PGA RESEARCH ARCHIVE

INSECT MANAGEMENT / CHEMICAL RESISTANCE

PGA
Est. 1966
POTATO GROWERS OF ALBERTA

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Agriculture and Agri-Food Canada
Research Branch

Agriculture et Agroalimentaire Canada
Direction générale de la recherche

Research Centre
P.O. Box 3000
Lethbridge, AB T1J 4B1

Telephone: (403) 327-4561
Facsimile: (403) 382-3156

December 15, 1999

Fund Centre SPA A01802

Mr. E. Van Dellen
Interim Manager/Technical Director
Potato Growers of Alberta
6008 - 46th Avenue
Taber, AB T1G 2B1

Dear Mr. Van Dellen:

Enclosed for your file is one fully executed copy of the Research Support Agreement between Her Majesty the Queen as represented by the Minister and Potato Development Inc., for Dr. Goettel's study entitled, "Development of microbial control of insecticide resistance management of the Colorado Potato Beetle".

We gratefully acknowledge receipt of your cheque for payment in the above-mentioned agreement.

We are pleased to be involved with you in this investigation.

Sincerely,

S. D. Morgan Jones, Ph.D.
Director

:wd
cc: M. S. Goettel
Finance

RESEARCH SUPPORT AGREEMENT

BETWEEN:

HER MAJESTY THE QUEEN IN RIGHT OF CANADA
as represented by the Minister of Agriculture and Agri-Food
("Canada")

AND:

Potato Development Inc., a company incorporated
under the laws of the Province of Alberta,
having its head office at 6008 - 46th Avenue, Taber, Alberta T1G 2B1
("the Company")

THE PARTIES HERETO COVENANT AND AGREE AS FOLLOWS:

1. The Project

Canada will conduct the research project entitled "Development of microbial control of insecticide resistance management of the Colorado Potato Beetle" ("the ***Project***"), described in detail in Appendix "A" hereto.

2. LOCATION AND DURATION

The ***Project*** will be carried out at Agriculture and Agri-Food Canada's Research Centre, Lethbridge, in the Province of Alberta, Canada, between the 1st day of January, 2000 and March 31, 2000.

3. CONTRIBUTION BY THE COMPANY

The Company's contribution for the ***Project*** shall comprise the items listed in Appendix "B" hereto and is estimated at CDN $5,300 dollars.

As part of this contribution, the Company agrees to provide Canada with a cash contribution in the amount of CDN $5,300 dollars, payable on signing to the Receiver General for Canada, to be deposited in a Specified Purpose Account and used by Canada to fund the items as listed in Appendix "B".

It is understood that upon termination of this ***Agreement*** any uncommitted funds (excluding any applicable interest) shall be returned to the Company. Upon expiration of this ***Agreement***, any unexpended funds shall vest in Canada unconditionally.

All goods and services purchased by Canada in connection with the ***Project*** with funds from the Company shall remain the property of Canada.

4. CONTRIBUTION BY CANADA

Subject to the availability of funding from the Matching Investment Initiative, Canada's contribution will not exceed the value of the cash plus in-kind contribution from the Company's contribution as shown in Appendix "B".

It is understood that Canada's contribution will be in kind and that no payments will be required to be made by Canada to the Company under this ***Agreement***.

5. REPORTS

Canada shall provide the Company with a copy of public reports arising from this ***Project***.

6. RELATIONSHIP

Nothing contained in this ***Agreement*** shall be considered or construed as creating a partnership or the relationship of principal and agent, lessor and lessee, licensor and licensee or of employer and employee between the parties.

7. INTELLECTUAL PROPERTY

All technical information, inventions, designs, methods and processes and other intellectual property rights related to the ***Project*** that are conceived, developed, or first reduced to practice in the carrying out of the ***Project*** (collectively, the ***"Intellectual Property"***) shall be the property of Her Majesty and, subject to the *Access to Information Act*, shall be treated as confidential.

8. TERMINATION

Canada may, by notice in writing to the Company, terminate this ***Agreement*** if it can no longer continue with the ***Project***, or if in Canada's opinion, the circumstances surrounding the ***Project*** have changed and are such that further support by Canada to the ***Project*** is not warranted.

9. NOTICE

Unless otherwise notified, the representative of the parties for the purpose of the ***Agreement*** shall be:

For Canada:
Dr. M. S. Goettel
Research Scientist
Agriculture and Agri-Food Canada
Lethbridge Research Centre
5403 1st Avenue South
Box 3000
Lethbridge, AB T1J 4B1
Telephone: (403) 317-2264
Facsimile: (403) 382-3156
Internet:goettel@em.agr.ca

For the Company:
E. Van Dellen
Interim Manager/Technical Director
Potato Growers of Alberta
6008 - 46th Avenue
Taber, AB T1G 2B1
Telephone: (403) 223-2262
Facsimile: (403) 223-268

10. ENTIRE AGREEMENT

This ***Agreement*** constitutes the entire agreement between the parties and sets forth all representations forming part of or in any way affecting or relating to this ***Agreement***. The parties acknowledge that there are no representations, either oral or written, between Canada and the Company, relating to this ***Agreement***, other than those expressly set out in this ***Agreement***.

11. GENERAL

a) This ***Agreement*** shall be governed, firstly, by applicable Canadian Federal laws, and secondly, by the laws of the Province of Alberta.

b) All amendments to this ***Agreement*** shall be in writing.

IN WITNESS WHEREOF this ***Agreement*** has been signed by duly authorized representatives of the parties.

Executed in duplicate this 3 day of December, 1999.

- For Her Majesty:

(Witness)

(Signature)
S.D. Morgan Jones, Ph.D., Director
Lethbridge Research Centre

- For Potato Development Inc.

Juanita Strange
(Witness)

(Signature)

ED VAN DELLEN
(Name in Block Letters)

TECHNICAL DIRECTOR
(Title)

AGRICULTURE AND AGRI-FOOD CANADA
Fund Centre SPA No. A01802

APPENDIX "A"

(to the Research Support Agreement)

DESCRIPTION OF RESEARCH PROJECT

Objective:
To determine the role that *Beauveria bassiana* could play in resistance management of chemical pesticides being used for control of the Colorado potato beetle.

This project addresses several key points. The immediate problem at hand is the development of resistance to chemical insecticides. Adoption of an alternative method in conjunction with the use of chemicals would defer development of resistance and provide the industry with a long term control method for this noxious pest. In the longer term, the fungus could be integrated into a more comprehensive IPM program for the beetle, eventually eliminating or reducing dependence on chemical pesticides. Reduction on the reliance to chemical pesticides will contribute to the sustainability and competitiveness of the potato industry in Canada.

This project will be the beginning of the development and implementation of a much more comprehensive IPM strategy for diseases and pathogens of potatoes. Results of these studies would also have direct applicability to development of IPM strategies for the Colorado potato beetle in eastern Canada. Other benefits of the proposed research include the possible feasibility of using this fungus for control of other soil-dwelling insect pests such as wireworms. It is hoped that it will result in more involvement and financial contributions from the industry in the near future.

Work Plan
Dec '99 - March, 2,000. Preliminary assays under soil conditions, Monitor field persistence, Laboratory Transmission studies. Carry out field-cage efficacy trials against larvae pupae and emerging adults. Monitor emergence of overwintered adults, Continue transmission studies, Carry out larger scale field trials. Conduct bioassays under different soil conditions.

This project is a part of the Lethbridge Research Centre study #387-2125-9706 entitled "Deveopment of new cultivars and sustainable disease and pest control strategies for the western Canadian potato industry". The principal investigator is Dr. M. Goettel.

APPENDIX "B"

(to the Research Support Agreement)

COMPANY'S CONTRIBUTION

Non-pay

NSERC PostDoc	$4,609
Materials & supplies	0
Administrative services	691
	$5,300

* Administrative costs will be deposited to a separate Specified Purpose Account reserved specifically for these expenditures.

Protected Business Information
Fund Centre SPA No. A01802

RESEARCH SUPPORT AGREEMENT

BETWEEN:

HER MAJESTY THE QUEEN IN RIGHT OF CANADA
as represented by the Minister of Agriculture and Agri-Food
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AND:

Potato Growers of Alberta, a company incorporated
under the laws of the Province of Alberta,
having its head office at 6008 - 46th Avenue, Taber, Alberta T1G 2B1
("the Company")

THE PARTIES HERETO COVENANT AND AGREE AS FOLLOWS:

1. **The Project**

Canada will conduct the research project entitled "Development of microbial control as a component of insecticide resistance management of the Colorado Potato Beetle" ("the ***Project***"), described in detail in Appendix "A" hereto.

2. **LOCATION AND DURATION**

The ***Project*** will be carried out at Agriculture and Agri-Food Canada's Research Centre, Lethbridge, in the Province of Alberta, Canada, between the 1st day of June, 2000 and March 31, 2001.

3. **CONTRIBUTION BY THE COMPANY**

The Company's contribution for the ***Project*** shall comprise the items listed in Appendix "B" hereto and is estimated at CDN $5,000 dollars.

As part of this contribution, the Company agrees to provide Canada with a cash contribution in the amount of CDN $5,000 dollars, payable on signing to the Receiver General for Canada, to be deposited in a Specified Purpose Account and used by Canada to fund the items as listed in Appendix "B".

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It is understood that Canada's contribution will be in kind and that no payments will be required to be made by Canada to the Company under this ***Agreement***.

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Canada shall provide the Company with a copy of public reports arising from this ***Project***.

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Canada may, by notice in writing to the Company, terminate this ***Agreement*** if it can no longer continue with the ***Project***, or if in Canada's opinion, the circumstances surrounding the ***Project*** have changed and are such that further support by Canada to the ***Project*** is not warranted.

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For Canada:
Dr. M. S. Goettel
Research Scientist
Agriculture and Agri-Food Canada
Lethbridge Research Centre
5403 1st Avenue South
Box 3000
Lethbridge, AB T1J 4B1
Telephone: (403) 317-2264
Facsimile: (403) 382-3156
Internet:goettel@em.agr.ca

For the Company:
E. Van Dellen
Interim Manager/Technical Director
Potato Growers of Alberta
6008 - 46th Avenue
Taber, AB T1G 2B1
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10. ENTIRE AGREEMENT

This ***Agreement*** constitutes the entire agreement between the parties and sets forth all representations forming part of or in any way affecting or relating to this ***Agreement***. The parties acknowledge that there are no representations, either oral or written, between Canada and the Company, relating to this ***Agreement***, other than those expressly set out in this ***Agreement***.

11. GENERAL

a) This ***Agreement*** shall be governed, firstly, by applicable Canadian Federal laws, and secondly, by the laws of the Province of Alberta.

b) All amendments to this ***Agreement*** shall be in writing.

IN WITNESS WHEREOF this ***Agreement*** has been signed by duly authorized representatives of the parties.

Executed in duplicate this 23 day of May, 2000.

- For Her Majesty:

(Witness)

(Signature)
S.D. Morgan Jones, Ph.D., Director
Lethbridge Research Centre

- For Potato Growers of Alberta.

(Witness)

(Signature)

ED VAN DELLEN
(Name in Block Letters)

TECHNICAL DIRECTOR
(Title)

AGRICULTURE AND AGRI-FOOD CANADA
Fund Centre SPA No. A01802

APPENDIX "A"

(to the Research Support Agreement)

DESCRIPTION OF RESEARCH PROJECT

Objectives

The Colorado potato beetle has developed resistance to almost all the insecticide classes used against it to date. It has become necessary to develop an insecticide resistance management program to help prolong the effectiveness of the only registered chemicals still effective against this pest and to allow producers to deal with the tremendous economic loss that this insect causes.

Our **objective** is to determine the effectiveness of an insect pathogenic fungus, *Beauveria bassiana,* in reducing beetle populations and to evaluate its use in an insecticide resistance management program. Implementation of such an alternative control method in the management of the Colorado potato beetle will cause minimal environmental damage, delay development of resistance to chemicals thus prolonging their efficacy, decrease dependency on use of chemical insecticides and eventually lead to an IPM strategy against this pest in Canada.

Research Plan

Hypothesis: Application of *Beauveria bassiana* to the soil at overwintering sites, or to adults as they emigrate from the fields will provide significant, longer term mortality to the overwintering populations, thereby contributing to overall population reductions and insecticide resistance management of the Colorado potato beetle.

Host- pathogen relationships and the effects of soil factors on fungal persistence and infectivity must be determined before larger scale field trials are initiated. The experiments are designed to obtain information on 1) the relationships between fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions,

4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions. Unless otherwise noted, all experiments will be arranged as complete randomised block designs with at least 3 replications of 50 beetles per treatment and control. Every experiment will be repeated and all field plot designs and statistical analyses will be performed in consultation with statisticians at the Lethbridge Research Centre.

Action Plan and Work Schedules

a) First year: 2000/2001

Virulence of the fungus will be determined. How many spores must the beetles contact in order to get infected? Persistence of the fungus under field conditions will be determined. Will enough spores remain viable from year to year to ensure continued efficacy? Will beetles be contaminated with an adequate amount of inoculum as they bury in the soil which contains spores?

This project is a part of the Lethbridge Research Centre study #387-2125-9706 entitled "Deveopment of new cultivars and sustainable disease and pest control strategies for the western Canadian potato industry". The principal investigator is Dr. M. Goettel.

APPENDIX "B"

(to the Research Support Agreement)

COMPANY'S CONTRIBUTION

Manpower	
COOP Student	
Connie Mayer (0.17FTE)	$3,650
FSWEP student (0.02 FTE)	450
Material/Supplies	250
Sub-Total	4,350
*Administrative Services	650
TOTAL	$5,000

* Administrative costs will be deposited to a separate Specified Purpose Account reserved specifically for these expenditures.

POTATO DEVELOPMENT, INC.

FUNDING APPLICATION

Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle

Submitted by

Mark S. Goettel
Lethbridge Research Centre

7 February 2000

Note to applicants:

Applicants who receive funding from PDI must get approval from the PDI chairman before reporting any findings.

POTATO DEVELOPMENT, INC.
FUNDING APPLICATION- SUMMARY PAGE

PROJECT TITLE: Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle

REASON FOR PROJECT (Objectives of project) (75 words.) Brief description of what this project is attempting to find out or accomplish.

In many parts of the world including most of eastern Canada, and most recently in Manitoba, the Colorado potato beetle has developed resistance to insecticides in almost all of the insecticide classes. Evidence has surfaced that resistance may also be developing in Alberta. With the increase in acreage and decrease in rotation intervals, it has become necessary to develop an insecticide resistance management program to help prolong the efficacy of the chemicals that are still effective against this pest and to allow producers to deal with the tremendous economic loss that this insect can cause in the absence of effective chemicals. The **objective** of the proposed study is to determine the role that *Beauveria bassiana* could play in suppression of beetle populations and especially in resistance management of chemical pesticides being used for control of the Colorado potato beetle.

PROJECT PLAN (What is going to be done - 50 words)

The experiments are designed to obtain information on 1) the relationships between fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions, 4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions.

BENEFITS TO ALBERTA'S POTATO INDUSTRY (50 words.)

This project addresses several key points. The immediate problem at hand is the development of resistance to chemical insecticides. Adoption of an alternative method in conjunction with the use of chemicals would defer development of resistance and provide the industry with a long term control method for this noxious pest. In the longer term, the fungus could be integrated into a more comprehensive IPM program for the beetle, eventually eliminating or reducing dependence on chemical pesticides. Reduction on the reliance to chemical pesticides will contribute to the sustainability and competitiveness of the potato industry in Alberta.

DURATION OF PROJECT. The project will start April, 2000 and run until March, 2003

FINANCIAL INFORMATION

	This year only	Total all years
Project cost	119,010	398,255
Amount requested from PDI	5,000	15,000
Amount from other sources	114,010	383,255

PRINCIPAL APPLICANT INFORMATION

Principal applicant's name	Phone
Mark Goettel	317-2264

Research agency or company

Lethbridge Research Centre

Mailing Address	Postal code
P.O. Box 3000 T1J 4B1	

Location of research project.(Research farm name or legal location.)

Vauxhall Research Substation and Lethbridge Research Centre

3C) PROJECT CONTINGENCIES

a) If you do not get grant monies from sources can this project be conducted as submitted?

Yes ____ No ____ Yes, with changes __X__

b) Modifications necessary: Amount of research will be severely curtailed. AAFC resources do not include a post-doctoral fellow nor summer technical help, both very important aspects of the proposed programme. Instead of the 3 years proposed, this research could take as long as 10 years without outside funding. Priorities (i.e. Alberta or Ontario) will be determined depending on funding sources. If PDI is the only source of industry funding, it will be matched either through AARI or MII and used to hire summer technical help.

BACKGROUND, OBJECTIVES, AND PLAN (Maximum of 3 pages for items 5A - 5D.)

5A) Background to the Proposed Project

The most important insect pest problem faced by potato producers is the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). This insect is considered the most destructive foliage feeding pest of potatoes in the world. Adults of this beetle emerge in the spring from diapause and begin feeding and laying eggs on young and newly emerged potato plants. The eggs hatch within one week and the larvae feed on the potato plant and pass through four instars. Once the fourth instar has completed development, it drops off and pupates in the soil at the base of the plant. After approximately 10 days, adults emerge and begin feeding on the plants in preparation for diapause. Later, these adults will either fly or walk out of the field, find a suitable location where they will dig into the soil to overwinter (Noronha and Cloutier 1999). In certain parts of the country, such as southern Ontario and Quebec, the insect undergoes 2 generations per season. Because both the adults and larvae feed on the same plants, they can cause extensive plant damage. If left uncontrolled they can destroy a producers crop. This beetle is known to attack not only potatoes but also tomato, eggplant and other solanaceous plants.

The Colorado potato beetle was first recorded in Canada in 1867 from Ontario. It is now present virtually everywhere potatoes are grown in Canada. Until recently, potato producers have relied solely on the use of insecticides to control this pest. However, this total dependence on insecticides has resulted in the rapid development of resistance. The first reports of Colorado potato beetle resistance in Canada were to organochlorine insecticides (Harris & Svec, 1979; McClanahan, 1975; McDonald, 1976). By 1981, populations showing resistance to organophosphates and carbamates were found in the province of Quebec (Harris & Svec, 1979). In 1979, most populations tested in Ontario were susceptible to pyrethroids, but by 1982 a 22-37 fold resistance was reported just two years after this class of insecticides started being used (Harris & Turnbull, 1986). In New Brunswick, an increase in population by 70% between 1974 and 1984 was attributed to the development of insecticide resistance in beetle populations (Boiteau et al., 1987). In the mid-90s, in some potato producing areas, control of the Colorado potato beetle had reached a critical point, with nearly all of the previously effective insecticides no longer able to reduce beetle populations and new insecticides losing their effectiveness within a few years because of cross resistance (Boiteau et al., 1987; Hilton et al., 1998; Stewart et al., 1997; Turnbull et al., 1988). This was especially true in southern Ontario where there are two and maybe three generations a year. A survey conducted in Ontario found populations that were resistant to all registered insecticides (Turnbull et al. 1988).

By 1995, beetle populations in Ontario and Québec were virtually uncontrollable which resulted in the emergency registration of a new insecticide, imidacloprid (Admire®). Although this product is 10 times more costly for a grower than previously used chemicals, the growers have had little choice but to use this, the only economically viable control method. Furthermore, there have already been reports of populations resistant to imidacloprid in the US (Grafius, 1999), and insecticide resistance will develop rapidly in a situation where only one chemical is used exclusively for control of this pest.

Until recently, in western Canada, potato beetle populations have been kept under control exclusively with the use of insecticides. However, in the last few years, problems began arising in Manitoba (Gavloski, 1997). In a recent survey conducted in western Canada, we found 90% of the populations tested from Manitoba were totally resistant to either one or more of the four insecticide classes (Noronha & Goettel, 1999). In Saskatchewan and Alberta, populations showing high levels of resistance to pyrethroids were found. Thus, if pyrethroids continue to be used, these populations will become totally resistant to this class of insecticide. There were also indications of low levels of resistance to organophosphates in Alberta. The situation in Manitoba has led to the registration of imidacloprid for use in that province. Consequently, although presently producers in eastern Canada and Manitoba have a reprieve, the beetle remains a major threat to the potato industry as development of insecticide resistant populations to this chemical is imminent, unless insecticide management strategies are immediately adopted.

In order to prolong the effective use of chemical methods for the economic control of the Colorado potato beetle, integrated pest management programs (IPM) must be implemented (Roush & Tingey, 1994). Several new products have become available for control of the Colorado potato beetle (i.e., imidacloprid and Bt transgenic potatoes), however, in order to delay the development of resistance to these new control agents, a resistance management strategy will also be needed. Several alternative strategies for control of the Colorado potato beetle are being developed (Cloutier et al., 1995). Some of the methods being implemented include propane flamers, plastic lined trenches, use of transgenic (Bt) cultivar borders, straw mulch, trap crops and crop rotation. Suppression of overwintering adults would provide another means for pest population management.

The Ontario Ministry of Agriculture vegetable production publication recommends: producers scout their fields and spray only when populations warrant by using the economic threshold of 14-24 overwintered adults per 50 plants or stems; use propane flamers (Weisz et al. 1994), plastic lined trenches (Boiteau et al., 1994), and trap crops (Hunt and Whitfield, 1996), which have been effective in reducing the population. However, often in Ontario the overwintering populations are especially large (Hilton et al., 1998) and threaten emerging potatoes and young tomato plants. Since a single female can lay between 350 and 400 eggs, it does not take long for a population to build up. Thus, control strategies implemented against the overwintering population would be beneficial. If we continue to have mild winters, survival within the Colorado potato beetle overwintering populations is expected to increase. This in turn will result in an increase in the number if spray applications needed to control this insect. Because of its adaptability and history of insecticide resistance a more balanced, sustainable and environmentally friendly approach to control the Colorado potato beetle is needed.

The entomopathogenic fungus, *Beauveria bassiana,* is pathogenic to the Colorado potato beetle and is capable of decimating Colorado potato beetle populations (Allee et al., 1990; Gaugler et al.,1989). It has recently been registered in the United States against a variety of pests, including the Colorado potato beetle. Foliar application for control of the Colorado potato beetle can be effective (Lacey et al., 1999, Poprawski et al., 1997) but the fungus is short lived when exposed to the sunlight on the leaf surface

(Hajek et al., 1987; Inglis et al., 1993). However, conidia persist much longer in the soil (Inglis et al., 1997) and a novel approach for its use against the Colorado potato beetle would be incorporation of conidia into the soil to target larvae pupating in the soil and adults as they emerge from the soil. Alternatively, since the fungal spores can germinate at temperatures as low as 2°C and in many areas adult beetles migrate out of the fields to high density overwintering sites (Noronha & Cloutier, 1999), treatment of the migrating adults or of the overwintering sites may be a method of contaminating the overwintering sites resulting in the long-term decimation of overwintering populations. Implementation of such an alternative control method in the management of the Colorado potato beetle would cause minimal environmental damage, decrease the possibility or delay development of resistance to chemicals and eventually lead to the implementation of an IPM strategy against this pest, thus reducing overall use of chemical insecticides.

In order to meet the increased demand for potatoes in southern Alberta, the next few years will require a rapid expansion of potato acreage and a reduction of rotations from 5 to 3 years. This will provide conditions that will increase the risk posed by the Colorado potato beetle. Reduced rotations and large acreages are ideal conditions that favour beetle outbreaks. Widespread use of chemical applications to stem such outbreaks will increase the likelihood that resistant beetle populations will be quickly selected, as has occurred in eastern Canada and the U.S.. **It is imperative that insecticide resistance management practices be implemented as soon as possible, to ensure the sustainability of potato production in southern Alberta.**

b) Objectives

The Colorado potato beetle has developed resistance to almost all the insecticide classes used against it to date. It has become necessary to develop an insecticide resistance management program to help prolong the effectiveness of the only registered chemicals still effective against this pest and to allow producers to deal with the tremendous economic loss that this insect causes.

Our **objective** is to determine the effectiveness of an insect pathogenic fungus, *Beauveria bassiana,* in reducing beetle populations and to evaluate its use in an insecticide resistance management program. Implementation of such an alternative control method in the management of the Colorado potato beetle will cause minimal environmental damage, delay development of resistance to chemicals thus prolonging their efficacy, decrease dependency on use of chemical insecticides and eventually lead to an IPM strategy against this pest in Canada.

5C) Research Plan

Hypothesis: Application of *Beauveria bassiana* to the soil at overwintering sites, or to adults as they emigrate from the fields will provide significant, longer term mortality to the overwintering populations, thereby contributing to overall population reductions and insecticide resistance management of the Colorado potato beetle.

Host- pathogen relationships and the effects of soil factors on fungal persistence and infectivity must be determined before larger scale field trials are initiated. The experiments are designed to obtain information on 1) the relationships between fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions, 4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions. Unless otherwise noted, all experiments will be arranged as complete randomised block designs with at least 3 replications of 50 beetles per treatment and control. Every experiment will be repeated and all field plot designs and statistical analyses will be performed in consultation with statisticians at the Lethbridge Research Centre.

5D) Action Plan and Work Schedules

a) First year: 2000/2001

Virulence of the fungus will be determined. How many spores must the beetles contact in order to get infected? Persistence of the fungus under field conditions will be determined. Will enough spores remain viable from year to year to ensure continued efficacy? Will beetles be contaminated with an adequate amount of inoculum as they bury in the soil which contains spores?

b) Second year: 2001/2002

How many of the inoculated adults that diapaused under natural conditions became infected and died before emergence in the Spring? How many spores persisted over the season? Should beetles be inoculated directly, or can they pick up an adequate amount of inoculum from the soil? How do soil conditions affect spore persistence and virulence?

b) Third year: 2002/2003

Do adults that succumbed in the soil produce adequate numbers of spores to ensure infection of the next generation? Should spores be reapplied yearly? Should we proceed with registration?

Final report and manuscripts will be prepared.

5E) **RELATED RESEARCH** (Literature review - Maximum of 2 pages.)

a) At your institution

The Lethbridge Research Station holds the mandate for potato research serving western Canada. Programs include breeding, genetic enhancement, pathology and pest management. This proposal will be undertaken under the pest management theme.

In addition to use of the fungus for management of the Colorado potato beetle, *Beauveria bassiana* has been evaluated as a potential microbial control agent of grasshoppers. It was found that the spores of the fungus were relatively short-lived on leaf surfaces, but long-lived in the soil. Contaminated grasshoppers exhibited a behavioural fever, whereby they basked in the sun and rose their body temperature to 42^{0}C. Through this behaviour, they were able to eliminate infection.

Newer initiatives at LRC. In collaboration with Dr. Hector Carcamo, we are investigating the role that *Beauveria bassiana* may play in the management of the cabbage seedpod weevil, a new threat to the canola industry. Initial studies have found weevils overwintering in hedgerows succumbing to the fungus.

This raises interesting possibilities of developing a management strategy using this fungus that could benefit both canola and potato industries.

b) At other institutions

Other AAFC Centres:

Charlottetown, Prince Edward Island. Dr. Jeff Stewart has been conducting research on insecticide resistance management of Bt transgenic potato.

Fredericton, New Brunswick. Dr. Gilles Boiteau has been developing IPM of the Colorado potato beetle. He has also evaluated *Beauveria bassiana* as a foliar spray.

Harrow, Ontario. Dr. David Hunt has been studying control of the Colorado potato beetle as a pest of tomatoes in southern Ontario. He has demonstrated that planting trap crops is helpful. We are trying to team up with Dr. Hunt so that we can develop a collaborative effort between Ontario and Albertan producers and researchers to maximize limited resources and chances of success.

United States:

Weslaco, Texas & Ithaca, NY. Drs. Tad Poprawski and Steven Wraight, USDA/ARS are evaluating *Beauveria bassiana* as a foliar application against the beetle. Although they have obtained significant reductions in beetle numbers, we feel that this method is presently not economically feasible for Alberta, because effective low cost chemicals are still available

Orono, Maine. Dr. Ellie Groden, University of Maine is conducting extensive studies on IPM of the beetle. In her studies with *Beauveria bassiana*, she found that control increased with each subsequent yearly application of the fungus. One hypothesis is that this could be the result of contamination of overwintering sites.

c) References. (List references cited in the above literature review.)

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6) BENEFITS OF PROJECT

What are the potential economic, marketing, and quality benefits to Alberta's potato producers and to Alberta's potato industry in general. Include economic analysis of increased production potential or changed management as a result of information found in this study.

a) To Alberta's potato producers.

This project addresses several key points. The immediate problem at hand is the development of resistance to chemical insecticides. On average, resistance to a newly registered insecticide for control of the Colorado potato beetle appears within 3.5 years. Adoption of an alternative control method in conjunction with the use of chemicals would defer development of resistance and provide the industry with a long term control method for this noxious pest. The only alternative to conventional chemicals, Admire, costs tens times more than the chemicals presently used. Reduced markets for transgenics, makes this alternate strategy not reliable in the short term at the very least, due to public concerns over transgenics. In the longer term, the fungus could be integrated into a more comprehensive IPM program for the beetle, eventually eliminating or reducing dependence on chemical pesticides. There is no guarantee that the newly registered pesticides now entering the market will stand the test of time as far as safety and long term sustainability is concerned. Exclusive reliance on any one method of control, as is presently the case in eastern Canada, be it chemical pesticides or transgenic potato, will eventually result in the development of resistance. Reduction on the reliance to chemical pesticides will contribute to the sustainability and competitiveness of the potato industry in Canada.

b) To Alberta's potato industry.

Availability of potatoes is imperative for the continuation of the potato industry in southern Alberta. With the public outcry over transgenic potatoes, and industry acceptance to give in to these public concerns, it will become imperative to adopt IPM for control of not only the beetle, but other pests and diseases. Once concerns over transgenics subside, it may be very possible that the next target may be chemical insecticides. Alberta's potato industry has everything to gain by adopting as many chemical-free management practices as possible and will be in a very enviable position, should public acceptance of pesticide-treated products take a sudden nose-dive.

7) BUDGET AND MANPOWER NEEDS *FOR 1 YEAR*[1]

7A) MANPOWER TO BE HIRED WITH PDI/OTHER FUNDS

NAME (If known)	POSITION	TIME REQUIRED	RATE OF PAY	AMOUNT REQUIRED
Professional and Technical manpower				
Dr. C. Noronha	Post-Doctoral	1 PY		38800
Technical	EG-02	1 PY		40280
Casual manpower				
Summer student	University Student	0.3 PY		8000
Summer students	Secondary students	0.6 PY		10000
		TOTAL LABOUR COSTS		**97,080A**

7B) TRAVEL EXPENSES TO BE PAID WITH PDI/OTHER FUNDS FOR 1 YEAR[1]

DESTINATION	PERSON(S)	PURPOSE	NUMBER OF TRIPS	TRAVEL COSTS	MEALS AND ACCOM.	TOTAL COST
Annual Growers meetings	MSG CN DH	Convey results, updates	2	2000	1000	3000
Ontario/Alberta	MSG DH	Visit test sites in Ontario/ Alberta	1	750	500	1250
				TOTAL TRAVEL COSTS		**4,500B**

[1] The budget presented above represents our attempt to undertake a collaborative programme with Dr. D.W.A. Hunt, at AAFC's Harrow Research Centre with joint funding from the the Alberta Agricultural Research Institute (AARI), AAFC Matching Investment Initiative (MII), Stuart Cairns Memorial Potato Research Fund (for fiscal years 2000/2001 -), Ontario Department of Agriculture (OMAFRA), the Ontario Potato Board (OPB) and Potato Growers of Alberta through PDI.. Such a larger study would provide us with the resources that can be shared to address both Ontario and Alberta's needs. If funding from all sources is not available, the programme will be scaled down accordingly, with researchers from Alberta and Ontario separately addressing their priorities and commitments to their respective regions accordingly.

7C) MATERIALS, SUPPLIES AND SERVICES TO BE PAID WITH PDI/OTHER FUNDS

DESCRIPTION	COST
Disposable labware, microbiological media	1800
Cages, traps, bioassay chambers	2205
Greenhouse/field services; publication costs	3200
TOTAL COST OF MATERIALS, SUPPLIES AND SERVICES FOR 1 YEAR	**7,205C**

7D) OTHER EXPENSES TO BE PAID WITH PDI/OTHER FUNDS FOR 1 YEAR

DESCRIPTION	AMOUNT
AAFC overhead .	10730
Other	
TOTAL OTHER EXPENSES	**10,730D**

7E) SUMMARY OF FUNDS REQUIRED FROM PDI AND OTHER SOURCES FOR 1 YEAR

DESCRIPTION	COST
Professional, technical, and casual labour	**97,080A**
Travel and accommodation	**4,250B**
Materials, supplies and services	**6,950C**
Other expenses	**10,730D**
TOTAL COSTS FOR WHICH FUNDING IS REQUESTED FROM ALL FUNDING SOURCES (A+B+C+D)	**119,010**

7F) FUNDING SOURCE SUMMARY FOR 1 YEAR

FUNDING SOURCE	AMOUNT
Amount requested from PDI in this application	5000
Other OMAFRA)(Ontario Department of Agriculture)	26000
other AARI (Alberta Agricultural Research Institute Direct funding)	47000
Other AARI Matching of OMAFRA	26000
Other OPB (Ontario Potato Board)	5000
Other AAFC MII (Agriculture Canada Matching of OPB and PDI	10000
TOTAL FUNDS APPLIED FOR (EQUAL TO E, ABOVE)	**119,000**

7G) VALUE OF "IN KIND" CONTRIBUTIONS BY RESEARCH AGENCY FOR 1 YEAR
Include estimated value of research staff time and operating budgets contributed by principal researcher's agency, or other cooperator's agency, towards this project in the period covered by this application. (Funding is not requested for these items.)

DESCRIPTION	PERSON YEARS	APPROX. VALUE
Professional, technical, and other staff		70,000
Materials and supplies		125,000
Travel		4,000
Overhead (estimate)		30,000
	TOTAL VALUE "IN-KIND" COSTS	**229,000F**

ESTIMATED TOTAL PROJECT COST FOR 1 YEAR

ESTIMATED TOTAL COST OF PROJECT (1 YEAR) E & F	348,000

8) APPROVAL BY PRINCIPAL APPLICANT'S EMPLOYER

The undersigned declare the approval and support of their organization for the research project as describe in this application. Signatures confirm that space and basic facilities for carrying out the proposed research are available for use and that the applicant is authorized to participate in this research project.

Peter Burnett	P A Burnett	Acting Director	Feb 10, 2000
Name	Signature	Position	Date

9) TERMS AND CONDITIONS

The applicant(s) agree that, upon acceptance of funding, a commitment is made to:

a) Conduct the research as laid out in the proposal, excepting changes mutually agreed upon by the applicant(s) and the Executive of the Alberta Potato Research Association.

b) Allow the Alberta Potato Research Association to use all information, data and results generated as a result of the research for extension purposes.

c) ***Not publish or present any data from this study without the written permission of the Chairman of the PDI.***

Mark Goettel

Principal Cooperator Signature

Feb. 11, 2000

Date

PDI Executive Committee Signature

Date

Agriculture and Agri-Food Canada
Research Branch

Agriculture et Agroalimentaire Canada
Direction générale de la recherche

Research Centre
P.O. Box 3000
Lethbridge, AB T1J 4B1

Telephone: (403) 327-4561
Facsimile: (403) 382-3156

April 25, 2001

RECEIVED MAY 0 2 2001
Paid ✓

Fund Centre SPA A01802

Mr. V. Warkentin
Potato Growers of Alberta
6008 - 46th Avenue
Taber, AB T1G 2B1

Dear Vern:

Enclosed are two original signed copies of the first Amendment to the Research Support Agreement signed May 23, 2000 between the Minister of Agriculture and Agri-Food for Canada and Potato Growers of Alberta for Dr. Goettel's study entitled, "Development of microbial control for insecticide resistance management of the Colorado Potato Beetle".

These amendments are as follows:

1. The Project will be expanded to cover the second year of the action plan as described in Appendix A (attached). The Company will contribute an additional $5,000 for the Project, to be made payable to the Receiver General for Canada, deposited into Specified Purpose Account No. A01802, and used as detailed in Appendix B (attached).

2. The expiration date of the Agreement is extended to March 31, 2002.

In all other respects the Research Support Agreement remains unchanged and in full force and effect.

If you agree with these amendments, please have the appropriate Company authority sign both copies of this letter in blue ink in the space below, keep one original for your records, and return the other to us for our files, together with your cheque for $5,000 CDN made payable to the Receiver General for Canada.

We are pleased to be involved with PGA in this project.

Appendix A
(to the Research Support Agreement)
Amendment #1
Project Description

Objectives

The Colorado potato beetle has developed resistance to almost all the insecticide classes used against it to date. It has become necessary to develop an insecticide resistance management program to help prolong the effectiveness of the only registered chemicals still effective against this pest and to allow producers to deal with the tremendous economic loss that this insect causes.

Our **objective** is to determine the effectiveness of an insect pathogenic fungus, *Beauveria bassiana,* in reducing beetle populations and to evaluate its use in an insecticide resistance management program. Implementation of such an alternative control method in the management of the Colorado potato beetle will cause minimal environmental damage, delay development of resistance to chemicals thus prolonging their efficacy, decrease dependency on use of chemical insecticides and eventually lead to an IPM strategy against this pest in Canada.

Research Plan

Hypothesis: Application of *Beauveria bassiana* to the soil at overwintering sites, or to adults as they emigrate from the fields will provide significant, longer term mortality to the overwintering populations, thereby contributing to overall population reductions and insecticide resistance management of the Colorado potato beetle.

Host- pathogen relationships and the effects of soil factors on fungal persistence and infectivity must be determined before larger scale field trials are initiated. The experiments are designed to obtain information on 1) the relationships between fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions, 4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions. Unless otherwise noted, all experiments will be arranged as complete randomised block designs with at least 3 replications of 50 beetles per treatment and control. Every experiment will be repeated and all field plot designs and statistical analyses will be performed in consultation with statisticians at the Lethbridge Research Centre.

Action Plan and Work Schedules

b) Second year: 2000/2001

How many of the inoculated adults that diapaused under natural conditions became infected and died before emergence in the spring? How many spores persisted over the season? Should beetles be inoculated directly, or can they pick up an adequate amount of inoculum from the soil? How do soil conditions affect spore persistence and virulence?

Appendix B
(to the Research Support Agreement)
Amendment #1
COMPANY'S CONTRIBUTION

	2001-02
Manpower	
Student (FSWEP)	
Loa Barendregt (0.18FTE)	$4,100
Material/Supplies	250
Sub-Total	4,350
*Administrative Services	650
TOTAL	$5,000

* Administrative costs will be deposited to a separate Specified Purpose Account reserved specifically for these expenditures.

Yours truly,

S.D. Morgan Jones, Ph.D.
Director

:wd
Encl.
c.c. J.G. Stewart, Head, Crops Sciences
M.S. Goettel, Research Scientist
Finance

Accepted and agreed to by
Potato Growers of Alberta

(signature)

May 3/01
Date

Appendix A
(to the Research Support Agreement)
Amendment #1
Project Description

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Agriculture and Agri-Food Canada
Research Branch

Agriculture et Agroalimentaire Canada
Direction générale de la recherche

Lethbridge Research Centre
5403 - 1st Ave. S.
P.O. Box 3000
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T1J 4B1
Tel.(general) 403-327-4561
Tel (direct): 403-317-2264
Fax.: 403-382-3156
Email: goettel@em.agr.ca

August 21, 2001

Mr. Vern Warkentin
Potato Growers of Alberta
6008 - 46th Avenue
Taber, AB
T1G 2B1

Return to Vern

Dear Vern

RE: Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle

For your perusal, enclosed please find a draft of a manuscript entitled "Damage potential and phenology of the Colorado potato beetle [Coleoptera: Chrysomelidae] in southern Alberta" which I plan to submit to the journal "Phytoprotection." I also plan to rewrite a shortened version for publication in "The Common Tater" in due course.

I would appreciate any comments you may wish to make on this paper before 6 September if at all possible.

This study was partially funded by PGA and this funding is greatly appreciated.

Sincerely,

Mark

Mark Goettel
Research Scientist
Insect Pathology

CC. Christine Noronha

RECEIVED AUG 2 3 2001

Canada

Present Version
21/08/01

Damage Potential and Phenology of the Colorado Potato Beetle [Coleoptera: Chrysomelidae] in Southern Alberta.

Christine Noronha,[1,2] Grant M. Duke[1] and Mark S. Goettel[1,3]

[1] Lethbridge Research Centre, Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta T1J 4B1

[2] Present Address: Crops and Livestock Research Centre, Agriculture & Agri-Food Canada, P.O. Box 1210, Charlottetown, Prince Edward Island (E-mail: NoronhaC@em.agr.ca)

[3] To whom correspondence should be addressed; e-mail: goettel@em.agr.ca

Running Title: Colorado potato beetle in Alberta

The phenology and damage potential of the Colorado potato beetle, *Leptinotarsa decemlineata* was studied in the heart of the potato producing area in southern Alberta. Experimental plots were established at two locations. At each site, one plot was protected against the beetle through application of insecticides while the other was "unprotected." Natural potato beetle populations quickly colonized unprotected plots in all years. Overwintered adults began colonizing the plots by mid June with mean densities reaching between 0.27 and 0.65 per plant. Eggs were laid on young emerging plants with mean densities reaching 2.14 egg batches per plant by late June. Maximum larval densities per plant reached 9.5 for 1^{st}, 2^{nd} and 3^{rd} instars and 13.6 for 4^{th} instars. Maximum density for newly emerged adults was 57 per plant recorded at the 2000 Lethbridge unprotected plot. Defoliation was very low at the beginning of the season but sharp increases were noted when 3^{rd} and 4^{th} instar populations peaked and continued to rise when new adults emerged. Maximum defoliation occurred at the Lethbridge plot in 2000 with 100% defoliation by 10 August. Total yields in all unprotected plots ranged from 10 to 40% lower than in the protected plots. Densities of overwintering adults averaged 76 per m^2 with a maximum of 232 per m^2. Overwintering mortality averaged 22% and the mean depth of beetles was 12 cm, with 63% of the beetles at depths ≤ 10 cm. Our results indicate that the phenology of the beetle is similar to areas where population buildups were rapid and devastating soon after insecticide resistant populations appeared. Consequently the beetle must be considered as a serious threat to potato production in southern Alberta.

INTRODUCTION

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) [Coleoptera: Chrysomelidae], is the most destructive foliage feeding pest of potatoes in North America (Hare 1990). In Canada and the northern part of the United States, overwintered adults emerge from the soil in the spring and either walk or fly in search of food plants and oviposition sites. Eggs are deposited on foliage of newly emerging plants in late spring. Larvae emerge and pass through four instars that feed voraciously on potato foliage. When ready, larvae burrow into the soil around the base of the plant and pupate. Approximately 10-15 days later, adults of the new generation emerge and begin feeding on the maturing potato plants. At this time the adults do not lay eggs, but feed mainly to store reserves in preparation for diapause (Dortland and Kort 1978). When ready to diapause, beetles either enter the soil within potato fields, or move out by walking or flying in search of peripheral overwintering sites offering potentially more protection such as forest borders and drainage ditches (Noronha and Cloutier 1998, 1999; Voss and Ferro 1990; Weber and Ferro 1993).

The CPB can cause heavy economic loss if left uncontrolled (Cranshaw and Radcliffe 1980; Ferro *et al.* 1983; Hare 1980). Severe defoliation by beetles can not only result in total yield loss but may also decrease the quality of potatoes (Senanayake and Holliday 1990; Shields and Wyman 1984). Insecticides have been used extensively to control the Colorado potato beetle, but their long term efficacy is threatened by multiple resistance (Roush et al. 1990). Widespread economic losses become apparent only when insecticide control measures begin to fail. In eastern Canada, loss of control efficacy due to resistance to all four chemical classes resulted in the emergency registration of imidacloprid, an insecticide within a new class. In western Canada, there have been recent reports of insecticide resistance from Manitoba (Gavloski 1997; Noronha *et al.* in press). In Alberta, CPB populations are still susceptible to conventional insecticides (Noronha *et al.* in press), making the cost of CPB control more economical as compared to areas that rely on the more expensive, newer chemical insecticides.

Potato agroecosystems and production practices differ between regions. Factors, such as the crops used in a rotation, distance of rotation, irrigation and cultivation influence the colonization and successful development of the beetle (Hough-Goldstein and Whalen 1996). There is also growing evidence differences exist between beetle populations from different regions (Senanayake *et al.* 2000). Potatoes in southern Alberta are grown principally for french fry and chipping under irrigation on sandy loam soil. Rotations are typically with cereals and sugar beets with potatoes grown every fourth year. The purpose of this study was to gain a better understanding of the phenology and damage potential of the CPB in the heart of the potato producing area in southern Alberta.

MATERIALS AND METHODS

Experimental plots were established at two different locations in southern Alberta; the Lethbridge Research Centre, approx 50 km from major potato producers and the Lethbridge Research Centre's Vauxhall Substation, which is in the heart of the potato growing region of southern Alberta. At each site, Russet Burbank potatoes were planted in two 10 x 10 m plots with 10 rows of 30 plants per row, with a 5 m buffer zone between plots. At each site one plot was designated as the "protected" plot and the other as an "unprotected" plot. In the protected plots, the seed was treated at planting with Thimet ™ (phorate), a systemic organophosphate insecticide, at a rate of 45.6 kg/ha. CPB infestations were subsequently controlled as required within these protected plots with foliar applications of an organophosphate, Monitor ™ (methamidophos) and a pyrethroid, Ambush ™ (permethrin) at rates of 3 L/ha and 172 ml/ha respectively. The other plots (unprotected) received no insecticide treatment.

At both sites, plots were established and monitored in 1998 and 1999; the Lethbridge plot was established and monitored for a third year in 2000. In 1998, 2 unprotected plots were established in Lethbridge: Plot A was planted 24 April and plot B and all other plots at Vauxhall were planted on May 7. In 1999, all plots were planted on 7 May, and in 2000, the plots at Lethbridge were planted on 27 April. In all years, a natural beetle population was allowed to colonize the unprotected plots.

Sampling Procedure Each plot was divided into three blocks consisting of 10 plants per row x 10 rows. Sampling for beetles began as soon as the plants emerged and adults were visible.

Twice weekly, two randomly selected plants per row per block were examined to estimate CPB population density and defoliation. The number of eggs, first, second, third and fourth instar larvae and adults were counted and the extent of defoliation noted for each sample plant from mid June to mid August. A defoliation index (**1**<10%; **2**. 11-25%; **3**. 26-39%; **4**. 40-50%; **5**. 50%; **6**. 51-74%; **7**.> 75%; **8**. 100%) was established to classify the extent of damage per plant over the summer (Boiteau 1994).

Yield At the end of August, potatoes from two plants per row from each block were harvested (total of 60 plants per plot per location). The total weight of all potato tubers from each block was determined. Tubers were then divided into unmarketable (<48 mm), marketable (48-88mm) and large (<88 mm) sizes. All the marketable tubers harvested from a block were weighed again to determine marketable yield.

Traps In 1998 and 1999, two 1 m long pitfall traps (Hunt and Vernon 2001) were placed approximately 1 m from the edge of the plot, along all four borders of each plot at both sites. At the Lethbridge site, two flight intercept traps per border were also set up. Each flight trap was 10 m high and 4 m wide and was placed 1 m above the surface of the soil, at approximately the canopy level. The edges of the trap were bordered with a strip of tangle trap and a trough was placed at the bottom of each side of the trap to catch any beetles that hit the trap and fell while flying either into or out of the plot (Noronha and Cloutier 1998). Both pitfall and flight traps were monitored bi-weekly throughout the summer. All adults caught in traps were returned to the laboratory and verified if they were ready for diapause. Adults were placed in containers with soil and potato foliage in the greenhouse. There was no additional lighting in the greenhouse so adults received natural day length. Adults that failed to feed (i.e., were satiated) or that entered the soil within 24-48 hours were considered ready to enter diapause.

Overwintering population mortality, densities and depth Overwintered beetle densities were monitored in the 2000 Lethbridge plot. Between 11 and 17 May, 2001, determinations of the numbers of live and dead beetles within the soil were made. Ten quadrants of 37.5 x 49.4 cm were excavated within each plot. Soil was carefully removed layer by layer until hard sub top soil clay was encountered (at approx 40 cm). Each centimetre of soil was sieved and presence of beetles was noted at each depth.

RESULTS

Protected plots Beetle densities in all protected plots remained under 2 per plant. In late summer of 2000, beetles began moving from the unprotected plot to the protected plots as defoliation of the unprotected plot reached 100%.

General phenology observed Natural potato beetle populations quickly colonized all unprotected plots in all years with the exception of Lethbridge plot B in 1998 (Fig 1). The phenology remained essentially the same in all these plots and years except for minor shifts in time between plots and sites. Overwintered adults began colonizing the plots by mid June with mean densities reaching between 0.27 and 0.65 per plant, however, no colonizing beetles were found in our traps at this time. Eggs were laid on young emerging plants with mean densities reaching 2.15 egg batches per plant by late June. Hatch was recorded within the first week following egg laying. First and second instar larvae were present in the field from late June to early July, third instar larvae were present in late June to mid July and 4th instar were present in early July to the third week of July. Maximum densities reached per plant were as follows: 1st & 2nd instar, 9.5, 3rd instar, 9.5 and 4th instar, 13.6. A considerable overlap in larval instars was observed from late June to mid July; at times all four larval instars were observed in the field. In plot B at Lethbridge, there was a delay in colonization and in emergence of third and fourth instars and new adults compared to plot A (Fig. 1 a & b).

New adults started emerging at the end of July to early August with a maximum density of 57 adults per plant recorded at the 2000 Lethbridge unprotected plot and 20 adults per plant at the 1999 Vauxhall plot. On emerging, these adults began feeding on the potato foliage. Very few eggs if any were laid by these females and by mid-August, the adults were observed leaving the plants. At this time, beetles were found in pitfall and flight intercept traps. In 1999, a total of 104 beetles were found in traps in plot A at the Lethbridge site; 73% of these beetles were satiated and ready for diapause. In Vauxhall, 51 beetles were found in the traps; 91% were satiated. In 1999, 34 beetles were collected from the traps at the Lethbridge site and 303 at the Vauxhall site. Of these beetles, 9% at the Lethbridge site and 46% the Vauxhall site were found to be satiated. Flight was not very extensive and in 1998, only 11 beetles were trapped and in 1999, only 5. All beetles trapped were satiated and were found to be moving out of the plots. Although traps were not set out in 2000, adults were seen moving from the unprotected plot into the protected plot in early August.

Defoliation Defoliation was very low at the beginning of the season but sharp increases were noted when the third and fourth instar populations peaked and continued to rise when new adults emerged from pupation (Fig.1). Defoliation was slower in Lethbridge plot B reaching only 30% by the end of the summer as compared to 75% in plot A. Maximum defoliation occurred at Lethbridge in 2000 with 100% defoliation by 10 August. Defoliation in protected plots was observed mainly in outer rows and this was later in the season when new adults were moving into these plots. However, defoliation did not exceed 10% in these plots.

Yield and Quality Total yields in all unprotected plots ranged from 10 to 49% lower than in the protected plots (Table 1). At the Vauxhall site, marketable yield was reduced by 38% and 24% in 1998 and 1999. At the Lethbridge site, a slight increase of 4% in marketable yield was recorded in plot B in 1998. Decreases in marketable yield of 13 and 17% were recorded in 1999 and 2000.

Overwintering population mortality, densities and depth A total of 141 beetles were collected from the soil in the protected plot and 25 from the unprotected plot. Densities of overwintering adults averaged 76 (±21.5 SEM) per m^2 with a maximum of 232 per m^2 in the protected plot and 13 (±3.4 SEM) per m^2 with a maximum of 27 per m^2 in the unprotected plot. Mortality in both plots averaged 22% with only a single beetle (3%) being diagnosed as infected by the fungus *Beauveria bassiana*. Mean depth of live beetles was similar to dead beetles with a mean of 12.3 (±0.95 SEM) cm for living beetles and 11.9 (±2.13 SEM) for dead beetles and ranges of 0 to 39 cm for both groups. For living beetles (n = 131), 63% were found at depths of ≤10 cm; 10 % between > 10 to 20 cm; 11 % between > 20 to 30 cm and 12 % at depths between > 30 and 39 cm. For dead beetles (n = 36), 67% were found at depths of ≤10 cm; 8 % between > 10 to 20 cm; 14 % between > 20 to 30 cm and 11 % at depths between > 30 and 39 cm. Minimum temperatures recorded at a turfed field at the Lethbridge Research Centre were as follows: -31.4 ^{0}C at the soil surface on 11 December, 2000; -8.8 and -7.2 ^{0}C at 5 cm; -7.6 and -6.7 ^{0}C at 10 cm; and -5.3 and -5.5 ^{0}C at 20 cm on 16 December, 2000 and 27 February, 2001 respectively. There was no appreciable precipitation during the winter and the plots remained essentially dry and without snow cover throughout the winter period.

DISCUSSION

Although the Lethbridge site was far removed from major potato production areas, while the Vauxhall site was in the heart of the potato growing area in southern Alberta, we found no difference in the timing of adult colonization in the spring between these two sites; overwintered adults were found at both sites by early to mid June. We suspect that home gardens and smaller potato plots around Lethbridge may have contributed to the density of colonizing beetles at this site. Hough-Goldstein and Whalen (1996) reported no linear correlation between distance to previous years' fields and colonization pressure.

The timing of plant emergence, however, played a role in beetle colonization. Plot B in Lethbridge, which was planted one week after plot A, had delayed colonization which resulted in reduced defoliation. Mean beetle numbers reached 7 4th instars per plant in plot A and 2 per plant in plot B and defoliation in these plots reached 60% and 30% respectively by the end of the summer. The lower beetle pressure in plot B may explain the smaller difference in total yield between the protected plot B and unprotected plot during this year. Thus a delay in planting date which results in later plant emergence may actually result in a lower infestation. A study in New Brunswick, however, found that beetles colonizing early emerging varieties will move into the later emerging varieties resulting in large populations in these fields (Boiteau 1986). We found no evidence of this in our small plots in the 3 years of this study.

In all 3 years we found an overlap of larval instars in the field, with overwintered adults, eggs, and all four instars found in the field at the same time at the end of June early July. Early in the season when 1st and 2nd instar larvae were present in the field, defoliation was found to be generally low. However, when larvae molted to 3rd and 4th instars, a very rapid increase in defoliation occurred, as was previously found in other areas (e.g. Boiteau, 1994). The economic injury level for Manitoba was determined to be 0.14- 0.82 larvae per plant (Senanayake and Holliday 1990). Populations in our plots were much higher with 3rd and 4th instars reaching over 6 larvae per plant.

Beetle infestations in all years resulted in a reduction in total yield. This decrease in yield was smaller in the plot that had low beetle populations as seen in plot B in 1998 at the Lethbridge site when compared to plot A. A decrease in marketable yield between 13 and 38% was recorded at both sites except at the Lethbridge site in 1998, where the difference was less than 5%. The phenological age of the plant and the extent of defoliation at that stage influences yield reduction. Boiteau (1994) found that defoliation during the bloom period plays a critical role in yield reduction. The difference in timing of major defoliation activity of the 4th instar larvae may be the reason why in 1998 we found a greater yield loss at our Vauxhall site as compared to the Lethbridge site (Fig 1).

Our overwintering survival data indicated that a lower number of beetles overwintered directly in the plot where they had fed and completely defoliated the plot (with a mean of 13 per m^2) than in an adjacent, protected plot (with a mean of 76 per m^2). This supports the findings of Hunt and Tan (2000), who presented evidence suggesting that beetles would remain in a tomato field if there was a food source available and of Noronha and Cloutier (1999), who demonstrated that protected potato plots may act as a sink for diapausing adults emigrating from defoliated plots. This is in contrast to the findings of Milner *et al.* (1992) who found that, in central Wisconsin, adults attracted to trap potatoes in the fall, did not remain in these plots to overwinter.

All adults intercepted in flight traps and the majority of adults intercepted in the pitfall traps were satiated indicating that movement in late summer was mainly in search of overwintering sites. In eastern North America, large numbers of pre-diapause beetles walk or fly from potato or tomato fields to surrounding forested or wooded edges for overwintering, resulting in higher densities of overwintering adults along these edges than in their host fields (Hunt and Tan 2000, Weber and Ferro 1993). Densities of overwintering beetles in our plots were much higher than those reported elsewhere in tomato fields in Ontario (2 - 5 per m^2, Hunt and Tan 2000) and in potato fields in Massachusetts (4.9 - 19.9 per m^2, Voss and Ferro 1992; Weber and Ferro 1993) suggesting that beetles on the prairies may have adapted to overwinter in the fields rather than emigrate to edges of wooded areas, which are scarce on the prairies. This hypothesis warrants further investigation.

Mean depth of beetles in our study was 12.3 cm with 63% being found at depths of ≤ 10 cm and this is similar to mean depths found for beetles overwintering in New Jersey (7.6-12.7 cm, Lashomb *et al.* 1984) and central Wisconsin (80.4% in top 15 cm, Milner *et. al.* 1992). However, this contrasts with the findings of Hunt and Tan (2000) who reported that 74-78% of beetles overwintered at depths between 10 and 25 cm in Ontario and of Webber and Ferro (1993) who reported 67.7% of the overwintering beetles at 10 to 20 cm depth with only 12% in the 0 - 10 cm depth. Body size and soil temperature, density and moisture affect digging behavior and depth of prediapausing Colorado potato beetles (Noronha and Cloutier 1998). The exceptionally mild and dry winter of 2000/2001 in southern Alberta may have contributed to this shallower overwintering depth.

Mean overwintering survival of beetles in our plot was 78%. Survival rates can vary tremendously with survivorship of 14 to 75% recorded in New Jersey (Lashomb *et al.* 1984), 55 to 91% in Massachussets (Weber and Ferro1993), 17 to 29% in central Wisconsin (Milner *et al.* 1992) and 0 to 100% in Ontario (Hunt and Tan 2000). In these studies, a direct relationship between decreasing mortality with increasing depth was found and is generally attributed with thermal shock (Milner *et al.* 1992, Kung *et al.* 1992). In our study, a depth-mortality relationship was not apparent, however, our sample size was relatively low. Kung *et al.* (1992) reported < 50% survival after exposure of diapausing beetles to 6 cold-shock exposures of ≤-4 ^{0}C, but much higher survivals after single thermal shocks. In our study, only two thermal shocks were experienced, temperature differences between the 5 cm and 20 cm depths differed by less than 4^{0}C and temperatures never dropped below 8.8 ^{0}C. The 78% survival compares favorably with the survival of 71% after a single shock of -8 ^{0}C reported by Kung *et al.* (1992) In Massachusetts, Weber and Ferro (1993) reported that over 70% of the dead beetles exhibited infection by *B. bassiana*. In our study, we found only one infected cadaver which represented less than 3% of the dead beetles.

In Alberta, beetle populations have been traditionally low, but with the presently expanding potato industry, an increase in the Colorado potato beetle population could result in the need for increased insecticide applications and the rapid development of resistance. The large numbers of beetles already demonstrating lower susceptibility to insecticides in southern Alberta are an indication that resistant populations are being selected (Noronha *et al.* in press). Our results indicate that the phenology of CPB is similar to areas where population buildups were rapid and devastating, soon after insecticide resistant populations appeared. Beetle populations rapidly increase following colonization by relatively few beetles and overwintering survival during the winter of 2000/2001 was estimated at 78%. Thus, caution must be exercised and a resistance management program should be implemented immediately in southern Alberta to prevent, or at least delay, further selection of insecticide resistant populations.

ACKNOWLEDGEMENTS

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Table 1. Yield of potato from protected and unprotected plots at Lethbridge and Vauxhaul, Alberta.

	Total Yield		Percent Change	Marketable Yield		Percent Change
Year/Site	Protected	Unprotected		Protected	Unprotected	
Lethbridge						
1998						
Plot A		20392			11996	
Plot B	27728	24877	- 10	12446	12816	+ 3%
1999	38919	30633	- 21	20672	18036	-13%
2000	78173	39904	- 49	22547	18692	-17%
Vauxhall						
1998	31798	18032	- 43	19767	12321	-38%
1999	27958	18602	- 33	18162	13786	-24%

Figure Caption

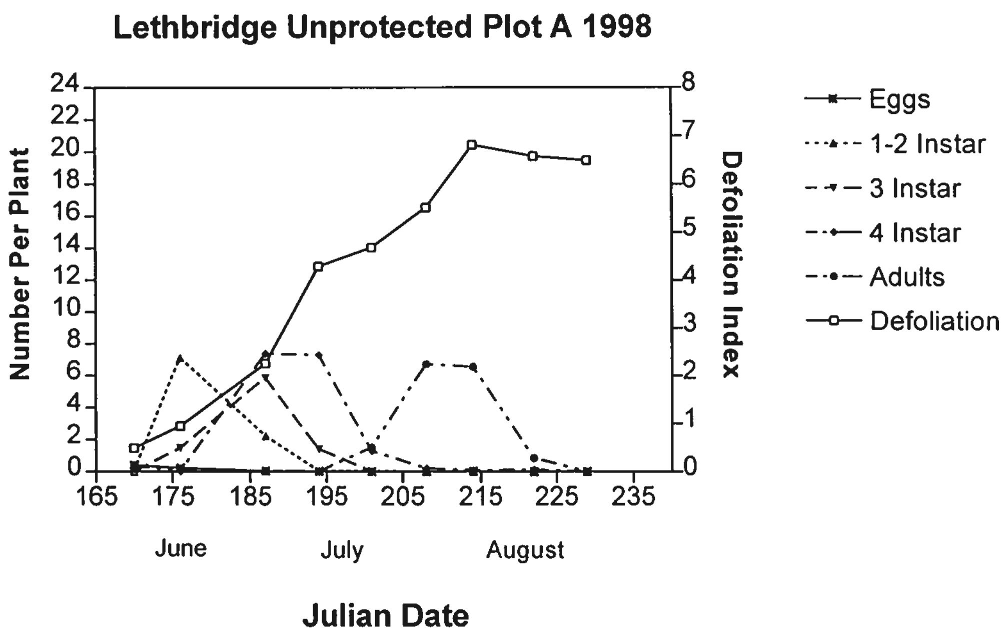
Figure 1. Mean beetle densities and defoliation index in unprotected potato plots. a) Lethbridge Plot A in 1998; b) Lethbridge Plot B in 1998; c) Lethbridge Plot in 1999; d) Lethbridge Plot in 2000; e) Vauxhall Plot in 1998; f) Vauxhall plot in 1999. Defoliation index according to Boiteau 1994: 1. <10%; 2. 11-25%; 3. 26-39%; 4. 40-50%; 5. 50%; 6. 51-74%; 7.> 75%; 8. 100%.

Figure Caption

Figure 1. Mean beetle densities and defoliation index in unprotected potato plots. a) Lethbridge Plot A in 1998; b) Lethbridge Plot B in 1998; c) Lethbridge Plot in 1999; d) Lethbridge Plot in 2000; e) Vauxhall Plot in 1998; f) Vauxhall plot in 1999. Defoliation index according to Boiteau 1994: 1. <10%; 2. 11-25%; 3. 26-39%; 4. 40-50%; 5. 50%; 6. 51-74%; 7.> 75%; 8. 100%.

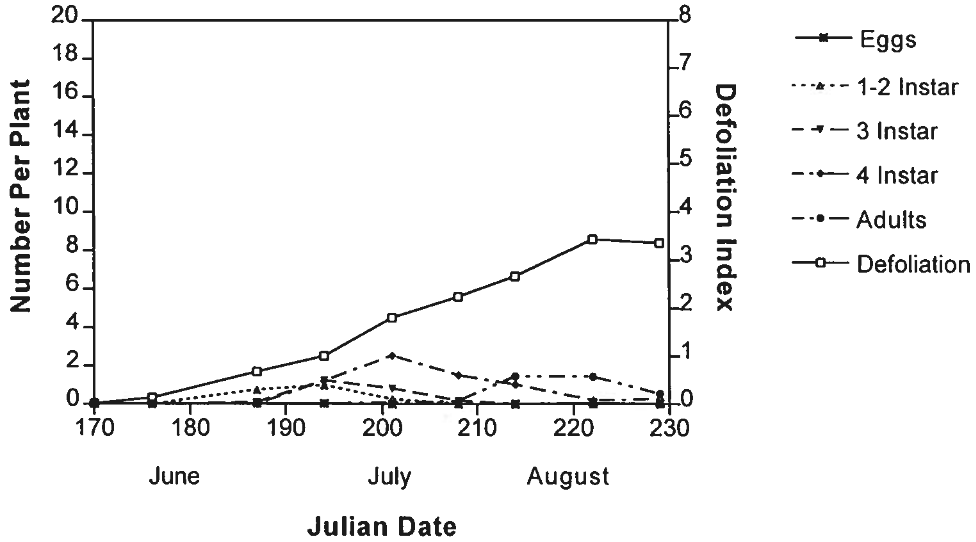
NOTE: FIGURES WILL BE ARRANGED IN A MATRIX OF 6 ON ONE PAGE.

a)


Lethbridge Unprotected Plot A 1998
Number Per Plant
24
22
20
18
16
14
12
10
8
6
4
2
0
Defoliation Index
.8
.7
.6
.5
.4
.3
.2
.1
0
165
175
185
195
205
215
225
235
June
July
August
Julian Date
Eggs
1-2 Instar
3 Instar
4 Instar
Adults
Defoliation

k)

Lethbridge Unprotected Plot B 1998



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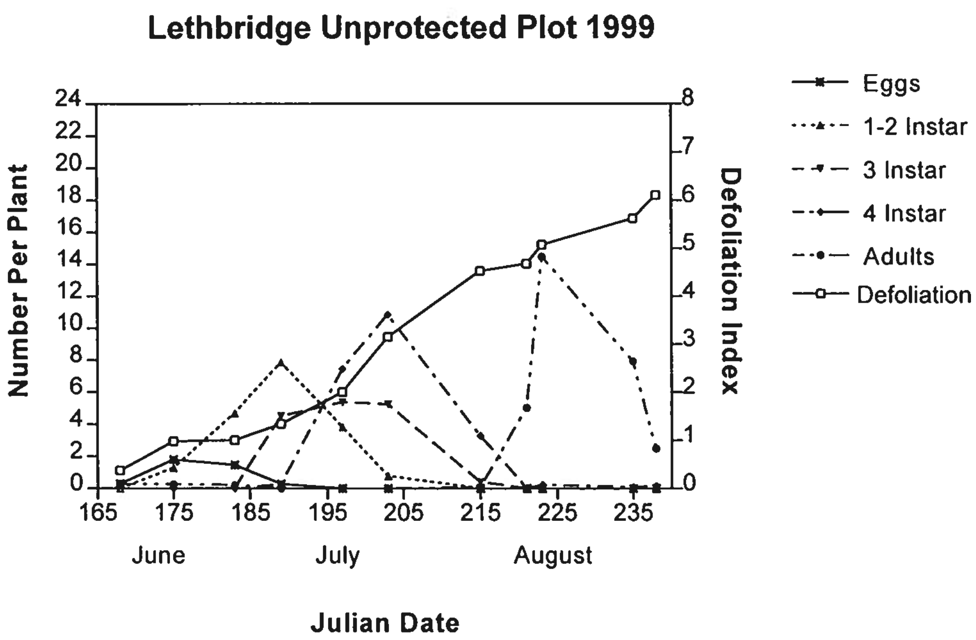
Lethbridge Unprotected Plot 1999
Number Per Plant
24
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20
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16
14
12
10
8
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2
0
Defoliation Index
.8
.7
.6
.5
.4
.3
.2
.1
0
165
175
185
195
205
215
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235
June
July
August
Julian Date
Eggs
1-2 Instar
3 Instar
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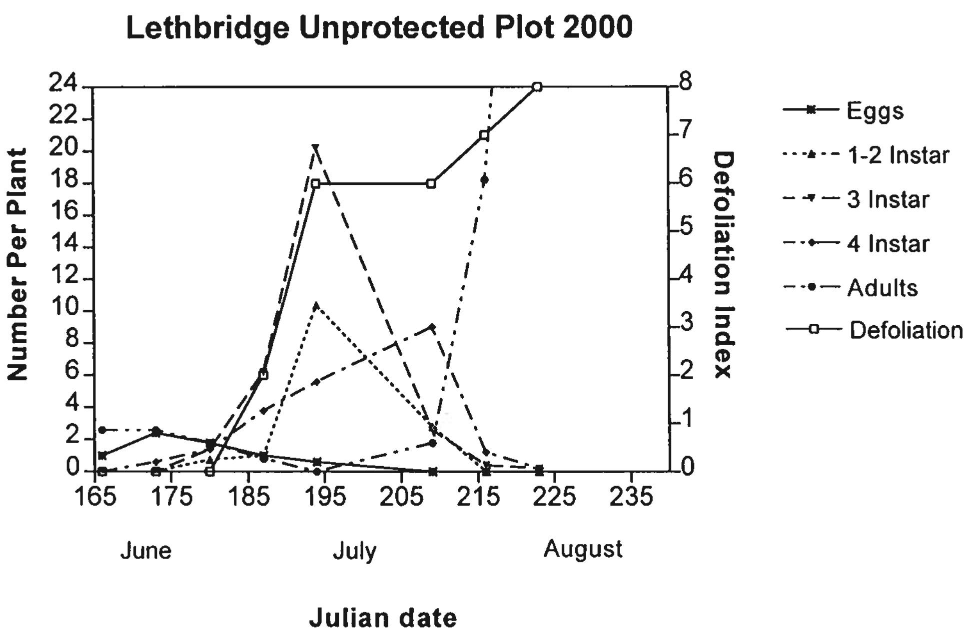
Adults
Defoliation

d)


Lethbridge Unprotected Plot 2000
Number Per Plant
Defoliation Index
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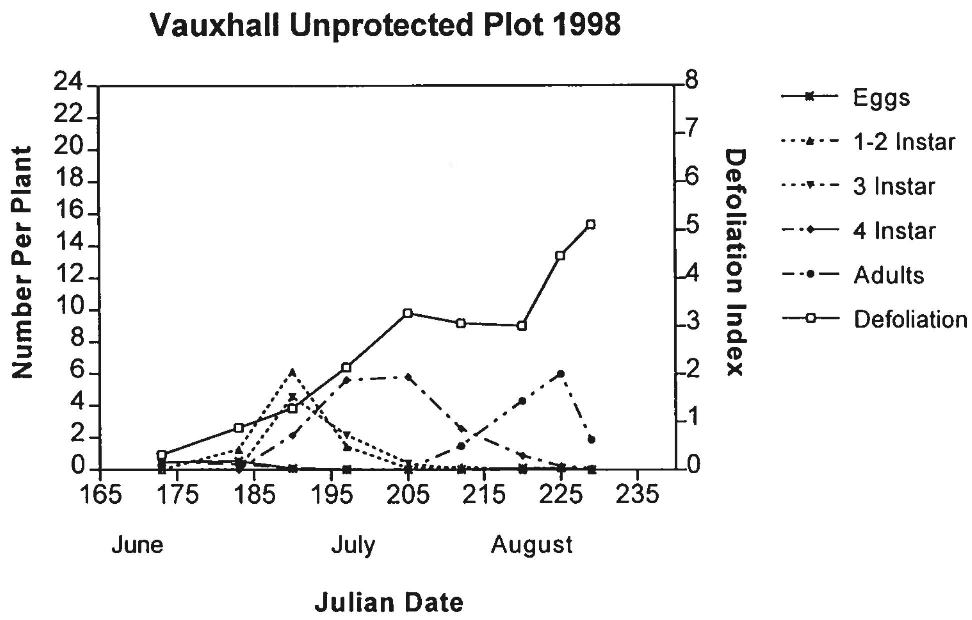
Julian date
Eggs
1-2 Instar
3 Instar
4 Instar
Adults
Defoliation

e)


Vauxhall Unprotected Plot 1998
Number Per Plant
24
22
20
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16
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12
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Defoliation Index
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Julian Date
Eggs
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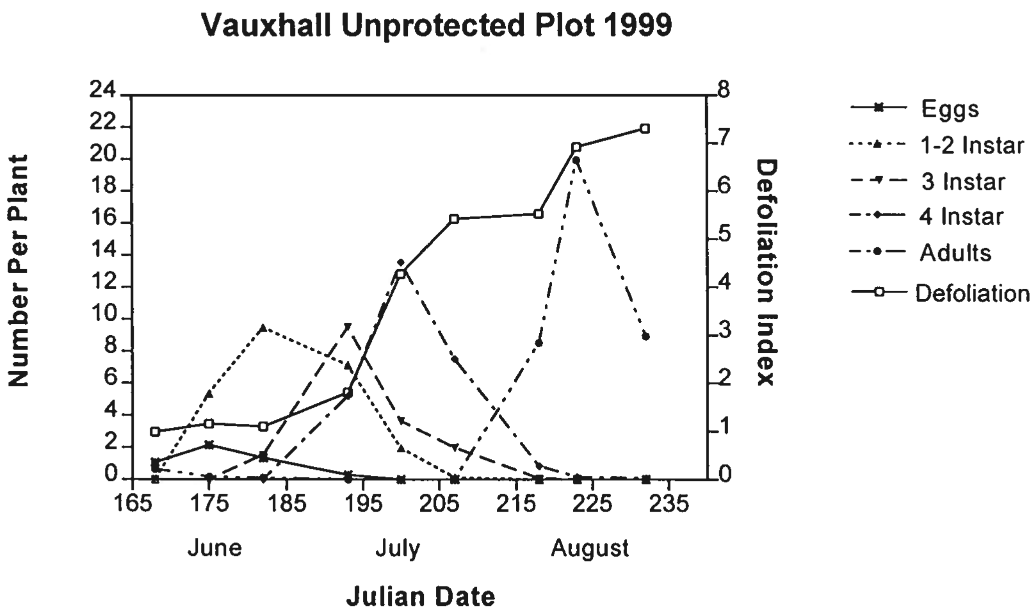
Adults
Defoliation

b)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Number Per Plant
Defoliation Index
Julian Date
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Adults
Defoliation
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Approved
$5000

Alberta Agricultural Research Institute (AARI)
Matching Grants Program Application - 2001/2002

Office Use Only: Date Received ______ Application Number ______

1. Project Title (maximum 15 words)

Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle

2. Commencement and Duration of Project

Expected commencement date for this request for funding: April 1, 2001

Anticipated duration of project is: 3 year(s)   Is this a renewal application? yes

If yes, state the first year the project was funded: 2000   and the current project #: 2000M701

3. Choice of Research Committee

Beef & Dairy		Pork, Poultry, Sheep & Other Livestock	
Cereals & Oilseeds		Forage, Pulse, Vegetable & Other Crops	X
Resource Conservation		Policy, Economics & Marketing	

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		Fax #/Email	519-738-2929/huntd@em.agr.ca

ii. *Objectives*

1. To determine the effectiveness of an insect pathogenic fungus, *Beauveria bassiana,* in reducing beetle populations.

2. To evaluate its use in an insecticide resistance management program.

Implementation of such an alternative control method in the management of the Colorado potato beetle will delay development of resistance to chemicals, decrease dependency on use of chemical insecticides, and allow development of an IPM strategy against this pest in Alberta.

iii. *Key Results Expected*

The objective is to determine the role that the fungus *Beauveria bassiana* could play in the resistance management of the pesticides being used for control of the Colorado potato beetle. Virulence of the fungus towards the soil stages of the beetle will be determined. Furthermore, it will be determined to what extent the fungus can affect overwintering adult beetle populations. The strategy would be to contaminate migrating adults with spores of the fungus as they leave the potato fields in late summer in search of overwintering sites. Results on persistence, virulence and effects of contamination of overwintering sites will provide information for use of this fungus in IPM.

2000 -2001: Virulence of the fungus will be determined. How many spores must the beetles contact in order to get infected? Persistence of the fungus under field conditions will be determined. Will enough spores remain viable from year to year to ensure continued efficacy? Will beetles be contaminated with an adequate amount of inoculum as they bury in the soil which contains spores?

2001- 2002: How many of the inoculated adults that diapaused under natural conditions became infected and died before emergence in the spring? How many spores persisted over the season? Should beetles be inoculated directly, or can they pick up an adequate amount of inoculum from the soil? How do soil conditions affect spore persistence and virulence?

2002-2003: Do adults that succumbed in the soil produce adequate numbers of spores to ensure infection of the next generation? Should spores be reapplied yearly? Should we proceed with registration? Final report and manuscripts will be prepared.

B. Progress to Date (renewal applications only)
Provide a concise report of the results achieved. It should contain a summary of the data collected and any preliminary conclusions made. The report should clearly state whether the results expected under the action plan for the preceding year have been achieved. If not, provide reasons. Include all changes or modifications to original expectations, citing reasons. One page may be added to this section if required.

We have been very fortunate to have been able to attract 2 excellent COOP students from the University of Lethbridge to work on this project during the last year. These students were highly motivated and are compensated at a very economical level. One of the students has since been hired under the Federal Student Work Experience programme and continues to work on the project on a part time basis (15 hrs/week). Consequently, we are able at this time to have proceeded more or less with the same basic research plan albeit at a reduced level. We have had to reduce some of our original studies as follows: 1) Field plots were not inoculated and monitoring for soil persistence was not carried out during the present summer season. 2) Field cage trials against larvae and summer generation emerging adults were not carried out. Nevertheless we have made much progress here in Alberta as well as with our studies in Prince Edward Island and Ontario. We were fortunate to have received funding from the Ontario Fresh Vegetable Producers Association, matched by AAFC's Matching Investment Initiative (Harrow), which funded our activities in Ontario. We have approached several new potential funding sources (McCain's, Ontario Potato Board, Potato Snackfood's

Host-pathogen relationships and the effects of soil factors on fungal persistence and infectivity must be determined before larger scale field trials are initiated. The experiments are designed to obtain information on 1) the relationships between fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions, 4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions. Unless otherwise noted, all experiments will be arranged as complete randomised block designs with at least 3 replications of 50 beetles per treatment and control. Every experiment will be repeated and all field plot designs and statistical analyses will be performed in consultation with statisticians at the Lethbridge Research Centre.

1) Susceptibility of the soil stages.

Hypothesis: Spores within the soil will incite disease and cause mortality in the soil dwelling stages of the insect.

Spores of *B. bassiana* will be mixed uniformly into sterile soil at several concentrations (approximately 10^4 to 10^8 conidia/g dry weight of soil). Water will be added to obtain a moisture level of 9% (w/w). Each soil/dose combination will be dispensed into three plastic containers. Fifty mature larvae will be placed onto the surface of each container and allowed to enter the soil and pupate. After incubation, emergent adults will be transferred to clean containers and observed daily for mortality. The soil will be examined for presence of dead prepupae, larvae or adults. Cause of death will be determined by examination of hemolymph for fungal blastospores or by incubating surface sterilized cadavers under high humidity and observing emergent fungus. Spore viabilities will be quantified at the start and end of the experiment by removing cores of soil from the containers and by standard dilution/plating techniques on selective media. Dose-mortality results will be analysed using probit analyses.

2) Influence of soil type

Hypothesis: Soil type will influence persistence and pathogenicity.

The experimental design will be similar to the one described above except that only one dose will be used. Different soil types and moisture levels will be incorporated into the bioassay design. Soil types to be tested will be those characteristic of habitats where pupation and overwintering occurs. Comparisons will also be made between sterilized and non-sterilized soils.

3) Spore persistence

Conidia will be incorporated into the top 25 cm layer of soil in 3-m^2 field plots in Alberta in field and overwintering habitats (e.g., windbreaks). The persistence of conidia will be determined by taking soil cores and determining viability using standard dilution plating techniques. Monitoring will continue for up to 24 months if warranted.

4) Spore transmission

Hypothesis: Emigrating diapausing adults inoculated with spores will contaminate overwintering sites.

The experimental design will be similar to the one described above (2), except that the soil will not contain spores. Forty diapausing adults will be allowed to enter the soil. Subsequently, 10 spore -treated adults (using a spray tower) will be added and allowed to enter the soil. The experiment will be conducted under several different cool temperature conditions. Following termination of diapause, adults will be held at 25°C and observed daily for mortality. The soil will be examined for cadavers. Presence of *B. bassiana* in cadavers will be verified as described above (2).

method of control, be it chemical pesticides or transgenic potato, will eventually result in the development of resistance. Reduction on the reliance to chemical pesticides will contribute to the sustainability and competitiveness of the potato industry in Alberta.

This project will be the beginning of the development and implementation of a much more comprehensive IPM strategy for pests of potatoes. Other benefits of the proposed research include the possible feasibility of using this fungus for control of other soil-dwelling insect pests such as wireworms. Results of this project could also be used in the development and implementation of insecticide resistance management and IPM programs for other economically important pest insects in Alberta and elsewhere.

F. Related Research Performed in Your Organization

1) Development for IPM against the Colorado potato beetle. Dr. Gilles Boiteau, Fredericton Research Centre. Dr Boiteau is evaluating *B. bassiana* as a foliar application. There is no direct overlap, however, results from our proposed study would be most beneficial for IPM programs throughout Canada.

2) Development for IPM against the Colorado potato beetle. Dr. Bob Vernon, Aggasiz Research Centre. Dr. Vernon is monitoring the recent introduction of the potato beetle in the Okanagan Valley and testing several IPM protocols, none of which include *B. bassiana* at this time.

3) Development of *Beauveria bassiana* for control of grasshoppers. Drs. Doug Inglis, Dan Johnson and Mark Goettel. Lethbridge Research Centre. For the past 5 years we have been studying the potential of this fungus for use against grasshoppers. Part off the project included testing the fungus against ovipositing females under a variety of soil conditions. It was found that females become infected after ovipositing in spore augmented soils. This has given us considerable experience which will be used in the proposed research.

We will collaborate closely with Drs. Gilles Boiteau and Bob Vernon, and other scientists studying IPM of the Colorado potato beetle in Canada.

G. Related Research Performed in Other Agencies

1) IPM of pests of potatoes in Maine. Drs. Ellie Groden and Frank Drummond, University of Maine, Orono. *Beauveria bassiana* has been studied extensively, but mainly as foliar application for use in IPM programs against the Colorado potato beetle. These researchers have found that populations drop significantly following the 3rd consecutive year of application. I am in close contact with this team and have discussed my proposed approach. Both Drs. Groden and Drummond have expressed interest and we will be working together so that there will not be any unnecessary duplication.

2) IPM of Colorado potato beetle. Dr. Tad Poprawski, Texas A & M University and USDA-ARS, Weslaco, Texas and Dr. S. Wraight, USDA/ARS, Ithaca, NY. *Beauveria bassiana* is being evaluated against the Colorado potato beetle when applied as a foliar microbial insecticide. Some very promising results have been obtained. This team has not studied the potential of this fungus as a soil amendment against pupating or diapausing populations.

3) Dr. Lerry Lacey, USDA, Yakima, WA, is evaluating *Beauveria bassiana* when used in conjunction with *Bacillus thuringiensis tenebrionis* as a foliar application against the Colorado potato beetle.

4) Commercialization of *Beauveria bassiana*. Mycotech Corp., Butte Montana. Mycotech Corporation has developed an innovative cost effective method for the mass production of this fungus. They will provide us with spores without cost, however, since they are a small company, they are unable to support our proposal financially at this time.

will also be used to obtain support for registration of the fungus in Canada. Results will be published in scientific journals for the benefit of the scientific community at large. It is expected that if the project demonstrates feasible use of fungus in a potato IPM program, private industry (e.g., Mycotech Corp., Butte MT and possibly other companies) will invest in the registration and commercialization of this fungus in Canada. Because registration requirements between the United States and Canada are being harmonized and the fungus is already registered in the United States, registration in Canada should be expedited requiring minimal new safety data. Once registration has been obtained, we will work closely with the Potato growers' association is to ensure that the technology is being adopted appropriately and effectively.

C. Supplies and Services

i. *Travel* (includes travel and accommodation costs)

a. Project Travel

Traveller's Name: Mark Goettel

Destination(s): PGA & Prairie Potato Council Annual Meetings

Number of Trips: 2

Mode of Travel: car or air, as warranted

Purpose: to confer with growers and inform them of progress

Cost: $1,500

b. Conference Travel

Traveller's Name: __________

Destination(s): __________

Number of Trips: __________

Mode of Travel: __________

Purpose: __________

Cost: __________

Justification is required for requests over $1,500:

ii. ***Materials/Supplies*** **(if you have more than six items, please attach a list)**

List Item	Quantity	$ Per Unit	Cost
Disposable Petri dishes	10 bx	$50/bx	$500
Agar	5 kg	$140/kg	$700
Crop Sciences Greenhouse/Field Services			$2,000
Miscellaneous laboratory supplies (glassware/disp)			1,500
		Total:	$4,700

Summary of Budgets for Anticipated Duration of Project

	RENEWALS A. Amount Approved in 2000-2001*	B. Amount Proposed for 2001-2002	C. Amount Proposed for 2002-2003	D. Amount Proposed for 2003-2004
Manpower	$8,500	$40,100	$40,100	
Benefits	0	0	0	
Capital Assets	0	0	0	
Travel	0	1500	1500	
Materials/Supplies	440	4,700	4,700	
Computer Cost	0	0	0	
Publication Cost	0	0	0	
Rentals & Leases	0	0	0	
Contract Personnel	0	0	0	
Overhead Cost	1060	5,560	5,560	
TOTAL	$10,000	$51,860	$51,860	

E. Total amount for this request and future requests anticipated for this project $103,720

F. Total amount approved in previous years for this project $25,000*

* Although $25,000 in matching funds were approved for 2000/2001, we were only able to obtain $5,000 of industry money to match. We are soliciting new industry funding and partners and are confident that we'll be able to obtain $25,000 to match for 2001/2002, in order to get the project back on track and to full speed for the next field season.

G. Approval in any year does not guarantee funding for subsequent years. Provide substantive reasons to justify proposals requesting multiple year funding.

Evaluation under field conditions requires multiple years of study. At least 3 field seasons are required in order to evaluate the proposed method of using this pathogen against the target insect. Laboratory studies will be conducted during the winter months.

12. *Terms and Conditions*

A. This application is submitted, and will be evaluated, under the authority of the Alberta Agricultural Research Institute Act and the Farming for the Future Matching Grants Program Guidelines. The applicant accepts the conditions specified in the guidelines.

B. All completed applications submitted to AARI become the property of AARI and will not be returned to the applicant. While every effort will be made to keep the information contained in the application form confidential, the application review procedures require that copies of the application be distributed to a number of reviewers. The contents of this application may also be subject to access under the Freedom of Information and Protection of Privacy Act.

C. The decision of AARI's Board of Directors regarding this application is final.

[signature]	20-10-2000
Principal Researcher's Signature	Date
Co-applicant's Signature	Date
Co-applicant's Signature	Date

Principal Researcher - Biographical Data

This personal information is being collected for the purpose of assessing the researchers' qualifications under the authority of the AARI Act. It is subject to the provisions of the Freedom of Information and Protection of Privacy Act.

Name (surname first):

GOETTEL, Mark S.

Post-Secondary Education and Training Relevant to Proposal:

Institution	Field Specialization	Degree/Diploma	Year Completed
Concordia	Biology	B.Sc. (Honors)	1975
Ottawa	Entomology	M.Sc.	1977
Alberta	Insect Pathology	Ph.D.	1987

Relevant Professional Experience (begin with present position):

Dates	Position or Function	Employer	Location
Nov. 1988-present	Research Scientist	Agric. & Agri-Food Canada	Lethbridge
Feb. 1987-Nov. 1988	Post-doctoral Fellow	Boyce Thompson Inst.	Ithaca

Research Activities Related to Research Proposal (list up to 4 projects):

Title	Date
Investigations into penetration processes of entomopathogenic fungi.	Feb. 1987-Nov. 1988
Investigations into the use of *Beauveria bassiana* for control of grasshoppers in Alberta	Jan. 1991 - present
Study of environmental constraints of entomopathogenic fungi	Aug. 1995- Sept., 1996
Development of microbial control for management of the Colorado potato beetle	Oct, 1977 - present

Relevant Articles Published in Refereed Journals and Other Relevant Works in the Last Three Years

Butt, T.M. & **M.S. Goettel**. 2000. Bioassays of Entomogenous Fungi in Bioassays of Entomopathogenic Microbes and Nematodes (A. Navon & K.R.S. Ascher, eds). CABI International Press, Wallingford, U.K. pp 141-195.

Inglis, G.D., D.L. Johnson, L.M. Kawchuk & **M.S. Goettel**. 1998. Effect of soil texture and soil sterilization on susceptibility of ovipositing grasshoppers to *Beauveria bassiana*. **J. Invertebr. Pathol.** 71: 73-81.

Goettel, M.S. & D.L. Johnson (eds.).1997. Microbial Control of Grasshoppers and Locusts. **Memoirs Entomological Society of Canada** 171, 400 pp.

Fargues, J., A. Ouedraogo, **M.S. Goettel** & C.J. Lomer.1997. Effects of temperature, humidity and inoculation method on susceptibility of *Schistocerca gregaria* to *Metarhizium flavoviride*. **Biocont. Sci. Technol.** 7: 345-356.

Inglis, G.D., D.L . Johnson & **M.S. Goettel**.1997. Use of pathogen combinations to overcome the constraints of temperature on entomopathogenic Hyphomycetes against grasshoppers. **Biological Control** 8: 143-152.

Inglis, G.D., D.L . Johnson & **M.S. Goettel**.1997. Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* against grasshoppers. **Canadian Entomologist** 129: 171-186.

Co-applicant (2) - Biographical Data

This personal information is being collected for the purpose of assessing the researchers' qualifications under the authority of the AARI Act. It is subject to the provisions of the Freedom of Information and Protection of Privacy Act.

Name (surname first):

HUNT, David W.A.

Post-Secondary Education and Training Relevant to Proposal:

Institution	Field Specialization	Degree/Diploma	Year Completed
University of Victoria	Biology	B.Sc.	1980
Simon Fraser University	Pest Management	MPM	1983
Simon Fraser University	Entomology	Ph.D.	1987

Relevant Professional Experience (begin with present position):

Dates	Position or Function	Employer	Location
April 1989-present	Research Scientist	Agric. & Agri-Food Canada	Harrow, ON
April 1987-April 1989	Postdoctoral Research Associate	University of Wisconsin	Madison, WI

Research Activities Related to Research Proposal (list up to 4 projects):

Title	Date
Investigations into the use of *Beauveria bassiana* for control of bark beetles	1980-1983
Study of the overwintering densities and survival of the Colorado potato beetle	1995-1999
Development of a device for trapping walking Colorado potato beetle	1995-1999
Potato trap crops for control of Colorado potato beetle	1993-1996

Relevant Articles Published in Refereed Journals and Other Relevant Works in the Last Three Years

Hunt, D.W.A, and C.S. Tan. 1999. Overwintering densities and survival of the Colorado potato beetle (Coleoptera: Chrysomelidae) in and around tomato (Solanaceae) fields. Canadian Entomologist. (In press).

Hunt, D.W.A. 1998. Reduced tillage practices for managing the Colorado potato beetle in processing tomato production. HortScience 33: 279-282.

Hunt, D.W.A., and G. Whitfield. 1996. Potato trap crops for control of Colorado potato beetle (Coleoptera: Chrysomelidae) in tomatoes. Canadian Entomologist. 128: 407-412.

Hunt, D.W.A., A. Liptay, and C.F. Drury. 1994. Nitrogen supply during production of tomato transplants affects preference by Colorado potato beetle. HortScience 29: 1326-1328.

Co-applicant's Organization

Co-applicant's Name Dr. Christine Noronha

A. Name Dr. J.A. Ivany — Title Research Scientist

Signature [signature] — Date Oct. 23/00

B. Name Dr. C. Deslauriers — Title Director

Signature C. Deslauriers — Date 00.10.23

C. Name Dr. Y. Martel — Title Director General, Eastern Region

Signature ______ — Date ______

Co-applicant's Organization

Co-applicant's Name Dr. David W.A. Hunt

A. Name Dr. G.H. Whitfield — Title Director

Signature ______ — Date ______

B. Name Dr. Y. Martel — Title Director General, Eastern Region

Signature ______ — Date ______

C. Name ______ — Title ______

Signature ______ — Date ______

Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

Research Branch / Direction générale de la recherche

Lethbridge Research Centre
PO Box 3000
LETHBRIDGE AB T1J 4B1
Telephone: (403) 327-4561
Facsimile: (403) 382-3156

January 24, 2001

POTATO GROWERS OF ALBERTA
6008 46 AVENUE
TABER AB T1G 2B1

RECEIVED JAN 3 0 2001

To Whom it May Concern:

Application for funding: "Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle"

Enclosed please find an AARI Matching Grants Application form requesting funding from PDI in the amount of $5,000 for 2001/2002. This form was submitted to AARI as a progress report for the first year of funding from PDI ($5,000; SPA A01802) which was matched by the AARI Matching Grants Program ($5,000), and as a request for a second year of funding.

In addition to AARI/PDI monies, we were fortunate to have received funding from the Ontario Fresh Vegetable Producers Association, which was matched by AAFC's Matching Investment Initiative (Harrow), which funded our activities in Ontario. We have approached several new potential funding sources and hope that this year, in addition to starting a research component in PEI, we will be able to significantly expand our activities at Lethbridge. Because winter weather is unpredictable and highly variable, we benefit greatly from performing overwintering mortality trials at 3 different ecozones.

Looking forward to receiving continued support from PGA/PDI for this important research on management of an important pest of potatoes worldwide.

Sincerely,

Dr. P.A. Burnett
Assistant Director

MSG:kg
cc: Dr. J.G. Stewart, Section Head, Crop Science Section
Dr. M.S. Goettel, Entomologist

Canada

Recycled Paper / Papier recyclé

Differential susceptibility to insecticides by *Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae] populations from western Canada

Christine Noronha[1,2], Grant M. Duke[1], Jason M. Chinn[1], and Mark S. Goettel[1,3]

Received 2001-06-03; accepted 2002-01-22

PHYTOPROTECTION 82 : 113-121

The susceptibility of Colorado potato beetles (*Leptinotarsa decemlineata*) (CPB) from three provinces in western Canada was measured using a filter paper bioassay to substantiate the reported insecticide resistance by the beetle in Manitoba, and to compare the situation there to beetle populations from Saskatchewan and Alberta. Susceptibility of beetles was measured against five insecticides: the organophosphates, azinphos-methyl (Guthion), and methamidophos (Monitor); the pyrethroid, permethrin (Ambush); the organochlorine, endosulfan (Endosulfan); and the carbamate, carbaryl (Sevin). All 12 populations tested from Manitoba were found to have resistance to one or more of the insecticides. All populations were classified as either having resistance or intermediate resistance to permethrin; two of the populations were classified as having resistance to azinphos-methyl and three to methamidophos. Two of four populations from Saskatchewan were classified as having intermediate resistance to azinphos-methyl and methamidophos. Intermediate resistance to permethrin was recorded in 12 of the 13 populations from Alberta, with only one being highly susceptible. Two populations showed evidence of intermediate resistance to azinphos-methyl and three to methamidophos. In all three provinces, survival rate from different egg masses within the susceptible populations ranged from 0-100%, indicating the presence of individuals with either resistance, intermediate or high susceptibility within these populations. With the expanding potato acreage in western Canada and the detection of the CPB populations with resistance to insecticides, a resistance management program must be implemented to prevent the rapid selection of resistant populations.

[Sensibilités variées aux insecticides de populations de *Leptinotarsa decemlineata* [Coleoptera : Chrysomelidae] de l'Ouest canadien]

La sensibilité du doryphore de la pomme de terre (*Leptinotarsa decemlineata*) de trois provinces de l'Ouest canadien a été mesurée par bioessai sur

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2. Present address: Crops and Livestock Research Centre, Agriculture & Agri-Food Canada, 440 University Ave., Charlottetown, Prince Edward Island, Canada C1A 4N6
3. To whom correspondence should be addressed; e-mail: goettel@em.agr.ca

papier filtre pour corroborer les comptes rendus faisant état de doryphores résistants aux insecticides au Manitoba, et pour comparer la situation au Manitoba à celles de la Saskatchewan et de l'Alberta. La sensibilité des doryphores a été mesurée pour cinq insecticides : les organophosphates azinphos-méthyl (Guthion) et méthamidophos (Monitor), le pyréthroïde perméthrine (Ambush), l'organochloré endosulfan (Endosulfan) et le carbamate carbaryl (Sevin). Les 12 populations du Manitoba examinées démontraient de la résistance à au moins un des insecticides. Toutes les populations ont été classées comme étant résistantes ou moyennement résistantes à la perméthrine; deux des populations ont été classées comme résistantes à l'azinphos-méthyl et trois au méthamidophos. Deux des quatre populations de la Saskatchewan ont été classées comme étant moyennement résistantes à l'azinphos-méthyl et au méthamidophos. Une résistance intermédiaire à la perméthrine a été trouvée dans 12 des 13 populations de l'Alberta, alors qu'une seule était très sensible. Deux populations se sont montrées moyennement résistantes à l'azinphos-méthyl et trois au méthamidophos. Dans les trois provinces, le taux de survie de diverses masses d'oeufs provenant de populations sensibles variait de 0 à 100 %, ce qui montre que des individus résistants, moyennement résistants et très sensibles coexistent dans ces populations. Avec l'expansion de la culture de la pomme de terre dans l'Ouest canadien et la détection d'une résistance aux insecticides dans les populations du doryphore de la pomme de terre, un programme de gestion de la résistance doit être mis en place pour éviter la sélection rapide de populations résistantes.

INTRODUCTION

Potato is the most important vegetable crop in Canada, making up 60% of all vegetable farm cash receipts (Statistics Canada 1999). The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) [Coleoptera: Chrysomelidae] is considered one of the most destructive foliage feeding pests of potatoes. The application of chemical insecticides has been the primary method used to control this insect in North America (Hare 1990; Martel 1987; Roush *et al.* 1990). However, widespread and repeated use of chemicals as a control method have resulted in the selection of insecticide resistant populations. In some potato producing areas in North America, nearly all the previously effective insecticides are no longer able to reduce beetle populations and new insecticides lose their effectiveness within a few yr because of cross resistance (French *et al.* 1992; Harris and Turnbull 1986). Thus, the selection of insecticide resistant populations is a major threat to the potato industry and a continuing problem in Colorado potato beetle management (Forgash 1981; Grafius 1997; Martel 1987).

In Canada, insecticide resistant populations have been reported from most of the eastern provinces where potatoes are grown (Boiteau 1988; Boiteau *et al.* 1987; Harris and Svec 1976; Stewart *et al.* 1997). The first reports of resistance were to organochlorine insecticides (Harris and Svec 1976; McClanahan 1975; McDonald 1976). By 1981, populations showing resistance to organophosphates and carbamates were found in Quebec. In 1979, most populations tested in Ontario were susceptible to pyrethroids (permethrin, fenvalerate and cypermethrin), but by 1982, a 22-37 fold resistance was reported after just 2 yr of use of these insecticides (Harris and Turnbull 1986). In New Brunswick, there was a 70% increase in beetle populations between 1974 and 1980 which coincided with an increase in insecticide resistant populations (Boiteau *et al.* 1987). Some populations in these areas have developed resistance to insecticides in all classes, resulting in the emergency registration in 1995 of imidacloprid (Admire™), which belongs to the new chloronicotinyl class of insecticide.

In western Canada there have been recent reports of insecticide resistance to three of the four classes of insecticides tested from Manitoba (Gavloski 1997). In Alberta, the last report of the presence of resistance was to DDT (McDonald 1976) but since then there have been no surveys conducted.

Over the last 10 yr, Canadian potato production has increased by 55% and the area planted by 40% (Statistics Canada 1999). The future growth of the potato industry is expected to be in western Canada, including Alberta, as more potato processors establish there. This increasing demand for potatoes requires an increase in acreage, and may result in a decrease in rotation, factors that will favour Colorado potato beetle populations and the need for an increase in the use of insecticide treatments. This will consequently provide ideal conditions for the selection of insecticide resistant beetle populations.

The objective of this study was to measure the susceptibility to insecticides, of beetles from three western Canadian provinces, to substantiate the reported occurrence of insecticide resistance to the beetle in Manitoba, to compare the situation there to beetle populations from Saskatchewan and Alberta, and to provide base-line data for future survey.

MATERIALS AND METHODS

Insecticide resistance in beetle populations from Manitoba, Saskatchewan, and Alberta was measured using a filter paper bioassay (French *et al.* 1992; Heim *et al.* 1990). Due to the low numbers of beetles found throughout parts of western Canada, we were unable to collect sufficient numbers of egg masses directly from the field. Consequently, during the summer of 1998, laboratory cultures of 35 egg-laying females and 15 males were established on potted "Russet Burbank" potato plants from field collected adults or fourth instar larvae from commercial fields in each province (Table 1). Beetles from each commercial field were considered separate populations. The egg masses from each population were collected over a 1 to 3-month period and used in the assays. For the most part, eggs were collected from adults that arose directly from the field collected larvae. However, for four populations (Portage la Praire, Selkirk 2, Winkler and Lake Diefenbaker), we had to add a few beetles from the first generation to the cage with the original population towards the end of experimentation because egg laying had slowed down considerably in these populations.

Each bioassay unit consisted of a filter paper (5.5 cm diam., Fisher P-5) that was pre-treated with a commercial insecticide dissolved in acetone using the diagnostic concentrations calculated by

Table 1. Collection localities for *Leptinotarsa decemlineata* from western Canada

No.	Alberta	Saskatchewan	Manitoba
1	Edmonton-1	Lake Dienfenbaker	Lauder
2	Edmonton-2	Outlook	Hartney
3	Nobleford	Saskatoon	Bagot
4	Lethbridge	Nipawin	Portage la Prairie-1
5	Cranford		Portage la Prairie-2
6	Enchant-1		Winkler
7	Enchant-2		Gretna
8	Taber-1		Selkirk-1
9	Taber-2		Selkirk-2
10	Vauxhall		Steinbach-1
11	Hays-1		Steinbach-2
12	Hays-2		Unknown
13	Grassy Lake		

Table 2. Insecticides tested for resistance in Colorado potato beetle populations from western Canada using a filter paper bioassay

Insecticide class	Insecticide	Diagnostic concentration[a] (μg ai cm⁻²)	Mean mortality of susceptible strain[b] (%)	Mean mortality of resistant strain[b] (%)
Organophosphate	Azinphos-methyl (Guthion™) Bayer	1.05	95 ± 5	61 ± 13
	Methamidophos (Monitor™) Bayer	1.05	97 ± 9	72 ± 10
Pyrethroid	Permethrin (Ambush™) Zeneca	0.16	93 ± 3	0
Organochlorine	Endosulfan (Endosulfan™) Aventis	0.11	100	0
Carbamate	Carbaryl (Sevin™) Aventis	30.00	100	92 ± 2

[a] As used by French *et al.* 1992.

[b] Susceptible and resistant laboratory strains were obtained from S. Hilton, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario (Hilton *et al.* 1998). Ten egg masses of each strain were tested against each insecticide.

French *et al.* (1992) (Table 2). A single egg mass, with the excess foliage removed, was placed on the insecticide treated filter paper in a Petri dish and incubated at 23°C and 16L:8D photoperiod. When at least 50% of the eggs hatched, the filter paper was moistened with about 0.3 mL water. The dish was sealed with parafilm, and the number of dead and live larvae were counted 24 h later. A larva that did not return to its upright position after having been placed on its dorsal side was considered dead. Egg masses for the controls were placed on acetone treated filter paper. Ten egg masses (> 15 eggs per egg mass) per population per insecticide were tested. We followed the classification scheme of Kennedy and French (1994); egg masses showing < 50% mortality were classified as resistant. The proportion of egg masses within each population showing resistance (*i.e.* < 50% mortality) to the diagnostic concentrations of French *et al.* (1992) was used to classify the populations according to their susceptibility as follows: resistant (> 80% of the egg masses had < 50% mortality); intermediate (between 20 to 79% of the egg masses had < 50% mortality); and susceptible (between 0 to 19% of the egg masses had < 50% mortality). Controls for all populations from the three provinces were run simultaneously.

To verify the diagnostic concentrations against known resistant and susceptible populations of the beetle, we tested 10 egg masses per insecticide each from susceptible and resistant populations originating from populations from southern Ontario (Hilton *et al.* 1998). The resistant population was resistant to organochlorine, pyrethroid and organophosphate insecticides, with the exception that it had lost its resistance to carbofuran and resistance to azinphos-methyl had decreased (S. Hilton, personal communication).

RESULTS

Populations from all three western provinces demonstrated presence of individuals with resistance to one or more of the insecticides (Table 3). Of the 12 populations tested from Manitoba, 6 were classified as having resistance and 6 showed intermediate susceptibility to permethrin (Fig. 1; Table 3). Popula-

Table 3. Mean percent mortality and range per egg mass of *Leptinotarsa decemlineata* for each insecticide and population tested. Numbers in bold representant resistant populations

	Mean percent mortality (range) per insecticide				
Population number	Azinphos-methyl	Methamidophos	Permethrin	Endosulfan	Carbaryl
Alberta					
1	81 (5-100)	99 (93-100)	77 (11-100)	100	100
2	84 (60-100)	86 (54-100)	55 (14-100)	100	100
3	92 (77-100)	71 (3-100)	80 (40-95)	99 (96-100)	99 (92-100)
4	97 (74-100)	99 (95-100)	82 (27-100)	100	100
5	78 (24-100)	74 (3-100)	27 (0-84)	80 (0-100)	92 (24-100)
6	80 (38-100)	90 (18-100)	69 (14-100)	99 (96-100)	100
7	88 (66-100)	88 (45-100)	62 (15-100)	97 (85-100)	99 (96-100)
8	86 (67-100)	93 (65-100)	68 (16-100)	99 (96-100)	100
9	83 (17-100)	70 (26-100)	49 (0-86)	76 (22-100)	98 (75-100)
10	87 (33-100)	67 (28-100)	42 (0-95)	85 (11-100)	99 (95-100)
11	92 (75-100)	82 (3-100)	73 (23-100)	98 (90-100)	97 (83-100)
12	90 (72-100)	71 (19-100)	63 (9-100)	95 (50-100)	99 (87-100)
13	83 (32-100)	74 (23-100)	54 (5-88)	93 (79-100)	97 (79-100)
Saskatchewan					
1	74 (41-100)	84 (30-100)	87 (38-100)	100	99 (97-100)
2	87 (60-100)	88 (66-100)	46 (0-100)	97 (83-100)	99 (96-100)
3	77 (47-97)	88 (45-100)	48 (0-95)	95 (80-100)	94 (48-100)
4	82 (40-100)	98 (92-100)	76 (46-100)	100	99 (94-100)
Manitoba					
1	87 (29-100)	92 (29-100)	64 (0-100)	82 (17-100)	99 (95-100)
2	57 (0-96)	69 (23-100)	39 (0-100)	85 (25-100)	95 (73-100)
3	78 (4-100)	78 (9-100)	**30 (0-69)**	56 (9-100)	99 (91-100)
4	78 (39-100)	69 (6-100)	**26 (0-52)**	**17 (0-93)**	83 (23-100)
5	74 (7-100)	82 (13-100)	**14 (0-67)**	**28 (0-94)**	97 (79-100)
6	39 (0-100)	43 (15-90)	**<1 (0-3)**	88 (39-100)	43 (0-89)
7	57 (21-92)	73 (0-100)	32 (5-67)	**35 (0-100)**	57 (0-95)
8	**19 (0-100)**	**17 (0-100)**	48 (0-98)	50 (19-86)	**29 (0-100)**
9	**28 (0-71)**	**30 (0- 67)**	**1 (0-7)**	**39 (6-82)**	40 (0-89)
10	52 (0-100)	**36 (0-93)**	38 (0-100)	57 (13-100)	57 (8-100)
11	39 (0-80)	34 (0-100)	45 (4-100)	37 (0-100)	**13 (0-71)**
12	40 (0-81)	47 (8-92)	**12 (0-67)**	77 (53-100)	46 (6-100)

tions showed highest susceptibility to carbaryl, with only two of the 12 populations classified as having resistance to this insecticide. Four of the 12 populations were classified as having resistance and 7 showed an intermediate level of susceptibility to endosulfan. Two populations showed resistance to azinphos-methyl and 3 populations to methamidophos, with 7 and 8 showing intermediate levels of susceptibility. One population (population 1) of the 12 tested was classified as highly susceptible to all three chemicals, azinphos-methyl, methamidophos, and carbaryl; no populations were found to be highly susceptible to permethrin. Several populations demonstrated multiple resistance with population 8 demonstrating resistance to azinphos-methyl, methamidophos, and carbaryl and population 9 to azinphos-methyl, methamidophos, permethrin and endosulfan.

From Saskatchewan, only two populations of the four tested showed intermediate susceptibility to permethrin and azinphos-methyl (Fig. 1). All four populations were highly susceptible to the methamidophos, endosulfan and carbaryl.

In Alberta, an intermediate level of susceptibility to permethrin was recorded in 12 of the 13 populations tested (Fig. 1). Of the two organophosphates tested, two populations showed intermediate susceptibility to azinphos-methyl and four populations to metha-

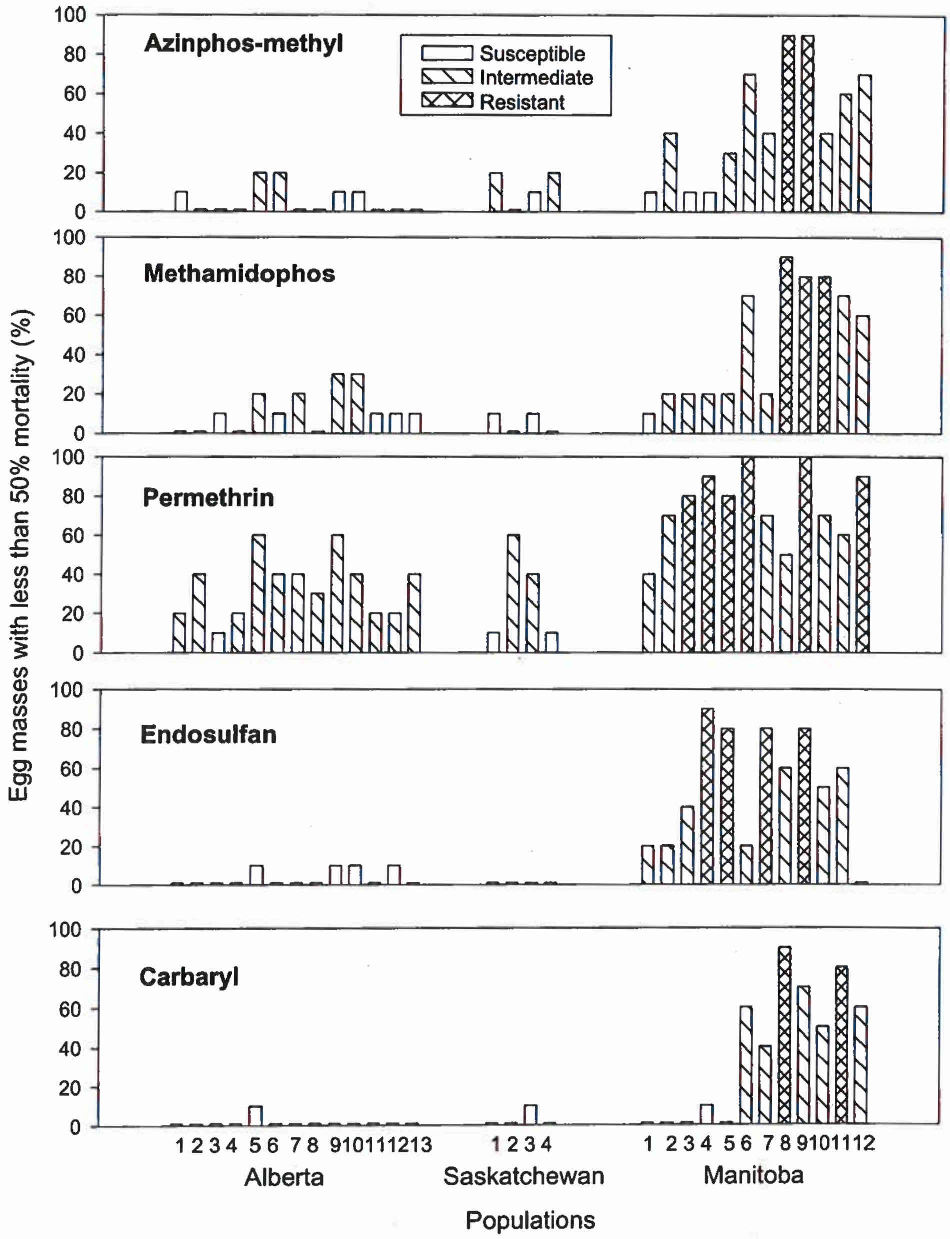

Figure 1. Proportion of egg masses (%) of *Leptinotarsa decemlineata* from the Canadian prairie provinces with less than 50% mortality after exposure to insecticides. Susceptibility categories are defined as follows: Resistant when > 80% of the egg masses had < 50% mortality; Intermediate when between 20 and 79% of the egg masses had < 50% mortality; and Susceptible when between 0 and 19% of the egg masses had < 50% mortality (see Kennedy and French 1994). Insecticide concentrations used were those previously determined as diagnostic for resistant and susceptible populations from North Carolina (French *et al.* 1992).

midophos. All 13 populations were classified as highly susceptible to endosulfan and carbaryl; 10 of the populations were highly susceptible to at least three insecticide classes.

Controls for all populations from the three provinces showed no mortality over the 24-hr assay period. The application of the French *et al.* (1992) diagnostic concentrations to laboratory-reared Canadian susceptible and resistant strains of potato beetles confirmed the suitability of these diagnostic concentrations used for this survey (Table 2). The intermediate resistance response of the resistant population to azinphos-methyl and methamidophos was as expected for this colony (S. Hilton, personal communication).

DISCUSSION

Populations from all three western provinces demonstrated some level of resistance to one or more of the insecticides tested. Resistance was most prevalent in populations from Manitoba, which is consistent with the results of a previous survey, where populations from 21 of 55 potato fields in Manitoba were found to be resistant to at least one of the nine insecticides tested (Gavloski 1997). Although the diagnostic concentrations used in this study were calculated for resistant and susceptible populations from North Carolina, they were verified by Stewart *et al.* (1997) for populations from Prince Edward Island and Ontario, and for a resistant and susceptible population from Ontario in this study. However, we have chosen to report our results according to level of susceptibility to French *et al.*'s (1992) diagnostic concentrations rather than to probability of field control (Kennedy and French 1994), because base-line data on the susceptibility of beetle populations from western Canada had not yet been determined and consequently, local resistant and susceptible populations were not available for us to verify these diagnostic concentrations. The results of the present study can now be used as a base-line to establish local dose: response regressions in order to fine tune, if necessary, the diagnostic concentrations we used for further monitoring of the evolution of resistance in the prairie provinces. These results can also be used as a base to measure the future development of resistance to insecticides by Colorado potato beetles in the Canadian prairie provinces.

In our study, populations most commonly showed some level of resistance to the pyrethroid, permethrin. Resistance to pyrethroids can develop quite rapidly. Harris and Svec (1981) reported low levels of resistance to pyrethroids in field populations of CPB from Sherbrooke, Quebec in 1979, however by 1982, greater than 23-fold resistance was reported in this population (Harris and Turnbull 1986). In a laboratory bioassay, CPB selected sequentially for fenvalerate exhibited a 1700 fold increase in resistance to this insecticide within eight generations (Huang *et al.* 1994, 1995). The prevalence of populations with low susceptibility to permethrin throughout all three western provinces and especially in Manitoba, should be a warning signal to producers that pyrethroids should be used cautiously.

Our data also shows that the development of resistance to organophosphate insecticides may soon occur in western Canada. In Manitoba, 17% of the populations were classified as having resistance to azinphos-methyl and 25% to methamidophos. However, 58 and 67% of the populations were classified as having intermediate levels of susceptibility to these two insecticides in Manitoba, and 15 and 23% had intermediate susceptibility in Alberta. This is an indication that the selection of resistant beetles has already started and these populations typify a "mixed" population.

In all three provinces, there were populations classified as highly susceptible to at least one or more insecticides. The numbers of highly susceptible populations were much greater in Alberta and Saskatchewan than in Manitoba. However, even within these susceptible populations, the range of survival within the bioassay units varied considerably, ranging from 0-100% for some insecticides. Although such natural variations are common, it underscores that there is always the potential for the rapid development of resistance, even in a highly susceptible population and that the development of resistance can be very localized. A similar variation was observed in the populations classified as having intermediate levels of susceptibility. Continuous pressure by the use of insecticides within the same class, especially on these intermediate populations, would result in the eventual selection of a resistant population.

The presence of larger numbers of populations with low susceptibility in Manitoba as compared to Alberta and Saskatchewan may be attributed to the recent rapid increase in potato acreage in Manitoba (62% since 1992) (Statistics Canada 1999) resulting in an increase in Colorado potato beetle populations, and the need to control these populations using insecticides (J. Gavloski, personal communication). In Saskatchewan and Alberta, Colorado potato beetle populations have been traditionally low, but with the expanding potato industry, an increase in the Colorado potato beetle population is expected, which in turn could result in increased insecticide applications and the eventual rapid development of resistance. Thus caution must be exercised and a resistance management program should be implemented immediately in order to prevent, or at least delay, further selection of insecticide resistant populations.

ACKNOWLEDGEMENTS

We thank the many potato producers and the following for providing us with beetles, insecticide or information: Connie Achtymichuk, Lake Diefenbaker Potato Cooperative; Jackie Bantle, University of Saskatchewan; David Feindel, Bayer Inc.; John Gavloski, Manitoba Agriculture; Denis Kirkham, Canadian Food Inspection Agency; Claire Martin, Agriculture & Agri-Food Canada; Don Pittman, Southern Agri-Services Ltd.; and Stephanie Hilton and Jay Whistlecraft, Agriculture and Agri-Food Canada, London, Ontario. The Potato Growers of Alberta provided financial assistance. Drs. Gilles Boiteau and Jeff Stewart provided comments on the manuscript. This is LRC contribution number 3870033.

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Agriculture and Agri-Food Canada
Research Branch

Agriculture et Agroalimentaire Canada
Direction générale de la recherche

Lethbridge Research Centre
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LETHBRIDGE AB T1J 4B1
Telephone: (403) 327-4561
Facsimile: (403) 382-3156

February 13, 2002

RECEIVED FEB 25 2002

Approved Mar 12/02
$5,000 —
Pd Mar 27/02

Fund Centre SPA A01802

MR. V. WARKENTIN
POTATO GROWERS OF ALBERTA
6008 - 46TH AVENUE
TABER AB T1G 2B1

Dear Vern:

Application for funding: "Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle"

Enclosed please find an AARI Matching Grants Application form requesting funding from PDI in the amount of $5,000 for 2002/2003. This form was submitted to AARI as a progress report for the second year of funding from PDI ($5,000; SPA A01802) and Perry Produce ($5,760 in kind) which was matched by the AARI Matching Grants Program ($10,760), and as a request for a third year of funding.

In addition to AARI/PDI monies, this past year we were fortunate to have received an in kind contribution from Perry Produce Ltd., which we hope to renew this year. Working directly with as commercial producer has enabled us to monitor beetle populations, as per PDI's request, and to help evaluate potential control methods in a commercial setting.

Looking forward to receiving continued support from PGA/PDI for this important research on management of an important pest of potatoes worldwide.

Sincerely,

for Peter Burnett, Ph.D.
Assistant Director

MSG:kg
Encl.

MARK GOETTEL
CROP SCIENCES SECTION
PO BOX 3000
LETHBRIDGE AB T1J 4B1
CANADA

Canada

Alberta Agricultural Research Institute (AARI)
Matching Grants Program Renewal Application - 2002/2003

Office Use Only: Date Received

1. *Project Title*

Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle
Application Number: 2000M701

2. *Principal Researcher*

Name	Dr. Mark S. Goettel	Mailing Address	P.O. Box 3000
Title	Insect Pathologist		Lethbridge, AB T1J 4B1
Organization	Agric. & Agri-Food Canada		
Department	Crop Sciences Section	Telephone #	403-317-2264
		Fax #/Email	403-382-3156/goettel@em.agr.ca

3. *Co-applicants*

Name	Dr. Christine Noronha	Mailing Address	P.O. Box 1210
Title	Research Scientist		Charlottetown, PEI, C1A 7M8
Organization	Agriculture & Agri-Food		
Department		Telephone #	902-566-6844
		Fax #/Email	902-566-6821/noronhac@em.agr.ca

Name	Dr. David W.A. Hunt	Mailing Address	2585 Highway 20 E
Title	Research Scientist		Harrow, ON
Organization	Agriculture & Agri-Food		N0R 1G0
Department		Telephone #	519-738-2251 ext 427
		Fax #/Email	519-738-2929/huntd@em.agr.ca

4. *Outline of Research Proposal (one page may be added to this block if required)*

A. Background, Objectives and Key Results Expected

i. *Background* (Provide a brief statement indicating what this research is about and why it is considered important)

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) is considered the most destructive foliage feeding pest of potatoes in the world. The beetle is now present virtually everywhere potatoes are grown in Canada. Until recently, potato producers have relied solely on the use of insecticides to control this pest. However, this total dependence on insecticides has resulted in the rapid development of resistance. By 1995, beetle populations in Ontario and Québec were virtually uncontrollable which resulted in the emergency registration of a new insecticide, imidacloprid (Admire®). Although this product is 10 times more costly for a grower than previously used chemicals, the growers have had little choice but to use this, the only economically viable control method. Furthermore, there have already been reports of populations resistant to imidacloprid in the US (Grafius, 1999), and insecticide resistance will develop rapidly in a situation where only one chemical is used

2. To evaluate its use in an insecticide resistance management program.

nplementation of such an alternative control method in the management of the Colorado potato beetle will delay development of resistance to chemicals, decrease dependency on use of chemical insecticides, and allow development of an IPM strategy against this pest in Alberta.

ii. Key Results Expected

The objective is to determine the role that the fungus *Beauveria bassiana* could play in the resistance management of the pesticides being used for control of the Colorado potato beetle. Virulence of the fungus towards the soil stages of the beetle will be determined. Furthermore, it will be determined to what extent the fungus can affect overwintering adult beetle populations. The strategy would be to contaminate migrating adults with spores of the fungus as they leave the potato fields in late summer in search of overwintering sites. Results on persistence, virulence and effects of contamination of overwintering sites will provide information for use of this fungus in IPM.

2000 -2001: Virulence of the fungus will be determined. How many spores must the beetles contact in order to get infected? Persistence of the fungus under field conditions will be determined. Will enough spores remain viable from year to year to ensure continued efficacy? Will beetles be contaminated with an adequate amount of inoculum as they bury in the soil which contains spores?

2001- 2002: How many of the inoculated adults that diapaused under natural conditions became infected and died before emergence in the spring? How many spores persisted over the season? Should beetles be inoculated directly, or can they pick up an adequate amount of inoculum from the soil? How do soil conditions .ffect spore persistence and virulence?

2002-2003: Do adults that succumbed in the soil produce adequate numbers of spores to ensure infection of the next generation? Should spores be reapplied yearly? Should we proceed with registration? Final report and manuscripts will be prepared.

B. Progress to Date

Provide a concise report of the results achieved. It should contain a summary of the data collected and any preliminary conclusions made. The report should clearly state whether the results expected under the action plan for the preceding year have been achieved. If not, provide reasons. Include all changes or modifications to original expectations, citing reasons. One page may be added to this section if required.

Despite difficulties in obtaining funding that would have adequately supported our research efforts at 3 centres in 3 provinces, we've been able to make very good progress, and overall, the project remains on track, although it is apparent that larger field trials will no longer be warranted, as we have not yet progressed to that stage, and don't expect to by the end of next year. Larger field trials would require regulatory approvals which would consume too much of our time at this point. We feel that concentrating on field cage trials will be more fruitful, especially due to difficulties encountered during the last overwintering season.

1) Laboratory assays were carried out against pupating larvae at 3 soil moistures replicating the results obtained and reported last year. High mortality (>80%) was obtained at all spore concentrations at 7% soil moisture, but at greater soil moistures (12 and 14%), lower mortalities were obtained at the lower spore concentrations. However at the highest spore concentration, over 90% mortality was obtained at all soil moisture levels, demonstrating the important effect of soil moisture (greater moistures are detrimental to infection).

2) Field cage trials against larvae and summer generation emerging adults were carried out. Fourth instar larvae

We are once again approaching several potential funding sources (McCain's, Keystone Vegetable Producers Association, Potato Snackfood's Association, and the PEI Potato Board) and are confident that this year we'll be le to fully match AARI's allotted contribution of $25,000. Full funding of this project will bring the program back on track and into full speed in the next year.

The benefits of the tripartite collaboration (PEI, Ontario, and Alberta) are evident. Evaluations with 3 different populations of beetles in 3 different soil types and under unique overwintering conditions provides us with information that will allow us to better understand the different parameters that are important in disease epizootiology and in development of this fungus as a management tool for the beetle. However, the potato wart hiatus in PEI prevented the PEI Potato growers from considering funding last year. Further studies at our field plots in Prince Edward Island, Ontario and Alberta will evaluate the effectiveness of *B. bassiana* for inducing mortality in overwintering beetle populations.

Laboratory assays will continue to evaluate the importance of soil type and moisture, dose, and temperature.

C. Research Plan

Hypothesis: Application of *Beauveria bassiana* to the soil at overwintering sites, or to adults as they emigrate from the fields will provide significant, longer term mortality to the overwintering populations, thereby contributing to overall population reductions and insecticide resistance management of the Colorado potato beetle.

Host-pathogen relationships and the effects of soil factors on fungal persistence and infectivity must be determined before larger scale field trials are initiated. The experiments are designed to obtain information on 1) the relationships etween fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions, 4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions. Unless otherwise noted, all experiments will be arranged as complete randomised block designs with at least 3 replications of 50 beetles per treatment and control. Every experiment will be repeated and all field plot designs and statistical analyses will be performed in consultation with statisticians at the Lethbridge Research Centre.

1) Susceptibility of the soil stages.

Hypothesis: Spores within the soil will incite disease and cause mortality in the soil dwelling stages of the insect.

Spores of *B. bassiana* will be mixed uniformly into sterile soil at several concentrations (approximately 10^4 to 10^8 conidia/g dry weight of soil). Water will be added to obtain a moisture level of 9% (w/w). Each soil/dose combination will be dispensed into three plastic containers. Fifty mature larvae will be placed onto the surface of each container and allowed to enter the soil and pupate. After incubation, emergent adults will be transferred to clean containers and observed daily for mortality. The soil will be examined for presence of dead prepupae, larvae or adults. Cause of death will be determined by examination of hemolymph for fungal blastospores or by incubating surface sterilized cadavers under high humidity and observing emergent fungus. Spore viabilities will be quantified at the start and end of the experiment by removing cores of soil from the containers and by standard dilution/plating techniques on selective media. Dose-mortality results will be analysed using probit analyses.

2) Influence of soil type

Hypothesis: Soil type will influence persistence and pathogenicity.

The experimental design will be similar to the one described above except that only one dose will be used. Different soil types and moisture levels will be incorporated into the bioassay design. Soil types to be tested will be those characteristic of habitats where pupation and overwintering occurs. Comparisons will also be made between sterilized term, the fungus could be integrated into a more comprehensive IPM program for the beetle, eventually eliminating or reducing dependence on chemical pesticides. There is no guarantee that the newly registered pesticides now entering the market will stand the test of time as far as safety and long term sustainability is concerned. Exclusive reliance on any one method of control, be it chemical pesticides or transgenic potato, will eventually result in the development of resistance. Reduction on the reliance to chemical pesticides will contribute to the sustainability and competitiveness of the potato industry in Alberta.

This project will be the beginning of the development and implementation of a much more comprehensive IPM strategy for pests of potatoes. Other benefits of the proposed research include the possible feasibility of using this fungus for control of other soil-dwelling insect pests such as wireworms. Results of this project could also be used in the development and implementation of insecticide resistance management and IPM programs for other economically important pest insects in Alberta and elsewhere.

ii. *Computer Cost*

	NIL

Justification is required for requests over $500:

iii. *Publication Cost* **(specifically for this project□s results)**

	NIL

Justification is required if request is over $700:

iv. *Rentals and Leases*

	NIL

v. *Contract Personnel*

	NIL
TOTAL C	$6,200
TOTAL A + B + C	$46,300

D. Overhead Cost

9% of total AARI project costs, excluding benefits	$2,060
15% of industry/grower costs ($23,360)	$3,500

Indicate how overhead costs were calculated : 9% of project costs, excluding benefits for AARI portion; %15 of project costs, excluding benefits for industry portion

TOTAL AMOUNT REQUESTED FOR 2002-2003 (A + B + C + D) $51,860

7. Funding Support Applied for, Granted, or Promised for This Project From Sources Other Than Alberta Government Departments, Agencies or Programs

	Funding Agency	Amount of Grant in Cash	Amount of Grant In-kind	Total Amount Applied for, Granted or Promised	Date Grant Received or Expected
A.	Potato Growers of Alberta	$5,000		$5,000	Renewal expected April, 2002
B.	Perry Produce		$6,000	$6,000	Renewal expected April, 2002
C.	McCain Foods	10,000		10,000	Spring, 2002
D.	Keystone Vegetable Producers Association	5,000		5,000	Spring, 2002
E.	Stuart Cairns Memorial Potato Reserch Fund	5,000		5,000	Applied for

Total Amount Requested From Other Agencies (A + B + C +D) 31,000

8. Amount Requested From AARI

Total amount requested from AARI for the 2002-2003 fiscal year is $__25,000___. This amount should not exceed one half of the total amount requested for 2001-2002 or the difference between the total shown in block 9 for 2002-2003 and the contributions from other sources, whichever is less.

9. Terms and Conditions

A. This application is submitted, and will be evaluated, under the authority of the Alberta Agricultural Research Institute Act and the Farming for the Future Matching Grants Program Guidelines. The applicant accepts the conditions specified in the guidelines.

B. All completed applications submitted to AARI become the property of AARI and will not be returned to the applicant. While every effort will be made to keep the information contained in the application form confidential, the application review procedures require that copies of the application be distributed to a number of reviewers. The contents of this application may also be subject to access under the Freedom of Information and Protection of Privacy Act.

C. The decision of AARI's Board of Directors regarding this application is final.

[signature] 27/11/01

Principal Researcher's Signature   Date

Co-applicant□s Signature   Date

Co-applicant□s Signature   Date

Acknowledgement of Receipt

Please fill out the name, address and title information and submit this form with your original application (14 copies of this sheet are not required). The form will be returned to you to acknowledge receipt of your Matching Grants Program application by the Alberta Agricultural Research Institute.

Principal Researcher Dr. Mark Goettel

Mailing Address Box 3000

Lethbridge, Alberta T1J 4B1

This is to acknowledge receipt of your proposal entitled:

For Office Use Only

Your application is:

______ **complete as received**

______ **incomplete. Please forward immediately:**

______ **14 photocopies of your application**

______ **original signatures in Blocks 11, 12 & Employers□ Approval Form:**

______ animal care certificate

______ completed biographical data for:

______

You will be provided with a written response regarding the status of your application when the evaluation process is completed. We expect the evaluation process to be completed by March, 2002.

Date Received: ______________________________

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada
Research Branch
Direction générale de la recherche

Lethbridge Research Centre
5403 1st Ave South
PO Box 3000
LETHBRIDGE AB T1J 4B1
Telephone: (403) 327-4561
Facsimile: (403) 382-3156

February 13, 2002

RECEIVED FEB 25 2002

MR. V. WARKENTIN
POTATO GROWERS OF ALBERTA
6008 - 46TH AVENUE
TABER AB T1G 2B1

Fund Centre SPA A01802

Passed accepted!

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Looking forward to receiving continued support from PGA/PDI for this important research on management of an important pest of potatoes worldwide.

Sincerely,

for Peter Burnett, Ph.D.
Assistant Director

MSG:kg
Encl.

MARK GOETTEL
CROP SCIENCES SECTION
PO BOX 3000
LETHBRIDGE AB T1J 4B1
CANADA

Alberta Agricultural Research Institute (AARI)
Matching Grants Program Renewal Application - 2002/2003

Office Use Only: Date Received

1. *Project Title*

Development of Microbial Control as a Component of Insecticide Resistance Management of the Colorado Potato Beetle

Application Number: 2000M701

2. *Principal Researcher*

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3. *Co-applicants*

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4. *Outline of Research Proposal (one page may be added to this block if required)*

A. Background, Objectives and Key Results Expected

i. *Background* (Provide a brief statement indicating what this research is about and why it is considered important)

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) is considered the most destructive foliage feeding pest of potatoes in the world. The beetle is now present virtually everywhere potatoes are grown in Canada. Until recently, potato producers have relied solely on the use of insecticides to control this pest. However, this total dependence on insecticides has resulted in the rapid development of resistance. By 1995, beetle populations in Ontario and Québec were virtually uncontrollable which resulted in the emergency registration of a new insecticide, imidacloprid (Admire®). Although this product is 10 times more costly for a grower than previously used chemicals, the growers have had little choice but to use this, the only economically viable control method. Furthermore, there have already been reports of populations resistant to imidacloprid in the US (Grafius, 1999), and insecticide resistance will develop rapidly in a situation where only one chemical is used

2. To evaluate its use in an insecticide resistance management program.

Implementation of such an alternative control method in the management of the Colorado potato beetle will delay development of resistance to chemicals, decrease dependency on use of chemical insecticides, and allow development of an IPM strategy against this pest in Alberta.

ii. Key Results Expected

The objective is to determine the role that the fungus *Beauveria bassiana* could play in the resistance management of the pesticides being used for control of the Colorado potato beetle. Virulence of the fungus towards the soil stages of the beetle will be determined. Furthermore, it will be determined to what extent the fungus can affect overwintering adult beetle populations. The strategy would be to contaminate migrating adults with spores of the fungus as they leave the potato fields in late summer in search of overwintering sites. Results on persistence, virulence and effects of contamination of overwintering sites will provide information for use of this fungus in IPM.

2000 -2001: Virulence of the fungus will be determined. How many spores must the beetles contact in order to get infected? Persistence of the fungus under field conditions will be determined. Will enough spores remain viable from year to year to ensure continued efficacy? Will beetles be contaminated with an adequate amount of inoculum as they bury in the soil which contains spores?

2001- 2002: How many of the inoculated adults that diapaused under natural conditions became infected and died before emergence in the spring? How many spores persisted over the season? Should beetles be inoculated directly, or can they pick up an adequate amount of inoculum from the soil? How do soil conditions affect spore persistence and virulence?

2002-2003: Do adults that succumbed in the soil produce adequate numbers of spores to ensure infection of the next generation? Should spores be reapplied yearly? Should we proceed with registration? Final report and manuscripts will be prepared.

B. Progress to Date

Provide a concise report of the results achieved. It should contain a summary of the data collected and any preliminary conclusions made. The report should clearly state whether the results expected under the action plan for the preceding year have been achieved. If not, provide reasons. Include all changes or modifications to original expectations, citing reasons. One page may be added to this section if required.

Despite difficulties in obtaining funding that would have adequately supported our research efforts at 3 centres in 3 provinces, we've been able to make very good progress, and overall, the project remains on track, although it is apparent that larger field trials will no longer be warranted, as we have not yet progressed to that stage, and don't expect to by the end of next year. Larger field trials would require regulatory approvals which would consume too much of our time at this point. We feel that concentrating on field cage trials will be more fruitful, especially due to difficulties encountered during the last overwintering season.

1) Laboratory assays were carried out against pupating larvae at 3 soil moistures replicating the results obtained and reported last year. High mortality (>80%) was obtained at all spore concentrations at 7% soil moisture, but at greater soil moistures (12 and 14%), lower mortalities were obtained at the lower spore concentrations. However at the highest spore concentration, over 90% mortality was obtained at all soil moisture levels, demonstrating the important effect of soil moisture (greater moistures are detrimental to infection).

2) Field cage trials against larvae and summer generation emerging adults were carried out. Fourth instar larvae

We are once again approaching several potential funding sources (McCain's, Keystone Vegetable Producers Association, Potato Snackfood's Association, and the PEI Potato Board) and are confident that this year we'll be able to fully match AARI's allotted contribution of $25,000. Full funding of this project will bring the program back on track and into full speed in the next year.

The benefits of the tripartite collaboration (PEI, Ontario, and Alberta) are evident. Evaluations with 3 different populations of beetles in 3 different soil types and under unique overwintering conditions provides us with information that will allow us to better understand the different parameters that are important in disease epizootiology and in development of this fungus as a management tool for the beetle. However, the potato wart hiatus in PEI prevented the PEI Potato growers from considering funding last year. Further studies at our field plots in Prince Edward Island, Ontario and Alberta will evaluate the effectiveness of *B. bassiana* for inducing mortality in overwintering beetle populations.

Laboratory assays will continue to evaluate the importance of soil type and moisture, dose, and temperature.

C. Research Plan

Hypothesis: Application of *Beauveria bassiana* to the soil at overwintering sites, or to adults as they emigrate from the fields will provide significant, longer term mortality to the overwintering populations, thereby contributing to overall population reductions and insecticide resistance management of the Colorado potato beetle.

Host-pathogen relationships and the effects of soil factors on fungal persistence and infectivity must be determined before larger scale field trials are initiated. The experiments are designed to obtain information on 1) the relationships between fungal dose and infection/mortality under soil exposure conditions, 2) the influence of soil type, temperature and moisture on fungal viability, persistence and pathogenicity, 3) persistence of fungal spores under field conditions, 4) transmission of spores by emigrating adults and subsequent contamination of overwintering sites, 5) efficacy of spores in soil against first generation and overwintering beetles under field conditions. Unless otherwise noted, all experiments will be arranged as complete randomised block designs with at least 3 replications of 50 beetles per treatment and control. Every experiment will be repeated and all field plot designs and statistical analyses will be performed in consultation with statisticians at the Lethbridge Research Centre.

1) Susceptibility of the soil stages.

Hypothesis: Spores within the soil will incite disease and cause mortality in the soil dwelling stages of the insect.

Spores of *B. bassiana* will be mixed uniformly into sterile soil at several concentrations (approximately 10^4 to 10^8 conidia/g dry weight of soil). Water will be added to obtain a moisture level of 9% (w/w). Each soil/dose combination will be dispensed into three plastic containers. Fifty mature larvae will be placed onto the surface of each container and allowed to enter the soil and pupate. After incubation, emergent adults will be transferred to clean containers and observed daily for mortality. The soil will be examined for presence of dead prepupae, larvae or adults. Cause of death will be determined by examination of hemolymph for fungal blastospores or by incubating surface sterilized cadavers under high humidity and observing emergent fungus. Spore viabilities will be quantified at the start and end of the experiment by removing cores of soil from the containers and by standard dilution/plating techniques on selective media. Dose-mortality results will be analysed using probit analyses.

2) Influence of soil type

Hypothesis: Soil type will influence persistence and pathogenicity.

The experimental design will be similar to the one described above except that only one dose will be used. Different soil types and moisture levels will be incorporated into the bioassay design. Soil types to be tested will be those characteristic of habitats where pupation and overwintering occurs. Comparisons will also be made between sterilized term, the fungus could be integrated into a more comprehensive IPM program for the beetle, eventually eliminating or reducing dependence on chemical pesticides. There is no guarantee that the newly registered pesticides now entering the market will stand the test of time as far as safety and long term sustainability is concerned. Exclusive reliance on any one method of control, be it chemical pesticides or transgenic potato, will eventually result in the development of resistance. Reduction on the reliance to chemical pesticides will contribute to the sustainability and competitiveness of the potato industry in Alberta.

This project will be the beginning of the development and implementation of a much more comprehensive IPM strategy for pests of potatoes. Other benefits of the proposed research include the possible feasibility of using this fungus for control of other soil-dwelling insect pests such as wireworms. Results of this project could also be used in the development and implementation of insecticide resistance management and IPM programs for other economically important pest insects in Alberta and elsewhere.

ii. *Computer Cost*

NIL

Justification is required for requests over $500:

iii. *Publication Cost* (specifically for this project's results)

NIL

Justification is required if request is over $700:

iv. *Rentals and Leases*

NIL

v. *Contract Personnel*

NIL

TOTAL C	**$6,200**
TOTAL A + B + C	**$46,300**

D. Overhead Cost

9% of total AARI project costs, excluding benefits	$2,060
15% of industry/grower costs ($23,360)	$3,500

Indicate how overhead costs were calculated : 9% of project costs, excluding benefits for AARI portion; %15 of project costs, excluding benefits for industry portion

TOTAL AMOUNT REQUESTED FOR 2002-2003 (A + B + C + D) $51,860

7. Funding Support Applied for, Granted, or Promised for This Project From Sources Other Than Alberta Government Departments, Agencies or Programs

	Funding Agency	Amount of Grant in Cash	Amount of Grant In-kind	Total Amount Applied for, Granted or Promised	Date Grant Received or Expected
A.	Potato Growers of Alberta	$5,000		$5,000	Renewal expected April, 2002
B.	Perry Produce		$6,000	$6,000	Renewal expected April, 2002
C.	McCain Foods	10,000		10,000	Spring, 2002
D.	Keystone Vegetable Producers Association	5,000		5,000	Spring, 2002
E.	Stuart Cairns Memorial Potato Reserch Fund	5,000		5,000	Applied for

Total Amount Requested From Other Agencies (A + B + C +D) 31,000

8. Amount Requested From AARI

Total amount requested from AARI for the 2002-2003 fiscal year is $__25,000___. This amount should not exceed one half of the total amount requested for 2001-2002 or the difference between the total shown in block 9 for 2002-2003 and the contributions from other sources, whichever is less.

9. Terms and Conditions

A. This application is submitted, and will be evaluated, under the authority of the Alberta Agricultural Research Institute Act and the Farming for the Future Matching Grants Program Guidelines. The applicant accepts the conditions specified in the guidelines.

B. All completed applications submitted to AARI become the property of AARI and will not be returned to the applicant. While every effort will be made to keep the information contained in the application form confidential, the application review procedures require that copies of the application be distributed to a number of reviewers. The contents of this application may also be subject to access under the Freedom of Information and Protection of Privacy Act.

C. The decision of AARI's Board of Directors regarding this application is final.

[signature] 27/11/01

Principal Researcher's Signature Date

Co-applicant□s Signature　　　　　　　　　　Date

Co-applicant□s Signature　　　　　　　　　　Date

Acknowledgement of Receipt

Please fill out the name, address and title information and submit this form with your original application (14 copies of this sheet are not required). The form will be returned to you to acknowledge receipt of your Matching Grants Program application by the Alberta Agricultural Research Institute.

Principal Researcher Dr. Mark Goettel

Mailing Address Box 3000

Lethbridge, Alberta T1J 4B1

This is to acknowledge receipt of your proposal entitled:

For Office Use Only

Your application is:

__________ **complete as received**

__________ **incomplete. Please forward immediately:**

__________ **14 photocopies of your application**

__________ **original signatures in Blocks 11, 12 & Employers□ Approval Form:**

__________ animal care certificate

__________ completed biographical data for:

You will be provided with a written response regarding the status of your application when the evaluation process is completed. We expect the evaluation process to be completed by March, 2002.

Date Received: ______________________________