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CHAPTER 3

Comparison of Russian wheat aphid (*Diuraphis noxia*) developmental studies on resistant and susceptible near-isogenic wheat (*Triticum aestivum*) cultivars

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3.1 Abstract

The Russian wheat aphid (*Diuraphis noxia*) is a serious insect pest of wheat in South Africa causing an average 60% losses in yield. Symptoms of infestation include leaf streaking, inward curling of leaves, leaf senescence and stunted growth. Russian wheat aphid feeding studies were conducted on two near-isogenic wheat lines, cvv. 'Tugela *Dn1*' (Tugela *5/SA 1684; Russian wheat aphid resistant) and 'Tugela' (Russian wheat aphid susceptible), and a third cultivar, 'Palmiet' (Russian wheat aphid susceptible). The development of first instar nymphs was monitored on wheat leaves of the three cultivars. Nymphs preferred the susceptible cultivars because more nymphs survived to reach reproductive maturity (80% for 'Palmiet' and 85% for 'Tugela') compared to the resistant cultivar (25% for 'Tugela *Dn1*'). However, those nymphs that survived to become reproductive adults showed no significant differences in their reproductive rates or life span on the three cultivars. The surviving aphids show no statistical differences when the reproduction and life span on resistant and susceptible wheat plants were compared. The resistance encountered by the RWA on the resistant 'Tugela *Dn1*' line showed that nymphs experienced difficulties in settling down and locating the phloem, causing 75% to die before becoming reproductively mature; indicating the antibiotic resistance of 'Tugela *Dn1*'.

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3.2 Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), was first recognized as a serious insect pest on wheat (*Triticum aestivum*) in South Africa during 1978. Feeding damage by the RWA results in chlorotic lesions on the leaves (Walters *et al.*, 1980) and reduction in growth and development of susceptible plants (Burd & Burton, 1992) because of a reduction in photosynthetic leaf area as a result of leaf stunting and new leaves not unfolding.

The first RWA resistant wheat cultivar (cv. 'Tugela*5/SA 1684'; 'Tugela *Dn1*') was produced in South Africa and released for commercial production in 1989 (Du Toit, 1989). Almost normal growth occurs on resistant wheat plants and only small isolated chlorotic spots are found. There is also a lower RWA colonization on these plants (Quisenberry & Schotzko, 1994).

Most studies on the development of the RWA on wheat have focused on the effect of plant growth stage and temperature (Aalbersberg *et al.*, 1987; Michels & Behle, 1988, 1989; Girma *et al.*, 1990). There have also been investigations into the development of the RWA when reared on resistant and susceptible genotypes of wheat (Quisenberry & Schotzko, 1994; Zwer *et al.*, 1994; Rafi *et al.*, 1996). No developmental studies have been conducted comparing the near-isogenic wheat lines, cvv. 'Tugela' (susceptible to the RWA), 'Tugela *Dn1*' (resistant), as well as the cultivar 'Palmiet' (susceptible).

The diet of an aphid consists mostly of the phloem sap from the host plant. The phloem sap consists largely of sucrose and amino acids, where the sucrose acts as an attractant (Dreyer &

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Campbell, 1987). When the RWA probes the plant, the stylet follows an intercellular path to the phloem (Fouché *et al.*, 1984). On resistant wheat lines, the RWA has difficulty in locating the phloem (Webster *et al.*, 1993). The probing time to reach the phloem was also found to be two times longer on the resistant lines (Kindler *et al.*, 1992; Webster *et al.*, 1993). This implies that the RWA encounters some resistance in the intercellular washing fluid (IWF), which is the path of the stylet. Aphid infestation of 'Tugela *Dn1*' results in the induction of four groups of proteins in the IWF (Van der Westhuizen & Pretorius, 1996). No induced proteins were found when the susceptible near-isogenic 'Tugela' cultivar was infested. Induced proteins were also found when a resistant barley line (Porter, 1992) and a resistant wheat line, PI 137739 (Rafi *et al.*, 1996) were infested with the RWA. These induced proteins in the infested resistant cultivar have been hypothesized to play a role in the resistance mechanism to the RWA. Studies comparing the RWA development and reproduction on susceptible and resistant wheat cultivars would help in understanding more about the resistance mechanisms of these plants.

The aim of this study was to infest the near-isogenic lines with the RWA and compare RWA development, longevity and fecundity. These lines were also compared to the susceptible 'Palmiet' cultivar which is not near-isogenic.

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3.3 Materials and Methods

3.3.1 Aphids

Wheat (cv. 'Palmiet') was grown in a greenhouse at a temperature of 25±1°C. This cultivar is susceptible to the RWA. RWA obtained from the Agricultural Research Council – Small Grain Institute (ARC-SGI) in Bethlehem, South Africa, was maintained on this wheat.

3.3.2 Developmental studies

Adult apterous aphids were removed from RWA colonies and placed on wheat leaves cut from the 'Palmiet' cultivar. The second and third leaves were used. Three aphids were placed on each leaf. These leaves were placed individually in a petri dish (Michels *et al.*, 1987). The cut ends were placed between moistened paper towels to prevent desiccation of leaves. These leaves were replaced every three to four days (Michels & Behle, 1989), or when they showed signs of desiccation. The petri dishes were placed at 25±1°C with a photoperiod of 12:12 (L:D). A fine horse-hair paint brush was used to transfer aphids (Aalbersberg *et al.*, 1987).

Nymphs produced by the adults were removed daily and placed individually on a wheat leaf as described above. The age of these nymphs (first instar) was therefore known (Aalbersberg *et al.*, 1987). The wheat leaves used here were either one of three cultivars; two cultivars that were susceptible to the RWA ('Palmiet' and 'Tugela') and the other was resistant to the aphid ('Tugela

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Dn1') (Tugela*5/SA 1684). The last two cultivars are near-isogenic lines. Each nymph/host-plant combination was replicated 20 times.

Individual nymphs were observed daily for moulting and survival. The presence of exuviae was used to establish that the aphid had moulted (Aalbersberg *et al.*, 1987). This indicated that the aphid had entered the next instar. When the aphids reached the adult stage (fifth instar), the number of progeny produced was also recorded.

3.3.3. *Statistical analyses*

Comparisons of the nymphal developmental period (before the onset of reproduction), progeny production and longevity was analyzed using a Student's t-Test (P = 0.05; Freund, 1976) with the Systat® 7.0.1 (1997) software.

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3.4 Results

The development of RWA nymphs were observed on the three wheat cultivars ('Palmiet', 'Tugela' and 'Tugela *Dn1*'). Their ability to survive and become reproductively active is presented in Table 3.1. Nymphal survival to reproductive maturity differed markedly on the susceptible ('Palmiet' and 'Tugela') and resistant wheat cultivars ('Tugela *Dn1*'). RWA survival on the two susceptible cultivars exceeded that of the resistant cultivar .

Table 3.1. Russian wheat aphid survival and reproductive maturity. Twenty nymphs were placed on each of three wheat cultivars ('Palmiet', 'Tugela' and 'Tugela *Dn1*') at 25±1°C with a 12:12 (L:D) photoperiod.

Cultivar	Percentage becoming reproductive adults
'Palmiet'	80%
'Tugela'	85%
'Tugela *Dn1*'	25%

Nymphal developmental time. Developmental time of the nymphal life stages was observed on all three cultivars and the results are presented in Table 3.2. The duration in days spent by the aphids in the II to IV instar was not significantly different on the three cultivars. However, significantly less time was spent in the first instar by aphids on the resistant cultivar ('Tugela

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Dn1'). The total duration spent in the developmental period was similar for the near-isogenic lines ('Tugela' and 'Tugela *Dn1*'), being 9.88 and 8.60 days respectively. Nymphs placed on 'Palmiet' remained in the developmental stage for a significantly shorter period (7.81 days) comparised to those on 'Tugela' (9.88 days).

Table 3.2. Duration of developmental stages in days for the Russian wheat aphid to reach the reproductive stage at 25±1°C with a 12:12 (L:D) photoperiod.

Cultivar	**Developmental Stages (Instars) (Days) ± SD**					**Total**
	1st*	**2nd***	**3rd***	**4th***	**5th***	
'Palmiet'	1.63±0.50^{a}	1.50±0.63^{a}	1.69±0.60^{a}	2.06±0.57^{a}	0.94±0.93^{a}	7.81±1.05^{a}
N	4	0	0	0	0	
'Tugela'	1.82±0.64^{a}	1.88±0.49^{a}	2.29±0.92^{a}	2.18±0.53^{a}	1.71±1.31^{b}	9.88±1.58^{b}
N	0	1	1	1	0	
'Tugela *Dn1*'	1.00±0.00^{b}	2.00±1.23^{a}	1.80±0.84^{a}	2.40±1.14^{a}	1.40±1.52^{ab}	8.60±1.52^{ab}
N	2	4	6	1	2	

*Means in the same column followed by the same letter are not significantly different (P>0.05).

N = the number of aphids out of the original 20 that died before reaching reproductive maturity.

Included in Table 3.2 is the instar life stage during which nonreproductively active nymphs died. On the susceptible cultivar ('Palmiet') all nymphal deaths occurred during the first instar. The

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other susceptible cultivar ('Tugela') had one nymphal death in each of the instars from II to IV. The resistant cultivar ('Tugela *Dn1*') had more nymphal deaths in most of the instars.

Reproduction. RWA reproduction on the three cultivars is given in Table 3.3. Results only include those nymphs that survived to become reproductive. There are no significant differences for the average number of nymphs produced on all three wheat cultivars. Comparatively 1.36 nymphs were produced per day on 'Palmiet' and this was no different to those on 'Tugela' and 'Tugela *Dn1*' (1.29 and 1.23 nymphs, respectively).

Table 3.3. Average number of nymphs produced per day, average number of nymphs produced per adult and average life span of the Russian wheat aphid on three wheat cultivars. Aphids were placed at 25±1°C with a 12:12 (L:D) photoperiod. (Table only includes nymphs that survived to become reproductive).

Cultivar	Average Nymphs/ Day ± SD*	Average Nymphs/ Adult ± SD*	Average Life Span (Days) ± SD*
'Palmiet'	1.36±0.38[a]	11.81±6.99[a]	15.13±4.49[a]
'Tugela'	1.29±0.61[a]	13.82±11.52[a]	18.47±6.18[a]
'Tugela *Dn1*'	1.23±0.55[a]	10.20±8.35[a]	14.80±4.92[a]

*Means in the same column followed by the same letter are not significantly different (P>0.05).

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Life span. Table 3.3 shows the average life span of the aphids on the three wheat cultivars tested. No significant differences are found amongst the three cultivars, suggesting that if a nymph survived to adulthood, the reproductive rate and average life span is not affected by the choice of the three host-plants used in this study.

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3.5 Discussion

RWA nymphs were placed on three wheat cultivars: two were susceptible to the RWA ('Palmiet' and 'Tugela') and the other was resistant ('Tugela *Dn1*'), with the last two being the near-isogenic lines. Nymphs were shown to prefer the susceptible cultivars in that more nymphs survived to reach reproductive maturity when compared to the resistant cultivar. Michels & Behle (1988) found a 74% survival rate when the RWA was placed on a susceptible 'Scout 66' wheat cultivar. This survival rate is similar to that found in this study on the two susceptible cultivars.

The total time spent in the developmental period, the reproduction and life span showed that there were no significant differences between the near-isogenic lines 'Tugela' and 'Tugela *Dn1*'. However, less aphids survived to become reproductively active on the resistant 'Tugela *Dn1*' cultivar. Michels & Behle (1988) found that the RWA spent on average 8.5 days in the prereproductive period on a susceptible wheat cultivar. They found the RWA to produce an average of 1.30 nymphs per day. Both these findings do not seem to differ markedly from the findings of this study. However, 16.90 nymphs were produced per adult and the RWA had a life span of 22.60 days (Michels & Behle, 1988), which is more than that observed in this study.

The RWA resistant cultivar, 'Tugela *Dn1*', displays moderate levels of resistance (antibiosis). Antibiosis is defined as the manner in which resistant plants adversely affect the biology of the insect (Painter, 1958). This resistance is conferred by the *Dn1* gene from the plant introduction line (PI) 137739 (Du Toit, 1989). Painter (1958) described some effects resulting from the

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feeding of insects on plants exhibiting antibiosis. These include death of the insect that often occurs during the first instar, abnormal life span, decreased fecundity and restlessness. When looking at the RWA on the resistant cultivar it was found that the aphid did not have a great likelihood of reaching adulthood (only 25%). However, death of the RWA only occurred 13% of the time in the first instar. The most deaths occurred in the third instar (40%). The life span and the fecundity were similar to those aphids on the susceptible cultivars. However, the aphids were more restless on the resistant cultivar. It has been shown that the probing time to reach the phloem is two times longer on resistant lines (Kindler *et al.*, 1992; Webster *et al.*, 1993). Aphids also made more brief probes on the resistant lines, although this was not found by Kindler *et al.* (1992). Therefore, aphids on the resistant lines had more difficulty in locating the phloem and less phloem sap was ingested. More time was spent on nonphloem feeding to survive.

In South Africa the RWA reproduces only by way of parthenogenesis (*i.e.* reproduction takes place without fertilization). Only winged (alate) or wingless (apterous) vivaparous females are found. Winged females are only produced on depletion of the food source or under adverse environmental conditions (Robinson, 1992). Countries where the RWA is indigenous have males and oviparae that mate to produce eggs. These eggs then overwinter during the harsh winters in these countries (Kiriac *et al.*, 1990). No sexual forms have been reported from South Africa.

All the RWA in South Africa are therefore asexual clones of each other. This then raises the question of how some individuals overcome resistance. The moderate levels of resistance (antibiosis) does not completely inhibit the aphid from feeding on the plant (Du Toit, 1987). One finds a lower colonization of aphids on infested resistant plants when compared to susceptible

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plants (Quisenberry & Schotzko, 1994). Only small isolated chlorotic spots are found and growth is almost normal. Therefore, some aphids in a population do manage to survive on resistant wheat plants. This is in agreement with results found in this study. Twenty-five percent of aphids placed on the resistant cultivar manage to overcome the resistance posed by 'Tugela *Dn1*' and show reproduction and longevity similar to aphids placed on the susceptible 'Palmiet' and 'Tugela' cultivars.

This is the first reported case that the surviving aphids show no statistical differences when comparing reproduction and life span on resistant and susceptible wheat plants. Rafi *et al.* (1996) found that there was a higher reproductive rate of the RWA on the susceptible cultivar compared to that of the resistant cultivar. However, in their experiment, the aphids were placed in leaf cages at densities of 20, 40 or 80 aphids. No comparison can be drawn when comparing aphids on excised leaves to those on plants as the physiology of the plant tissue changes after leaf excision. Aphids often accept excised leaves more readily; they develop more quickly and are larger than those on whole plants (Blackman, 1988). However, the excised leaves of all three wheat cultivars in this study were subjected to the same environmental conditions and thus comparisons can be drawn about the aphid's life span and reproductive rates on the three cultivars tested.

This study has shown that when the RWA was placed on the near-isogenic lines ('Tugela' and 'Tugela *Dn1*') the aphid encountered some resistance in the resistant line. This resistance was encountered when the aphid started to probe the leaf initially (Kindler *et al.*, 1992; Webster *et al.*, 1993) and resulted in the death of the nymph before the aphid became reproductive. A small percentage of nymphs survived and displayed similar development, fecundity and life span to

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those aphids on the susceptible cultivar. Aphids on resistant cultivars have difficulty in locating the phloem and then turn to nonphloem feeding to survive (Webster *et al.*, 1993). This does not have the same nutritional benefit as the phloem sap. Aphid infestation on 'Tugela *Dn1*' resulted in the induction of four groups of proteins in the intercellular washing fluid (Van der Westhuizen & Pretorius, 1996). These induced proteins have been hypothesized to play a role in the resistance of 'Tugela *Dn1*' to the RWA. On the resistant cultivar, the aphid will encounter these induced proteins when feeding on the nonphloem to survive. Further research would concentrate on identifying these induced proteins and determining if they play a role in the resistance that these plants pose to the RWA.

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3.6 References

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