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JUL 21 2000

LCMR Work Program 1997

I. PROJECT TITLE: Biological Control of Agricultural Pests

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Total Biennial Project Budget: 200,000

\$ LCMR: 200,000

\$ LCMR Amount Spent: 200,000

\$ LCMR Balance: 0

A. Legal Citation: ML 1997, Chap. 216, Sec. 15, Subd. 7(a)

BIOLOGICAL CONTROL OF AGRICULTURAL PESTS \$200,000

This appropriation is from the trust fund to the University of Minnesota to accelerate using biological control of pests in agricultural production systems. This appropriation is available until June 30, 2000, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. PROJECT SUMMARY AND RESULTS:

The four subprojects selected for funding focus on insect, plant pathogen, and weed pests of crop (corn, potato and cabbage) and animal (cow-calf, dairy and confined livestock) production systems in Minnesota. The ultimate goal of these subprojects is to reduce reliance upon chemical control for key pest species in selected agricultural production systems. A summary of results for each subproject is reported below.

III. SUMMARY of PROGRESS (by subproject)

Subproject A: Biological control of four introduced filth flies

Two collecting trips in Russia and Kazakhstan were conducted during the life of this project, one each in 1998 and 1999 (Obj. 1). I met and worked with Drs. Oleg Kovalev, Alexey Solodovnikov and Alexey Kuprijanov, all of which are entomologists associated with the Russian Academy of Sciences' Institute of Zoology. Kovalev helped me acquire copies of ca. 30 previously unavailable papers (in Russian) on flies and associated parasites. Over 15,000 fly puparia were obtained from pen and pasture habitats at 49 dairy farms throughout Russia and Kazakhstan. Identification of this material is mostly complete and candidate biological control agents are being reared at a USDA quarantine facility located in Florida. Thousands of *Spalangia*, *Muscidifurax* and *Trichopria* were obtained from field collected house fly and stable fly puparia at 16 dairies in each region in 1999. To transport parasites we used irradiated house flies (so no accidental release of flies would occur in Russia) produced in Gainesville. Upon arrival back in the US in 1999, I worked Dr. Chris Geden (ARS, Gainesville, FL) to transfer emerging parasites into rearing quarters within the ARS quarantine facility. Progress to date indicates at least one species of *Muscidifurax* and three of *Spalangia* have been established, but final identifications will not be obtained until the material is released from quarantine for lab propagation in Minnesota. A significant finding from the field work was that the geographic ranges of face fly and horn fly extend farther east and south than was previously recorded in the scientific literature. Work is continuing on this project using funds obtained from the MDA biological control program in 2000.

Subproject B: Interference of potato fungicides used for late blight control with parasitic fungi that kill aphids.

Field experiments were conducted for three consecutive field seasons to evaluate effects fungicides have on a group of beneficial fungi that help suppress green peach aphids (Result 1B). Research plots were located at the Rosemount Agricultural Experiment Station (Dakota County) and at the Central Lakes Ag Center, Staples (Todd County). Laboratory studies (Result 2B and 4B) were conducted to document the effects various fungicides have on the most common entomopathogenic fungus, *Conidiobolus thromboides*. The findings were that all commercially available fungicides have a detrimental effect on sporulation, germination, and the ability of *C. thromboides* to grow in culture. The goal of this study was to determine how best to conserve these naturally occurring beneficial fungi. Unfortunately, all potato fungicides tested were detrimental to a wide range on entomopathogenic fungi. In the course of this study we did discover that overuse of common insecticides was the key in triggering aphid outbreaks in potato. We have developed recommendations for growers to follow to prevent aphid outbreaks and using funds obtained from a USDA competitive grant we developed a web site for rapid dissemination of this information. An example of the type of information available to anyone can be found on:
<http://ipmworld.umn.edu/aphidalert/alert4.htm>.

Conclusions: We were hopeful that we would be able to advise potato producers which fungicides or fungicide combinations were the least detrimental to the beneficial fungi. However, our data indicate that all commonly used fungicides adversely impact several species of beneficial fungi. The conclusions are based on both laboratory and field experiments.

Based on the experiments conducted with this project, we have discovered that what flares green peach aphids in potato is multiple applications of broad spectrum insecticides targeted for other mid-season insects pests such as the potato leafhopper or Colorado potato beetle. By using no more than 2 insecticide applications in July, growers can maintain the natural enemy complex (predators and parasites) that will keep green peach aphids populations in check. Without these natural enemies, green peach aphids will build to damaging levels. We have also discovered that a key mid-season insect pest, potato leafhopper, is easily controlled by using ¼ the recommended rate, reducing insecticides costs while at the same time preserving natural enemies that keep green peach aphids under control.

Subproject C: Genetic engineering cover crops for improved biological weed management.

The goal of this project was to create a weed control system by genetically engineering weed-suppressing cover crops that can be controlled by safe and inexpensive compounds. We investigated approaches to improve the usefulness of cover crops in weed management. Cover crops suppress weeds thereby reducing herbicide use while decreasing soil erosion. But cover crops often compete with the crop for water and nutrients decreasing crop yield precluding their widespread use. We made progress towards developing a genetic switch called a promoter that may allow farmers to eliminate cover crop competition by application of an inexpensive, nontoxic chemical. During the grant period, we evaluated different plant promoters to assess their inducibility by nontoxic compounds. None of the candidate plant promoters tested appeared to provide sufficient inducibility. Therefore, we constructed a synthetic iron-inducible promoter based on a bacterial protein that binds to a specific DNA sequence in response to elevated cellular iron content. Our aim was to develop an iron-inducible promoter that "turns on" gene expression when plants are sprayed with a dilute iron solution. Iron is ubiquitous in nature and is nontoxic. The synthetic promoter was fused to a reporter gene called GUS that allows easy detection of gene expression. Transgenic tobacco plants were produced using this test system as a model for cover crops. The plants were treated with dilute iron solutions and tested for GUS expression. We observed iron induction of GUS activity in the plants and progeny of some of these plants suggesting that the synthetic promoter was iron inducible. In the second phase of the experiment, we fused the synthetic iron-inducible promoter to a senescence gene and genetically engineered tobacco plants again as a model for cover crops. Some progeny of the transgenic plants exhibited iron-inducible senescence. However, it was clear from these first tests that further modifications to the promoter were necessary to improve iron-inducibility and reduce background expression of the promoter in non-iron treated plants. Recombinant DNA modifications of the synthetic iron-inducible promoter were carried out and the modified synthetic promoters were tested using GUS in genetically engineered tobacco plants. While the modified promoters exhibited some improvement in iron-inducibility, it was apparent that further work is necessary to produce an iron-inducible genetic system that would confer inducible senescence. In summary, progress was achieved in producing a synthetic promoter that has the potential, with further recombinant DNA modification and testing, to be useful in conferring iron-inducible expression of senescence genes in cover crops. Although the project has terminated, we plan to continue this work to develop a useful promoter.

**Subproject D: Making Biological Control Work in Insecticide-Intensive Crops:
Cabbage in Minnesota**

Spinosad, a fermentation-derived toxin from an *Actinomycete* bacterium, provided excellent results in small-plot trials, compared with the standard insecticide, permethrin (as shown in previous LCMR reports; see also Hines 1998). Specifically, efficacy of Spinosad was excellent against all three caterpillar pests, and most importantly against the cabbage looper, the most damaging pest in Minnesota. In addition, Spinosad at ½ the maximum rate, continued to control the larval pests, yet also allowed for some conservation of the naturally occurring beneficial insect fauna (predators and parasites). Results of these experiments were disseminated to growers through meetings, field demonstrations, extension publications, and incorporated into recommendations available on a vegetable pest management web site (<http://www.vegedge.umn.edu/>).

IV. OUTLINE OF PROJECT RESULTS: - Organized by SUBPROJECTS A, B, C & D

Subproject A Title: Biological control of four introduced filth flies.

Overall Budget: \$22,080

Amount Spent: \$22,080

Result 1A: Examine museum material and add new specimens from Kirgiz Steppe, Russia for parasitoids of dung fauna.

Two collecting trips were conducted and over 15,000 specimens returned for identification.

Result 2A: Collect and ship live parasitoid material to USDA quarantine laboratories in Gainesville, Florida

Thousands of specimens of parasitized pupae were shipped to the USDA quarantine facility in Gainesville.

Result 3A: Establish parasitoids in culture in Gainesville, Florida to determine the parasites' host range and life history

At least four parasite species have been successfully reared at the quarantine facility. Release of reared material will occur if no animal pathogens are recovered from these specimens for detailed laboratory studies and host testing required before parasites can be cleared by USDA for release in the field.

Result 4A: Final report written and data presented to appropriate audiences

This report and presentations at a scientific meeting have occurred and will continue as new information is obtained from further analysis.

Subproject B Title: Interference of potato fungicides used for late blight control with parasitic fungi that kill aphids.

Overall Budget: \$68,459

Amount Spent: \$68,459

Result 1B: Establish field plots and test commercially available fungicides used on potato for effects on entomopathogenic fungi of the green peach aphid.

Objective completed by conducting experiments in three field seasons.

Result 2B: Culture entomopathogenic fungi in the laboratory and evaluate effects of fungicides *in vitro* and disseminate preliminary results of field studies to potato growers.

We thoroughly tested 10 fungicides on their *in vitro* effects on the fungus *C. thomoboides*.

Result 3B: Validate previous field studies by conducting a second year of trials to determine effects of potato fungicides on entomopathogenic fungi of the green peach aphid.

We were able to conduct three field studies, one each in 1997, 1998 and 1999.

Result 4B: Evaluate additional materials in laboratory assays

We tested the new fungicide, Quadris, when it became available to growers in 1999 using our laboratory bioassay.

Result 5B: Conduct data analysis and report preliminary findings to potato growers.

Many presentations were given to potato growers at field days, annual meetings and through articles written for the principal trade magazine, Valley Potato Grower. Our research group has been invited to speak to the National Potato Council annual meeting (December 2000) as our research has impacted potato production on a national level.

Subproject C Title: Genetic engineering cover crops for improved biological weed management.

Overall Budget: \$99,019

Amount Spent: \$99,019

Result 1C: Characterization of promoter sequences that are induced by biologically derived chemicals in genetically engineered *Arabidopsis* plants.

The synthetic promoter was tested in plants for iron-inducibility. We constructed a transformation vector consisting of the iron-inducible promoter controlling a reporter gene called b-glucuronidase (GUS). Expression of GUS activity in plant tissues was tested using histochemical staining and spectrophotometric assays. This vector was genetically engineered into tobacco for evaluation of iron-inducibility. More than 20 transgenic plants were analyzed for histochemical GUS activity. Various treatments of iron application to these transgenic plants such as soaking leaf tissue or spraying whole plants identified numerous plants that exhibited iron induction of reporter gene expression. We also determined iron-inducibility in progeny of these plants to be certain that inducibility is heritable. Iron-inducibility was observed; however, reproducibility of induction was low indicating that further research was necessary to produce a useful iron-inducible promoter.

Result 2C: Produce genetic engineering constructs to place senescence genes under the control of the inducible promoter(s) found in Result 1C.

The iron-inducible promoter was fused to the senescence gene, barnase, which in combination with barstar, an inhibitor of barnase, was genetically engineered into the model plant tobacco. We observed variability in inducible senescence in the progeny of transgenic plants that was similar to the variation we saw in the iron-inducible promoter-GUS plants. These results indicate the need for further modification of the promoter before it will be useful for controlling inducible senescence.

Result 3C: Test for inducible senescence by genetically engineering constructs produced in 2C into *Arabidopsis*.

The synthetic promoter was re-engineered to simplify its use of the promoter in recombinant DNA work and to reduce non-induced background expression. The modified versions of the iron-inducible promoter were fused to the reporter gene and genetically engineered into tobacco. Tests of reporter gene induction indicate that further modifications of the promoter are required to provide adequate inducibility characteristics for iron-inducible senescence to cover crop.

Result 4C: Genetically engineer a dwarf *Brassica* cover crop for future field testing of inducible senescence.

This result was dependent upon obtaining useful promoters which unfortunately we were unable to obtain during the funding period. Work is continuing using federal and other funds to improve performance of the iron inducible promoters we discovered during the project period with the ultimate goal of moving these genes into *Brassica* cover crops for testing under field conditions.

Result 5C: Complete data analysis and report findings to appropriate audiences.

A patent application was filed for the iron inducible promoter and students and others in the laboratory continue to publish scientific papers on these results and present those results at meetings.

**Subproject D: Making Biological Control Work in Insecticide-Intensive Crops:
Cabbage in Minnesota**

Overall Budget: \$10,442

Amount Spent: \$10,442

Result 1D: Determine whether reduced rates of Spinosad 4SC (= Spintor) will facilitate increased levels of parasitism by a diverse native parasitoid fauna (9 species total), and therefore greater control of the Lepidopteran pest complex overall (cf. full-rate Spinosad alone).

Given the positive results from the small plot studies in 1996-1997, we developed and implemented an IPM program with one of our leading fresh-market grower farms in 1998. Our purpose was to compare an IPM program, based on all previous experiment station (small plot) research, including: presence/absence action threshold, use of at least 2 applications of SpinTor (labeled in 1998 for U.S. cole crops), and CL pheromone traps to guide the initial sampling effort. Treatments included the IPM plots, conventional grower practices and untreated checks. The grower program relied primarily on the use of Warrior and Lannate, conventional broad-spectrum insecticides, and approx. 7-10 day spray schedule. Because this was the first year of the project, we selected the more conservative 10% CL Egg/Larval action threshold for our IPM program (based on previous small plot results). Once CL moths arrived in late July, sampling was done twice per week, with a fixed sample of 40 plants/treatment in IPM and Conv. Plots; 20 plants/check; and 6 replications/treatment.

Table 1 summarizes final treatment dates, and treatments applied to the IPM and Conventional plots; 4 total sprays in the IPM vs. 7 by the grower reflect a 43% reduction. As in previous years, CL was the dominant pest species in 1998 (only these data are summarized for this report). Cumulative late instar CL infestations were significantly lower, and marketability was higher, in the IPM vs. conventional plots (Table 2). Cumulative larval infestation, marketability, and contaminants at harvest in the IPM and conventional treatments were superior to the untreated check. Yield, as in previous studies (Hines 1998) indicated numerical trends, but no significant effects (Table 2). In summary, these results have lead to an expansion of the IPM program for on-farm implementation in 1999.

Result 2D: The targeted parasitoids (e.g., *Costesia rubecula*) that we had planned to collect for release in Minnesota, were at low population densities this past year. Thus, we were not able to release new parasitoids in 1998. Plans are underway to do so in 1999. Timing of release must coincide with peak infestations of imported cabbageworm populations.

Table 1. Spray dates and product rates for comparison of pest management programs: cabbage on-farm trial, Apple Valley, Minnesota, 1998

IPM	<u>Conventional</u>	Check
7/24 Spintor-0.094 lbs AI/ac (6 oz./ac)	7/19 Warrior-0.025 lbs AI/ac (3.20 oz./ac)	--
7/31 Spintor-0.094 lbs AI/ac (6 oz./ac)	7/27 Lannate-0.9 lbs AI/ac (1.0 lbs/ac)	--
8/11 Warrior-0.025 lbs AI/ac (3.20 oz./ac)	7/31 Warrior-0.0125 lbs AI/ac (1.60 oz./ac)	--
8/21 Warrior-0.025 lbs AI/ac (3.20 oz./ac)	8/10 Warrior-0.025 lbs AI/ac (3.20 oz./ac)	--
	8/17 Lannate-0.45 lbs AI/ac (0.50 lbs/ac)	--
	8/21 Warrior-0.0125 lbs AI/ac (1.60 oz./ac)	--
	8/28 Lannate-0.9 lbs AI/ac (1.0 lbs/ac)	--

Table 2. Summary of results for cabbage on-farm trial: insect pest pressure, marketability, and yield, Apple Valley, Minnesota, 1998

	IPM	Conventional	Check
Cumulative season avg. (% of plants infested with late instar cabbage looper)	4.29 a	10.46 b	21.04 c
Marketability (Green's scale; 1-6)	1.17 a	1.53 b	2.21 c
Contaminants (# of larvae and/or pupae per head)	0.00 a	0.005 a	0.11 b
Yield (lbs./5 heads)	10.10 a	9.41 a	9.01 a

ANOVA based on 6 replications; means in a row, followed by same letter are not significantly different; REGWF (P=0.05).

V. Dissemination.

Publications from funded research projects.

- Ragsdale, D. W. and E. B. Radcliffe. 1999. A new threat from an old pest: green peach aphid. *Valley Potato Grower* 64(110): 12, 14.
- Suranyi, R., C. Longtine, D. Ragsdale, and E. Radcliffe. 1999. Controlling leafhoppers with below-label rates. *Valley Potato Grower* 64(113): 11-13, 16.
- Radcliffe, E. B. and D. W. Ragsdale. 1998. Aphids cause big problems for industry. *Valley Potato Grower* 63(110): 4, 6, 31.
- Lagnaoui, A. and Radcliffe, E. B. 1998. Potato fungicides interfere with entomopathogenic fungi impacting population dynamics of green peach aphid. *Amer. J. Potato Research*. 75: 19-25.
- Hines, R.L. 1998. Biologically intensive integrated pest management of the Lepidopteran pest complex in Minnesota cabbage, 131 pp. M.S. Thesis, University of Minnesota, St. Paul, Minn.
- Ragsdale, D. W. 1997. Late Blight Fungicides Linked to Increases in Green Peach Aphids in Potato, Feature Article. *Midwest Biological Control Newsletter* 4(12): 1-3, 7.
- DiFonzo, C. D., D. W. Ragsdale, E. B. Radcliffe, N. C. Gudmestad, and G. A. Secor. 1997. Seasonal abundance of aphid vectors of potato virus Y in the Red River Valley of Minnesota and North Dakota. *J. Econ. Entomol.* 90: 824-831.
- Ted Radcliffe & Dave Ragsdale. 1996. Late blight fungicides have an impact on aphid control. *Valley Potato Grower* 61: 16-17, 21.
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Andow, D., D. Ragsdale, & B. Nyvall, eds. 1997. Ecological Interactions and Biological Control, Westview Press, 334pp.

Andow, D. A., D. W. Ragsdale, R. F. Nyvall. 1997. Biological control in cool temperate regions, pp. 1-28. *In* D. Andow, D. Ragsdale, & B. Nyvall, [eds.], Ecological Interactions and Biological Control, Westview Press, Boulder.

Public presentations of research projects:

Suranyi, R. A., E. B. Radcliffe, and D. W. Ragsdale. Seasonal abundance of aphid vectors of potato viruses in the Red River Valley. Entomological Society of America – North Central Branch, Des Moines, March 1999. NOTE: First Place Award, Ph.D. presentation.

Aphid Biology and Virus Spread. Red River Valley Potato Growers Annual Meeting and Scientific Workshop. 19 November 1999. Fargo, ND. (D. W. Ragsdale [presenter] with E. B. Radcliffe, and R. Suranyi).

Monitoring Aphid Flight Activity in Minnesota and North Dakota. Red River Valley Potato Growers Annual Meeting and Scientific Workshop. 19 November 1999. Fargo, ND. (R. Suranyi [presenter], D. W. Ragsdale, E. B. Radcliffe, and B. Lockhart).

Aphid Alert. Red River Valley Potato Growers Annual Meeting and Scientific Workshop. 19 November 1999. Fargo, ND. (E. B. Radcliffe [presenter], R. Suranyi, D. W. Ragsdale, and B. Lockhart).

Novy, R., Ragsdale, D. W., Radcliffe, E. B., and Nasruddin, A. Introgression of Virus and Aphid Resistance From *Solanum etuberosum* to Cultivated Potato. Potato Association of America Annual Meeting, New Jersey, July 1999.

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Hines, R.L. & W.D. Hutchison. 1998. Enhanced biological control of Lepidopteran pests of cabbage with spinosad. (Poster), Annual meeting of Entomol. Soc. of Am., N. Central Branch, Sioux Falls, SD (March).

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- Ragsdale, D. W. 1998. Interactions of Late-Blight Fungicides with Entomopathogenic Fungi. Department of Plant Pathology, University of Minnesota, October, 1998.
- Ragsdale, D. W. and E. B. Radcliffe. 1998. Becker Field Day, 16 July 1998, Area II Potato Growers Association, Becker, MN
- Ragsdale, D. W. and E. B. Radcliffe. 1998. Minnesota Seed Growers Association, Summer Meeting, 20 July 1998, Fergus Falls
- Ragsdale, D. W. and E. B. Radcliffe. 1998. Red River Valley Potato Growers Association, 20 August 1998, Grand Forks, ND
- Ragsdale, D. W. and E. B. Radcliffe. 1998. Minnesota Seed Growers Association, Annual Business and Update Meeting, 20 November 1998, Fargo, ND
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Hutchison, W.D. and E.C. Burkness. 1999. On-farm experience using action thresholds and Spintor™ for management of Lepidoptera in cabbage: steps to implementation, pp. 35-39. *In* Proc. International Workshop for Integrated Pest Management of Cole Crops, May 20-21, 1999, University of Celaya, Celaya, Guanajuato, Mexico.

(Invited Presentation for The Association of Processors and Exporters of Fruits and Vegetables, Mexican Frozen Foods Council, Pillsbury-Green Giant and Birdseye).