# Factors affecting the resistance mechanisms of the Russian wheat aphid (*Diuraphis noxia*) on wheat.

BY

## LIESCHEN BAHLMANN

A thesis submitted in partial fulfillment of the requirements for the degree of

## **MAGISTER SCIENTIAE**

In the Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria

June 2002

Study Leaders:

Prof. Anna-Maria Oberholster
Prem Govender

## **DECLARATION**

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Magister Scientiae to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other University.

Bahlmann

Lieschen Bahlmann

June 2002

## **ACKNOWLEDGEMENTS**

I wish to thank the following persons and organizations:

- This work was supported by the National Research Foundation (NRF) and the University of Pretoria.
- Prof. A.-M. Oberholster and Prem Govender for critical review of manuscripts, valuable advice and support.
- Dr. Ben Eisenberg for statistical help.
- Dr. M.C. Oosthuizen and Brigitta Steyn (Department of Microbiology, University of Pretoria)
   for assistance with the two-dimensional gel electrophoresis.
- Jacques Berner (Department of Genetics, University of the Free State) for assistance with the Russian wheat aphid.
- Albé van der Merwe for computer assistance and motivation.
- My parents, brother, sister and grandmother for your continued support during this period.
- Gavin, for your encouragement and patience.



To my brother Etienne: you are always in my thoughts, I will never forget you.

## List of abbreviations

RWA Russian wheat aphid

D. noxia Diuraphis noxia

L:D Light:Dark

SD Standard deviation

PI Plant Introduction

kDa Kilo Dalton

ARC-SGI Agricultural Research Council-Small Grain Institute

IWF Intercellular washing fluid

SDS-PAGE Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

T Percentage of the total for acrylamide and bisacrylamide

C<sub>bis</sub> Concentration of bisacrylamide

MDH Malate dehydrogenase

IEF Isoelectric focusing

SEM Scanning electron micrograph

TEMED N, N, N, 'N'-tetramethyl-ethylenediamine

NADH Nicotinamide adenine dinucleotide, reduced state

pI Isoelectric point

## List of figures

- Figure 2.1.
- (A) The Russian wheat aphid.
- (B) A viviparous female Russian wheat aphid.

p. 7

Figure 5.1.

SDS-PAGE profiles of intercellular protein from (A) noninfested leaves of 'Tugela *Dn1*' and (B) RWA infested leaves of 'Tugela *Dn1*'. The vertical axis represents molecular masses (kDa). Black arrows indicate unique proteins in each profile. Red arrows indicate proteins that are overexpressed during RWA infestation. Blue arrows indicate proteins that are underexpressed during RWA infestation.

n. 89

Figure 5.2.

Protein profiles after two dimensional gel electrophoresis of intercellular protein from (A) noninfested 'Tugela Dnl' and (B) RWA infested 'Tugela Dnl'. The horizontal axes represents pl's of the isoelectric focusing gradients. The vertical axes represents molecular masses (kDa). Diamonds indicate unique proteins. Ovals indicate proteins that are overexpressed. The protein marker is shown on the left and the pl points to the top of each gel.

p. 90

Figure 6.1.

Scanning electron micrographs of the position of leaf trichomes on the adaxial and abaxial leaf surfaces of three wheat cultivars (60X; bars =  $100 \mu m$ ). (A) adaxial surface of 'Palmiet'. (B) abaxial surface of 'Palmiet'. (C) adaxial surface of 'Tugela'. (D) abaxial surface of

'Tugela'. (E) adaxial surface of 'Tugela DnI'. (F) abaxial surface of 'Tugela DnI'.

p. 109

Figure 6.2.

Scanning electron micrographs of epicuticular wax ultrastructure on the adaxial and abaxial surface of the 'Palmiet' wheat cultivar (9 500X; bars =  $1\mu m$ ). (A) adaxial surface. (B) abaxial surface.

p. 110

Figure 6.3.

Scanning electron micrographs of epicuticular wax ultrastructure on the adaxial and abaxial surface of the 'Tugela' wheat cultivar (9 500X; bars =  $1\mu m$ ). (A) adaxial surface. (B) abaxial surface.

p. 111

Figure 6.4.

Scanning electron micrographs of epicuticular wax ultrastructure on the adaxial and abaxial surface of the 'Tugela Dn1' wheat cultivar (9 500X; bars = 1 $\mu$ m). (A) adaxial surface. (B) abaxial surface.

p. 112

## List of tables

		1301	nts	
Tab	ıle	3.	1	į
H 64 K				•

Russian wheat aphid survival and reproductive maturity. Twenty nymphs were placed on each of three wheat cultivars ('Palmiet', 'Tugela' and 'Tugela DnI') at  $25\pm1$ °C with a 12:12 (L:D) photoperiod.

n. 48

Table 3.2.

Duration of developmental stages in days for the Russian wheat aphid to reach the reproductive stage at 25±1°C with a 12:12 (L:D) photoperiod.

p. 49

Table 3.3.

Average number of nymphs produced per day, average number of nymphs produced per adult and average life span of the Russian wheat aphid on three wheat cultivars. Aphids were placed at 25±1°C with a 12:12 (L:D) photoperiod. (Table only includes nymphs that survived to become reproductive).

p. 50

Table 4.1.

Composition of Diet A and Diet B.

p. 66

Table 4.2.

Russian wheat aphid adults surviving to become reproductive. Twenty adults were placed on each of the five diets at 25±1°C and a 12:12 (L:D) photoperiod.

p. 68

Table 4.3. Average number of nymphs produced per Russian wheat aphid, average number of nymphs produced per day and average life span on each of the five diets at 25±1°C at a 12:12 (L:D) photoperiod.

p. 68

Table 5.1. Malate dehydrogenase assay (MDH) for IWF and total proteins from RWA infested 'Tugela Dn1'.

p. 86

Table 6.1. Trichome length and density in three wheat cultivars examined. Two cultivars ('Palmiet' and 'Tugela') are susceptible to the RWA and the third is resistant to the RWA ('Tugela *Dn1*').

p. 107

Table 6.2. Position of trichomes on the adaxial and abaxial surfaces of three wheat cultivars examined. Two cultivars ('Palmiet' and 'Tugela') are susceptible to the RWA and the third is resistant to the RWA ('Tugela Dn1').

p. 108

## **Index**

Chapter 1		
PRI	EFACE	1
Chapter 2	Abatract	
LIT	TERATURE REVIEW	
2.1	Introduction	3
2.2	Russian wheat aphid description and biology	4
2.3	Feeding	8
2.3.	1 Examination, probing and selection of the feeding site	8
2.3.	2 Location of the food source	10
2.3.	3 Ingestion	13
2.3.	4 Withdrawal of stylets	15
2.4	Methods of curbing Russian wheat aphid damage	16
2.4.	1 Cultural practices	16
2.4.2	2 Insecticides	17
2.4.	3 Biological control	17
2.4.4	4 Planting of resistant cultivars	19
2.4.4	4.1 Antibiosis	20
2.4.4	4.2 Antixenosis (nonpreference)	21
2.4.4	4.3 Tolerance	22

23

2.5 Induced protein alterations

26
26
43
44
46
46
46
47
48
52
56
60
61
64
64
64

	4.3.2.1	Testing optimal sucrose concentrations	64
	4.3.2.2	Testing diet A and diet B	65
	4.3.3	Statistical analysis	66
	4.4	Results	67
	4.5	Discussion	70
	4.6	References	72
Chapt	ter 5		
		IAN WHEAT APHID (DIURAPHIS NOXIA) INDUCED	
	PROT	EIN ALTERATIONS IN WHEAT	
	5.1	Abstract	77
	5.2	Introduction	78
	5.3	Materials and Methods	80
	5.3.1	Plants	80
	5.3.2	Intercellular washing fluid (IWF) extraction	80
	5.3.3	IWF contamination with cytoplasmic constituents	81
	5.3.4	Protein determination	82
	5.3.5	SDS-PAGE analysis	82
	5.3.6	Two-dimensional gel electrophoresis	83
	5.3.7	Analysis of protein profiles	84
	5.4	Results	86
	5.5	Discussion	91
	F (	Deferences	9:

Chapter 6	)
-----------	---

I	6		
	LEAF I	EPICUTICULAR WAX ULTRASTRUCTURE AND	
	TRICH	IOME EFFECT ON RUSSIAN WHEAT APHID	
	(DIUR	APHIS NOXIA) FEEDING	
	6.1	Abstract	99
	6.2	Introduction	100
	6.3	Materials and Methods	102
	6.3.1	Plants	102
	6.3.2	Leaf trichome examination	102
	6.3.3	Leaf epicuticular wax ultrastructure and trichome position	103
	6.3.4	Statistical analysis	104
	6.4	Results	105
	6.5	Discussion	113
	6.6	References	110

## Chapter 7

#### SUMMARY

		118
7.1	Summary	
	Oino	121
77	Opsomming	