Maine Agricultural Center Integrated Research and Extension Projects: 2011–2012

MAC123: Non-Potato Sources of PVY Inoculum

Principal investigator: Andrei Alyokhin, Gary Sewell. James Dwyer

Background:

Potato virus Y (PVY) is an important aphid-borne potato pathogen that can cause very significant crop losses. PVY infection rates have been increasing across North America in the recent years. Results of the 2010 post-harvest testing revealed 4.7-fold increase in percent seed lots rejected due to the PVY infection compared to average rejection rate during the preceding 10 years (Johnson, Spudlines 49(1):6, 2011). Furthermore, increase in the incidence of strains that cause tuber necrosis present an additional threat to potato production.

PVY transmission is non-persistent, i.e., the mouthparts of the aphid may get contaminated with viral inoculum in the brief process of probing the epidermal tissues of infected plants. There is no latent period between acquisition and inoculation, and the entire transmission process may take only a few seconds. However, infectivity is lost after several probes.

PVY is transmitted by at least 50 different aphid species. The majority of those do not colonize potato plants because they are unsuitable hosts for their development. However, rejection of non-host plants does not take place until aphids probe them with their mouthparts. As a result, dispersing winged adults of non-colonizing species commonly land on potato plants, insert their stylets into plant tissue, and then leave in search of a more appropriate host. Direct damage caused by probing is negligible. However, probing may result in the transmission of certain viruses to healthy plants.

Farmers have historically relied on insecticides to protect their crops, but even the most effective compounds take minutes if not hours to kill exposed aphids. As a result, insecticides that are highly efficient in eliminating colonizing aphids often fail to suppress PVY spread by non-colonizing aphids. Furthermore, intoxication might actually encourage aphids to leave plants of their residence and erratically probe adjacent plants in search of a more suitable place to settle.

A better approach to PVY management is to focus on reducing the amount of initial virus inoculum within or near potato fields. Much can be done by planting certified seed tubers. However, PVY has a wide host range, being transmissible to approximately 120 plant species in five families. Currently, surprisingly little is known about infestation rates of non-crop hosts. In a preliminary survey conducted by James Dwyer in 2010, between 2-10% of the collected nightshade samples, 2-10% of the collected lambsquarter samples, and 1-5% of the collected American germander samples tested positive for PVY. A more comprehensive survey will allow identifying potential sources of infection within and around potato fields and their subsequent elimination by farmers.

Brief description of research and/or extension education activities:

Fifteen commercial potato fields will be selected across Maine. Non-crop vegetation on these fields and within 10 m radius around their perimeter will be surveyed. Foliage samples will be taken from the common (>5% of individual non-crop plants within the fields and >5% canopy cover around field perimeters) non-host vegetation. Twenty plants of each species will be sampled at each field. Volunteer potato plants germinating from unharvested tubers left from previous years will be also sampled in a similar manner if found. Collected samples will be brought to our laboratory in Presque Isle and analyzed for PVY using ELISA (Agdia Inc., Elkhart, IN).

Findings of this study will be presented at the potato Pest Management Conference and Annual Potato Conference. They will also be published in Spudlines, the UM Cooperative Extension newsletter widely circulated among potato growers, and posted on the Maine Potato Program website.

Expected outcomes and method for sharing outcomes:

We expect to identify potential non-crop sources of potato virus Y that are likely to be at least partially responsible for the current epidemic. Managing these sources will improve crop protection. It will also reduce the amount of

insecticides that farmers use in an attempt to protect their crops. We will also use our results as preliminary data to apply for the USDA Pest Management Alternatives Program grant.

Results will be shared through presentations and publications (see above).

Final Report

Original project objectives that were met and significant findings:

We have successfully identified non-crop sources of potato virus Y that are likely to be at least partially responsible for the current PVY epidemic. Common uncultivated plant species (dandelion, *Taraxacum officinale*, plantain, *Plantago major*, red clover, *Trifolium pratense*, white clover, *Trifolium repens*, burdock, *Arctium minus*, Canada thistle, *Cirsium arvense*, raspberry, *Rubus* sp., cow vetch, *Vicia* sp., lupine, *Lupinus polyphyllus*, wild rose, *Rosa acicularis*, corn spurry, *Spergula arvensis*, American germander, *Teucrium canadense*, lamb's quarters, *Chenopodium album*, hairy nightshade, *Solanum sarrachoides*, pigweed, *Amaranthus* spp., daisy fleabane, *Erigeron annuus*, bindweed, *Convolvulus arvensis*, goldenrod, *Solidago* sp., and bedstraw, *Galium* sp.) were sampled on ten potato farms in northern Maine. Between three and 17 composite samples of five plants each were collected on each farm, homogenized in the laboratory, and analyzed for the presence of PVY by ELISA (Agdia Inc., Elkhart, IN).

Four of the surveyed plant species tested positive for PVY (Table 1). On one of the farms, up to 90% of collected dandelions contained the virus. While this certainly provides serious grounds for concern, it is also worth noticing that on most of the surveyed farms we detected no PVY in any of the sampled vegetation.

Table 1. PVY infection of surveyed plants (all farms combined). Low estimate assumes that only one plant in a composite sample was infected. High estimate assumes that all plants in a composite

Original project objectives that were not met:

None

Methods used to evaluate outcomes:

Outcomes were evaluated based on the feedback received from growers following our presentations and publications in *Spudlines*, as well as on the anonymous speaker evaluation forms distributed by Cooperative Extension. We also presented our results to the Research Committee of the Maine Potato Board.

Integration of research and extension activities:

Findings of this study were presented at the Potato Pest Management Conference, Annual Potato Conference, Potato Expo, and Annual Meeting of the Potato Association of America. They were also published in Spudlines, the UM Cooperative Extension newsletter widely circulated among potato growers.

Educational material, publications, and programs:

- Alyokhin, A., J. Dwyer, and G. Sewell. 2011. Potato virus Y epidemiology: Looking beyond a potato field. *Spudlines* 49(3): 1-2.
- Alyokhin, A. 2011. Potato virus Y transmission by aphids. *Spudlines* 49(2): 5-6.

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- Alyokhin, A., J. Dwyer, and G. Sewell. 2012. Non-crop hosts of Potato Virus Y in the vicinity of potato fields in Northern Maine. Presentation at the Annual Meeting of the Potato Association of America, Denver, CO.
- Alyokhin, A., J. Dwyer, and G. Sewell. 2012. Vegetation surrounding potato fields as a reservoir for Potato Virus Y. Invited Presentation at the Potato Expo, Orlando, Florida.
- Alyokhin, A. 2012. Aphids: the new face of an old enemy. Presentation at the 27th Annual Maine Potato Conference, Caribou, ME.
- Alyokhin, A. 2011. What can be done about Potato Virus Y? Presentation at the 2011 Potato Pest Management Conference, Presque Isle, ME.

Research publications and abstracts:

None

MAC124: Alternative Susceptibility Trends for Microbial Isolates from Organic Dairy Herds with Mastitis in the Northeast

Principal investigator(s): Gary W. Anderson, RobertC.Causey, Beth Calder, Richard Kersbergen

Background:

Objectives:

- To determine the antimicrobial action of herbal tinctures or essential oils *in vitro.*
- To determine the Minimal Inhibitory Concentrations (MIC) for the herbal tinctures or essential oils against common mastitis causing bacterial species: *i e*, *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis Staphylococcus epidermis Staphylococcsu xylosus* and *Staphylococcus intermedius.* using a disc diffusion approach.
- Utilize the MIC findings to aid in defining dose, efficacy, safety and treatment course of the herbal treatment used for further experiments.

Mastitis is the most prevalent and costly disease affecting the dairy industry. In the US and Canada, it is thought that 50% of cows have one or more infected quarters (Duval, 1997). Estimated monetary loss in 1998 based on somatic cell counts (SCC) was $222/cow/year in Maine (Miller and Norman, 1998). “Mastitis is inflammation of the mammary gland due to either infection or trauma and typified by one or all of the following: loss of milk production, increase in inflammatory cells, abnormal milk, changes in gland size and/or consistency and occasionally systemic illness or death of the animal” (Rainard and Riollet, 2006).

Mastitis costs accumulate due to reduced production, potential source of contagious organisms for other cattle in the herd, loss of milk sales, cost of treatments, cost of veterinary assistance and increased labor to treat animals. Mastitis in dairy cows can cause major economic losses through reduction in milk yield and milk discarded not safe for human consumption. Prevention and treatment of mastitis is the most prominent issue facing dairy farmers whether they are organic or conventional producers. Treatment and prevention of mastitis is of especially great concern to organic dairy producers because antibiotic use in organic livestock removes the animal from the organic herd (Duval, 1997; MOFGA Certification Services, LLC. 2006). Treatment is important for organic dairy farmers to be able to remain profitable and ecologically sound. While conventional producers have some antibiotic treatments available to them, the organic producer has other preventative and curative measures available, but most lack data concerning efficacy or safety. Many of the treatments that are available are reported to enhance the activity of the immune system, but lack data on their efficacy.

Herbal remedies are being administered for mastitis by practicing naturopathic veterinarians and organic dairy farmers. Most of the evidence that these treatments are effective is anecdotal through centuries or decades of practical use and not scientifically based.

Research and/or extension education activities:

This project is one of discovery. It is very similar to basic discovery work of the the largest pharmaceutical companies in their search for natural products with pharmacologic activity. In the proposed study, the antimicrobial action *in vitro* of various herbal preparations will be studied. The attached proposal provides more specific detail. Results of this work will provide a base for more detailed work in designing non-antibiotic treatment protocols.

Research:

The purpose of the proposed research is to determine *in vitro*, the effects of various herbal extracts on growth of the major mastitis causing organisms. This project will attempt to thoroughly investigate three or four of the most commonly used herbal substances currently used for treatment of mastitis. These are all Generally Recognized as Safe (GRAS) compounds such as garlic, aloe, wintergreen, licorice and thymol.

We will prepare saturated extracts of several herbs using available scientific information such as the Merck Index, USP Pharmacopeia and the National Formulary for our initial standard. There are no saturated extracts in the marketplace nor are there good analytical techniques to quantify concentration of these herbal extracts. We will use the initial standard as a 100% solution and then dilute it serially to prepare 50%, 25% and 12.5% standards. These four dilutions will then be used in screening various herbal extracts on the sensitivity/resistance to growth of mastitis causing organisms. At this time, we are most interested in their action against the environmental organisms *Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus epidermis, Staphylococcus xylosus* and *Staphylococcus intermedius.* While we are most interested in environmental organisms, we will evaluate activity against the contagious organism, *Streptococcus agalactiae*. We do not plan to evaluate any activity against *Staphylococcus aureus* or any coliforms since they are very difficult to treat even with antimicrobial therapy. This strategy will give a wide range of herbal extract concentration tested and will provide a standard curve of concentration against millimeters of inhibition of growth.

We plan to use a modification of the Kirby-Bauer method to determine anti-microbial resistance as a screening tool. Minimum inhibitory concentrations (MIC) will be assessed using the Kirby-Bauer disc diffusion method on Mueller Hinton agar plates. Paper discs, 6mm in size, will be infused/impregnated with 0.02 milliliters of each of the herbal standards at 100%, 50%, 25%, 12.5% and 0% concentration. *In vitro* sensitivity/resistance will be evaluated by the absence of growth in zones around these discs and will be measured in millimeters. Sensitivity is displayed by the lowest concentration of the extract infused in the disc. Sensitivity/resistance measures will be determined by assessment of the proportion of the amount of the extract infused in the disc, the solubility of the agent and its diffusion rate. (Madigan and Martinko,2006) These data will be collected on standard cultures obtained from the American Type Culture Collection (ATCC) as well as isolates from milk samples submitted to the University of Maine Veterinary Diagnostic Lab. The most promising herbal or homeopathic extracts will be further assessed by developing a more accurate *in vitro* method to determine effectiveness.

These data will be important base information for organic farmers and their veterinarians to make informed decisions as to what future treatments might be most effective for these types of mastitis.

Those individuals that would benefit most would be the organic farmers and practicing naturopathic veterinarians. Conventional farmers that are considering changing over to organic dairy practices or conventional farmers who just want to use less antibiotics and more natural remedies could also benefit. Extension and the private sector will be able to benefit from educational opportunities through research and workshops. These programs will improve their understanding of the biology of the cow, the development of mastitis and the availability of alternative treatments.

Expected outcomes and method for sharing outcomes:

These are preliminary data investigations, but still will provide a base of new information for further work on nonantibiotic treatment of mastitic infections in cattle.

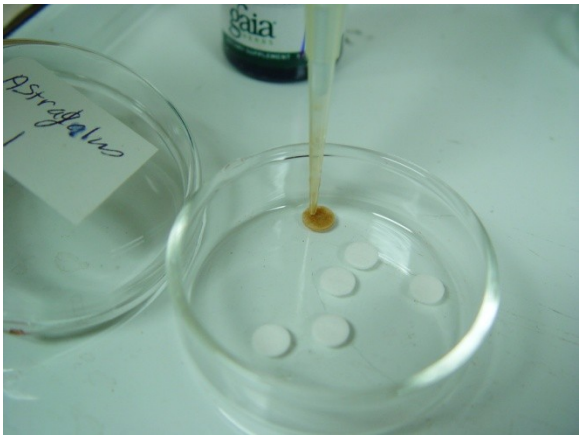
Outcomes:

These data can be substantial scientific evidence for the entire organic dairy industry on the possible effectiveness of herbal treatments for mastitis. If the results are positive they can serve as a starting point on how to treat mastitis using herbals. This data will be published in existing newsletters such as Northeast Organic Dairy Producers Assoc. (NODPA) and Maine Organic Milk Producers (MOMP). Extension outreach will be conducted in Maine to the dairy community at the Maine Agricultural trade show, Maine Organic Farmers and Gardeners Assoc. (MOFGA), and New England Spring Dairy Seminars. A research bulletin will be created as a guideline for dairy farmers on how to treat organic cows with mastitis using herbal remedies.

Final Report

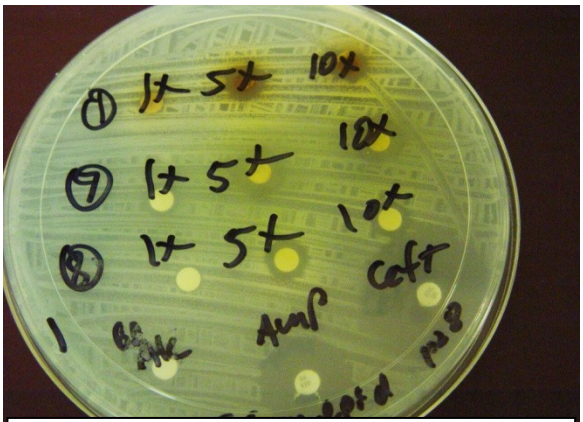
Original project objectives that were met and significant findings:

1. To determine the antimicrobial action of herbal tinctures or essential oils in vitro.



Disk infusion of sterile disks with herbal extracts (Photo credit Brenda Kennedy-Wade 2012)

Commercially available herbal extracts of unknown concentration (astragalus, benzoin resin, calendula, cinnamon bark, *Echinacea purpurea*, panax ginseng, goldenseal, licorice root, pine needle essential oil, sacred basil, thyme and turmeric root) were tested for antimicrobial activity against 6 American Type Culture Collection species *Staphylococcus epidermidis* ATCC# 12228, *Streptococcus agalactiae* ATCC#13813, *Streptococcus uberis* ATCC#9927, *Staphylococcus xylosus* ATCC# 29971, *Streptococcus dysgalactiae* ATCC#12394, *Staphylococcus intermedius* ATCC#51874 and 4 mastitis isolates from local organic dairy farmers (*Coagulase Negative Staphylococcus*,#12653-3, *Coagulase Negative Staphylococcus*,#12551-5, *Non Streptococcus agalactiae* #12493, *Non Streptococcus agalactiae* #12529-2). Of the herbal extracts tested, astragalus, goldenseal and licorice root showed the most promise and were tested at 1X, 5X and 10X does to determine if there was a dose/response effect. A dose/response was not seen with commercial extracts of these three herbs.



Zones of Inhihition of bacterial growth of commercially available herbal extracts (Photo credit Brenda Kennedy-Wade 2012)

2. To determine the Minimal Inhibitory Concentrations (MIC) for the herbal tinctures or essential oils against common mastitis causing bacterial species: *i e*, *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis Staphylococcus epidermis Staphylococcsu xylosus* and *Staphylococcus intermedius* using a disc diffusion approach.

Laboratory extracts of goldenseal and licorice root were prepared with five different mixtures of sterile water and 95% ethanol (0%, 25%, 50%, 75% and 100% ethanol) to vary the polarity and determine the best solvent for extraction; extractions were done for a 2 week period. Extracts were tested for antimicrobial activity against the same 10 bacterial isolates as above at 1, 7, and 14 days of extraction. Zones of inhibition of bacterial growth illustrated that the 50% ethanol solvent at day 7 of extraction was most effective with no increase in activity at day 14. Astragalus did not show a significant effect with in-house extracts and was removed from further study. A dose response effect of goldenseal and licorice root at 1X, 2X and 3X the initial extraction concentration was evaluated; goldenseal was the most effective and equaled or exceeded the inhibition zones of the antibiotic controls, ceftiofur (30µg) and ampicillin (10µg) for *Staphylococcus epidermidis* and two of the organic farm coagulase negative staphylococcal isolates, but none of the streptococcal species.

Original project objectives that were not met:

3. Utilize the MIC findings to aid in defining dose, efficacy, safety and treatment course of the herbal treatment used for further experiments.

This objective was beyond the scope of the project and should not have been included in this request. The data from the present study will be used to inform further study on this objective. Due to the federal approval process for treatment, clearance rates in milk, safety to the animal, etc., this will take much more time than one year.

Methods used to evaluate outcomes:

The results of these basic studies provide a tremendous base of information for further work in developing a non-antibiotic treatment for mastitis. These results will be valuable information to organic dairy producers and those conventional producers who seek a method of treatment that does not use antibiotics. While we are in no way recommending a treatment protocol, the present data is highliy important in the development of new protocols. Due to the lengthy approval process, the release of a treatment protocol will only occur after several years of work to evaluate safety to the animal, safety to the consumer of food products from treated cows, a withdrawal period for the treatment and elucidation of the active compounds.

Inhouse Laboratory Herbal Extracts (Photo credit Brenda Kennedy-Wade 2012)

Integration of research and extension activities:

A field problem, the non-antibiotic treatment of mastitis, was brought to University staff. While this project was very simple in design and basic in conduct, it provided tremendous results for future work. The fact that the goldenseal extract equaled or exceeded the antimicrobial effect of control antibiotics on staphylococcal organisms is tremendous. Future work will be directed at the mechanism of action of this extract.

Educational material, publications, and programs:

- Possible future Master's projects in Food Sciences related to identifying the active components of the most promising herbal extracts utilizing HPLC and also evaluating the microbiological basis for the difference in susceptibility between Staphylococcus and Streptococcus genera.
- Through Dave Marcinkowski, a student in the Animal & Veterinary Science department (Rebekah Wheaton) completed a similar project on one commercial product for her Senior Capstone project. She tested a commercially available herbal product that was used on the organic dairy farm where she was employed.
- These data will be used to provide an update on the effect of various herbal extracts on mastitis causing organisms. The present study illustrated the beneficial effect of a goldenseal extract on staphylococcal species, but not streptococcal species. Further work by would look at fractions of the crude extract we produced to elucidate the compounds responsible for this action.

MAC125: Effect of Sterol Inhibitor Fungicides on Honey Bees

Principal investigator(s): Frank Drummond, David Yarborough

Background:

Honey bees are exposed to many pesticides when pollinating crops. Most fungicides have always been considered to have low animal toxicity. Recently, several laboratory studies have shown that honey bee larvae are sensitive to certain groups of fungicides. The sterol inhibitor fungicides are one group that has been shown to synergize insecticide-induced mortality in honey bee larvae. Insecticides such as acetamiprid that has very low toxicity to honey bees have a 1000 fold increase in toxicity when honey bees are also exposed to propiconazole. This is very disturbing because propiconazole is the main fungicide used to protect wild blueberry from infection by *Monilinia vaccinii-corymbosi* which causes mummy berry disease AND we have shown that even when propiconazole is applied correctly PRIOR to bloom, honey bees are exposed to considerable residues by coming in contact with the outer surface of the corolla and it ends up contaminating pollen that is fed to the bee larvae.

The objective of our proposed research project is to assess the potential effects of propiconazole (both alone and in conjunction with insecticide exposure) to honey bee colonies under FIELD conditions. This is important since all published studies to this point have been based upon laboratory feeding studies with individual larvae. Laboratory studies on individual animals are not always corroborated by studies designed to measure these effects in the field on whole colonies.

Research and/or extension education activities:

The research will involve selecting two populations of honey bee colonies from migratory beekeepers that bring their colonies to Maine. Currently I have commitments of cooperation from Mr. David Mendez (president of the American Beekeeping federation) and Mr. David Hackenberg, a large migratory beekeeper from Pennsylvania. The first population will be eighteen randomly selected colonies that are divided and aportioned to three large (> 200 acres) blueberry fields that have been treated with the fungicide propiconazole (Orbit) just prior to bloom for control of mummy berry. The second population will be eighteen randomly selected colonies that are apportioned to 3 large (> 200 acres) blueberry fields that have NOT been treated with any fungicide. Two of the large blueberry companies in Maine have expressed interest in cooperating in this study. Each one of these populations in a field would be split into two groups of colonies. One group would be fed pollen patties with acetamiprid mixed in at the dose that has been reported to synergize insecticide effects. The other group would be fed uncontaminated pollen patties. The colonies would have pollen trapped from them during blueberry bloom to assess how much propiconazole is being brought into the hive. In addition, colony strength (adults and brood) and queen status (supercedure, quality of egg pattern, etc) and disease symptoms and mite numbers would be assessed monthly (May, June, July, August, September, - moved to a good bee pasture after blueberry bloom) and then overwintered and colony survival and strength would be assessed in the following April. The experimental design will be a completely randomized analyzed as a mixed model ANOVA since colonies nested within fields and fields nested within treatment are random effects. Extension presentations will be made at the 2011 annual summer field day in Jonesboro, ME and the winter blueberry schools in March 2012.

Expected outcomes and the method for sharing the outcomes:

The expected outcome of this research is to determine the level of risk that honey bee colonies are exposed to when propiconazole fungicides are used in blueberry fields either alone or in an environment where insecticide exposure is an additional risk to honey bees. If risk levels are high then more effort will be expended to find alternatives to this important plant protection chemical that many blueberry growers currently rely upon to prevent crop loss due to mummy berry disease.

We plan to share our research results with Maine wild blueberry growers at the 2011 annual wild blueberry summer field day and the 2012 Maine Blueberry Schools.

Final Report

Original project objectives that were met and significant findings:

Objectives addressed in this MAC project: Determine if detrimental effects exist for honey bee colonies exposed to the sterol inhibiting fungicide, propiconazole, during bloom. Criteria evaluated were: colony survival both during the spring and summer and over the winter; parasitic mite levels; colony strength (population estimates); queen egg laying; larval survival; worker longevity; and hypopharyngeal gland mass.

The objectives of the funded MAC project were originally for a single year (2011). However, because the PI and co-PI received additional funding (see below) to support a two year study, the objectives were investigated over two full field seasons (2011 and 2012). Because of this, the results are not all complete. The results from the second year of the study (2012) are not fully analyzed, but will be complete in the spring of 2013. However, the objectives did not change. The experiment was repeated over two years.

Findings in 2011 study.

Flowers and pollen that we collected in 2011 suggested that the control bees (no propiconazole exposure) had exposure to the fungicide propiconazole, but at very low levels. Flower residues were 0.0 ppm and pollen brought into the hive had levels of 7.0 ppm. Exposure for the colonies placed in the propoiconazole treated field was 3,987.7 ppm on flowers and 555.3 ppm on pollen brought into the hive. During the summer of 2011, 20% (n = 4) of the hives were lost. This is not atypical and did not appear to be due to the fungicide exposure treatment as 2 colonies were lost in each of the two groups (untreated check and fungicide exposure). Colony strength measured as both worker population and brood population were not affected by fungicide exposure ($F_{(1,14)} = 0.044$, $P = 0.837$ and $F_{(1,14)} = 0.119$, $P = 0.736$; workers and brood respectively). In addition there was no significant change in the difference between the two treatments over time ($F_{(4,56)} = 1.181$, $P = 0.329$ and ($F_{(4,56)} = 1.007$, $P = 0.412$; workers and brood respectively). However, Fig 1 shows that there was a decline in colony strength during bloom and that this appears sharper in the fungicide exposed group than the untreated check group; although, as stated previously this is not a significant trend. Figure 2 suggests that this trend is similar in the brood population; although, it appears even more during the bloom period. Again, however, these trends are not significant.

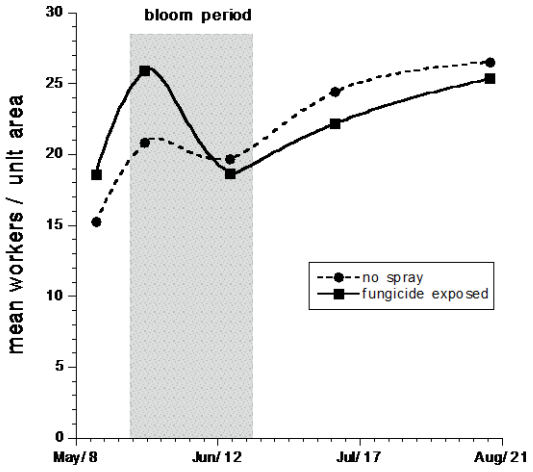


Fig. 1. Worker honeybee strength per colony (measured as the cumulative percent comb area occupied by workers) for the two treatment groups. Shaded rectangle represents period of time when bees were moved to their respective blueberry foraging fields.

Queen egg-laying was also not significantly affected by exposure to propiconazole. During the period that hives were in blueberry during bloom queens in the untreated check field had a 2-day egg-laying rate of 1466.4 compared to the fungicide-exposed queens with a rate of 750.6. Despite almost twice the rate in the non-sprayed field, the variation was extremely high and this resulted in a lack of evidence suggesting a fungicide effect, $F_{(1,8)} = 1.859$, $P = 0.209$. A second trial of queen egg laying was conducted after the hives were moved back to Orono. In this trial again there was no evidence to suggest an adverse effect of fungicide exposure to queen egg laying with a 2-day day egg laying rate of 1366.6 in the untreated check field compared to the fungicide-exposed queens with a rate of 1372.8, $F_{(1,8)} = 0.0002$, $P = 0.989$. Even if our experiment failed to pick up a true effect of fungicide exposure on queen egg laying while the hives were in blueberry, it appears that by the time the hives had been moved off of the blueberry fields, any potential deleterious effects had disappeared.

Larvae reared during blueberry bloom did not experience differential survival due to fungicide exposure ($F_{(1,8)} = 2.087$, $P = 0.187$). Mean survival for brood reared in the non-sprayed field was 85.9% while those reared on the fungicide-exposure field had a survival rate of 95.6%. The longevity of young worker bees that were reared as larvae when the hives were on the

blueberry fields and then emerged in the laboratory did appear affected by fungicide exposure (Mantel test: χ^2 = 14.647, *P* = 0.0001; Proportional Hazards model: χ^2 = 12.349, *P* = 0.0004).

Figure 3 shows the longevity of the bees in the two groups. While the median (50%) longevity is not different, it can be seen that the bees that foraged on the non-sprayed field lived several days longer than the bees exposed to fungicide. On average a bee that foraged on the fungicide sprayed field had a relative risk of dying at a rate 1.41 times that of a bee that had foraged on the non-sprayed field.

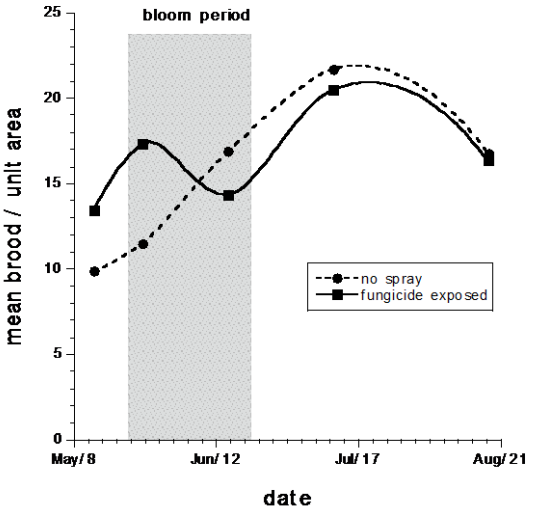


Fig. 2. Honeybee brood strength per colony (measured as the cumulative percent comb area occupied by capped brood) for the two treatment groups. Shaded rectangle represents period of time when bees were moved to their respective blueberry foraging fields.

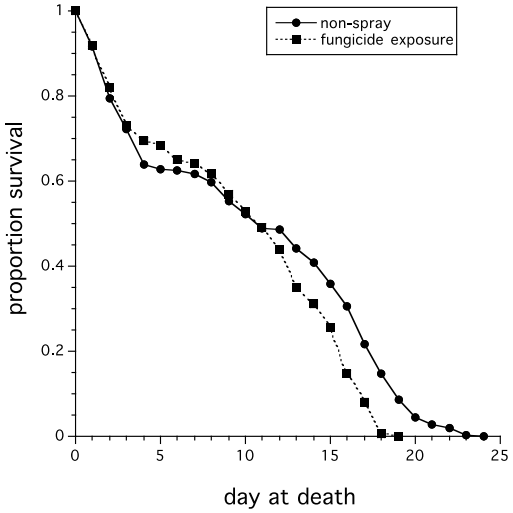


Fig 3. Longevity of newly emerged worker bees held in the laboratory.

Another measure that we made on bee health was the size of the hypopharyngeal glands of worker bees reared as larvae while the hives were in blueberry fields during bloom. The hypopharyngeal glands are extremely important neuroendocrine glands that are involved in several physiological processes in bee development and feeding of larval bees by nurse worker bees. We found that two-dimensional areas of these glands calculated with digital photography resulted in acini (the subunits of the gland) size that were significantly different due to fungicide exposure ($F_{(1,8)}$ = 8.191, *P* = 0.019). We are not clear what these results mean, but the pattern was characterized by glandular acini being 8% larger in honeybees exposed to fungicide compared to those not exposed. This is not an atypical response. Many toxicants can result in enlarged organs. The implications for honeybee health are unknown and could have no effect on colony health.

Nosema cerani disease levels in worker bees did not differ between experimental groups (MANOVA: $F_{(1,8)}$ = 0.0231, *P* = 0.671). We also measured *Varroa* mite infestation in July and August and we found no significant differences in parasitic mite levels due to fungicide exposure (MANOVA: $F_{(1,8)}$ = 0.0014, *P* = 0.971). Figure 4 shows the mite densities for the two sample dates. Figure 4 shows the mite densities for the two sample dates.

Conclusions:

Our first year of research shows that the honeybee health affects of the commonly used fungicide propiconazole needs further investigation. We found that overall exposure of honeybee foragers to residues on flowers does not reduce colony strength of worker or capped brood populations. Queen laying and capped brood survival also does not appear to be affected by exposure to sub-lethal doses of this fungicide. We did find evidence to suggest that workers reared as larvae during bloom result in young nurse bees whose longevity is shortened by about 20%. Accompanying this finding is that the workers exposed to the fungicide had larger hypopharyngeal gland acini than those not exposed to the fungicide. We are not sure at this time whether gland enlargement has honeybee health ramifications. Results of the 2012 field season will determine if the effects of honey bee exposure to propiconazole are consistent and of concern.

Original project objectives that were not met:

The original objectives in the MAC proposal was to assess the potential deleterious effects of the combined residues of acetamiprid (a neo-nicotinoid insecticide) and the sterol inhibiting propiconazole. However, we decided first to test the assumption that propiconazole was safe for bees before testing the more complex hypothesis of a synergistic effect of the two pesticides. This objective will be developed into a future grant proposal for submission for a USDA/NESARE project.

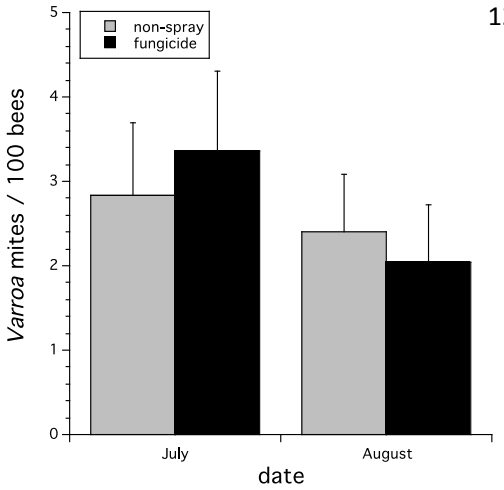


Fig. 4. *Varroa* mite infestation (per 100 bees) sampled in July and August.

Methods used to evaluate outcomes:

Methods for evaluating outcomes of our research will be targeted at both blueberry growers and honey bee keepers. Dr. Drummond presents several extension talks to both audiences each year. He will present the results of this MAC funded research to both groups and later survey the growers and beekeepers on their knowledge of the subtle effects of this important group of pesticides used in blueberry production just prior to bloom.

Integration of research and extension activities:

Dr. Drummond presented the results of the 2011 portion of this study to the Maine State Beekeepers Association in Fall 2011. Currently, there are three activities that are underway to integrate the results of our research with extension. First, Dr. Drummond and Dr. Yarborough will provide cautionary notes in the 2013 Wild Blueberry Insecticide Recommendations so that blueberry growers and honey bee keepers have knowledge regarding the effects sterol inhibitor fungicides on honey bees when these pesticides are applied just prior to bloom. Second, Dr. Drummond is a member of a national panel for pesticide education for pesticide applicators and he will incorporate the findings of this research into national testing. The third aspect of our activities is to present the results of our research on the CAPS pollinator eXtension honey bee web page.

Educational material, publications, and programs:

None as of yet, but plan to add conclusions of study to 2013 maine Insect Pest Control Recommendations for Wild Blueberry. This objective will be conducted both by Dr. Drummond and Dr. Yarborough.

Research publications and abstracts:

None as of yet, but plan to publish results of the TWO year study in a peer reviewed honey bee journal.

MAC126: Expanding Spelt Production in Maine

Principal investigator(s): Richard Kersbergen, Ellen Mallory, Tom Molloy

Background:

Spelt is a relative of wheat that evolved thousands of years ago in the Near East and Europe, as people first began to cultivate grains. European settlers brought the grain to the U.S. in the late 1800s, and it remained popular for decades. By the 1920s, however, spelt had fallen out of favor as a food for human consumption for several reasons, including its inconsistent yields and need to be dehulled. Experts disagree on whether to classify spelt as its own species of wheat, scientifically known as *Triticum spelta*, or to define it as a subspecies of common wheat, *Triticum aestivum* subspecies *spelta.*

More recently, spelt has re-emerged as a viable product in the health food market, both in the U.S and in Europe. On a recent trip to Denmark to visit organic wheat growers and processors we were amazed at the number of spelt products and popularity of the grain with farmers and consumers. Many consumers say it's much easier to digest than wheat and its nutrients are more "bioavailable," that is, more readily accessed during digestion. While it does contain gluten and is not suitable for people with Celiac's disease, many people who have sensitivity to wheat can digest spelt products (See attached support letter from Beth George at Pelt Right Baking Co.). We have been asked repeatedly at outreach events for Maine's local bread wheat project if we also have information about spelt as a potential Maine-grown grain to meet this demand.

Spelt has been grown in Maine and has been investigated as a possible grain for feeding organic dairy cows. (SARE Project Number: FNE06-587 *Growing winter spelt as an organic grain or forage for dairy cows* http://sare.org/MySare/ProjectReport.aspx?do=viewProj&pn=FNE06-587 and Project Number: LNE06-240 *Expanding grain production and use on organic dairy farms in Maine and Vermont* http://www.sare.org /MySare/ProjectReport.aspx?do=viewProj&pn=LNE06-240

Spelt has not, however, been evaluated as a food-grade grain in Maine or Northern New England. Using data from trials conducted in other regions is insufficient because, and much existing research illustrates, different soil and environmental conditions, as well as different varieties of spelt, impact the quality and nutritional profile of the grain. Research in Saskatchewan, Canada has indicated that spring spelt varieties may also have a place in Maine if sown early in the season (http://library2.usask.ca/theses/available/etd-10212004-001220/). There is a need to evaluate suitable varieties of spelt for Northern New England, especially varieties of winter spelt that are winter hardy and can potentially resist *Fusarium* infection. *Fusarium* has been a major issue in wheat production in the more humid Northeast.

Spelt production has the potential to fill a market niche in Maine for growers and processors of locally grown products. Interest in local small grain production is evident by the recent development of a mill in Skowhegan and the growth of local bakery industries such a Borealis Breads and Spelt Right Bakery. Attached letters of support document the growing interest by consumers and producers.

Research and/or extension education activities:

We are proposing to evaluate both spring and winter varieties of spelt in replicated plots at the Rogers Farm in Orono. Working with Don Stinchcomb, President of Purity Foods, (http://www.purityfoods.com/) we will select 6-8 potentially suitable varieties to trial in Maine (Oberkumeratkorn, Roquin, Franckenkorn, Comet, Sungold, Maverick, Sammi). Winter varieties will be sown in the fall of 2011, and spring varieties will be sown in late April or early May of 2012. For the fall sowing, we will also evaluate the impact of delayed planting on the yield and tillering ability of spelt to compensate for later plantings, by staggering planting dates (2-week intervals) from mid September through October. This will be done to see if spelt can better compensate for the reduced yield we see in other winter grains sown after September 15th.

These trials will follow a similar format to ongoing wheat trials currently managed by Ellen Mallory and Tom Molloy as part of a funded USDA/OREI project. We will be using similar equipment for tillage, fertility, weed control and harvest of the spelt. Additional work will be needed to de-hull the spelt before final analysis of the product for nutrient content and suitability for baking. We will work with existing mills (Webb farm in Pittston, or Amber Lambke in Skowhegan) to dehull and process the harvested spelt.

Additional evaluations of the baking qualities of the varieties tested will be done by local bakers, including Beth George of Spelt Right, who currently buys all her spelt flour from Purity Foods.

Expected outcomes and method for sharing the outcomes:

A production guide suitable for Maine will be produced to assist growers interested in spelt production. In addition, an excel budget for spelt will be produced for growers to evaluate the potential economic returns if they invest in spelt as a part of their crop rotation.

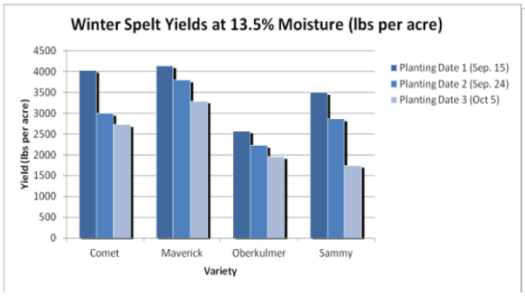
Data from the trials will be publicized through existing outreach efforts, including but not limited to the Rogers Farm Sustainable Agriculture Field day, the Maine Grains Facebook page http://www.facebook.com/pages/Maine-Grains/110335742314892 , the Maine Kneading conference, and the Northern New England Local Bread Wheat Project website https://sites.google.com/site/localbreadwheatproject/ .

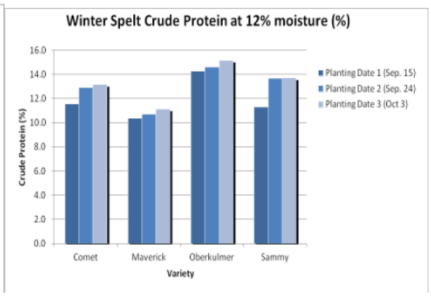
A final report will be submitted to MAC with high resolution photographs related to the project

Final Report

Original project objectives that were met and significant findings:

We tested four varieties of Winter Spelt in 2012, each planted at three different dates in the fall of 2011. The plots were managed organically with dairy manure as the primary source of fertility applied prior to planting. Overall yields and protein levels were good. The Oberkulmer did have some lodging issues and would probably require a lower fertility rate. We had to combine each variety across planting date to get enough flour to mill/bake with. The crude protein levels for the combined samples are calculated weighted averages and are; Comet – 12%, Maverick – 11%, Oberkulmer – 15% and Sammy – 13%. The DON levels were all low, below the 0.5 detectable limit of the test we use.





All varieties displayed higher crude protein (often a criterion for baking quality) in later plantings. Oberkulmer tested significantly higher in crude protein against all varieties but significantly higher in planting dates 2 and 3.

Spring Spelt (common, not a named variety) was planted in two trials, both in association with the organic bread wheat variety trials conducted by Ellen Mallory. One trial was conducted on a farm in Sidney, Maine, while the other was conducted at the University Smith Farm. On the Sidney farm, yields were considerably lower (average 1401 lbs/acre) as compared to the Smith farm (average 3027 lbs per acre). Nitrogen fertility levels were considered to be the reason for the difference. Both yields were respectable as compared to the spring wheat varieties planted in the same trial.

The summer Rogers Farm Field Day featured a stop at the Spelt variety trial (see attached pictures) and growers were able to see the spelt varieties just before they matured and evaluate the grains based on maturity, height and lodging and disease susceptibility.

The harvested spelt was milled and sifted at the Somerset Grist mill in Skowhegan. This company is interested in obtaining more locally produced spelt to process and sell. Spelt Right Foods LLC (Beth George) conducted baking tests. Originally in Yarmouth Maine, Spelt Right has recently moved to NJ. Below is the report from Spelt Right Baking.

The first trial was on November 29, 2012 in which we used the Maverick spelt. We used 50% hydration, and the same proprietary formula and procedure that we use with our whole grain spelt from other sources. Interestingly, in this trial, the bread structure held up better as compared to our standard bread. The structure of the crumb held up very well, and the texture was light. Persons familiar with the standard whole grain Spelt Right bread consistently said that they thought Maverick had a taste and texture more aligned with "whole wheat" rather than the "whole spelt" to which they were accustomed. The overall response, however, was very positive and we were extremely pleased with the commercial appeal of the bread.

Maverick Spelt Bread 11-29-12—Spelt Grown in Maine

We conducted the second trial on December 3, 2012. We made two separate formulas using 10lbs of Obkerulmer and 10lbs of Comet. The dough was very heavy for both of these varieties, so I added an extra 6 ounces (2.87%) of water to the Olberkumer and an extra 12 ounces (5.75%) to the Comet. Both varieties were in the proofer for the same amount of time, but were proofed at a temperature that was slightly too high (as we were trying to hasten the proofing time given time constraints). The Olberkumer structure fell (you will see by the flat tops on the loaves), and the Comet held up relatively well. The crumb of the Olberkumer was frail and could not hold up to spreads like the cooled butter or peanut butter. The crumb of the Comet was sturdy and would make perfect sandwich bread. (The picture does not do justice to the quality of the Comet bread). The resounding response from six individuals who tried all three breads was that the taste and the texture of the Comet was by far the best. Also, the six who were testing the bread all were surprised as to how different each variety tasted. The only change in any of the formulas was the spelt variety; all other ingredients remained consistent.

On December 13, 2012, we did a trial bake with Sammy and then with a mix of all the varieties. The bakes failed dismally, because we changed a variable we should not have changed: the bread pans. We were trying new bread pans and the gauge was not heavy enough and the breads were not able to proof or bake properly. The failed breads will be used for breadcrumbs or other non-sandwich uses.

Oberkulmer Spelt Bread 12-3-12
Spelt Grown in Maine
(weak structure – flat top and brittle crumb likely from overproofing)

Comet Spelt Bread 12-3-12
Spelt Grown in Maine
(This photo does not do the bread justice. Light but strong crumb; nice crust; great taste; good for sandwiches. May have been slightly overproofed, but structure held up well.

On December 18, 2012, we did another trial run with the remaining 2.5 pounds of Sammy that we had. The breads came out well with a good structure, nice crumb, and sturdy crust. We have three of those breads (but no photo), which we will provide to the University of Maine System. We have frozen the sample breads and will send 3-5 loaves of each to Richard Kersbergen and his crew to try.

Conclusion:

Depending upon multiple factors, including demand, quality, availability and price, Spelt Right would be interested in continued discussions of the possibility of working with the State of Maine through the University of Maine Extension program and the Maine Department of Agriculture in developing spelt as a staple crop in Maine.

Original project objectives that were not met:

The data from the trial has yet to be posted on the websites and facebook pages, but they will be soon.

Methods used to evaluate outcomes:

The varieties tested were evaluated for winterhardiness, ability to withstand late planting, yied potential, crude protein, and Deoxynivalenol (DON). DON, commonly referred to as vomitoxin, is a mycotoxin that may be produced in wheat and barley grain infected by Fusarium head blight (FHB) or scab. FHB may infect grain heads when wet weather occurs during the flowering and grain filling stages of plant development. DON is one of the contributing factors for why wheat is difficult to grow in this region. If spelt is more resistant to the fungus, then it may be a more viable crop to grow. All out varieties tested below detectable limits.

Our baking tests indicate that bakers would be able to work with any of the varieties we tested with some modifications of recipes based on the analysis from Spelt Right LLC. Some of the issues we faced in our trial and evaluation of the results include finding appropriate facilities (infrastructure) to de-hull the spelt before it can be milled for flower, or used as a seed source for future use by the grower. We had to modify our research combine to de-hull the spelt and run it through the combine several times. Somerset grist mill does have a de-hulling machine, but it was not available at the scale we needed for our trial. After the baking tests, Beth George's comments included: "What would be our next step to start getting some spelt in quantities grown in Maine?"

Integration of research and extension activities:

Research was conducted at the University of Maine Rogers Farm and was conducted using organic techniques. The seed we used was not certified organic, but was not treated with a fungicide.

The project was highlighted in the summer 2012 field day at Rogers farm with about 45 people in attendance.

Processors and bakers from Maine were involved in helping to find varieties and evaluate the end product for commercial use.

Data and other information will be posted on websites and Facebook pages sponsored by Cooperative Extension. An organic cereal production fact sheet was produced for growers and includes information concerning production practices for winter spelt. A winter grain production budget was also produce as part of this and the OREI bread wheat project led by Ellen Mallory.

Educational material, publications, and programs:

- A fact sheet was developed to assist growers interested in all winter and spring organic grains (http://umaine.edu/publications/2207e/) Growing Organic Cereals, Extension Educator Richard Kersbergen, Sustainable Agriculture Specialist Ellen Mallory, and Research Associate Tom Molloy
- Additionally, a budget for winter grains was developed that can be modified for any winter grain, including spelt. This budget is available on the Northern New England Bread wheat project website at https://sites.google.com/site/localbreadwheatproject/enterprise-budget

MAC127: Designing Small Ruminant Parasite Control Programs for Maine

Principal investigator(s): Anne Lichtenwalner, James A. Weber

Background:

The goal of this project is to expand ongoing work to help Maine's small ruminant producers detect and treat drug-resistant intestinal parasites, and develop strategies to decrease development of anthelmintic (dewormer) resistance in small ruminant flocks. Outcomes will include: 1) Implementation and validation of a diagnostic method to identify parasite species in sheep, goat and camelid fecal samples, 2) Implementation and validation of a diagnostic method to identify resistance of these parasite larvae to commonly used anthelmintic drugs and 3)Presentation of these methods and other parasite control methods to producers via UMCE web publications, producer meetings, and peer-reviewed publications. At least 50 regional small ruminant producers will provide samples for this project, will receive recommendations for treatment, and will be re-contacted for followup samples to evaluate the effectiveness of this program.

Research and/or extension education activities:

From 2008-11, most necropsy cases of small ruminants at the UMaine Animal Health Laboratory have involved heavy intestinal parasite burdens. Increasing resistance to commonly used dewormers has been well documented in small ruminants worldwide, and in the United States. In spite of this, small ruminant producers continue to use conventional dewormers due to the high prevalence of intestinal parasites. Because genetic resistance to dewormers may be strengthened by using marginally effective dewormers, some producers have quit using these conventional dewormers altogether and rely on "all natural" methods, such as herbal remedies or high-tannin forages. These methods vary in efficacy, but usually do not approach the potential efficacy of properly used conventional, commercially available dewormers. While there may be a need for using alternative therapies, *if* there is an effective dewormer for the particular parasites in those animals, the farm will benefit. Even "alternative" strategies such as pasture rotation are more effective if starting out with parasite-free livestock. Given the high prevalence of intestinal parasites in small ruminants of Maine, reputed resistance of these parasites to anthelmintics, and the short grazing season in Maine, it is critical for producers to be able to design parasite control for their particular setting. Producers need to know the species of parasites carried by their animals, and the anthelmintic resistance patterns of those parasites, to effectively use a combination of strategies to avoid heavy parasitism in their livestock. In this project, we will enroll 50 of the approximately 500 farms in our client list to send in composite fecal samples from small ruminants. The samples will be tested for the presence of parasites using a standard egg count method. Positive samples will be further tested using a larval identification method (FAO manual), and by a modification of an established anthelmintic susceptibility assay. Results and recommendations will be provided to each farmer. Each farm will document how treatment/rotation guidance was followed, and will submit a followup sample. This project will provide a basis for further adaptations of this technique to predict resistance patterns in parasites, will augment our current outreach to small ruminant producers in our region, and will help farmers using alternative strategies assess their programs. We expect to develop a SARE project working with Maine small ruminant farmers based on implementation of this assay in our lab. We will report to regional small ruminant owners at regional workshops and fairs, and will post results on industry websites (ASI, sheepandgoat.com, UMCE) and submit results to the J of Extension or other peer-reviewed journal. Besides the primary benefits of using this assay to help regional producers, this assay can be used to further develop the monoclonal antibody projects begun by Dr. Weber, with the ultimate goal of partnering with one of Maine's biotech companies to produce a species-specific quick test for on-farm use. This could allow producers to easily test for the most problematic parasites in their animals (which can look identical to less pathogenic parasites using current test methods). Again, this would allow producers to worm only when it is most needed, saving them money/

Expected outcomes and method for sharing the outcomes:

We expect to be able to measurably reduce parasitism in the 50 enrolled farms. As an example, a family-owned small ruminant dairy that produces artisanal cheese has lost several goat kids during the winter. Investigation of the case reveals a heavy load of nematode ova, despite a history of regular use of conventional dewormers on the farm. With

this assay, a fecal sample can be tested to reveal the types of worms present, and which wormers kill them. The dairy then uses an effective wormer that a veterinarian has prescribed, allowing safe and legal utilization of the right treatment. Money is saved by increasing feed efficiency and milk yield, and by reducing deaths of young kids. As well, food safety is increased by having a veterinary recommendation regarding proper withdrawal times for milk and meat. Establishment of this method in our lab will assist many other Maine small ruminant producers in keeping their flocks of goats, sheep or llamas/alpacas healthy. The results of this trial will be communicated via Extension seminars, the Extension webpage and producer-oriented publications (see above). Dr. Lichtenwalner has given multiple small ruminant health seminars around the state in the last 3 years, is working on a funded SARE grant with Richard Brzozowski. Dr. Weber owns a dairy sheep herd and is active in the small ruminant community.

Final Report

Original project objectives that were met and significant findings:

Objective 1) Implementation and validation of a diagnostic method to identify parasite species in sheep, goat and camelid fecal samples. Objective was met through incorporation of a previously reported fluorescent lectin binding assay into Dr. Weber's laboratory, and by the development of a novel non-fluorescent nematode speciation assay that could potentially be used on-farm to speciate small ruminant nematodes. Objective 3) Presentation of new diagnostic methods to producers. Currently, 12 producers with over 1000 small ruminants have participated in a 2012 survey of farm practices and an analysis of parasite burdens and species for individual farms. We expect that an additional 12 producers will participate in this year's survey. Results of the survey will be shared with individual producers, and will be presented to additional Maine producers at an annual meeting in October 2012.

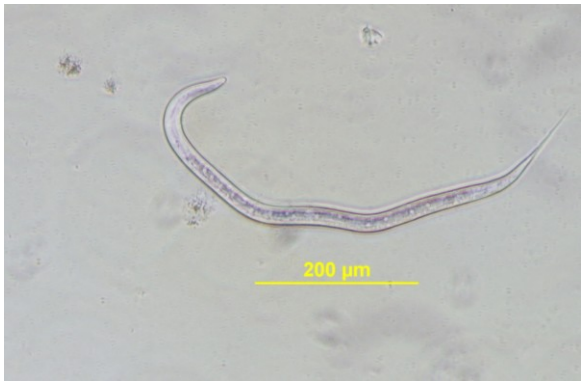


Figure 1- Haemonchus Contortus L3 larvae.

Original objectives that were not met:

Objective 2) Evaluation of the level of antihelmintic resistance in Maine sheep and goats, was not met. After consultation with the technical staff at the University of Georgia, the only group in the U.S. who offer a drug resistance assay, we determined that the cost of individual assays (>$400 for each farm) was not within the budget of this project. 2012 survey results from several conventional Maine farms indicates that currently available drugs for parasite control are not adequately controlling parasitism, so we can assume that Maine's drug resistance problem is similar to what has been reported in other parts of the U.S. In addition, over half of our surveyed farms either followed organic practices or raised dairy animals, so did not use drugs or were limited in their ability to use drugs during the milking season.

Methods used to evaluate outcomes:

As of September 1, 2012, 12 farms had participated in the survey of parasite management methods and had submitted two or more pooled fecal samples from their sheep / goats for parasitological analysis. In addition, several clinical cases accessed at the UMAHL received species-specific parasitological information that was used to help the farmers more effectively manage their animals' parasites. We expect that these no-cost analyses will foster discussion among farmers, practicing veterinarians and University experts that will improve management of internal parasites in Maine. Case reports from this project will be presented to a larger (40+ farms) at the annual small ruminant management seminar in Fairfield in October 2012, and will likely result in the adoption of improved parasite management strategies by other Maine sheep or goat producers.

Integration of research and extension activities:

Both applied research and Extension-type activities were completed within this project. New and existing methodologies for analysis of parasite burdens in small ruminants were incorporated into Dr. Weber's research lab, and these assays will be adopted by the UMAHL technicians in the coming year. Several clinical cases accessed by the UMAHL during 2011-2012 were supported by parasite speciation results from this study. We expect that the results of the Maine parasitism survey will be communicated to producers through both traditional (at meetings) and digital means.

External funding both sought and received:

Drs. Weber and Lichtenwalner applied for a $148,000 NorthEast SARE grant based on the work from this MAC Grant in the summer of 2012. The pre-proposal for the SARE grant was recently approved, so we are currently in the process of developing a full proposal for review in November 2012.

Educational material, publications, and programs:

Small ruminant producers who participated in the 2012 survey of Maine parasitism were provided detailed results regarding the type and amounts of internal parasites present in their animals. As a result of this new information, we expect that many of these producers will be prompted to improve their parasite management strategies. Producers were encouraged to work on management plans with either their practicing veterinarian or Umaine Extension. Results of this MAC project will be communicated to the sheep and goat producers at the annual small ruminant workshop in Fairfield, Maine in October 2012. Several producers who submitted fecal samples were assisted by new technology used in this grant, and this diagnostic work enabled more targeted management suggestions for the affected farms.

Several undergraduate students benefitted from participation in the laboratory work associated with this grant. Two Senior AVS students completed both their Capstone experiences and Honors theses based on parasite diagnostic work, and one poster was completed that participated in the Undergraduate Research Exhibition in 2012.

Research publications and abstracts:

No publications at present. We expect that these results will be reported as an Experiment Station Publication, or possibly as peer-reviewed publication.