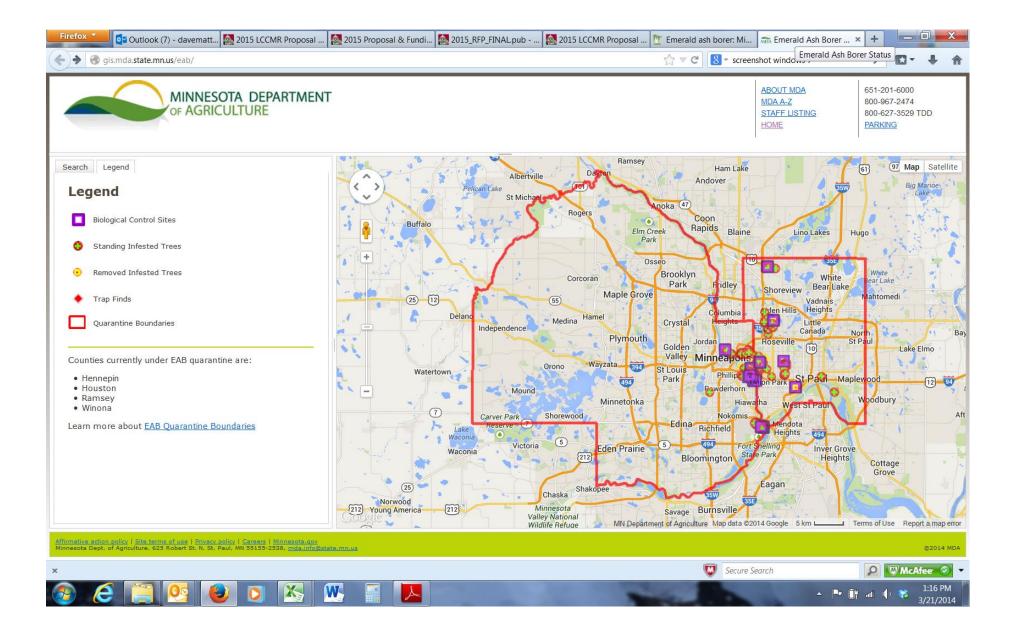
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IV. TOTAL ENRTF REQUEST BUDGET 1 year

| BUDGET ITEM | AMOUNT | |
|---|----------|---------|
| Personnel: | | |
| Primary Investigator: Thomas D. Matthews 1/1/16-3/31/17 (17% towards salary) | \$20,000 | |
| Temporary technicians (5): 4/1/16-11/30/16 (25% towards salary) | \$30,000 | |
| Contracts: Aerial spraying service for application of boreGONE! [™] . | \$65,000 | |
| Equipment/Tools/Supplies: Saws, knifes and other tools for branch collection and analysis; safety | \$5,000 | |
| equipment; tarps to collect dead insects; office supplies. | | |
| TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST = | \$ | 120,000 |

V. OTHER FUNDS

| SOURCE OF FUNDS | AMOUNT | <u>Status</u> |
|--|-----------|------------------------------------|
| Other Non-State \$ To Be Applied To Project During Project Period: | N/A | Indicate: |
| | | Secured or |
| | | Pending |
| Other State \$ To Be Applied To Project During Project Period: | N/A | Indicate: Secured or Pending |
| In-kind Services To Be Applied To Project During Project Period: Phyllom BioProducts Corp. will supply the boreGONE! TM and support out-of-state travel. | \$ 70,000 | Secured |
| Funding History: | N/A | |
| Remaining \$ From Current ENRTF Appropriation: | N/A | |



Project Manager Qualifications & Organization Description

Dr. Dave Matthews has over 25 years of experience in the biotech industry and academia, with expertise in the areas of microbial genetics and physiology, and molecular biology. He is currently serves on the Scientifc Advisory Board of Phyllom BioProducts Corp., a small agricultural biotech company that produces a biopesticide specific for beetle pests that is based on the bacterium Bacillus thuringiensis (Bt). He started his career with Sandoz Agro working on the insecticidal crystal proteins of Bt. In addition to genetic modification of production strains, he also improved insecticidal activity through mutagenesis of various crystal protein genes. These efforts culminated with the development of a Bt strain with increased activity against the beet armyworm (Spodoptera exigua), an important crop pest. After Sandoz, Dr. Matthews joined a team at Quidel that successfully developed a rapid diagnostic test for influenza that is currently in wide use. Subsequently, he worked for Monsanto where he developed a transformation system for mircroalgae to increase yields of omega-3 fatty acids via metabolic engineering. At CP Kelco he modified production strains through metabolic engineering for increased yields of commercially valuable bacterial polysaccharides, developed an expression system for Xanthomonas campestris, and a transformation system for Sphingomonas. He also developed an assay that identified numerous genes affecting phenotypic phase shifting and polysaccharide production in Sphingomonas.

While in academia, Dr. Matthews studied the evolutionary role of large-scale chromosomal rearrangements that occur in host-specific serovars of *Salmonella enterica*. His research showed that these rearrangements were the consequence of a host-restricted lifestyle lowering selective pressure to maintain gene order, and supports current paradigms of pathogen evolution. He is also compared the genome sequences of strains belonging to three closely related *Salmonella* serovars that vary in host range and virulence, and has identified differences in gene and prophage content that at least partially explains this variation.

Dr. Matthews received his bachelor's degree from San Jose State University, his master's degree from San Diego State University, and his doctoral degree from the University of California, San Diego/San Diego State University Joint Doctoral Program in Biology. His post-doctoral work was also done at San Diego State University.

Phyllom BioProducts Corp. is a small agricultural biotech company that produces a biopesticide specific for beetle pests that is based on a proprietary strain of the bacterium *Bacillus thuringiensis* (*Bt*). Last year the company received EPA registrations for beetleGONE!TM and grubGONE!TM, products for the agricultural, and turf and ornamental markets. Phyllom also received an Experimental Use Permit from the EPA to test boreGONE!TM in field trials against the Emerald ash borer.