6-1-09 **Proposal for "Grow Iowa Values Fund" Grants Program**

Transgenic Plant Resistance to Invertebrate Pests

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Company Partners: Pioneer Hi-Bred International, a DuPont Company, with headquarters in Johnston, Iowa: Pioneer has been providing growers with leading seed products for over 80 years. With annual sales of more than $4 billion dollars and over 8,000 people, Pioneer is a worldwide leader in discovering and commercializing novel genetic solutions for growers and users of seeds and grain products worldwide. Pioneer brand corn and soybeans are the market leaders in North America and Pioneer has a significant and rapidly expanding international seed business, providing services to customers in nearly 70 countries.

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EXECUTIVE SUMMARY

Invertebrate pests (insects and nematodes) cause devastating agricultural losses worldwide and are estimated to consume 15% of total agricultural output. Overall yield losses to food and fiber crops resulting from invertebrate pest damage in the U.S. alone are well over a billion dollars per year. Current management relies primarily on the use of chemical pesticides, the efficacy of which can be short-lived as pesticide resistance develops. Hence new tools for invertebrate pest management are essential for sustained and environmentally sound agricultural productivity. A single technology effective for control against multiple pests would have significant value when introduced into leading germplasm that has other major traits and attributes demanded by growers and the marketplace. We have developed a novel transgenic technology for plant resistance to aphids, and propose to test this technology for efficacy against a broad range of invertebrate pests, including economically important plant bugs, moth pests, and nematodes. This technology consists of a plant virus coat protein fused to a toxin that acts within the body cavity. The coat protein enters the insect body cavity, thereby delivering the toxin to its target site. We expect to demonstrate resistance to a broad range of invertebrate pests, thereby providing an environmentally benign alternative technology to chemical pesticides. The development of transgenic plants resistant to a broad range of invertebrate pests would contribute to environmentally sustainable pest management with reduced yield losses and chemical inputs for sustainable food and biomass production. This technology would confer significant economic benefit both to Iowa and to U.S. agriculture as a whole.

PROJECT DESCRIPTION

Technical Objectives We have developed a new transgenic technology for management of aphid pests (U.S. Patent 7,312,080. Plant Resistance to Insect Pests Mediated by Viral Proteins. W.A. Miller and B.C. Bonning). While this in itself is a significant achievement, this technology may have broader commercial application for other economically important pests including plant bugs and nematodes. The objectives of this proposal for research at ISU are to (1) test the resistance technology against a broad range of invertebrate pests, and (2) construct transgenic plants and determine the extent of pest resistance. Attainment of these goals will demonstrate the breadth of application of this new technology for invertebrate pest management. Our industrial partner, Pioneer Hi-Bred International, Inc., a DuPont Company, will then proceed with construction of transgenic soybean for commercialization.

Scope of the Work: Sap-sucking insects (order Hemiptera) including the pervasive aphids and plant hoppers, cause significant economic loss on a wide range of agricultural and horticultural crops. Current management of such insect populations depends primarily on application of classical chemical insecticides, with relatively few resistance genes available to plant breeders. We have developed a novel transgenic means for hemipteran pest control: we showed that the coat protein (CP) of an aphid-vectored plant virus, *Pea enation mosaic virus* (a luteovirus), can deliver an insect-specific toxin (ω-atractoxin-Hv1a) that acts within the aphid body cavity (hemocoel) (Miller and Bonning 2007). Aphid feeding on the recombinant CP-P-Hv1a fusion, either in membrane feeding assays or via transiently transfected plants (*Nicotinia benthamiana*), resulted in significant mortality of the pea aphid, *Acyrthosiphon pisum* and the green peach aphid, *Myzus persicae* (Fig. 1). While this strategy shows great potential for management of aphid pests, there are several questions that need to be addressed to clearly define the commercial prospects for this new technology. Specifically, the mechanism of uptake of CP into the aphid hemocoel is poorly understood, and hence it is unknown whether the CP-P-Hv1a fusion protein would also be effective against other invertebrate pests. It has been postulated that glycan (D-mannose) binding plays a role in plant virus uptake into the aphid hemocoel, in which case other invertebrates with D-mannose in the gut may be susceptible to CP-P-Hv1a. In addition, the toxin Hv1a alone has been shown to be orally active against moth pests and arachnids (spiders, mites) (Khan, Zafar et al. 2006; Mukherjee, Sollod et al. 2006). We propose to explore the extent to which CP-P-Hv1a can be applied for pest control, by testing the range of pests susceptible to the recombinant fusion protein, CP-P-Hv1a, and by constructing transgenic *Arabidopsis thaliana* for *in planta* bioassays.

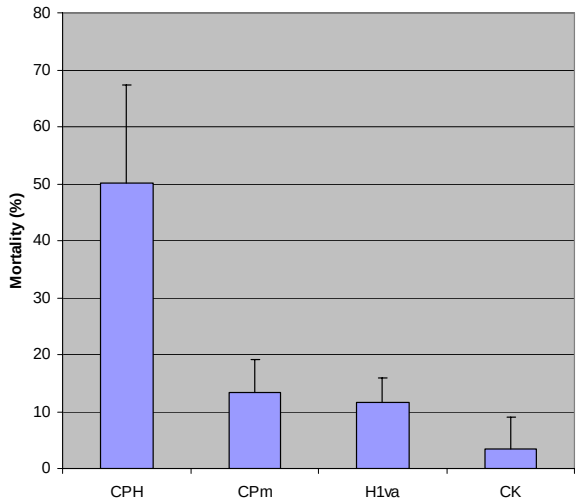

Fig. 1. Bioassay of *E. coli*-produced fusion proteins against the pea aphid by membrane feeding. Mortality (%) is shown for CP-P-Hv1a (CPH); CP-P-Hv1am, a fusion with a modified, inactive toxin (CPm); toxin alone (Hv1a), and 25% sucrose alone (CK).

Objective 1 ***Test efficacy of CP-P-Hv1a against invertebrate plant pests*** We will produce and purify recombinant CP-P-Hv1a in *E. coli* using the pGEX expression system (GE Life Sciences). We will conduct bioassays to assess the efficacy of CP-P-Hv1a against key invertebrate pests of significant economic importance, including the cotton aphid (*Aphis gossypi*), soybean aphid (*Aphis glycines*), plant bug (*Lygus spp.*), and tomato hornworm (*Manduca sexta*).

Objective 2 ***Assess resistance of transgenic Arabidopsis to invertebrate pests*** We will construct transgenic *Arabidopsis* expressing CP-P-Hv1a and test for resistance to pests that readily feed on this plant, including the green peach aphid (*Myzus persicae*), the tobacco hornworm (*M. sexta*), and soybean cyst nematode (*Heterodera glycines*).

Project Deliverables: Upon completion of these studies, we expect to have (1) more clearly defined the range of invertebrate pests that are susceptible to CP-P-Hv1a, and (2) demonstrated the efficacy of CP-P-Hv1a against invertebrate pests when delivered from transgenic plants. This research will provide the foundation for development of agronomically relevant transgenic plants (soybean) for agricultural use by our industry partner.

Benefits and Sustainability: Invertebrate resistant transgenic plants will benefit U.S. agriculture by enhancing agricultural productivity for both food and biofuels, and will reduce environmental contamination resulting from use of classical chemical insecticides for pest management. This technology would confer additional economic and environmental benefits as follows: (1) The proposed technology is less likely to impact nontarget organisms than use of chemical insecticides, which deleteriously affect all beneficial insects including insect natural enemies (e.g., lacewings and ladybugs that help reduce pest aphid populations), bees, and butterflies. Many chemical sprays can also impact vertebrate populations (Flickinger, Juenger et al. 1991). (2) Costs to the grower associated with application of chemical insecticides for protection against pests would also be avoided. (3) The use of fossil fuels required for transportation and aerial or ground spray application of chemical insecticides will be mitigated. (4) The efficacy of chemical insecticides can be short-lived with the rapid evolution of insecticide resistance (Devonshire 1989). In the event that insect resistance arises to the toxin, an alternative toxin with a different target site could be employed to overcome the resistance. The invertebrate resistance technology will provide a useful alternative tool to growers for pest management.

References

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Devonshire, A. L. (1989). Resistance of aphids to insecticides. Aphids, their biology, natural enemies and control. A. K. Minks and P. Harrewijn. Amsterdam, Elsevier. **C.:** 123-139.
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Commercialization Plan

Business case for the project Based on preliminary technical data and the economic importance of invertebrate pests, this project warrants further development: Economic losses resulting from insect pest damage are second only to losses resulting from natural disasters. Among the most economically important invertebrate pests are aphids, plant bugs, and soybean cyst nematodes. Aphids affect almost all agricultural crops and invasive species such as the recently introduced soybean aphid have had a particularly severe impact on Iowa agriculture. In 2003, the soybean aphid *Aphis glycines* exceeded 3,000 aphids per plant, and cost farmers an estimated $80 million in Minnesota alone (Marking 2000; Associated-Press 2003). In Iowa, soybean yields were reduced by 32% compared to the 2002 season. In 2007, an estimated 50% of the nearly 8.5 million acres of soybean grown in Iowa was treated with a foliar insecticide targeting the soybean aphid at a cost of $68 million. Sap-sucking plant bugs within the genus *Lygus* (Miridae) are also significant agricultural pests. The tarnished plant bug for example feeds on more than half of all commercially grown crop plants. The soybean cyst nematode is a devastating pest of soybean worldwide. This nematode has resulted in annual losses of at least $500 million and has reduced yields by as much as 75% (Davis and Tylka 2000). Clearly, a plant resistance technology that confers resistance to multiple invertebrate pests would be of significant commercial and economic value for protection of agricultural yields.

Given that *there are currently no competing technologies for management of sap-sucking insect*s, this technology could result in Pioneer developing and commercializing new products that bring value to customers worldwide, thereby boosting the Iowa economy and benefiting both national and international agriculture. Pioneer is interested in licensing the patent for this technology, U.S. Patent 7,312,080 Plant Resistance to Insect Pests Mediated by Viral Proteins, W.A. Miller and B.C. Bonning, which is available for licensing, along with others that may result from the proposed research. The potential impact of this technology is considerable. We envisage the success of this technology to be comparable to that of transgenic plants expressing herbicide resistance (Roundup Ready) that are now widely used for management of weeds within the United States. In 2008, 92% of the soybean acres planted in the United States was transgenic. With a $10 technology fee per bag of soybean seed for the herbicide resistance trait, this amounts to generation of over $680 million per year for soybean seed companies in the United States. If the CP-P-Hv1a trait controls aphids and soybean cyst nematodes, a trait value similar to Roundup Ready is expected. As an additional benefit, reduced insecticide use will reduce environmental impacts and producer contact with potentially harmful chemicals.

ISU research capabilities: In the event that the fusion protein is not toxic to any of the invertebrates tested, funding will be sought by Bonning for directed modification of the fusion protein to broaden the host range based on the physiological basis for the lack of toxicity. Following development of agriculturally relevant transgenic plants (soybean) by Pioneer, Bonning will apply for funding from the USDA Biotechnology Risk Assessment Research Grants Program to examine the potential impact of the fusion protein on non-target, beneficial invertebrates such as aphid natural enemies (ladybugs, parasitoids, lacewing larvae). Such data would facilitate EPA registration of products carrying the toxin fusion system.

List of competitors Competitors for production of transgenic pest resistance in crops include Bayer CropScience, Dow Agrosciences, Monsanto, and Syngenta.

Budget

CATEGORY	AMT REQUESTED	ISU cost share (in kind)	Pioneer cost share (cash)	USDA cost share (in kind)	TOTAL
Salaries	45,675	6,360	45,675		97,710
Benefits	10,505	1,730	10,505		22,740
Graduate student stipends				19,570	19,570
Benefits				2,270	2,270
Tuition				4,466	4,466
Undergraduates					
Personnel Sub-total	$56,180	$8,090	$56,180	$26,306	146,756
Equipment	25,000		9,200		34,200
Lab Supplies	18,000		25,000		43,000
Field Supplies					
Other Supplies & Services	5,000		5,000		10,000
Travel	2,500		2,500		5,000
Publication	1,000				1,000
Miscellaneous			2,120		2,120
TOTAL	$107,680	$8,090	$100,000	$26,306	$242,076

Budget Justification

Personnel: Funds are requested for a postdoctoral research associate for a period of 24 months (benefits 23%, 3% pay increase for Yr 2). A two year funding period is requested due to the time taken for production of transgenic *Arabidopsis*.

Equipment: Funding is requested for a Percival PGC15.5 plant growth chamber. This estimate includes the 10% discount provided by Percival to ISU.

ISU Cost Share (in kind): Bonning will spend 6% time on this project (benefits 27.2%).

Pioneer Hi-Bred International Cost Share (cash): Funding provided by industry (subject to conditions specified in the supporting letter) will also include purchase of an Eppendorf refrigerated microcentrifuge (5417R: $8,000) and associated rotor ($1,200).

USDA NRI Cost Share (in kind): USDA NRI grant "Aphid-luteovirus interaction"
B.C. Bonning and S. Liu (3-1-09 to 2-28-12).

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GIVF FY10-9

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May 22, 2009

Dr. Bryony C. Bonning
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Ames, Iowa 50011-3222 USA

Dear Dr. Bonning:

We understand that you are submitting a grant proposal to the Grow Iowa Values Fund ("GIVF") program entitled *Transgenic Plant Resistance to Invertebrate Pests*. Because Pioneer believes this subject has scientific, economic, and commercial merit, Pioneer is interested in providing scientific and monetary support should your grant be funded by GIVF.

Pioneer Hi-Bred International, Inc., a DuPont Company, headquartered in Johnston, Iowa, is a worldwide leader in discovering, developing and commercializing novel genetic solutions for growers and users of seeds and grain products worldwide. Pioneer's annual revenues exceed $4 billion dollars and Pioneer brand corn and soybeans are the market leaders in the North America. We have a significant and rapidly expanding international seed business.

Pioneer encourages the discovery and development of innovative science and technology throughout the global scientific community for crop yield improvement, crop protection against weeds, insects and environmental stresses, and crop utilization for industrial applications. Technical success in your project of *Transgenic Plant Resistance to Invertebrate Pests* would not only be of interest to Pioneer, but when combined with leading germplasm containing a suite of important traits, would also have significant positive economic and societal impact throughout the world.

Pioneer is willing to provide financial support for the proposed project in the form of a matching fund of US$100,000 for two year, subject to: (1) your success in obtaining funding from the GIVF program; (2) execution of an exclusive commercial license to Pioneer for U.S. Patent 7,312,080; and (3) execution of a research collaboration agreement between Pioneer and the ISU Research Foundation. Pioneer wishes you success in obtaining funding from the GIVF program and in conducting a successful project.

Sincerely,

Robert T. Giaquinta
Director, Technology
Transfer and Licensing

GROW IOWA VALUES FUND GRANT COMPETITION
OLLEGE REVIEW FORM

PI Name: Bryony Bonning

Project Title: Transgenic Plant Resistance to Invertebrate Pests

College Ranking _6 of 6__

Recommendation: Fund ___ Fund if Possible __ Do Not Fund _X__

SECTION I: TECHNICAL MERIT (60% of recommendation)

4.5

Considerations:

- What is the scientific merit of the proposed project?
- Is the project technically feasible to accomplish in the listed time frame?
- Does the budget seem reasonable?
- Does the PI/team of researchers have the qualifications necessary to carry out the work?

Justification:

High scientific importance with a highly qualified team of researchers, but it is premature to seek GIVF because the concept needs to be proven and then the steps towards commercialization would be clear. Modest and appropriate budget for a 2-yr project.

SECTION II: BROADER IMPACTS (30% of recommendation)

3.5

Considerations:

- What is the probability that this project could be used to leverage future funding from non-ISU sources?
- What is the potential to increase ISU's research capabilities or capacity?
- What is the potential to enhance learning opportunities for students?

Justification:

Very strong research connection with the potential industry connection unclear. Clear leveraging of industry-sponsored research. Unclear connection with graduate students.

SECTION III: COMMERCIAL POTENTIAL (10% of recommendation)

2.5

Considerations:

- What is the likelihood that new intellectual property will be generated?
- What is the likelihood of eventual commercial success?

Justification:

Uncertain likelihood of new IP and eventual commercial success. Project still at the early to mid-research phase.

Proposal Number: FY10-9

PI Name: Bryony Bonning

Project Title: Transgenic Plant Resistance to Invertebrate Pests

College Ranking:

College Rating:

Commercial Recommendation: **Fund**

Project would seek to extend IP of GMO based insect resistance from Aphids to other insects

107,680, with only 8k of ISU cost share and $100k of cash (under contingency)

Commercial Potential:

1) Will this project lead to a new Iowa business/company, or increase the sales/profitability of a recently started Iowa company?

   No

   Please comment on the above:

2) Will this project increase the sales or profitability of an existing Iowa business/company ?

   Yes

   What is the probability of commercial success: High quality commercial partner (Pioneer) that is investing significant cash dramatically increases the probability.

3) Are competitors identified, is the advantage of the proposed technology clear.

   Yes

   Please comment on the above: Chief competitor is the use of pesticides, however, the proposed $10 increase in seed bag value is not compared to the cost of pesticide application across comparable acreage.

4) Is there a clear strategy for entry to the market, start up or existing Iowa business/company.

   Yes

   Please comment on the above: Pioneer Hybrid intends to commercialize but their willingness to contribute cash is contingent on exclusive licensing of background IP.

5) Please make any other comments related to the commercial potential of this proposal:

   Good market evaluation and demonstration of need. Excellent partner and cost-share. Is ISURF agreeable to the licensing?

Intellectual Property Evaluation

1. Have any current ISURF invention disclosures been identified as background IP?

Patent has been awarded.

Pioneer's funding is contingent on an exclusive license to background IP – which is available for licensing. Therefore, funding must be contingent on a successful license negotiation.

If Yes:

a) What is/are the ISURF number(s)?

b) What is the IP/patent status?

c) What is the licensing status?

2. Does this project have the potential to generate new intellectual property? Yes

   (please explain)

3. Based on your current knowledge, without having the opportunity to receive input from the principle investigator or to conduct a market or technology assessment, what will be the barriers to commercializing this technology?

   Reasonable opportunity for new IP.