

## PROJECT DETAILS

- **Title:** Optimizing canola production through biological control of Blackleg and Insect pests of canola
- **Funders:** Manitoba Canola Growers and SaskCanola
- **Research program:** Canola Agronomic Research Program
- **Principal investigator:** Dilantha Fernando
- **Collaborators/additional investigators:** Neil Holliday
- **Year completed:** 2007

### Final report

The design behind this project was to see if both insects and pathogens of canola could be reduced with an application of one of the biological control agents. This is to optimize canola production without the use of chemicals, thus improving the plant health while protecting the environment. The project investigated the nature of biological control of one of the most important canola pathogens, the blackleg pathogen by using naturally occurring microorganisms. The study found several bacterial agents (mostly *Pseudomonas* and *Bacillus* spp.) to be excellent biological control agents against blackleg at the seedling and adult plant stage in the field (Biocontrol. Sci. and Tech. 2006: 16:567-682). Bacteria isolated from within the plant (inside) known as endophytes were the best biological control agents. Several of these biocontrol agents produced antibiotics such as Phenazine, Pyrrolnitrin, and Zwittermycin. The identification consisted of a PCR-based marker technique to precisely identify the secondary metabolites produced by each bacterium against the Blackleg pathogen control. We were then able to for the first time identify *Bacillus* spp that were able to produce novel secondary metabolites (antibiotics) that are known to be antagonistic to plant pathogens, using PCR and GC/MS MALDI-TOF analysis (Canadian Journal of Microbiology 2007: 53:901-907).

Strains of some plant growth promoting rhizobacteria (PGPR) can inhibit plant pathogens. Two PGPR strains, *Pseudomonas chlororaphis* (PA23) and *Bacillus amyloliquifaciens* (BS6) have been shown to control some fungal diseases of canola. PGPR control pathogens through the production of bacterial metabolites and volatile compounds and through induced systemic resistance, which is initiated by the signalling molecule jasmonic acid, and can protect plants against multiple pest threats. Direct application of jasmonic acid has been shown to activate defense compounds and influence insect herbivory in canola. Field and laboratory studies were carried out to investigate the effects of the two bacteria and of jasmonic acid on insects of canola.



Field studies did not find any significant effects of treatment on any of the insects sampled or on level of flea beetle injury, cabbage maggot parasitism or canola yield or quality. However, laboratory experiments found that jasmonic acid significantly affects oviposition and larval feeding in the diamondback moth (*Plutella xylostella*) and development and reproduction in the turnip aphid (*Lipaphis erysimi*).

In addition to constitutive defences, defences can also be induced in many plants in response to pathogen infection insect herbivory or other elicitors. The objective of this research was to investigate the effects of two types of elicitors, the plant growth-promoting rhizobacteria (PGPR) *Pseudomonas chlororaphis* strain PA23 and *Bacillus amyloliquifaciens* strain BS6 and jasmonic acid, on insect pests of canola (*Brassica napus* L.). The first part of this research was a field experiment that investigated the effects of treatment on insect populations, flea beetle injury, cabbage maggot pupal parasitism and canola seed yield and quality.

In the field study, it was expected that there would be an effect of the jasmonic acid treatments on diamondback moth (*Plutella xylostella* L.) populations, based on results seen in laboratory experiments conducted prior to the start of the field season. In the laboratory, choice experiments were conducted using PA23, BS6, jasmonic acid and control treatments to investigate oviposition preference of *P. xylostella*. The results showed that jasmonic acid affects diamondback moth oviposition preference, with greater numbers of eggs laid on the jasmonic acid treated plants. Larval experiments on feeding and growth rate were also conducted prior to the field season, and showed that larvae feeding on jasmonic acid treated plants consume less plant material and have lower growth rates. Despite these significant laboratory results, there was no evidence of any effects of jasmonic acid on *P. xylostella* in the field. The field experiment would have been more likely to detect treatment effects on oviposition preference than on feeding rate, because numbers of *P. xylostella* were examined through beat cloth and sweep net sampling, rather than the amount of damage caused by larval feeding. The treatment application may not have been timed appropriately to test for this effect.

Diamondback moths are not believed to overwinter in Canada, but are thought to be blown north in the spring. In the field, *P. xylostella* development from egg to pupa can take approximately 25–51 days. The second application of treatments was made on 16 July 2006 at the 30–50% bloom stage, as this time was used in earlier studies with the bacterial treatments to control *Sclerotinia sclerotiorum*. This application date appears to have been after eggs had been laid on the canola, as there were many large larvae present during the first sampling date (26 July), one week after the application of treatment. On the second sampling date, (9 August) there were few very few larvae present, indicating that the majority of oviposition had occurred prior to the second application of treatments, and could not influence *P. xylostella* oviposition. Had the first and second treatment applications both been made to the same plants (5 June field), as had originally been planned, the treatment may have had an effect on oviposition preference; however, problems with the weather conditions and the type of seeder used for planting prevented this.

In canola, laboratory studies have also shown that jasmonic acid treatments can induce defense compounds and reduce feeding by insect herbivores. Similarly, laboratory and field studies on tomato have found that jasmonic acid treatments can also induce defense compounds and negatively affect insect herbivore populations. Based on these examples of insect control, jasmonic acid treatments were predicted to induce defense compounds in *B. napus* and negatively affect insect herbivores of canola, yet there were no significant effects on any of the insects sampled, or on flea beetle injury, cabbage maggot pupal parasitism, canola seed yield or quality.

The analysis of seed quality did show that there was a nearly significant effect of treatment on glucosinolate levels, with greatest levels in the jasmonic acid treated plants. In the laboratory studies, the jasmonic acid treatments also had greater levels of glucosinolates, and the test of systemic influences and the experiments on plant chemistry demonstrated a systemic effect of treatment. These results indicate that jasmonic acid treatments may have an effect on plant chemistry in the field as well as the laboratory, but further examination of plant chemistry is needed to more fully understand the effects.

In the laboratory studies, we found that jasmonic acid treatments increased glucosinolates, important defense compounds found in canola and other Brassicaceae. When plants are damaged, glucosinolate hydrolysis occurs in the presence of the enzyme myrosinase, producing several toxic compounds that can have negative effects on insects and pathogens. Although glucosinolates are believed to have evolved as a method of plant defense, specialist pathogens and insects are not deterred by these defenses and many insect specialists have adapted to use them as cues host plant identification.

In this research, laboratory studies found that there were significant effects of jasmonic acid on *P. xylostea* oviposition preference and larval feeding and growth rate. There were also effects of jasmonic acid on *Lipaphis erysimi* (Kaltenbach) development and reproduction. From the studies on *P. xylostea* larval feeding and growth rate and the analysis of plant chemistry, the effects of jasmonic acid were attributed to systemic responses induced in the plants. There were no effects of either bacterial treatment or of the PA23+pathogen treatment on either of the insects examined.

The level of peroxidase activity increased in the PA23+insect treatment and phenol concentration increased in the PA23+insect and PA23+pathogen+insect treatments. These results indicate that the bacterial treatments may be priming the plants to activate enhanced defences after the challenge of insect feeding.

**Conclusions:** Plant-growth promoting rhizobacteria (PGPR) are potentially important biocontrol agents because they can protect plants from pathogens through the production of various bacterial metabolites and the activation of induced systemic resistance. The use of PGPR in the control of pathogens is highly desirable because there is a decreased risk of pests developing resistance to bacterial-induced plant defences than chemical measures such as fungicides. Although the effects of these bacterial species on naturally occurring bacterial communities can be difficult to predict, research has shown that negative effects can be less, comparable to the effects of chemical fungicides. Although we did not find any significant effects of the PGPR on the insect herbivores investigated, we also did not find any significant negative effects on beneficial insect species. This provides additional safety information for the use of PGPR and may be important if products are registered for use in agriculture in the future, especially as these PGPR (biocontrol) agents have continuously proven to be excellent biocontrol agents agents Sclerotinia and blackleg disease pathogens of canola/rapeseed.

Through the research presented and work done by others, jasmonic acid treatments have been shown to control numerous insect herbivores in a number of plant species. Research on transgenic plants, and the analysis of genes activated by different elicitors has given us a greater understanding of induced plant defences, and in the future there may be potential to exploit these defences in an agricultural setting. Although these treatments may not provide complete control of any insect pest or pathogen, this research makes an important contribution to the development of alternative approaches to sustainable agriculture by enhancing our understanding of the interaction between microbial agents, pests, elicitors and plants.