THE INFLUENCE OF ENVIRONMENT ON THE EXPRESSION OF RUSSIAN WHEAT APHID; *Diuraphis noxia* (KURDJUMOV) RESISTANCE

BY

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DECLARATION

I hereby declare that the submitted thesis for the degree *Magister Scientiae Agriculturae* at the University of the Free State, handed in by myself, is my own work and has not previously been submitted for attaining a degree at another university/faculty. I hereby relinquish my author's rights in favour of the University of the Free State.

5th May 2008

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“To study nature was, to me, to ramble through her domains late and early. If I observed all as I should, I knew that the memory of what I saw would be of service to me”.

(John James Audubon, Wildlife Artist and Naturalist, 1795-1851)

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CHAPTER 1

General introduction

The FAO (Food and Agriculture Organization of the United Nations) reports that Africa will have to import an additional 36 367 million tons of cereals (wheat, coarse grains and rice) in 2007 despite the continent's forecasted production of 117.9 million tons for this period. The bill for covering the imports of the 44 LIDC's (Low-Income Food-Deficit Country) in Africa is estimated to cost approximately 14 640 million US$ (GIEWS, 2007). Wheat imports are mainly the result of insufficient production resources, poor rural infrastructure and high marketing costs. Climate change through increasing aridity in North- and Southern Africa is furthermore expected to halve food production in Africa by 2020 (IRIN, 2007) and may eventually result in the discontinuation of wheat production on the continent by 2080. WASDE (World Agricultural Supply and Demand Estimates) of the United States Department of Agriculture expects 2007/08 world stocks of wheat to be the lowest in 30 years at 112.4 million tons, stimulating a worldwide climb of wheat prices that will eventually also affect trading of the commodity (WASDE-450, 2007). Sustainable production of wheat capable of reducing and eventually replacing imports into sub-Saharan Africa is biologically achievable and makes economic sense. The major wheat producing country in Southern Africa is the Republic of South Africa where production is concentrated on large, highly mechanized farms. Fluctuating weather patterns, however, result in varying wheat harvest volumes and in 1991 for example, roughly 2.1 million tons were produced compared to only 1.3 million tons in 1992. South Africa is also a net importer of wheat as approximately 1.5 million tons is produced against the domestic demand of about 3.1 million tons per annum. Imports for 2008 will again be necessary as the national wheat yield for 2007/08 is estimated at 1.77 million tons (fifth forecast) compared to 2.11 million tons in 2006/07 (Crop Estimates Committee, 2008). Wheat production in South Africa can, however, expect an upward trend during 2008/2009 as low world wheat stocks will stimulate the on-farm price of wheat. The price per ton for wheat imports to South Africa docked in Durban and transported to Randfontein during middle-December 2007 is currently estimated at ZAR 3648.16 for US Hard Red wheat, ZAR 2978.06 for Argentinean Trigo Pan and ZAR 4203.61 for Canadian Western Red Spring wheat (SAGIS, December 2007).

Russian wheat aphid (RWA), *Diuraphis noxia,* feeding damage can result in severe yield losses in wheat and reductions of between 35-60% have been recorded in

South Africa (Robinson, 1992). RWA is a phloem feeding species that is commonly found on adaxial surfaces, in the axils of young growth, or within rolled-up leaves (Girma *et al.*, 1992) causing typically white, yellow and purple to reddish-purple longitudinal streaks on leaves. Tillers of seedlings subjected to heavy infestations become prostrate, whereas rolled-up flag leaves at later growth-stages trap the emerging wheat spikes (Walters *et al.*, 1980). Although RWA shows preference for stressed host plants, the percentage infestation, growth stage of the host plant and duration of the infestation ultimately determines severity of feeding damage. Wheat plants at growth stages between flag leaf and flower initiation are most vulnerable to *D. noxia* damage (Du Toit and Walters, 1984). A biotype, for the purpose of this study, is a strain of Russian wheat aphid, *D. noxia* different from the original strain in its ability to damage resistant wheat plants (Smith, 1994). Differences between biotypes can also include different requirements in regard to biology, adaptation and environment (Ogecha *et al.*, 1992; Webster *et al.*, 1992). The definition proposed by the WERA-066 Aphid Ecology and Insect-Plant Interaction Subcommittee is: "A population (independent of geographic location) that is able to injure a cultivated plant containing a specific gene(s) that was previously resistant to known aphid populations" (SAES-422, 2006). In this definition of *D. noxia* biotypes there is no presumption of the genetic basis within an aphid, nor evolutionary or taxonomic status implied except providing a convenient way of describing an array of resistant- and susceptible plant responses. In June 2003 a new resistance-breaking biotype of *D. noxia* designated as biotype B was reported in Colorado and proved to be more damaging than the previous "biotype A" (Peairs *et al.*, 2003; Haley *et al.*, 2004). Virulence reports on all wheat cultivars containing the resistance gene *Dn-4* (Smith *et al.*, 2004) and *Dn-y* based resistance sources (Jyoti *et al.*, 2006) confirm the existence of the new biotype. Discovery of *D. noxia* biotypes virulent to *Dn-4* and *Dn-y* resistance sources were also reported for RWA populations from Chile, the Czech Republic, and Ethiopia (Smith *et al.*, 2004). In Argentina, Almaraz *et al.* (2003) reported failure of resistance in genotypes containing *Dn-2* and *Dn-4* to RWA populations collected in the region. In South Africa, host plant resistance of 80% of winter and intermediate dryland wheat varieties released up to 1998 (Tolmay *et al.*, 2005) and 82% of varities released up to 2005 (ARC–SGI, 2005) consisted mainly of the dominant single genes, *Dn-1* or *Dn-2* from donor accessions PI 137739 and PI 262660. Alternative donors such as PI 294994 containing *Dn-5, Dn-8 and Dn-9* (Liu *et al.*, 2001) were introduced into a number of varieties but the possibility for development of a new biotype of *D. noxia* always existed. Tolmay *et al.* (2006) reported that wheat farmers in the eastern Free State of South Africa observed population build-ups of *D. noxia* and consequent damage on resistant wheat cultivars during the 2005 season. Confirmation of virulence of RWASA2 on seedlings of most commercial wheat varieties (Tolmay *et al.*, 2007) has urgently prompted breeders to locate alternative genetic sources resistant to RWASA2 as well as likely future biotypes. Collapse of resistant crop varieties due to biotype development is a major threat to food security and an even greater catastrophe would be caused by the unavailability of advanced breeding lines containing genetic variability potentially resistant to future biotypes. An ARC–SGI initiative implemented in 2006 based on international shuttling breeding anticipates improved effectiveness by identifying and selecting genotypes with wide resistance against RWA for use in the ARC-SGI pre-breeding programme. A set of ARC–SGI wheat genotypes screened at USDA, Stillwater; Oklahoma in 2005 against US biotypes RWA1 (A) and RWA2 (B) identified SA breeding lines exhibiting host plant resistance to both South African and US biotypes (Dr V. Tolmay, ARC–Small Grain Institute, Bethlehem, personal communication, 2005). The current objective in RWA resistance breeding is to develop a strategy employing pre-emptive measures for host plant resistance against RWA in South Africa.

Climate change is a reality. On average, Africa's climate is 0.5°C warmer than a century ago although some regions (eg. Kenya) have experienced temperature increases of more than 3.5°C. Arid or semi-arid areas across the expanse of Africa are becoming drier, while equatorial Africa and regions in southern Africa are getting wetter. As insects are poikilothermic, their development is strongly influenced by external temperatures. Higher temperatures increase metabolic rates and decrease population doubling times (Gullan and Cranston, 2005) and will affect insect pest abundance by shortening generation time, unsettling predator/prey relationships (Lawton, 1995), and shifting pest distribution (Porter, 1995). In temperate agricultural areas aphids are regarded as among the most important pests (Minks and Harrewijn, 1987) and increases in temperature of 0.4°C to 1.0°C have already advanced spring migrant flights in the United Kingdom by 6 to 14 days (Fleming and Tatchell, 1995; Harrington *et al.*, 1995). Although effects of climate change are currently speculative with little evidence for validation, general scenarios are projected through use of various climatic models. Adaptation is seen as a major option for countering effects of climate change on agricultural production. Introducing traits such as increased heat-, drought- and pest resistance and the ability of plants to utilize higher CO_2 levels will become future selection criteria in breeding programmes. Germplasm containing required traits are already available in international gene banks and need to be explored for use in breeding programmes. Climatic patterns in the wheat-producing areas of South Africa can become increasingly unstable in the near future resulting in genotype x environment interaction that will also affect expression of host plant resistance to RWA. The first objective was to address the effects of changing climate on expression of host plant resistance by investigating the effects of vernalization and temperature on host-plant resistance of four wheat varieties infested with RWASA2. The information obtained will be used to accordingly adjust the current RWA mass screening protocol but will also determine long-term approaches for the selection of host plant resistance to RWASA2. Higher temperatures may directly impact on the frequency of biotype development and also increase RWA damage due to higher populations and increased metabolic rates. A strategy supporting the broadening of genetic diversity of resistance to RWASA2 will be obtained by developing advanced breeding lines with stable expression of host plant resistance against RWASA2 over various environments in South Africa. In this context the second objective was to attempt to establish an effective pre-breeding strategy for developing resistance to RWASA2 by firstly identifying commercial wheat varieties with stable expression of RWA resistance over environments and secondly, identifying donor accessions with resistance to RWASA2.

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CHAPTER 2

Literature review

2.1. Bread wheat (*Triticum aestivum* L.)

The world population currently obtains more than 50% of its vital calories from only four crops: rice, maize, wheat and potato (Webb, 2000). Due to wheat being the most widely produced cereal crop in the world, global world trade of this commodity is currently greater than for all other crops combined (Curtis, 2002). Cultivated wheat originated in the Fertile Crescent of the Middle East (Pagesse, 2000) and is divided by Feldman (2000) into three main groups: diploids [2n=2x=14] (einkorn), tetraploids [2n=4x=28] (emmer, durum, rivet, Polish and Persian wheat), and hexaploids [2n=6x=42] (spelt, bread, club and Indian shot wheat). Popular opinion suggests that bread wheat, *Triticum aestivum L.,* originated in the northern areas located between modernday Iran and Turkey, as the product of hybridization between tetraploid wheat and diploid *Aegilops tauschii*.

Average global wheat production between 1995 and 1999 was 584 million tons per annum but is expected to increase to 860 million tons per annum by 2030 (Maratheè and Gomez-MacPherson, 2000). The worldwide consumption of wheat per person averages 73 kg per year compared to the mean per capita consumption of 76 kg per year in South Africa (Payne *et al.*, 2000). The annual consumption of wheat in South Africa is approximately 2.8 million tons per annum and with a national production average of 1.7–2.7 million tons per annum (NDA, 2000), depending on the season, South Africa is a net importer of wheat. Fluctuating annual rainfall, and wheat prices determined primarily in a free market environment strongly influenced by the Rand/USD exchange rate, renders profit margins very slim for most producers. In South Africa, wheat is cultivated in three distinctly different environments. The mediterranean climate, winter rainfall region in the Western Cape contributes 30% of the annual yield; 20% of the annual yield is produced in the high-yielding central irrigated areas including the Northern Cape and the remaining 50% consist of dryland winter and intermediate or facultative wheat, grown in the summer rainfall region on conserved soil moisture which accumulates during the previous late summer and autumn. This production system is characterized by low seeding rates of 15-30 kg per ha using cultivars with long coleoptiles (>6cm) that produce a large number of tillers. Wheat is planted from May to the beginning of August and harvested from late

November to January, depending on the season. Typical abiotic stress factors encountered by wheat producers can include aluminium toxicity due to acid soils and pre-harvest sprouting after wet spells during wheat ripening. Biotic stresses include plant diseases, such as stripe rust (*Puccinia* Westend *f. sp. striiformis* Eriks.), leaf rust (*Puccinia triticina* Eriks.), take-all (*Gaeumannomyces graminis* var. *tritici*), glume blotch (*Septoria nodorum* Berk.) and crown rot (*Fusarium* spp) and a number of insect pests. The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) is the most economically important insect pest on wheat in South Africa's wheat producing areas.

2.2 Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae)

The region between the Caucasus Mountains and the Tien Shan or "Mountains of Heaven" that divides China's Xinjiang Province from Kyrgyzstan and Kazakstan in Central Asia, is considered to be the native range of the Russian aphid (Gonzàlez *et al.*, 1990). The Kyrgyz territory has an average altitude of 2750 metres above sea level and a continental climate with maximum summer temperatures ranging between 26–30ºC and a minimum of -5ºC in the lowlands during winter. The aphid was first described, and originally identified as the barley aphid, *Brachycolus korotnewi*; by Mordvilko (1901). He identified and described various differences between collected samples of the barley aphid, and thereby convinced Kurdjumov to acknowledge that the aphid was in fact an independent species (Kurdjumov, 1913). Kurdjumov consequently renamed the aphid to *Brachycolus noxius,* Mordvilko, and A.K. Mordvilko granted permission that the name *B. noxius* be used in a new identification key published by Kurdjumov (1913). With the new identification key, *B. noxius* was morphologically separated from other aphids infesting small grains. According to literature several synonyms and taxonomic changes occurred since the original identification (Eastop and Hille Ris Lambers, 1976; Durr 1983). The current authorship of *D. noxia,* Kurdjumov was however determined by Kovalev *et al.* (1991), according to the provisions of the International Code of Zoological Nomenclature.

> *"… At first clouds of alatae (winged forms) as dense as smoke flew over the ground, then they went higher and higher forming real clouds which could be so large that in the steppe they were able to mask the disc of the setting sun, as if they were coated with smoke. If a traveler happened to encounter such a cloud, he could, for 10 or 20 minutes, walk in the steppe in the flow of live creatures; tiny insects covered his coat, eyes, ears and nose, making it difficult to breath…" (S. A. Mokrzhetski, quoted in Grossheim 1914, translated by Poprawski et al., 1992).*

This early description of *D. noxia* in flight during sunset probably refers to the major outbreak of the pest in Moldova and the southern Ukraine between 1912 and 1913. Mokrzhetski (1914) estimated that in the 1912 outbreak, *D. noxia* reduced harvests in the affected areas by 75%. Recent damage to wheat crops in the former USSR was restricted to the steppe zone of the Ukraine and Russian Soviet Federated Socialist Republic (Voronin *et al.*, 1988). On the African continent, the Russian aphid initially was reported in Ethiopia but progressively spread to other parts of the continent. *D. noxia* was first reported in the Wukro (Atsbi) and Adigrat regions of Ethiopia during the 1972/1973 season and then in the western Welo region in 1974 (Haile, 1981). By 1976 *D. noxia* was occurring on a widespread scale throughout Ethiopia resulting in barley grain yield losses of 41-71% (Miller and Adugna, 1998). *D. noxia* was identified on dryland wheat in South Africa in 1978 (Walters, 1984) and caused severe damage to wheat fields between Bethlehem and Senekal in the eastern region of the Free State province. By September 1979 *D. noxia* had spread over the greater part of the Free State and nation of Lesotho and isolated infestations were already found in the former Transvaal and Natal. Insecticides registered for control of the existing aphid species of the time were found to be ineffective and extensive losses were experienced by wheat producers. The next outbreak of *D. noxia* on wheat and barley in Africa was reported from Egypt in the Beni-Suef Province in 1985 (Attia and El-Kady, 1988), gradually spreading to all the other small grain producing areas in Egypt. In 1995 *D. noxia* was also reported from Kenya where farmers experienced yield losses of between 25-90% (Kiplagat, 2005).

Diuraphis noxia feeding damage can inflict severe yield losses and in wheat, losses of 68% in Ethiopia and between 35-60% in South Africa have been recorded (Robinson, 1992a). *Diuraphis noxia* is a phloem feeding species (Girma *et al.*, 1992) and typical white, yellow and purple to reddish-purple longitudinal streaks appear on the leaves of infested plants. Aphid feeding increases free amino acids in phloem contents (Telang *et al.*, 1999) and inhibits synthesis and accumulation of proteins essential for normal plant metabolism (Porter and Webster, 2000). Aphids are found on adaxial surfaces or in the axils of young growth, or within rolled-up leaves. During early growth stages tillers of seedlings subjected to heavy infestations become prostrate, whereas rolled-up flag leaves trap the wheat spikes when the growth stage has become more advanced (Walters *et al*., 1980). Different opinions exist about the origins of damage caused by the Russian aphid. A widely acknowledged fact is that *D. noxia* shows preference for stressed host plants but factors such as the percentage infestation, the growth stage of the host plant and duration of the infestation ultimately influence the severity of feeding damage. Wheat plants at growth stages between flag leaf and flower initiation, are most vulnerable to *D. noxia* damage (Du Toit and Walters, 1984).

2.3 Reducing the impacts of Russian aphid, *Diuraphis noxia* (Kurdjumov) on wheat

2.3.1 Chemical control

Rolled leaves are a common damage symptom indicating host plant susceptibility. A characteristic behaviour of *D. noxia* is to feed and develop inside the rolled leaf whorl confining insecticide options to active ingredients with systemic action able to penetrate the rolled leaf. Systemic insecticides containing disulfuton, dimethoate and demeton-S-methyl; or vapour-action insecticides with chlorpyriphos or parathion have proven to be effective against RWA. A more recent addition to the array of chemical control methods against *D. noxia*, although expensive, is pre-plant treatment of seed with imidacloprid or thiamethoxam (Nel *et al*., 2002). As current research is substantiating the existence of a new biotype, identified in South Africa in 2005, a major collapse of host plant resistance in commercial wheat fields will justify chemical control measures in coming seasons until effective host plant resistance has been identified. In contrast to genetic resistance, indiscriminate applications of Aphidicides without consideration of the economic threshold levels for wheat in a specific field will undoubtely overshadow the benefits thereof.

2.3.2 Non-chemical control

Host plant resistance in wheat has been introduced and applied successfully as a control measure against *D. noxia* in South Africa (Van Niekerk, 2001; Tolmay and Van Deventer, 2005). This alternative control method has proven to be more cost effective and has contributed largely to the decline in usage of insecticides over the past ten years (Marasas, 1999). Natural enemies and host plant resistance are considered as more desirable alternatives to insecticides because of their low cost and environmentally friendly action (Burton *et al*., 1991; Quisenberry and Schotzko, 1994). Wiseman (1999) reports that host plant resistance can generally be regarded as an effective, environmentally responsible, economically and socially acceptable method of pest control, playing an integral part in sustainable agricultural systems. The most important attribute of host plant resistance is that pest control occurs independently of the managerial ability, skill and resource level of the producer (Tolmay, 2001). Consideration of all different aspects of host plant resistance will assist greatly in the identification and incorporation of the most effective type of resistance in a sustainable agricultural production system. Pest adaptation to resistant cultivars can, however, result in the loss of the particular resistance. A typical solution to the problem is the continual development and sequential release of crop varieties with new genes that confer resistance to the adapted pest. Sequential release is effective in the short term but costly and not always sustainable in the long term, as the number of suitable resistance donor accessions eventually becomes depleted. Waller *et al*. (1983) defines durability or effective lifetime of pest-resistant crop varieties as a major challenge in resistance breeding and recommends that plant breeders develop new innovative methods to extend the duration thereof.

2.4 Implementation of host plant resistance against RWA in bread wheat

2.4.1 Nature and definition of host plant resistance

The origin and functioning of host plant resistance within the plant has been researched extensively (Agrawal *et al*., 2000). Gatehouse (2002) distinguished between constitutive resistance, consisting of morphological- and chemical factors present in a plant prior to attack and induced resistance, defined as the active response of the plant to attack. Morris and Dwyer (1997) have, however, discovered that constitutive resistance affects the rate of herbivore invasion by having a major influence on spatial dynamics of herbivore populations. In the case of the birth-, growth- and survival rate, both constitutive and induced resistances are capable of influencing demographically important rates.

Kennedy and Barbour (2001) stated that context determines the definition of resistance and must discern between non-host immunity and host resistance. Non-host immunity refers to the number of qualities possessed by a plant species that places it outside the host range of potential insect pests species to which it may be exposed.

- Non-host immunity could result from either the presence of genes conditioning plant qualities that actively interfere with the ability of a herbivore to recognize or utilize the non-host. This type of non-host immunity can be available for transfer to other plant species.

- Non-host immunity can also result from the absence of genes conditioning qualities necessary for a herbivore to recognize or utilize the plant. With the latter, non-host immunity cannot be transferred genetically to other plants.

Host resistance, in contrast, refers to genetically controlled qualities possessed by some individuals, clones, populations, races, or varieties of a plant species that result in less damage by a particular herbivore species than other individuals, clones, populations, races, or varieties of the same plant species within the host range of the herbivore (Eigenbrode, 2002). In the agricultural context, Painter (1951) established his classical definition for resistance as: "the relative amount of heritable qualities possessed by the plant that influences the ultimate degree of damage done by the insect" and explained host plant resistance by using three functional mechanisms namely antixenosis, antibiosis and tolerance.

Antixenosis or non-preference

Refers to plant characteristics that lead insects away from a particular host and in a crop variety may have either an allelochemical or morphological basis.

Antibiosis

Antibiosis refers to all adverse effects on the insect life history that result when a resistant host plant variety or species is used for food.

Tolerance

Includes all plant responses resulting in the ability of a plant to withstand insect infestation and yield satisfactorily in spite of injury levels that would significantly injure susceptible plants. Unlike antixenosis and antibiosis, only plant response is involved in tolerance.

2.4.2 Genetics of host plant resistance

Flor (1956) established the hypothesis of a gene-for-gene relationship in which a gene for resistance in the host is matched by a gene for virulence in the pathogen.

This concept was predominant until researchers were able to differentiate between vertical- and horizontal resistance (Table 2.1). In vertical resistance a single- or major gene controls the inheritance in the progeny. This type of resistance is usually manifested dramatically and plants either exhibit total resistance or no resistance at all. Horizontal resistance is controlled by multiple genes, all having a cumulative effect. The numerous advantages and disadvantages associated with each are tabled below.

Table 2.1 Advantages and disadvantages of vertical- and horizontal resistance in agricultural plants (Robinson, 1997)

Vertical Resistance		Horizontal Resistance	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Provides complete protection	Temporary by nature as it breaks down to new strains or biotypes	Displays wide resistance to many or all biotypes	It is difficult to measure and work with
Compatibility with breeding for wide climatic adaptation	Horizontal resistance is lost in the process of breeding for vertical resistance	Creates less selective pressure in the parasite	
In plant diseases can only control allo-infections	Single genes for host plant resistance against pests are not found readily for all crops	Reduces allo-infection and auto-infections	

2.4.3 The origin and genetic sources for host plant resistance to Russian wheat aphid

Russian aphid resistance in wheat (*Triticum monococcum*, *Triticum timopheevi*, *Triticum dicoccoides* and *Aegilops squarossa*) was initially reported in South Africa in the early 1980's (Butts and Pakendorf, 1984; Du Toit and Van Niekerk, 1985). Resistance was identified in *Triticum aestivum* lines from the former Soviet Union (PI 262660) and Iran (PI 137739) (Du Toit, 1987) and Bulgaria (PI 294994)(Du Toit, 1988). Souza (1998) listed 98 accessions of *T. aestivum* and related species expressing resistance against *D. noxia*. Host plant resistance has, however, not been confined to wheat; resistance in *Secale cereale*, X *Tritosecale* crosses, *Hordeum* *vulgare*, *Hordeum bulbosum*, *Hordeum bogdani* and *Hordeum brevisubulatum* has also been documented. *Diuraphis noxia* resistance identified in *T. aestivum* is, however, the most widely utilised source of resistance currently being used in breeding programmes. Souza (1998) found that accessions collected from the ancestral *D. noxia* boundaries of Central Asia had the highest frequency of resistance. From the total number of resistant accessions that was discovered, 38% originated from Iran and 35% from the adjacent countries of Afghanistan and the former Soviet Union. Mexico was a secondary center for diversity for resistance with 13% of the resistant material originating from there, primarily resulting from improved triticale carrying resistance genes from the *Secale cereale* genome and the moderate levels found in Mexican barley breeding lines. Relatively few new sources were added to the existing resistant germplasm list in recent years, with the exception of the above-mentioned triticale and barley developed at CIMMYT. The majority of resistance sources were identified in tertiary germplasm of landraces and old local cultivars (62% for wheat and 70% for barley).

The primary objective of any breeding programme involved in the development of host plant resistance is to release cultivars that are resistant to an insect pest while still maintaining or improving basic agronomic characteristics when compared to the susceptible equivalent.

2.5 Breeding for host plant resistance to RWA

2.5.1 Identifying genetic resistance to RWA

Souza (1998) recommended four areas that should be constantly reviewed in order to optimise a breeding programme involved with host plant resistance breeding for *D. noxia*. The areas are 1) Identification of host plant resistance, 2) Characterisation of host plant resistance*,* 3) Resulting utilisation of resistant genotypes and 4) Potential for *D. noxia* biotype development. Butts and Pakendorf (1984) implemented mass screening of genotype seedlings for vertical resistance in the greenhouse with a set number of aphids reared on seedlings from susceptible cultivars. Although they also screened germplasm for horizontal resistance (HR), their attempts proved unsuccessful due to low detection levels and heritability associated with HR. Initial crosses between resistance donors and adapted South African bread wheat cultivars were made in 1986, first field evaluations of the backcross progeny carried out in 1989 (Du Toit, 1993) and the first *D. noxia* resistant cultivar in South Africa, Tugela-Dn, released in 1992 (Van Niekerk, 2001). Numerous efforts by researchers all contributed toward the development of a general protocol in order to obtain optimal results when screening for host plant resistance. The general protocol for mass screening of seedlings can be adapted in several ways to meet specific requirements as long as reliability of the test is not reduced. A general screening process for the identification of resistance against *D. noxia* will include the following steps:

> <u>Step 1. Precondition aphids before infestation of test plants.</u> Preconditioning refers to the uniform rearing of aphids on a susceptible host (Figure 2.1) of *D. noxia* and is essential for uniform host plant resistance evaluations (Schotzko and Smith, 1991). *Diuraphis noxia* virulence is dependent on the specific type of nurse plant used for rearing cultures (Worral and Scott, 1991) as well as the physical condition of the aphids.

> <u>Step 2. Infest and screen seedlings.</u> Seedling evaluations (Figure 2.1) produce a consistent response and are the preferred growth stage for mass evaluation (Porter *et al.*, 1993; Robinson, 1992b). Rating of resistance levels takes place two to four weeks after infestation of plants.

Figure 2.1 Uniform rearing and seedling evaluation for identification of *Diuraphis noxia* resistance

> <u>Step 3. Score seedlings for resistance.</u> Simple scoring systems are used to determine resistance. In some instances researchers have applied independent scoring to separate the symptoms of infestation (leaf rolling, chlorosis, stunting, and hindered organ emergence) with the scoring level depending on the percentage of leaf area with a symptom (Formusoh *et al.*, 1992). Each plant symptom would then have a separate 1-9 or 1-6 score. Miller *et al*. (1994) concluded that chlorosis was the visual symptom of *D. noxia* damage that had the best correspondence to physiological measures of susceptibility.

Step 4. Field evaluations. Field evaluations have been used to identify host plant resistance (Calhoun *et al*., 1991, Robinson and Skovmand, 1992). Hill plots are used for uniform genotypes, or spaced planting for segregating generations.

2.5.2 Characterization of resistance to RWA

The nature of resistance in a host plant can be characterized by categorizing the genetics of resistance, the effects of resistance on the insect or evaluation of the plant mechanism involved in the particular resistance. Genetics of resistance involves determination of the effective gene number. Monogenic resistance can sometimes be determined in the F2 segregating populations. Polygenic resistance on the other hand, is characterized through evaluation of F3 families or requires a backcross generation if multiple host plant resistance genes are expected to be involved. Segregation studies using crosses of resistant genotypes with susceptible genotypes will indicate the number of genes carried by the resistant lines. In order to test for allelism, crosses of resistant by resistant cultivars are necessary to confirm the novelty of the gene identified in segregation studies. After the initial identification of resistant accessions in South Africa, Du Toit (1989a) reported resistance in both PI 137739 (*Dn-1*) and PI 262660 (*Dn-2*) to be controlled by single dominant genes. Linkage and cytogenetic studies are used to characterise the chromosome position of resistance loci. Schroeder-Teeter *et al*. (1994) used cytogenetic analysis to identify chromosome 7D as the location of *Dn-1*, the host plant resistance factor in PI 137739. As utilized by Nkongolo *et al*. (1990), cytogenetic studies indicate that the genetic nature of the host plant response may be complex and affected by background factors that may not be identified in simple segregation studies. In the consequent search for additional resistance sources, researchers were able to identify ten resistance genes and determine their chromosome location. *Diuraphis noxia* resistance genes generally occur either on the D chromosome or a rye translocation of wheat (Lage *et al*., 2004). Seven of the genes, *Dn-1* (Marais and Du Toit, 1993; Schroeder-Teeter *et al*., 1994), *Dn-2* (Ma *et al*., 1998), *Dn-5* (Marais and Du Toit, 1993), *Dn-6* (Liu *et al*., 2002), as well as *Dn-8*, *Dn-9* and *Dn-x* (Liu *et al*., 2001), have been located on the 7D chromosome of wheat. Ma *et al*. (1998) reported that *Dn-4* occurs on the 1D chromosome while the recessive gene, *dn-3*, is found on a diploid D-genome *Aegilops tauchii* line. *Dn-7* was found to occur on the 1BL/1RS translocation from rye, *Secale cereale* (Marais and Du Toit, 1994). In a recent attempt, aimed at broadening the genetic variation of *D. noxia* resistance, resistant synthetic hexaploid wheat was obtained from an interspecific cross between *Triticum* *dicoccum* and *Aegilops tauchii*. In contrast to previously identified resistance genes, these genes were located on the A and/or B genomes (Lage *et al*., 2004). Plant resistance to an insect can be categorized as antibiosis, antixenosis, tolerance or combinations of the three categories (Kogan and Ortman, 1978). Of particular interest in regard to host plant resistance to *D. noxia*, are the accessions identified as strong sources of tolerance with limited or no antibiosis such as PI 266260 (Du Toit, 1989b; Tolmay, 2007), CI 15465 (Formusoh *et al*., 1992), Sando collection accessions SS36 and SS385 (Formusoh *et al*., 1994), and PI 366447 (Webster *et al*., 1991). Tolerance may provide yield protection without forcing biotype development associated with the widespread use of a single antibiosis mechanism. Multiple mechanisms of resistance to *D. noxia* occur in 72% of the accessions examined by Souza (1998). Numerous accessions were identified with either antibiosis or tolerance without significant levels of antixenosis. However, one accession (PI 225217), has been identified where antixenosis was independent of the occurrence of antibiosis (Baker *et al*., 1994a). The mechanism of resistance in PI 137739 and PI 262660 are expressed through antibiosis and antixenosis (Du Toit, 1987, 1989b). According to Smith *et al.* (1992) both of these lines possess a significant level of tolerance when resistance is based on the percentage reduction in plant height. Reduced reproductive rates of *D. noxia* occurring after 21 days indicate the presence of low-level antibiosis in both lines. Quisenberry and Schotzko (1994) reported that *D. noxia* occurring on PI 137739 have significantly lower reproduction rates than on PI 262660 and “Stephens”, the susceptible control. This indicated that PI 137739 expressed antibiosis, in contrast to PI 262660 which had higher plant growth, dry weight and moisture while expressing reduced leaf chlorosis and mid-leaf rolling indicating tolerance. Both PI 137739 and PI 262660 are used extensively in breeding programmes in South Africa. A popular hypothesis concludes that *D. noxia* damages plants by injecting phytotoxins into the plant during feeding resulting in the increased release of free amino acids in the phloem content of plants (Telang *et al*., 1999). These phytotoxins simultaneously inhibit synthesis and accumulation of proteins essential for normal plant metabolism (Porter and Webster, 2000) and eventually result in the degradation of chloroplasts and reductions in plant photosynthetic rate and osmotic pressure (Burd and Burton, 1992). Damage in susceptible wheat plants result from increased *Mg*-dechelatase activity accelerating chlorophyll catabolism, leading to bleaching and premature leaf senescence (Ni *et al*., 2001). Van der Westhuizen *et al.* (2002) reported that β-1.3-glucanases accumulate where tissue is damaged most by aphids, indicating a possible role of this enzyme in the resistance mechanism against RWA. Botha and Matsiliza (2004) found that feeding by *D. noxia* results in the redirection of the assimilate flow through formation of local sinks and results in significant damage to cells and tissue. In the resulting feeding-related pressure loss, the carbohydrate translocation capacity of the phloem is reduced and ultimately affects plant development.

2.5.3 Utilisation of RWA resistance in a breeding programme

Although numerous advantages result from exploiting naturally occurring plant defences in a plant breeding programme, an enormous challenge remains in combining resistance with high yield and acceptable grain quality (Tolmay, 2001; Van der Westhuizen, 2004). Most accessions resistant to *D. noxia* originate from germplasm or wild species lacking important agronomic traits. Rare desirable traits do, however, exist in some *D. noxia* resistant germplasm such as the improved seedling emergence and early growth vigour of PI 294994, PI 47545 (Zwer *et al.*, 1994), and Yilmaz-10 (Martin and Harvey, 1995). In most cases acceptable resistance must first, through a pre-breeding programme, be incorporated into an acceptable agronomic background. Backcrossing is the most commonly used method of transferring *D. noxia* host plant resistance genes into an acceptable and adapted agronomic background. The backcross method effectively conserves favourable linkages and produces progeny that closely resemble the recurrent parent with the addition of the host plant resistance factor. However, recurrent backcrossing does not produce cultivars with traits other than the targeted resistance trait and top - crossing the initial F1 plants with a second elite cultivar has been used to address this shortcoming. “Halt”, the first resistant wheat cultivar released in the US, is a topcross produced by Colorado State University and carries the *Dn-4* gene from PI 372129. Single crosses made between elite lines of resistant sources, combined with selection and inbreeding have been used by breeders from the United States of America’s Department of Agriculture (USDA) in order to produce improved germplasm (Baker *et al.*, 1994b; Mornhinweg *et al.*, 1995). This material however, requires additional mating and selection before resistance in the improved germplasm can be used in the final stages as a potential cultivar.

2.5.4 Russian wheat aphid biotype development

Widespread collapse of resistant crop varieties due to biotype development is a major threat to food security, especially in third world countries. An even greater catastrophe can develop should no advanced breeding lines with effective resistance genes against the new biotype exist to replace the failing varieties. Puterka and Burton (1991) outlined three criteria that are essential for the development of biotypes in crop pests. 1) Genetic mutation or existing variability within the pest population, 2) sexual recombination and 3) host plant resistance selection pressure must exist or have a high probability of occurrence, if biotype development is likely to occur. Quick (1989) considered the development of new biotypes of *D. noxia* unlikely in the US due to lack of variation in US populations and the gynocyclic sexual cycle (producing oviparae with no males and no viable eggs) of the pest in the US. In South Africa host plant resistance of 80% of dryland wheat varieties released up to 1998 (Tolmay and Van Deventer, 2005) and 82% up to 2005 (ARC–SGI, 2005) consisted mainly of the dominant single genes, *Dn-1* or *Dn-2* from donor accessions PI 137739 and PI 262660. Alternative donors such as PI 294994 containing *Dn-5, Dn-8 and Dn-9* (Liu *et al.*, 2001) were introduced in a number of varieties but the possibility for development of a new biotype of *D. noxia* always existed.

In June 2003, a new resistance-breaking biotype of *D. noxia* was reported in Colorado. RWA2 appears to be much more damaging than the previous RWA1 (Haley *et al.*, 2004) and is virulent on all wheat cultivars containing the resistance gene *Dn-4* as well as “Stanton” previously considered to have another type of resistance. Jyoti *et al.* (2006) confirmed virulence of the new biotype, and stated that biotype 2 has overcome both *Dn-4*- and *Dn-y* based sources of resistance. The new biotype has proven to be more virulent and induces plant injury more rapidly than RWA1. Virulence of *D. noxia* biotypes to previously resistant wheat varieties was also reported in Argentina, Chile, the Czech Republic, and Ethiopia (Smith *et al.*, 2004). In Argentina, wheat cultivars with resistance genes *Dn-2* and *Dn-4* failed against a new biotype (Almaraz *et al.*, 2003). Occurrence of a new biotype has also been reported from South Africa. Tolmay *et al.* (2007) reported in 2005 that wheat farmers in the eastern Free State of South Africa observed population build-ups of *D. noxia* and consequent damage on resistant wheat cultivars. As the existence of RWASA2 *D. noxia* in South Africa becomes more definite, researchers are aiming toward identifying novel sources of resistance against RWASA2, as well as sources of resistance against likely future biotypes.

2.6 Exploring sources for host plant resistance to RWASA2; *Diuraphis noxia* (Kurdjumov)

South African wheat breeders are in certain aspects facing a similar redundant situation in regard to Russian wheat aphid host plant resistance. Screening assays with rapid throughput of potential resistance sources without compromise of accuracy is the primary focus in most resistance breeding programmes. Delicate alteration of the methodology in a screening assay may be required to achieve a high probability of selecting vital genotypes. The first part of this study therefore investigates the influence of vernalization and fluctuating temperatures on expression of host plant resistance to RWA during the seedling stage.

After the initial release of RWA resistant wheat varieties in South Africa, breeders have for some years been occupied with increasing the genetic diversity of host plant resistance to RWA in breeding material. Promising genotypes, often totally unadapted to local conditions, were evaluated in greenhouse seedling tests against the predominant biotype of the time. Germplasm with satisfactory resistance levels was then integrated into an acceptable agronomic background and entered into the main breeding programme. These lines, however, often failed to make the final selection lists as they lacked important traits in regard to quality or disease resistance and therefore were often neglected as potential sources of wider genetic variability in RWA resistance. With the introduction of biotype RWASA2, these locally adapted lines have now become major sources of resistance against the new biotype. The second part of this study will therefore investigate the stability of RWA resistance of wheat cultivars in different environments, as commercial cultivars are commonly used in crossing combinations with unadapted donor accessions. The latter part of the study will also identify and evaluate foreign germplasm for host plant resistance against RWASA2 in different environments.

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CHAPTER 3

The effect of vernalization on resistance to RWA biotype RWASA2

3.1 Introduction

In 2005, wheat farmers reported considerable field infestations of Russian wheat aphid; *Diuraphis noxia* (Kurdjumov) in commercial wheat fields of the eastern Free State of South Africa (Tolmay *et al.*, 2006; 2007). This was a concern in the industry because all wheat varieties planted for commercial purposes had for previously exhibited efficient resistance against Russian wheat aphid (RWA). Significantly warmer than average temperatures experienced during the wheat production months of 2005 gave rise to a prominent hypothesis stating that warmer winter temperatures reduced vernalization periods of winter wheat varieties, ultimately reducing immunity of plants to RWA (Lindeque, 2006). Entomologists and plant breeders from the Agricultural Research Council's Small Grain Institute (ARC – SGI) in Bethlehem suspected a new biotype to be the cause. The peril of wide-scale collapse of resistant germplasm and the economic implications to seed companies warranted an investigation into the validity of both hypotheses.

In most plant genera, climate has a profound influence on initiation and development of reproductive structures; in wheat, reproductive structures are influenced by time of vernalization, temperature and photoperiod. Worland *et al.* (1998) confirms that the life cycle of wheat is controlled by three sets of genes for 1) vernalization, 2) photoperiod and 3) earliness *per se* where vernalization and photoperiod genes react to environmental stimuli. Photoperiod and vernalization are considered by Slafer (1996) to account for most, if not all of the different development rates of wheat. Crofts (1989) calls vernalization "springization" implying that a plant is converted from a winter growth habit into a spring growth habit during which floral development is initiated. In a study completed on the effect of vernalization on resistance to the cereal aphid; *(Metopolophium dirhodum)* and oat aphid *(Sitobion avenae),* Kay *et al.* (1981) concluded that aphids were smaller when reared on vernalized plants and contained fewer embryos than on non-vernalized genotypes. Their results also showed that resistance rankings of tested wheat varieties changed markedly. Significant resistance between vernalized cultivars at the extreme of the resistance sequence became insignificant when not vernalized. Quick *et al.* (1991) found contradicting results and reported no marked effect of seedling vernalization on RWA resistance ranking and concluded that either vernalized or non-vernalized plants can be used, depending on user need. They screened seedlings in a greenhouse test without prior vernalization and removed RWA from wheat seedlings after evaluation. Two of the four replications were then vernalized for eight weeks and were transplanted, while the other two remained unvernalized and were not transplanted. Although seedling survival after RWA removal was significantly reduced by vernalization and transplanting, it did not, irrespective of eight weeks of vernalization and consequent transplanting, correlate with RWA damage ratings. An important consideration currently at the ARC-SGI is that mass screening of seedlings under controlled conditions are conducted without vernalizing seedlings beforehand, due to time constraints. If the vernalization hypothesis proved to be valid, current mass screening of seedlings for RWA resistance at ARC-SGI without prior vernalization may discriminate against RWA resistance in some winter genotypes.

The objective of this study was firstly to determine the effect of vernalization on the expression of host plant resistance to RWASA2 in seven wheat genotypes differing in vernalization requirements and secondly to confirm virulence of RWASA2 on *Dn-1* wheat varieties.

3.2 Material and methods

3.2.1 RWASA2 colony

RWASA2 (*D. noxia,* Kurdjumov) was collected in 2005/2006 from the Reitz district in the eastern Free State from infested plants within commercial farmer fields. A colony was established by isolating a clone from the original field sample and rearing it on seedlings from the RWA susceptible wheat cultivar Betta. The RWASA2 colony was maintained under controlled conditions in aphid cages (2.4 m^3) at constant day/ night temperatures of 20ºC/12 ºC (±2) through re-infestation of Betta seedlings once susceptible host plants start to deteriorate.

3.2.2 Experimental selection of wheat varieties

Betta (RWA susceptible check), Elands, Gariep, Hugenoot, Limpopo and Molen vary in vernalization requirements and are grouped in four vernalization similarity groups (Müller, 2004) with only Molen being regarded as a true winter wheat (Table 3.1). The resistant check, CItr 2401, is a genotype described by Porter *et al*. (1993) to have originated from the former Soviet Union and having a winter growth habit. The genetic response of the seven wheat genotypes to RWA damage (Tolmay and Van

Deventer, 2005) can be grouped in three categories (Table 3.1). Betta, Hugenoot and Molen do not have any resistance against *D. noxia* whereas Elands, Gariep and Limpopo contain resistance from PI 137739. CItr 2401 was used as resistant check and Voothuluru *et al.* (2006) reported that two genes are expressed in CItr 2401 as dominant traits and may be controlling expression of antibiosis and tolerance in this genotype.

Table 3.1 Vernalization and RWA resistance characteristics of seven South African wheat varieties

Wheat variety	Type	Vernalization requirement (Müller, 2004)	RWA resistance donor accession	Postulated resistance Gene
Betta	Intermediate	No vernalization	Susceptible (Check)	None
CItr 2401	Winter	3 - 6 weeks	Resistant (Check)	*Dn-4 and another gene different from Dn-5 or Dn-6*
Elands	Winter	2 or more weeks*	PI 137739	*Dn-1*
Gariep	Intermediate	No vernalization	PI 137739	*Dn-1*
Hugenoot	Winter	3 - 6 weeks	Susceptible	None
Limpopo	Intermediate	No vernalization	PI 137739	*Dn-1*
Molen	True winter	≥6 weeks	Susceptible	None

* Elands is a winter wheat but clusters separately from other winter groups and needs at least two weeks of vernalization.

3.2.3 Application of vernalization and aphid treatments

The different vernalization treatments were established according to Müller (2004) and include a non-vernalized control (0-weeks) and 2-week-, 4-week- and 6-week vernalization treatment. Seedlings were vernalized for respective periods at 3°C (± 1°C) with a 10-hour light and 14-hour dark photoperiod regime provided by cool fluorescent tubes. The 6-week vernalization period material was planted and vernalized first with other treatments following in descending order so that all six vernalization periods were concluded simultaneously. Vernalized seedlings were removed from the cold room to two separate plant cubicles and maintained at 18°C/12°C day/night (± 2°C) and 26°C/18°C day/night temperatures (± 3°C) respectively. Two seeds were planted in each cell ($20cm^3$) containing a sandy-loam soil and cells were placed inside a waterproof pan for the duration of the experiment. The pan allowed sub-irrigation of plants through capillary movement in soil, preventing direct water on leaves that may affect aphid activity and encourage fungal infection of both plants and aphids.

Seedlings were allowed to recover from vernalization treatment for one week (Nkongolo *et al.*, 1990) before infestation with RWASA2. An adequate amount of aphids providing four to five aphids per plant was weighed and sprinkled evenly over each tray.

3.2.4 Experimental layout and measurements

The experiment was designed as a split-plot with vernalization as main plots and varieties as sub-plots cultured at two different temperature regimes. Treatments were replicated twice and consisted of 10 plants for each treatment. Effect of vernalization and temperature on resistance to RWASA2 was determined three weeks after infestation. Seedlings were scored according to the 1 to 10 visual rating scale (1 to 3.5 indicating highly resistant - and 6.5 to 10 completely susceptible plants) developed by Tolmay (1995) and fresh plant biomass determined by clipping two plants from each cell at soil level and weighing both together. Data was analysed with Genstat 8th Edition (GENSTAT, 2005).

3.3 Results

3.3.1 ANOVA of main effects and interaction of variety, temperature and vernalization

The mean squares of varieties was highly significant for both visual damage and fresh plant biomass and interaction of vernalization x variety was highly significant for fresh plant biomass (Table 3.2).

Table 3.2 Main and interaction effects of different variables

	Visual Damage Rating			Fresh Plant Mass		
	df	ss	ms	df	ss	ms
Variety	6	31548.7	5258.1***	6	1279993	21332***
Temperature	1	23.260	23.260	1	517	517
Vernalization	3	76.814	25.605	3	34624	11541
Vernaliz. x var.	18	214.78	11.932	18	155510	8639***
Temperature x var.	6	33.481	5.580	6	32417	5403
Temperature x var. x vernalization	18	151.92	8.440	18	30763	1709

Vernaliz. – vernalization, var. – variety. ***P≤0.001

3.3.2 Visual damage rating

Visual damage ratings at respectively 18°C/12°C and 26°C/18°C of Betta (8.8 and 8.9), Hugenoot (8.7 and 8.9), Molen (8.7 and 8.9), Gariep (8.2 and 8.3), Limpopo (7.9 and 8.2) and Elands (7.4 and 7.6) indicate high virulence of RWASA2 on seedlings three weeks after infestation (Table 3.3). On the 1 – 10 rating scale damage ratings higher than 6.5 are considered as susceptible and only CItr 2401 (4.2 and 4.4) exhibited a resistant score against RWASA2. No change in ranking of varieties occurred between varieties developing at the two temperatures

Table 3.3 Main effects of varieties on visual damage of RWASA2 at 18°C/12°C day/night (± 2°C) - and 26°C/18°C and as a percentage of Betta

Variety	18°C/12°C	26°C/18°C	% of Betta (temperatures combined)
Cltr 2401[a]	4.2a	4.4a	48.84a
Elands	7.4b	7.6b	84.58b
Limpopo	7.9c	8.2c	91.97c
Gariep	8.2c	8.3c	92.61c
Molen	8.7d	8.9d	99.05d
Hugenoot	8.7d	8.9d	99.12d
Betta[b]	8.8d	8.9d	100.00d
Mean square	21.01***	20.32***	5258.11***

[a]Resistant check; [b]Susceptible check; ***P≤0.001. Values followed by different letters in each column differ significantly at p≤0.05.

Visual damage ratings of Hugenoot and Molen expressed as a percentage of the susceptible control, did not differ significantly as neither contained genetic resistance to RWA and were significantly more susceptible than Gariep, Limpopo, Elands and CItr 2401. Percentage damage of Elands though was significantly less than Gariep and Limpopo but significantly more compared to CItr 2401 containing alternative *Dn*-genes.

3.3.3 Fresh plant biomass

Values for this variable were expressed as a percentage of the susceptible check Betta in order to illustrate the effects of variety, vernalization period and temperature on expression of host plant resistance more accurately.

Fresh plant biomass results indicate trends similar to those observed with visual damage ratings. “Varieties” was the only main to significantly (<0.001) affect fresh plant biomass (Table 3.4). Biomass of Betta, Molen and Hugenoot did not differ significantly from each other but was significantly less than the four, less susceptible genotypes. Plants of Elands and CItr 2401 (resistant check) weighed significantly more than Gariep and Limpopo although susceptibility of Elands to RWASA2 is visible in a significantly lower biomass than CItr 2401.

Table 3.4 Main effects of variety and vernalization x variety interaction expressed as a percentage of Betta, on fresh plant biomass (grams)

	Variety main effect	Variety x vernalization interaction			
Variety		**0 weeks**	**2 weeks**	**4 weeks**	**6 weeks**
Cltr 2401[a]	420.0a	336.4c	344.8bc	414.5b	584.4a
Elands	231.2b	181.1b	278.1a	228.8ab	237.0ab
Limpopo	146.8cd	144.4a	162.1a	130.1a	150.7a
Gariep	151.5c	138.7a	146.3a	171.2a	149.9a
Molen	113.4de	110.6a	92.5a	122.4a	128.1a
Hugenoot	96.2e	83.2a	126.2a	83.9a	91.6a
Betta[b]	100.0e	100.0a	100.0a	100.0a	100.0a
Mean square	213332***	8639***			

[a]Resistant check; [b]Susceptible check; ***P≤0.001. Values followed by different letters in each column differ significantly at p≤0.05

Vernalization period interacted significantly with Varieties but only affected fresh biomass of Elands and CItr 2401 (Table 3.4). Biomass of CItr 2401 plants vernalized for 0 weeks (336.4), 2 weeks (344.8) and 4 weeks (414.5) was significantly less than CItr 2401 vernalized for 6 weeks (584.4). Plants of Elands vernalized for 2 weeks (278.1) had significantly more biomass than unvernalized plants (181.1) but increasing vernalization with an additional 2- or 4 weeks did not improve fresh biomass of Elands significantly (Figure 3.1).

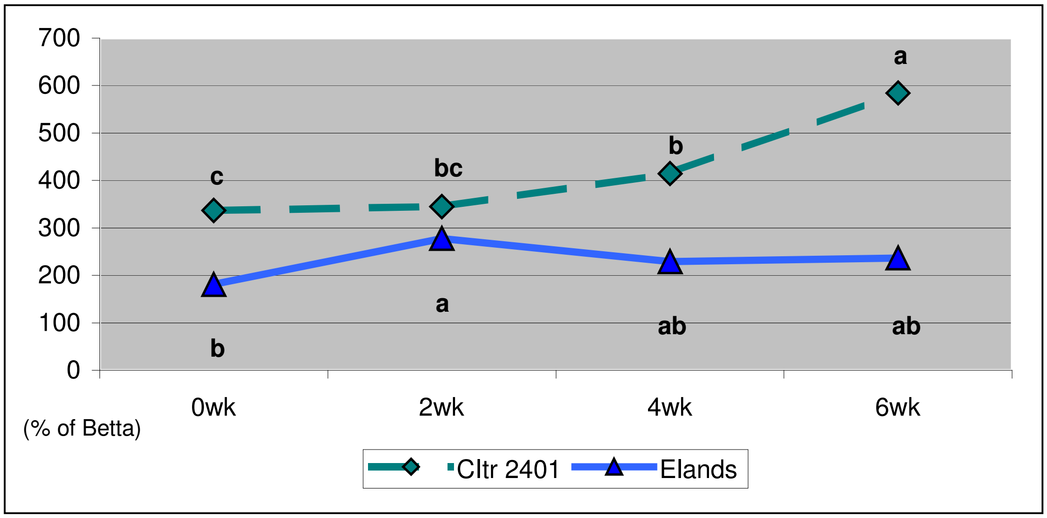

Figure 3.1 **Effect of vernalization x variety interaction on fresh biomass of Elands and CItr 2401 (CI 2401=PI 9781), expressed as percentage of Betta (susceptible check)**

3.4 Discussion

The most important observation from this experiment was the high virulence level of RWASA2 on six of the seven wheat genotypes tested and confirms preliminary results reported by Tolmay *et al.* (2007). Both visual seedling score and fresh biomass of Elands, Limpopo and Gariep were significantly below levels recorded for the resistant check CItr 2401 at both temperatures. Vernalization interacted significantly with varieties and influenced fresh biomass of Elands and CItr 2401. Müller (2004) remarked that although winter-wheat varieties require 3- to 6 weeks vernalization, Elands grouped differently and need only be vernalized for 2 weeks. Application of this vernalization period in our experiment resulted in a higher plant biomass by Elands and corresponded with the vernalization recommendation for this variety. CItr 2401 (CI 2401=PI 9781) is reported by Porter *et al.* (1993) to have a winter growth habit and the vernalization periods of 4- or 6-weeks increased fresh biomass significantly.

Increased resistance to aphids on vernalized plants (Belizin, 1936) is attributed to accelerated plant development and more vigorous growth. Our findings consequently also suggest that CItr 2401 benefited from extended vernalization periods as the 6 weeks period produced a significantly higher percentage biomass than 0 weeks, 2 weeks and 4 weeks periods. Biomass of CItr 2401 at these three periods did not differ significantly from each other. Different vernalization periods are theoretically different growth stages complicating separation of the effect of plant stage from the influence of the vernalization process. In this regard CItr 2401 plants from the 6 weeks vernalization period can be considered as more mature and should exhibit better resistance to RWA damage although one would then also have expected similar effects on other test varieties as well. Voorthuluru *et al.* (2006) speculated that the significantly reduced root dry weight loss in CItr 2401 might result from transfer of fresh weight from shoots to roots in response to *D. noxia* herbivory. Dying shoots would deter aphid development and eventually initiate plant recovery from stored carbohydrates in the root system.

Observations by Belizin (1936) indicate that vernalized wheat and barley were more resistant to *Toxoptera graminum* (Rondani) and *Brachycolus noxius* (Mordvilko) = *Diuraphis noxia* (Kurdjumov). Kay *et al.* (1981) remarked that vernalization resulted in smaller adults of *Metopolophium dirhodum* with a lower embryo complement than those reared on unvernalised material. They speculated that in vernalized wheat levels of soluble nitrogen in plants increase and have been shown to affect aphid growth and reproduction (Van Emden, 1972; Dixon, 1973). We conclude that our results for Elands, Gariep and Limpopo support findings by Gray *et al*. (1990) and Nkongolo *et al*. (1990) reporting no effect of non-vernalized or vernalized plants on expression of RWA plant resistance ranking. RWA resistance in Elands, Gariep and Limpopo was derived from a similar donor accession PI 137739 and other resistance donors and –mechanisms may prove more sensitive to vernalization treatment.

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CHAPTER 4

The effect of temperature and rainfall on host plant resistance of four wheat varieties to Russian wheat aphid, *Diuraphis noxia*; biotype RWASA2

4.1 Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov) is a worldwide pest of wheat and was first identified in South Africa in 1978 (Walters *et al*., 1980) causing significant damage on wheat crops. Sporadic reports of RWA virulence on commercial wheat were received from localized areas in the central Free State in 1996 and eastern Free State in 1998; both years exhibited a wet summer followed by a mild winter (Tolmay *et al*., 2006). After field reports of RWA virulence received from the eastern Free State in 2005, South African scientists anticipated development of a new RWA biotype as genetic variance for resistance to RWA in commercial wheat varieties was generally based on *Dn-1* from donor PI 137739 and *Dn-2* from PI 262660 (Tolmay and Van Deventer, 2005). Virulence of a new biotype was confirmed after the outbreak in 2005 by mass screening seedlings of all commercial varieties under controlled conditions and designating the biotype as RWASA2 (Tolmay *et al*., 2007). Smith (1994) defines a biotype as a strain of Russian wheat aphid, *D. noxia* different from the original strain in its ability to damage resistant wheat plants. Differences between biotypes can also include different characteristics with regard to biology, adaptation and environment (Ogecha *et al*., 1992; Webster *et al*., 1992). The definition proposed by the WERA-066 Aphid Ecology and Insect-Plant Interaction Subcommittee is: “A population (independent of geographic location) that is able to injure a cultivated plant containing a specific gene(s) that was previously resistant to known aphid populations”. This definition describes numerous resistant- and susceptible plant responses without putting emphasis on either genetic composition or evolutionary- or taxonomic status of the aphid population.

Changing climates are a worldwide phenomenon generally attributed to global warming. Several authors have reported on the effects of higher temperatures on aphid development and virulence. Gullan and Cranston (2005) stated that a rise in temperature would probably increase the metabolic rate and reduce population-doubling times of aphids. Green peach aphid, *Myzus persicae* (Sulzer) is becoming more abundant in Europe and the expansion is directly correlated with temperature and changes in crop species (Cocu *et al*., 2005). Davis *et al.* (2006) concluded in their study that the green peach aphid would benefit from an increase in temperature of 2.5 to 3.5°C. Southern Africa has experienced the warmest and driest decade on record between 1985 and 1995 with an average temperature increase of 0.56°C (Kluger, 2006; Rosenzweig and Hillel, 2004). In South Africa an apparent link exists between increased damage and climate, as deviations from long-term average temperatures and rainfall coincided with lost *Dn-1* resistance in the eastern Free State during 2005. This study attempted to investigate validity of the hypothesis by determining firstly if climate change is quantifiable through comparison of temperature and rainfall records between three different periods. Data from 2004/2005 when RWASA2 was identified was compared with the preceding five-year period of 1999 to 2003 and long-term averages for the Free State (1961 to 1991). Secondly, visual damage rating of seedlings at three temperatures to RWASA1 and RWASA2 was determined and put into context with climatic variations in the eastern Free State. Thirdly, influence of RWASA2 on leaf area, leaf roll and leaf chlorosis of Komati, Matlabas, SST 966 and SST 399 were determined at high developmental temperatures.

4.2 Material and methods

4.2.1 Aphid colony

A sample of *D. noxia* was collected from commercial wheat during the winter of 2005 from the Reitz district in the eastern Free State. A colony was established from a clone isolated from the original field sample and reared under controlled conditions on susceptible "Betta". Subsequent seedling screening tests on all prominent wheat varieties confirmed virulence and the new biotype was designated as RWASA2 (Tolmay *et al.*, 2007). Both RWASA1 and RWASA2 were maintained on Betta seedlings but were isolated from each other with aphid cages placed in different cubicles. Temperatures in both cubicles were maintained at day/ night temperatures of 20°C/15°C (± 3°C) under natural light conditions.

4.2.2 Temperature and rainfall averages

Variation in patterns for rainfall (mm) and minimum- and maximum temperatures (°C) were determined by comparing records for the average of January - December over the two-year period of 2004 – 2005, five-year period from 1999 – 2003; obtained from the ARC – Small Grain Institute weather station and long term monthly averages for the Free State (1961 to 1991) obtained from the National Weather Buro (South

African Weather Services, 2007). Temperature- and rainfall means relevant to wheat development in the summer rainfall regions of South Africa were compared and put into context with RWA- and wheat development during 2005.

4.2.3 Visual damage rating at fluctuating temperatures

The experiment was designed as a non-replicated trial determining rankings of varieties according to visual damage ratings against RWASA1 and RWASA2 at three fluctuating temperature regimens namely 18°C/12°C, 22°C/16°C and 26°C/20°C respectively (Table 4.1). The experiment was concluded and analyzed separately in two runs although results were expressed as means for each entry over both runs. Seedling trays contained six entries with ten plants for each entry and all trays included Betta (susceptible) and Halt (resistant) checks. Four commercial varieties, Komati, Matlabas, SST 399 and SST 966 were evaluated and expressed RWA resistance through *Dn-1* from donor accession PI 137739. Once seedlings reached the two-leaf stage, plants were infested with an adequate amount of aphids for an infestation level of approximately five aphids per seedling. Infestations developed for three weeks before seedlings were visually rated on the 1 - 10 scale developed by Tolmay (1995).

Table 4.1 Temperature regimens used in visual screening, leaf area, percentage leaf roll and percentage leaf chlorosis evaluations

Temperature regimen (°C)		Mean temperature (°C)	Hours at maximum	Hours at minimum
Visual screening	18 – 12	15.12	2 (± 1)	4 (± 2)
	22 – 16	17.52	2 (± 1)	4 (± 2)
	26 – 20	24.87	2 (± 1)	3 (± 2)
Plant variables	26 – 12	21.85	2 (± 1)	2 (± 1)

4.2.4 Effects of RWASA2 on leaf area, percentage leaf roll and percentage leaf chlorosis of Komati, Matlabas, SST 966 and SST 399

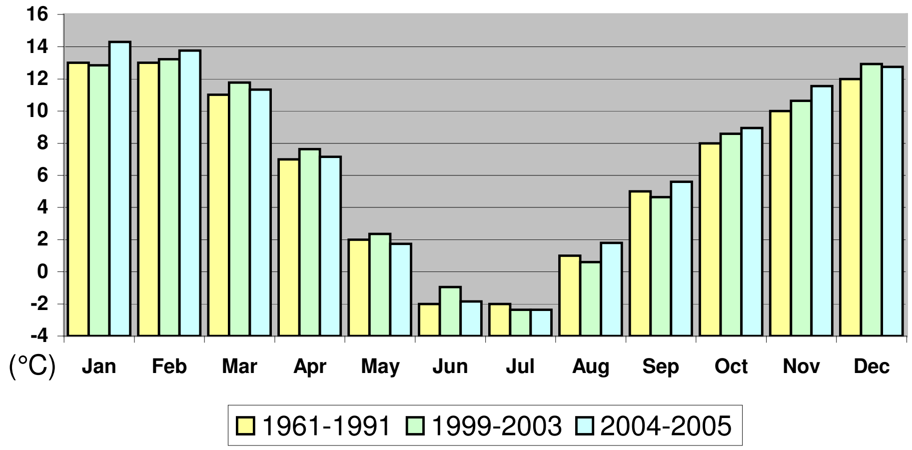
The experiment was executed in two separate runs each designed as a randomized block with four replications for each variety. In each replication, consisting of two aphid cages, entries in one cage were infested with RWASA2 at the two-leaf stage (five aphids per plant) and the other cage was maintained free from aphids. Seed of each variety was planted in two-litre pots filled with a sandy-loam soil placed in aphid cages in a greenhouse with set temperature of 26°C/12°C (± 3°C). Cage covering of

325 - micron mesh prohibited contamination of the aphid colony by natural enemies or foreign aphids but enabled access to pots through a funnel opening. Dry plant leaves and no disturbance of aphid colonies was obtained by placing aphid cages with pots inside water trays in which water levels were maintained at a constant level. After three weeks of infestation two seedlings from each pot were cut at soil level for determination of leaf area (cm^2) with a LI – 3100 Area Meter (Li-Cor, inc. Lincoln, Nebraska, USA). Leaf roll (%) and chlorosis (%) were determined by counting the number of leaves exhibiting damage rating 5 or 6 (chlorosis) and 7 or 8 for leaf roll according to a standard rating scale (Tolmay, 1995). The ARC - Biometry Unit in Pretoria, South Africa analyzed results from the experiment with the Genstat 8th Edition (GENSTAT, 2005) program.

4.3 Results

4.3.1 Variation in minimum temperature (°C)

Mean monthly minimum temperatures (Fig 4.1.1) for January to December in 2004 - 2005 compared with 1999 - 2003 varied more than 1°C for January (+1.5), for August (+1.2), September (+1.0) and November (+1.0) and were colder for June (-1.0).



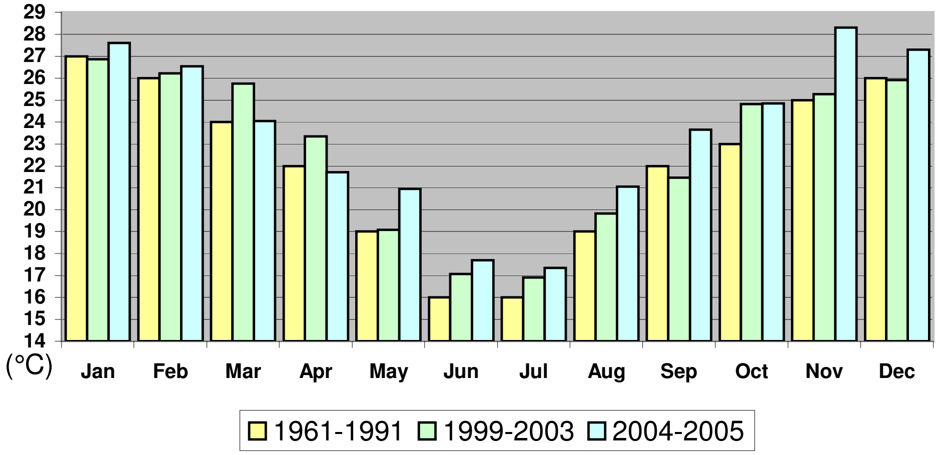
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2004-2005	14.3	13.8	11.4	7.2	1.8	-1.9	-2.4	1.8	5.6	9.0	11.6	12.8
1999-2003	12.8	13.2	11.8	7.6	2.4	-0.9	-2.3	0.6	4.6	8.6	10.6	12.9
1961-1991	13.0	13.0	11.0	7.0	2.0	-2.0	-2.0	1.0	5.0	8.0	10.0	12.0

Figure 4.1.1 Mean minimum temperatures (°C) for January to December for 2004 - 2005, 1999 - 2003 and 1961 - 1991

Minimum monthly temperatures for 2004 - 2005 were also warmer with 1°C or more for January (+1.3), October (+1.00) and November (+1.55) compared to long term averages for the Free State from 1961 - 1991.

4.3.2 Variation in maximum temperature (°C)

Mean monthly maximum temperatures (Fig 4.1.2) for January to December in 2004 - 2005 varied with more than 1°C for March (-1.6), April (-1.7), May (+1.9), August (+1.3), September (+2.2), November (+3.0) and December (+1.4) compared to the preceding five years from 1999 - 2003. Differences between maximum temperature means of 2004 - 2005 and the long term for the Free State (1961 - 1991) are even more dramatic and vary with more than 1°C for May (+2), June (+1.7), July (+1.4), August (+2.1), September (+1.7), October (+1.9), November (+3.3) and December (+1.3).



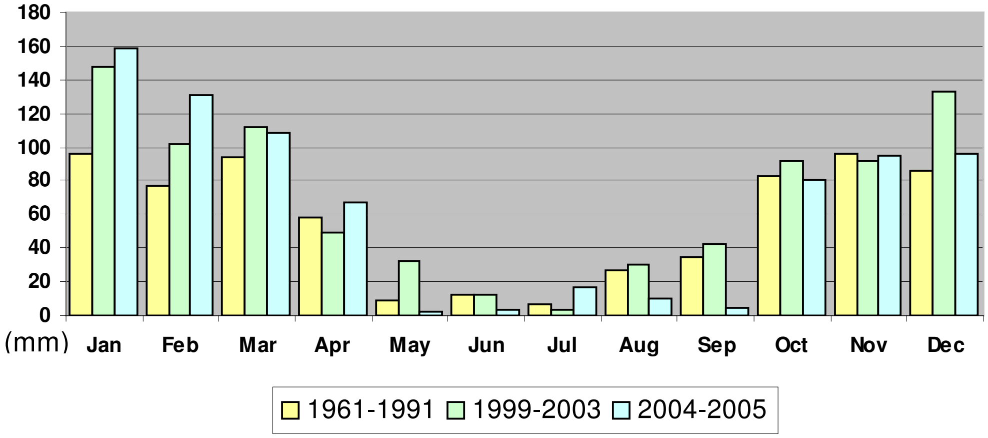
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2004-2005	27.6	26.6	24.1	21.7	21.0	17.7	17.4	21.1	23.7	24.9	28.3	27.3
1999-2003	26.9	26.2	25.7	23.4	19.1	17.1	16.9	19.8	21.5	24.8	25.3	25.9
1961-1991	27.0	26.0	24.0	22.0	19.0	16.0	16.0	19.0	22.0	23.0	25.0	26.0

Figure 4.1.2 Mean maximum temperatures (°C) for January to December for 2004 - 2005, 1999 - 2003 and 1961 - 1991

4.3.3 Variation in rainfall (mm)

Mean total rainfall (Figure 4.1.3) for January to December during 2004 - 2005 was 772.8mm compared to 844.6mm for the same months during 1999 - 2003 and long-term average of 680.0mm for the Free State. Mean monthly rainfall recorded in

January (+11.4), February (+29.5), April (+7.2), July (+13.5) and November (+2.5) of 2004/2005 was more than the average for the corresponding month in 1999 - 2003. Totals of mean monthly rainfall for 2004 - 2005 in March (-3.1), May (-29.6), June (-9.1), August (-19.2), September (-37.2), October (-11.0) and December (-36.6) was less than for the corresponding month in 1999 - 2003.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2004-2005	158.7	130.9	108.6	66.7	2.3	3.3	16.3	10.5	5.0	80.2	94.6	95.9
1999-2003	147.3	101.4	111.7	49.5	31.9	12.4	2.8	29.7	42.2	91.2	92.1	132.5
1961-1991	96.0	77.0	94.0	58.0	9.0	12.0	7.0	27.0	35.0	83.0	96.0	86.0

Figure 4.1.3 Mean monthly rainfall (mm) for January to December for 2004 - 2005, 1999 - 2003 and 1961 - 1991

4.3.4 Visual damage ratings at fluctuating temperatures

Visual damage ratings of the susceptible check Betta (Table 4.2) and SST 966 showed consistent susceptibility irrespective of biotype or development temperature. Halt was medium-resistant against RWASA1 at 22°C/16°C and 26°C/20°C and against RWASA2 at 18°C/12°C and 22°C/16°C but highly resistant against RWASA1 at 18°C/12°C and against RWASA2 at 26°C/20°C. Komati and Matlabas exhibited medium resistance against RWASA1 at 18°C/12°C but was susceptible as developmental temperature increased to 22°C/16°C or 26°C/20°C. SST 399 was medium resistant against RWASA1 at all three temperatures but susceptible to RWASA2 regardless of the development temperature. Increasing temperature regimens resulted in changes in ranking for SST 966 (5 to 3 to 5) against RWASA1 and Komati (3 to 6 to 5) and SST 399 (6 to 3 to 2) against RWASA2.

Table 4.2 **Visual seedling damage of biotypes RWASA1 and RWASA2 on six wheat genotypes at three different temperatures**

			Rating[a] (Ranking[b])		
Test variety	***Dn*-gene (Tolmay, 2005)**	**RWA Biotype**	**18°C/ 12°C**	**22°C/ 16°C**	**26°C/ 20°C**
Betta	**None**	**SA1**	S (6)	S (6)	S (6)
		SA2	S (5)	S (5)	S (6)
Halt	***Dn-4*** **(PI 372129)**	**SA1**	HR (1)	MR (1)	MR (1)
		SA2	MR (1)	MR (1)	HR (1)
Komati	***Dn-1*** **(PI 137739)**	**SA1**	MR (4)	S (5)	S (4)
		SA2	S (3)	S (6)	S (5)
Matlabas	***Dn-1*** **(PI 137739)**	**SA1**	MR (3)	S (4)	S (3)
		SA2	S (2)	S (2)	S (3)
SST 399	***Dn-1*** **(PI 137739)**	**SA1**	MR (2)	MR (2)	MR (2)
		SA2	S (6)	S (3)	S (2)
SST 966	***Dn-1*** **(PI 137739)**	**SA1**	S (5)	S (3)	S (5)
		SA2	S (4)	S (4)	S (4)

Rating[a]: S (Susceptible), MR (Medium resistant) and HR (Highly Resistant); Ranking[b]: 1 = excellent rating, 6 = poor rating

4.3.5 Effects of RWASA2 on leaf area, percentage leaf roll and percentage leaf chlorosis of Komati, Matlabas, SST 966 and SST 399

The main effect of RWASA2 was significant for the second run as mean leaf area was reduced by 60.6% from 120.4 cm^2 for aphid-free plants to 47.5 cm^2 for infested plants (Table 4.3). RWASA2 feeding also resulted in 40.6% leaf-roll and 58.6% chlorosis in the first run compared to 66.6% leaf-roll and 23.68% chlorosis in the second run. Effect of variety x aphid interaction on leaf-roll was significant for the first run and indicates that Matlabas (21.2%) had significantly less leaf-roll than Komati (52.7%) and SST 966 (48.6%) but SST 399 (39.8%) did not differ significantly from Matlabas, Komati or SST 966 (Table 4.3). In run 1 variety x aphid interaction showed that Matlabas (78.4%) had significantly more chlorosis compared to SST 399 (56.7%), SST 966 (51.5%) and Komati (47.9%) although the difference between SST 399, SST 966 and Komati was insignificant. Percentage chlorosis in run 2 of Matlabas (29.37%) was significantly more than for Komati (16.35%) although SST

966 (24.61%), SST 399 (24.38%) and Komati (16.35%) did not differ significantly from each other.

Table 4.3 **RWASA2 damage on Komati, Matlabas, SST 966 and SST 399 measured by leaf area, percentage leaf roll and percentage leaf chlorosis**

		Leaf area (cm^2)		Leaf roll (%)		Chlorosis (%)	
TMT [a]		1st Run	2nd Run	1st Run	2nd Run	1st Run	2nd Run
Aphid-free		-	120.4a	0.0b	0.0b	0.0b	0.0b
RWASA2 infested		-	47.5b	40.6a	66.6a	58.6a	23.68a
VAR x TMT[b]							
Komati	Aphid-free	-	-	0.0c	-	0.0c	0.0c
	RWASA2	-	-	52.7a	-	47.9b	16.4b
Matlabas	Aphid-free	-	-	0.0c	-	0.0c	0.0c
	RWASA2	-	-	21.2b	-	78.4a	29.4a
SST 966	Aphid-free	-	-	0.0c	-	0.0c	0.0c
	RWASA2	-	-	48.6a	-	51.5b	24.6ab
SST 399	Aphid-free	-	-	0.0c	-	0.0c	0.0c
	RWASA2	-	-	39.8ab	-	56.7b	24.4ab
Mean squares	TMT[a]	ns	42535.0***	13171.9**	35517.1***	27510.9**	4484.99**
	VAR x TMT[b]	ns	ns	391.14***	ns	374.23***	58.32*

TMT[a] (Treatment): Aphid – free, Infected with RWASA2; VAR x TMT[b] (Variety x Treatment Interaction); ns – not significant, *-P≤ 0.05, **-P≤ 0.01, ***-P≤ 0.001, Values followed by different letters within columns are significantly different at P≤ 0.05

4.4 Discussion

Subtle changes in climate occurred during 2004 - 2005 if monthly minimum- and maximum temperature and rainfall are measured against averages for the same months in 1999 - 2003 and long-term for the Free State from 1961 - 1991. Peacock *et al.* (2006) found that monthly summer temperature and soil moisture closely related to rainfall provided a more accurate classification for presence of the Aphididae-family in New Zealand. In agreement with their findings, variability found in mean monthly minimum – and maximum temperatures and rainfall for 2004 - 2005 in the eastern Free State may well be the cause for increased occurrence of RWA during the same period. The recorded climatic fluctuations will have had direct effects on RWA life cycles by affecting host availability and host nutritional quality as well as affecting the developmental rates of the aphids themselves, influencing aphid populations size. Abiotic stress in regard to above-average temperatures and reduced rainfall during early developmental stages of dryland-wheat in August and September would also have reduced plant growth vigour and favoured RWA population growth, ultimately increasing population development and probably increasing damage on wheat. Higher mean rainfall during the off–season in January, February and April ensured alternative host availability and increased over-wintering survival rate of RWA.

For extrapolation of effects of climatic variation on expression of host plant resistance, seedlings of four wheat varieties were screened in three cubicles in a greenhouse against RWASA1 and RWASA2 at three different temperature regimens. RWASA1 resistance ratings of Halt, Komati and Matlabas shifted to less resistant- or susceptible categories as developmental temperature increased and changed the ranking of SST 966 substantially. An increase in temperature shifted the visual rating of Halt to RWASA2 to a more resistant category and changed rankings of Komati and SST 399 markedly. An important observation in the visual screening experiment is the increase in RWASA1 damage on Halt (*Dn-4*), Komati (*Dn-1*) and Matlabas (*Dn-1*) at higher temperatures. Davis *et al*. (2006) reported that increased high temperatures coupled with low temperature recoveries allowed the green peach aphid, *Myzis persicae* to increase more rapidly with better survival and higher fecundity. They speculated that endosymbionts (Dadd, 1985) flourish at higher temperatures, resulting in more amino acid production for the aphid while fluctuations to cooler temperatures allow aphid recovery from high temperature exposure. Kieckhefer and Elliot (1989) found the highest intrinsic rate of increase for RWA at a fluctuating temperature regimen of 20.0 – 33.4ºC (mean of 26.7ºC) putting the highest temperature regimen in our experiment (18.0 – 34.0ºC; mean of 24.9ºC) still below the threshold level. Another significant observation is the high virulence of RWASA2 on seedlings of all varieties except Halt, regardless of temperature regimen. This finding confirms the existence of RWASA2 as the cause for lost resistance of wheat varieties under field conditions. A link may, however, still exist between virulence of RWASA2 on wheat varieties and increased temperature. Jyoti *et al.* (2006) reported virulence of RWA2 from eastern Colorado, USA (Haley *et al.*, 2004) to be related to higher colony growth rates and more rapid development of damage symptoms that were even more pronounced at higher temperatures.

Komati, Matlabas, SST 966 or SST 399 did not express any significant resistance to RWASA2 through leaf area, leaf roll and chlorosis and support results obtained for the same varieties in the visual screening test. Reduction of leaf area and percentage induced leaf roll and - chlorosis were significant between aphid-free - and infested plants. Though significantly higher chlorosis appears on leaves of Matlabas compared to Komati, visual damage was similar than the other three varieties. Jyoti and Michaud (2005) found that neither overall plant damage nor leaf rolling is a simple function of aphid numbers but rather reflect cultivar-specific responses to *D. noxia* feeding.

In conclusion, warmer winters and milder but drier summers mirrored in higher temperatures and reduced rainfall during 2004 and 2005 contributed to increased damage of the ancestral RWASA1 biotype on wheat in the eastern Free State. The new biotype RWASA2 appeared to be more tolerant to climatic fluctuations and caused severe damage on all resistant varieties containing *Dn-1* resistance. *Dn-4* based resistant check Halt and CItr 2401, however, expressed good levels of resistance to RWASA2. The effect of climate change on biotypic evolution and frequency is still an open discussion and warrants further investigation. Programmes for resistance breeding and pre-breeding may well have to adapt in order to ensure that host plant resistance in varieties is still effectively expressed under unstable environments, especially in regard to fluctuating temperature and rainfall.

4.5 References

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CHAPTER 5

Expression of Russian Wheat Aphid host plant resistance in South African wheat cultivars under different environmental conditions

5.1 Introduction

Russian wheat aphid (RWA); *Diuraphis noxia* (Kurdjumov) is the most important insect-pest of wheat in South Africa. A considerable effort was invested in the development of lasting control strategies against RWA after the initial outbreak in the eastern-Free State of South Africa in 1978. Du Toit (1989) pioneered host plant resistance in South Africa and first identified the dominant single genes *Dn-1* and *Dn-2* from donor accessions PI 137739 and PI 262660. Alternative donors such as PI 294994 containing *Dn-5, Dn-8 and Dn-9* (Liu *et al.*, 2005) were also introduced into a number of breeding lines and varieties in South Africa, though general deployment was limited. The first RWA resistant cultivar; Tugela-Dn was released in 1992 (Van Niekerk, 2001) and in 2007, fourteen of the nineteen wheat cultivars recommended for production in the summer rainfall region contained resistance to RWA (ARC-SGI, 2007). The *Dn-1* and *Dn-2* resistance to RWA express as a qualitative trait from a single dominant gene (Du Toit, 1989) implying that expression will only be marginally affected by the environment. In contrast Liu *et al.* (2005) suggested that *Dn-1*, *Dn-2*, *Dn-5*, *Dn-6*, *Dn-x* and uncharacterised genes in PI 47545, PI 222666 and PI 225245 are either allelic at the same locus on wheat chromosome arm 7DS, or are tightly linked to one another and located in a gene cluster. Since application of host plant resistance for prevention of RWA damage, varying resistance levels within resistant cultivars or between different resistant cultivars containing resistance from the same donor remains unexplained. Tolmay and Van Deventer (2005) researched the percentage yield retained after RWA infestation in 19 wheat cultivars and determined the reaction of cultivars in different environments by using Additive Main effects and Multiplicative Interaction (AMMI) analyses. The results concluded that cultivars varied dramatically in levels of resistance under severe RWA infestations and did not react similarly over environments, even when resistance genes originated from the same donor accession.

Genotype x Environment interaction (G x E) in wheat is the varying response of wheat genotypes under different environmental conditions. In regard to host plant resistance to RWA, the ideal resistant cultivar would express the same level of resistance under different environmental conditions and at varying RWA infestation levels. Seed dressings and particularly the chloronicotinyl insecticide imidacloprid (Gaucho™), has been used successfully for control of early-season infestations of cereal aphids in wheat (Wilde *et al.*, 2001) whereas foliar insecticides are more effective against late-season infestations. The hypothesis for plants expressing host plant resistance to an insect firstly "reduces the probability of successful utilization of that plant by an insect species or biotype" (Beck, 1965) and secondly "influences the ultimate degree of damage done by insects" (Maxwell *et al.*, 1972). This study investigated this hypothesis by assuming firstly that application of seed-dressings always result in aphid-free plants during the vegetative growth period or first two-months of plant development, preventing any RWA damage. Secondly, foliar-insecticides applied between GS12 and GS13 (Joubert, 1974) always result in aphid-free plants up to harvesting and thirdly, a combination of the two control strategies will always result in aphid-free plants throughout the duration of the wheat season.

5.2 Material and methods

Field trials were planted in the Free State province of South Africa from 2003 - 2006 at Bethlehem, Glen, Ladybrand and Qwaqwa (Table 5.1). Localities were selected in areas with little commercial wheat production to reduce effects from neighbouring farmer's fields. Localities also had different climatic -, soil - and production characteristics.

Table 5.1 Characteristics of the localities used from 2003 to 2006

Locality (Seasons used)	Weather station	Longitude/latitude of weather station	Elevation (m above sea level)	Dominant soil type[a]
Bethlehem (2003-2006)	ARC-SGI	28.2953/-28.1626	1660	Avalon
Ladybrand (2003)	Clocolan-Lanark	27.6589/-29.0193	1584	Westleigh
Glen (2004)	Glen College	26.3529/-28.9208	1344	Bainsvlei
Qwaqwa (2005, 2006)	UniQwa	28.8252/-28.4829	1696	Avalon

[a] - Purchase *et al.* (2000a)

Wheat cultivars in each of the four seasons consisted of Komati and SST 966 (2003 - 2004) replaced with Matlabas and SST 334 for 2005 - 2006 and Betta (susceptible) and PAN 3364 (Table 5.2). The experimental design was a split-split plot with four replicated treatments in which primary blocks consisted of foliar sprayed (FI)- or non-sprayed treatments (CT) and secondary blocks of seed treatment (SD) or -untreated seed of four different commercial wheat cultivars in each season. The standard seeding rate of 20 kg ha^{-1} and - fertilizing rates of 40 kg N ha^{-1} were applied during planting. The foliar insecticide treatment consisted of a Demeton-S-Methyl EC/Parathion EC tank mixture (125 g.a.i. ha^{-1}/325 g.a.i. ha^{-1}) applied with a backpack sprayer delivering 177 l ha^{-1} at a constant pressure. The number of RWA infested tillers was determined on GS12 (Joubert, 1974) just before application of the foliar insecticide treatment and GS20 with onset of flowering. Each plot consisted of five 5-meter rows of which the inner three rows were harvested for yield determination.

Table 5.2 Agronomic characteristics of wheat cultivars used from 2003 to 2006

		Resistance characteristics		**Cultivar characteristics (ARC-Small Grain Institute, 2006 and 2007)**	
Cultivar	**Season**	**RWASA1 resistance gene**	**Resistance donor (Tolmay, 2008)**	**Wheat-type**	**Yield potential conditions**
Betta*	2003 to 2006	Susceptible	None	Winter	Medium
Komati	2003, 2004	*Dn-1*	PI 137739	Intermediate	Low to high
Matlabas	2005, 2006	*Dn-1*	PI 137739	Winter	Medium to high
SST 966	2003, 2004	*Dn-1*	PI 137739	Winter	Low to high
SST 334	2005, 2006	*Dn-1*	Intelectual property	Intermediate	Low to high
PAN 3364	2003 to 2006	*Dn-1*	Intelectual property	Winter	Medium to high

* Removed from the cultivar recommendation list for summer rainfall area

To be able to interpret the AMMI analyses accurately, mean monthly minimum and – maximum temperatures and total monthly rainfall were recorded simultaneously with RWA infestation data for every locality in each season. The seasonal infestation intensity was derived from the percentage RWA infested tillers at GS20 determined for the susceptible cultivar Betta, although infested tillers were determined for all the cultivars. Climatic data for Bethlehem was sourced from the ARC-Small Grain Institute weather station, Qwaqwa data was supplied by the AgroMet section of the ARC-Institute for Soil, Climate and Water in Pretoria and data for Glen College, Clocolan and Ficksburg was received from the South African Weather Service's historical database. No data was available for the Ladybrand area and rainfall data from Clocolan and temperature data from Ficksburg, respectively situated 35 kilometers (km) and 70 km from Ladybrand, was used as substitution. Long-term climatic data for the Bethlehem area was obtained from the SGI weather station and for Glen from the South African Weather Service's historical database. Yield data was analyzed by using the Additive Main effects and Multiplicative Interaction (AMMI) analyses available in Genstats (Genstats, 2005).

5.3 Results and discussion

5.3.1 Abiotic- and biotic stress, 2003 to 2006

5.3.1.1 Climatological trends

Monthly total rainfall in the 2003 season was marginal with total rainfall for Bethlehem during the pre-season period of January to May and in-season period of June to December showing a considerable deviation from the 1999 - 2003 average (Figure 5.1.1) for Bethlehem (Bhm). Total monthly rainfall for Ladybrand (Ldb) was normal during the pre-season and provided optimal planting conditions, though the below-normal total rainfall for the in-season period indicates that wheat plants probably were subjected to moisture stress at advanced growth stages.

A similar trend was observed for 2004 at Bethlehem and Glen (Gln), although the total rainfall in-season in Bethlehem was even lower than during 2003 (Figure 5.1.1). Glen is situated in the central Free State region and characterized by a lower annual rainfall compared to the eastern Free State. The pre-season rainfall for Glen in 2004 was below normal, similar to Bethlehem in 2003 and together with reduced total in-season rains, resulted in moisture-stressed wheat plants. Mean total rainfall for the pre-season and in-season periods at Bethlehem during 2005 - 2006 increased considerably from the tendency observed in 2003 - 2004. Although the total rainfall for the pre- and in- seasons in Qwaqwa (Qwa) during the 2005- and 2006 wheat seasons was less compared to Bethlehem the climatic trend for Qwaqwa, situated 75 km from Bethlehem was in accordance with the general pattern for Bethlehem.

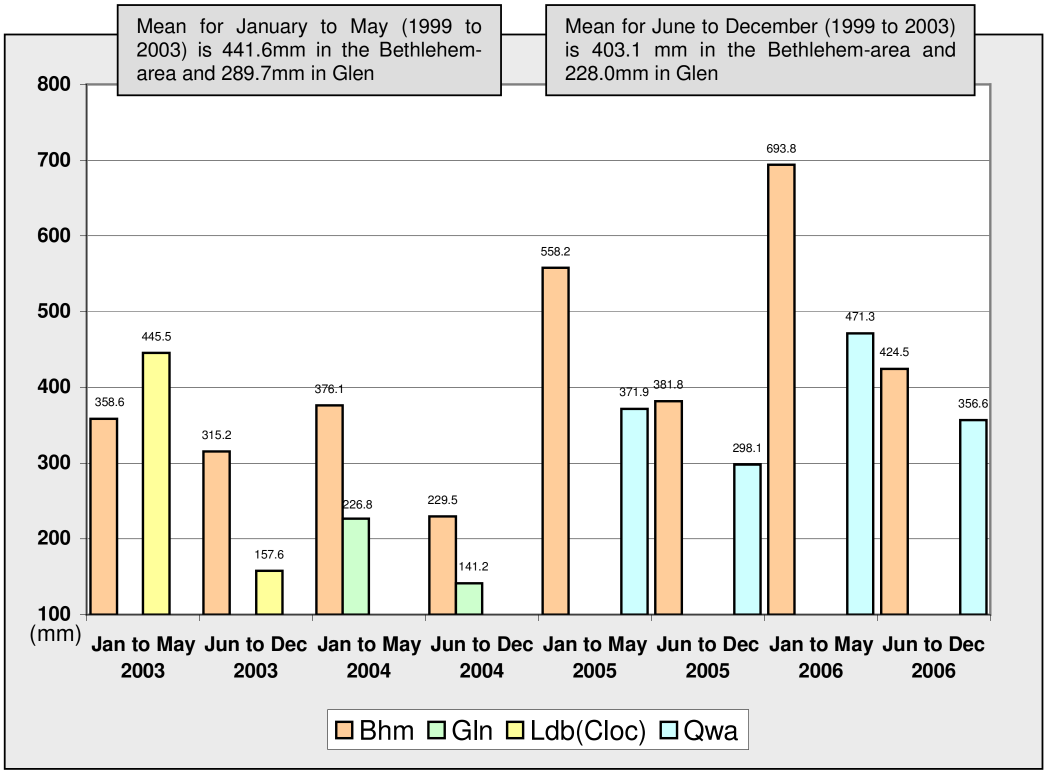
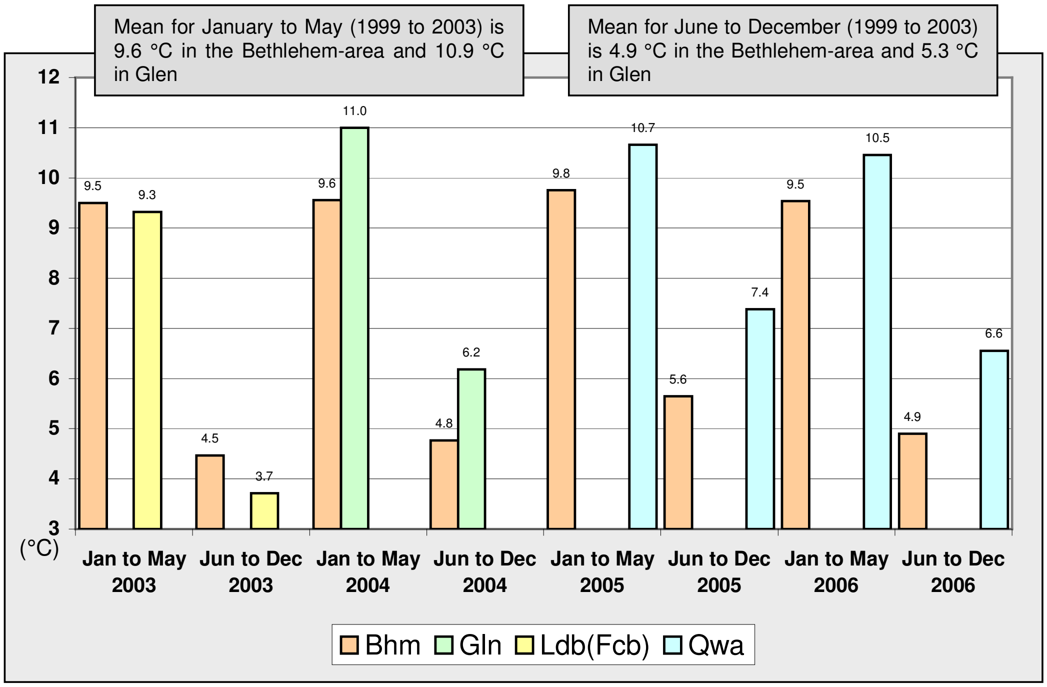


Fig 5.1.1 **Total monthly rainfall for 2003 to 2006 in Bethlehem, Glen, Ladybrand (Clocolan) and Qwaqwa**

Bethlehem and Ladybrand are in close proximity to the snow-covered peaks of Lesotho that significantly influence the minimum temperatures of the eastern Free State. The low mean monthly minimum temperatures in 2003 as a result, appear normal for January to December (Figure 5.1.2) although mean maximum temperatures for both localities were markedly higher than the 1999 - 2003 average in both pre-season and in-season periods (Figure 5.1.3).

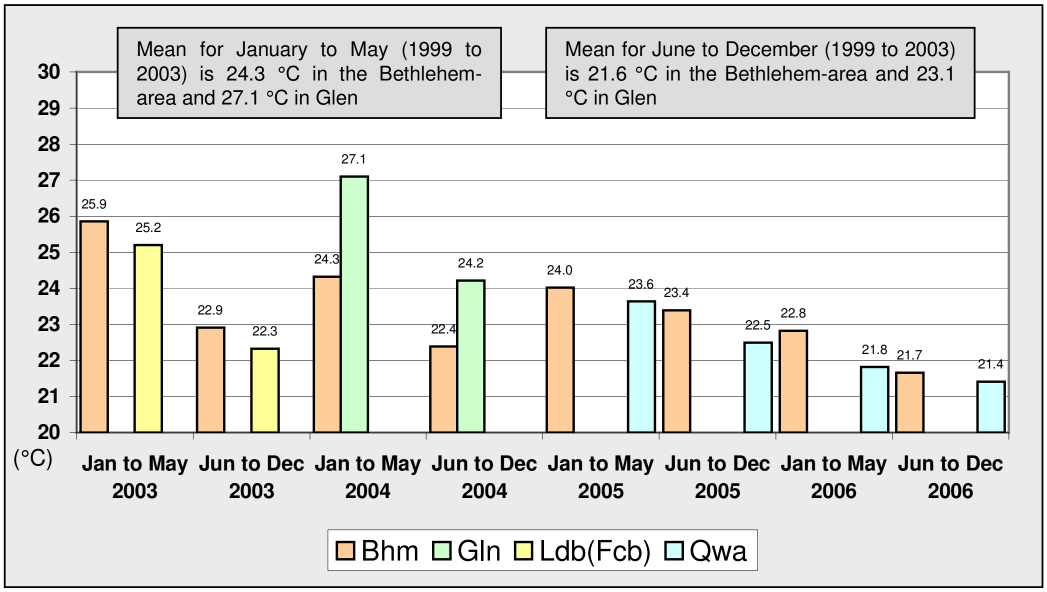
Mean monthly minimum- and maximum temperatures for Bethlehem in 2004 were very similar to the previous season (Figure 5.1.2) though pre-season maximum temperatures were cooler compared to the same period in 2003 (Figure 5.1.3). Mean monthly minimum- and maximum temperatures at Glen were significantly warmer than Bethlehem but below the 1999 - 2003 mean for the Glen area. In 2005 - 2006 the mean minimum temperatures during the pre-season for Bethlehem stayed similar to 2003 - 2004 but minimum temperatures for Qwaqwa during both pre- and in-seasons were warmer, especially during 2005 (Figure 5.1.2).



Mean for January to May (1999 to 2003) is 9.6 °C in the Bethlehem-area and 10.9 °C in Glen

Mean for June to December (1999 to 2003) is 4.9 °C in the Bethlehem-area and 5.3 °C in Glen

Fig 5.1.2 **Mean monthly minimum temperature for 2003 to 2006 in Bethlehem, Glen, Ladybrand (Clocolan) and Qwaqwa**



Mean for January to May (1999 to 2003) is 24.3 °C in the Bethlehem-area and 27.1 °C in Glen

Mean for June to December (1999 to 2003) is 21.6 °C in the Bethlehem-area and 23.1 °C in Glen

Fig 5.1.3 **Mean monthly maximum temperature for 2003 to 2006 in Bethlehem, Glen, Ladybrand (Clocolan) and Qwaqwa**

In 2005 and 2006, the mean maximum temperatures for both localities dropped in the pre-season, increased in the in-season compared to 2003 - 2004 and decreased more in 2006 compared to the previous pre- and in-season in 2005 (Figure 5.1.3).

5.3.1.2 RWA infestation intensity, 2003 to 2006

The percentage-infested tillers on Betta were used as an indicator of the RWA infestation intensity during each of the four seasons but were not analyzed statistically. During 2003 RWA occurrence at Ladybrand resulted in a 68.8% infestation rate of tillers of untreated Betta compared to 33.3% of tillers from seed-treated Betta (Figure 5.2.1). In Bethlehem RWA infested 41.6% of tillers of untreated Betta compared to 41.7% of seed-treated Betta. The high infestation levels on untreated and seed-treated Betta indicated significant RWA populations throughout early plant development augmented by late-season infestations as indicated by high infestation of seed-treated Betta.

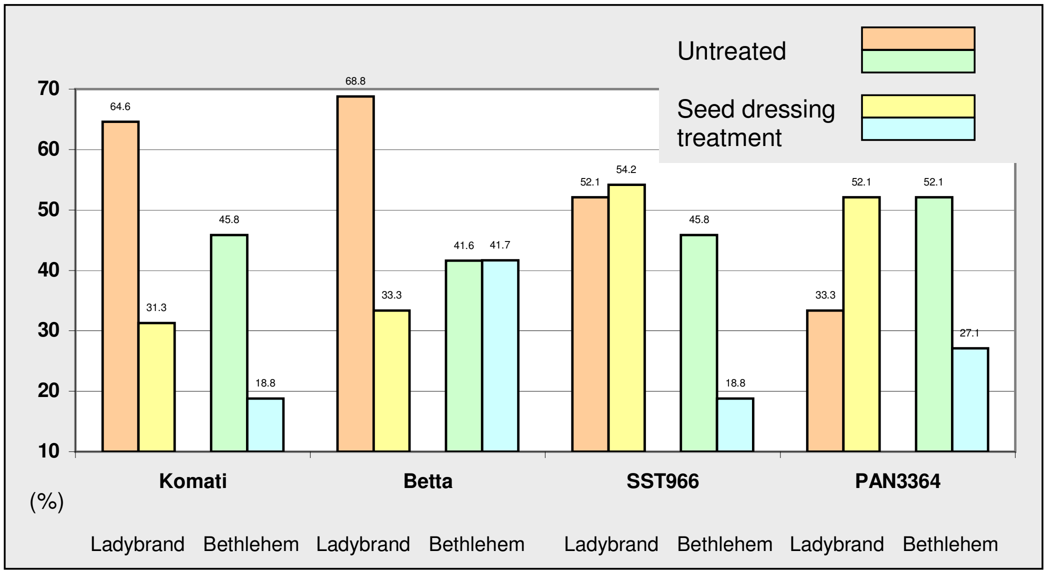


Fig 5.2.1 **Percentage infested tillers at GS20 during 2003**

In 2004 a similar trend was observed at Glen with 45.8% and 37.5% infestation levels on tillers of untreated- and seed treated Betta respectively (Figure 5.2.2). Percentage infested tillers of Betta in Bethlehem during 2004 was markedly lower at 29.2% for untreated and 8.3% for seed-treated Betta compared to 2003.

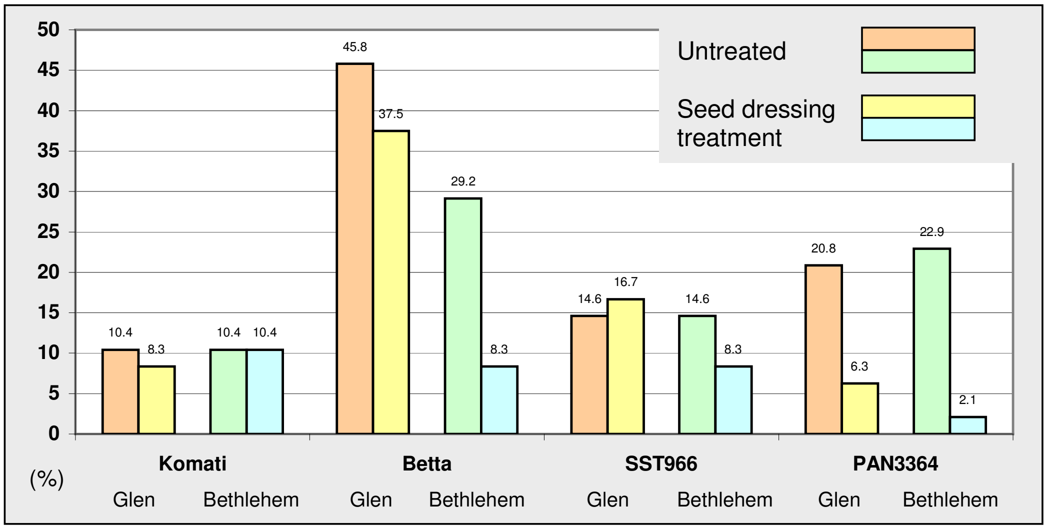


Fig 5.2.2 Percentage infested tillers at GS20 during 2004

RWA infestation during 2005 was notably lower than the previous two seasons with a 22.0% infestation of tillers for untreated Betta and 16.4% for seed-treated Betta in Qwaqwa whereas the infestation of untreated and seed-treated Betta at Bethlehem was nearly non-existant (Figure 5.2.3). The Qwaqwa infestation was relatively low and it would be expected that with an infestation level not as heavy as in the 2003 season host-plant resistance of the different cultivars would possibly express more effectively.

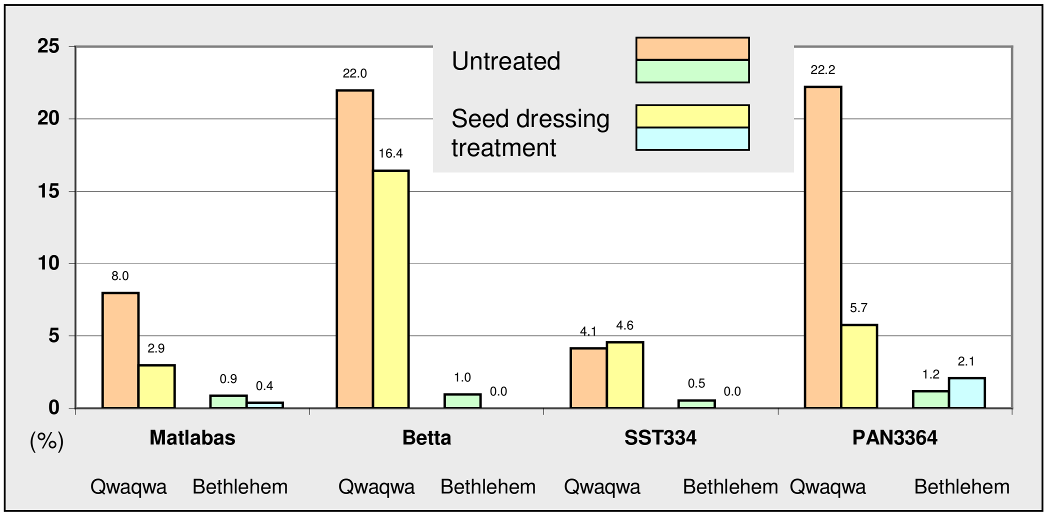


Fig 5.2.3 Percentage infested tillers at GS20 during 2005

In the 2006 wheat season 50% of tillers of untreated Betta and 22.2% of seed-treated Betta planted in Qwaqwa were infested by RWA indicating a high incidence of RWA throughout the plant development stages of wheat (Figure 5.2.4). Russian Wheat Aphid infestation of untreated (9.7%) and seed-treated Betta (13.9%) at Bethlehem was slightly higher compared to 2005.

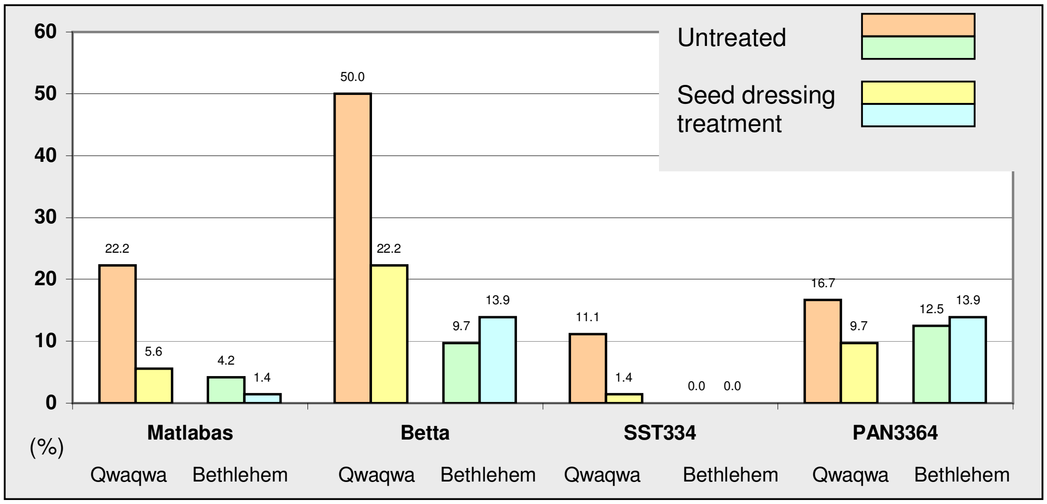


Fig 5.2.4 **Percentage infested tillers at GS20 during 2006**

Normal pre-season rainfall and minimum temperatures from January to May and above-average maximum temperatures and reduced in-season rainfall from June to December can describe the general climatic pattern during the 2003 and 2004 wheat seasons at Bethlehem, Ladybrand and Glen. Above average to high rainfall during the pre- and in-season together with milder pre-seasonal temperatures from January to May and warmer in-seasonal temperatures from June to December can categorize the climatic pattern for Bethlehem and Qwaqwa in 2005 and 2006. This trend would strongly indicate warmer winter months and cooler summer months to have occurred during 2005 and 2006 combined with relatively high rainfall. In order to obtain a more descriptive image of the rainfall and temperature trends during the four seasons each element was divided into three different categories.

The pre-seasons and in-seasons for 2003 – 2006 of the Eastern – and Central Free was categorized according to the 1999 to 2003 averages for Bethlehem and Glen. Deviations of 50mm for total monthly rainfall and 1°C for minimum – and maximum temperatures were applied to distinguish between above normal, normal and below normal conditions (Table 5.3).

Table 5.3 **Categorizing montly rainfall (mm), minimum temperatures (°C) and maximum temperatures (°C) based on the pre-season and in-season averages for 1999 - 2003**

		Pre-season (January to May)			In-season (June to December)		
Region		**Below normal**	**Normal**	**Above normal**	**Below normal**	**Normal**	**Above normal**
Eastern Free State **(Bethlehem, Ladybrand and Qwaqwa)**	Total montly rainfall	<392mm	≤492mm≥392mm	>492mm	<353mm	≤453mm≥353mm	>453mm
	Minimum montly temperature	<8.6°C	≤10.6°C≥8.6°C	>10.6°C	<3.9°C	≤5.9°C≥3.9°C	>5.9°C
	Maximum monthly temperature	<22.3°C	≤25.3°C≥22.3°C	>25.3°C	<20.6°C	≤22.6°C≥20.6°C	>22.6°C
Central Free State **(Glen)**	Total montly rainfall	<240mm	≤340mm≥240mm	>340mm	<178mm	≤278mm≥178mm	>278mm
	Minimum montly temperature	<9.9°C	≤11.9°C≥9.9°C	>11.9°C	<4.3°C	≤6.3°C≥4.3°C	>6.3°C
	Maximum monthly temperature	<26.1°C	≤28.1°C≥26.1°C	>28.1°C	<8.6°C	≤10.6°C≥8.6°C	>10.6°C

The occurrence of generally high RWA infestations during 2003 decreased towards 2005 but increased slightly in 2006. In most seasons and with the majority of cultivars, percentage infested tillers did not necessarily indicate yield loss from RWA feeding. A summary of the RWA infestation trends during the four seasons (Table 5.4) is based on the percentage infestations occurring on Betta not treated with any insecticide. The percentage RWA infested tillers were determined in field trials by counting the actual number of tillers exhibiting damage from RWA feeding and putting the data into context with the recommended economic injury level for dryland wheat. Du Toit (1986) determined the economic injury level (lowest infestation level at which economic damage will be caused) at 14% on Zadoks growth stage 59 (Tottman and Makepeace, 1979) and an economic threshold of 4% to 7% infested plants at Zadoks growth stage 31. The economic threshold refers to the infestation level at which chemical control will prevent an increasing pest from attaining the economic injury level. Current recommendations for wheat producers in South Africa are to spray for RWA at an infestation level of 14% for a crop with a yield potential of 1.0 to 1.5 tons ha^{-1} and 7% for a yield potential of 2.0 to 2.5 tons ha^{-1}. Categorization of RWA infestations during the field experiments are based on these recommendations and specify that the infestation is very high when the percentage infested tillers are >28%; a doubling of the recommended 14%. Percentage infested tillers are regarded as high when the infestation is between 14% and 28%, medium when between 7% to 14% and low when less than 7%.

Table 5.4 Intensity of abiotic- and biotic stress in 2003 to 2006

			Abiotic stress			Biotic stress	
						% RWA infested tillers	
Year	Locality	Time of season	Rainfall	Min temp	Max temp	GS 12	GS 20
2003	Bethlehem*	Pre-season	Below-normal	Normal	Above-normal	Very high	Very high
		In-season	Below-normal	Normal	Above-normal		
	Ladybrand	Pre-season	Normal	Normal	Normal	Very high	Very high
		In-season	Below-normal	Below-normal	Normal		
2004	Bethlehem	Pre-season	Below-normal	Normal	Normal	Very high	Medium
		In-season	Below-normal	Normal	Normal		
	Glen	Pre-season	Below-normal	Normal	Normal	Very high	Very high
		In-season	Below-normal	Above-normal	Above-normal		
2005	Bethlehem	Pre-season	Above-normal	Normal	Normal	Low	Low
		In-season	Normal	Normal	Above-normal		
	Qwaqwa	Pre-season	Below-normal	Above-normal	Normal	High	High
		In-season	Below-normal	Above-normal	Normal		
2006	Bethlehem	Pre-season	Above-normal	Normal	Below-normal	Medium	Medium
		In-season	Normal	Normal	Normal		
	Qwaqwa	Pre-season	Normal	Normal	Below-normal	Very high	High
		In-season	Normal	Above-normal	Below-normal		

[a] according to the Joubert (1974) growth point scale

Two important and major conclusions emerging from Table 5.4 are firstly that the 2003 and 2004 seasons differed distinctly from 2005 and 2006 due to the fact that abiotic- and biotic stresses occurred more frequently and at higher intensity in 2003 and 2004. A second trend is that below-normal rainfall in both in-seasons of 2003 and 2004 simultaneously occurring with above-normal maximum temperatures in Bethlehem and Glen invariably resulted in very high occurrences of RWA throughout the duration of the wheat season. These trends will be most important for the interpretation of the AMMI analysis.

5.3.2 AMMI (Additive Main effects and Multiplicative Interaction) analyses

5.3.2.1 Aphidicide and environmental effects

Analysis of variance presented in Table 5.4.1 indicate main effects of Aphidicide (A), Environment (E) and interaction Aphidicide x Environment (A x E) to have a significant effect on variance components of yield during the 2003- and 2004 wheat seasons. The main effect of environment contributed 93.6%, the aphidicide contributed 3.03% and the interaction of A x E contributed 3.41% to the total variation of treatment sum squares.

Table 5.5.1 ANOVA for main effects of aphidicides on stability of RWA resistance from 2003 to 2006

Source	2003/ 2004			2005/ 2006		
	df	ss	ms	df	ss	ms
Total	63	8.915	0.1415	63	39.53	0.627
Treatment	15	8.210	0.5474	15	35.52	2.368
Aphidicide	3	0.249	0.0830**	3	0.38	0.125
Environment	3	7.681	2.5604**	3	34.88	11.628**
Interaction	9	0.280	0.0312*	9	0.26	0.029
IPCA1	5	0.263	0.0525**	5	0.220	0.044
Residual	4	0.018	0.0045	4	0.04	0.010
Error	36	0.434	0.0121	36	2.24	0.062
Noise (%)	0.2% of the Treatment SS			0.1% of the Treatment SS		

* $P \leq 0.05$, ** $P \leq 0.01$

Interaction Principal Component 1 (IPCA1) was significant ($P \leq 0.05$) and this component accounted for 93.9% of the observed A x E interaction. In 2005 and 2006 only main effect of “E” significantly affected yield variance components from the four different aphidicide treatments and contributed 98.2% to the total variation of treatment sum of squares.

The 2003 and 2004 wheat seasons were subjected to adverse abiotic- and biotic stresses during which seed dressing + foliar insecticide (SD + FI) provided the best aphid control under high-yielding conditions. Foliar-applied insecticide (FI) and untreated control (CT) grouped into the low-yielding conditions with SD and FI providing the most stable yields (Figure 5.3). The prominence of seed dressing alone or in combination with foliar insecticides in establishing stable yield results against RWA accentuates the importance of protecting wheat plants during the early vegetative phases of plant development. Seed dressing treatment prevents RWA population build-up in wheat plants during the early spring months of August and September when populations on dryland wheat in the Free State increase sharply.

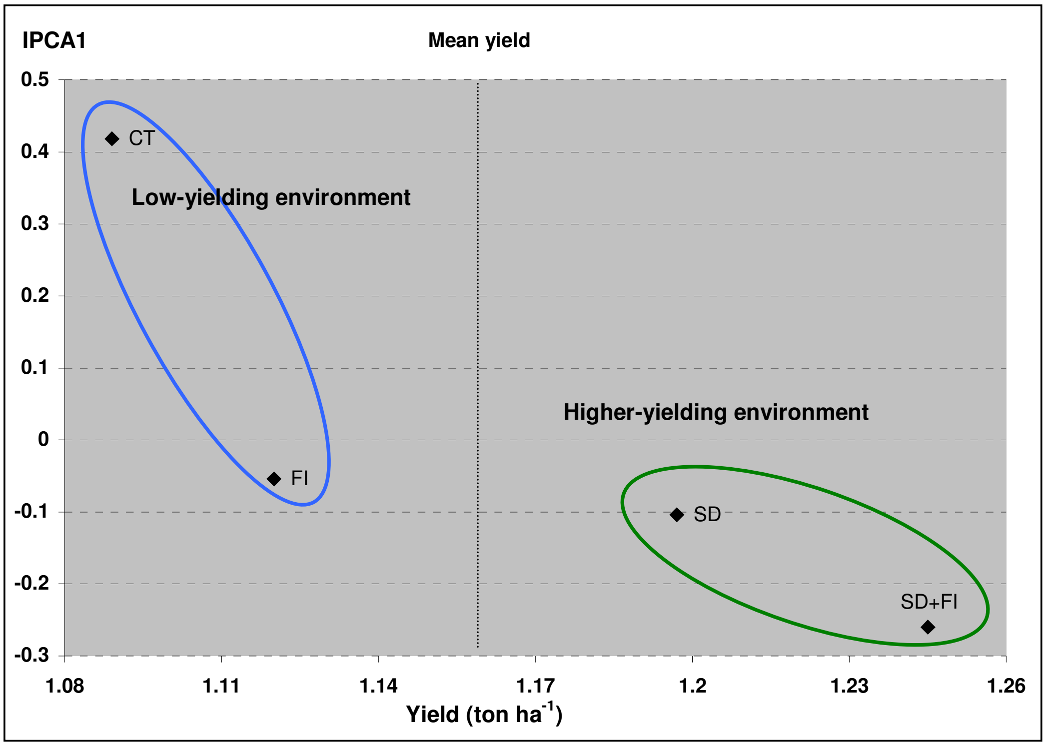


Figure 5.3 **Yield and stability of aphidicides during 2003 and 2004. CT-untreated control, FI-foliar-applied insecticide, SD-seed dressing and SD+FI-seed dressing and foliar insecticide**

In 2005 and 2006 aphidicides and the interaction of A x E were insignificant, probably resulting from optimal conditions for plant development occurring throughout the season. In an optimal season with very few abiotic constraints host plant resistance can prevent yield loss from RWA even when high infestation levels of the pest occur on wheat plants.

5.3.2.2 Cultivar and environment effects

During 2003 and 2004, cultivars showed no significant interaction with environment; the main effects of environment (E) contributed 94.92% and cultivars (C) 3.69% to the variance components. Marginal conditions for plant development in 2003 and 2004 arrested adaptation competence of cultivars in specific environments consequently also reducing the ability to obtain optimal yields.

The 2005 and 2006 wheat seasons provided favourable conditions for optimal plant development and much higher yields were obtained by the four cultivars (Table 5.4.2). Stability or adaptive ability of each cultivar over environments was an important factor with interaction of C x E being significant and contributing 14.3% to the total sum of squares. Main effects of cultivar and environment were also significant with cultivar contributing 1.3% and environment contributing 84.5% to the total variation sum of squares.

Table 5.5.2 ANOVA for main effects of cultivars on stability of RWA resistance from 2003 to 2006

Source	2003/ 2004			2005/ 2006		
	df	ss	ms	df	ss	ms
Total	63	8.759	0.1390	63	44.41	0.705
Treatment	15	8.011	0.5341	15	41.33	2.756
Genotype	3	0.296	0.0987**	3	0.52	0.172**
Environment	3	7.604	2.5346**	3	34.91	11.637**
Interaction	9	0.111	0.0123	9	5.91	0.656**
IPCA1	5	0.263	0.053	5	4.62	0.924**
IPCA2				3	1.23	0.410**
Residual	4	0.020	0.005	1	0.06	0.057
Error	36	0.473	0.013	36	1.320	0.037
Noise (%)	0.3% of the Treatment SS			0.1% of the Treatment SS		

* $P \leq 0.05$, ** $P \leq 0.01$

Although no significant C x E interaction occurred, SST 966 and PAN 3364 were more stable than Betta (susceptible to RWA) and Komati (Figure 5.4.1) and yielded above the mean of the four cultivars. This tendency can be the result of effective expression of host plant resistance to RWA under optimal growing conditions, particularly in comparison with Komati which also contains *Dn-1* resistance to RWA.

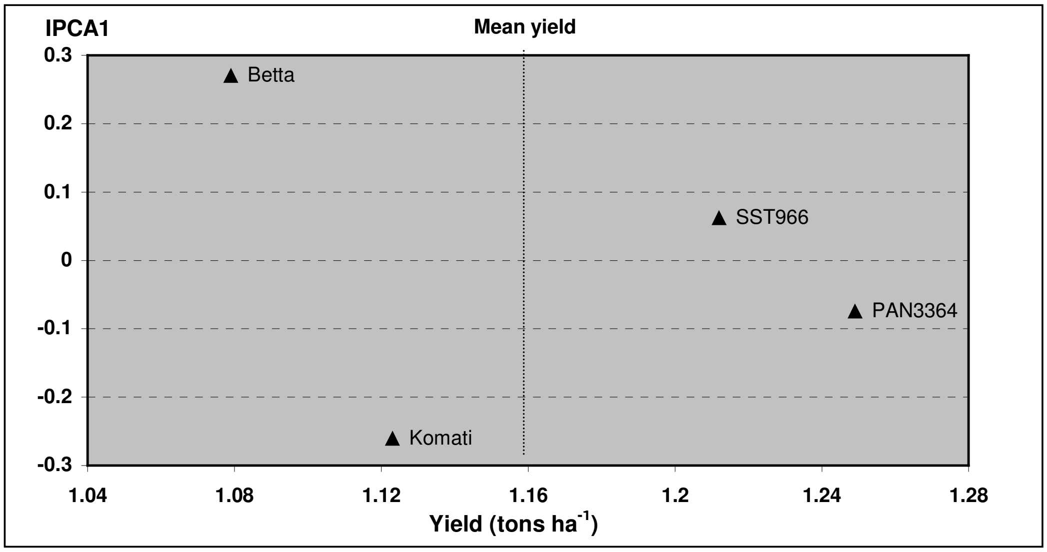


Figure 5.4.1 Yield and stability of cultivars during 2003 and 2004

During 2005 and 2006 ASV (AMMI Stability Value) was used to interpret the effect of the C x E interaction on yield, as both IPCA1 (<0.001) and IPCA2 (<0.001) were significant. ASV introduced by Purchase *et al.* (2000b) is defined as the distance from zero on a two-dimensional scattergram of IPCA1 against IPCA2 scores. ASV correlates well with the stability measures of Eberhart and Russel (1966), Wricke (1962) and Shukla (1972) and scores close to zero are an indication of good stability.

Optimal plant development during 2005 and 2006 was reflected by satisfactory grain yield of the susceptible cultivar Betta during low abiotic stress, particularly in combination with chemical control strategies. According to the ASV, Betta and SST 334 were the two most stable varieties (Figure 5.4.2). Matlabas is a new, facultative-type cultivar with a planting period from the second week in May to the end of June. The tendency of this cultivar to be unstable and perform below the mean yield of the four cultivars for 2005 and 2006 emphasizes the specific adaptability requirement for certain environments, particularly in optimal seasons such as 2005 and 2006.

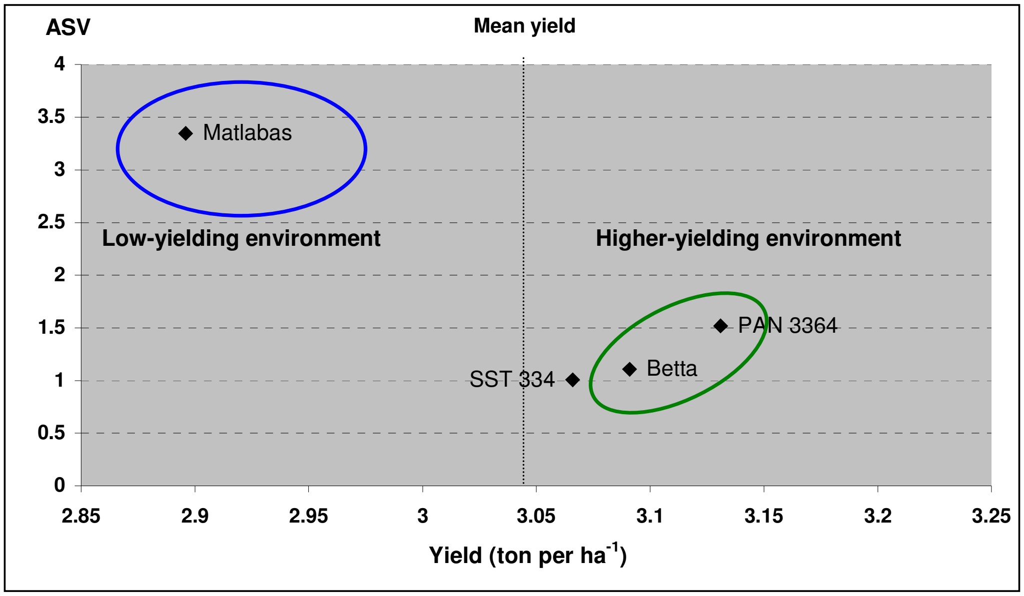


Figure 5.4.2 Yield and stability of cultivars during 2005 and 2006

5.3.2.3 Genotype (cultivars/aphidicides) and environment interaction

The AMMI analyses of variance for G (cultivar/aphidicide) x E combinations indicated that genotype; G (<0.001) and environment; E (<0.001) main effects and G x E (<0.05) interaction were significant for the 2003 and 2004 seasons (Table 5.4.3). The main effect of environment contributed 82.3% to the total variation in contrast to genotype and G x E interaction each contributing 8.9% to the total variation. During the 2005 and 2006 seasons, the same tendency was observed with the effect of environment being highly significant and contributing 80.5% to the total variation of sum of squares compared to 2.6% of genotype and 16.9% of G x E interaction. IPCA1 explained 60.5% and IPCA2 30.4% of the G x E interaction sum of squares in 2003 and 2004. As IPCA2 was insignificant in explaining G x E interaction during 2003 and 2004, IPCA1 would provide the major interpretation of G x E interaction. During 2005 and 2006 IPCA1 explained 69.85% and IPCA2 22.22% of the G x E interaction. Both IPCA1 and IPCA2 were significant and ASV was used to determine the most stable cultivar/treatment combination, as both IPCA1 and IPCA2 were significant (Table 5.4.3). In the context of the previous discussion, the combination of RWA resistant cultivars and aphidicides is expected to stabilise yields during 2003 and 2004 whereas RWA resistant cultivars should be more stable in the more favourable conditions in 2005 and 2006. The large effects of environment in 2003/2004 (82.3%) and in 2005/2006 (80.5%) indicate the enormous influence of the environment on the effectiveness of the different cultivar/aphidicide treatments during both periods. The contribution of 16.9% of G x E interaction during 2005 and 2006 signifies the positive response of cultivar adaptation to the more favourable growing conditions that prevailed during both seasons.

Table 5.5.3 ANOVA of RWA resistance of cultivars/aphidicides from 2003 to 2006

Source	2003/ 2004				2005/ 2006		
	df	ss	ms		df	ss	ms
Total	255	46.66	0.183		255	218.61	0.857
Treatment	63	37.14	0.590		63	173.81	2.759
Genotype	15	3.29	0.220**		15	4.59	0.306
Environment	3	30.56	10.187**		3	139.83	46.611**
Interaction	45	3.29	0.073*		45	29.39	0.653**
IPCA1	17	1.99	0.117**		17	20.53	1.208**
IPCA2	15	1.00	0.067		15	6.53	0.435*
Residual	13	0.29	0.022		13	2.33	0.179
Error	180	8.41	0.047		180	37.78	0.210
Noise (%)	0.8% of the Treatment SS				1.3% of the Treatment SS		

* P≤0.05, ** P≤0.01

In Figure 5.5.1 quadrant III describes yield and stability under higher-yielding conditions and quadrant I yield and stability under lower-yield conditions. SST 966/Seed Dressing + Foliar Insecticide, PAN 3364/Seed Dressing and Komati/Seed Dressing + Foliar Insecticide performed best under higher-yielding conditions with PAN 3364/Seed Dressing being the most stable. Understandably Betta, in combination with a foliar insecticide or untreated, grouped together in the lower-yielding environment as it does not contain any host-plant resistance to RWA and, as discussed previously, showed the effect of RWA feeding damage during the two seasons. Komati/Untreated Control also grouped in the low-yielding environment and although containing *Dn-1* resistance, was unable to express host-plant resistance to the same extent as SST 966 and PAN 3364. Betta treated with a foliar insecticide was the most stable combination in 2003 and 2004, probably due to the fact that primary damage from RWA on susceptible cultivars occurs between growth stages 13 to 15 rendering foliar-applied insecticides a more effective option. Expression of host-plant resistance was the most stable in PAN 3364 and yield of PAN

3364/Untreated Control was above the mean for all the cultivar/aphidicides combinations. Under the specific environmental conditions prevailing during 2003 and 2004, resistance of PAN 3364 would have been effective under higher-yielding conditions and confirm the ability of resistant cultivars to achieve yields comparable to chemical treatment of RWA. However, the grain yield increases achieved by the modernday, high-yielding varieties PAN 3364 (19.84%) and SST 966 (11.73%) containing RWA resistance compared to susceptible Betta implies that yield increase is the result of collective genetic progress comprising improvement of yield components but also expression of host plant resistance.

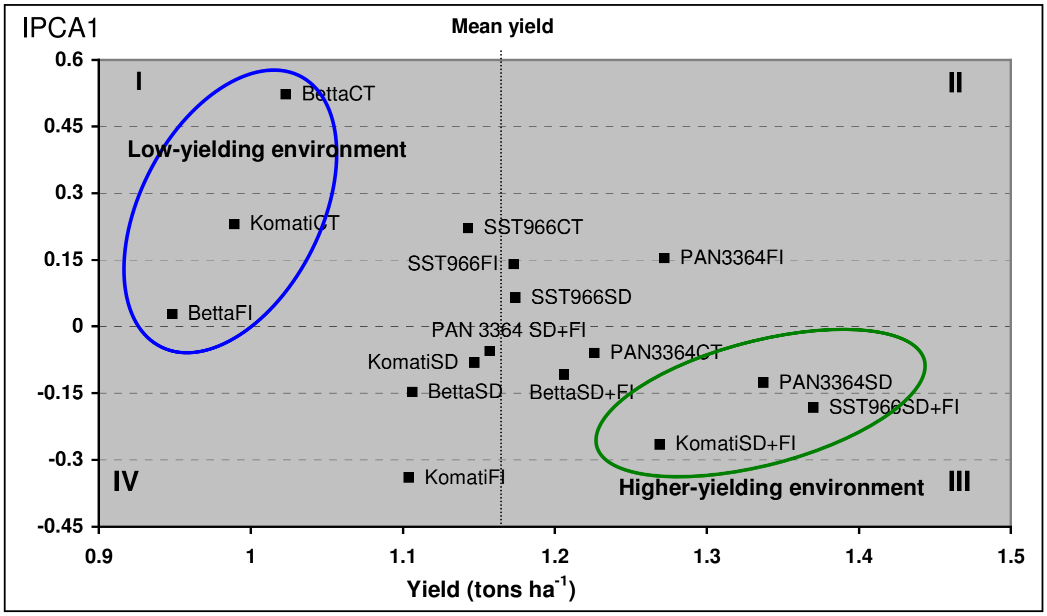


Figure 5.5.1 **Yield and stability of genotypes (cultivars/aphidicides) in 2003 and 2004. CT- untreated control, FI-foliar-applied insecticide, SD-seed dressing and SD+FI-seed dressing and foliar insecticide**

ASV scores for 2005 and 2006 indicate a very similar trend as in 2003 and 2004 with the susceptible check Betta in combination with chemical insecticides, performing similarly to wheat cultivars with genetic resistance against RWA (Figure 5.5.2). Favourable conditions are reflected by the mean yield increasing from 1.16 ton ha^{-1} in 2003 and 2004 to 3.05 ton ha^{-1} in 2005 and 2006. Matlabas generally grouped into the low-yielding environment regardless of aphidicide treatment because of specific adaptation requirements. This response emphasizes the important fact that a cultivar not adapted for a specific environment will probably exhibit a reduced level of plant resistance to RWA. PAN 3364 SD+FI, Betta SD+FI and Betta SD achieved the highest yields whereas SST 334 SD and Betta FI were the two most stable combinations over environments. A general conclusion of the 2005 and 2006 seasons is that the difference between the yields of SST 334 (9.16%) and PAN 3364 (7.59%) and RWA susceptible Betta were less than in 2003 and 2005 under severe RWA infestation.

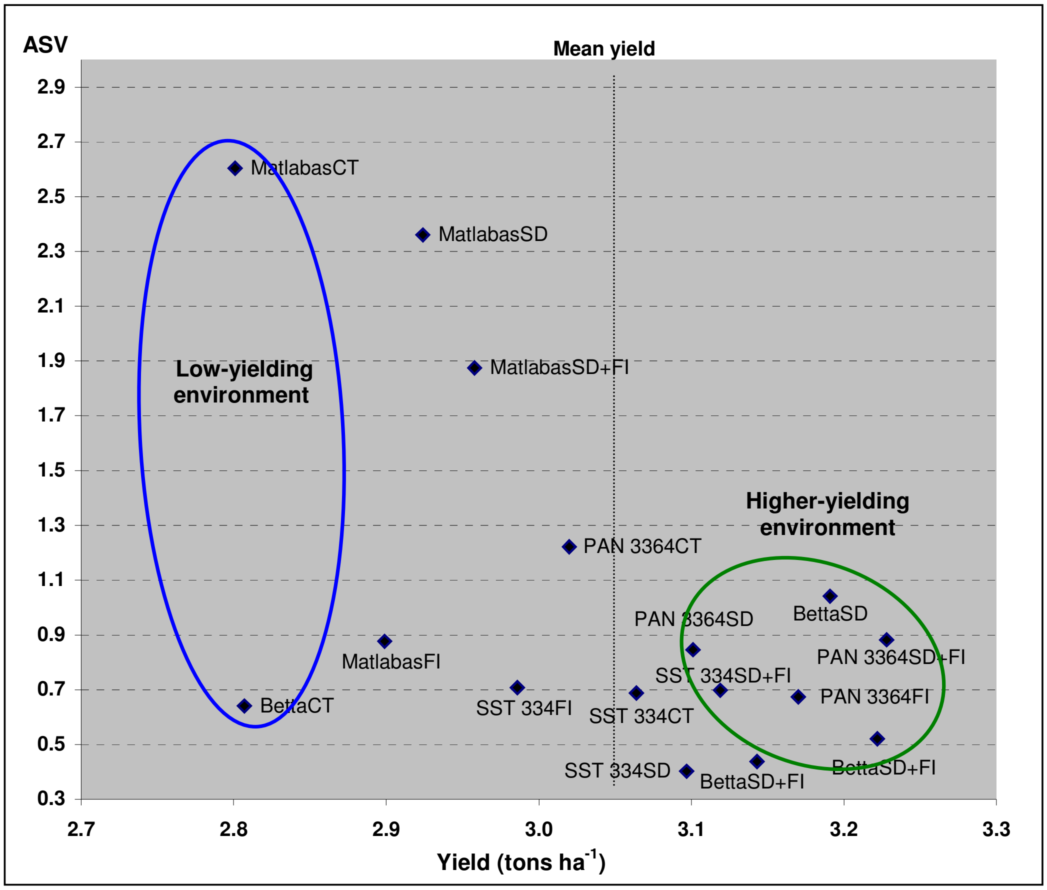
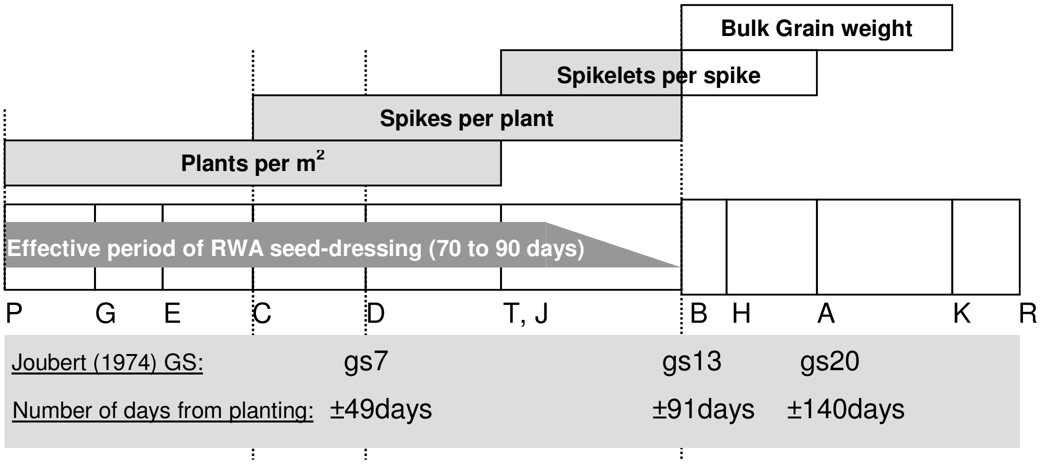


Figure 5.5.2 **Yield and stability of genotypes (cultivars/aphidicides) in 2005 and 2006. CT- untreated control, FI-foliar-applied insecticide, SD-seed dressing and SD+FI-seed dressing and foliar insecticide**

5.4 Conclusions

AMMI results of aphidicide- and cultivar/aphidicide treatments indicated the ability of seed dressing (Imidacloprid) to increase yields under higher-yielding conditions and also stabilize yield performance of cultivars irrespective of whether or not they contain genetic resistance against RWA. An obvious deduction will be that the two- to three month protection period provided by imidacloprid during the initial stages of plant development indicates that it is more critical to prevent RWA damage in the early stages of plant growth in the latter part of plant development. Though production conditions are very different in the Eastern Free State than production conditions in Colorado, Montana, Texas and Washington in the USA, our results followed the trend reported by Archer *et al.* (1998) who concluded that infestation in the fall in these four states resulted in reduced bulk grain weight and number of spikes compared to the reduction in number of seeds per spike, 200-seed weight and foliage dry weight when wheat plants were only infested in the spring. Though plants infested in spring were capable of producing the same number of spikes as uninfested plants, the quantity and quality of seed were reduced. Wilde *et al.* (2001) confirmed this tendency and found that imidacloprid and thiamethoxam can provide excellent early season (fall) control of greenbugs *(Schizaphis graminum)* and RWA *(Diuraphis noxia)* but was less effective and inconsistent for spring infestations. For a possible explanation, the various yield components of wheat and their relation to seed dressing must be brought into this discourse (Figure 5.6).



P = planting
G = germination
E = emergence
C = tillering begins
D = double ridge
T = terminal spikelet
J = jointing
B = boot begins
H = heading begins
A = anthesis
K = grain fill ends
R = harvest ripe

Figure 5.6 Developmental events in summer rainfall wheat in relation to seed-dressing efficacy (adapted from Müller, 2004 and ARC-SGI Production guide, 2008)

Early infestations of RWA or the absence thereof will not affect plant density per square meter but indications from the schematic diagram (Figure 5.6) are that number of spikes per plant and, to a lesser extent, number of spikelets per spike will be affected. Trends from the AMMI results, particularly during 2003 and 2004, indicate that wheat cultivars are generally more prone to yield reductions from RWA damage at an early growth stage when spikelet initiation is determined, eventually determining bulk grain weight at harvest.

The significance of aphidicide x environment interaction during incidence of high abiotic stresses in 2003 and 2004 indicate that host plant resistance will be more essential for shielding grain yield under suboptimal growing conditions (Figure 5.7). Starý and Lukášová (2002) discovered that RWA not previously considered as an injury-increasing factor in barley, contributed significantly to yield loss in drought-stressed barley.

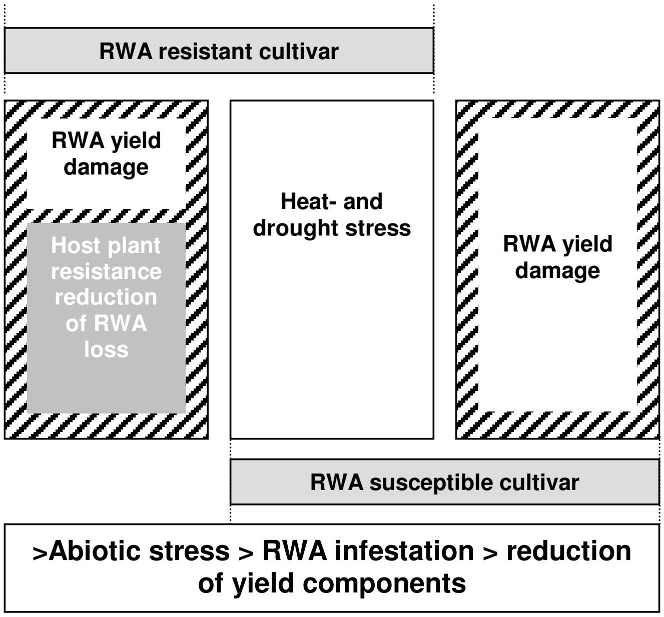


Figure 5.7 **Schematic presentation of the role of host plant resistance during drought seasons in prevention of RWA damage**

Also supporting this finding is that, in contrast to 2005 and 2006, Bethlehem, Ladybrand and Glen experienced very high RWA infestation levels throughout the duration of abiotically stressed plant development in 2003 and 2004. Though moisture- and heat stress in wheat is conducive to RWA population development and consequent plant injury resulting in grain yield loss, host plant resistance maintains its ability to reduce RWA damage under conditions when efficacy of insecticides and particularly foliar applications become inconsistent due to large-scale moisture stress.

To conclude, a strategy for resistance breeding against RWA will benefit from investigating genetic variance and expression of host plant resistance to RWA during the first half of the reproductive phase in plant development. Evaluation of RWA resistance donors for adaptation in diverse environments can also ensure effective expression of RWA resistance during seasons exposed to abiotic stresses.

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CHAPTER 6

Identification and exploitation of germplasm with resistance against Russian wheat aphid *(Diuraphis noxia)*, biotype RWASA2

6.1 Introduction

Russian wheat aphid (RWA) can cause significant losses to the general wheat industry; e.g. Morrison and Peairs (1997) assessed related costs from the introduction of RWA in the US from 1986 to 1994 at $1 billion. Consistent genetic susceptibility of the North American wheat cultivars instigated an intensive campaign by the Western Regional Coordinating Committee No. 66 (WRCC-66) to coordinate the identification and development of RWA resistance in wheat cultivars. Screening of the USDA – wheat germplasm collection (WERA-066, 2006) indicated that the bulk of accessions with high incidence of repeatable resistance to RWA originated from Central Asia and particularly Iran (38%), the adjacent countries of Afghanistan and the former USSR (35%) and Mexico providing 13% of the total RWA resistance accessions; largely resulting from improved triticale genotypes; carrying resistance from the rye (*Secale cereale* L.) genome and barley breeding lines (Souza, 1998). The most resistant sources were identified in tertiary germplasm of landraces and old local cultivars (62% for wheat and 70% for barley) but species of wild and related crops are likely additional resistance sources not yet fully exploited. The original outbreak of RWA in 1978 in Bethlehem, South Africa required extensive insecticide treatment to prevent crop failure (Walters, 1984) as all cultivars were highly susceptible. Du Toit and Van Niekerk (1985) first reported host plant resistance to RWA in South Africa by identifying resistance in *Triticum monococcum* (Einkorn), line A 544 and *T. monococcum*/*T. durum* amphiploids. Du Toit (1987) extended this work and identified *D. noxia* resistance in PI 137739, PI 262660 and PI 294994. Seven of the ten resistance genes identified to date (Table 6.1); *Dn-1* (Marais and Du Toit, 1993), *Dn-2* (Ma *et al.*, 1998), *Dn-5* (Marais and Du Toit, 1993), *Dn-6* (Liu *et al.*, 2002) and *Dn-8*, *Dn-9* and *Dn-x* (Liu *et al.*, 2001) are located on the 7D chromosome of wheat. *Dn-4* is located on 1D (Ma *et al.*, 1998), *dn-3* was found in an *Aegilops tauschii* diploid D-genome line and *Dn-7* on a 1BL/1RS translocation from rye (Marais *et al.*, 1998).

Table 6.1 **Wheat accession numbers and origin of *Diuraphis noxia* (Kurdjumov) resistance reported in literature**

Proposed gene	Accession (S. African identity)	Origin	Resistance level	Source species	Remarks	Literature reference
Dn-1	PI 137739 (SA1684)	Iran	MR/R	*T. aestivum*	AB/AX	Du Toit, 1989
	PI 140207	Iran	MR	*T. aestivum*	AB/T (Baker *et al.*, 1992)	Porter *et al.*, 1997
Dn-2	PI 262660 (SA2199)	USSR	R	*T. aestivum*	AB/T	Du Toit, 1989
dn-3	SQ 24			*T. tauschii*		
Dn-4	PI 372129	USSR	R/MR	*T. aestivum*	Single gene	Saidi and Quick, 1996
Dn-5	PI 294994 (SA463)	Bulgaria	R	*T. aestivum*	Hetero-zygous accession	Zhang *et al.*, 1998
Dn-6	PI 243781	Iran	MR/R	*T. aestivum*		Saidi and Quick, 1996
	Yilmaz-10	Turkey		*T. aestivum*	Single gene	Dong and Quick, 1995
Proposed *"Dn-7"*	Shz.W-104	Iran	R	*T. aestivum*	T (AB, AX)	Assad and Dorry, 2001
Dn-7	Turkey 77 (GamtoosR)			*Secale cereale*	Single gene	Marais *et al.*, 1994
Dn-8	PI 294994 (SA463)	Bulgaria	R	*T. aestivum*		Liu *et al.*, 2001
Dn-9	PI 294994 (SA463)	Bulgaria	R	*T. aestivum*		Liu *et al.*, 2001
Dn-x	PI 220127	Afghanistan	R	*T. aestivum*		Liu *et al.*, 2001
Dn-y	PI 220350	Afghanistan	MR	*T. aestivum*		Harvey and Martin, 1990

Adapted from Souza (1998), Lage (2003) and Tolmay (2006); AB-antibiosis, AX-antixenosis, T-tolerance.

Farmer reports of failing host plant resistance to RWA in the eastern Free State in 2005 lead to the discovery of a new biotype virulent on all commercially released wheat cultivars in the summer rainfall production area (Tolmay *et al.*, 2007). The introduction of the new RWA biotype designated as RWASA2 compelled the redirection of the entire RWA pre-breeding programme at the ARC-Small Grain

Institute (ARC-SGI), commencing with identification of new resistant donor accessions. The first of three major donor pools available shortly after identification of RWASA2 were ±200 breeding lines selected from the ARC-SGI pre-breeding programme for evaluation against prevalent US RWA biotypes in a collaborative shuttle-breeding project at Stillwater, Oklahoma. Several of these lines resulted from combinations of South African cultivars/lines with Halt (*Dn-4*), CI 6501(*Dn-6*), Stanton (*Dn-y*), CItr 2401 and OR52 with added advantages that adaptability was largely established and some of these lines may exhibit resistance against North American biotypes. The second pool became available in the latter part of 2007 in the form of the Russian Wheat Aphid Screening Nursery (RWASN) received from the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico with 55 entries including synthetic hexaploids and combinations with *A. tauschii*. Lage *et al.* (2004) reported that as resistance in the synthetic hexaploids is derived from *T. dicoccum*, resistance gene(s) must be located on either the A- or B genome or both and suggests new types of resistance to RWA. The third pool was a consignment of donor accessions received from Iran in exchange for South African breeding material. Unfortunately detailed information stipulating genetic pedigree and make-up of these RWA resistance accessions was not available.

This study had the objectives to 1) subject seedlings from all entries of the three pools to a standard seedling-screening test for resistance against RWASA2 and 2) divide all entries from the three pools into adapted- or unadapted categories through field evaluation under prevalent conditions of the eastern Free State in 2007.

6.2 Material and methods

6.2.1 Greenhouse seedling screening test

6.2.1.1 Plant material

Entries were sown inside plastic seedling trays filled with a sandy-loam soil mixed beforehand with the appropriate amount of fertilizer for optimal germination and plant growth. At planting the soil surface was thinly covered with a layer of moist fine sand to facilitate drilling of eighty holes (eight by ten rows) in each tray. Each of the holes was ±20mm in diameter and 10mm deep and received a single seed that was covered with moist sand after planting. The screening was unreplicated and every tray consisted of six entries, a susceptible (Betta) - and resistant (CItr 2401) check each comprising one row of ten seeds each. All trays were placed inside water flats filled with an adequate amount of water to enable sub-irrigation of plants through capillary movement in the soil. Seedlings were grown under controlled environmental conditions of 20°C/18°C at a 16h/8h day/night regime with natural light and infested at the one- to two leaf stage with four – to five aphids per seedling.

6.2.1.2 Aphid populations

RWASA2, *Diuraphis noxia* (Kurdjumov) was collected from the Reitz district in the eastern Free State, during the 2005 wheat season. A colony was established in the greenhouse from cloning aphids from original field samples on susceptible wheat cultivar Betta. Enough aphids to provide an infestation level of four- to five aphids per plant (mean of 0.0408 grams for 400 aphids) were weighed on a laboratory scale and sprinkled evenly over each tray. Seedlings were rated three weeks after infestation and scored visually according to the scale of 1 to 10 (Figure 6.1) developed by Tolmay (1995). Seedlings with a score of $> 1 \leq 3.5$ is considered as highly-resistant, $>3.5 \leq 6.5$ as medium-resistant and > 6.5 as susceptible to RWASA2.

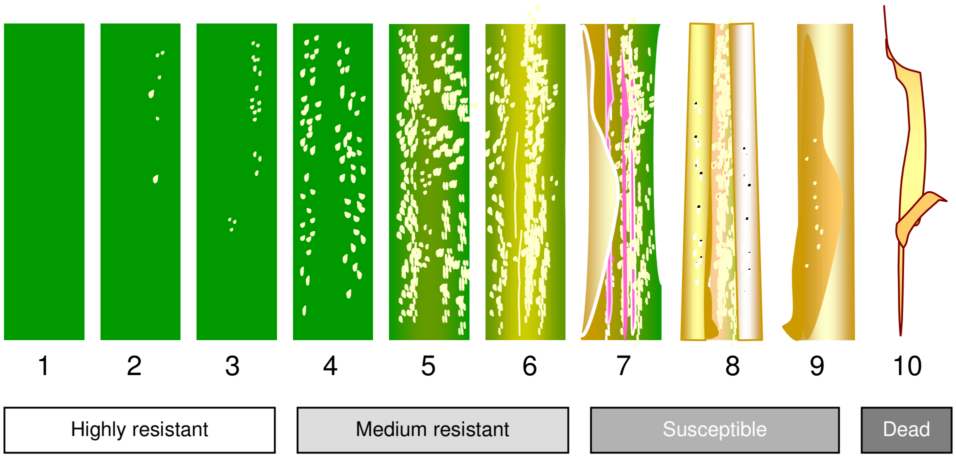

Figure 6.1 **Evaluation scale for visual damage of RWA to seedlings (Tolmay, 1995)**

6.2.2 Field trials

Non-replicated field trials were planted in the eastern Free State on a farm in the Reitz district, at ARC-SGI near Bethlehem and in Lesotho at Makoabating in close proximity to Mokhotlong. The eastern Free State trials were planted with a modified commercial planter adapted for planting of experimental plots or in this case, single rows of five-metres. Standard seeding rate of 20 kg ha^{-1} and fertilizing rate of 40 kg N ha^{-1} were applied during planting. Plant diseases and RWA were allowed to develop without any pest control during the season. Experimental blocks at both localities were covered with plastic netting for protection against bird damage (Knittex 30 white, with a shade factor of 12% and a UV block of 30) from the onset of anthesis. Low seed quantity and remoteness of the Makoabating locality hampered transport of planting equipment therefore entries were planted as hill plots and only used as seed increase. In all three localities RWA infestations developed from natural infestations enabling determination of percentage of tillers infested with RWA. General agronomic characteristics and adaptability were gathered throughout plant development at all three localities until rows were harvested by hand, machine-threshed and cleaned for determination of seed quality and grain mass.

6.3 Results and discussion

6.3.1 Resistant- and susceptible checks

The three checks consisted of the susceptible commercial wheat cultivar Betta and two RWA resistant cultivars, Komati and Elands (respectively resistant checks 1 and 2). Seed quality and grain yield obtained at both localities were high for all three checks and any negative impact of biotic stresses such as RWA, as indicated by the relatively high percentages of infested tillers were compensated for by favourable growing conditions (Table 6.2).

Table 6.2 Seedling resistance and field performance of the RWASA1 resistant- and susceptible checks

Entry	Seedling evaluation (RWASA2)[a]		Field evaluation					
			% Infested tillers		Seed quality		Grain mass (grams)	
	Score	Rating	Bethlehem	Reitz	Bethlehem	Reitz	Bethlehem	Reitz
Susceptible check	9.21	S	40	40	Good	Good	1320.36	1785.41
Resistant check 1	7.9	S	60	80	Good	Good	827.23	1501.31
Resistant check 2	7.2	S	60	60	Good	Good	1439.68	1251.76

[a] -Tolmay *et al.* (2007)

6.3.2 Stillwater, Oklahoma/SGI RWA resistance pool

6.3.2.1 Seedling evaluation

Table 6.3 lists the 21 entries that expressed a resistant response against RWASA2 in the seedling test. Entry 67 contains *Dn-1* resistance through donor accession PI 137739 combined with resistance from AUS22498; a *T. vavilovii* derived RWA resistance donor that is medium-resistant to RWASA2. Entry 74 has *Dn-1* resistance from PI 137739 combined with *Dn-2* resistance through PI 262660 but is the only remaining entry from the bulk of the specific crossing combination expressing resistance to RWASA2. Entries 120 to 194 and 720 and 726 are from different crossing combinations between similar RWA resistance sources, PI 294994 (*Dn-5*) and CItr 2401. Of the total number of entries in this resistance pool 61.9% expressed resistance to RWASA2. The four entries of 1106 to 1113 from a crossing combination utilizing RWA resistance from PI 137739 (*Dn-1*) and CItr 2401 provided an additional 19% of resistance to RWASA2 in this pool. A crossing combination with resistance from OR52 (OSU ID2808) and CItr 2401 formed the RWA resistance donors for the two remaining entries; 1472 and 1474; which both express medium resistance against RWASA2.

6.3.2.2 Field evaluation

If grain mass (vegetative growth stages) and seed quality (reproductive growth stages) are considered as indicators of occurrence of abiotic- and biotic stress in a season, more favourable climatic conditions existed at the Reitz locality than Bethlehem (Table 6.3). The detrimental effect of above-normal precipitation at Bethlehem resulting in leaching of nutrients, plant lodging and high incidence of wheat diseases; in particularly stripe rust caused by *Puccinia striiformis Westend f.sp tritici Eriks.,* which was evident in the seed quality and grain mass of several entries. Though no data was gathered in this regard, a number of entries resulted in generally tall plants that in the case of the Bethlehem locality were prone to lodging. Considered overall, enough seed was generated for all entries resistant to RWASA2 to allow new crosses or direct inclusion into the main breeding programme.

Table 6.3 **Seedling resistance and field performance of the Stillwater, Oklahoma/SGI pool**

Entry	Seedling evaluation (RWASA2)		Field evaluation					
			% Infested tillers		Seed quality		Grain mass (grams)	
	Score	Rating	Bethlehem	Reitz	Bethlehem	Reitz	Bethlehem	Reitz
67	5.47	MR	0	20	Good	Good	270.24	934.79
74	5.68	MR	20	0	Medium	Good	217.49	492.19
120	5.50	MR	0	0	Good	Good	872.91	853.00
123	5.10	MR	0	0	Good	Good	1330.25	1162.77
124	4.95	MR	0	0	Good	Good	802.84	1134.70
182	5.19	MR	0	0	Good	Good	794.56	1068.99
183	5.44	MR	40	0	Medium	Good	189.06	1141.20
184	4.05	MR	0	0	Good	Good	421.94	1278.44
185	6.00	MR	0	20	Good	Good	391.83	1069.59
188	4.14	MR	0	0	Good	Good	735.45	1345.06
189	5.38	MR	0	0	Good	Good	186.06	1029.28
192	5.95	MR	0	40	Medium	Good	361.09	590.82
194	3.90	MR	0	0	Good	Good	1270.14	481.97
720	5.94	MR	0	0	Good	Good	565.53	1132.08
726	5.63	MR	0	0	Medium	Medium	574.84	1083.97
1106	4.61	MR	0	20	Good	Good	665.87	680.15
1111	5.98	MR	40	0	Good	Good	904.89	1083.75
1112	5.95	MR	20	0	Good	Good	807.30	697.45
1113	4.79	MR	0	0	Missing	Good	Missing	948.96
1472	6.07	MR	20	0	Good	Good	1005.51	1145.10

6.3.3 CIMMYT RWASN RWA resistance pool

6.3.3.1 Seedling evaluation

Seedling evaluation of the CIMMYT pool revealed 31% of the entries with acceptable resistance to RWASA2 (Table 6.4). A general overview of the donor accessions accountable indicates a number of unfamiliar accessions not previously utilized in RWA pre-breeding at the SGI. Accession donors PI 221699 from the former Soviet Union (Harvey and Martin, 1990), PI 222666 (Gandum No. 1657) reputed to be a single gene but different from *Dn-4*, *Dn-5* and *Dn-6* (Dong and Quick, 1995), PI 222671 from Iran (Harvey and Martin, 1990), PI 94355 (syn. 'Reasante') from Armenia (Smith *et al.,* 1991), PI 94365 from Iran (synonym 'Dickinson No. 60) and CI

9358 from Finland (Harvey and Martin, 1990) are all new donors identified for utilization in the RWA pre-breeding programme. Eight entries with IWA (International wheat accessions) designations, presumably originating from Iran, constitute the majority of resistance to RWASA2.

6.3.3.2 Field evaluation

As already discussed in 6.3.2.2, conditions at the Bethlehem locality were favourable for development of plant diseases and susceptibility of entries without genetic resistance to disease, particularly stripe rust, was evident. Personal observations during plant development, together with seed quality and grain mass, indicated entries 17, 19, 42, 44, 45, 46, 47, 52 and 53 to be medium-susceptible to susceptible to stripe rust (Table 6.4).

Table 6.4 Seedling resistance and field performance of the CIMMYT RWASN pool

	Seedling evaluation (RWASA2)		**Field evaluation**					
			% Infested tillers		**Seed quality**		**Grain mass (grams)**	
Entry	**Score**	**Rating**	**Bethlehem**	**Reitz**	**Bethlehem**	**Reitz**	**Bethlehem**	**Reitz**
7	5.23	MR	0	10	Medium	Good	1173.27	1084.14
8	5.38	MR	0	0	Good	Good	539.42	407.88
11	4.80	MR	0	70	Good	Good	827.05	769.12
12	5.34	MR	0	40	Good	Good	1202.51	778.26
13	4.09	MR	0	80	Good	Good	940.60	584.54
14	3.93	MR	0	60	Good	Good	1082.55	814.75
17	5.42	MR	0	40	Good	Good	615.23	1754.85
18	5.05	MR	0	10	Good	Good	830.19	1185.86
19	4.16	MR	0	30	Poor	Good	400.99	1201.81
42	5.63	MR	0	40	Medium	Good	466.75	768.02
43	5.41	MR	0	30	Good	Good	378.52	661.18
44	3.62	MR	0	10	Poor	Good	191.21	1003.03
45	4.85	MR	0	20	Poor	Medium	209.24	854.27
46	4.32	MR	0	40	Medium	Good	101.81	405.91
47	5.75	MR	40	0	Poor	Good	75.76	790.91
52	6.13	MR	20	0	Medium	Good	222.39	853.59
53	6.32	MR	0	0	Poor	Poor	0	158.62

6.3.4 Iran RWA resistance pool

6.3.4.1 Seedling evaluation

Twenty entries were identified with resistance to RWASA2 (Table 6.5) though no indication of the donor accessions or *Dn*-genes could be determined due to the unavailability of a pedigree list. Inclusion of these entries in the RWA pre-breeding programme will solely be based on reports verifying high incidence of genetic RWA resistance in germplasm from Central Asia and specifically Iran (Souza, 1998).

6.3.4.2 Field evaluation

As in the case with the CIMMYT RWASN, unadaptibility of entries was evident in susceptibility of a majority of entries to the races of stripe rust *(Puccinia striiformis f.sp tritici)* currently occurring in the eastern Free State (Table 6.5).

Table 6.5 Seedling resistance and field performance of the Iran pool

			Field evaluation					
	Seedling evaluation (RWASA2)		**% Infested tillers**		**Seed quality**		**Grain mass (grams)**	
Entry	**Score**	**Rating**	**Bethlehem**	**Reitz**	**Bethlehem**	**Reitz**	**Bethlehem**	**Reitz**
IR3	6.01	MR	0	0	Medium	Good	46.70	579.26
IR4	4.60	MR	0	0	Medium	Medium	185.21	190.52
IR12	5.23	MR	0	40	Poor	Good	39.19	795.89
IR13	5.34	MR	0	0	Medium	Good	157.48	810.31
IR14	5.65	MR	20	0	Poor	Good	39.92	509.01
IR15	6.22	MR	0	20	Good	Good	1326.56	986.84
IR18	4.36	MR	0	0	Medium	Good	119.29	484.36
IR19	5.31	MR	0	0	Good	Good	264.75	977.21
IR20	4.88	MR	40	0	Medium	Good	4.56	455.55
IR27	5.92	MR	0	0	Medium	Good	1153.07	627.46
IR31	4.45	MR	0	0	Good	Good	348.99	801.08
IR36	5.66	MR	0	0	Medium	Good	68.36	412.39
IR37	4.69	MR	0	0	Good	Good	130.08	215.35
IR38	5.33	MR	0	0	Good	Good	1180.72	456.05
IR39	6.08	MR	20	0	Good	Good	234.80	483.60
IR41	5.86	MR	0	0	Good	Good	143.19	548.60
IR43	4.75	MR	0	0	Poor	Good	49.18	560.45
IR44	4.96	MR	0	0	Poor	Good	73.95	568.49
IR49	6.46	MR	0	0	Good	Good	23.48	305.31
IR53	5.62	MR	40	40	Good	Good	700.90	732.88

6.4 Conclusions

Easier accessibility to international development of all three pools will contribute markedly towards broadening of the existing genetic variation of RWA resistance in the RWA pre-breeding programme of the SGI. AUS 22498, CItr 2401, CI 6501, OR52 (OSU ID2808) and PI 294994 are all globally recognized resistance donor accessions with diverse genetic variance to RWA and in the case of the Stillwater, Oklahoma/SGI pool are already incorporated into local wheat backgrounds. In addition, new sources not previously exploited in South Africa have become available through the CIMMYT and Iranian pools for use as future crossing parents. Germplasm in cultivated crops can be represented as a pyramid (Figure 6.2) where the apex is formed by adapted cultivars and the base by unadapted, broader material. The further germplasm is removed from the apex the more intense breeding effort is required to bring the gene of importance into an acceptable background (Souza, 1998).

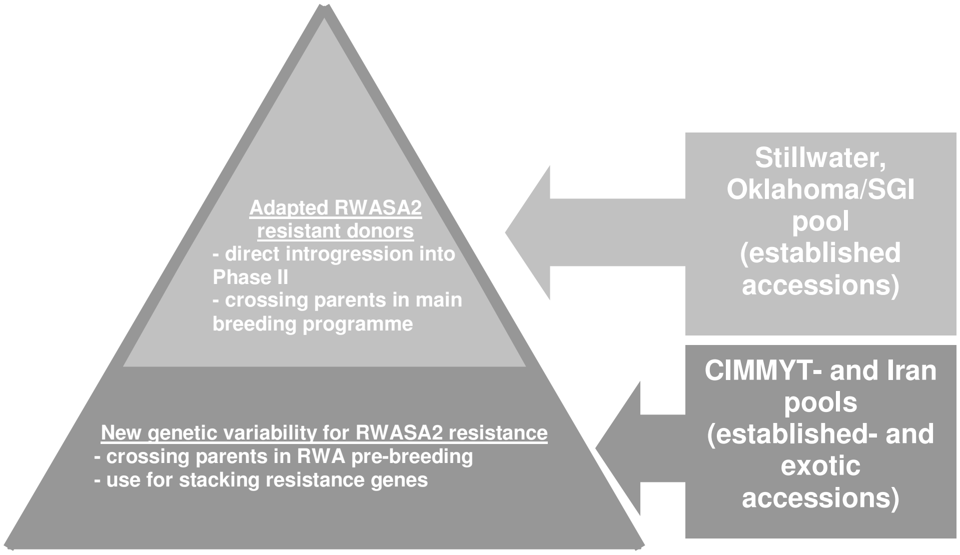

Figure 6.2 Breeding pyramid for RWA resistance in South Africa (adapted from Souza, 1998)

The next challenge after identification will be the wise employment of resistance genes in local crossing programmes to form the foundation of RWA resistant wheat cultivars in South Africa thereby ensuring prolonged resistance against future biotype development.

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CHAPTER 7

General conclusions and recommendations

7.1 Climate and RWASA1 and RWASA2

Vernalization period, minimum- and maximum temperatures and monthly rainfall are just three meteorological components forming part of a much more complex climatic system influencing all forms of life on our planet. As this study focused on climatic factors affecting the expression of host plant resistance to RWA, the primary emphasis was on the response of wheat plants in different environments and not RWA. A main observation, particularly in Chapters 3, 4 and 6, is the ability of the newly identified biotype RWASA2 to overcome host plant resistance in established commercial wheat cultivars released for the summer rainfall production region in South Africa prior to 2005. RWASA2 is capable of overcoming *Dn-1* and *Dn-2* resistance in cultivars but seems avirulent on *Dn-4* based resistance partly present in CItr 2401 (other resistance gene still unknown) and Halt, confirming results by Tolmay *et al.* (2007). RWASA1, though, will probably remain in the Eastern Free State wheat fields. Together with low precipitation and high maximum temperatures RWASA1 will continue to inflict significant damage to wheat (Chapters 4 and 5). Jyoti *et al.* (2006) found similar results in a study on virulence of two North American RWA biotypes at two temperatures and suggested that higher virulence of biotype 2 results from higher colony growth rates and rapid development of damage symptoms more prominent at higher temperatures. The adaptation of RWA to changing climatic patterns worldwide, through biotype development, should be expected to positively correlate with increased virulence on existing resistant wheat cultivars.

7.2 Climate and expression of host plant resistance to RWA

Weather defined by Maarten (2003) as short-term variations in the atmosphere, consisting mainly of:

1. Moisture stress – caused by drought, floods or inconvenient rainfall.
2. Temperature stress – caused by temperatures that are too cold or too hot for optimal plant growth.
3. Natural disasters such as cyclones, hurricanes and hailstorms.

Moisture- and temperature stress are generally accepted to be conducive to RWA colonization and consequent plant damage and yield loss in wheat crops. This trend was also observed during the wheat seasons of 2003 and 2004 in the summer rainfall region of the Eastern Free State and particularly Bethlehem, Glen and Ladybrand (Chapter 5). Frequent rainfall and moderate maximum temperatures in the growing season strengthens tolerance levels of dry land wheat to endure high aphid numbers without affecting yield components negatively. Johnson *et al.* (1998) investigated the impact of RWA on drought-stressed wheat. They concluded that RWA exploits drought-stressed wheat plants more successfully than other aphids and their existence inside longitudinally rolled wheat leaves assists in attaining optimum developmental and reproductive rates through maintenance of higher body temperatures. Smith (2005) reported that environmental factors such as light intensity, temperature, soil moisture, relative humidity and atmospheric fluctuations may affect the expression of host plant resistance in plants often culminating in frequently observed Genotype x Environment interaction. He defined useful arthropod resistance as genotypes able to remain stable over several years in a broad range of environmental conditions. The ability of RWA to thrive on stressed wheat plants may build a strong case for the development and selection of stable expression of host plant resistance to RWA, especially in environments prone to frequent abiotic stress. Another important aspect emerging from Chapter 5 and the consequent work with seed dressing is that host plant resistance should be stable and effectively expressed, particularly during the vegetative growth period. The conceptual model devised for drought adaptation in wheat by Reynolds *et al.* (2005) identified four traits that may also prove to be decisive in effective expression of RWA resistance; 1) efficient vegetative growth expressed through stem carbohydrate remobilization and percentage transpiration increase, access to water as a result of 2) increased root depth and 3) increased root density and 4) water-use- and transpiration efficiency of canopy growth. All of these traits contributed significantly to adaptation to post-anthesis drought stress. Various authors have supported the importance of these traits; particularly root development in expression of host plant resistance to RWA. Zwer *et al.* (1994) recommended that root measurements, in conjunction with measurements of leaf damage, would provide more accurate identification of promising RWA resistant genotypes. Voothuluru *et al.* (2006) found reduction of root dry weight of CItr 2401 (=CI 2401) by both RWA biotypes 1 and 2 to be insignificant in comparison to other wheat genotypes and suggest measurement of root- and shoot dry weights as a more accurate measurement of plant tolerance to RWA. Identification of important physiological traits linked to the effective expression of resistance to RWA will assist in defining a “wheat ideotype” containing characteristics essential for effective and long-term expression of host plant resistance to this pest in dryland wheat in South Africa.

7.3 Donor accessions resistant against RWASA2

The Russian wheat aphid originates from Central Asia and numerous researchers have, in searching for broader genetic resistance to the pest, adhered to the principle of evaluating accessions originating from the area of origin of the pest. Souza (1998) reported that of the 86 accessions with reproducible RWA resistance compiled in reports by the WRCC-66 (Western Regional Coordinating Committee - 066), 73% had origins from Central Asia. Likewise PI 137739 (*Dn-1*), PI 262660 (*Dn-2*) and PI 294994 (*Dn-5*, *Dn-8* and *Dn-9*) identified by Du Toit (1989a;b) resistant to the original RWA biotype in South Africa, also originated from germplasm from Central Asia.

AUS 22498, CItr 2401, CI 6501 and PI 294994 from the Stillwater, Oklahoma/SGI pool and PI 221699, PI 222666, PI 222671, PI 94355 and PI 94365 from the CIMMYT pool and all 20 entries in the Iran pool with resistance against RWASA2 derive from Central Asia. For our purposes, the main constraint with a majority of these accessions is that they are unadapted for production conditions in the summer rainfall wheat production region of South Africa.

7.4 Recommendations

In order to determine stability of expression of host plant resistance to RWA, resistant cultivars, advanced breeding lines and resistant donor accessions identified with seedling resistance to RWASA2 must be subjected to environments exhibiting diverse climatic conditions (Figure 7.1). Firstly, the percentage yield retained selection will then be based on comparison between seed-dressed treatment and untreated control of all entries with acceptable levels of resistance to RWA in all three environments. This mode of selection, though, will not assist in specifying the particular plant components responsible for increased resistance; scientific apparatus and advanced field techniques enabling measurement of canopy-reflectance, root development and water-use efficiency will have to be employed for this purpose. Secondly, yield results for every two seasons over the three localities will be analyzed with AMMI in order to identify entries exhibiting stable expression of RWA resistance and drought-tolerance over the three environments (Figure 7.1). Only donor accessions already incorporated into a local agronomical background will be included in the field trials.

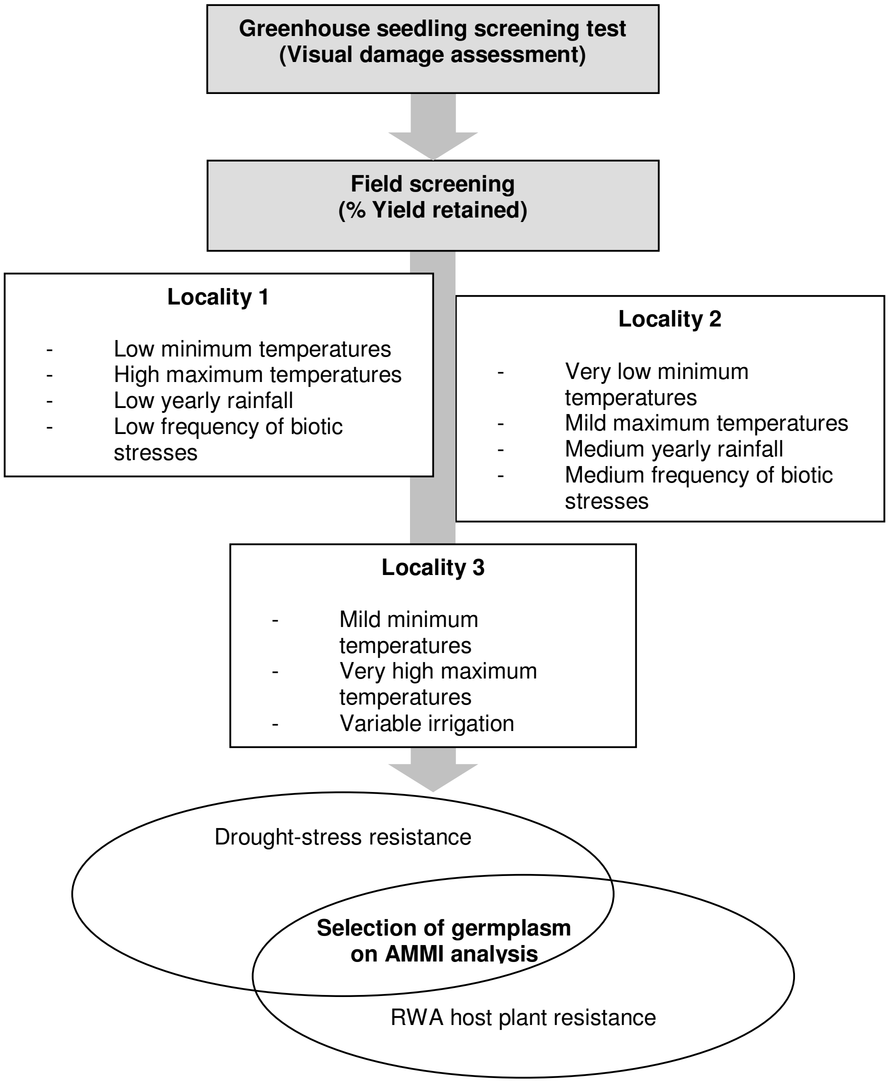

Figure 7.1 Diagram for selecting wheat genotypes with resistance against RWA

Adapted germplasm identified in the Oklahoma/SGI pool can be immediately incorporated into the main breeding programme of the ARC-SGI as breeding parents whereas unadapted germplasm from the CIMMYT and Iran pools first have to be introgressed into backgrounds with acceptable agronomic characteristics. The three pools described here provide an excellent opportunity to exploit novel germplasm with potential new genetic variance for RWA resistance but also contain numerous other beneficial traits such as drought-tolerance and disease-resistance.

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CHAPTER 8

Summary

The purpose of this study was to investigate the effect of variations in climatic factors such as vernalization periods, minimum- and maximum temperatures and rainfall on the expression of host plant resistance to RWASA1 (original biotype) and RWASA2 (newly identified biotype) in the eastern Free State of South Africa between 2003 and 2006. Climatic records indicate above-average temperatures and reduced rainfall occurred in the Eastern Free State in 2003 to 2005 probably contributing to widespread RWA damage on dryland wheat crops. Greenhouse experiments confirmed that vernalization did not alter RWA plant resistance ranking of wheat entries. However, infestation did produce significant damage by RWASA2 on Elands, Gariep and Limpopo but not on CItr 2401 (containing *Dn-4* and another unidentified *Dn*-gene) and Halt (*Dn-4*). Resistance rating to RWASA1 of Halt, Komati and Matlabas in greenhouse tests at 18°C/12°C, 22°C/16°C and 26°C/22°C shifted to less resistant- or susceptible categories and changed the ranking of SST 966 as temperature increased. Also, RWASA2 ratings at increased temperature shifted Halt to a more resistant category and changed rankings of Komati and SST 399. Komati, Matlabas, SST 966 or SST 399 did not express any host plant resistance to RWASA2 through leaf area or leaf roll and chlorosis supporting the susceptible ratings that were given to the same varieties in the visual screening test. Seed-dressing in AMMI results for 2003 to 2006 were able to increase yields under higher-yielding conditions in seasons when moisture- and heat stress occured and the yield performance of cultivars was stabilized irrespective of whether they contained genetic resistance to RWA. Identifying new RWA resistance donors provide an excellent opportunity to exploit novel germplasm with potential new genetic variance for RWA resistance, as well as other beneficial traits such as drought-tolerance. Three germplasm pools were evaluated for this purpose. Twenty-one entries from the Stillwater, Oklahoma/SGI RWA resistance pool expressed resistance against RWASA2 in the seedling test and where generally well adapted to local conditions. Seedling evaluation of the CIMMYT pool revealed twenty entries with acceptable resistance to RWASA2 though field adaptability particularly to biotic stress was occasionally lacking. Twenty entries from the Iranian pool were resistant to RWASA2 though unadapted for resistance to stripe rust; *Puccinia striiformis Westend f.sp tritici Eriks.,* occurring in the Eastern Free State.

Opsomming

Die doel van hierdie studie was om die invloed van variasie in vernalisasie, minimum- en maksimum temperature en reënval op uitdrukking van gasheerplant weerstand teen RWASA1 (oorspronklike biotipe) en RWASA2 (nuut geidentifiseerde biotipe); *Diuraphis noxia* (Kurdjumov) in die Oos Vrystaat tussen 2003 en 2006 te bepaal. Klimaatsrekords dui aan dat bogemiddelde temperature en laer reënval in die Oos Vrystaat in 2004 en 2005 voorgekom het en waarskynlik bygedra het tot die algemene voorkoms van RKL skade in droëlandkoring. Glashuisproewe bevestig dat ongevernaliseerde- of gevernaliseerde plante nie die RKL weerstandsrangorde verander nie maar RWASA2 wel hoë skadelikheid op Elands, Gariep en Limpopo maar nie CItr 2401 (bevat *Dn-4* en 'n ander ongeidentifiseerde *Dn*-geen) veroorsaak. Weerstandwaardebepaling van Halt, Komati en Matlabas teen RWASA1 in glashuisevaluasies by 18°C/12°C, 22°C/16°C en 26°C/22°C verskuif na matige weerstand of vatbare kategorieë en verander die rangorde van SST 966 tydens temperatuur verhoging terwyl weerstandsbepaling van Halt teenoor RWASA2 by hoër temperatuur na 'n meer weerstandbiedende kategorie verskuif en die rangorde van Komati en SST 399 verander. Geen gasheerweerstand uitgedruk in blaaroppervlak, blaarrol en chlorose teenoor RWASA2 kom voor by Komati, Matlabas, SST 966 en SST 399 nie en bevestig vatbaarheid van hierdie kultivars in die visuele evaluasies. AMMI resultate vir 2003 tot 2006 toon dat saadbehandeling opbrengs verhoog onder hoë-opbrengs omstandighede in seisoene waarin vog- en hitte stremming voorkom en koringopbrengs van kultivars stabiliseer ten spyte daarvan of die kultivars RKL weerstand bevat of nie. Identifikasie van nuwe RKL weerstandsbronne bied 'n uitstekende geleentheid vir die ontginning van nuwe kiemplasma met potensiële nuwe genetiese variasie vir RKL weerstand maar ook ander belangrike eienskappe soos droogteweerstand en drie kiemplasmapoele is vir hierdie doel geevalueer. Een-en-twintig inskrywings uit die Oklahoma/ARC-SGI poel het saailingweerstand teenoor RWASA2 getoon en is algemeen goed aangepas vir plaaslike toestande. Saailingevaluasie van die CIMMYT poel het twintig inskrywings geidentifiseer met weerstand teenoor RWASA2 alhoewel aanpassing van hierdie inskrywings teenoor veral biotiese stremming wisselvallig is. Twintig inskrywings uit die Iranese poel toon weerstand teenoor RWASA2 maar is veral gevoelig vir streeproesinfestasie deur *Puccinia striiformis f.sp tritici* wat huidiglik in die Oos-Vrystaat voorkom.