Russian wheat aphid biotypes in Lesotho: distribution, impact on wheat production and the role of phytohormones in host resistance

By

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Dedication

I dedicate this thesis to my lovely wife Senate Rosemary Masupha

My wife, my best friend, thank you for standing by my side when times get hard.

Declaration

I declare that the dissertation submitted by me for the degree Philosophiae Doctor at the University of the Free State, South Africa is my own independent work and has not previously been submitted by me to another University. I furthermore concede copyright of the dissertation in favour of the University of the Free State.

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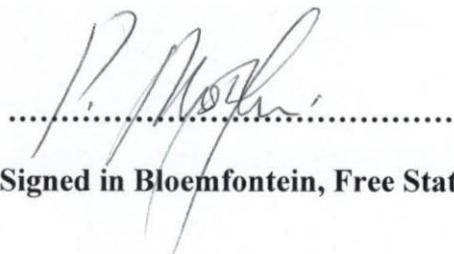
Signed in Bloemfontein, Free State, South Africa

Abbreviations

A

ABA	Abscisic acid
ARC-SGI	Agricultural Research Council- Small Grain Institute

B

BSA	Bovine serum albumin

C

CIMMYT	The international maise and wheat improvement centre

D

DAR	Department of Agricultural Research
DTT	Dithiothreitol
Dn	Diuraphis noxia

E

EDTA	Ethylenedinitrilotetraacetic acid
EPG	Electrical Penetration Graph
ET	Ethylene

G

GLC	Glucanase

H

hpi	Hours post infestation
HPLC	High performance liquid chromatography
HR	Hypersensitive response

I

ICS	isochorismate synthase
IR	Infested resistant
IS	Infested susceptible
IWF	Intercellular washing fluid

J

JA	Jasmonic acid

M

MeJA	Methyl jasmonate

MR		Medium resistant
MRM		Multiple reaction monitoring
	N	
NO		Nitric oxide
N: P: K		Nitrogen: Phosphorus: Potassium
	P	
PR		Pathogenesis related
POD		Peroxidase
PAL		Phenylalanine ammonia lyase
	R	
ROS		Reactive oxygen species
R		Resistance
RWA		Russian wheat aphid
RWASA		Russian wheat aphid South Africa
	S	
S		Susceptible
SA		Salicylic acid
SAGL		Southern African Grain Laboratories
SAR		Systemic acquired resistance
	T	
Tris		Tris(hydroxymethyl)aminomethane

Conferences contributions and published papers originating from this thesis

Conference contributions:

- **Masupha, P**., Jankielsohn, A. and Mohase, L. 2016. Factors affecting the production of wheat (*Triticum aestivum* L.) in Mokhotlong and Thaba-Tseka in Lesotho. South African Journal of Botany. 103:330.

- Jankielsohn, A. and **P. Masupha**. 2016. Field screening of Lesotho and South African wheat cultivars for Russian wheat aphid resistance. Combined Congress, UFS, Bloemfontein, 18-21 January.

- **P.V. Masupha**, L. Mohase, & A. Jankielsohn. 2017. Variation in Russian wheat aphid (*Diuraphis noxia*) resistance between South African wheat (*Triticum aestivum*) cultivars and cultivars grown in the mountains of Lesotho. Poster presented at the Entomological Society of Southern Africa and the Zoological Society of Southern Africa (ESSA & ZSSA) Combined Biennial Congress, CSIR ICC, Pretoria, South Africa, 3-7 July.

- Mohase L, **Masupha, P**. & Jankielsohn A. 2017. South African Russian wheat aphid biotypes induce differential responses in farmers' wheat varieties grown in the highlands of Lesotho. Paper delivered at the Entomological Society of Southern Africa and the Zoological Society of Southern Africa (ESSA & ZSSA) Combined Biennial Congress, CSIR ICC, Pretoria, South Africa, 3-7 July.

Published papers:

- **Masupha, P**., Jankielsohn, A. and Mohase, L. 2018. Assessment of cultivation practices of wheat and knowledge of Russian wheat aphid (*Diurphis noxia*), in Mokhotlong and Thaba Tseka districts of Lesotho. *International Journal of Agricultural Extension and Rural Development Studies*. 5 (3): 13-23.

- Jankielsohn, A., **Masupha, P**. and Mohase, L. 2016. Field screening of Lesotho and South African wheat cultivars for Russian wheat aphid resistance. *Advances in Entomology*. 4: 268-278

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Chapter One

Background

1.1 Lesotho: Agro-geographical information

Lesotho is an enclave within the Republic of South Africa, located between 28° and 31° south of the equator, and 27° and 30° east of the Greenwich meridian. It is situated on the Drakensburg escarpment, falls between 1500 and 3482 m altitudes (Chakela, 1999). The country has ten administrative districts and four agro-ecological zones (Fig.1.1), characterised by distinctive ecological and climatic differences (Bureau of statistics, 2008). The climate in the lowlands region is suitable for maise, beans, sorghum, winter wheat and vegetables. The foothills zone rises from 1800 to 2400 m above sea level. Sorghum, maise, winter wheat, beans, vegetables and summer peas are also grown in this zone. The "mountains" (highlands) is a region characterised by chilly winters. The area elevates to 3500 m; wheat and peas are grown in this zone. The fourth zone is the Senqu River Valley. It is a steep basin along the Senqu River, running from east to west across the country. The valley receives the lowest annual rainfall, and typical crops are winter wheat and maise.
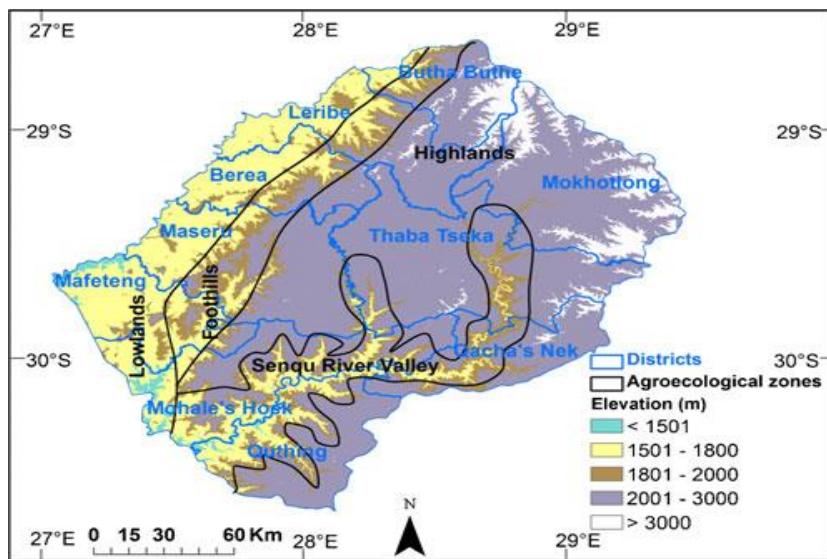
Figure 1.1 Lesotho map showing the ten districts and four agro-ecological zones

The climate is temperate with hot, wet summers and cool to cold winters. Average annual rainfall is 788 mm varying from less than 300 mm to 1 600 mm in the western lowlands and the northeastern highlands, respectively. There is a considerable seasonal distribution of rainfall, and as much as 80% of the total precipitation occurs from October to April (FAO, 2016). The mountains usually receive the highest rainfall. However, due to the early onset of frost, the cropping season is much shorter. The mountainous regions also receive snow during winter (LMS, 2000).

There is a significant variation in temperature on daytime, monthly and annual time scales. Minimum temperatures in winter, usually range from -6.3 °C in the mountains to 5.1 °C in the foothills and lowlands on a monthly bases. However, daily temperatures in winter can drop as low as -21 °C at some places in the mountains and the average minimum monthly temperatures of 10.7 °C can be reached in winter (LMS, 2000). Subzero daily minimum temperatures are recorded even in summer, in the mountains as well as in the lowlands. The hottest month is January with the lowlands exceeding 30 °C during the day (FAO, 2016).

Short seasons characterised by an early frost, snowfall and icy conditions are unsuitable for maise, which is the primary staple food for most parts of the mountain districts as shown in Fig. 1.2 below.
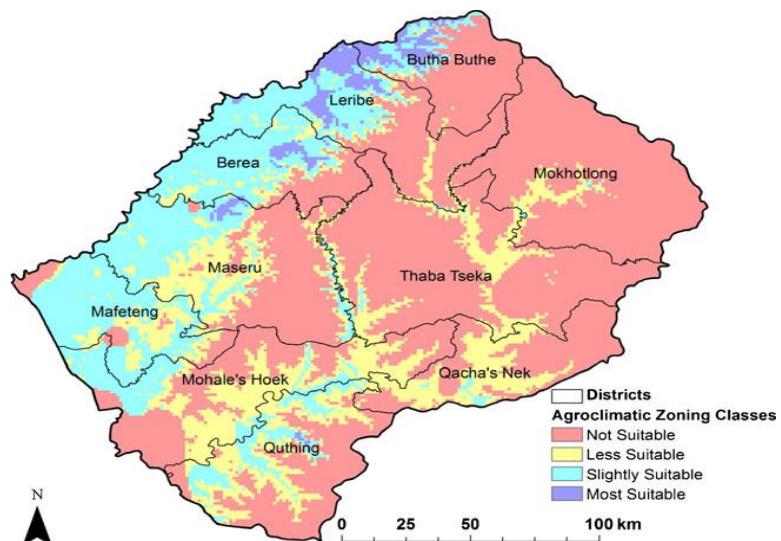
Figure 1.2 Lesotho map showing maize suitability in different districts (Moeletsi and Walker 2013)

1.2 Wheat Production in Lesotho

Wheat (*Triticum aestivum* L.) is a universal cereal crop cultivated in the four agro-ecological zones of Lesotho. It comes third after maise and sorghum as a staple food. Winter wheat is grown in the three agro-ecological zones (Lowlands, foothills and the Senqu River Valley) of the country. Spring wheat is grown in mountain areas (Moremoholo, 2000). There are places in the mountain region like Thaba-Tseka (Lesobeng and Mantšonyana) and Mokhotlong (Malefiloane) where climatic conditions do not support maize production, and farmers mainly rely on wheat as a cereal crop. The unconducive weather conditions make Mokhotlong and Thaba Tseka districts leaders in the overall wheat production in the country. To support this, Mofoka (1985) reported that wheat production for nine years from the growing season 1973/74 to 1981/82 showed Mokhotlong average yield (1241kg/ha) to be higher than all the other districts of Lesotho. The recent Agricultural crop production surveys also revealed that Mokhotlong and Thaba Tseka have the highest area planted with wheat in the country (BOS, 2015; 2017).

Wheat cultivars that were commonly grown because of their adaptability and high yield in Lesotho in the '80s were Schepeers 69, Wilge, SST 102, Flamink and Betta. These were winter cultivars grown mainly in the lowlands, while Flamink, Kenya sokkies, Bolane, and Gamka were grown in the mountain areas and the foothills (Mofoka, 1985). The survey conducted by Rosenblum, *et al*., (1999) showed that the most commonly used cultivars in Lesotho in the '90s were Bolane, Mantša Tlala (Tugela) and Mohohlotsane.

Bolane is a tall cultivar that was introduced in Lesotho in the 1960s (Weinmann, 1966). The grains are relatively white, and farmers prefer it for bread making, and its large straws, favoured for roofing. The cultivar is still grown in different parts of Mokhotlong and Thaba Tseka districts. Farmers in this region also cultivated Mantša-Tlala (meaning expelling hunger), which was released as Tugela in South Africa in 1985 and promoted in Lesotho. The cultivar has an intermediate canopy with high tillering capacity. The third cultivar Mohohlotsane is awnless with median canopy height, dense spikes and comfortable grain thrashing. Durum cultivar Telu-Ntšo (meaning black beard) was another cultivar that was widely grown in the mountains. The cultivar has distinctive black spikes with prominent

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awns. Farmers no longer cultivate this cultivar because the grains are hard to mill (Tolmay and Mare, 2000).

A survey was conducted in February and March (2015) in Mokhotlong (Libibing and Malefiloane regions) and ThabaTseka (Mantšonyane and Lesobeng regions). These areas represent strategic production areas within the districts. The survey revealed that farmers still depend on recycled seed, plant without fertilisers and still use ox-drawn equipment to prepare the seedbed. Some farmers broadcast the seed and plough it under; while others plough their fields, broadcast the seed and then harrow to cover it. The seed is prepared by first winnowing to remove spikelets and other debris. A special sieve ensures that small grains and the weed seeds are filtered, and only the large grains are retained and used as seed. The survey also discovered that farmers never monitor their fields for any potential pest or disease attacks. They only go in the end when the crop is ready for harvesting (Masupha, *et al.,* 2018).

Cultivars that are currently used by farmers in the mountains are Tšolooa, Gariep, Puseletso (Tugela *Dn*) and Bolane. Tšolooa means spilling out, and farmers gave this cultivar the name because of its high yield. This cultivar is also suitable for roofing and livestock feeding as it grows tall. Gariep has the highest yield, but farmers complain about its short straw that is unsuitable for roofing (Masupha, *et al.,* 2018). Bolane, the third cultivar is also preferred for yield and straw. In Malefiloane, an area in Mokhotlong, only one cultivar Bolane, which the farmers believe adapts to their high altitude, is grown. Even though farmers in this region have confidence in their seed cultivars, these seeds still have to be tested as they may be some of the old cultivars bred in South Africa given the indigenous names by the farmers. Evaluation of cultivar reaction to different RWA biotypes and yield potential is necessary. The assumption is that cultivars available in the market outperform the farmers' in-house cultivars in terms of tolerance to pests and yield.

Lesotho shares the border with one of the major wheat-producing areas of South Africa, the Eastern Free State, where the production of winter wheat is under dryland conditions

(Purchase, *et al.,* 1995). The environment for wheat production in Lesotho is favourable. Wheat average yield per ha in the country, as shown in Fig. 1.3 for the past five years has been higher than the other five major crops grown in Lesotho (BOS, 2015). The report further indicated that Mokhotlong and Thaba Tseka were the leading districts in wheat production with the average yield of 1.7 t/ha and 1.05 t/ha respectively, in 2013-2014 cropping season. This higher wheat production occurs even though these districts do not use improved cultivars and fertilisers. However, these yields are far below those of the Free State in South Africa (2.90 t/ha), in the same year, where modern cultivars are used (SAGL, 2015). The production of wheat in the country is relatively insignificant when compared to South Africa, SADC, Africa and the whole world (Table 1.1).
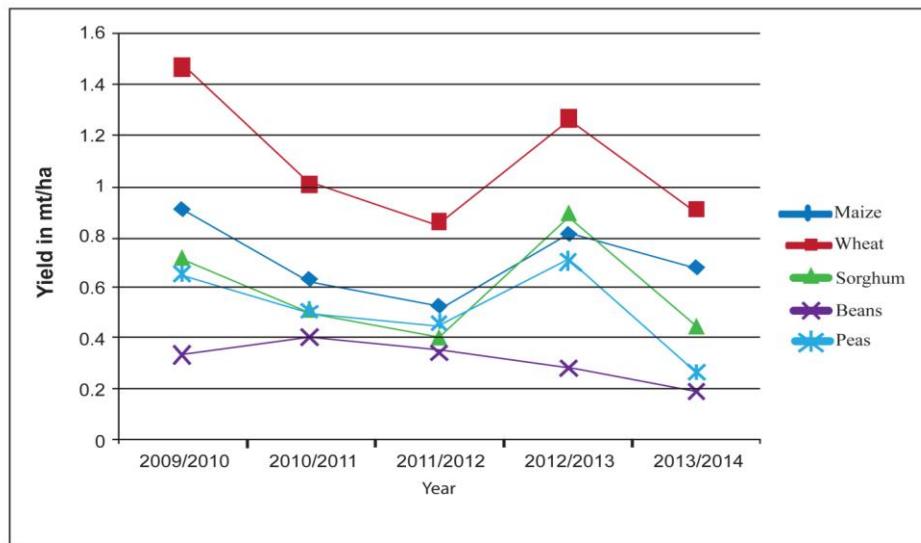
Figure 1.3 Yield (t/ha) for the five major crops grown in Lesotho from 2009/2010 to 2013/2014 (BOS, 2015)

Table 1.1: Wheat production (tons) in Africa, SADC, South Africa and Lesotho from 2013 to 2017 (Source: FAOSTAT)

Region	2013	2014	2015	2016	2017
Africa	28 060 897	25 440 497	29 123 992	23 319 715	27 153 529
SADC	1 899 192	1 773 282	1 459,725	1 929 130	1 557 930
South Africa	1 870 000	1 750 000	1 440 000	1 910 000	1 535 000
Lesotho	13 472	12 592	7 069	4 690	8 851

1.3 Russian Wheat aphid

Russian wheat aphid (*Diuraphis noxia*, Kurdjomov) has been reported as a pest of small grains in the USSR since the early 1900s. It subsequently spread to several Mediterranean and Middle East countries, but its population never reached epidemic status because of the unfavourable climate (Dick and Moore, 1987). In 1978, Russian wheat aphid (RWA) became established in South Africa, apparently due to a more favourable climate; severe damage resulted (Walters, 1984). The aphid damages most of the small grain cereals, like barley, wheat, Triticale, rye and oats.

The RWA has an elongated, spindle-shaped body; it is pale to light green and grows up to 2 mm long. The antennae are short, with rounded and almost invisible cornicles (Gary and Leon, 1987). Feeding, which is accompanied by injection of salivary toxins results in white or yellow longitudinal bands which appear on the leaves. These symptoms differ from those of other grain aphids such that one can easily recognise its infestation through the resulting damage. In colder climates, the bands become pinkish or purplish due to the existence of anthocyanin pigments. The RWA feeds on the upper surfaces of curled leaves. Young host plants become stunted, and massive RWA attacks and pre-panicle infestations usually result in panicle deformations and curling of flag leaves (Kazemi, *et al.,* 2001).

Russian wheat aphid is capable of surviving under icy conditions. Harvey and Martin (1988) reported that the RWA survived the two coldest months (January and February) -20.8°C and -21.5°C in Hays, Kansas. The temperatures in Lesotho are colder than the Free State in South Africa where this pest is abundant. The climatic data for Lesotho as analysed by Moeletsi (2004) from 1990 to 2004 shows the average minimum temperatures for Lesotho in June and July to be -6.1 and -6.5°C respectively. The temperatures in Lesotho, therefore, cannot impede the survival of RWA.

The control of RWA, especially in the Eastern Free State was mainly large scale aphicide applications (Du Toit, 1987). Report by Du Toit (1992) shows that the RWA seriously hampered the production of wheat in the Free State and drastic reductions in yield occurred if insecticides were not used. Lesotho started experiencing low wheat production since the

introduction of the RWA from about 1979 (Purchase *et al.,* 1993). Unfortunately, farmers in Lesotho are resource-poor and do not have the expertise and economic power to control RWA using insecticides (Purchase *et al.,* 1993; Moremoholo and Purchase, 1999).

The use of resistant cultivars is considered an economical, effective and environmentally safe management option for RWA management (Bregitzer, *et al.,* 2015). Agricultural Research Council – Small Grain Institute (ARC-AGI) identified the sources of genetic resistance and through backcrossing of cultivars with acceptable agronomic background, such as Tugela (pedigree: Kavkaz/jaral "S") produced resistant cultivars. The first of such cultivars was Tugela-DN, released in 1992 in South Africa, and donated to the Ministry of Agriculture in Lesotho (Moremoholo and Purchase, 1999). The farmers renamed the cultivar "Puseletso", which means "the recovering of that which we have lost" (due to RWA infestation). It carried the highly effective RWA resistance gene *Dn1* (SA 1684), was tolerant of low soil pH and had good yellow rust resistance.

While it is essential to know the distribution of RWA in the country, it is even more critical to know the 'biotypes' of the aphid present in the country for the development of sustainable integrated management strategies. There are three biotypes reported in Lesotho (Jankielsohn, 2011) and five in South Africa (Jankielsohn, 2019). Jankielsohn (2011) suggested that South African biotypes, RWASA2 and RWASA3 were possibly introduced at the same time with RWASA1. They probably survived and diverged on the diverse alternative host plants in Lesotho and the Eastern Free State and from there attacked cultivated wheat fields.

In the USA, eight RWA biotypes exist since 2003 (Puterka, *et al.,* 2014). The rate at which new RWA biotypes evolve globally and in South Africa in wheat producing areas illustrates the importance of constant monitoring of the diversity and distribution of RWA biotypes to manage this aphid successfully. Collection of RWA samples for biotypic diversity determination in an area should target not only cultivated wheat but also alternative host plants used for over-summering (Jankielsohn, 2011).

1.4 Plant Defence

The first line of plant defence is its surface. Presence and amount of surface cuticles, needles, thorns, trichomes, and waxes influence feeding and oviposition behaviour of the aphid and may repel the aphid (Smith and Boyko, 2007). Aphid probing and feeding in plants activate a suite of host responses, such as the expression of genes in different defence signalling pathways, which are crucial to resistance or susceptibility during insect-plant interactions. The phytohormonal signals include endogenous molecules such as jasmonic acid (JA), gibberellic acid (GA), abscisic acid (ABA), ethylene (ET), salicylic acid (SA) and free radicals like nitric oxide (NO) and hydrogen peroxide (H_2O_2), which individually or collaboratively, affect natural chemical resistance (Figure 1.4, Morkunas, *et al.*, 2011). Salicylic acid, for instance, induces the expression of defence response genes, which include pathogenesis-related (PR) proteins, and mediates systemic acquired resistance (SAR) and localised plant tissue hypersensitive (HR) responses.

Plant hormones play essential roles in regulating reproduction, growth, and development in plants. Furthermore, they are cellular signal molecules essential in the responsible for plant immune responses to herbivores, pathogenic and beneficial microorganisms. The signalling pathways of these hormones are organised in a complex system, providing plants with enormous potential to regulate and adjust to the biotic environment quickly and to use their limited resources for development and survival cost-efficiently (Corne, *et al.,* 2012).
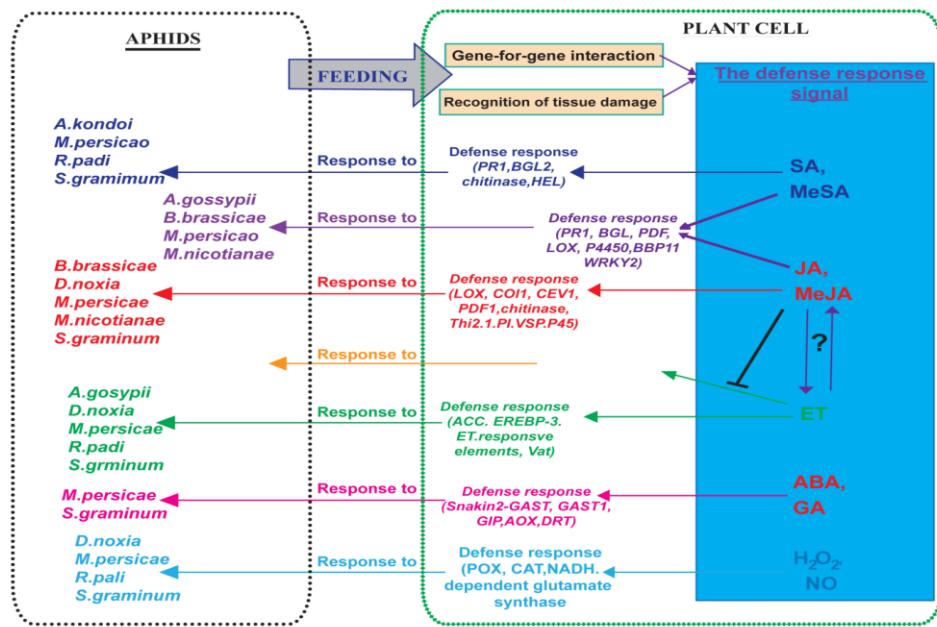
Figure. 1.4 Plant signalling pathways involving defence responses to aphid attack. Arrows show pathway activation. Bidirectional arrows signify positive regulatory interactions amongst these signalling pathways, and lines indicate antagonistic interactions (Morkunas, *et al.,* 2011).

Advances in plant immunity studies support the crucial role of cross-talk in hormones in the regulation of plant defence signalling (Spoel and Dong, 2008; Pieterse, *et al.,* 2009). Hormonal cross-talk involves a process whereby various hormonal signalling pathways act synergistically or antagonistically to provide powerful regulatory potential. This interaction allows plants to adapt to a range of environmental conditions. Salicylic acid, ethylene and jasmonic acid signalling pathways cross-talk are central in the regulatory mechanism of plant immunity (Verhage, *et al.,* 2010). Evidence from several unrelated plant species shows that there can be evolutionarily conserved SA- and JA-signaling cross-talk, resulting in mutual antagonism between the JA and SA signalling pathways (Glazebrook, 2005). Pieterse, *et al.*, (2012) pointed out that an overall knowledge on the temporal and spatial dynamics of hormone production and signalling as plants and other organisms interact remains deficient, particularly about how the interplay in hormones directs plant defence response.

Host plant resistance is an environmentally friendly, economical and effective method of controlling RWA. The implementation of this method requires information on the distribution and diversity of RWA biotypes that are present in the environment. Russian wheat aphid population densities fluctuate with the change in climatic conditions but persist in the major wheat-producing areas, and the population and the subsequent damage can vary (Jankielsohn, 2017). Wheat cultivation in the lowlands districts of Lesotho has been declining, and this too may have an effect of the population density, distribution and diversity of RWA.

The mountains districts, especially Mokhotlong and Thaba Tseka, lead in wheat production. Our preliminary survey on the cultivation practices in these districts revealed that farmers still use their recycled seed without any fertilisation of the soil. The most cultivated cultivars are Bolane and Makalaote. Farmers have been using these cultivars for over 50 years. The resistance status of these cultivars towards RWA is unknown. Furthermore, there is evidence that RWA prevails and affect yield in these districts (Moremoholo & Purchase, 1999; Makhale, Moremoholo and Mohammed 1999; Jankielsohn, 2011). Yield performance of these cultivars compared to those from South Africa recommended for Lesotho, and the Eastern Free State is not known.

1.5 Objectives of study

The study aims to establish occurrence, distribution and biotype status of the Russian wheat aphid in Lesotho, its impact on wheat yield and the role of phytohormones in host resistance. The objectives are, therefore:

1. To investigate the occurrence and distribution of RWA in Lesotho.
2. To determine different biotypes of RWA in Lesotho.
3. To establish the level of resistance against RWA in different cultivars in the greenhouse.
4. To determine the level of resistance against RWA in different cultivars in the field.
5. To determine the involvement of jasmonic, salicylic, and absiscic acids in the resistance response of wheat to the RWA.
6. To determine the activities of key enzymes in the biosynthetic pathways of specific hormones during infestation under greenhouse conditions.

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Chapter Two

Literature Review

2.1   Introduction

Wheat is a vital crop in Lesotho; however, its production has been declining. Russian wheat aphid is one of the biggest challenges in areas of the world where wheat is cultivated (Morrison and Peairs, 1998). The review addresses i) the state of wheat production in Lesotho, ii) Russian wheat aphid (RWA) origin, description and distribution, iii) RWA as a pest in cereal production and iv) inducible host plant resistance in RWA management. This review was id guided by analysis of journal articles, technical reports, books and theses.

Wheat Production in Lesotho

Wheat is the third most important cereal crop in Lesotho, after maise and sorghum (Bureau of Statistics, 2014). However, its production is continually declining in terms of yield and area planted. Morojele and Sekoli (2016) reported a sharp decline in wheat production from 57 540 tons from 1961 to 13 000 tons in 2013, revealing a decrease of 77%. Similarly, trends in areas planted wheat decreased dramatically from 39 119 hectares (ha) in 1962 to 7 000 ha in 2013, resulting in a decrease of 82%. Late planting time, low seeding rate, low soil fertility, poor seed-bed preparation, inadequate harvesting machinery and adverse climatic conditions such as hail storm during the growing season are cited as some of the critical factors affecting wheat production in Lesotho (Central Bank, 2012; Bureau of Statistics, 2014; Lesotho review, 2015).

Wheat cultivars used in Lesotho

Lesotho does not have any wheat breeding programs. The majority of cultivars planted in the country are from South Africa. The Department of Agricultural Research (DAR) is screening seven sets of germplasm; the International Center for Agricultural Research in Dry Areas (ICARDA) provided four of these, and the International Maize and Wheat Improvement Center (CIMMYT) supplied the other three (DAR, 2016). These cultivars will

be released to the farmers once adaptable, and high yielding ones have been identified. However, cultivars released from DAR are hardly adopted by the farmers, who are always ahead with the knowledge of best-performing cultivars from South Africa.

Collaborative research between DAR and Agricultural Research Council – Small Grain Institute (ARC-SGI) in the late 90s yielded cultivars such as Tugela *DN,* commonly known as Puseletso (Moremoholo and Purchase, 1999). The research extensively focused on evaluating and introducing in Lesotho several new wheat cultivars from South Africa. In the trials, Scheepers 69, an old cultivar of wheat grown in Lesotho for many years, was considered as the standard. From these trials, Tugela, Karee, SST 107, and Gamtoos, emerged as significantly outperforming (20 – 50%) the standard cultivar (Scheepers 69) grown in the country in terms of yield (Ntokoane, 1992). Recent studies have revealed Bolane, Mohohlotsane (Mother of the birds), Mantša-Tlala (Tugela) and Puseletso (Tugela *DN1*) as the most preferred among the smallholder farmers in the principal wheat production areas of the mountain districts of Lesotho (Rosenblum, *et al.,* 1999; Boshoff, *et al.,* 2002; Jankielsohn, *et al.,* 2016; Masupha, *et al.,* 2018). The performance of these cultivars in terms of yield, RWA resistance and rust tolerance has not been compared to the current South Africa cultivars.

2.2 The Russian wheat aphid

Origin and distribution

The Russian wheat aphid *Diuraphis noxia* (Kurdjumov, Hemiptera: Aphididae), was first reported in 1900 Southern Russia and the Mediterranean region (Elsidaig and Zwer, 1993). Mordvilko identified and named this aphid *Brachycolus korotnewi* in 1990, which is native to the Steppe county of Southern Russia. In 1912, Kurdjumov recognised that the barley species was a different species, which he named *B. noxia,* and subsequently, the RWA genus was renamed *Diuraphis* (Robinson, 1994). In America, the RWA was first detected in the Texas Panhandle of the USA in 1986 (Nkongolo, *et al.,* 1989). The aphid spread from west of Asia to the USA and Canada through South Africa and Mexico (Saidi and Quick, 1996).

In several European countries where RWA is endemic, it was first reported in the late 80s and early 90s. For example, in Yugoslavia, it was in discovered in 1989, Serbia in 1990, Hungary in 1990 and the Czech Republic in 1993 (Stary, 1999). This aphid first appeared in Australia in 2016, in wheat sown early, in the Mid North of South Australia (Perry and Kimber, 2016).

In the African continent, RWA was detected in the Atsbi and Adigrat areas of Tigray, Ethiopia, in 1972 (Haile and Megnasa, 1987). In Kenya, it was discovered in 1995, where affected areas experienced a 90 – 100% crop loss (Macharia, *et al.,* 2017). In South Africa, RWA was identified in 1978 (Walters, *et al.,* 1980). Its distribution was initially confined to in the Eastern Free State, around Bethlehem, but by 1979, the RWA had dispersed to other wheat-producing areas in the country. South Africa landlocks Lesotho, and one of the significant wheat-producing areas in the mountains region (Mokhotlong) is close to Bethlehem in South Africa. Reports by Purchase, *et al.,* (1995) indicate that RWA was introduced in Lesotho in 1979. In agreement, wheat trials conducted in Lesotho in 1993 showed that late plantings in June suffered more RWA attack resulting in significant yield reduction than early plantings (Moremoholo and Purchase, 1999). Jankielsohn (2011) further reported that the three RWA biotypes recorded in South Africa also existed in Lesotho.

Morphology

Russian wheat aphid has a small, elongated cigar or spindle-shaped body, about 2 mm long, and it is light greyish-green except for dark endings on the antennae and legs. Unlike the corn leaf aphid, *R maidis* and green bug, *S graminum,* its cornicles (tailpipes) are nearly invisible (Dick and Moore, 1987). The extremely short antenna and the existence of an appendage (supracaudal process) on the dorsum of the eighth abdominal tergite, provides a distinguishing feature from other cereal aphids. The supracaudal process is approximately the length of the cauda in aptera but only a short knob in alata, giving an impression of two tails on the RWA (Stoetzel, 1987; Karren, 1989).

Feeding and symptoms

Feeding and the resultant symptoms help in the identification of RWA infested plants. The RWA prefers to feed at the base of the young and tender leaves of the plant, which are strong sinks for phloem-mobile carbohydrates, mineral nutrients and amino compounds (Macedo, *et al.,* 2003). Aphids usually use their stylets to attempt to penetrate the leaf surface irrespective of the plant species. Seemingly, high viral disease transmission rates by aphids on non-host plant species result from probing behaviour in aphid-non-host interactions (Powell, *et al.,* 2006; Jaouannet, 2014). Electrical Penetration Graph (EPG) has been used to explain the feeding mechanism and behaviour of aphids and EPGs are obtained through completing the electrical circuit by passing electrodes through the insect (Tjallingii, 1978). Results of EPG show that the stylet pathway is through the intercellular spaces, probing through the middle lamella between cells and secondary wall material, via intercellular air spaces or between plasmalemma and the cell wall, that is intramural and extracellular (Botha, *et al.,* 2017).

The release of watery saliva and chemical compounds (Miles, 1999) accompanies RWA feeding. Digestive enzymes in RWA saliva cause break down in leaf chloroplasts, resulting into white, purple, yellow, or reddish-purple longitudinal streaks on infested plant leaves (Pike, *et al.,* 1991). Feeding by RWA also results in redistribution of the assimilate movement through the formation of local sinks. It also causes vast, probably long-term, injury to cells and tissues, through enhanced callose deposition in the damaged functional phloem in wheat plants that are not resistant (Botha and Matsiliza, 2004).

Symptomatic leaves have low photosynthetic efficiency, resulting in reduced vigour and increased susceptibility to environmental stresses (Seheed, *et al.,* 2007). In young plants, high infestations lead to tillers being prostrate; in matured plants, tillers become trapped in the rolled flag leaf, and severe outbreaks result in the death of the plant (Walters, *et al.,* 1980). Smith, *et al.*, (1992) further showed that infestation in wheat induces two forms of leaf rolling, that is, leaf folding in completely expanded leaves and deterrence of unfolding in developing and immature leaves. In mature leaves, the leaf edges start to roll inward, enclosing in the aphids in a tubular structure that protects aphid colony as it develops.

Lodging of aphids in rolled leaves reduces the effectiveness of chemical and biological control methods.

Host Plants

The RWA is polyphagous, feeding on barley and wheat in winter and spring and surviving on non-cultivated grass hosts in summer (Burd, *et al.,* 1998). Alternative host plants are crucial in the survival and life cycle of the RWA by acting as a source of food when there is a deficiency of suitable host, between harvest and planting of new crop. Suitable hosts in South Africa comprise volunteer wheat (*Triticum aestivum*), oats (*Avena sativa*), wild oats (*Avena fatua*), rescue grass (*Bromus catharticus*), barley (*Hordeum vulgare*), and false barley (*Hordeum murinum*). These plants grow immensely in and around South African wheat fields and in the road reserves close to main routes; resulting from spilled seeds from trucks carrying grains to the silos (Jankielsohn, 2013). Weiland (2009) assessed the non-cultivated grass hosts in four Colorado counties and found that RWAs were consistently collected from downy brome (*Bromus tectorum* L.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), Canada wild rye (*Elymus canadensis* L.), and intermediate wheatgrass [*Thinopyrum intermedium* (Host)]. Barkw, *et al.,* (1989) additionally found that, of the 65 warm and cold season grass species that the RWA survived on, jointed goatgrass was the most preferred host, followed by barley, European dune grass, and little barley. Host preference of South African RWA biotypes (RWASA1, RWASA2 and RWASA3) tested on different host plants found mainly in the Eastern Free State revealed that different biotypes have different abilities to survive on diverse host plants (Jankielsohn, 2013).

Effect on yield

Yield reduction is closely related to the proportion of infested tillers and the duration of infestation (Archer and Bynum, 1992). However, Burd and Burton (1992) showed that the duration of infestation, rather than the level of infestation, might be more critical in damaging the host plant. Akhtar *et al.,* (2010) found that there was a decline in grain yield (7.9 to 34.2%) associated with increasing aphid infestation in various genotypes. Tesfay and Alemu, (2015) further reported a massive reduction in wheat grain yield (68%), biomass (55%), weight per 1000 seeds (20%), and delayed heading and maturity as infestation

intensity increased. The differences in yield loss were associated with host resistance, as Mornhinweg *et al.,* (2005) found that highly resistant cultivars increased or maintained yield components and grain yield (5% average grain yield increase) under high RWA feeding intensity. Susceptible cultivars, on the other hand, had significant yield components and grain yield reduction (56% average reduction). The responses of moderately resistant or susceptible cultivars were intermediate and continuous, and the average grain yield reduction was 20%. In South Africa, RWA reduced average yield by about 48% (Du-Toit and Walters, 1984). In Lesotho, there are no statistics on the effect of RWA on wheat yield. However, Purchase, *et al.,* (1995) reported that Lesotho had a thriving wheat industry until RWA decimated the industry from about 1979.

Effects of RWA infestation on susceptible plants also vary with climatic conditions. Ntokoane (1992) reported that in Lesotho, droughty springs were associated with heavy RWA infestations, which destroyed wheat. Riedell (1989), on the other hand, showed that RWA infestation caused drought-stress like symptoms in leaves of infested plants even in the presence of ample root moisture.

Development and Reproduction

Development and population growth of RWA rapidly proceed if a suitable host and favourable climatic conditions are present. The environment should also be free of constraining biological factors such as parasitoids, predators and fungi. Reproduction is highest at 18-21 °C on wheat in developmental stages from stem elongation to heading (Behle and Michels, 1990; Kaplin, *et al.,* 2015). This temperature requirement does not have to be persistent, even if it occurs only for a part of the day, some development still occurs (Pike, *et al.,* 1991). The RWA is sufficiently cold tolerant of enduring winters in the Great Plains, (Colorado and Nebraska) and in the Pacific Northwest (Elliott, *et al.,* 1998). Jankielsohn (2011) reported the presence of RWA in Lesotho as far as Mokhotlong (about 3000 m above sea level) where winters are often freezing (-21 °C, LMS, 2000).

Aphids have two principal life cycles which are holocyclic and anholocyclic. The genotype x environment interaction influence these life cycles (Blackman, 1974). During the

holocyclic life cycle (cyclical parthenogenesis), aphids alternate annually from asexual in summer to sexual in autumn; they lay eggs that overwinter in freezing winter. In spring, aphids hatch from eggs as fundatricies (stem mothers), which develop to reproduce asexually. The clonal lineages continuously expand through parthenogenesis in summer, resulting in clone populations.

In contrast, the anholocyclic life cycle is based on asexual reproduction. Furthermore, anholocyclic forms produce adults capable of overwintering and continuing to feed during winter. In environments where RWA feeds outside its natural range, it reproduces anholocyclically.

The genetic make-up of RWA determines the co-occurrence of holocyclic and anholocyclic clones in populations of the same species in temperate regions (Blackman, 1972). Aphids with an anholocyclic life cycle only produce asexual females throughout the year in warm climates that have mild winters. This life cycle rapidly increases aphid clone populations to the levels that they become critical pests of agriculture.

The anholocyclic life cycle has two categories, the facultative parthenogenesis (produce asexually in warm climates, cyclically parthenogenetic in cold environments) and the obligate (permanently asexual) parthenogenesis (Blackman, 1974; Dixon, 1985). Females have a life-span of 60-80 days, reproduce asexually throughout the year, and give birth to live young ones.

The holocyclic RWA is present in Hungary and Russia (Basky and Jordaan, 1997) and anholocyclic ones in South Africa (Aalbersberg *et al*., 1987).

Biotypes
The term biotype refers to a population of insects that is capable of damaging specific plant cultivars that are resistant to other populations of the same insect species. The physical characteristics of RWA cannot distinguish between the biotypes. The differences may be

physiological and biochemical/molecular, and can best be identified by damage symptoms on cultivars with specific resistance genes (Jankielsohn and Tolmay, 2006). About 11 resistance genes (Dn1, Dn2, Dn3, Dn4, Dn5, Dn6, Dn7, Dn8, Dn9, Dnx, and Dny) have been identified in wheat and its related plants. Most of these genes occur on either 1D or 7D chromosomes in hexaploid wheat (Liu, *et al.,* 2002; 2005; Fazel-Najafabadi, *et al.,* 2015; Fatma, *et al.,* 2016). In South Africa, a differential of designated *Dn* genes 1-9, *Dn*x and *Dn*y, a susceptible wheat cultivar Betta and a resistant RWA matrix, Cltr2401 which is resistant to all known South African RWA biotypes, designates the biotypes (Jankielsohn, 2014).

In the US, RWA biotypes (RWA1, RWA2, RWA3/7, RWA6, and RWA8) distinct from those recorded in South Africa, can be determined using four wheat genotypes having *Dn3*, *Dn4*, *Dn6*, and *Dn9* (Purteka, *et al.,* 2014). There are currently five RWA biotypes reported in South Africa (Jankielsohn, 2019). The first biotype, designated *RWASA1,* was recorded in 1978. The second biotype, *RWASA2* which is virulent against the *Dn1* resistance gene in wheat, was reported in 2005 on wheat in the Eastern Free State (Tolmay, *et al.,* 2007). The third biotype, *RWASA3*, virulent to the *Dn4* resistance gene in wheat, was reported in 2009, also mainly in the Eastern Free State. During 2011 another biotype, *RWASA4*, virulent to the *Dn5* resistance gene was also documented in the Eastern Free State, near Bethlehem (Jankielsohn, 2014). The presence of the majority of these biotypes, RWASA1, 2 and 3, was also reported in areas of Lesotho bordering the Eastern Free State province of South Africa (Jankielsohn, 2011). No studies have been conducted to establish the occurrence of RWASA4 or 5 in Lesotho since their discovery in South Africa.

2.3 Russian wheat aphid control

knowledge of the biology and ecology of the RWA is essential for the successful management of the aphid. Monitoring of the host for infestation symptoms is vital to the control of any pest, including the RWA. Various approaches, such as biological, chemical and host resistance, can be followed to manage aphid populations and their impact on wheat production.

Biological control

Different types of natural enemies attack the aphids, including predators, parasitoids, and fungi. In greenhouse crops, natural enemies used against aphids include eight parasitoids of the *Braconidae* and *Aphelinidae* (Hymenoptera), fifteen predator species of the *Coccinellidae*, *Chrysopidae*, *Syrphidae, Cecidomyiidae, Anthocoridae,* and *Miridae,* and some insect-pathogenic fungi (Yano, 2006). Rapid reproduction rate in aphids, which is characteristic of parthenogenesis, viviparity and polymorphism, allows overlapping of generations and provides the preferred aphid developmental stages to the predators and parasitoids. Their honeydew is an attractive food source for many entomopathogens (Joshi, 2010). Wright, *et al.,* (1993) found that RWA was the most commonly parasitised aphid, and *Diaeretiella rapae* (M'Intosh) was the most regular parasite. They also reported that the predator syrphid fly larvae were consistently found preying on aphids within the RWA rolled-leaves. Nonetheless, syrphid populations were low, less than 0.3 larvae in the aphid-infested tillers. Therefore their effectiveness in reducing aphid populations was not convincing.

In South Africa, *Aphelinus hordei,* a parasitoid imported from Ukraine for the control of RWA was introduced in 1991. The parasitoid was released in 1993 and 1994 in the Eastern Free State, and the highest percentage of parasitism recorded was 83.3% (Prinsloo, 1998). However, this was not sustainable, as more releases were required every year. Mycoinsecticides such as Mycotrol® ES, containing the hyphomycete *Beauveria bassiana*, have been used with some success to control RWA infestation of resistant wheat cultivars in South Africa (Hatting, *et al*., 2004). However, 65% of control seemed insufficient to recommend the use of *B. bassiana* alone as an aphid control agent.

Chemical control

The use of insecticides is one of the most efficient strategies in managing pests throughout the world; insecticides are readily available, induce a rapid effect, and are highly reliable. A single insecticide application may control several pest species and usually develops a persistent residue that continuously kills the insects for hours or even some days after application (Meyer, 2003). However, the aphid feeding habit and its seclusion within rolled

leaves, which renders contact with insecticides problematic (Robinson, 1994), limits the chemical option to manage RWA. Nonetheless, Umina, *et al.,* (2017) showed that chlorpyrifos (contact and stomach insecticide) could be the most effective foliar spray for control of RWA in barley and wheat. Doses of 150-600 grams of chlorpyrifos active ingredient per hectare were tested, and 300 g ai/ha (600 ml/ha of a 500 g/L formulation) consistently provided high levels of RWA control. Further tests showed that reduced rates of chlorpyrifos, 240 g ai/ha gave 99% control of RWA on winter wheat in 14 days (Hill, *et al.,* 1993). However, Umina *et al.,* (2017) cautioned that chlorpyrifos should only be applied under higher pest infestation pressure or later during the growing season due to possible adverse effects on beneficial insect species, especially if higher doses are to be used.

Tesfay and Alemu (2015) also found that Fenitrothion 50 EC, which is a contact insecticide, controlled RWA and prevented 67% decline in grain yield and 44% biomass yield reduction. On the other hand, foliar spray with Demeton-S-Methyl Parathion resulted in a yield increase of a resistant wheat cultivar in both dry and wet years. However, it was not effective on susceptible wheat under drought conditions (Tolmay, *et al.,* 1997). The use of insecticides in the control of RWA is based on economic thresholds. Control is necessary when RWA infestations reach thresholds of 20% seedling infestation at the beginning of tillering and 10% of plants through a critical period of jointing to a soft dough (Umina *et al.,* 2017).

Tolmay and Mare (2000) showed that even though the application of insecticides increase grain yields but, more than often these increases are not economically justifiable; because the cost of buying the insecticide and its application is not always recovered. Factors such as RWA infestation levels and input cost compared to the wheat grain market price are key profit determinants. The use of insecticides to control RWA also kill non-target organisms and beneficial insects such as ladybird which predates on aphid (Ozkara, *et al.,* 2016). Furthermore, residues of organophosphates (e.g., parathion and fenitrothion) mostly used against aphids, do not affect non-target organisms. However, they have resulted in disequilibrium in the ecology of microorganisms degrading the pesticides (Ghorab and

Khalil, 2016).   Lastly, long term use of chemicals is not desired because of insecticide resistance development (Yu, *et al.,* 2014)

Host plant resistance

The most sustainable, effective and environmentally safe management option for RWA is the cultivation of resistant wheat cultivars. These cultivars do not exhibit the typical RWA associated damage symptoms. Even though resistant cultivars are important, RWA has a characteristic capacity to overcome the inherent resistance developed through plant breeding. It should be clear that host resistance is just one important tool that may be used to manage the pest (Umina, *et al.,* 2017). Messina and Sorenson (2001) found that the effects of plant resistance and reduction in intrinsic rate of aphid population growth were synergistic; lacewing larvae reduced aphid density more on a tolerant resistant cultivar (with the *Dn*4 gene) than on its near-isogenic less susceptible parent plant. Higher predation levels on the resistant line continued over a wide range of prey/predator ratios.

Plant breeding for RWA resistance in South Africa started at the Small Grain Institute – Agricultural Research Council (SGI-ARC) in 1985. The institute released the first RWA resistant cultivar in 1993 (Marasmas, *et al.,* 1998). The first sources of RWA resistance were found in wheat in countries where the pest is widespread, namely Iran, the Balkans, the former Soviet Union, Turkey and the rest of the Middle East (Du Toit, 1992). Even though there is significant progress in resistance breeding, Jankielsohn (2016) cautions that the plasticity nature of the RWA will continue to be a challenge to the development of resistant cultivars. She indicated that continued monitoring and evaluation of the genetic and biotypic structure of aphid populations are essential for integrated protection of wheat.

2.4 Plant defence

Plants use two distinct strategies to fend off insect herbivores: induced direct defence, which deals with the attacker and indirect defence, which attracts the natural enemies of the attacker (Howe and Jander, 2008; Dicke and Baldwin, 2010; Wu and Baldwin, 2010). Plant characteristics that affect insect biologies such as trichomes, hairs, spines, thorns, and thicker leaves mediate direct defences. These structures affect insect feeding, oviposition

and movement (Pedigo, 1996). Production of toxic chemicals such as phenols, terpenoids, anthocyanins, alkaloids, and quinones that either kill or retard the growth of the herbivores (Hanley *et al.,* 2007; War *et al.,* 2012) also moderate the direct defences. These chemicals are broadly categorised as anti-nutritive or toxic. Anti-nutrition occurs before ingestion to limit food supply and after ingestion to reduce nutrient quality to the attacking insect. Toxicity involves physical damage and chemical disruptions to the invading insect by specific plant traits (Chen, 2008). A combination of volatiles released by plants, which mainly attract natural enemies of the insect pest, or provide food (for instance extrafloral nectar) and shelter to enhance the efficiency of the natural enemies, confer indirect defences against herbivores (Arimura, *et al.,* 2009).

Elicitors originating from the pests or the interaction of the plant and the pest activate biochemical pathways leading to the production of hormones, such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET). The accumulation of these hormones mediates the production of a broad spectrum of volatiles. These volatiles include indoles, aldehydes, terpenes, esters, alcohols, ketones, and nitrogenous compounds (Fig 2.1). These compounds attract natural enemies, including parasitoids, predators, and omnivores, resulting in the reduction of the pest population (Ajibory and Chen, 2018).
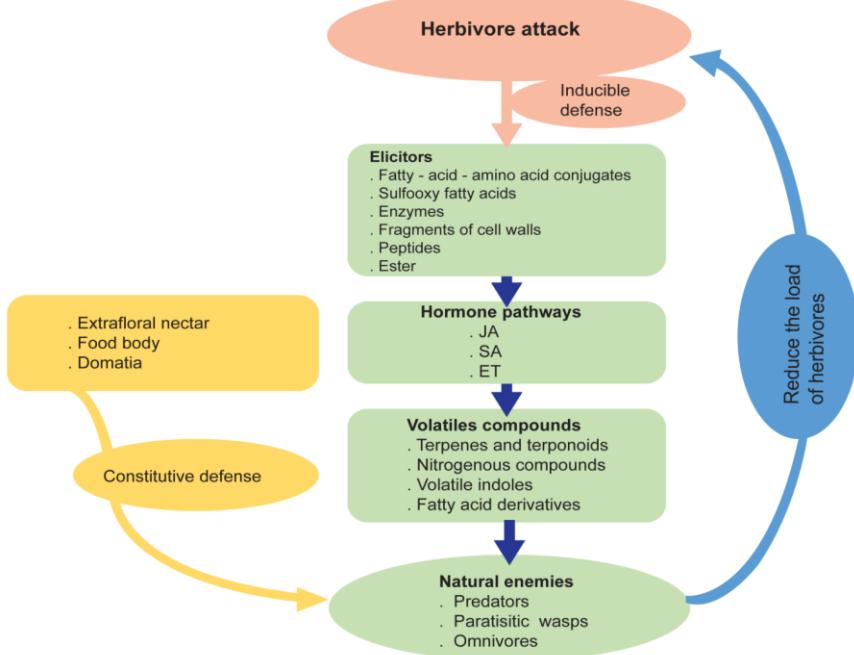
Figure 2.1 Indirect Plant Defences (Ajibory and Chen, 2018).

2. 5 Biochemical pathways modified during induced defence responses

Lipoxygenases pathway

Plant lipoxygenases (linoleate, LOXs: oxygen oxidoreductase, EC 1.13.11.12) contain a big gene family of nonheme iron having fatty acid dioxygenases, which are abundant in animals and plants (Brash, 1999). They catalyse hydroperoxidation of polyunsaturated fatty acids leading to the development of fatty acid hydroperoxides. The latter are chemically or enzymatically broken down to unstable and highly reactive γ-ketols, epoxides or aldehydes (Bruinsma, *et al.,* 2009). Linoleic and linolenic acids are significant substrates of LOX in plants. One of the most critical effects of LOX in plant defence is the oxidation of linolenic acid in the jasmonic acid signalling pathway, which subsequently plays a leading role in enhancing activation of plant defences (Mao, *et al.,* 2007). Williams and Harwood (2008) added that the significant roles for products of LOX pathways in plants are in defence against pathogen attack and herbivore wounding. Berner and Van Der Westhuizen (2015) reported a differential increase in LOX activity in resistant but not susceptible wheat plants after infestation with RWA. Zhao (2009) also reported that volatiles produced from aphid-

infested plants induced the activity of LOX, which was followed by activation of the JA-signaling pathway and the accumulation of transcript levels of associated multiple defence genes. On the other hand, Botha, *et al.,* (2014) showed that RWA infestations in the resistant near the isogenic line, Betta, when compared with susceptible Tugela increased LOX activity, but this did not show any significant differences in defence response.

Jasmonic acid and its derivatives (jasmonates) are phytohormones with essential roles in plant defence against pathogens and herbivorous arthropods (Okada, *et al.,* 2014). The jasmonates are linoleic acid-derived cyclopentanone-based compounds and essential molecules of the octadecanoid signalling pathway (Meyer, *et al.,* 1984). The Jasmonic acid/ethylene pathway induces indirect defences through the development and discharge of plant volatiles that attracts both parasitoids and predators of the insect pest (Kessler and Baldwin, 2002). Morkunas, *et al.,* (2011) observed that the application of MeJA or JA exogenously results in wound-induced defence. The resultant high amount of endogenous JA is similar to induced defence responses. Similarly, the low production of the JA pathway does not allow the initiation of defence responses. Plants treated with MeJA or JA produce volatile emissions comparable to insect attack. Furthermore, the volatiles induce copious floral nectar production, synthesis of secondary metabolites, decreased development and oviposition of herbivores, the increased attraction of predators and parasitoids, and more excellent parasitism rates of herbivores for a variety of plant species. (Bruinsma, 2009; Thaler, *et al.,* 2012).

Tolerance to RWA in resistant barley (Stoneham) was found to be linked to greater constitutive expression of JA-, ET- and auxin-biosynthetic pathway, than in susceptible barley, indicating the likelihood of immediate plant adjustments in response to RWA feeding (Marimuthu and Smith, 2012).

Jasmonic acid and SA are known for their antagonistic cross-talk. Rising SA levels are associated with down-regulation of the JA/ethylene-regulated defence-response genes, and JA-regulated wound responses (Walling, 2000; Erb, *et al.,* 2012; Thaler, *et al.,* 2012). In *Arabidopsis*, SA down-regulates the expression of JA-responsive marker genes like

PDF1.2, VSP1 and the genes encoding main enzymes in the JA biosynthesis pathway, such as *AOS, LOX2, OPR3* and *AOC2 (*Leon-Reyes, *et al.,* 2010). Eichenseer, *et al.,* (2010) demonstrated that glucose oxidase activity in saliva secretions of lepidopteran insect pest and aphids induces SA signalling, resulting into the suppression of JA dependent defences, which ultimately promotes the performance of herbivores. Zang, *et al.,* (2013) also found that whitefly nymph feeding suppresses downstream JA defences by initiating SA induction, and these changes increase nymph performance. On the other hand, exogenous application of JA on rice dramatically decreased SA in response to exogenous JA, indicating that JA suppresses SA signalling (Tamaoki, *et al.,* 2013).

Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL), is a crucial enzyme in the phenylpropanoid pathway, which catalyses the deamination of phenylalanine to *trans*-cinnamic acid (Berner and Westhuizen, 2010). The key enzymes in the biosynthetic pathway of salicylic acid (SA), isochorismate synthase and PAL are vital regulators of SA-mediated responses. Different abiotic and biotic stress factors modulate them. Abiotic stress, wounding, and infection, amongst others, induce the expression of PAL (Khan, *et al.,* 2015). Russian wheat aphid infestation also induces an increase in PAL activity and phenolic content of wheat (Mohase and Van der Westhuizen, 2002; Chaman, 2003; Berner and van der Westhuizen, 2010).) Han, *et al.,* (2009) reported an increase in PAL activity in the resistant cultivars but not in the susceptible ones when barley flag leaf and ear stages were invaded by *Sitobion avenae*.

A wide range of prokaryotic as well as eukaryotic plant and animal species produce secondary metabolites such as salicylic acid (SA). Chemically, SA is a phenolic compound that has an aromatic ring having a hydroxyl group or its derivatives (An and Mou, 2011). Mammals have some levels of SA in their blood, besides the original one from plant material; there is also evidence that ingested benzoic acid produces SA (Paterson, *et al.,* 2008). Kastner, *et al.,* (2014) found that the locomotion secretions of one slug, *Deroceras reticulatum*, had significant amounts of SA. However, no other slug or snail species have been reported to express SA or any hormone in their locomotion secretions.

Salicylates, discovered as early as the 5th century B.C., have medicinal powers. The Hippocrates realised the therapeutic powers from the leaves and the bark of the willow tree; rich in salicylates, which eased childbirth pains (Rainsford, 1984; Weissman, 1991). Salicylic acid is synthesised though isochorismate and the phenylpropanoid pathways. Chorismate, which is a primary plant metabolite is vital in these pathways.

Salicylic acid promotes the induction of systemic acquired resistance (SAR), a response to a wide range of pathogenic microorganisms. The hormone is also crucial for localised plant hypersensitive response (Walling, 2000). In addition to SA derivatives, chemicals such the lipid-derived molecule (glycerol-3-phosphate)-dependent factor, a lipid transfer protein (DIR1), dehydroabietinal, pipecolic acid, and azelaic acid also mediate SAR (Liu, *et al.,* 2011).

The accumulation of SA in RWA resistant but not susceptible wheat cultivars indicates the involvement of SA in the resistance mechanism of wheat against the RWA (Mohase and van der Westhuizen, 2002). Morkunas, *et al.,* (2011) also demonstrated that SA induces resistance to RWA in wheat. Salicylic acid-dependent responses additionally use the methyl-conjugated form to stimulate expression of defence-related genes, including the apoplastic pathogenesis-related (PR) genes (Smith and Boyko, 2006).

Salicylic acid is also involved in reducing the adverse effects of a variety of abiotic stresses in plants by increasing the level of other plant growth regulators in plants (Sakhabutdinova, *et al.,* 2003). For instance, Wang and Li (2006) reported that SA regulated increased Ca^{2+} homeostasis and associated antioxidant defences in grapevine under heat and cold stress. Alavi, *et al.,* (2016) also observed that exogenous application of SA reduced chlorophyll degradation, membrane instability, H_2O_2 generation and lipid peroxidation induced by osmotic stress, which was associated with the enhancement of antioxidant defence. Overproduction of SA protects plants against environmental stresses (Khan, *et al.,* 2015). For instance, SA antagonises metal toxicity by either acting directly as an antioxidant or enhancing the activity of antioxidant systems of plants, and indirectly by inhibiting the uptake of metals from the environment (Wani, *et al.,* 2017). In this regard, treating wheat

plants with SA also prevented the harmful effects of salt stress, and SA gained potential as a possible growth regulator or antioxidant to improve plant growth under moderate salt stress (Barakat, *et al.,* 2013).

Abscisic Acid

Abscisic acid (ABA) is widely known as a stress hormone that responds to a wide range of environmental stresses including both abiotic and biotic stress factors (Zhang, 2014; Vishwakarma, *et al.,* 2017). It is involved in a wide variety of developmental and physiological processes such as transpiration, germination, dormancy, and in-plant adaptation to various environmental stresses. It is a crucial component in responses to water stress involving drought, salinity, and low temperature (Pospısilova, *et al.,* 2005).

Traditionally ABA was known to be an abiotic stress hormone, but there is a positive relationship between ABA and plant disease resistance. Absiscic acid is not only involved in modulating plant defences against plant pathogens, but it can also act directly to inhibit microbial activity (Flors, *et al.,* 2009). It positively regulates plant defence system at the early stages of infection by induction of callose deposition or by enhancing stomatal closure against invaders (Alazem and Lin, 2014). Iriti and Faoro (2008) demonstrated that exogenous ABA application attenuates infection symptoms and reduces viral spreading. However, if induced at later infection stages, ABA can suppress SA or JA signal transduction and ROS induction, thereby interfering with the defences controlled by these two pathways (Alazem and Lin, 2014). Miller, *et al.,* (1994), on the other hand, found that application of ABA to plants infested by RWA did not show any effect on the development of damage induced by the aphid.

Pathogenesis related proteins

Pathogenesis-related proteins (PR Proteins) refers to a group of proteins in a plant induced in response to bacterial, viral, fungal, and viroid diseases, and some chemicals. Expression of PR genes is not only due to pathogen attack but is also by other stress factors such as wounding, drought, UV light and oxidation. In most cases, Induction of PR proteins occurs via the activity of some growth hormones, which include JA, ET, and SA, whose levels

increase in infected plant tissues (Durrant and Dong, 2004). There are 17 families of PR proteins, classified according to their properties and functions (Saboki, *et al.,* 2016). β-1, 3-Glucanases, chitinases and peroxidases are some of the PR proteins involved in defence response against pathogens and herbivore attack.

Peroxidases (POD, EC 1.11.1.7) are some of the PR proteins, induced by pathogen infection in host plant tissues. They belong to group 9 of the PR proteins (Almagro, *et al.,* 2008). Peroxidases are part of defence-related processes that are outside the matrix of the cell. They are involved in the strengthening of the cell walls by lignifications, intermolecular cross-linking of cell wall sugars and suberin synthesis. Peroxidases also facilitate the production of reactive oxygen species involved in signalling events associated with defence responses (Bowles, 1990; Minibayeva, *et al.,* 2015). Consequently, ROS generated by peroxidases activates NADPH oxidases, which also generate a plasma membrane-associated oxidative burst (Bindschedle, *et al.,* 2006).

β-1, 3-Glucanases (E.C. 3.2.1.39) belong to group 2 of PR proteins (Klein, *et al.,* 2004). These proteins are categorised into various classes. Class I encompasses vacuolar proteins that accumulate in roots and mature leaves in response to pathogen infection. The class II and III proteins are acidic and excreted in the extracellular space. Members of class IV are similar to those of class II but are not inducible upon pathogen attack (Minic, 2008). β-1,3-glucanases have a direct effect on fungal resistance since they hydrolyze fungal cell walls. β-1,3-Glucanases also induce the production of oligosaccharide, which initiates the production of some PR proteins and some antifungal chemical. An example of such chemicals are phytoalexins (Saboki, *et al.,* 2016).

Various reports have shown that PR proteins are not only involved in defence against phytopathogenic species but also defend plants against herbivore attack (van der Westhuizen, *et al.,* 1998; Mohase and van der Westhuizen, 2002; Botha, et al 2014). Van der Westhuizen, *et al.,* (1998) reported that infestation selectively induced peroxidase activity in all resistant cultivars infested with RWA. There was a minor increase in the peroxidase activity in the infested 'Tugela', a susceptible cultivar. In contrast, infested

'Molopo' and 'Betta,' both susceptible to RWA, expressed a delayed increase in peroxidase activity.

Mohase and van der Westhuizen (2002) showed varying levels of increase in apoplastic β-1,3-glucanase and peroxidase activities of RWA infested wheat. The induced enzyme activity was higher in resistant than susceptible plants. Mohase and Taiwe (2015) also reported elevated activities of β-1,3-glucanase and peroxidase in resistant Tugela *Dn1* challenged with RWASA1, which showed the involvement of these enzymes in the resistance response of wheat to RWA. Additionally, Moloi and van der Westhuizen (2005) confirmed the association of β-1,3-glucanase and peroxidase with RWA resistance in wheat.

2.6 Conclusion

The state of wheat production as the third most essential staple food (after maize and sorghum) in Lesotho needs an integrated approach. Firstly, surveys intended to generate preliminary information from farmers on the challenges surrounding wheat production need to be initiated. There is no doubt that Lesotho is equally faced with the problem of RWA as other wheat-producing regions, but are farmers aware and employing appropriate mitigation strategies? Famers still use cultivars that were introduced in Lesotho more than 40 years ago. The resistance status of these cultivars against RWA is not known. Therefore, research must focus on laboratory and field studies to establish the potential sources or mechanisms RWA resistance in various wheat cultivars. The performance of the cultivars in terms of yield and bread-making qualities should also be tested against the modern South African cultivars.

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Chapter Three

Russian wheat aphid diversity and tolerance by wheat cultivars grown in Lesotho

3.1 Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop grown in all agro-ecological zones of Lesotho. It comes third in importance after maize and sorghum. It is grown over two planting seasons. Wheat grown in winter is adaptable to the Senqu River Valley, the foothills and the lowlands while wheat planted in spring is common in the mountains (Moremoholo, 2000). Rosenblum, *et al.,* (1999) indicated that cultivars that were most common in Lesotho were Bolane (Ou Boland), Mantša-Tlala (Tugela) and Mohohlotsane (which by its awnless character is probably Makalaote). The recent study conducted in Thaba Tseka and Mokhotlong indicated that Bolane and Makalaote are still the most preferred cultivars in the mountains of Lesotho (Masupha *et al.,* 2018).

Russian wheat aphid (*Diuraphis noxia,* Kurdjumov, Hemiptera: Aphididae) is a destructive pest in many areas of the world. It is significantly destructive to the wheat grown in winter under dryland conditions of North America (Morrison and Peairs, 1998) and both winter and spring wheat in South Africa (Walters, *et al.,* 1980). The RWA was first identified in 1978 in South Africa. Initially, the distribution was restricted in the Bethlehem area Free State. However, in 1979, the RWA had extended to the other parts of the country where wheat is produced (Walters, *et al.,* 1980). In the immediate proximity, Lesotho had a thriving wheat industry until the introduction of RWA in 1979 (Purchase, 1999). Makhale, *et al.,* (1999) affirmed the constraining impact of RWA by reporting over a two-fold decline in wheat production in Lesotho.

The Spread and record of RWA biotypes is a challenge to wheat farming in Lesotho. There are five RWA biotypes currently reported in South Africa (Jankielsohn, 2019). The first biotype designated South African biotype1 (RWASA1) was confirmed in 1978 (Walters, *et al.,* 1980). The subsequently reported biotypes were named chronologically. The second

biotype, RWASA2, was reported in 2005, and it was comparatively unaffected by the *Dn1* resistance gene (Tolmay, *et al.,* 2007). Then in 2009 in the Eastern Free State and northern and central parts of Lesotho mountains RWASA3 was reported. This biotype is relatively virulent to *Dn4* gene in wheat (Jankielsohn, 2011). Within two years, RWASA4, which is unaffected by the *Dn5* resistance gene, was recorded in 2011 in the east of the Free State (Jankielsohn, 2014). The newly reported RWASA5 with additional virulence to the *Dnx* resistance gene is the most virulent biotype recorded. So far, it has only been recorded in the Eastern Free State (Jankielsohn, 2019). The Eastern Free State has more RWA biotype complex with all the five biotypes recorded than other major wheat-producing areas; the Western Cape and the Northern Cape (Jankielsohn, 2017).

 The Eastern Free State lies very close to Mokhotlong and Thaba-Tseka, which are major wheat-producing areas of Lesotho. Three of these biotypes (RWASA1, 2 and 3) have been recorded in Lesotho (Jankielsohn, 2011). The short interval between the discovery of RWASA2 and RWASA4 shows that field evaluations for aphid infestation should be a routine process to ensure timely reporting of any new biotypes. The ever-changing RWA biotype complex is influenced by the host genotype as well as environmental factors like moisture, temperature and altitude. It can change over time, leading to variations in population composition over localities and years. Detecting new biotypes is therefore critical in the developing and utilizing the resilient sources of wheat resistance (Jankielsohn, 2016).

One of the effective ways of establishing the presence of RWAs in the field is by evaluating induced host damage symptoms. Aphid cause induced damage to the wheat by injecting toxic saliva into while feeding. These prevent or degrades chlorophyll production and causes curls the leaves (Karren, 1989). The toxin initially causes white longitudinal streaks in the leaves, which may ultimately turn to a bright purple discolouration depending on the level of infestation and the climatic conditions (Girma, *et al.,* 1992). Symptoms of severe infestations include stunted growth, bleached spikes containing poorly formed grain, and partially emerged spikes forming a "gooseneck" due to trapped awns in the firmly rolled flag leaf. Infestations result in the reduction of grain quality and yield (Pears, 1989).

Mokhotlong and Thaba Tseka are leading Lesotho districts in wheat production (BOS, 2015); despite cultivation devoid of resistant RWA cultivars, fertilizers, or pesticides. The geographical position of these districts, cold temperatures (12 - 22 ℃) and relatively higher precipitation (900 – 1000 mm/annum) (LMS, 2000) create humid conditions favourable for RWA multiplication. The most recent study on the diversity and distribution of RWA was in 2011. This study was a general survey that evaluated RWA diversity and distribution. The study reported that RWASA1 to 3 were present in the mountains of Lesotho and RWASA1 and 3 were the most predominant. The reporting of RWASA3 and 4 in South Africa within four years, (2011 and 2014) shows that RWA biotype evaluations may be long overdue in Lesotho. Therefore the purpose of this study was to expand on the previous investigations by evaluating the diversity and distribution of RWA in the lowlands districts (Maseru and Leribe) and Mountain districts (Mokhotlong and Thaba Tseka) of Lesotho. Additionally, the reaction (resistance/susceptible) of Lesotho farmers' wheat cultivars and some commercially available ones from South African to the RWA were determined.

3.2 Materials and Methods
Description of study sites in Lesotho
The survey was carried out in the Mokhotlong and Thaba Tseka, representing the mountain districts as well as Leribe and Maseru, two of the lowlands districts in Lesotho.
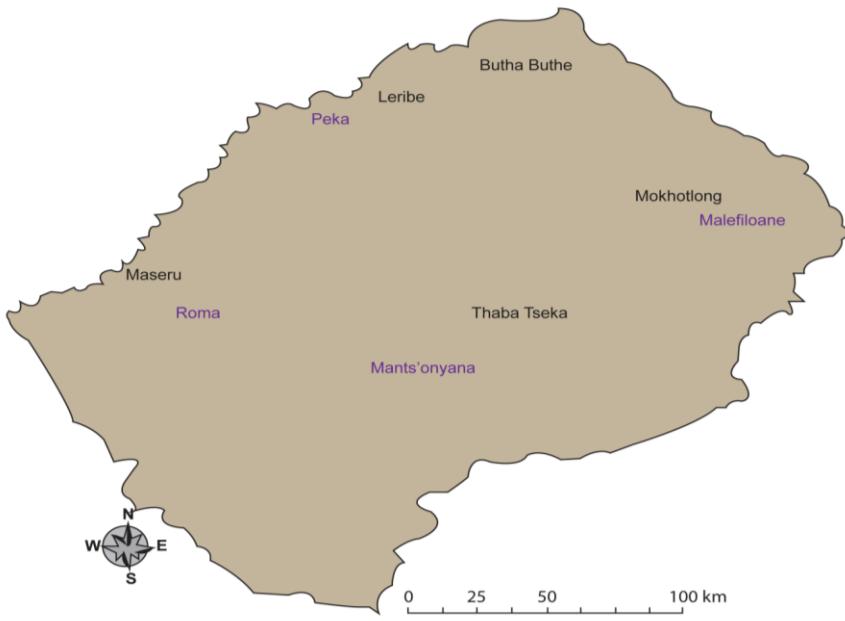
Figure 3.1 Study sites in Lesotho (Districts are printed in black and the actual study sites in purple)

Lesotho has different growing seasons for winter and spring wheat. Surveys were therefore conducted in September 2015 in the lowlands (winter wheat) and February 2016 and 2018 in the mountains (spring wheat). Screening and biotyping of the collected aphids were performed at the Agricultural Research Council –Small Grains (ARC-SG), Bethlehem in South Africa.

Sampling of Russian wheat aphid in the field

The samples of the Russian wheat aphid were collected from the farmers' fields in Thaba Tseka and Mokhotlong and winter wheat trials in Maseru, (Roma) and Leribe (Peka) in October 2015 during the winter wheat growing season. Russian wheat aphid samples for Mokhotlong and (Thaba Tseka) were collected from the cultivated crops, volunteer crops, and RWA trials in February 2018 during November 2017 – April 2018 spring wheat season. Collections were made in Malefiloane and along the route (Linakaneng) that connects to Mantsonyana (Thaba Tseka). The sites were 10 to 15 km apart depending on availability of fields with wheat or the presence of other host plants. Accessibility of some fields due to

rough terrain was rugged; therefore, collection sites were fields lying along the main road and other roads that transect the principal wheat, barley or oats areas.

Sampling mainly occurred along the field edges up to about 5 m into the interior of the field. The length of the fields ranged from 50 m to 100 m. Striped or rolled wheat leaves were inspected for the presence of aphids. The number of aphid samples collected per field ranged from 5 to 10, depending on the field size and level of infestation. The percentage of plants with aphids and the degree of damage on each plant was recorded. The recorded data included district and village names, geographical elevation, and coordinates. The information created an aphid distribution map. The leaves with aphids were cut and placed into 90 mm Petri dishes lined with moistened filter paper. Petri dishes were then sealed with parafilm and placed in a cooler box with ice packs and transported to the laboratory.

Evaluating Russian wheat aphid Biotype status

Aphid cloning and biotyping were conducted according to the guidelines described by Jankielsohn (2014), at the greenhouse of ARC-SG, Bethlehem. The aphids were initially transferred to a potted wheat plant with a soft camel hairbrush (no 2). The infested plants were isolated in cages covered with gauze (315-micron Nylon). A single aphid from the culture was then removed and reared on a new plant in a separate cage to start a new clone colony. The colonies were then maintained in cages in the greenhouse. The temperatures were 16˚C/22˚C, at night/day and aphid colonies were reared on different cultivars. The changing of cultivars prevented adaptation to any particular cultivar. The culture conditions were maintained until a population size required for screening was achieved. Each clone colony was cultured one month before the screening. The biotypic determination of each RWA clone was achieved through an evaluation of the induced damage on 11 sources of host resistance established previously (Table 3.1 containing the resistance genes designated as Dn1 to Dn9, Dnx and Dny). Tugela, a universally susceptible cultivar, was used as a susceptible check and RWA Matrix 2401 as a resistant check.

Table 3.1: Host/genotype differential used to designate new Russian wheat aphid biotypes in South Africa (original seed obtained from USDA-ARS, Stillwater, OK).

no	Gene	ID	Resistance source
1	Dn1	CO-03797	PI127739
2	Dn2	CO-03804	PI262660
3	Dn3	CO-03811	Triticum tauschii line SQ24
4	Dn4	Yumar	PI372129
5	Dn5	CO-950043	PI294994
6	Dn6	CI 6501	PI243781
7	Dn7	2003-1378027 Winter	94M370
8	Dn8	Karee-Dn8	PI294994
9	Dn9	Betta-Dn9	PI294994
10	Dnx	2006 RWA-1	PI220127
11	Dny	2006 RWA-1 Stanton	PI220350
12	Susceptible check	Tugela Susceptible	-
13	Resistant check	RWA Matrix 2401	CItr2401

Experimental design and data collection

Seedling trays containing previously sterilized sand were each planted with ten seeds and thinned to seven plants for each wheat crop entry in a randomized complete block design. Each biotypic determination was replicated four times. Wheat plant entries were allocated randomly to the rows. The RWA susceptible Tugela was used as border rows to separate plant entries. Plants were maintained in greenhouse cages at night and day temperatures of 12 °C and 22 °C respectively, and natural light (14 h) and dark (10 h) periods. The seedling trays were put in 315-micron gauze cages immediately after planting to prevent contamination by other aphids. At the two-leaf stage, each plant was infested with 5 RWA (adult apterous female).

The RWASA1 infestation produces symptoms of damage on wheat entries with the *Dn2* and *Dn3* resistance genes. Biotype 2 (RWASA2) causes susceptibility symptoms on entries with the *Dn1, Dn2, Dn3*, *Dn8* and *Dn9* resistance genes. RWASA3 is virulent to *Dn4* sources of resistance. RWASA4 differs from RWASA3 by its additional virulence to *Dn5*. (Jankielsohn, 2019).

1 – 4.5 resistant (R), 4.6 – 6.5 medium resistant (MR) and 6.6 – 10 susceptible (S)

Figure 3.2: Greenhouse damage rating scale (Tolmay, 1995)

Russian wheat aphids induced injury on the various entries was rated on a scale of 1-10, shown in Fig. 3.2. Rating based on infested plants with 5 RWA apterae, adult females on each plant occurred immediately when susceptible wheat Tugela showed the signs of damage. The four biotypes of RWA were categorized according to rated damage obtained in each wheat plant entry. A biotype designation for each clone was based on the differential profile of virulence to the resistance genes (*Dn1* to *Dn9*). A two-way (clone, plant entry)

analysis of variance (ANOVA) analysed the biotype groups (RWASA1 – 4) for all plant differentials. The mean damage rating entries that showed a significant difference ($P < 0.05$) were further separated using the protected least significant difference (LSD) (proposed by Fisher, 1939) test at the 5% level.

Screening the reaction of Lesotho and South Africa dryland wheat cultivars to RWA infestation

Two wheat cultivars from Lesotho, Makalaote and Bolane were both screened with the commonly grown wheat cultivars (dryland seeds) from South Africa. These were Matlabas, Elands, PAN3379, PAN3118, SST387 and Senqu all with proofed RWA resistance. This screening was done in the glasshouse at ARC-SG in Bethlehem against RWASA1 to 4. Elands, SST387, Senqu and Matlabas contain the *Dn1* resistance gene; therefore, they are resistant to RWASA1. PAN3379 is resistant to South African biotypes 1 - 4 while PAN3118 is susceptible to the four RWA biotypes (RWASA1 – 4).

As was the case in the biotypic determination in the previous section ten seeds were also grown in each plant entry (thinned to seven plants) in seedling trays with sterilized sand laid in a randomized complete block design. Each cultivar was replicated four times to yield a sample size of n = 40. At the two-leaf stage, plants were infested with four different RWA biotypes (RWASA1 – 4), and one tray, which was the control was left uninfested. Infestation with 5 RWA (adult apterae), was done on each plant. Induced plant response that included leaf rolling and chlorosis were rated on a ten-point damage rating scale (Fig 3.2). A score from 1 - 4 assigned to leaves expressing chlorosis; 5 – 6: for leaves showing striping and 7 – 10: for leaf rolling. As soon as susceptible damage symptoms were observed on the Tugela cultivar, all entries were rated.

3.4 Results
Survey results from the lowlands districts

South African biotype 1 and 3 were found in Maseru while RWASA2 and RWASA4 were present in Leribe. The relatively recent biotype RWASA4, dominated in Leribe as well as the surrounding areas of the Eastern Free State like Ficksburg and Fouriesburg (Figure 3.3).

The biotypes identified in this study were the same as those found in the Free State around Fouriesburg, Ficksburg and Clocolan. This observation suggests a possible movement of RWA between Lesotho and neighbouring Free State farms. Generally, the direction of the wind in Lesotho is north-westerly or north-easterly (Lesotho Meteorological weather updates). Therefore, the wind blowing from the Eastern Free State especially Bethlehem, which has all the RWA biotypes, being located north of Lesotho serves as a possible supplier of the aphids to the lowlands and the mountain areas of Lesotho.
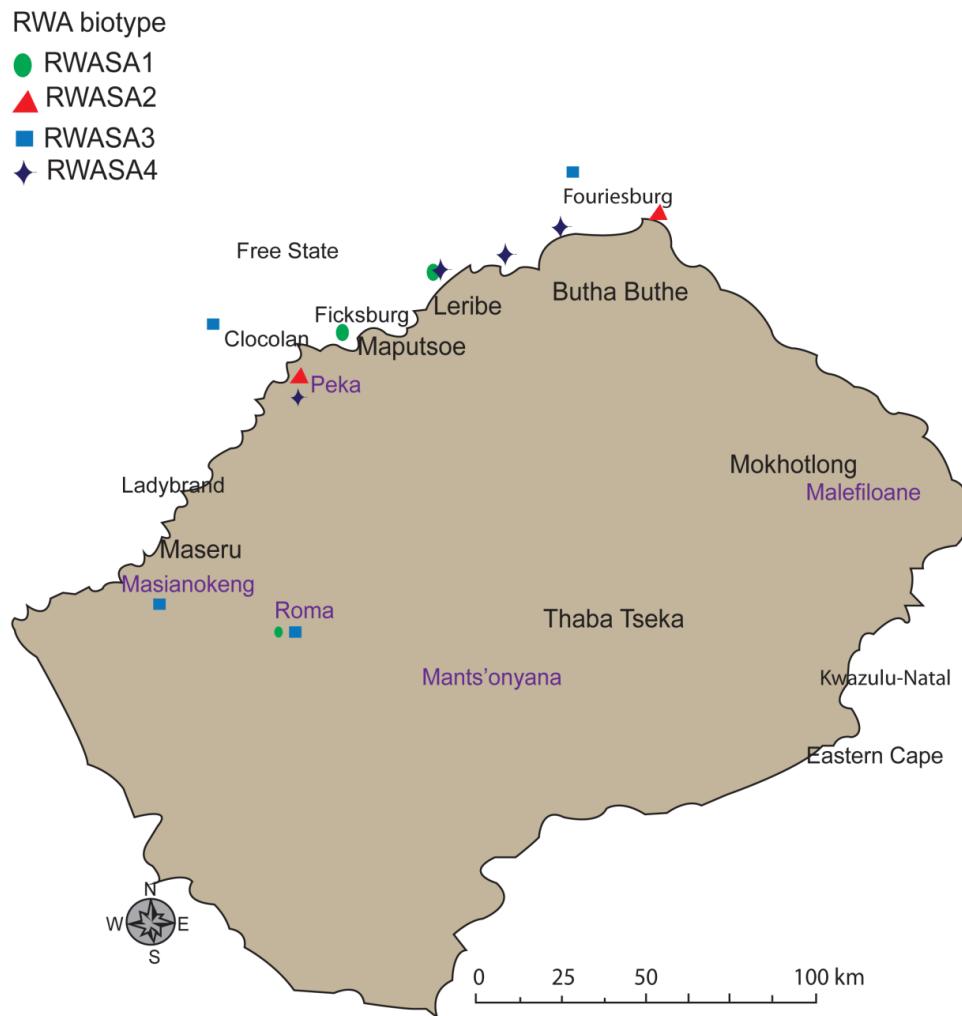
Figure 3.3: Russian wheat aphid distribution in Maseru, Leribe and the South African Surrounding areas (Ficksburg and Fourisburg).

Survey results from the mountain districts

In Mokhotlong and Thaba Tseka, 56 aphid samples were collected. The majority (63%) of samples were from farmers' tall cultivars (Bolane, Makalaote and T'soloha) whereas 34% were from the South African short cultivars and 3% from wild oat grown for livestock feeding. Samples were also collected from oat and barley that are grown for livestock feeding. The most dominant biotype in the two Mountain districts was RWASA4. South African Biotype 1 (RWASA1) occurred only in Mokhotlong while RWASA2 presence was very scarce, found only at one field in Thaba Tseka.
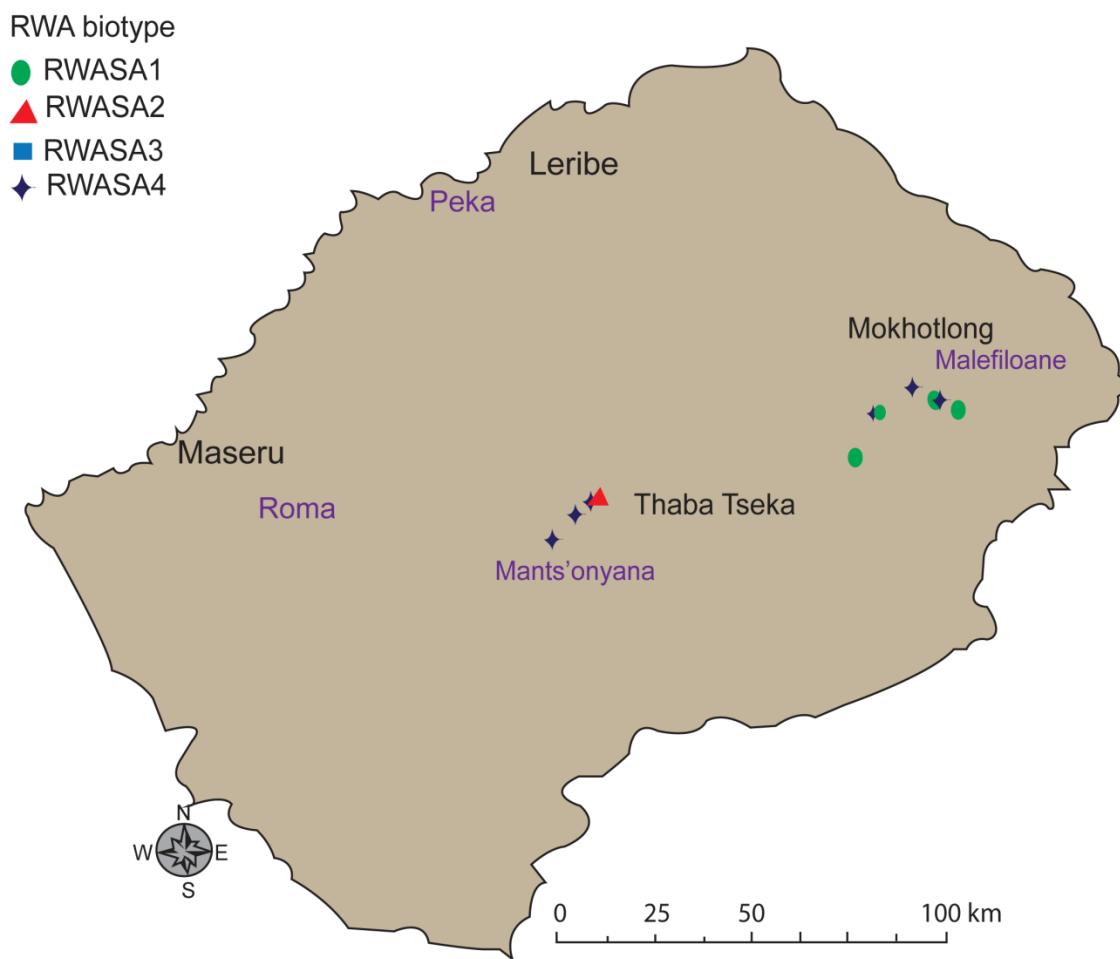
Figure 3.4: Russian wheat aphid diversity and distribution in Mokhotlong and Thaba Tseka

Glasshouse screening

Glasshouse results showed that the Lesotho cultivars Bolane and Makalaote and a South African cultivar PAN 3118 were as susceptible to RWASA1 as Tugela, the susceptible check. South African biotype 1 (RWASA1) was more rampant in Mokhotlong where Bolane and Makalaote are widely cultivated. The South African cultivars Elands, Gariep, Puseletso (Tugela DN), Matlabas, Senqu and SST387, showed resistance to RWASA1. PAN3379 known to express resistance against the four biotypes showed medium resistance.

Table 3.2: Resistance status of Lesotho and South African cultivars commonly grown in Lesotho towards RWASA1: R= Resistant, MR = Medium resistant, S = Susceptible, AVG = Average

| | R (AVG<=4.5); MR(AVG<=6.5); S(AVG<=10) | | | | | | | | | | Seedling | |
Entry	1	2	3	4	5	6	7	8	9	10	AVG	R/MR/S
Elands				5	2						4.29	R
Gariep				7							4.00	R
Senqu			3	4							3.57	R
Matlabas				4	3						4.43	R
Tugela									7		9.00	S
Tugela DN (Puseletso)				7							4.00	R
PAN3118							2	2	4		8.25	S
PAN3379				4	2	1					4.57	MR
SST387				7							4.00	R
Makalaote									7		9.00	S
Bolane									7		9.00	S

The results from the greenhouse trial show that the majority of South African cultivars were resistant to RWASA1. However, PAN3379 showed medium resistance, while PAN 3118 was susceptible to RWASA1. The two Lesotho cultivars Makalaote and Bolane were both susceptible to RWASA1. Mokhotlong district where Bolane and Makalaote are the main cultivars used had the highest incidence of RWASA4, followed by RWASA1 (Fig 3.4).

Table 3.3: Resistance status of Lesotho and South African cultivars commonly grown in Lesotho towards RWASA2: R= Resistant, MR = Medium resistant, S = Susceptible, AVG = Average.

| | R (AVG<=4.5); MR(AVG<=6.5); S(AVG<=10) | | | | | | | | | | Seedling | |
Entry	1	2	3	4	5	6	7	8	9	10	AVG	R/MR/S
Elands								3	4		8.57	S
Gariep							1	2	4		8.43	S
Senqu						1	2	1	3		7.86	S
Matlabas								1	6		8.86	S
Tugela									7		9.00	S
Tugela DN (Puseletso)									7		9.00	S
PAN 3118							3	1	3		8.00	S
PAN3379				7							4.00	R
SST387							2	2	3		8.14	S
Makalaote									7		9.00	S
Bolane				2	3	2					5.00	MR

Results from the greenhouse trial indicate that all the South African cultivars used except PAN3379 were susceptible to RWASA2, which is somewhat unaffected by the *Dn1* resistance gene found in wheat. Makalaote was also susceptible, but Bolane was medium resistant. RWASA2 is prevalent in the lowlands, particularly in Leribe, where South African cultivars dominate wheat cultivation (Figure 3.2). The low incidence of RWASA2 in 2018 in Mokhotlong and Thaba Tseka (Figure 3.4) where Bolane is mostly cultivated, rendered the medium resistance it displays insignificant to wheat production in the mountain districts.

Table 3.4: Resistance status of Lesotho and South African cultivars commonly grown in Lesotho towards RWASA3: R= Resistant, MR = Medium resistant, S = Susceptible, AVG = Average

| Entry | R (AVG<=4.5); MR(AVG<=6.5); S(AVG<=10) | | | | | | | | | | Seedling | |
	1	2	3	4	5	6	7	8	9	10	AVG	R/MR/S
Elands									7		9.00	S
Senqu									7		9.00	S
Matlabas									7		9.00	S
Tugela									7		9.00	S
Tugela DN (Puseletso)									7		9.00	S
PAN3118									7		9.00	S
PAN3379				7							4.00	R
SST387									7		9.00	S
Makalaote									7		9.00	S
Bolane									7		9.00	S

Results of damage induced by RWASA3, virulent to the *Dn5* resistance gene, showed a similar pattern to those of RWASA2. However, both Bolane and Makalaote were susceptible to RWASA3 (Table 3.4). The biotype (RWASA3) is most abundant in the lowlands (Fig 3.2) where South African cultivars dominate production.

Table 3.5: Resistance status of Lesotho and South African cultivars commonly grown in Lesotho towards RWASA4. R= Resistant, MR = Medium resistant, S = Susceptible, AVG = Average

| Entry | R (AVG<=4.5); MR(AVG<=6.5); S(AVG<=10) | | | | | | | | | | Seedling | |
	1	2	3	4	5	6	7	8	9	10	AVG	R/MR/S
Elands									7		9.00	S
Gariep									7		9.00	S
Senqu									7		9.00	S
Matlabas									7		9.00	S
Tugela									7		9.00	S
Tugela DN (Puseletso)									7		9.00	S
PAN3118									7		9.00	S
PAN3379				7							4.00	R
SST387									7		9.00	S
Makalaote									7		9.00	S
Bolane					1	1	5				6.57	S

All the seven plants used per cultivar showed susceptibility except PAN3379 and Bolane. However, two of Bolane's plants showed moderate resistance, but the majority were susceptible, although the symptoms were not as appalling as in other cultivars. This biotype (RWASA4) was most prevalent in both Thaba Tseka and Mokhotlong (Fig. 3.3) where Bolane and Makalaote dominate wheat production.

Table 3.6: Reaction of Lesotho and South Africa wheat cultivars to Russian wheat aphid

Cultivar	RWASA1	RWASA2	RWASA3	RWASA4
Elands	R	S	S	S
Gariep	R	S	S	S
Senqu	R	S	S	S
Matlabas	R	S	S	S
Tugela	S	S	S	S
Tugela *DN* (Puseletso)	R	S	S	S
PAN3118	S	S	S	S
PAN3379	MR	R	R	R
SST387	R	S	S	S
Makalaote	S	S	S	S
Bolane	S	MR	S	S

(Resistant: R; Susceptible: S; Medium resistant; MR)

The summary in Table 3.6 shows that the majority of cultivars (>90%) from South Africa and Lesotho cultivated in Lesotho, do not have resistance against three (RWASA2, 3 and 4) of the tested four RWA biotypes.

3.5 Discussion

The widespread distribution of RWASA4 in the mountains (Thaba Tseka and Mokhotlong) and the lowlands (Leribe) suggests that it has been in existence for years in Lesotho. It was first identified in the Eastern Free State in South Africa in 2011 (Jankielsohn, 2014) and since then, it has become more prevalent in survey studies in South Africa. The surveys conducted between 2011 and 2014 showed that RWASA3 and RWASA4 were dominant in the eastern areas of the Free State. Samples collected from the survey all had RWASA4 while RWASA3 was identified in 54% of the samples (Jankielsohn, 2016). Jankielsohn (2017) reported that RWASA4 was so widespread that it could dominate the other biotypes. It persists in the environment and populations increase when conditions become more favourable, and it increases with the area cultivated with wheat. The planting of susceptible wheat cultivars and the cold and the relatively humid conditions in the mountain districts of

Lesotho, favour the population increase of this biotype. The Eastern Free State and the mountain districts of Lesotho planting dates for wheat ensure continuous host availability for RWA. The mountain districts in Lesotho grow spring wheat while winter wheat dominates the Eastern Free State. Therefore, aphids can easily migrate between the seasons, increasing their numbers. Sydenham and Tolmay (2017) reported that RWASA4 is highly virulent. It can overcome other RWA resistance genes, including the *Dn5*; this could have negative implications for the wheat-growing industry in South Africa in the summer rainfall areas. The limited number of resistant cultivars towards RWASA3 and RWASA4 could be due to the shorter time interval these biotypes were discovered as breeding programmes usually take time to develop a cultivar.

Russian wheat aphids should be monitored for biotypic diversity because new biotypes can potentially disrupt the progress of plant breeding programs aimed at developing resistance. An insect biotype refers to the population of insects that is capable of surviving, reproducing, or causing injury to cultivated plants that are resistant to other species of the same population (Shufran and Payton, 2009). In a survey conducted by Burd *et al.,* (2006) in Kansas, Nebraska, Texas and Wyoming, three RWA biotypes were discovered. These were found in cultivated wheat and barley, and they were designated as RWA3, RWA4 and RWA5. In a later survey conducted in Colorado State in the Montane region (Chapela, 2013), two previously unknown biotypes were discovered. These were isolate M5, collected from a montane site, and isolate P14, collected from a prairie site. Both isolates produced more damage than RWA2. Weiland *et al.,* (2008) showed that the development of aphid biotypes results from the use of resistant cultivars. They apply high selective pressure on the biotypes that already exist, resulting in new virulent biotypes. However, Jankielsohn (2011) suggests that South African biotypes 2 and 3 were possibly introduced at the same time as RWASA1. She further argued that they might have survived on other host plants in Lesotho and the eastern areas of the Free State, and infested cultivated wheat fields. This suggestion may be valid as few surveys were done in South Africa on wheat and alternative host plants from 1978 to 2005; when the second biotype then designated, RWASA2 was discovered.

Our survey also showed that RWASA2 and RWASA3 populations were scarce in the mountains, only found in two isolated fields in Thaba Tseka (Fig. 3.3). These biotypes were not found in Mokhotlong, which is closer to the Eastern Free State and believed to be the source of biotypic diversity for the RWA. Jankielsohn's survey (Jankielsohn, 2011) reported RWASA2 as the most predominant RWA biotype in the collection area, constituting roughly 43% of the total biotype complex in the Free State and Lesotho. Jankielsohn (2017) further showed a time-dependent shift in RWA biotype composition in South Africa, notable in the Eastern Free State from 2012. A substantial decline is observed in RWASA2, making it the lowest in the RWA complex of 2016 while RWASA3 is increasing.

The reproductive capacity and growth rates of RWASA2 are faster than those of RWASA1 in both susceptible and resistant wheat and barley cultivars (Walton and Botha, 2008; Jimoh, *et al.,* 2010). This rapid reproduction led to Jimoh *et al.,* (2011) to suggest that the only possible biological difference between RWASA1 and RWASA2 is the higher reproductive rate. However, the continuing decrease of RWASA2 population size and distribution, and the concurrent increase in RWASA1 population in South Africa and Lesotho is contrary to the suggestion. Amongst the USA RWA biotypes, fecundity was significantly higher for RWA2 than for RWA1 at the lower temperature regime (Randolph, *et al*., 2008). They concluded that fecundity rates between different biotypes were a result of temperature differences and not the resistance status in the wheat cultivar. Joyti *et al*., (2006) affirmed by establishing that significant differences existed in the number of aphids for RWA1 and RWA2 per plant under two different temperature regimes.

The differences in the diversity of RWA biotypes between and within the mountain and the lowlands districts of Lesotho and the Eastern Free State in South Africa might be due to temperature differences in these areas. Reproduction and survival influenced by the effects of climate change (prolonged period of drought, heavy rainfall and too high and low temperatures) mostly lower temperature may be a critical factor in the distribution of these biotypes. Merril *et al.,* (2009) found that RWA2 in the United States had overwintering competitive advantage over RWA1. Ahmad *et al.,* (2016) also found that Aphid population

density increased in cold and humid climatic conditions and declined as temperatures increased.

The greenhouse results revealed that almost all the South African and Lesotho cultivars cultivated in Lesotho, except PAN3379, are susceptible to RWASA2, 3 and 4 (Table 3.6). However, the majority of the South African cultivars were resistant to RWASA1. Our survey showed that Bolane (spring wheat) was the most preferred cultivar in Mokhotlong and Thaba Tseka for its soft white grains, tall straws used for thatching traditional houses and livestock feed (Rosenblum *et al.,* 1999; Masupha, *et al.,* 2018). This cultivar was introduced in Lesotho in the 1960s (Weinmann, 1966). Bolane is resistant to RWASA2, a biotype that was only found in Thaba Tseka in one field. Therefore, Bolane's medium resistance to RWASA2 has minimal relevance to farmers growing wheat in Mokhotlong, where Makalaote (Malinonyana, mother of birds, 410) an awnless cultivar, is the second widely planted cultivar, which is unfortunately susceptible to all the four biotypes.

Host plant resistance is the most promising tool for managing the RWA. Tactics such as biological and chemical control have limited use because aphid damage causes leaf rolling, which shield the aphids from contact insecticides or biological control agents (Du Toit, 1989; Fikru, *et al.,* 1999). Systemic insecticides may be effective against RWA. However, the use of insecticides to control RWA has many risks which include resistance development and natural enemies' destruction. Furthermore, the use of insecticides has also been proven to be expensive (Macharia, *et al.,* 2017). Nkongolo *et al.,* (1990) highlighted that resistant cultivars provide the opportunity for both economical and effective management of the aphid. Agricultural Research Council – Small Grains (ARC-SG) identified sources of genetic resistance, and through backcrossing with cultivars such as Tugela, improved cultivars with a resistance gene *Dn1* were developed (Du Toit, 1989).

According to ARC-Small Grains Institute (2016), 12% of the wheat cultivars recommended for dryland conditions in the summer rainfall region, were susceptible to RWASA1, 65% to RWASA2 and RWASA3 and 76% to RWASA4. These statistics show a lag between breeding programs and the development of new biotypes. Breeding programs can be quite

lengthy and costly, and the price for the newly released cultivars resistant to the new biotypes is often not affordable to subsistence farmers. This high cost poses a challenge to resource farmers from underdeveloped countries like Lesotho, as they cannot afford the price of the resistant cultivars. Tolmay *et al.,* (1999) indicated that none of the "farmers' cultivars" in Mokhotlong were screened for Russian wheat aphid resistance. However, visual observations indicated that they were susceptible to aphids. Alarmingly, the high rainfall and low-temperature regime in Mokhotlong make the RWA a severe pest.

3.6 Conclusion

Makalaote is susceptible to the four RWA biotypes (RWASA1 to 4) whereas Bolane is only medium resistant to biotype 2 (RWASA2) and susceptible to the other three tested. However, this may not help in the management of RWA as RWASA2, which Bolane is medium resistant to was found in only one field in Thaba Tseka. The widespread distribution of RWASA4 in the mountain districts shows that the biotype might have occurred in Lesotho at around the same time it was discovered in South Africa. Biotype 1 (RWASA1) is prevalent in the mountains (Mokhotlong) where cultivars with *Dn1* gene are rarely planted.

3.7 References

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Chapter Four

Differential impact of Russian wheat aphid in various wheat cultivars

4.1 Introduction

Wheat is the most important cereal crop that is adapted to all Lesotho agro-ecological zones. It is the primary provider of carbohydrates for the people living in the mountain districts of Lesotho (Mokhotlong and Thaba Tseka). In Lesotho, there are two wheat planting seasons, winter and spring. Wheat is grown in dryland conditions on residual moisture of autumn rainfall and winter precipitation (snow) (Moremoholo and Purchase, 1998). Wheat in the lowlands can be planted from April until the end of June. However, early plantings in the lowlands have shown a tendency for the higher incidence of Russian wheat aphid (RWA) infestation (Ntokoane, 1992). In the mountains, wheat is planted from October to November.

There has been a significant decline in wheat production and area planted to wheat in Lesotho. Morojele and Sekoli (2016) reported a sharp decline of about 77% since 2013. A dramatic decrease in area planted to wheat from 39 000 ha (1962) to 7 000 ha (2013) resulted in 82% decrease in wheat production. Bureau of Statistics (2015) further reported a decrease in wheat yield from 1.47 t/ha (2009/2010) to 1.00 t/ha (2010/2011), followed by another decline from 1.00 t/ha (2010/2011) to 0.86 t/ha (2011/2012). Although sporadic increases in yield occurred, such as 0.86 t/ha (2011/2012) to 1.27 t/ha (2012/2013), the overall trend is a decline in wheat production. South Africa also reported a significant decrease in the total area planted to wheat, from 1 627 000 ha to 748 000 ha (Smit, *et al.,* 2013), but relative to Lesotho, a significant increase in wheat yield (Agricultural Statistics, 2009; van Lill and Purchase, 1995) was reported.

Late planting time, low seeding rate, low soil fertility, poor seedbed preparation and adverse climatic conditions during the growing season have been implicated as the major factors affecting wheat in Lesotho (Central Bank, 2012; Bureau of Statistics, 2014; Lesotho review, 2015). None of these reports, nonetheless, mentions the effect of RWA on wheat yield. According to Smit *et al.,* (2013), in South Africa, increase in wheat production and quality

is due to committed scientific inputs from different research disciplines including crop physiology, plant breeding, crop protection and agronomy. The combined effort of these disciplines in the development of cultivars with agronomic traits including high yielding, aluminium tolerance, pest and disease (RWA and rust) resistance, development of production manuals and dedicated farmers, are key to the success of South African wheat industry. Unfortunately, farmers in Lesotho, especially those in the mountain districts where wheat production is relatively high, use their traditional cultivars, which are recycled every year.

Russian wheat aphid (*Diuraphis noxia,* Kurdjumov) is a significant wheat and barley pest in the world (Webster, *et al.,* 1996; Starry, 1999). Economic importance is attributed to a reduction in grain and loss of kernel weight and quality (Marasas, *et al.,* 1997). Walters (1984) showed that yield losses due to RWA are very high with possible individual plant losses as high as 90%. Russian wheat aphid infestation can be distinguished from other cereal aphids by its characteristic damage symptoms. Infested leaves have longitudinal white, purple or yellowish streaks. When the temperatures are low, infested wheat tillers become a purplish colour.

Moreover, infestation during the flowering stage results in twisted or distorted heads, which assume a bleached appearance (Chemeda, 2015). Heads often assume a "fishhook" shape caused by trapped awns in tightly curled flag leaves. At this time, most RWAs feed on the stem in the flag leaf sheath or on the developing kernels (Pears, *et al.,* 2006). Stary and Lukasova (2002) further reported that drought-stressed plants irrespective of plant resistance status are conducive hosts, allowing the RWA to reach the highest population densities.

Chemical control is an integral part of most Integrated Pest Management (IPM) programs. However, Tolmay and Mare (2000) argued that even though the use of insecticides can increase grain yield in RWA infested fields, this increase is not always economically justifiable; the combined cost of buying the insecticide and its application is not always considered. Du Toit (1988) also highlighted that RWA damage to wheat could be limited by insecticidal control. However, the cost is expensive, significantly where unfavourable

climatic conditions reduce the effectiveness of the insecticides. Leaf rolling, characteristic of RWA susceptible host plants, reduces the efficacy of some management strategies, as it limits insecticide contact with the aphids and biological control agents (Gutsche, *et al.,* 2009). Even though chemical control might be useful in the control of many pests, it might not be the best in RWA management.

Host plant resistance is a sustainable, environmentally safe and cost-effective approach for managing the RWA (Bouhssini, *et al.,* 2011). Genetic plant-based resistance has been used as an effective control strategy in various areas where RWA is a severe risk in wheat production (Umina, *et al.,* 2017). Resistant wheat cultivars do not show the characteristic symptoms of RWA induced injury. Nonetheless, it is essential to note that resistance level can differ and it is vital to classify resistance in breeding so that combination of resistance mechanisms can be used in the development of new wheat cultivars (Randolph, *et al.,* 2005). Mornhinweg (2005) demonstrated that resistant and moderately resistant cultivars increased or maintained yield components despite heavy RWA feeding pressure while the susceptible ones had lower yield attributes and grain yield.

South Africa began breeding for RWA resistance in wheat in the 1980s, and the two resistance genes *Dn1* and *Dn2* were identified (Du Toit, 1988). Research progressed, and there are currently eleven wheat *Dn* genes (*Dn1-Dn9*, *Dnx* and *Dny*), which confer resistance against RWA (Botha, *et al.,* 2014). Nevertheless, breeders have a challenge of new RWA biotypes rapid development (Botha, *et al.,* 2006). Jankielsohn (2016) also warned that the plasticity of the RWA would persistently challenge the development of RWA resistant wheat cultivars.

Lesotho does not have its wheat breeding programmes, but there has always been research collaboration between Lesotho and South African professionals. Several research initiatives led by ARC-Small Grains Institute have been conducted (Moremoholo and Purchase, 1998; Tolmay and Mare, 2000; Jankielsohn, 2011). The majority of wheat cultivars planted in Lesotho (Elands, Gariep, Matlabas, PAN3118, PAN3379, SST387 and Senqu) are from South Africa. These cultivars are resistant to RWASA1 only except for

PAN3118, which is susceptible to four South African Biotypes (Biotypes 1- 4) and PAN3379, which is resistant to all four biotypes. Farmers in Lesotho, especially in the mountain districts, mostly use their recycled cultivars. Bolane, a cultivar that was introduced in Lesotho in the early 1960s (Weinmann, 1966), is preferred for its large white grains, large straws suitable for traditional roofing of houses, and livestock feeding (Masupha, *et al.,* 2018). Another widely used cultivar is Makalaote, which is probably the same cultivar as Mohohlotsane or Malinonyana (which means mother of the birds due to its awnless characteristics, making it prone to bird attack). The origin of Mohohlotsane is not known (Rosenblum, *et al.,* 1999) and farmers have over the years been recycling this cultivar.

Wheat is a typical cereal crop in Lesotho, and the RWA has been noted for over 28 years (Ntokoane, 1992). However, there has never been any field studies to investigate the reaction of the main adapted cultivars and the commonly cultivated South African cultivars under the local environments to the RWA. The comparative performance of these cultivars in terms of yield is therefore not recorded. Consequently, this study determines the resistance/susceptible reaction to RWA infestation and other pests as well as yield performance under different environmental conditions.

The primary objectives were:

1. To determine resistant/susceptible host reaction to RWA (genotype interaction) in various wheat cultivars planted in Lesotho.
2. To determine the yield performance of identified cultivars under different environmental conditions.

4.2 Materials and Methods
Study sites

Experiments were conducted in Lesotho in four different districts at planting seasons 2015/2016 to 2017/2018. The first locality was Leribe (Peka: S29.03115 °E27.74461°) at an altitude of 1652 m and an annual rainfall of 700 – 800 mm. The minimum and maximum

temperatures during wheat planting period were 7 ℃ and 30 ℃, respectively. The second locality was Maseru (Roma: S29.44447º E27.71944 º) at an altitude of 1610 m, annual rainfall of 800 – 900 mm and minimum and maximum temperatures of 6 ℃ and 30 ℃, respectively. The third locality was Mokhotlong ('Malefilone: N -29.2037º E29.1024º) in the mountains region where the altitude is 2581 m, and annual rainfall was 900 – 1000 mm. The temperatures ranged at minimum 12 ℃ and maximum 22 ℃. The last locality was Thaba Tseka (Mantsonyana - Ha Long: N29.3040º E28.1422º) on the eastern region of the mountains where the altitude is 2463 m, and annual rainfall was 600 – 700 mm, with minimum and maximum temperature of 12 ℃ and 19 ℃, respectively. The trials were conducted in fields owned by farmers except at Roma where they were planted at the National University of Lesotho farm.

Germplasm

The Agricultural Research Council - Small Grains (ARC-SG) in Bethlehem, provided wheat seeds from South Africa (Elands, PAN3118, PAN3379, Matlabas, SST387 and Senqu). These are the commonly cultivated dryland cultivars in Lesotho, especially in the lowlands districts. Makalaote and Bolane, which are the most planted cultivars in the mountain districts of Lesotho, were collected from the farmers. These farmers recycle seed, and their seed might not always be pure. Therefore, the seed was cleaned by removing weed seeds, small seeds and seeds that were suspected to be from other cultivars.

Experimental Layout

The trials were laid out as a randomized complete block design (RCBD) with four replications. The plot size was 5 m × 5 rows with 0.4 m in between the rows. Eight cultivars were planted in 2015/16 (Table 4.1), and four in 2016/17 and 2017/18 (Table 4.2). Since only a small portion of the farmer's fields was used for the trials, the rest of the fields were planted any other wheat cultivars. Wheat, especially Bolane, was mostly planted in the surrounding fields.

Table 4.1 Experimental layout for the trials in 2015/16 wheat cropping season

Bolane	PAN3379	Makalaote	Elands	Senqu	SST387	Matlabas	PAN3118
Matlabas	Elands	Bolane	PAN 3118	Makalaote	PAN3379	Senqu	SST387
SST387	Senqu	PAN3379	Matlabas	Bolane	PAN3118	Elands	Makalaote
Makalaote	PAN3118	Elands	Senqu	SST387	Bolane	PAN 3379	Matlabas

Table 4.2 Experimental layout for the trials in 2016/17/18 cropping seasons

Makalaote	PAN3379	Elands	Bolane
Bolane	Makalaote	PAN3379	Elands
Elands	Bolane	Makalaote	PAN3379
PAN 3379	Elands	Bolane	Makalaote

Management

Trials in the lowlands were fertilized [N: P: K (6:2:1) (31%)] at the rate of 300 kg/ha, while in the highlands no fertilizer was used. These simulated the local cultivation practices. Farmers in the mountains of Lesotho do not use any management practices like fertilization, herbicide, insecticide or fungicide applications on wheat. In the same manner, no other management practices were employed during the growing season.

Planting Dates for the field trials in the four locations

The dates for planting the trials were based on the recommendations made by the Department of Agricultural Research (DAR), Lesotho (Table 4.3).

Table 4.3 Trial planting and DAR recommended dates

Site	Planting season	Recommended planting dates *	Actual planting dates	
			2015	**2016**
Leribe (Peka)	Winter	15 May – 15 June	22/05	29/05**
Maseru (Roma)	Winter	15 May – 15 June	20/05	30/05
Mokhotlong ('Malefiloane)	Spring	October to Nov	-	29/10
Thaba-Tseka (Mantšonyane)	Spring	October to Nov	-	26/10

*Recommended planting dates by the Lesotho Department of Agricultural Research
**Hailstorm destroyed the trial

Data Collection and Analysis of RWA damage

Data on RWA damage was collected once in a season at anthesis. Russian wheat aphid induced leaf damage, degree of damage (%), RWA number, other pests, diseases and predators were recorded. A four-point damage rating scale (Fig. 4.1) was used for RWA induced damage where: 1-No visible damage (Escape/Resistant); 2-chlorotic spots on leaves (Resistant); 3-chlorotic striping on leaves (medium Susceptible); 4-Longitudinal rolling of leaves (Susceptible). Each plot was analysed according to this scale.

1- No visible damage:
Escape (E)Resistant (R)

2- Chlorotic spots:
Resistant (R)

3- Chlorotic Striping:
Medium susceptible (MS)

4- Leaves rolled:
Susceptible (S)
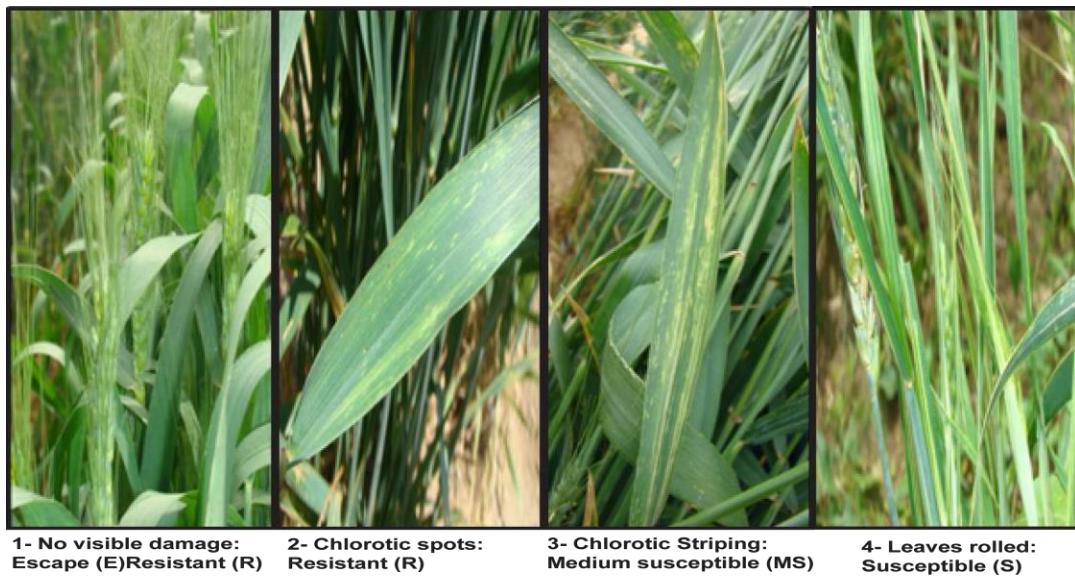
Figure 4.1 Four-point damage rating scale for RWA resistance in adult wheat plants under field conditions

Yield

The yield was assessed at physiological maturity, and data were collected from the three middle rows to avoid border effects. The harvest was transported to the National University of Lesotho, thrashed and weighed. Data on yield and RWA damage rating were analysed using a two-way (damage rating, cultivar) analysis of variance (ANOVA). Mean damage rate entries with significant differences were separated by Fisher's protected least significant difference (LSD) test at the 5% confidence level.

4.3 Results

Soil analysis and Environmental conditions

Data on the temperature regimes and precipitation (Table 4.5 and 4.6) were obtained from the Lesotho Meteorological Services (LMS). Lesotho Meteorological Services has substations placed strategically across the country to cater for different localities. Soil samples in the four research sites were collected and analysed before planting. Soil analysis was done at Agricultural Research Council-Small Grains (ARC-SG) soil laboratory according to the following methods. Potassium, calcium, magnesium and sodium were analysed through ammonium acetate test, phosphates according to Bray 2, pH by potassium chloride and nitrogen according to Kjeldahl soil testing methods. ARC-SG also supplied

soil nutrient guide for wheat production. Nitrogen content is the only component that was measured at the National University of Lesotho, Maseru, Lesotho. The results of the soil analysis are plotted against the soil Nutrient and pH guide for wheat production (Table 4.4).

Table 4.4 Results of the soil analysis for Mokhotlong, Thaba Tseka, Leribe and Maseru performed at ARC-SG in Bethlehem, Free State

Trial site	pH (kcl)	Phosphorus (mg/kg)	Potassium (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)	Sodium (mg/kg)
Mokhotlong ('Malefiloana)	5.1	6.5	183.5	7204	2255.8	68.2
Thaba Tseka (Mantšonyana)	5.0	24.9	283.5	4006	1144.7	40.3
Leribe (Peka)	4.5	3.7	65.1	301	42	8.9
Maseru (Roma)	4.7	80.7	134.7	545	146.1	8.6
Soil nutrient guide for wheat production						
Low	-	<15	<60	<200	<40	<1.5
Medium	-	15-25	60-80	200-400	40-80	1.5-2.0
Medium-high	-	25-35	80-120	400-800	80-120	2.0-6.0
High	-	>35	>120	>800	>120	6.0
Soil acid classification						
Extremely acid	<3.5	-	-	-	-	-
Very strong acid	3.5-3.8	-	-	-	-	-
Strong acid	3.8-4.0	-	-	-	-	-
Acid	4.0-4.3	-	-	-	-	-
Moderate acid	4.3-4.5	-	-	-	-	-
Slightly acid	4.5-5.0	-	-	-		

Nitrogen content was analysed at the National University of Lesotho for the two locations, Maseru (Roma) and Mokhotlong (Malefiloane). The results were 280 mg/kg for Mokhotlong and 0 mg/kg for Maseru.

The mountain districts (Mokhotlong and Thaba Tseka) had high amounts of the main soil elements required for crop production except for phosphorus, which was low in Mokhotlong. Soils in these districts had relatively high calcium and magnesium content. Such concentrations are characteristic of calcimorphic clay loam soils developed from basaltic lava in some parts of Mokhotlong and Thaba Tseka (Carroll and Bascom 1967). The pH range for all sites was in the category of slightly acidic, although that of Leribe could be moderately acidic.

Leribe and Maseru had relatively low amounts of critical elements. However, some of these elements Maseru were in the high range (potassium, magnesium and sodium) while calcium was medium-high. Although measurements on nitrogen content were not performed at all locations, looking at the other nutrients in Table 4.4, it was deduced that results from Thaba Tseka might be similar to those of Mokhotlong and nitrogen could equally be high. At the same time, the nutrient profile in Leribe may be as low as in Maseru. Yield has been relatively high in the mountain districts than the lowlands, even though no fertilizer was used in the mountains to simulate farmers' practices.

Table 4.5 Precipitation and average temperatures from 2015 to 2018 in Mokhotlong and Thaba Tseka during the cultivation of spring wheat

Year	Month	Mokhotlong			Thaba Tseka		
		Temperature Min (ºC)	Temperature Max (ºC)	Precipitation (mm)	Temperature Min (ºC)	Temperature Max (ºC)	Precipitation (mm)
2015	10	8.99	20.82	23.2	9.35	24.45	24.9
2015	11	6.43	20.63	0.4	8.01	23.51	45.3
2015	12	9.69	23.75	26.5	12.41	27.31	18.3
2016	1	9.78	21.32	142.4	12.43	24.55	87.5
2016	2	9.35	20.63	96.3	11.32	24.77	79
2016	3	7.3	19.06	46.1	9.98	22.35	54.4
2016	4	5.45	20.25	24.1	7.9	20.2	50.5
2016	10	7.55	23.08	35.9	6.62	22.13	47.6
2016	11	9.54	22.54	78.4	9.50	21.96	96.3
2016	12	11.64	25.51	72.4	11.5	25.48	62
2017	1	11.75	26.08	176.8	11.33	25.92	183.09
2017	2	10.59	20.17	150.4	10.21	19.06	193.2
2017	3	8.49	28.09	44.3	8.93	22.45	47.2
2017	4	5.43	19.95	19.8	6.03	18.77	28.2
2017	10	6.38	20.56	52.1	5.79	19.89	39.6
2017	11	7.02	21.6	61.4	6.6	21.87	32.9
2017	12	9.751	23.59	65.5	9.54	22.65	66.3
2018	1	10.25	26	82.3	10.570	25.57	113
2018	2	10.32	21.78	67.8	9.88	20.78	58.1
2018	3	8.35	20.27	209	9.15	19.58	204.5
2018	4	6.8	19.1	17.9	7.1	18.6	9.5

Table 4.6 Precipitation and average temperatures from 2015 to 2018 in Leribe and Maseru during cultivation of winter wheat

		Leribe			Maseru		
Year	Month	Temperature Min (ºC)	Temperature Max (ºC)	Precipitation (mm)	Temperature Min (ºC)	Temperature Max (ºC)	Precipitation (mm)
2015	5	3.0	21.62	6.4	5.20	17.98	0
2015	6	0.3	8.25	10	1.80	14.69	31.8
2015	7	0.0	0.31	0	3.41	12.77	1.3
2015	8	2.8	19.75	5.6	0.50	17.95	0.5
2015	9	7.4	19.08	29.2	8.59	22.99	12.2
2015	10	11.3	25.00	25.1	12.19	28.64	32.2
2015	11	14.2	31.08	0	9.28	33.30	28.5
2016	5	4.71	19.25	32	6.09	15.52	76.9
2016	6	0.83	16.19	19.4	3.37	15.60	17.5
2016	7	0.65	18.07	42.6	1.65	16.80	110.1
2016	8	2.47	20.50	47.9	4.13	19.56	24.8
2016	9	7.92	23.79	2.5	8.63	22.28	18.8
2016	10	9.10	21.11	59.4	6.97	20.15	30.4
2016	11	12.61	22.10	113.2	12.26	20.33	124.9
2017	5	3.32	19.05	19.2	5.06	18.60	9.1
2017	6	-0.13	16.34	0	2.48	16.52	2.4
2017	7	-0.29	17.65	1.9	2.53	18.31	0
2017	8	1.37	18.98	0	3.29	19.05	0
2017	9	7.65	23.00	16.7	8.95	24.44	2.6
2017	10	8.38	17.74	50	9.33	23.24	79.4
2017	11	10.4	26.6	64.1	11.5	25.2	35.9

There was an El-Niño-induced drought in 2015, which immensely affected winter wheat production in Leribe and Maseru. Yields obtained from Leribe (Table 4.7) reflect the severity of the situation, compared to Maseru trials (Table 4.8), which were irrigated four times. The mountain districts growing spring wheat had relatively good rains, and their yield was comparatively higher than the lowlands.

Leribe field trials 2015

Eight cultivars were grown in Leribe in the 2015 growing season. The analysis of variance between cultivars showed no significant difference in the damage caused by RWA (F = 1.03, df = 7, n = 8, p < 0.05). However, the damage rating scale showed resistance/susceptibility differences between the cultivars (Table 4.7). Senqu and SST387 showed no visible damage, which was suspected as "escape" considering the low level of infestation on these cultivars. The level of damage was about 5%, and the number of aphids per leaf ranged between 1-10 on the susceptible cultivars Makalaote, PAN3118 and Bolane. Elands, PAN3379 and Matlabas only had chlorotic spots, indicating resistance. The damage and the presence of aphids were relatively more visible on the local cultivars, Makalaote and Bolane. They expressed chlorotic stripping and were classified as medium susceptible. PAN3118 expressed similar symptoms.

The analysis of variance did not show any significant differences in yield (F = 0.157, df =7, n = 8, p < 0.05). There was almost no rainfall (Table 4.5) for the entire growth period, which negatively affected wheat growth and yields presented in Table 4.7 below. The Lesotho cultivars nonetheless had the lowest yield. Drought stress affects most of the functions of plant growth (photosynthesis, transpiration, translocation and respiration). Stress intensity depends on drought duration, plant growth stage, and the plant genotype.

Table 4.7 Analysis of RWA damage and wheat yield in Leribe (2015)

Cultivar	RWA damage score (Means)	Rounded means scores	Resistance or	Yield kg/ha
Senqu	1.00	1	Escape/ Resistant	346
SST387	1.25	1	Escape /Resistant	473
Elands	1.50	2	Resistant	406
PAN3379	1.50	2	Resistant	475
Matlabas	2.00	2	Resistant	352
PAN3118	2.50	3	Medium susceptible	364
Makalaote	2.50	3	Medium susceptible	289
Bolane	2.50	3	Medium susceptible	217
Mean	**1.78**			**365**
LSD(P≤0.05)	**1.617**			**340**

Maseru Trials 2015

The same cultivars used in Leribe were also planted in Maseru. The analysis of variance (Table 4.8) shows significant differences between RWA damage means (F = 0.77, df = 7, p < 0.05). The damage analysis based on the damage rating scale shows no visible damage on Senqu, Elands, and PAN3379, implying either resistance or escape. Matlabas and SST387 had chlorotic spots signifying resistance. PAN3118, Bolane and Makalaote displayed chlorotic stripping and were classified as medium susceptible.

Trials in Maseru were irrigated four times during their growth period (once at the vegetative stage, once at stem elongation and twice at heading) to reduce the El Niño induced drought stress. The analysis of variance showed no significant difference (F = 0.68, df = 7, n = 8, p < 0.05) between the yield means. However, there was a difference in yield realized between the cultivars. PAN3379 and 3118 had a higher yield than the other cultivars. Bolane had the lowest yield. These results are comparatively higher than those from Leribe are (Table 4.7). Although yields were still low compared to the other subsequent years because of the magnitude of drought, irrigation was able to induce improvement.

Table 4.8 Analysis of RWA damage and wheat yield in Maseru (2015)

Cultivar	RWA damage Score (Means)	Rounded means scores	Resistance or susceptibility status	Yield kg/ha
Senqu	1.00a	1	Resistant or escape	858.1
Elands	1.079ab	1	Resistant or escape	842.7
PAN3379	1.250abc	1	Resistant or escape	1.412
Matlabas	1.500abc	2	Resistant	1069
SST387	1.500abc	2	Resistant	931
PAN3118	2.500bc	3	Medium susceptible	1165
Bolane	2.500bc	3	Medium susceptible	655
Makalaote	2.553c	3	Medium susceptible	940
Mean	**1.750**			**987**
LSD(P≤0.05)	**1.465**			**829**

Means sharing the same letter denote that there is no significant difference at p<0.005.

Other Pests observed in Maseru and Leribe in 2015/16

Bagrada bug (*Bagrada hilaris*)

Bagrada bug is a pest that was observed on Bolane, PAN3379, PAN3118 and Senqu plots. This bug induced white rosette-shaped markings on both sides of the leaves (Fig. 4.2). Amongst the cultivars, PAN3379 and PAN3118 were not as seriously affected as Bolane. Infestation on Bolane was so high that some leaves were chlorotic, affecting photosynthesis and ultimately yield. Bagrada bug damage on Bolane was probably due to physical damage caused by its piercing-sucking mouthparts. Bolane suffered more damage than other cultivars, and yield in Leribe (Table 4.7) and Maseru (Table 4.8) was the lowest. This damage may have affected photosynthesis and hence yield of Bolane. The damage occurred in both Maseru and Leribe trials.

El-Niño-induced drought in 2015 characterized by abnormally low rainfall and scorching weather conditions discouraged farmers from engaging in vegetable production. Bagrada bug usually feeds on vegetables like English giant, Florida broadleaf and cabbage; due to the absence of such suitable host, wheat became the alternative host.

Figure 4.2 Bagrada bug (*Bagrada hilaris*) feeding damage on wheat

Loose Smut (*Ustilago tritici*)

Loose smut (Fig. 4.3) was observed only on Lesotho cultivars Bolane and Makalaote. Smut forms on the inflorescence of infected wheat. The spores completely displace the grain in the head so that there is no grain to be harvested on infected plants. Infections occurred in every experiment from 2015 to 2018. However, the fungus was common in the lowlands districts (Maseru and Leribe).

Figure 4.3 Loose smut (*Ustilago tritici*) on Bolane in Maseru, 2015

Field trials in 2016/17

The trials for 2016/17 were conducted in all the four sites: Mokhotlong, Thaba Tseka, Leribe and Maseru. However, a hailstorm damaged the Leribe trial, and no data was collected. In the remaining trials, the Lesotho cultivars Makalaote and Bolane were grown with PAN3379 which is resistant to RWASA1 - 4 and Elands, which is only resistant to the South African RWA biotype 1 (RWASA1).

Thaba Tseka trials 2016/17

Russian wheat aphid Damage rating

The analysis of variance (Table 4.9) shows that RWA induced damage in PAN3379 did not differ significantly from that in Elands and Bolane. Damage on Makalaote differed significantly from that of PAN3379. The damage rating scale results separate the four

cultivars into two resistance categories; two Lesotho cultivars were medium susceptible, and two South African cultivars were resistant. The infestation level based on the number of plants that were symptomatic of RWA damage in Thaba Tseka was around 20% with the infested plants having an average of between 10 – 100 aphids per leaf. The infestation was more on Bolane and Makalaote.

Elands had the lowest yield while PAN3379 had the highest. However, Elands, Makalaote and Bolane did not show any significant differences. PAN3379 had a significantly higher yield than Elands (Table 4.8)

Table 4.9 Analysis of RWA damage and wheat yield in Thaba Tseka (2016/17)

Cultivar	RWA damage score (Means)	Rounded means scores	Resistance or susceptibility status	Yield kg/ha
PAN3379	1.50a	2	Resistant	2520b
Elands	2.25ab	2	Resistant	1280a
Bolane	2.75ab	3	Medium susceptible	2180ab
Makalaote	3.25b	3	Medium susceptible	2240ab
Mean	**2.438**			**2055**
LSD(P≤0.05)	**1.58**			**1096**

Means sharing the same letter denote that there is no significant difference at p<0.005.

Mokhotlong Trials 2016/17

The infestation level in Mokhotlong based on the number of plants that were symptomatic of RWA damage was around 40% with an average of 10 – 100 aphids per leaf. The percentage given relates to Bolane, which had the highest rate of infestation. Although the analysis of variance shows no significant differences between Makalaote, Elands and Bolane, the rounded scores and the resistance or susceptibility status classify the four cultivars into four different categories (Table 4.9). Makalaote had the highest yield, and it was significantly higher than the Elands. Yield in Elands was lower than in Makalaote by 82%. PAN3379, which is resistant to the four RWASA biotypes, had a 35% lower yield than Makalaote.

Table 4.10 Analysis of RWA damage and wheat yield in Mokhotlong (2016/17)

Cultivar	RWA damage score (Means)	Rounded means scores	Resistance or susceptibility status	Yield kg/ha
PAN3379	1.750a	2	Resistant	1470bc
Elands	2.850ab	3	Medium susceptible	400a
Bolane	3.550b	4	Susceptible	1180ab
Makalaote	2.950ab	3	Medium susceptible	2280c
Mean	2.775			1332.5
LSD(P≤0.05)	1.507			465

Means sharing the same letter denote that there is no significant difference at p<0.005.

Maseru Trials 2016/17

Russian wheat aphid damage

The trial was free from RWA throughout the growth stages. Therefore, all the cultivars probably escaped the RWA attack. The results presented below (Table 4.10) therefore, reflect the performance of cultivars without RWA damage. Bolane had the lowest yield. Even though there was a high infestation of rose aphid (*Macrosiphum rosae*), mainly on PAN3379, there was no significant damage observed.

Table 4.11 Analysis of RWA damage and wheat yield in Maseru (2016)

Cultivar	RWA damage score (Means)	Rounded means scores	Resistance or susceptibility	Yield kg/ha
PAN3379	1	1	Escape	1720ab
Makalaote	1	1	Escape	1690ab
Elands	1	1	Escape	1440bc
Bolane	1	1	Escape	1046c
Mean				1474
LSD (0.05)				512

Means sharing the same letter denote that there is no significant difference at p<0.005.

Figure 4.4 Rose aphid on PAN3379 in 2017

Mokhotlong Trials 2017/18

The 2017/18 trials consisted of two Lesotho cultivars Bolane and Makalaote and two South African cultivars PAN3379 and PAN3118, where data were collected at anthesis. However, PAN3118 did not flower due to insufficient vernalization. The rate of infestation was nonetheless higher than the previous years; the susceptible cultivars Makalaote and Bolane had 60% infection levels and the average of 10 – 100 aphids per leaf. Relatively higher rainfall (Table 4.11) resulting in humid and cooler conditions were conducive for RWA multiplication. Leaf rolling signifying susceptibility occurred in the Lesotho cultivars and PAN3118.

The performance of PAN3379 (Table 4.11) was significantly lower than that of other cultivars (F = 2.98, df = 2, p = 0.1015). The first quarter of 2018 was characterized by floods, which resulted in water logging in the fields. Part of the vegetative and the entire reproductive stage were affected. PAN3379, which has been the highest yielding over the study period, had the lowest yield.

Table 4.12: Analysis of RWA damage and wheat yield in Mokhotlong (2017/18)

Cultivar	RWA damage score (Means)	Rounded means scores	Resistance or susceptibility status	Yield kg/ha
PAN3379	1.25a	1	Resistant	631.25b
PAN3118	4b	4	Susceptible	-
Bolane	3.5b	4	Susceptible	808.75ab
Makalaote	3.75b	4	Susceptible	1043.75a
Mean LSD	**3.125**			**827.92**
(0.05)	**1.491**			**383.38**

Means sharing the same letter denote that there is no significant difference at p<0.005.

Other Pests

Wheat rust

Wheat leaf rust (*Puccinia triticina*) was observed in all the experimental blocks, including PAN3118, which did not turn reproductive due to lack of vernalization. The surrounding fields were also heavily infected.

4.4 Discussion

The occurrence and numbers of the RWA were relatively low in the lowlands districts. There was even no record of RWA in 2017 in the Maseru trials. The area planted to wheat in the lowlands districts of Lesotho, especially Maseru, has been declining while the area remained constant or increased in the mountain districts (Bureau of Statistics, 2015). This declining cultivation area translates into absence or lack of primary host plants for RWA, and hence its low occurrence. Jankielsohn (2017) also noted that the decrease in dryland wheat cultivation in the Free State Province due to the late rains, poor growing conditions and increased costs of production, has a direct influence on the distribution and the population density of RWA. Similar production factors, exacerbated by inadequate harvesting machinery, also affect farmers in the lowlands districts of Lesotho. Macharia *et al.,* (2016) also reported that continuous cropping of wheat in Mt Kenya region and West Mau enabled the cereal aphids to multiply, move from one field to another and survive from one cropping season to the next. Few farmers in the lowlands of Lesotho are habitual

wheat producers. The scarcity of wheat farms directly reduces the size of RWA populations.

The few farmers growing wheat in the lowlands districts mostly use the *Dn1* resistant cultivars like Matlabas, Elands and Gariep. The low population density recorded in the lowlands districts can also be attributed to the use of resistant cultivars. Schotzko and Bosque-Pérez (2000) support this idea as they showed that resistance in a cultivar reduces RWA population development. Messina and Bloxham (2004) also observed that planting resistant cultivars reduced the performance and population density of RWA, and loss in biomass significantly increased in susceptible barley and wheat than on resistant oats and wheat.

Bolane, which is susceptible to RWA biotypes (1, 3 and 4) but moderately resistant to biotype 2, grows and matures relatively slower. Makalaote, on the other hand, is susceptible to RWA biotypes 1 – 4 (Table 3.2) and matures faster than most South African cultivars. In the trials, Makalaote attained yield with insignificant differences from that realised by South African cultivars. The minor impact of aphid infestation on yield could be associated with the rapid growth of Makalaote; probably higher aphid densities coincided with the time when it had almost reached physiological maturity. The RWA population densities reach their peak in late February to March in Mokhotlong and Thaba Tseka, and this coincided with the time when Makalaote had almost reached physiological maturity. Hein (1991) also found that RWA damage rating declined from an early heading, and there was no significant difference between the susceptible and resistant cultivars from this stage. Chemeda (2015) also reported that Ethiopian farmers in RWA hot spots like North Shewa adopted growing early maturing cultivars of barley to help the crop escape pest damage. The relatively slow growth and maturation of Bolane allow full exposure to RWA infestation.

Russian wheat aphid populations varied in different years and localities from 2015 to 2018. The RWA population densities in 2018 were relatively higher than the previous years following higher precipitation in February to March in Mokhotlong and Thaba Tseka. High rainfall creates humid and cooler conditions conducive to RWA multiplication, while the

dry, hot conditions from August to December characteristic of the wheat-growing season in Maseru and Leribe do not favour RWA development. Studies conducted in the Arid zone of Bhakkar in Pakistan with similar climatic conditions to Lesotho, also revealed that RWA breeds at a faster rate during the cold weather conditions and reaches the highest population density at the end of February to early March when ears begin repining (Khan, *et al.,* 2011). Ahmad *et al.,* (2016) also found that the population density of aphids increased in cold and humid climatic conditions and declined as temperatures increased. Merrill, *et al.,* (2009) showed that RWA has the highest fecundity around 18.5 ^{o}C. Longevity increases as temperatures decrease by approximately 1°C toward the developmental threshold.

The average wheat yield for Lesotho in 2015-2016 was 0.5 t/ha and 1.29 t/ha for 2016-2017 planting seasons (Bureau of Statistics, 2017). The yield is lower than that of the neighbouring Free State province where cultivars that are resistant to RWA grow under the same dryland conditions. The yields were 1.08 t/ha and 2.14 t/ha, respectively, in the same seasons (SAGL, 2017). Mokhotlong, followed by Thaba Tseka, had the highest area planted to wheat because of their geographical location, which limits the production of other grain crops (Bureau of Statistics, 2015). Farmers in these districts persistently use recycled seeds, mainly Bolane, for its large straws for roofing and livestock feeding (Rosenblum, *et al.,* 1999; Masupha, *et al.,* 2018). The high scale production of Bolane, which is a low yielding cultivar, is one of the critical factors responsible for the average low wheat production in Lesotho. In addition to conditions that do not favour maize and sorghum production, wheat performs better in the mountains than the lowlands. Our results indicate that yield in the lowlands districts (Maseru and Leribe) has never been more than 2 t/ha (Tables 4.7, 4.8, and 4.12). However, in 2016 when there was no record of RWA (Table 4.10) yield was better than in the past years in Maseru and Leribe. Makalaote yield in Mokhotlong was 2.3 t/ha, and in Thaba Tseka it was highest at 2.2 t/ha (Tables 4.8 and 4.9), while PAN3379 yielded highest in Thaba Tseka with 2.5 t/ha and dropped in Mokhotlong to 1.5 t/ha. Our results confirm the Bureau of Statistics reports (Bureau of Statistics, 2015; 2017) that Mokhotlong and Thaba Tseka are the leading districts in wheat production in Lesotho. However, high RWA infestation and rust infection probably reduced yield in Mokhotlong in 2018 (Table 4.11).

Soil analysis results revealed that soils in all the four sites were slightly acidic, and the highest was Mokhotlong with pH 5.1. Mohebbi and Mahler (1989) found that the lowest acceptable pH value for wheat production was 5.2. Acidic soils adversely affect wheat production by making other soil nutrients to be fixed and become unavailable to the crop. Wheat requires a pH of 6.0 – 7.5 (DAFF, 2016). The effect of fertilizer application in the lowlands districts (Maseru and Leribe) may be negatively affected by the acid soils resulting in reduced yield. The common practice by farmers in Mokhotlong and Thaba Tseka of planting wheat without the use of fertilizer affects the yield and quality of wheat. The primary effect is likely to come from nitrogen deficiency. Nitrogen increases rapid growth, tiller formation, green leaf duration, grain size, grain protein and grain quality in wheat (Roman *et al.,* 2018). Reduced soil fertility may be a reason for low RWA infestation despite conditions which are favourable for their survival.

Mokhotlong recorded a high incidence of rust on all the cultivars in 2017/18. The infection was associated with the humid conditions induced by heavy rains in January to March, which started when plants were still in their vegetative to reproductive stages. Mokhotlong is assumed a possible over-summering zone and source of inoculum for wheat rusts in South Africa because wheat is commonly planted late in the wheat-growing period in South Africa (Terefe *et al.,* 2009). Boshoff *et al.,* (2002) found that Bolane and Mohohlotsane (believed to be makalaote) showed medium susceptibility to yellow rust (*Puccinia striiformis* f. sp. *tritici* pathotypes 6E16A and 6E22A). Nonetheless, Bolane had the lowest infection (30%) while Mohohlotsane had 80%. PAN3379 is susceptible/medium susceptible to leaf, stem and stripe rusts (PANNAR 2014). The low yield in PAN3379 in 2018 might have been a direct effect of rust on the cultivar. Leaf rust causes yield losses irrespective of the level of RWA resistance possessed by the cultivar. Completely susceptible cultivars suffer more loss than resistant ones (Murray *et al.,* 1994). Ochoa and Parlevliet (2007) reported that yield loss due to barley leaf rust was correlated strongly with an area under disease progress curve, which implied that high levels of partial resistance are needed to prevent significant yield loss.

In addition to leaf rust, farmers' stored Bolane and Makalaote seed had loose smut infection.

Loose smut is a fungal seed-borne disease of wheat caused by *Stilago tritici*. It is a threat to the production of seed in developing countries where small-holder farmers recycle their seed. The degree of seed infection is often more than the standard for any categories of certified seed where no infections exist (Bishaw *et al.,* 2013). Yield loss of up to 40% due to loose smut has been recorded (Quijano *et al.,* 2016) and it is highly probable that farmers' continued use of untreated seed is one of the contributing factors to reduced yields.

Furthermore, seed recycling establishes a loose smut inoculum source. Bishaw *et al.* (2013) also observed that farmers' recycled seed had loose smut, while no infection existed in the certified seed. Planting certified pathogen-free seed eliminates the inoculum source, which can be removed by applying systemic fungicides or planting pathogen-free seed (French and Schultz, 2009).

4.5 Conclusion

The high yield of Makalaote, despite its susceptibility to RWA biotypes 1 – 4 shows how its rapid growth assists in avoiding RWA infestation. Bolane, on the other hand, susceptible to the dominant RWA biotypes 1, 3 and 4 grows, relatively slower and becomes the most suitable host for aphids. Cooler temperatures and humid conditions characteristic of the mountain districts support RWA development. In contrast, the inherent delayed rains, dry and hot conditions prevailing in the lowland districts negatively affect RWA population densities.

Climatic and soil conditions in Mokhotlong and Thaba Tseka favour wheat production. Overall, Makalaote outperformed most South African cultivars, especially in the mountain districts. Bolane consistently performed lower than other cultivars in terms of yield. Farmers prefer for this cultivar more for its large straws used for animal feeding and roofing than yield.

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Chapter five

Biochemical components of the resistance response in wheat against Russian wheat aphid

5.1 Introduction

Russian wheat aphid (RWA) is a severe pest of barley, *Hordeum vulgare* L. and wheat, *Triticum aestivum* L. The aphid severely hampers the production of wheat in Lesotho and South Africa. A decline in grain yield (up to 34%) associated with increasing RWA infestation in various wheat genotypes has been reported (Akhtar, *et al.,* 2010). Tesfay and Alemu (2015) further reported a massive reduction in wheat grain yield (68%), biomass (55%), weight per 1000 kernels (20%), and delayed heading and maturity as infestation intensified. The use of insecticides in the '80s was perceived as the most effective method of controlling the RWA (Du Toit and walters, 1984; Aalbersberg, *et al.,* 1989). However, RWA induced leaf rolling; its primary characteristic symptom makes this control ineffective as it prevents contact of insecticides with the aphids (Gutsche, *et al.,* 2009; Turanli, *et al.,* 2012). The efficiency of insecticides can further be compromised by drought, which is common in the Free State, South Africa (Du Toit, 1992).

Plant resistance, therefore, presents a viable, cheaper, and ecologically desirable alternative to chemical control. The use of insect-resistant cultivars could be an essential tool in the management of RWA in Lesotho, as no pesticides are used in wheat production.

Plants react to insect pest damage through an intricate and dynamic defence system, which includes the production of toxic secondary metabolites, remodelling of the cell wall, and emission of volatiles which attract natural enemies of aphids (Hanley, *et al.,* 2007). These direct and indirect defence responses may be present constitutively or induced after injury by herbivores. Induced plant responses form an essential component of pest control in crop production, and can regulate insect herbivore populations (Sharma, 2009; War, *et al.,* 2012).

The interaction between plants, pathogens and herbivores, activates a wide variety of defence responses. Russian wheat aphid induces the activities of pathogenesis-related proteins such as β-1,3- glucanase and chitinase (Mohase and Van der Westhuizen, 2002; Moloi and Van der Westhuizen, 2005). Further defence-related enzymes, including peroxidase, lipoxygenase, and phenylalanine ammonia-lyase (Botha, *et al.,* 2014; Berner and Van Der Westhuizen, 2015) also increase during aphid infestation. Wan, *et al.,* (2002) also reported the production of signalling compounds such as salicylic acid (SA), reactive oxygen species (ROS), ethylene (ET), nitric oxide (NO) and jasmonic acid (JA), which activate expression of downstream defences in plants.

The continued use of wheat cultivars introduced in Lesotho before identification of the RWA, despite the availability of improved higher-yielding and RWA resistant cultivars remains a challenge to wheat production in Lesotho. The use of these old than newer cultivars is more rampant in the mountain districts, which are significant areas of wheat production (Masupha, *et al.,* 2018). The RWA resistance status of these cultivars remains unknown. Therefore, this study investigated the involvement of defence-related enzymes (LOX, POD, PAL, and GLC) and some hormones (SA, JA and ABA), in the resistance of South African cultivars (PAN3379 and Elands) and the Lesotho cultivar Bolane, to the two RWA biotypes RWASA1 and 3. Specifically, the study aimed to:

1. Determine changes in apoplastic enzyme activities of pathogenesis-related proteins in wheat (peroxidase and β-1, 3-glucanase) during RWA infestation.
2. Measure changes in activities of key enzymes (PAL and LOX) associated with biosynthesis of certain hormones (SA and JA).
3. Measure content of salicylic, jasmonic and abscisic acids in wheat during RWA infestation.

5.2 Materials and methods

Plant material and infestation procedure

Wheat seeds, cultivar PAN3379, which is resistant to South African Russian wheat aphid Biotypes 1 - 4, Elands (resistant to RWASA1) and Bolane, were used. Bolane was introduced in Lesotho in the early 1960s (Weinmann, 1966). Farmers in the mountain districts of Thaba Tseka and Mokhotlong prefer the cultivar for its larger white grains and large straws used for roofing and livestock feeding (Masupha *et al.,* 2018). Elands and PAN3379 were obtained from ARC-SG in Bethlehem while the farmers in Mokhotlong provided Bolane.

Soaked seeds were pre-germinated in a growth chamber at 24ºC for 24 hours. The germinated seeds were transplanted [eight pots (1 L, 15 cm diameter) per cultivar] and transferred to the greenhouse maintained at 18 °C (night) and 24 °C (day). The eight pots per cultivar, each containing sixteen seedlings, were further divided into two sets: control and infested. At the three-leaf stage, two South African RWA biotypes: RWASA1 and RWASA3 (30 aphids per plant) were each separately used to infest the plants. All pots remained in cages (315-micron nylon mesh) throughout the experiment. The arrangement of experimental units (pots) followed a completely randomized design with three replicates.

Aphid multiplication and rearing

Agricultural Research Council-Small Grains (ARC-SG), Bethlehem, RSA, originally supplied the two biotypes: RWASA1 and RWASA3, in sealed Petri dishes. Each biotype was maintained and multiplied on the susceptible wheat cultivar, Tugela, in different cages under similar greenhouse conditions. Damaged Tugela plants were periodically replaced with fresh ones to ensure adequate food supply for aphid colonies.

The second and third leaves of five randomly selected wheat seedlings were harvested (0, 3, 6, 9, 12, 24 and 48 hours post infestation) by quick chilling in liquid nitrogen, and stored in falcon tubes at -20 °C until extraction of enzymes and hormones. The same number of leaves were randomly selected and harvested from either control or infested plants (0 and

48 hpi), for collection of intercellular washing fluid (IWF).

The IWF was extracted according to the method of van der Westhuizen *et al.,* (1998). The leaves were cut into about 8 cm pieces, rinsed twice in distilled water and vacuum infiltrated in a thick-walled glass tube with 50 mM Tris buffer at pH 7.8 for 5 minutes. The leaves were dried with a blotting paper and placed into a centrifuge tube with a perforated disc at the bottom, then centrifuged (500 *xg*) for 10 minutes at 4 °C. The IWF was collected from the tube; the collection procedure was repeated with the same samples. The extracts were combined, frozen in liquid nitrogen, and kept at -20 °C until peroxidase and β-1,3-glucanase activities assays.

The bioassays for the determination of enzyme activities and hormone levels, and the entire experiments, were each replicated three times.

Determination of enzyme activities

β-1,3-glucanase activity

β-1,3-glucanase (EC 3.2.1.39) activity was determined using a modified procedure from Fink, *et al*., (1988). The mixture for the assay, consisting of 10 μl enzyme extract, 250 μl laminarin (2 mg ml^{-1}, Sigma), and 240 μl 50 mM sodium acetate buffer, pH 4.5, was incubated for 10 min at 37 °C in a water bath. After that, 500 μl of Somogyi's reagent (Somogyi, 1952) were added. Samples were then boiled at 100 °C for 10 min and subsequently cooled under tap water cooling before adding 500 μl of Nelson's reagent (Nelson, 1944). Finally, the samples were vigorously shaken before reading absorbance at 540 nm (Cary 100 Bio UV-VIS) against a blank developed using the same procedure, but without the addition of the enzyme extract. The β-1,3-glucanase activity was determined using a calibration curve created with different glucose concentrations and expressed as mg glucose mg^{-1} protein min^{-1}.

Peroxidase activity

A modified method of Zieslin and Ben- Zaken (1991) was used to determine Peroxidase (EC 1.11.1.7) activity. The peroxidase assay mixture contained 840 μl of 40 mM potassium

phosphate buffer, pH 5.5 containing 2 mM EDTA, 100 µl of 5 mM guaiacol, 10 µl of enzyme extract and 50 µl of 82 mM H_2O_2. The change in absorbance of the assay mixture was measured at 470 nm using a spectrophotometer (Cary 100 Bio UV-VIS) at 30 ⁰C for 3 min. The molar extinction coefficient of guaiacol (2.66 $mM^{-1}cm^{-1}$) was used to calculate peroxidase activity and specific activity was expressed as µmol tetraguaiacol mg^{-1} protein min^{-1}.

Lipoxygenase activity

Substrate preparation

The linoleic acid substrate [2.5 mM linoleic acid in 0.15% (v/v) Tween 20] was prepared according to Ocampo, *et al*. (1986). Linoleic acid (400 µl), Tween 20 (768 µl) and 40 ml methanol were mixed and subjected to rotary evaporation at 60 ⁰C until dry. The dried material was then re-dissolved in 500 ml of 0.05 M sodium phosphate buffer, pH 9. The prepared substrate was stored in 5 ml aliquots under nitrogen at -20 ⁰C. During assays, the substrate was kept on ice.

Enzyme extraction

Enzyme extracts were prepared on ice from infested and uninfested leaves of Elands, PAN3379 and Bolane. The plant tissue (0.3g) was ground in liquid nitrogen using a cooled pestle and mortar. The leaf powder was homogenized in 3 ml of 0.2 M sodium phosphate buffer (pH 6.5), and the homogenate was centrifuged at 12000 *xg* for 20 min at 4°C.

Lipoxygenase activity was determined following methods modified of Ocampo *et al*. (1986) and Grossmann and Zakut (1997). The reaction mixture consisted of 1000 µl of 0.1 M sodium citrate phosphate buffer (pH 6.2), 50 µl of enzyme extract and 150 µl of 2.5 mM linoleic acid. The change in absorbance was measured at 30 ⁰C for 10 minutes at 234 nm. Molar extinction coefficient (9.6 x 10^{-7} $mM^{-1}cm^{-1}$) of hydroperoxide (HPOD) was used to calculate lipoxygenase activity, and specific activity was expressed as nmol HPOD mg^{-1} protein min^{-1}.

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity

Phenylalanine ammonia-lyase activity was determined on a spectrophotometer as outlined by Green *et al.* (1975), with some modifications. Polyvinylpyrrolidone (50 mg) and acid-washed sand (Sigma) were added to leaf tissue (0.5 g), which was ground to a fine powder using a pestle and motor. The tissue was homogenised in 0.1 M sodium borate buffer, pH 8.8, with 1 mM EDTA (freshly added), 1 mM dithiothreitol, and 25 mg Dowex (1 x 4 Cl^{-1}, Sigma). The ratio of leaf tissue to buffer was 1:4, and the homogenate was centrifuged at 15 000 *xg* at 4 ℃ for 20 min. The supernatant was used as an enzyme extract for the determination of PAL activity and protein concentration.

The assay mixture had 400 µl enzyme extract, 500 µl of 0.1 M sodium borate buffer, pH 8.8, and 100 µl of 60 mM L-phenylalanine. The change in absorbance was measured at 290 nm for 20 min at 40 ℃, with a 1 min delay. The amount of cinnamic acid liberated was calculated from a standard curve, and PAL activity was expressed as µg cinnamic acid mg^{-1} prot min^{-1}.

Protein concentration

The protein content of enzyme extracts was determined according to a modified method of Bradford (1976), using bovine serum albumin (0.5 mg ml^{-1}) as a standard. The absorbance was measured at 595 nm using a microplate reader (Anthos, Zenyth 3100).

Hormone extraction and analysis

The hormones (absiscic, jasmonic and salicylic acids) were extracted using a combination of modified methods of Forcat, *et al.,* (2008) and Segarra, *et al.,* (2006), with an addition e of solid-phase extraction (SPE) cartridges. The standards used were absiscic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and prednisolone (internal standard) at 1µg/µl suspended in MeOH. The induced hormones were expressed relative to the reference hormone (standard).

Frozen leaf tissue (250 mg) was crushed to a fine powder in liquid nitrogen and extracted

in 400 µl extraction solvent [MeOH/H_2O/acetic acid (10:89:1; v/v/v)] in a reaction tube containing two silver beads (4.8 mm). The homogenate was vortexed for 2 min, incubated for 30 min on ice and then centrifuged at 12 000 xg for 10 min. The extraction process was repeated and the supernatants combined. An internal standard (IS, prednisolone) was added to each sample. Two random samples were spiked with the hormonal standards (ABA, JA and SA).

The combined supernatants were separated by solid-phase extraction (SPE) cartridges (3 ml, 500 mg supelclean™ LC-SCX SPE tubes, Supelco). These cartridges were conditioned with 6 ml MeOH (100%) and equilibrated with 12 ml MeOH/H_2O/acetic acid (10:89:1; v/v/v) before sample application. Cartridges were washed with 3 ml MeOH/H_2O/acetic acid (10:89:1; v/v/v), dried and the trapped analytes were eluted with MeOH/H_2O (80:20; v/v). The samples were dried in a Savant SC 210 SpeedVac concentrator before reconstitution and separation.

Analysis of hormones

Samples used for hormones determination were analysed using an AB SCIEX 4000 QTRAP hybrid triple quadrupole ion trap mass spectrometer with a Shimadzu HPLC as a front end. The data acquisition and processing were conducted with Analyst 1.5.2 (AB SCIEX) software.

The samples were separated on a C18 (Restek Allure PFP propyl, 5µm, 50 x 2.1 mm) column at a flow rate of 300 µL/min using a fast 1 min gradient from 1% to 100% mobile phase B (80% Acetonitrile containing 7.5 mM ammonium formate). An additional 1min at 100% B was included, followed by column re-equilibration for a total of 7 min analysis time in negative ionisation mode. Eluting analytes were ionised by electrospray in the TurboV ion source at 550 ℃ to evaporate the excess solvent. The optimised instrument settings were 40 psi nebuliser gas, 40 psi heater gas, 25 psi curtain gas, and -4500 V ion spray voltage.

Sample analysis followed a targeted Multiple Reaction Monitoring (MRM) workflow on

the instrument. Throughout an MRM scan, the apparatus operated in triple quadrupole mode where every ionised analyte (the precursor) eluting off the column is fragmented in the collision cell to produce fragment masses. A set of masses, the precursor mass and one fragment mass create a transition. The instrument jumps between different transitions in an MRM transition list during an analysis cycle; each cycle was typically lasting less than a second. If a transition is detected, the instrument reaction is registered, and this ion intensity value is plotted as a chromatogram.

The targeted analyses for the analytes consisted of two transitions each (jasmonic acid: 209.1>59.0, 209.1>165.2; abscisic acid: 263.1>153.1, 263.1>219.1; salicylic acid: 136.9>93.2, 136.9>75.2; prednisolone (IS): 359.2>329.1, 359.2>259.3). The peak area on the chromatogram generated from the first and most sensitive transition was utilized as the quantifier while the second transition acted as the qualifier. The qualifier works as a different level of confirmation for the analyte presence. The withholding time for these three transitions has to be the same. The integrated peak area of each quantifier was normalised to the integrated peak area of the IS (prednisolone). The normalised peak area values were used in a relative quantitative fashion for comparing analyte levels in the different treatments.

5.3 Results

The results presented in this chapter are representative of 3 independent experiments. In the first part of the section, the effect of two RWA biotypes (RWASA1 and RWASA2) on apoplastic β-1,3-glucanase and peroxidase activities, was determined in all the three cultivars.

At the beginning of the experiment (0 hpi), there were no significant differences in β-1,3-glucanase activity between the control and RWASA1-infested seedlings of all the three cultivars (Fig. 5.1). The activity between control and infested plants at 48 hpi was significantly different in the resistant cultivar Elands (p = 0.0001) but not in the resistant cultivar PAN3379 (p = 0.2386) or the susceptible Bolane (p = 0.091).
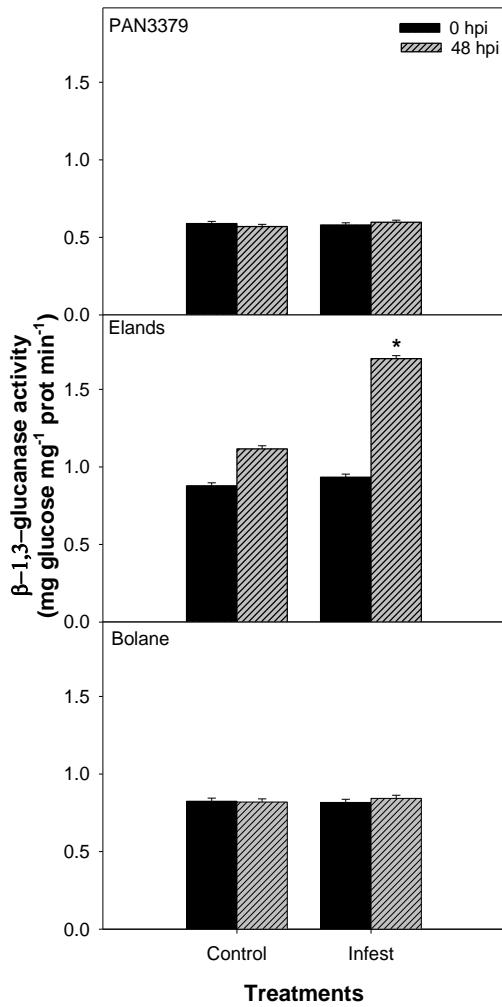
Figure 5.1 Effect of Russian wheat aphid (RWASA1) infestation on β-1,3-glucanase activity in PAN3379, Elands, and Bolane. PAN3379 and Elands are resistant, while Bolane is susceptible to RWASA1. The values are means, and the error bars indicate standard error. (*) indicates significant differences ($p < 0.05$), means were separated using the least significant difference (LSD) at 5%.

The effect of RWASA3 infestation on β-1,3-glucanase of the three cultivars differed from that of RWASA1. The cultivar PAN3379 is resistant to RWASA3, which significantly induced β-1,3-glucanase activity at 48 hpi ($p = 0.0001$). Elands and Bolane, both susceptible to RWASA3, showed some notable, but not significant ($p = 0.2202$ and $p = 0.442$, respectively) increases in β-1,3-glucanase activity (Figure 5.2).
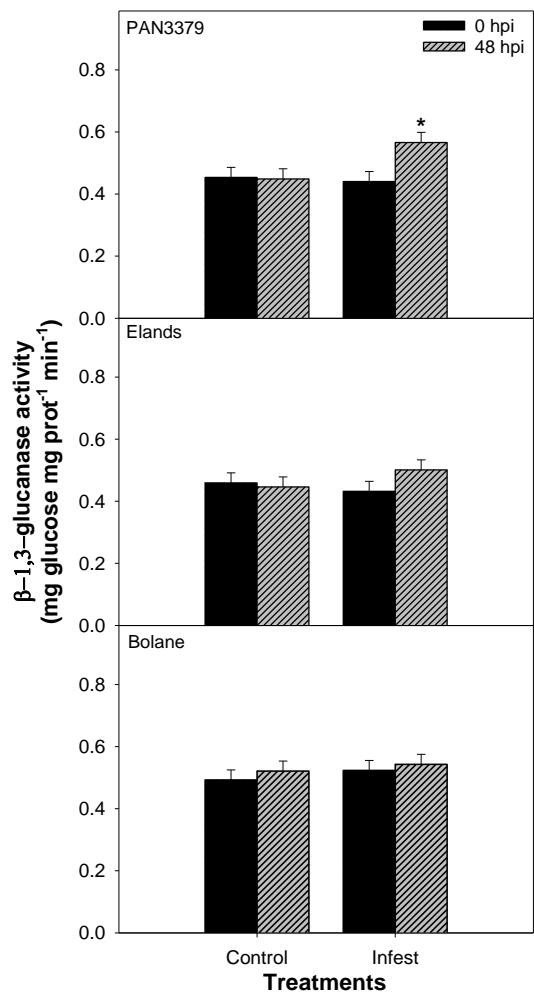
Figure 5.2 Effect of Russian wheat aphid (RWASA3) infestation on β-1,3-glucanase activity in PAN3379, Elands, and Bolane. PAN3379 is resistant while Elands and Bolane are susceptible to RWASA3. The values are means, and the error bars indicate standard error. (*) indicates significant differences ($p < 0.05$), means were separated using the least significant difference (LSD) at 5%.

Russian wheat aphid induced peroxidase activity, also determined from the apoplast of control and infested wheat seedlings, showed no significant differences between control and infested plants in all the cultivars (0 hpi). However, at the end of the experiment (Fig. 5.3, 48 hpi) RWASA1 infestation significantly induced peroxidase activity in the resistant cultivars, PAN3379 (p = 0.0001) and Elands (p = 0.0001) while there was no significant increase in Bolane (p = 0.152).
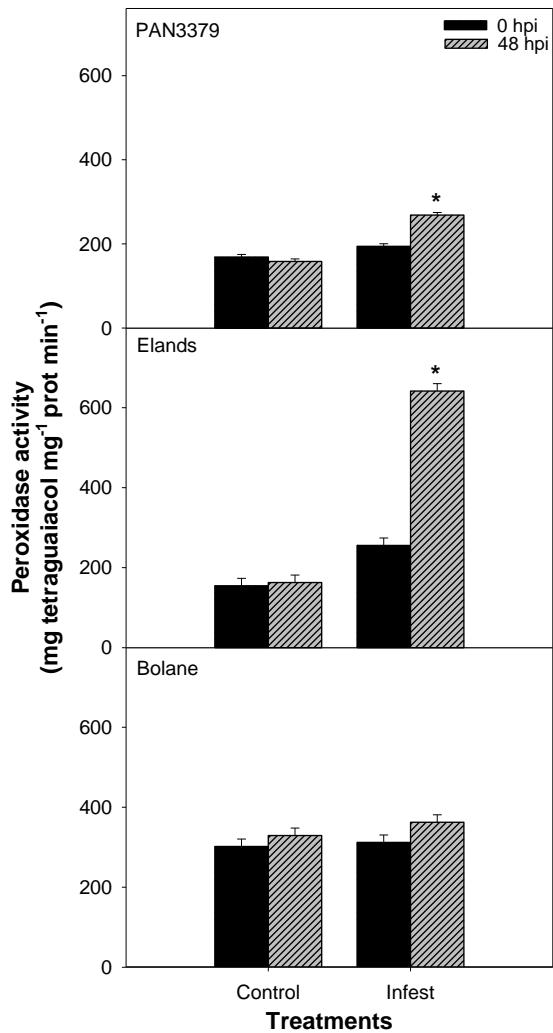
Figure 5.3 Effect of Russian wheat aphid infestation (RWASA1) on peroxidase activity in PAN3379, Elands, and Bolane. PAN3379 and Elands are resistant, while Bolane is susceptible to RWASA1. The values are means, and the error bars indicate standard error. (*) indicates significant differences (p<0.05). Means were separated using the least significant difference (LSD) at 5%.

Bolane is susceptible to RWASA3. However, infestation (48 hpi) induced a significant increase in peroxidase activity (Figure 5.4; $p = 0.0001$). Increased peroxidase activity was also recorded in PAN3379 ($p = 0.0001$). Elands, which is susceptible to RWASA3, did not show any significant induction in peroxidase activity ($p = 0.2134$).
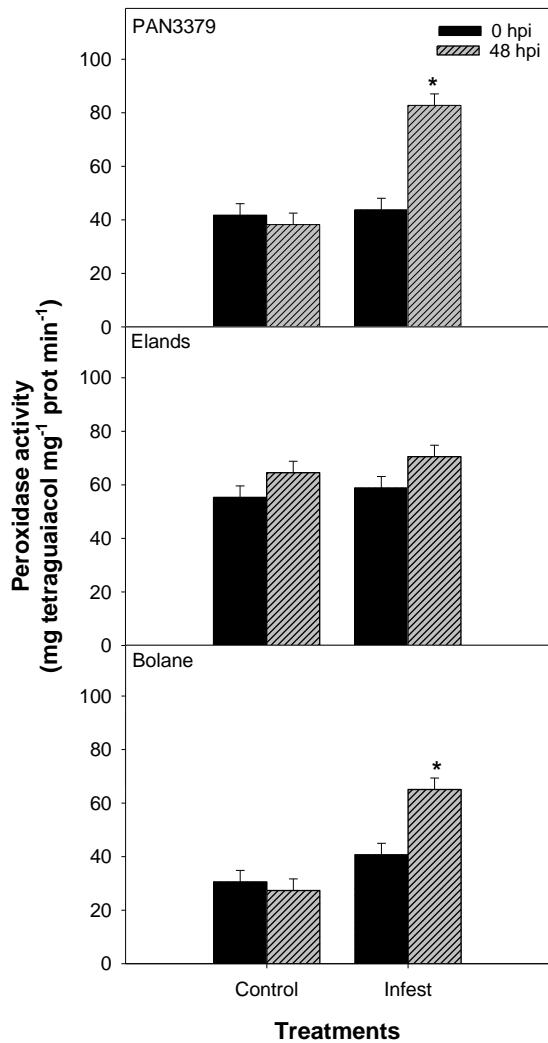

Figure 5.4 Effect of Russian wheat aphid (RWASA3) infestation on peroxidase activity in PAN3379, Elands, and Bolane. PAN3379 is resistant while Elands and Bolane are susceptible to RWASA3. The values are means, and the error bars indicate standard error. (*) indicates significant differences ($p<0.05$), means were separated using the least significant difference (LSD) at 5%.

The effect of RWA infestation on the activity of key enzymes (LOX and PAL) in the biosynthesis of salicylic and jasmonic acids was determined. Russian wheat aphid infestation induced various changes in LOX activity in the three wheat cultivars (Fig. 5.5). Biotype 1 and 3 infestation of PAN3379 significantly induced LOX activity ($p = 0.0001$). Biotype 1 induced LOX activity was significantly higher at 12 and 48 hpi ($p = 0.0115$) whereas RWASA3 increased LOX activity was maintained from 6 to 24 hpi ($p = 0.0001$).
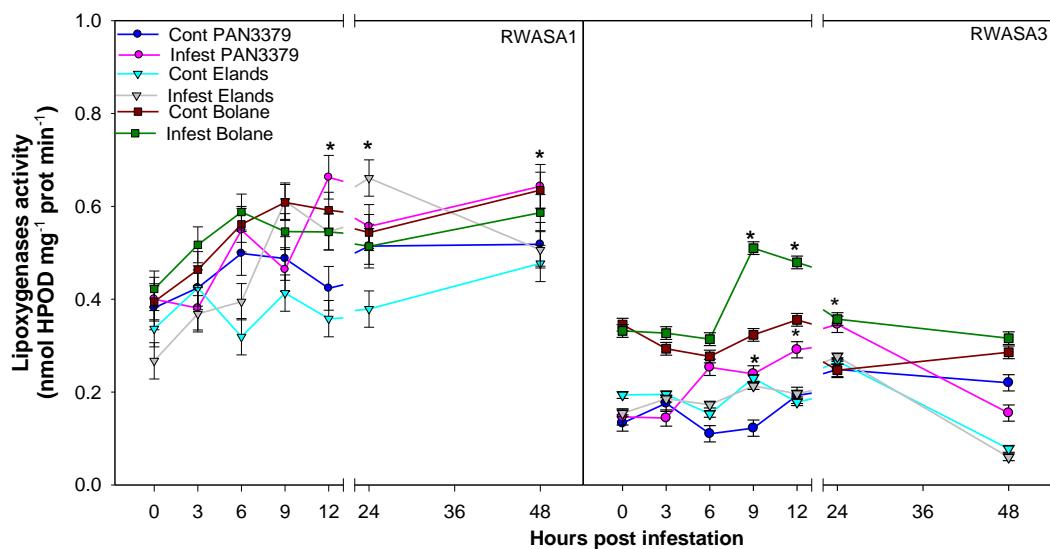
Figure 5.5 Effect of RWASA1 and RWASA3 infestation on lipoxygenase activity in PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences ($p<0.05$), means were separated using the least significant difference (LSD) at 5%.

Biotype 1 (RWASA1) infestation of Elands significantly induced LOX activity, beginning from 9 up to 24 hpi ($p = 0.0001$). However, RWASA3 did not induce any significant increase throughout the duration (48 hpi) of the experiment ($p = 0.3810$). Biotype 1 infestation on Bolane, a susceptible cultivar, did not induce any significant increases in LOX activity ($p = 0.379$) throughout the 48 h infestation period. However, RWASA3 induced significantly higher activity from 9 to 24 hpi ($p = 0.0001$). There was a noticeable decrease in LOX activity from a peak at 9 hpi until there was no significant difference at 48 hpi.

Aphid infestation (RWASA1 or RWASA3) of PAN3379 induced significant changes in PAL activity (Fig. 5.6). The rate of the response, however, differed; RWASA1 induced an earlier increase in PAL activity. Significant increases were measured as early as 9 hpi and the activity peaked 12 hpi (p = 0.0001). Biotype 3 (RWASA3) on the other hand, induced a relatively delayed but persistent increase in activity. Higher PAL activity was measured from 12 hpi (p=0.0001) and was sustained up to 48 hpi.
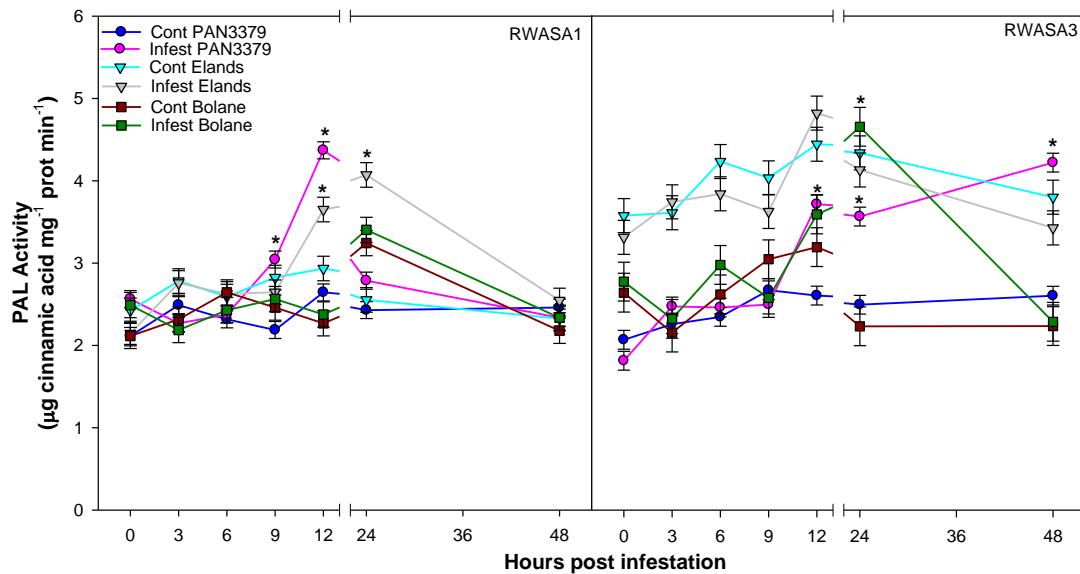

Figure 5.6 Effect of RWASA1 and RWASA3 infestation on phenylalanine ammonia-lyase (PAL) activity in PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences (p<0.05), means were separated using the least significant difference (LSD) at 5%.

Biotype 1 (RWASA1) infestation induced a delayed but significant increase in PAL activity of Elands, beginning at 12 hpi and peaking 24 hpi (p = 0.0001). The increase in PAL activity dropped at 48 hpi, where it was no longer significant. Biotype 3 infestation did not induce any significant increase in PAL activity throughout the experiment (P = 0.1762).

Biotype 1 infestation did not induce any significant increase in PAL activity of Bolane. The levels were almost the same from 0 to 12 hpi (p = 0.1762). Relative increases in PAL activity were noticed 24 hpi (Fig 5.6) but were not significant as both control and infested plants experienced an increase in PAL activity. Biotype 3 infestation induced a delayed transient but significant induction in PAL activity at 24 hpi (p = 0.004).
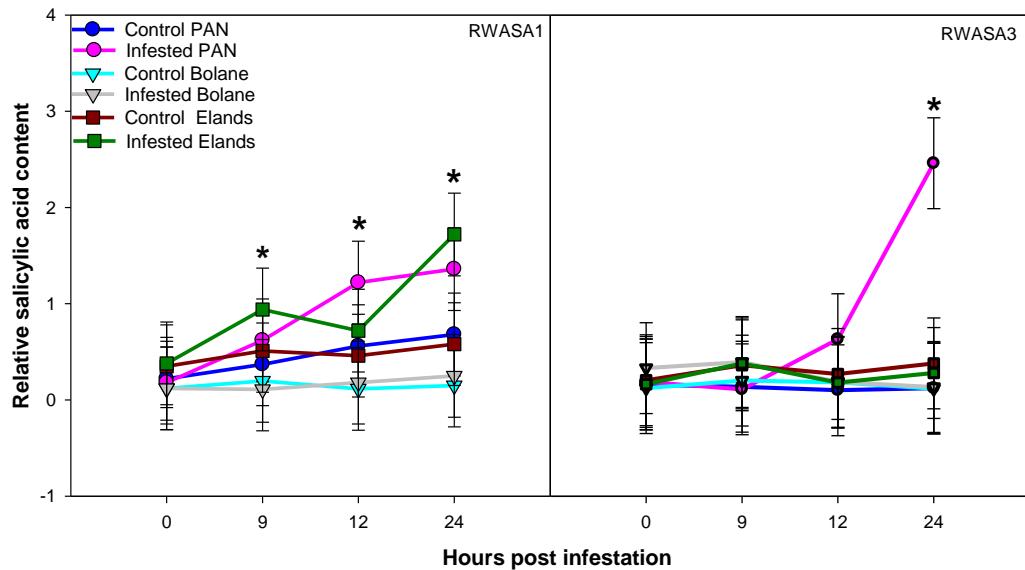

Figure 5.7 Effect of RWASA1 and RWASA3 infestation on salicylic acid content of PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences (p<0.05).

Biotype1 infestation significantly induced salicylic acid (SA) accumulation in Elands and PAN3379 but not in Bolane (Fig. 5.7). Induced SA accumulated with the duration of infestation. At 12 and 24 hpi, the induced SA level was significantly higher than in control. At 24 hpi, the highest SA level (p = 0.000), which was 50% higher than in control was measured. Infested Elands expressed the most significant degree of SA accumulation (Fig. 5.7). At 9 hpi, SA levels were significantly different between control and infested plants. Even though there was a dip at 12 hpi, SA levels further intensified and at 24 hpi were 66% higher than in control plants (p = 0.000).

Biotype 3 triggered a significant increase in SA accumulation only in PAN3379. In Bolane and Elands, SA levels in control and infested plants were almost the same throughout the 24 h period. At 12 hpi in PAN3379, SA concentration in the infested plants was significantly higher than all other treatments. The highest level of SA (94%) relative to control was measured in PAN3379 at 24 hpi (p = 0.0000).
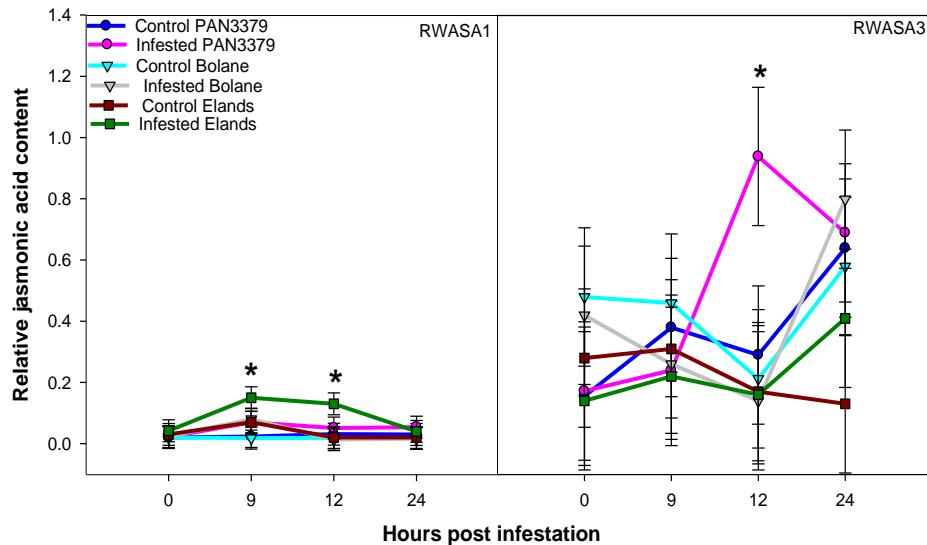

Figure 5.8 Effect of RWASA1 and RWASA3 infestation on jasmonic acid content of PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences (p<0.05).

Biotype 1 infestation induced a relatively lower JA accumulation than RWASA3 (Fig. 5.8) in all the cultivars. In Elands, induced JA accumulation was significantly higher than in control at 9 (85%, p = 0.0001) and 12 hpi (50%, p = 0.0001). Infestation did not induce any significant changes in JA concentration in both PAN3379 and Bolane.

Infestation by RWASA3 induced a significant but transient JA increase in PAN3379 at 12 hpi. There was a sharp decline in JA level from 12 to 24 hpi. At 24 hpi, there was a concurrent JA increase in both control and infested Bolane, as well as in unifested PAN3379.
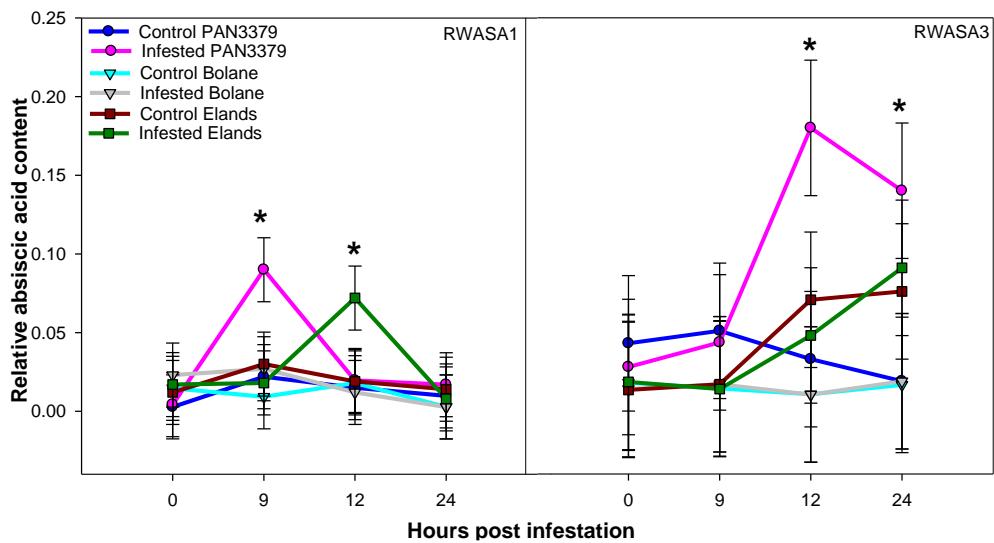
Figure 5.9 Effect of RWASA1and RWASA3 infestation on absiscic acid level of PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences (p<0.05).

Russian wheat aphid (RWASA1) infestation significantly induced ABA accumulation in PAN3379 and Elands at different times after infestation. Absiscic acid levels in PAN3379 peaked 9 hpi (p = 0.0000) while in Elands the greatest levels were 12 hpi (p = 0.0001). The highest aphid induced ABA content was in PAN3379 at 76%, followed by Elands at 71% relative to controls (Fig. 5.9).

Biotype 3 infestation only induced significant ABA accumulation in PAN3379. Accumulation of ABA was highest at 12 hpi and dropped by 22% at 24 hpi though still significant (p = 0.0000). Both control and infested Elands accumulated ABA (p = 0.1762) from 9 hpi to 24 hpi while the levels in both the control and infested Bolane remained low (p = 0.4664).

5.4 Discussion

The study focused on the involvement of jasmonic acid (JA), salicylic acid (SA) and absiscic acid (ABA) in the defence responses of wheat against the RWA. The work also investigated the changes in activities of some key enzymes in the biosynthetic pathways of JA and SA during RWA infestation. This chapter was a comparative study of wheat cultivars (PAN3379 and Elands) with known resistance status to the RWA, and the cultivar, Bolane, where the reaction to different RWA biotypes is unknown.

Bolane is a cultivar that has been in Lesotho since the 1960s (Weinmann, 1966). Farmers prefer it for its long straw used for roofing of traditional houses and livestock feeding, and it is extensively cultivated in different parts of Mokhotlong and Thaba Tseka, in the Lesotho highlands (Masupha, *et al.,* 2018). It is soft white wheat preferred for excellent qualities in bread making. The two South African RWA biotypes (RWASA1 and 3), which were the most prevalent in Lesotho during the preliminary survey, artificially infested wheat in this study.

Russian wheat aphid is a severe pest of barley and wheat in various production areas (Berner and van der Westhuizen, 2010). The cultivation of resistant wheat cultivars is the sustainable, environmentally safe and effective management option for RWA (Dogimont, *et al.,* 2010; Umina, 2017). Breeding of these cultivars involves the introduction of pathogen or pest resistance genes into plants through breeding or some other biotechnological techniques. Plant defence responses switch-on when specific receptors recognise the presence of pathogens, pests, induced damage, or even the existence of volatiles produced as plant-plant cues (Diaz, 2018).

Elands is resistant to RWASA1 and susceptible to RWASA3. PAN3379, on the other hand, is medium resistant to RWASA1 and resistant to RWASA3 (ARC-SGI, 2016). Even though the resistance status of Bolane to RWA biotypes is not known, phenotypic studies (Table 3.6) have classified Bolane as susceptible to South Africa biotypes 1, 3 and 4, and moderately resistant to biotype 2. We, therefore, expected RWASA1 infestation to induce increased POD, GLC, PAL and LOX activities in PAN3379 and Elands, while RWASA3 infestation was expected to enhance these activities only in PAN3379. On the other hand, the two biotypes were not expected to induce any of the

enzyme activities associated with defence responses to RWA in Bolane. The significantly higher RWASA1-induced POD activity as opposed to GLC activity in PAN3379, probably explains the moderate resistance status of the cultivar.

On the other hand, significantly higher activities of both GLC and POD in RWASA1 infested Elands (Fig. 5.1 and 5.3) may be necessary for the resistance response against the RWA. Conversely, RWASA3 induced significantly higher POD but not GLC activity in Bolane (Fig. 5.3 and 5.4). Contig1639, one of the β-1,3-glucanase sequences, was induced in susceptible lines in the interaction between barley and bird cherry-oat aphid (*Rhopalosiphum padi*) suggesting that it is related to barley susceptibility to *R. padi* (Merhabi, *et al.,* 2016). Nonetheless, there is some evidence that RWA induces GLC activity in resistant lines as a defence response during pathogenesis, and forms part of defence responses like the hypersensitive reaction (Van der Westhuizen, *et al.,* 1998; Saheed, *et al.,* 2009).

These findings may also imply that the induction of both enzymes is required to confer resistance to aphid infestation. A synergistic increase in the level of pest control occurs when two or more PR proteins are co-expressed (Hammond-Kosack and Jones, 1996). These results, therefore, suggest that the coordinated activity of several PR genes complements RWA resistance. Failure of Bolane to express resistance may be associated with the lack of dual expression of the enzyme activities. Our results are in agreement with Botha, *et al.,* (2014), who also found differentially higher POD activity in infested resistant than susceptible wheat. The increased peroxidase activity in the infested wheat crop that is resistant could be involved in a range of related defence reactions, which jointly contribute to RWA resistance (van der Westhuizen, 1998). Furthermore, Mohase and Van der Westhuizen, (2002) also recorded an induction in β-1,3-glucanase activity in elicitor-induced IWF of RWA infested wheat, in both resistant and susceptible plants.

Phenylalanine ammonia-lyase (PAL) is an important enzyme involved in the biosynthesis of phenolic compounds, including SA (Khan, *et al.,* 2015). Salicylic acid enhances the development of systemic acquired resistance, broad-range resistance against pathogenic microorganisms and some aphid species, and is essential for

localized plant hypersensitive response (Smith and Boyko, 2006). We hypothesized that both biotypes (RWASA1 and 3) would induce higher PAL activity and SA content in the resistant PAN3379. Similarly, we expected that RWASA1 would effect elevated SA content and PAL activity in Elands. Our results showed a positive correlation between PAL activity and SA accumulation, in agreement with our hypothesis. The significant increase in PAL activity coinciding with the increase in SA accumulation corresponded with the findings of Chaman *et al.,* (2003) in aphid-infested barley. They found that significant increase in PAL activity, as a function of aphid infestation, also induced significant SA accumulation. Mai *et al.,* (2014) also observed that pea aphid induced increase of SA in pea, which was associated with high activities of PAL and benzoic acid-2-hydroxylase. Berner and van der Westhuizen, (2010), additionally, observed that RWA infestation induced higher PAL activity and accumulation of some phenolic compounds in resistant than susceptible wheat cultivars. They found that the peak levels of these phenols corresponded to peak PAL activity. Similarly, Kaur, *et al.,* (2017) found that wheat infested with an aphid complex (*Sitobion miscanthi, Sitobion avenae, Rhopalosiphum maidis* and *Rhopalosiphum padi*) significantly induced activity of PAL and total phenols. Higher PAL activity and SA accumulation following RWA infestation are probably associated with defence response in wheat against aphid feeding activity.

Lipoxygenase (LOX) is a crucial enzyme in the biosynthesis of JA. Jasmonic acid and its derivatives, like methyl jasmonic acid (MeJA), are produced from linoleic or linolenic acids by consecutive actions of 13-lipoxygenase (13-LOX), allene oxide synthase, allene oxide cyclase, 12-oxophytodienoic acid reductase, and *β*-oxidative enzymes (Yang, *et al.,* 2009). Jasmonic acid induces production of benzoxazinoids, a class of metabolites that provides protection against insect herbivores, pathogens, and competing plants (Wouters, *et al.,* 2016).

Our results show that aphid (RWASA1, RWASA3) infestation induced significant increases in LOX activity in PAN3379 (Fig. 5.5). However, only RWASA3-mediated increase in LOX activity resulted in JA accumulation (Fig. 5.8). We expected that PAN3379 being medium resistant to RWASA1 will lack one or more of the biochemical defence-related components and failure to induce JA accumulation

probably added to the insignificant induction of GLC activity. Infestation by RWASA3 induced both LOX activity and JA accumulation. However, as expected, there was no increase in LOX activity and JA accumulation in RWASA3 infested Elands. Intriguingly, RWASA3 but not RWASA1 stimulated significant LOX activity in Bolane, which nonetheless did not promote JA accumulation. Our results confirm those of Berner and van der Westhuizen (2015) who found that RWA attack selectively increased LOX activity in resistant than susceptible wheat cultivars.

Boyko, *et al.,* (2006) suggested that RWA uses wheat jasmonates and terpenes as cues to upregulate the production of cytochrome P450. These products are crucial for detoxification of growth inhibitors and toxins that are absent or produced at low levels in susceptible plants. The RWASA1-mediated increase in LOX activity with no resultant JA accumulation in PAN3379 may imply that LOX may be involved in the biosynthesis of other defensive products other than JA. This implication may also be real for RWASA3-mediated LOX activity in Bolane. In support of these findings, Dicke and van Poecke (2002) showed that related oxylipins, besides jasmonic acid, also appear to act as defence signalling molecules and examples are dinor-oxo-phytodienoic acid and 12-oxophytodienoic acid.

Biotype 1 and 3 (RWASA1 and 3) induced differential ABA accumulation in PAN3379: RWASA1 activated ABA accumulation in Elands (Fig 5.9). Bolane did not express any significant differences in ABA content following infestation by the two biotypes. Abscisic acid is associated with adverse effects on plant immunity; it antagonises SA, JA and ethylene-mediated biotic stress signalling (Jiang, *et al.,* 2010, Yasuda, *et al.,* 2008, Mauch-Mani and Mauch, 2005). However, in support of our results, there is evidence that ABA plays a role in aphid defence responses. Morkunas, *et al.,* (2011) showed that application of ABA on barley protected the leaves from RWA induced rolling or streaking. Phenotypic studies to determine the resistance status of wheat to RWA usually, are based on induced damage symptoms. Therefore, this suggests that ABA might have a defensive role in aphid infestation on PAN3379 and Elands. Hillwig *et al.,* (2017) found that ABA content significantly increased in the aphid (*Myzus persicae*) infested *Arabidopsis* leaves compared with non-infested leaves.

The accumulation of SA, ABA and JA, in the resistant cultivars PAN3379 and Elands showed both synergistic and antagonistic crosstalk. Crosstalk among individual hormonal pathways allows plants to adjust their inducible defence arsenal to the nature of the attacker faced with and to use their limited resources cost-effectively (Pieterse, *et al.,* 2009). The aphid biotypes (RWASA1 and 3) induced higher SA content in PAN3379, while RWASA1 increased SA accumulation but suppressed JA accumulation in Elands.

These findings are supported by Thaler (2012), who showed that SA induction frequently suppresses JA accumulation and mediated responses, and plants are assumed to prioritize SA over JA induction. Equally, Giordanengo *et al.,* (2010) demonstrated that aphids like *M. persicae* and *S. graminum* inhibited the expression of JA-dependent genes while significantly inducing up-regulation of the SA-dependent pathway. The high levels of ABA in PAN3379, induced by both biotypes, which coincided with increased accumulation of SA, may suggest synergistic crosstalk between ABA and SA. In a related aphid study, Chapman *et al.,* (2018) also observed that soybean aphids induced ABA-related transcript expression when SA-mediated defences accumulated.

5.5 Conclusion

Russian wheat aphid (RWASA1) infestation induced neither defence related enzyme activities nor accumulation of the studied hormones in Bolane. The absence of inducible biochemical defences confirms the phenotypic screening results (chapter 3) that Bolane is susceptible to RWASA1. This biotype (RWASA1) was predominant in Thaba Tseka and Mokhotlong, where Bolane is the most cultivated wheat. On the other hand, RWASA3 infestation on Bolane induced POD, LOX and PAL activities. These inductions, however, did not lead to in any accumulation of JA, SA or ABA. Our findings, therefore, strongly indicate that Bolane is susceptible to RWASA3. Our results also affirm that PAN3379 is resistant to both RWASA1 and 3 while Elands is resistant to RWASA1 and susceptible to RWASA3.

5.6 References

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Chapter Six

General discussion

Mokhotlong and Thaba Tseka districts situated in the mountains region of Lesotho are the major wheat-producing areas in terms of yield and area planted in the country (Bureau of Statistics, 2015). Bolane and Makalaote continue to be the most preferred cultivars by farmers in these districts. Studies conducted in 2015 in Mokhotlong and Thaba Tseka (Masupha, *et al.,* 2018) confirmed earlier reports by Rosenblum, *et al.,* (1999) that farmers prefer Bolane for its unique characteristics, which are tall and broad straws used for roofing and livestock feeding. The grains are relatively white, and the bread-making quality is superior. The study also revealed that farmers do not monitor their crop for diseases and pests until the crop is ready for harvesting. There is no pest and disease management programme, nor the use of resistant cultivars or application of insecticides.

Russian wheat aphid is an economically important pest of barley and wheat in Lesotho and South Africa. Mokhotlong and Thaba Tseka lie close to one of the significant area of South Africa producing wheat, Eastern Free State. Our findings reveal that four South African RWA biotypes (RWASA1 – 4) found in South Africa are also present in Lesotho. Surveys undertaken by Jankielsohn (2011) in South Africa and Lesotho before the discovery of RWASA4 showed that all the three biotypes that existed in South Africa were also present in Lesotho.

Insects do not know political borders; therefore, there is always a high likelihood that new RWA biotypes found in South Africa, especially in the Eastern Free State, also exist in Lesotho. For instance, RWASA4 detected in South Africa in 2011 (Jankielsohn, 2014), was recorded in the mountains and lowlands districts of Lesotho in 2016 and 2017. This distribution suggests that the introduction of RWASA4 occurred almost at the same time in Lesotho and the Eastern Free State in South Africa. It is likely that the newly observed RWASA5, which so far has been recorded in the Eastern Free State and not in other wheat-producing areas in South Africa (Jankielsohn, 2019), is also present

in the areas of Lesotho bordering the Eastern Free State. The occurrence of new and virulent RWA biotypes like RWASA5 will continue to taint the already sinking wheat production in Lesotho.

Host plant resistance is practical and economically viable means of controlling RWA. We investigated the resistance status of Bolane, Makalaote and some commonly grown South African cultivars against the four biotypes (RWASA1 - 4) under greenhouse conditions. The results showed both Lesotho cultivars to be susceptible to all biotypes except for Bolane, which expressed medium resistance to RWASA2 (Chapter 3). Results of field studies (chapter 4) showed these two cultivars as either susceptible or medium susceptible. In years and areas where RWA infestation was low, these cultivars expressed medium susceptibility and susceptibility when the infestation was high. We also observed that Makalaote grew faster, and reproduced grains earlier, and escaped the peak period of RWA populations. Bolane, on the other hand, grows slower and takes longer to reach physiological maturity. This slow growth rate exposes the cultivar to RWA population peaks. Turanli, *et al.,* (2012) also observed that the degree of RWA induced damage on wheat was associated with aphid population levels; on fields that were highly infested damage was more than on those with a minimal infestation. Environmental factors, the scale of wheat production and the resistance status of the host plant all affect RWA seasonal abundance. The RWA populations were higher in the mountains where temperatures were relatively cooler than in the lowlands. In the lowlands, the aphid (Maseru district) populations were so low that in 2017 there was no RWA record, and for the first time, all the cultivars had yields higher than 1 t/ha (Chapter 4; Table 4.10). Jankielsohn, (2017) noted that RWA populations change with fluctuations in environmental conditions but persist in major wheat-producing areas of South Africa. Farmers' decision to plant maize, sorghum and beans and occasionally wheat or barley in the lowlands districts denies RWA food and overwintering sites. Climatic conditions prevailing in some parts of the mountains force farmers to grow wheat as the primary cereal grain. Colder conditions, stable availability of host plants and use of susceptible cultivars by farmers make the mountain districts a suitable habitat for RWA growth and development.

Other pests than the RWA existed throughout the study. However, significant damage was recorded only in 2018 when all the cultivars, including the surrounding fields, were infected with leaf rust (*Puccinia triticina*). PAN3379, which is resistant to the four RWA biotypes (RWASA1 – 4) was severely affected, and for the first time in all areas of the study, its yield was lower than the farmers' cultivars (Bolane and Makalaote). PAN3379 is susceptible to leaf, stem and stripe rusts (PANNAR 2014) while Makalaote and Bolane are medium susceptible (Boshoff, *et al.,* 2002). Cold temperatures, rainfall and altitude in Mokhotlong and Thaba Tseka favour leaf rust epidemics. Altitude ranging from 1800 – 2600 m, humid conditions and temperatures between 15 and 20 °C, support leaf rust epidemics (Teferi, 2015; GRDC, 2016). Geographical and environmental conditions stated above prevail in Mokhotlong and Thaba Tseka, which make these districts areas of high rust incidence. Rains that usually come in February to March coinciding with stem elongation and heading create the humid conditions that are required by the pathogen.

The most practical and economical method of controlling rust is growing resistant cultivars. Although it is tough for farmers to abandon their traditional cultivars and adopt modern RWA and rust-resistant cultivars, use of resistant cultivars is most relevant to them based on their resources and expertise.

Russian wheat aphid biotyping and field studies were accompanied by greenhouse trials that evaluated host resistance mechanisms at the biochemical level. These investigations evaluated responses of only Bolane, Elands and PAN3379 towards RWASA1 and 3. The question was if farmers' cultivars displayed medium susceptibility in the field, could there be some correlating biochemical indicators of the defence responses to RWA in Bolane? RWASA3 induced all the biochemical defence-related responses measured (PR proteins, enzymes and hormones), except JA in PAN3379. PAN3379 is medium resistant to RWASA1 (ARC-SGI, 2016). Probably minor accumulation of JA induced by RWASA1 affects the resistance mechanism of this cultivar, or the sum of other responses compensate for the slight accumulation of JA. Jasmonic acid triggers wound-induced resistance responses, which involve chemical defences such as the production of anti-nutritive, toxic, or repellent compounds, which protect plants against herbivore attack (Howe and Jander 2008). Elands, on the other hand, is resistant to RWASA1 but

susceptible to RWASA3, correspondingly expressed responses against RWASA1 but not RWASA3.

Biotype 1 (RWASA1) infestation of Bolane did not induce any increase in the activity of defence enzymes or content of hormones. This failure to activate defence-related responses then confirms the phenotypic results (chapter 3; Table. 3.4) that Bolane is susceptible to RWASA1. However, results in RWASA3 challenged plants showed an increase in POD, LOX and PAL activity, but these inductions did not result in accumulation of any of the hormones. These were also in line with phenotypic results in chapter 3, which describe Bolane as susceptible to RWASA3. Russian wheat aphid survey in the mountains shows that RWASA1 is the most dominant RWA biotype in Mokhotlong district (chapter 3; Fig 3.4). This presence of RWA in the highlands has a severe impact on wheat yield in Mokhotlong, which is the leading district of Lesotho in wheat production.

Conclusion and recommendations

The majority of farmers and extension staff in the mountain areas of Lesotho where wheat production is typical do not consider RWA as a deadly pest of wheat as shown by the preliminary survey. Awareness of RWA biotypes and the link between its damage and reduction in yield should help in improving wheat production in Lesotho. Transfer of this information to extension staff and farmers is crucial. Farmers in the mountain districts of Lesotho continue to use their recycled cultivars like Bolane and Makalaote, which are susceptible to Russian wheat aphid biotypes (RWASA1 - 4). Because these varieties were introduced in Lesotho more than 50 years ago, convincing farmers to use RWA resistant varieties will require concerted efforts by the government, non-governmental organizations and research institutions. Lesotho researchers must initiate efforts to collaborate with South African research institutes to develop breeding programs that improve Russian wheat aphid resistance in these adapted cultivars.

Peak periods for RWA boom in the mountains of Lesotho were mid-February to March. Makalaote grows fast and reaches physiological maturity before periods of high RWA infestations. Bolane, on the other hand, grows relatively slower and the highest RWA populations in February colonise the beginning of heading stage. Studies on re-

evaluating time of planting to evade periods of high RWA densities should therefore be conducted, especially in the mountains where weather conditions shorten the growing season. We suspect that any new RWA biotypes observed in the Eastern Free State in South Africa may probably be present in Lesotho. Regular monitoring programs on the diversity and distribution of the RWA conducted in South Africa, especially in the Eastern Free State should also include Lesotho, and both counties should share information. The recent discovery of new Russian wheat aphid (Jankielsohn, 2019) in the Eastern Free State in South Africa further emphasizes the need for regular monitoring.

In conclusion, Lesotho has a high potential for improving wheat production in the mountains districts, which act as the wheat-producing hub for the country. The current yields obtained using recycled RWA susceptible cultivars with no fertilizer application, poor seedbed preparation and no disease control demonstrate the prospects of yield increase if these cultivation practices are improved.

The Ministry of Agriculture and Food Security and researchers should devise an integrated action plan. The strategy should include a comprehensive soil analysis review in the major wheat-producing areas of the mountain districts, RWA population dynamics and the impact on wheat production. Furthermore, the plan must introduce alternative varieties with preferred agronomic traits and RWA resistance. Lastly, the plan must facilitate farmers' access to fertilisers, seed, and the market share in the lowlands at the national flour mills.

The Lesotho farmers' varieties, Bolane and Makalaote, are susceptible to South African RWA biotypes (RWASA1- 4), except for Bolane, which is medium resistant to RWASA2. Despite susceptibility to the aphid, these varieties are well-adapted to the mountain districts, and Makalaote fairly competes with South African commercial cultivars in terms of yield. Bolane, on the other hand, has low yield, but its other uses (roofing and livestock feeding) overrule that of yield alone. Breeding programs to improve the resistance status and yield of these varieties are necessary, but such programs should conserve the other good characteristics key to farmers. Breeding programs have always focused on few accessions to improve yield; this has led to a loss

of biodiversity to achieve future agricultural crop demands since genetic variability to cater for climatic, pest and disease adaptation is lost (Jankielsohn and Miles, 2017). Therefore, improvement of Bolane as a multipurpose cultivar through breeding programs should not affect its large straws crucial to the lives of the mountain districts.

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Chapter Seven
Appendix

7.1 Results of independent replicate experiments
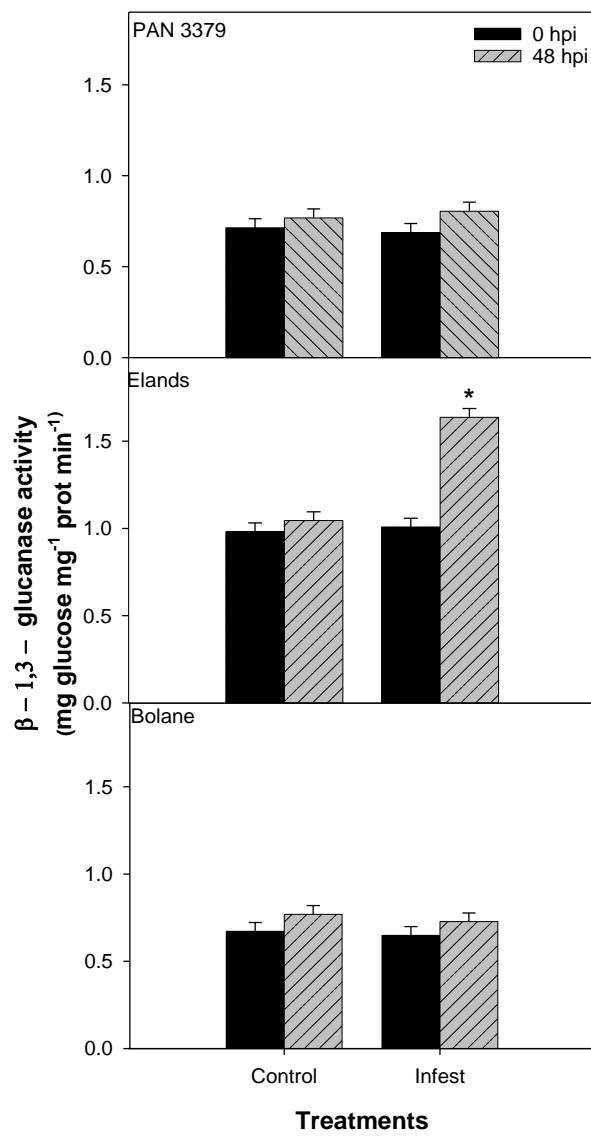
Figure 7.1: Effect of Russian wheat aphid (RWASA1) infestation on β-1,3-glucanase activity in PAN3379, Elands, and Bolane. PAN3379 and Elands are resistant, while Bolane is susceptible to RWASA1. The values are means, and the error bars indicate standard error. (*) indicates significant differences ($p<0.05$), means were separated using the least significant difference (LSD) at 5%.
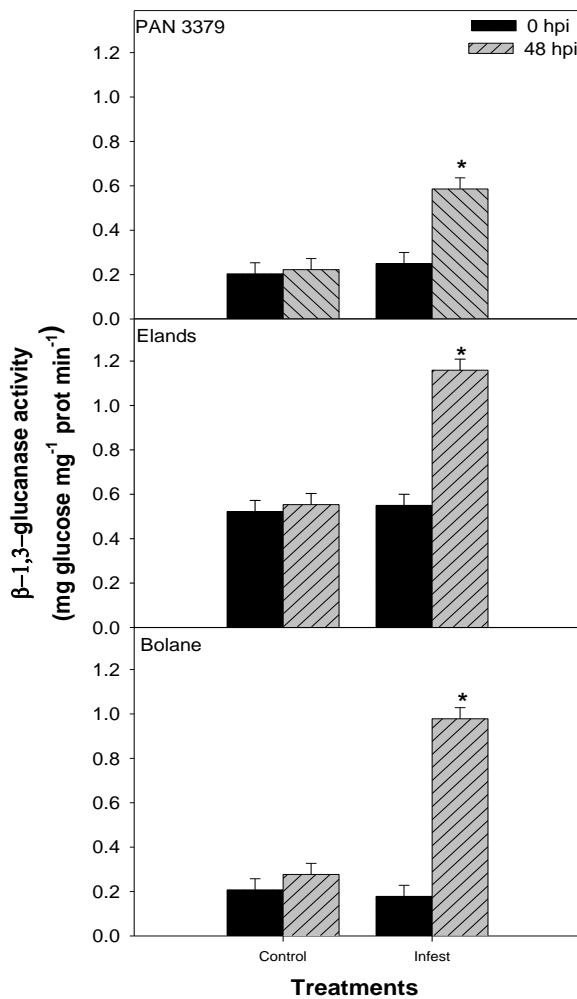
Figure 7.2: Effect of Russian wheat aphid (RWASA3) infestation on β-1,3-glucanase activity in PAN3379, Elands, and Bolane. PAN3379 is resistant while Elands and Bolane are susceptible to RWASA3. The values are means, and the error bars indicate standard error. (*) indicates significant differences ($p<0.05$), means were separated using the least significant difference (LSD) at 5%.
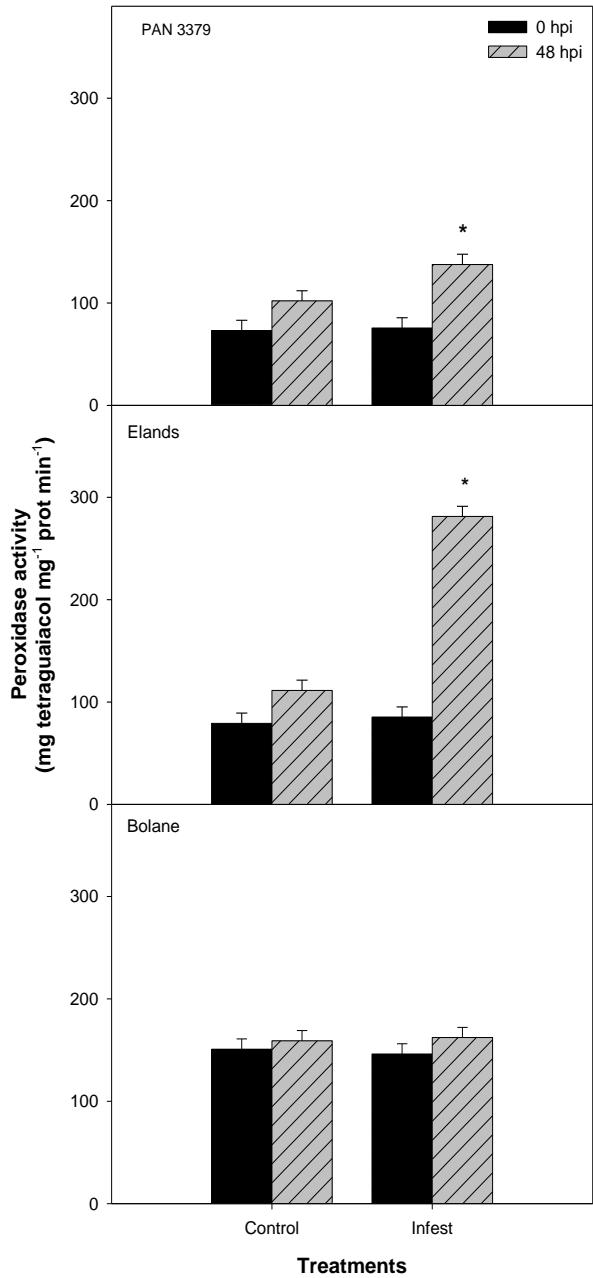
Figure 7.3: Effect of Russian wheat aphid infestation (RWASA1) on peroxidase activity in PAN3379, Elands, and Bolane. PAN3379 and Elands are resistant, while Bolane is susceptible to RWASA1. The values are means, and the error bars indicate standard error. (*) indicates significant differences (p<0.05). Means were separated using the least significant difference (LSD) at 5%.
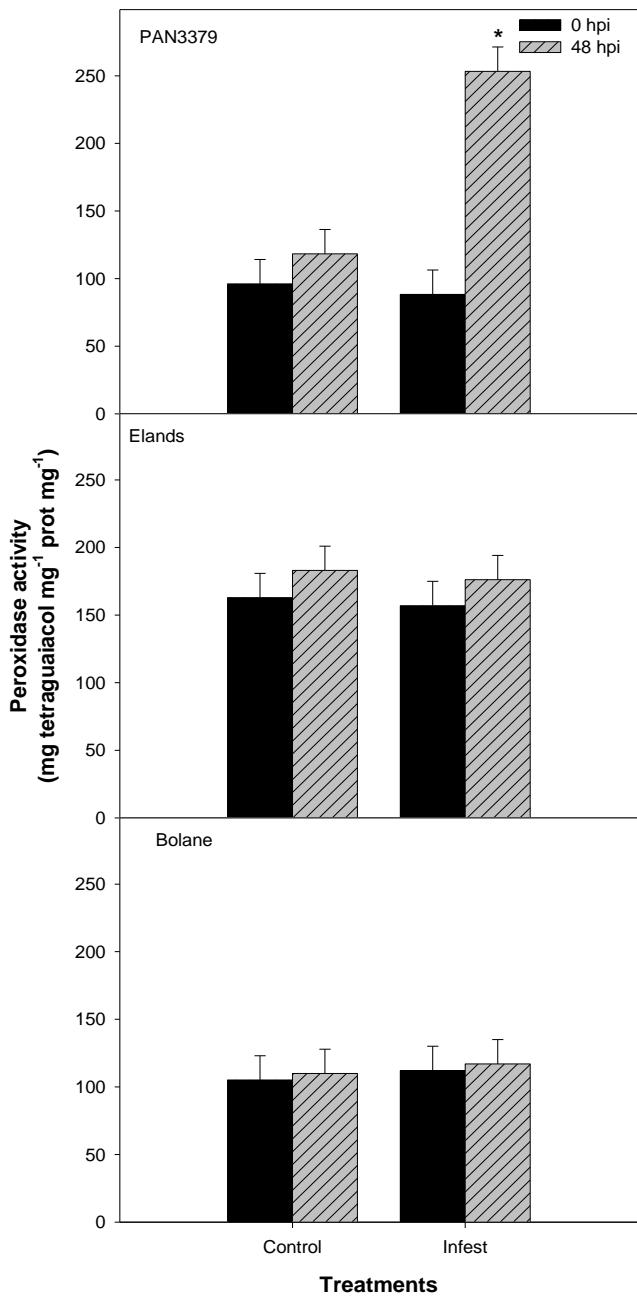
Figure 7.4: Effect of Russian wheat aphid (RWASA3) infestation on peroxidase activity in PAN3379, Elands, and Bolane. PAN3379 is resistant while Elands and Bolane are susceptible to RWASA3. The values are means, and the error bars indicate standard error. (*) indicates significant differences (p<0.05), means were separated using the least significant difference (LSD) at 5%.
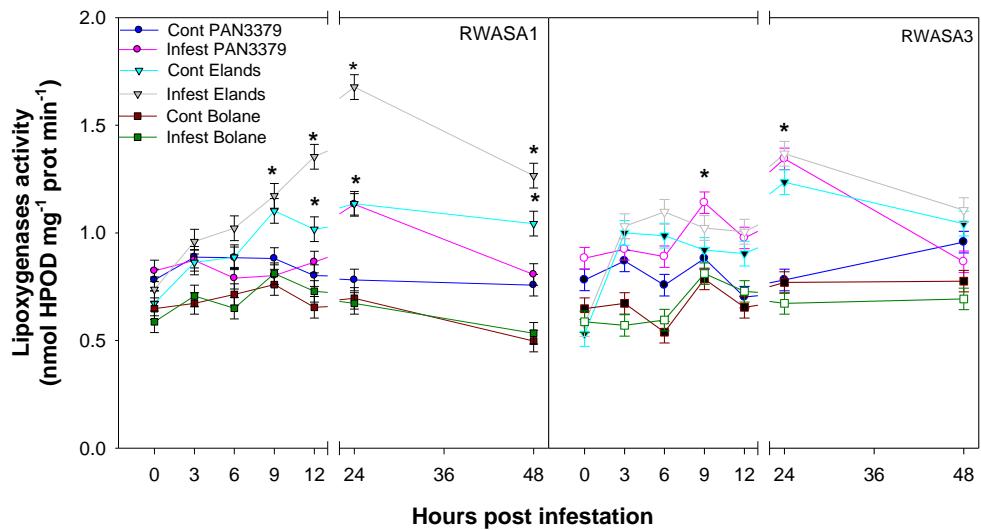
Figure 7.5: Effect of RWASA1 and RWASA3 infestation on lipoxygenases activity in PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences ($p<0.05$), means were separated using the least significant difference (LSD).
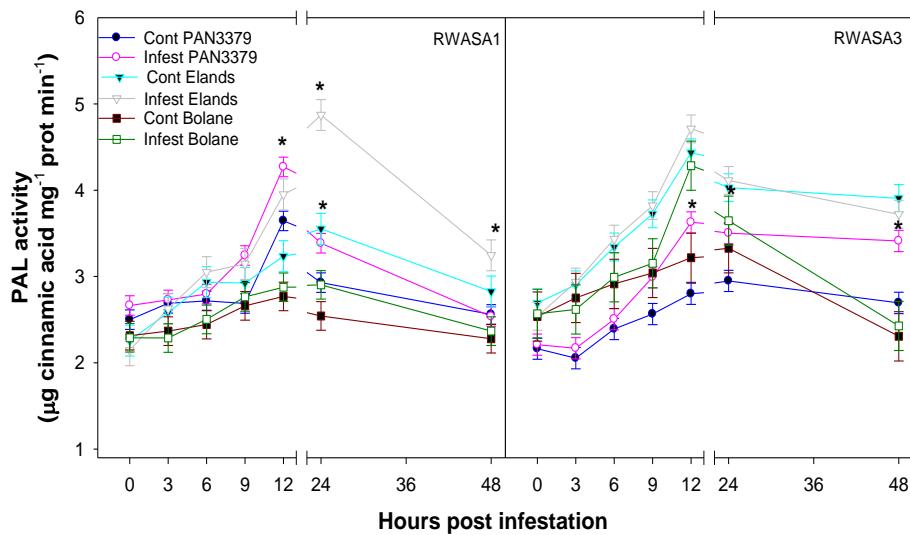
Figure 7.6: Effect of RWASA1 and RWASA3 infestation on phenylalanine ammonia-lyase (PAL) activity in PAN3379, Elands and Bolane. Values are means, and error bars indicate standard error. (*) indicates significant differences (p<0.05), means were separated using the least significant difference (LSD).

7.2 Preliminary survey in Mokhotlong and Thaba Tseka districts significant findings extracted from Masupha, Jankielsohn and Mohase, (2018).
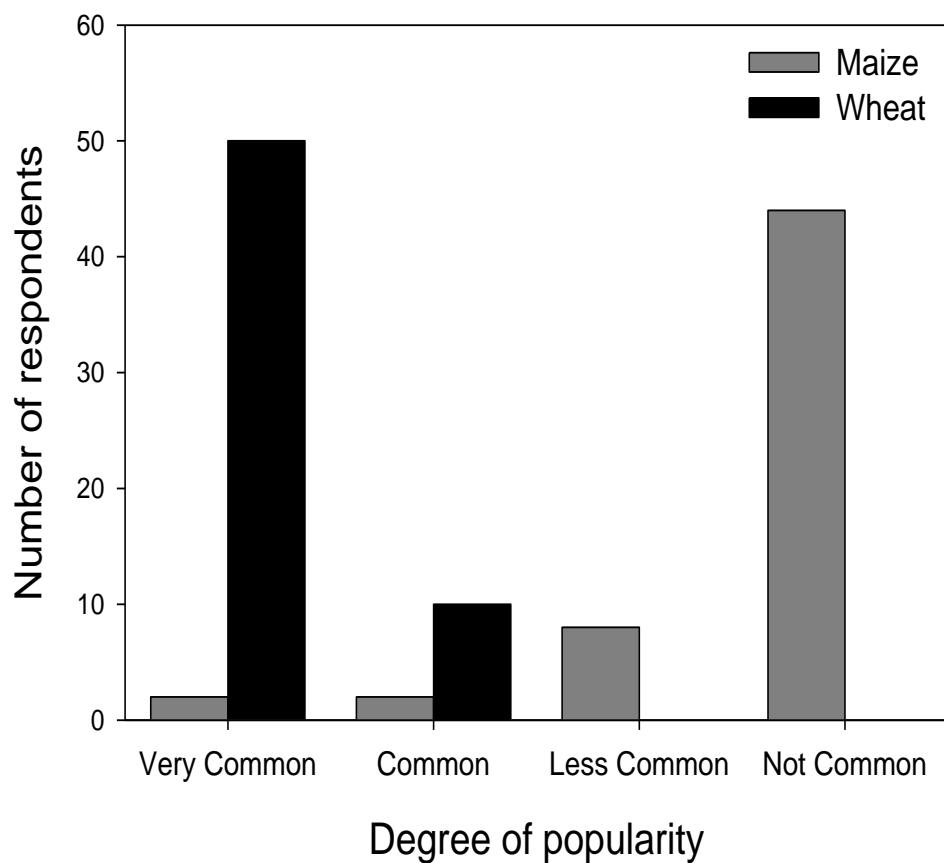


Figure 7.7: Popular crop between maize and wheat
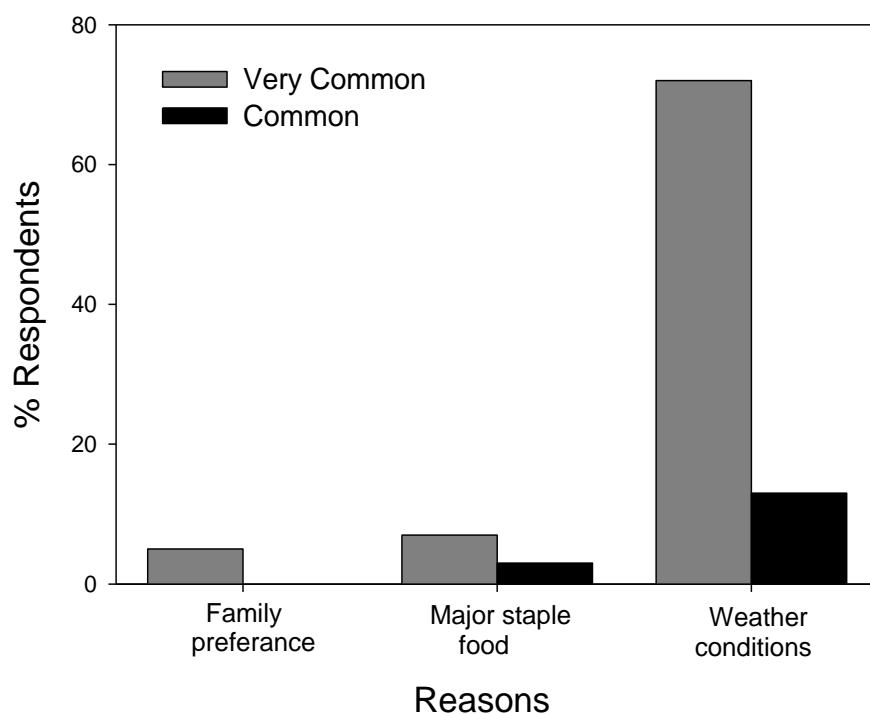
Figure 7.8: Farmers reasons for growing wheat

Table 7.1: Farmers cropping practices

Practices	Responses	Percentage
Land Preparation		
Ox-drawn plough	57	95
Tractor	3	5
Seed Source		
Selected from the previous harvest	31	51.7
Agro shops	2	3.3
Donors	1	1.7
Government	1	1.7
Other farmers	25	41.7
Seeding		
Broadcast by hand	58	96.7
Animal drawn planter	2	3.3
Fertiliser Application		
Yes	1	1.7
No	59	98.3
Cropping Challenges		
Diseases	4	6.7
Insects	1	1.7
Weather	50	83.3
Market	1	1.7
Weeds	1	1.7

Table 7.2: Extension staff response on farming systems

| Practices	Responses	Percentages
Fertiliser use		
Yes	10	32.3
No	13	41.9
Some farmers	8	25.8
Reasons for not fertilising the soil		
Expensive	10	50
Inaccessible	4	20
Destroys the soil	6	30
Common wheat pests/pathogens/weeds		
Smut	21	67.7
Wild oat	10	32.3
Availability of farmers' fields acreage database		
Yes		
No	30	96.8
	1	3.2
Farmers' knowledge on the acreage of their fields		
Yes		
No	14	45.2
Not all	7	22.6
	10	32.3
Reasons for not knowing		
They usually use their traditional ways of measuring their fields.	6	35.3
They use their cattle for operations. Never hire machinery.	11	64.7
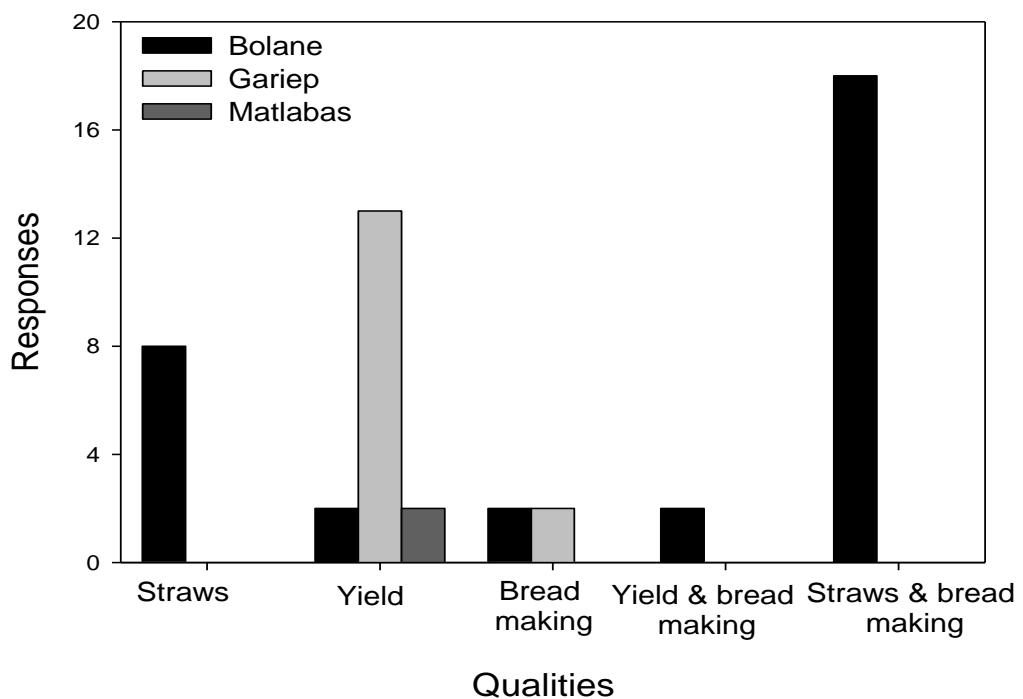
Figure 7.9: Extension staff perception of Common wheat varieties and their preferred characteristics
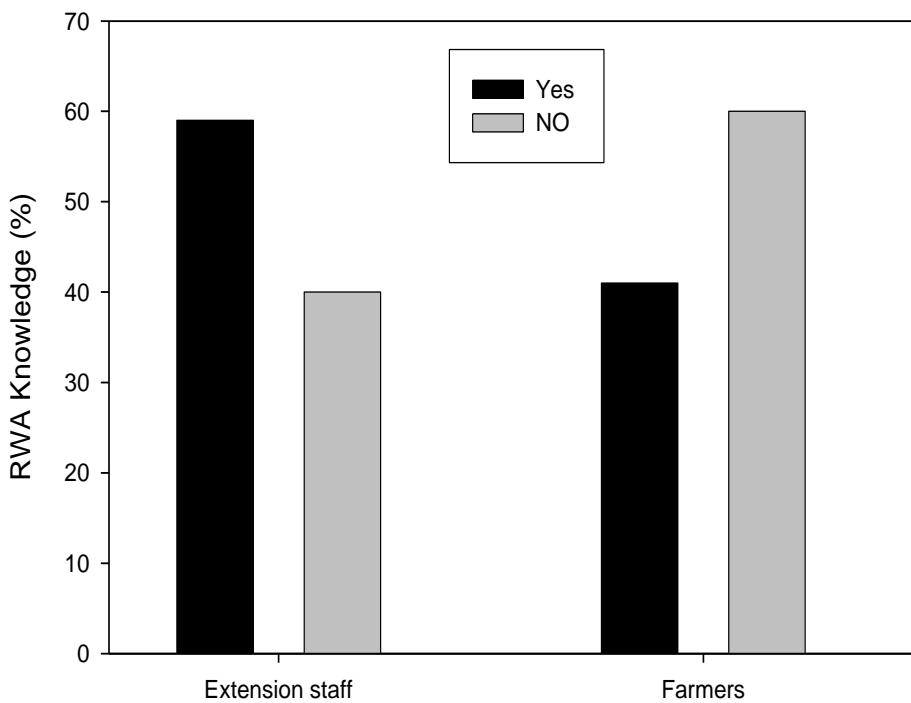
Figure 7.10: Knowledge of Russian wheat aphid by extension staff and farmers

Summary of the significant findings of the preliminary survey

i) Farmers still use traditional farming methods like an ox-drawn plough, planting without fertilisers, broadcasting and recycling the seed.

ii) Bolane, a variety that was introduced in Lesotho in the early 1960s, is preferred over the modern varieties because of its additional benefits like roofing, livestock feed and fuel.

iii) Both the farmers and extension staff do not know Russian wheat aphid despite its well-documented impact and distribution in Lesotho (Moremoholo & Purchase 1998; Makhale, Moremoholo, and Mohammed 1999 and Jankielsohn 2011).

7.3 Farmers' cultivars in Lesotho

Name	Notes
Kenasokies	Kenyasokies named Kenasokis, probably imported from Kenya, and introduced in Lesotho in the 1980s.
Bolane	Tall variety introduced in Lesotho in the 1960s; probably originates from South Africa as Boland. It is preferred for its large straws for roofing and livestock feeding.
Manthoba	Bolane referred to as Manthoba in some parts of Thaba Tseka district.
Tsoloha	Tsoloha which means "spilling out". Farmers gave it this name because of its high yield. Its agronomic characteristics are similar to that of Bolane, but farmers say they are different.
Mantša-tlala	Mantša-tlala, which means "driving out hunger" is Tugela. The name was assigned after farmers appreciated its relatively high yield compared to other farmers' varieties.
Puseletso	Puseletso which means "Regaining that which we used to have" is Tugela *Dn*. Russian wheat aphid introduction in Lesotho affected the yield of Tugela, and the release of Tugela *Dn* with its resistance to the aphid and tolerant to aluminium toxicity came as a relief to farmers who were loosing.
Phallelo	Phallelo, which means "donation" is Gariep. It was given to farmers as a donation by the government.
Mathethebale	Mathethebale refers to a stagnant person. It is a short cultivar, which grows very slowly, as the name implies.
Telu-Ntšo	Telu-Ntšo, which means "black beard". The name comes from its characteristic black awns. However, farmers no longer grow it because it is hard to mill with the grinding stones.
Mohohlotsane	Tall awnless variety introduced in Lesotho in the 1990s.
Malinonyana	Malinonyana, which means "mother of the birds" is an awnless cultivar, which makes it appealing to the birds. Same variety as Mohohlotsane.
Makalaote	Mohohlotsane referred to as Makalaote in some parts of Thaba Tseka.

Summary

Lesotho has over the years been experiencing a decline in wheat yield. The dynamic nature, rapid shifts in the environment, and devastating impacts of Russian wheat aphid (RWA) likely contribute to the low wheat yields in Lesotho. The study aims to investigate RWA biotypic diversity, distribution, impact on yield and the role of phytohormones in Lesotho farmers' cultivars and those imported from South Africa, in the mountains and lowlands districts of Lesotho.

The status of RWA distribution and diversity in Lesotho was evaluated by collecting and analysing samples from wheat fields in parts of Mokhotlong, Thaba Tseka, Maseru and Leribe. Aphid samples were cloned and screened for biotype status at the ARC-SG greenhouses (Bethlehem, South Africa). Our results revealed that four of the five South African biotypes, RWASA1 – 4, also exist in Lesotho. We further investigated the reaction of some of the farmers' wheat cultivars (Bolane and Makalaote) and South African dryland cultivars grown in Lesotho to RWA infestation. This part of the study occurred under greenhouse conditions where different biotypes (RWASA1 – 4) artificially infested the cultivars. A damage rating scale evaluated induced damage, and the Lesotho farmers' cultivars Bolane and Makalaote expressed susceptibility to all four biotypes except Bolane, which conferred medium resistance to RWASA2. Similar cultivar evaluations under natural infestation were carried out under field conditions in the mountains and lowlands of Lesotho. In contrast to the greenhouse results, both Makaote and Bolane expressed medium susceptibility; however, Bolane yielded lower than all other cultivars in almost all sites. Despite the reaction to aphids, Makalaote a relatively fast-grower escaped peak periods of RWA infestation and outperformed most of the South African cultivars.

The underlying resistance mechanism of these wheat genotypes was investigated by determining some of the induced biochemical changes during RWA infestation. In this regard, three cultivars (Bolane, Elands and PAN3379) with differential resistance to RWA (RWASA1 and 3) were evaluated. Biotype 1 infestation did not induce any of the pathogenesis-related enzyme activities (GLC and POD) or defence related enzymes (LOX and PAL) associated with biosynthesis of hormones in Bolane. However, RWASA3 infestation induced all the enzymes except GLC. In agreement with the

phenotypic studies, RWASA1 but not RWASA3, induced all enzyme activities in Elands. The two biotypes induced almost all the enzyme activities in PAN 3379, except GLC, which did not respond to RWASA1 infestation.

The involvement of defence modulating hormones salicylic, jasmonic and absiscic acids during the resistance response was also studied. In contrary to our expectations, RWA-induced PAL and LOX activities in Bolane did not positively correlate with salicylic or jasmonic acid accumulation, and no increases in absiscic content were recorded. According to phenotypic studies, PAN 3379 confers medium resistance to RWASA1 and resistance to RWASA3, and we anticipated some shortfall in the accumulation of one or two hormones. Levels of RWASA1-induced LOX activity did not elicit jasmonic acid accumulation, but PAL activity led to higher salicylic and absiscic acids content. Biotype 3 activated accumulation of all the three hormones.

Phenotypic studies show Bolane as susceptible to RWASA3, however induction of some defence enzymes in Bolane mandates additional studies that could elucidate defences determining resistance in wheat. Additionally, the biochemical studies could be complemented by full metabolite profiling during plant-aphid interaction. Perhaps some metabolite combinations could assist in elucidating the resistance mechanisms to aphid infestations and provide a clue to differential responses to the different biotypes.

Keywords: Biotypes, Russian wheat aphid, β-1,3-Glucanase, Peroxidase, Lipoxygenases, Phenylalanine ammonia-lyase, Salicylic acid, Jasmonic acid and Absiscic acid.