EXECUTIVE SUMMARY OF PROGRESS TO WINTER CEREAL TRUST: JULY 2013

PROJECT TITLE:
Biochemistry of resistance in wheat to biotic stresses (Russian wheat aphid)

PRINCIPLE APPLICANT:

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CO-WORKERS:

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DURATION:
2012 -2013

AMOUNT REQUESTED FOR 2013:
R60 000

OBJECTIVES:

- Identification and characterization of gene products involved in the resistance response of wheat to the Russian wheat aphid (RWA) and wheat rusts.
- Elucidation of the biochemical resistance mechanism in wheat against the RWA and wheat rusts.
- Development of alternative simple environmental friendly control methods for RWA and wheat rusts.

MOTIVATION:

The aim of the project is to 1) provide plant breeders or plant molecular biologists information on the wheat plant's biochemical resistance mechanisms against the RWA and rusts. The information includes the identities of defense events, resistance genes and resistance inducing compounds. 2) screening for natural plant activators/elicitors and products with bio-pesticidal activity. Plants respond to attacks by insects or diseases by mobilizing an array of compounds that inhibit plant diseases, or reduce feeding by insects. Elicitors are natural or synthetic compounds that initiate plant induced responses to herbivory when applied to foliage or roots. With the use of elicitors of plant resistance, we may be able to tailor the resistance profile of plants to suit the pest pattern of the particular region and the particular year in which the crop is being grown.

The emphasis of this research is the eliciting and signalling events during resistance responses of wheat against the RWA and stem rust with emphasis on the resistance breaking phenomenon as experienced currently with the new RWA biotype (2 & 3) and the stem rust race, Ug 99. Another aspect of the research is the development of plant activators which can

induce resistance. In view of the serious threats these pathogens/pests pose to wheat production the importance of the research is obvious.
Currently, limitations to fast development of effective resistance is the lack of information on the biochemical mechanisms of resistance, identity of genes involve in resistance and identity of possible resistance markers.

GENERAL OUTCOMES:

The gathered information would contribute a better understanding of the resistance mechanisms and positive spin-offs hereof could be faster and more directed breeding of new resistant cultivars, creation of new resistant transgenic plants and development of new strategies to control the pest e.g. chemical inducers of resistance or other biological control measures. All is aimed at using the plant's defence mechanisms to control the pest /disease and to decrease the pesticide/fungicide dependency. This will increase the profitability in the wheat industry and will make wheat cultivation more simple and affordable. In addition, new scientists in this field are trained in South Africa.

SUMMARY:

Signaling events can be regarded as putative switches for activating defense arsenals. The identification and the understanding of the action of signal molecules will be a major breakthrough for future resistance manipulation, especially in view of the creation of broad spectrum and durable resistance. Discovering alternative natural or chemical compounds that initiate plant induced responses when applied to foliage or roots, we may be able to tailor the resistance profile of plants to suit the pest pattern of the particular region and the particular year in which the crop is being grown. The development of natural products with repellent or insecticidal activity will be less harmful to the environment.

RESULTS:

Biochemistry of resistance in wheat to the Russian wheat aphid:

a) Identification of early resistance relating and signaling events

The lipid oxygenases play an important role in the production of lipid signal molecules e.g. oxylipids. The identification of such signal molecules would be a major breakthrough for future manipulation of resistance. The main objective of the study is to identify oxygenated lipids (novel compounds) during the early defence responses of wheat against the Russian wheat aphid. Previous research on susceptible Tugela wheat seedlings indicate the presence of a number of oxygenated fatty acids, however very low levels were detected.

A method developed as part of Ph.D study by Sculze, 2005 titled "Oxylipins and their involvement in Plant responses to biotic and abiotoc stress", was initially used as blueprint for extraction of these compounds. Some minor adaptations have been made to this method to suite the extraction of these compounds from wheat. However, this method proved to be very difficult in identifying lipids present in wheat. The so called pentafluorobenzyl (PFB) esters could not be qualitatively and quantitatively detected by using a GCMS equipped with only an EI+ ionization mode. When carboxyl groups of the Oxylipins are derivatized or estrified to PFB esters, they can only be detected by negative chemical ionization (NCI) or chemical ionization (CI). Therefore, a slightly modified method of Weber *et al*. (1997) was used as alternative as described in a review article by Mueller *et al.* (2006). This proved to be a better quantitative and qualitative method that can be used to detect Oxylipins in wheat. Extracts were methylated as well as silinated before GC/MS analysis. Differences in Oxylipin expression levels as well as unique oxylipins in control and infested resistant,Tugela Dn1 wheat, 0 h and 96 h.p.i. with RWA biotype 1were distinguished. Compounds were identified by their characteristic fragmentation pattern by using the NIST 2.0 library (2008).

Table 1. Identified compounds

Retention time (RT) min	**Compound identified**	**Chemical formula**
6.86	Nonanoic acid, allyl ester	$C_{12}H_{22}O_2$
7.07	BHT	
9.44	Methyl jasmonate (STD)	$C_{13}H_{20}O_3$
9.90	Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester	$C_{13}H_{20}O_3$
10.78	Methyl tetradecanoate	$C_{15}H_{30}O_2$
12.49	Dodecanoic acid, 3-hydroxy-methyl ester	$C_{12}H_{24}O_3$
13.05	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$
14.95	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$
15.63	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
16.31	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$
17.53	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	$C_{20}H_{38}O_2$
20.29	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (Linolenic acid, methyl ester)	$C_{19}H_{32}O_2$
21.00	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$
23.58	Octadecanoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester	$C_{22}H_{46}O_3Si$
24.27	Hexadecanoic acid, tert-butyldimethylsilyl ester	$C_{22}H_{46}O_2Si$
25.74	Octadecanoic acid, 2-oxo-, methyl ester	$C_{19}H_{36}O_3$
28.02	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$
28.40	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$
30.74	Eicosane, 7-hexyl-	$C_{26}H_{54}$
31.11	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$

The methyl esters of Hexadecanoic acid (Rt = 15.63) and Linolenic acid (Rt = 20.29) as well as the silyl ester of Hexadecanoic acid (Rt = 24.27) was differentially induced to much higher levels in infested resistant wheat 96 h.p.i than in control plants. Other methyl esters (Rt= 10.78; 14.95; 23.58) were also induced.

b) The role of polyphenol oxidases (PPO's) during the Dn 1 and Dn 5 resistance responses towards biotype 1 and biotype 2 RWA

In a previous study, 3-leaf stage susceptible and resistant Tugela wheat seedlings were infested with biotype 1 RWA. Plants were harvested at 0, 24, 48, 72 and 96 hours post infestation. Wheat leaves were ground to a fine powder in liquid nitrogen and subsequently homogenised in 1 ml extraction buffer. PPO activity was measured spectrophotometrically by following the conversion of L-DOPA (3,4-dihydroxy-L-phenylalanine) to dopaquinone at 478nm. No PPO activity was detected in any of the wheat samples. Apple and potato material were used as positive controls to check if the extraction and assay procedures were correct and we found PPO activity in both these samples.

The same protocol was used for follow-up experiments. We included more cultivars, susceptible and resistant Tugela, Thatcher and PAN, while apple and potato material were again used as positive controls. PPO activity was measured in the seeds, seedlings and flag leaves of the various cultivars. The results showed an increase in PPO activity in seeds, but very low concentrations were present in the leaves of all the cultivars even after infestation with RWA. It was concluded that PPO levels in wheat leaves were too low to play a role in the defence response of wheat against the RWA.

Plant activators / extracts:

a) The role of chitosan oligomers to act as elicitors in the RWA resistance response.

The study was part of a collaborative research project between the Department of Plant Sciences at the University of the Free State in Bloemfontein (South Africa) and the Department of Plant Biochemistry and Biotechnology, Westfälische Wilhelms-Universität *Münster* (Germany). The study was performed in collaboration with Dr Astrid Jankielsohn at the ARC-SGI.

The focus of this study was to investigate the role of chitosan oligomers to act as elicitors in the RWA resistance response. Previous results indicated that application of chitosan oligomers to infested susceptible and resistant wheat seedlings and adult plants, had a notable impact on the resistance response of susceptible plants after just one treatment. It is also clear that seed, as well as a combination of seed & foliar application were more effective in seedlings than just foliar treatment. In adult plants all treatments were effective.

The experiment was repeated and two chitosan oligomers were applied to seedlings of Tugela, Tugela Dn1 and Tugela Dn 5. Two hours, 24 h, 48 h and 2 weeks after foliar and seed treatment, plants were infested with the Russian wheat aphid biotype RWASA3 at a rate of ±3 aphids per plant.

Symptom development on plants was analyzed by scoring the plants two weeks after infestation according to a method described by Tolmay (1995), using a scoring system whereby a plant with a score of $1 \leq 3.3$ represents a highly resistant plant with very little chlorosis and no rolling of leaves, $3.6 \leq 6.5$ represents a medium resistant plant with chloretic spots, and $6.6 \leq 10$ represents a susceptible plant with striping or/and rolled leaves.

Results indicated that seed, foliar and seed & foliar chitosan applications to infested susceptible Tugela wheat seedlings decrease symptoms from susceptible to medium resistant after only 2 h exposure to the chitosans. It is also clear that seed, as well as a combination of seed & foliar application were more effective than just foliar treatment. A priming response was noticed after 24 h and 48 h of treatment. Symptom development decreased after infestation and plants show resistance against RWA3.

b) The effect of *Artemesia afra* extracts on RWA attraction / repulsion

Objectives:

a) Identification of classes of compounds present in the polar, non-polar and essential oil extracts of *A. afra.*
b) Bioactivity of extracts
c) Screening for initial aphid repellency of polar, non-polar and essential oil extracts using a four-arm olfactometer
d) Screening for aphid / plant acceptance with a no-choice aphid-settling test on plants sprayed with the polar, non-polar and essential oil extracts.

Botanical insecticides that are isolated from plants have long been noted as attractive alternatives to synthetic chemical insecticides due to the small threat they pose to the environment and human health. Some European countries are already successfully using botanical insecticides in pest management with the interest in plant essential oils gaining momentum (Isman 2006).

Artemesia afra, which is rich in terpenes, is known as a traditional medicinal plant that most likely has valuable biological activities (Van Wyk *et al*., 2000). These plants grow in the same areas where wheat is produced.

Constituents in the polar extracts were identified mainly as phenolic compounds, which also have anti-oxidant activity. The chemical composition of the essential oil was determined by GC-MS and the major compounds were thujone, eucalyptol and camphor.

The hexane and essential oil fractions show initial repellency against the Russian wheat aphid.
In the glasshouse trails, exposure to the essential oil resulted in significant reductions in aphid settling on both resistant and susceptible wheat plants. The number of aphids settling on the water extract was also significantly less on the resistant wheat than on the control plants indicating a possible induced resistance response in the plants towards the Russian wheat aphid.

Business plan (materials and methods)

1 Biochemistry of resistance in wheat to the Russian wheat aphid.

1.1 Eliciting and signaling events during the RWA resistance response.

Objective 1: Identification of early resistance relating and signalling events:

Investigating a number of methods during 2012, a slightly modified method of Weber *et al*. (1997) proved to be the better quantitative and qualitative method to detect oxylipins.
Future: The research will continue and concentrate on Dn-1 and Dn-5 resistance responses against the RWA after infection with biotypes1 and 2. The induced oxylipins will be monitored and identified by GC-MS at different time intervals after infestation.
Completion date: Dec 2014

Responsibility: H Pretorius (PhD student)

1.2 Biochemistry and molecular biology of resistance to the RWA

Objective 2: The role of polyphenol oxidases (PPO's) during the Dn 1 and Dn 5 resistance responses towards biotype 1 and biotype 2 RWA.

Polyphenol-oxidase oxidises phenolic compounds to quinones, which are often more toxic to micro-organisms than the original phenols, therefore greater resistance to infection. The role of PPO's in defence is not so clear, therefore we want to establish the involvement of PPO's in the RWA-wheat interaction.
PPO levels of seeds, seedling leaves and flag leaves were determined in susceptible and resistant Tugela, Thatcher and PAN wheat cultivars before and after infestation with RWA biotype 1. The results showed an increase in PPO activity in seeds, but very low concentrations were present in the leaves of all the cultivars even after infestation with RWA. It was concluded that PPO levels in wheat leaves were too low to play a role in the defence response of wheat against the RWA.
Completed

1.3 Plant activators / elicitors

Objective 3: The role of chitosan oligomers to act as elicitors in the RWA resistance response.

Oligochitosan is effective at eliciting plant innate immunity against plant diseases in lots of plants such as tobacco, rapeseed, rice and grapevine. It has a potent protective effect on several kinds of plants such as crops, fruits, vegetables and trees. It is used as bio-pesticide on crop protection in several provinces in China.

The German partners have done extensive research on the defense inducing capacities of chitins and chitosans. During 2012/13 results confirm that a seed and foliar application of chitosan was the most effective treatment on seedlings and adult plants to increase resistance in plants to RWA infestation. Symptom development decreased in susceptible plants to medium resistant to resistant.

Future: Field trials must be done to investigate symptom development and yield. It will also be interesting to look into the effect of these products on wheat rusts.

Completion date: Dec 2014

Responsibility: Dr A Jankielsohn, Dr ME Cawood

Objective 4: The effect of *Artemesia afra* extracts on RWA attraction / repulsion

This study focus on the behavioural response of *D. noxia* to polar and non-polar extracts of *A. afra*. During 2013, the hexane and essential oil fractions show initial repellency against the Russian wheat aphid. In the glasshouse trails, exposure to the essential oil resulted in significant reductions in aphid settling on both resistant and susceptible wheat plants. The number of aphids settling on the water extract was also significantly less on the resistant wheat than on the control plants.

Future: The roles of *A. afra* extracts in the activation of plant defences in susceptible and resistant wheat, as well as the possible involvement of PR-proteins in the resistance response of those plants during Russian wheat aphid infestation, will be investigated.

Completion date: Dec 2014

Responsibility: L Saba (MSc student), Dr M Cawood

Objective 5: The role of calcium and kinases in the RWA resistance response

Calcium and kinases are involved in very early responses in plant defense and play an important role in the induction of defense genes. Calcium functions amongst others as a co-factor of protein kinases involved in phosphorylation events. The study will be performed on a molecular-biochemical level and should shed light on the very early events of the RWA resistance response.

Completion date: Dec 2017