

## Influence of Environmental Fluctuation on the Russian Wheat Aphid Biotype Distribution in South Africa

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### Abstract

Because the Russian wheat aphid (RWA) pose a threat to the wheat industry in South Africa, it is important to understand the key factors affecting population density fluctuations in the RWA biotype complex. RWA were sampled in the wheat production regions of South Africa from 2010 to 2016 during the wheat growing season in spring and summer. Changes in the climatic environment have a significant impact on agricultural production as well as the suitability of an area for the production of a specific crop. Factors such as increasing production costs and decreasing prices for crops will influence the type of crop and the area planted to that specific crop. Not only are there constant changes in the natural environment but the agricultural landscape is also changing all the time. Different environmental conditions, including the availability of host plants play an important role in the population growth and distribution of RWA populations. Because the agricultural landscape changes from year to year and between different areas, the distribution of RWA biotypes will vary over years and also between different geographical areas. Since the monitoring of RWA biotypes started in South Africa in 2010 there were also shifts in dominance between the different biotypes in the complex. RWA populations fluctuate with the changing environment, but persists in the wheat production areas of South Africa, sometimes at low population levels and minimal to no damage to wheat crops. During the past couple of years, the agricultural landscape in South Africa, especially in the Free State, has changed dramatically. With a decrease in wheat cultivation in the summer rainfall areas of South Africa, the habitat for RWA became fragmented. This resulted in a decrease of RWA populations, limited to habitat patches. These observations emphasize the value of intercropping and crop rotation in managing insect pests and can serve as model for RWA management.

**Keywords:** Russian wheat aphid; Biotypes; Distribution; Wheat area; Environment

### Abbreviations

RWA: Russian wheat aphid

### Introduction

Russian wheat aphid (RWA), *Diuraphis noxia* (Kurdjumov), originates from central Asia [1] and has spread from here to other major wheat (*Triticum aestivum* L.) producing countries in the world to become a wheat pest on a global scale. It is considered a primary pest of dryland winter wheat in North America [2] and South Africa [3]. RWA, like other exotic aphid species, is capable of surviving at low numbers for a relatively long time, and can cause sudden population outbreaks in new areas [4]. The recent outbreak of RWA in Australia is a case in point. Australia is a major wheat producing country since 1839 [5], but the first outbreak of RWA was only detected in South Australia in 2016 [6]. The first record of

RWA outside its original area of distribution was in South Africa in 1978. Initially the distribution was confined to the Bethlehem area in the Eastern Free State, but by 1979, the Russian wheat aphid had spread to other wheat-producing areas in the country [3]. The first record of RWA in the United States was in 1986 [2]. RWA invaded all the Central European countries from the south-east [7] and was first detected in the Czech Republic in 1993 [8,9]. Resistant wheat cultivars are the most effective management options, saving insecticide application costs and reducing environmental risks associated with pesticide use. The first RWA-resistant cultivar was released in South Africa in 1992, and more than 70% of the wheat production area in South Africa was planted with Russian wheat aphid-resistant cultivars [10]. The durability of resistant cultivars was, however, challenged by the occurrence of RWA biotypes, first in Colorado in 2003 [11], and in South Africa in

2006 [12]. Russian wheat aphid biotypic variation was also found in Hungary [13] and Chile [14]. Currently there are four different RWA biotypes present in South Africa, RWASA1, RWASA2, RWASA3 and RWASA4 [3,12,16,17]. These biotypes vary in their damage potential to wheat cultivars containing different RWA resistant genes.

Climate change has a direct, but also an indirect impact on crop yield by affecting the prevalence and distribution of pest insects, diseases and weeds. Not only are there constant changes in the natural environment but the agricultural landscape is also changing all the time. Changes in the climatic environment have a significant impact on agricultural production as well as the suitability of an area for the production of a specific crop. Other factors such as increasing production costs and decreasing prices for crops influence the type of crop and the area planted to a specific crop. During the past couple of years, the agricultural landscape in South Africa, especially in the Free State, has changed dramatically. The area utilized for wheat production in South Africa showed a declining trend, decreasing by almost 43% from the 2004/2005 season and by 6% compared to the 2013/2014 season [15]. The decrease in wheat cultivation is mainly a result of dryland wheat producers in the summer rainfall area (Free State Province), shifting from wheat to summer crops like maize and soybeans. There are numerous factors influencing this shift, from poor growing conditions and late rains to increased production costs and low wheat prices. These fluctuations in wheat production and area planted with wheat will influence the distribution of RWA in South Africa. Because the Russian wheat aphid pose a threat to the wheat industry in South Africa, it is important to understand the key factors affecting population density fluctuations in the RWA biotype complex. This can be achieved by monitoring the Russian wheat aphid biotype distribution in the wheat production areas to detect shifts in biotype composition.

**Materials and Methods**

**Sampling**

RWA samples were collected from 2010 to 2016 during the wheat growing season in spring and summer (August to January). All main wheat production areas within the common known distribution of the RWA were sampled. There are two main dryland wheat production areas in South Africa, the Western Cape and the Free State, with irrigated wheat production areas in the central and western Free State and Northern Cape. The Western Cape is a winter rainfall area, while the Free State is a summer rainfall area. Winter wheat is planted in all these areas. Sampling sites were selected off primary or secondary roads that transacted major wheat or barley production areas. Sites were 10 - 20 km apart with distances depending on the continuity of the wheat fields. Samples were collected from cultivated wheat, barley and oats as well as volunteer wheat, wild oats, rescue grass and false barley in road reserves and around cultivated fields. Infested leaves were placed in Petri dishes containing

moist filter paper and stored in an icebox for transportation to the glasshouse. The number of aphids per plant, percentage plants infested, growth stage of the plants and damage on the plants were recorded. The geographical co-ordinates and elevation where the samples were collected were also captured on a GPS and plotted on a map (Google Earth). All the information of each sample collected was entered into a data base (Windows Office -Excel).

**Determination of biotypes:**

An individual aphid from each sample collected in the field was transferred to a wheat plant and caged (gauze size: 315 micron) to produce a clone colony. Colonies were kept in a greenhouse cubicle at night/day temperatures of 16°C/22°C. Clone colonies were maintained on different cultivars to avoid pre-adaptation to a specific cultivar until they multiplied sufficiently to be used for screening. Each clone colony was cultured for an average period of two to three months before screening. Founder colonies of RWASA1, RWASA2, RWASA3 and RWASA4 are kept and maintained at ARC-Small Grains, Bethlehem, South Africa.

The biotype of each RWA clone was determined by screening its feeding damage on 11 previously established plant resistant sources containing designated resistance genes *Dn1* to *Dn9* and *Dnx* and *Dny* (Table 1).

No	Gene	ID	Resistance source
1	<i>Dn1</i>	CO-03797	PI127739
2	<i>Dn2</i>	CO-03804	PI262660
3	<i>Dn3</i>	CO-03811	<i>Triticum tauschii</i> line SQ24
4	<i>Dn4</i>	Yumar	PI372129
5	<i>Dn5</i>	CO-950043	PI294994
6	<i>Dn6</i>	CI 6501	PI243781
7	<i>Dn7</i>	2003-1378027 Winter	94M370
8	<i>Dn8</i>	Karee- <i>Dn8</i>	PI294994
9	<i>Dn9</i>	Betta- <i>Dn9</i>	PI294994
10	<i>Dnx</i>	2006 RWA-1 PI 586955-23 KS94WGRC30	PI 220127
11	<i>Dny</i>	2006 RWA-1 Stan- ton	PI220350
12	-	Betta Susceptible	-
13	-	RWA Matrix 2401	CItr2401

**Table 1:** Differential used to designate new Russian wheat aphid biotypes in South Africa (seed obtained from USDA-ARS, Stillwater, OK).

Ten seeds of each plant entry were planted in a seedling tray filled with sterilized sand in a randomized complete block design with four replications for each biotype determination. Plant entries were randomly assigned to rows and were separated by border rows planted with RWA susceptible Tugela. Plants were kept in glasshouse cubicles at night/day temperatures of 16°C/22°C, natural light. Immediately after planting, the seedling trays were placed in gauze (315 micron) cages to avoid contamination by secondary aphids. Plants were infested at the two-leaf stage with RWA clone colonies. Plants were rated with a ten-point damage rating scale, which included leaf chlorosis and leaf rolling [18]. A score from 1 - 4 describes leaf chlorosis, 5 - 6 striping on the leaves and 7 - 10 rolling. Once the susceptible wheat Tugela showed susceptible damage symptoms, all plants were rated. RWA biotypes were classified by using damage ratings for each plant entry where the plant was considered resistant (R) if the damage rating was 1 - 6.5 and susceptible (S) if the damage rating was 6.5 - 10. Each clone was given a biotype designation based on the differential virulence profile to the *Dn1* to *Dn9* resistance genes.

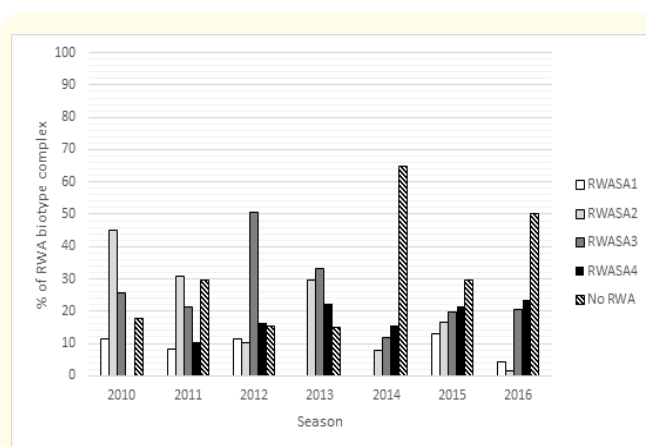
Biotype (clones) groups across all plant differentials were analysed using a two-way (clone, plant entry) analysis of variance (ANOVA). Mean damage rate entries with significant ( $P < 0.05$ ) clone-by-plant interactions were separated by Fisher’s protected least significant difference (LSD) test at the 5% level (SAS Institute 2003).

### Results and Discussion

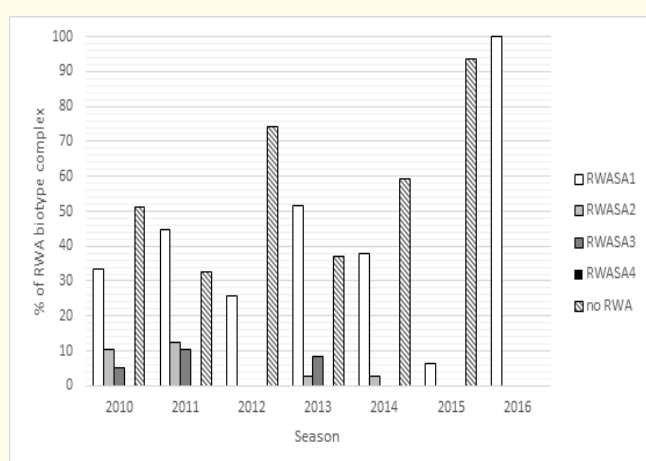
Analysis of the main effects of damage rating for the four Russian wheat aphid biotype clone colonies indicated a significant clone ( $F = 117.48$ ;  $df = 3$ ;  $P < 0.0001$ ), plant entry ( $F = 133.59$ ;  $df = 11$ ;  $P < 0.0001$ ) and clone-by-plant entry interaction ( $F = 12.82$ ;  $df = 33$ ;  $P < 0.0001$ ), suggesting that the plant entries responded differently to the different aphid clones. Infestations of RWASA1 caused susceptible damage symptoms on the wheat entry containing the *Dn2* and *Dn3* gene. RWASA2 caused susceptible damage symptoms on wheat entries containing *Dn1*, *Dn2*, *Dn3*, *Dn8* and *Dn9* resistance genes. RWASA3 is distinguished from RWASA2 by its added virulence to *Dn4* and RWASA4 is distinguished from RWASA3 by its added virulence to *Dn5*. RWASA4 was the most virulent biotype in South Africa with susceptible responses to eight plant differentials containing eight different *Dn* genes. Randolph, *et al.* (2009) found the American RWA2 to be the most virulent biotype tested with susceptible responses to 12 plant differentials [19].

RWASA2 and RWASA3 predominated in South Africa from 2010 to 2013. During 2010 and 2011, RWASA2 was the dominant biotype in the summer rainfall area, but during 2012, the dominance of RWASA2 dropped and RWASA3 became the dominant biotype in this area during 2012 and 2013 (Figure 1). From 2014 to 2016

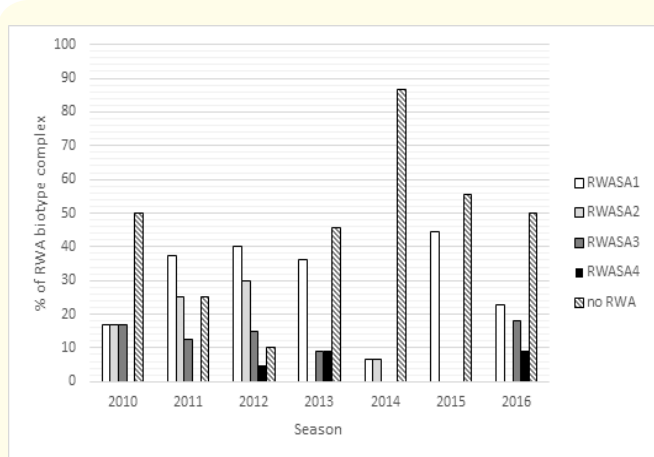
RWASA4 was the dominant biotype in the summer rainfall area (Figure 1). Since the first record of RWASA4 in 2011, RWASA4 was only recorded in the Eastern Free State and there was a gradual increase in the dominance of RWASA4 from 2011 to 2016. RWASA4 has the potential to displace other biotypes in this area and since this is the most virulent RWA biotype in South Africa this increase might have serious consequences for the wheat industry in the Eastern Free State. This situation will have to be monitored in case of further increases in this biotype and spreading of this biotype to other areas. From 2010 to 2016 RWASA1 was the dominant RWA biotype in the winter rainfall area of South Africa, with the other biotypes only occurring in small isolated populations from 2010 to 2014 (Figure 2). RWASA1 was also the dominant biotype in the irrigation area from 2011 to 2016 (Figure 3).



**Figure 1:** Variation in the Russian wheat aphid biotype complex in the summer rainfall area (Free State) of South Africa from 2010 to 2016.



**Figure 2:** Variation in the Russian wheat aphid biotype complex in the winter rainfall area (Western Cape) of South Africa from 2010 to 2016.

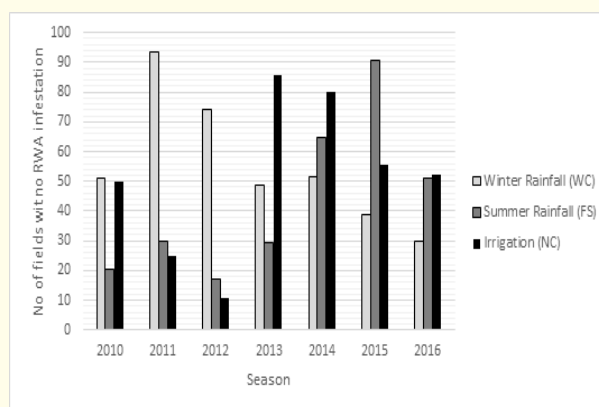


**Figure 3:** Variation in the Russian wheat aphid biotype complex in the irrigation area (Northern Cape) of South Africa from 2010 to 2016.

The surveys suggest that the Russian wheat aphid biotype complex was more diverse in the Eastern Free State than in the other wheat production areas, with the Eastern Free State supporting all the known biotypes, while RWASA1 was most prevalent in the Western Cape. There was also a shift in Russian wheat aphid biotype composition over time, with a notable change in biotype composition in the Eastern Free State during 2012 with an increase in RWASA3 and a decrease in RWASA2. Puterka, *et al.* (2014) reported that RWA6 was the dominant biotype infesting wheat on the Colorado Plateau and Great Plains during 2011 and 2013, and that Biotypes 1, 2, and 3/7 were consistently represented in both regions in smaller numbers [21]. In South Africa, RWASA1 was the dominant biotype infesting wheat in the Western Cape from 2011 to 2014, with smaller numbers of the other biotypes occurring during 2011, 2013, and 2014. In the Eastern Free State, no one biotype was consistently dominant from 2011 to 2014, but RWASA2, RWASA3, and RWASA4 were represented in varying numbers over time, while RWASA1 was represented in smaller numbers. RWASA4 was first recorded in the Eastern Free State in 2011, and it was the only biotype restricted to the Eastern Free State and not found in other wheat production areas. In the Eastern Free State, wheat cultivars with the *Dn1* gene were exclusively planted since 1992 [10], all of them resistant against RWASA1. Cultivars with the *Dn1* gene were not planted in the Western Cape. Hence, selection pressure on Russian wheat aphid was likely stronger in the Eastern Free State than in the Western Cape, resulting in biotypic diversity. Puterka, *et al.* (2014) reported that concentrated use of RWA 1-resistant wheat in areas of Colorado most infested by Russian wheat aphid could have imposed directional selection pressure toward biotypes that could overcome *Dn4* resistance and affect biotypic diversity at a localized level, which can influence biotype composition at a regional level [20]. The adaptive ability of the Russian wheat aphid to overcome

plant resistance through biotypic differentiation has prompted efforts to diversify Russian wheat aphid resistance genes in wheat breeding programs [21]. The genes in South African wheat cultivars confer resistance through antibiosis. Wheat with the *Dn6*, *Dn7*, and *Dnx* resistant genes are still resistant to all four known biotypes in South Africa. The practice of breeding for high levels of antibiosis, however, promotes development of aphid biotypes and diverse aphid resistance genes and genes that control tolerance resistance or more moderate levels of antibiosis resistance should be investigated [22]. Aphid-tolerant cultivars are often more stable than those that have antibiotic properties [23].

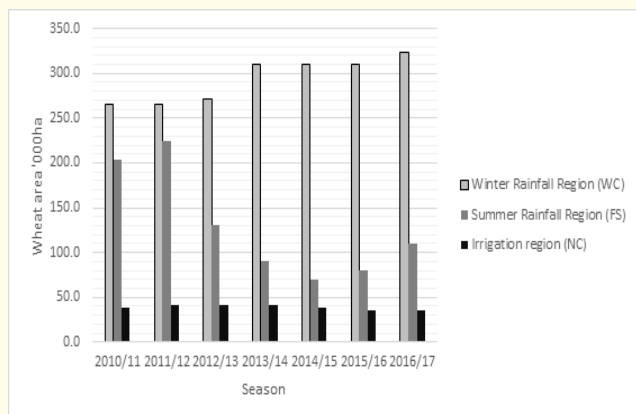
RWA utilizes wheat as its main host, with a limited number of alternative hosts. Monitoring of the four RWA biotypes in South Africa throughout the wheat production areas from 2010 to 2016 showed that there has been a steady decline in RWA infestation of wheat in the summer rainfall region (Free State), as can be seen by the increased percentage fields monitored that had no RWA infestation (Figure 4). The decline of RWA in these areas can be attributed to the decline of the prevalence RWASA1, RWASA2 and RWASA3, while there was a steady increase in the prevalence of RWASA4 over the seasons in these areas. This biotype, however, is limited to a few areas in the Eastern Free State. In the winter rainfall region (Western Cape), however, the percentage of fields with no RWA infestation decreased from 2010 to 2016, indicating an increase in RWA infestation in these areas (Figure 4), notably by RWASA1. In the irrigation areas (Northern Cape) the percentage of fields surveyed with no RWA infestation increased drastically during 2013 and then decreased gradually from 2014 to 2016 (Figure 4), implying an increased aphid prevalence recently. The main biotype identified in these areas was RWASA1.



**Figure 4:** Number of wheat fields not infested by Russian wheat aphid over seasons in the winter rainfall region (Western Cape), summer rainfall region (Free State) and irrigation region (Northern Cape).



There was a decrease in the area planted with wheat in the summer rainfall area (Free State) over seasons, with a drastic decrease in the 2013 season, onwards (Figure 5). This coincided with the drastic increase in the percentage of fields not infested by RWA in these areas during the next season in 2014 (Figure 4). There was a slight increase in the area planted with wheat in the winter rainfall region during the 2014 season (Figure 5). This coincided with a gradual decrease in fields not infested by RWA from 2014 to 2016 (Figure 4).



**Figure 5:** Area planted per production area over seasons, winter rainfall region (Western Cape), summer rainfall region (Free State) and irrigation region (Northern Cape). (Data-SA Grain).

The arrangement of habitat patches in landscapes plays an important role in determining the abundance and diversity of insects. Insects will increase within an area that contain the most suitable host plant, and decrease with isolation of the patch. RWA in South Africa rely mainly on cultivated wheat and barley as host plants for population increase. RWA can survive on alternative host plants, including oats, wild oats, false barley and rescue grass. The ability of different RWA biotypes to survive on these limited alternative hosts differ between biotypes [24]. RWA cannot survive on any of the other crop plants commonly cultivated in South Africa. With a decrease in wheat cultivation in the summer rainfall areas of South Africa, the habitat for RWA became fragmented. Not only is there a spatial fragmentation, but also a temporal fragmentation when other crops are planted during different seasons. This resulted in RWA populations being limited to certain habitat patches. At the same time, the beneficial insect complex associated with a wider crop spectrum increases, thereby exerting additional pressure on the survival of the pest species. The different biotypes, however, have different abilities to survive these changes in the environment. RWASA4 over time became the most dominant RWA biotype in the summer rainfall area of South Africa. Different survival abilities might give one

biotype a competitive advantage over the others. Merrill, *et al.* (2014) found that the American biotype RWA2 had an overwintering competitive advantage over RWA1, which enabled this biotype to displace RWA1 [25]. RWASA4 may be able to displace other biotypes, persist in the environment and increase their populations when the conditions become more favourable, as will be the case with an increase in the area of wheat cultivation. Since this biotype is the most virulent, able to overcome most RWA resistant genes in wheat, this may have serious consequences for the wheat industry in the summer rainfall areas of South Africa. These observations emphasize the value of intercropping and crop rotation in managing insect pests and can serve as model for RWA management in areas where wheat and barley are the predominant crops.

## Conclusion

The plasticity of the Russian wheat aphid will continue to challenge the development of Russian wheat aphid-resistant wheat cultivars in South Africa. RWA populations fluctuate when the environment changes, but does not disappear and persists in the wheat production areas of South Africa, sometimes at low population levels and minimal to no damage to wheat crops. This may change with changing environmental condition because RWA is capable of surviving at low numbers for a relatively long time, and can cause sudden population outbreaks with conditions beneficial for population growth. The continued monitoring of the biotypic and genetic structure of Russian wheat aphid populations to determine known biotypes and to detect new biotypes and population increase of these biotypes is therefore an important feature of RWA management.

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