CHAPTER 6

Leaf epicuticular wax ultrastructure and trichome effect on Russian wheat aphid (*Diuraphis noxia*) feeding

6.1 Abstract

The Russian wheat aphid (*Diuraphis noxia*) was first discovered on wheat in South Africa during 1978. It has since become a serious pest. The leaf epicuticular wax ultrastructure and leaf trichomes were examined on two Russian wheat aphid susceptible wheat cultivars ('Palmiet' and 'Tugela') and a Russian wheat aphid resistant wheat cultivar ('Tugela *Dn1*'). The lengths of the trichomes showed no significant differences in the three wheat cultivars examined. The resistant cultivar ('Tugela *Dn1*') had a significantly greater trichome density than the susceptible cultivars. Examination of the position of the trichomes revealed that there were differences for the adaxial and abaxial surfaces. Trichomes on all three wheat cultivars were found to occur mostly on the leaf veins of the adaxial surfaces, and on the leaf veins as well as between them on the abaxial surfaces. Leaf trichome density and position may act as physical obstacles to Russian wheat aphid feeding on leaf veins of adaxial leaf surfaces. The high trichome density on the leaf veins found in the resistant 'Tugela *Dn1*' cultivar could prevent the Russian wheat aphid from finding a suitable feeding site. Comparison of the scanning electron micrograph photos showed that the epicuticular wax structure was found to be similar for both the adaxial and abaxial surfaces amongst the three wheat cultivars. The wax structure was similar in the Russian wheat aphid resistant and susceptible cultivars and does not seem to affect Russian wheat aphid feeding.

6.2 Introduction

During its search for a potential food source, the Russian wheat aphid (*Diuraphis noxia*) (RWA) settles on a plant and comes into contact with the thin layer of lipids found on the surface known as the epicuticular wax. This covers the entire leaf surface. The chemical composition of these waxes are distinctive for each plant species and could play a role in RWA acceptance of the host plant (Dillworth & Berberet, 1990).

For the aphid *Sitobion avenae* feeding on wheat, cuticular waxes of leaves from wheat cultivars that were dark green and glossy (non-glaucous) were implicated in conferring resistance to this aphid. Conversely, glaucous (pale blue-green) plants were not found to be resistant to *S. avenae*. Fluorescent chromatography revealed that non-glaucous cultivars lacked diketones. Scanning electron microscopy revealed that non-glaucous leaves had a nearly smooth surface when compared to the glaucous leaves (Lowe *et al.*, 1985). They postulated that since diketones absorb ultraviolet light strongly, their absence in non-glaucous wheat would result in a visual deterrence to aphids. Also, the aphids may have difficulty clinging and probing the relatively smooth surface of the non-glaucous plants.

Ni & Quisenberry (1997) examined the epicuticular wax ultrastructure of two wheat cultivars, plant introduction (PI) 137739 (RWA resistant) and 'Arapahoe' (RWA susceptible). They found the wax ultrastructure to be similar in the two wheat cultivars and not to play a significant role in host preference. Ni *et al.* (1998) also found that leaf epicuticular waxes of different cereal crops had little effect on the feeding of the RWA.

Leaf trichomes have also been implicated in the resistance of plants to aphids. Ni and Quisenberry (1997) postulated that leaf trichomes play a role as a physical obstacle to RWA feeding. RWA that fed on the resistant PI 137739 cultivar spent more time probing the leaves before penetration, than on the susceptible 'Arapahoe' cultivar. The RWA also probed less and the feeding duration was shorter on the resistant cultivar compared to the susceptible cultivar. The resistant cultivar was found to be the least preferred amongst the two cultivars. Examination of the leaf surface structure revealed that leaf trichomes of PI 137739 were more than six times longer than 'Arapahoe', although 'Arapahoe' had a higher trichome density (Ni & Quisenberry, 1997). Trichomes are found on or near leaf veins. Subsequently, long trichomes are likely to be an important physical obstacle to aphids that probe close to leaf veins during phloem feeding.

This study investigated the effect of the leaf epicuticular wax structure and trichomes on RWA feeding. Three wheat cultivars were examined; two were susceptible to the RWA ('Palmiet' and 'Tugela') and the other was resistant to the RWA ('Tugela *Dn1*'; 'Tugela*5/SA 1684'). 'Tugela' and 'Tugela *Dn1*' are near-isogenic lines, differing only by the dominant RWA resistant gene, *Dn1*. The cultivar SA 1684 is also known as PI 137739; PI 137739 and 'Tugela' are the parental lines of 'Tugela *Dn1*' (Du Toit, 1989).

6.3 Materials and Methods

6.3.1 Plants

Wheat (*Triticum aestivum*) was grown in a greenhouse at a temperature of 25±1°C. Three wheat cultivars were grown; two were susceptible to the RWA ('Palmiet' and 'Tugela'), while the third was resistant to the RWA ('Tugela *Dn1*').

6.3.2 Leaf trichome examination

The RWA feeds mostly on the adaxial surfaces of wheat leaves (Ni & Quisenberry, 1997), hence the adaxial surfaces of the second and third wheat leaves were examined for trichomes.

Trichomes were measured using a stereo microscope (5X magnification) that was attached to an AxioCam. Measurements were done using Axiovision 2.0.5.3 (1999). Twenty-one leaves were examined for each of the three cultivars. The length of four randomly selected trichomes was measured on each leaf.

The trichome density was recorded by counting the number of trichomes in a 3 x 2mm^2 area (Ni & Quisenberry, 1997). The area examined was in the center of the leaf. The trichomes were counted using a microscope at 10X magnification. Twenty-one leaves were examined for each of the three cultivars.

6.3.3 Leaf epicuticular wax ultrastructure and trichome position

The epicuticular wax ultrastructure of the second and third wheat leaves of the three cultivars were examined using a scanning electron microscope (SEM) (Jeol JSM-840 Scanning Microscope). Air-dried leaves were used (Gülz *et al.*, 1992) as the standard plant tissue fixation and dehydration process affects the leaf epicuticular wax structure by partially removing the wax (Ni & Quisenberry, 1997; Ni *et al.*, 1998). Ten leaves of each cultivar were placed in a sealed container containing silicon gel. The leaves were taped to the petri dish to prevent curling during drying. The leaves were allowed to air dry for seven days. Ten segments were taken from the center of each leaf for each of the adaxial and abaxial surfaces of each of the three cultivars. These were then mounted on aluminum stubs and sputter-coated with a gold-palladium alloy. The epicuticular wax ultrastructure was then examined on the SEM at 9 500X magnification and photographed.

The position of the trichomes on the adaxial and abaxial surfaces of the wheat leaves was also examined. Leaf segments prepared and used to examine the epicuticular wax ultrastructure (above) were examined at 60X magnification and photographed. Twenty photographs were taken for each wheat cultivar; ten for each of the adaxial and abaxial surfaces. Trichomes were counted between and on veins for both the adaxial and abaxial surfaces for the three wheat cultivars in a 460 x 550 μm^2 area that was randomly chosen.

6.3.4 Statistical analysis

Trichome length, trichome density data and the position of the trichomes were subjected to an Analysis of Variance (ANOVA) ($P = 0.05$) using the SYSTAT® 7.0.1 (1997) software.

6.4 Results

The leaf trichomes and the structure of the leaf epicuticular wax of three wheat cultivars were examined to determine if they could affect RWA food selection and feeding.

Leaf trichome examination. Leaf trichome data is given in Table 6.1. Trichome density was significantly different in the three cultivars ($F = 40.67$; $df = 2, 249$; $P < 0.05$). The resistant 'Tugela *Dn1*' had the most trichomes per mm^2 compared to the susceptible cultivars. The susceptible 'Tugela' cultivar had 1.7 times less trichomes per mm^2 in comparison to its near-isogenic line, 'Tugela *Dn1*'. 'Palmiet' was found to have 1.37 times less trichomes per mm^2 than 'Tugela *Dn1*'. Of all three wheat cultivars examined, 'Tugela' had the lowest trichome density, having an even lower trichome density than the other RWA susceptible cultivar ('Palmiet').

No significant differences were found for the length of the trichomes ($F = 2.38$; $df = 2, 60$; $P < 0.05$) in the three wheat cultivars examined. The average length of a trichome was 197.67 µm on the resistant 'Tugela *Dn1*' cultivar, which was comparable to the two susceptible cultivars (233.19 and 215.57 µm for 'Palmiet' and 'Tugela', respectively). High standard deviations were found due to the differences in sizes of the randomly selected trichomes.

Leaf trichome position. SEM examination at 60X magnification revealed that trichomes on the adaxial surfaces were mostly located on the leaf veins (Fig. 6.1). Conversely, the trichomes on the abaxial surfaces were located on leaf veins as well as between leaf veins. This was statistically similar for all three wheat cultivars examined. For 'Palmiet', 'Tugela' and 'Tugela *Dn1*' the number of trichomes between the veins on the adaxial surfaces was significantly less to the number of trichomes between the veins on the abaxial surfaces for 'Palmiet' ($F = 26.55$; $df = 1, 18$; $P < 0.05$), 'Tugela' ($P = 22.46$; $df = 1, 18$; $P < 0.05$) and 'Tugela *Dn1*' ($F = 22.62$; $df = 1, 18$; $P < 0.05$). The number of trichomes occurring on the veins of the adaxial and abaxial leaf surfaces were statistically similar for each of 'Palmiet' ($F = 0.86$; $df = 1, 18$; $P < 0.05$), 'Tugela' ($F = 0.01$; $df = 1, 18$; $P < 0.05$) and 'Tugela *Dn1*' ($F = 1.95$; $df = 1, 18$; $P < 0.05$).

Leaf epicuticular wax ultrastructure. Visual examination of the SEM photos (9 500X magnification) of the wheat leaves showed that the epicuticular wax ultrastructure was very similar amongst the three wheat cultivars on both the adaxial and abaxial surfaces examined (Fig. 6.2, 6.3, 6.4). Photos of the ultrastructure of the epicuticular waxes were examined and compared to existing data (Ni & Quisenberry, 1997; Ni *et al.*, 1998). The structure of the epicuticular waxes was found to occur as an irregular mixture that consisted mostly of curved rod-shaped waxes with few flakes. The density of the epicuticular wax was similar for both the adaxial and abaxial leaf surfaces, as well as for the three different cultivars.

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

Cultivar	Number of trichomes, mm^2		Trichome length, μm	
	Mean ± SD*	n	Mean ± SD*	n
'Palmiet'	20.24±7.54^a	84	233.19±59.30^a	21
'Tugela'	16.39±6.90^b	84	215.57±42.94^a	21
'Tugela *Dn1*'	27.73±11.49^c	84	197.67±54.70^a	21

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

n = the number of wheat leaves examined.

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

Cultivar	**Number of trichomes between veins, 253 μm²**			**Number of trichomes on veins, 253 μm²**		
	Adaxial Mean±SD	**Abaxial Mean±SD**	**n**	**Adaxial Mean±SD**	**Abaxial Mean±SD**	**n**
'Palmiet'	0.40 ± 0.52^{a}	7.50 ± 4.33^{b}	10	6.90 ± 3.18^{a}	5.30 ± 4.45^{a}	10
'Tugela'	0.10 ± 0.32^{a}	6.60 ± 4.33^{b}	10	7.60 ± 4.01^{a}	7.80 ± 3.99^{a}	10
'Tugela *Dn1*'	0.40 ± 0.52^{a}	6.20 ± 3.85^{b}	10	9.70 ± 3.59^{a}	6.60 ± 6.04^{a}	10

[*] Means in the box followed by the same letter are not significantly different ($P<0.05$).

n = the number of wheat leaves examined.

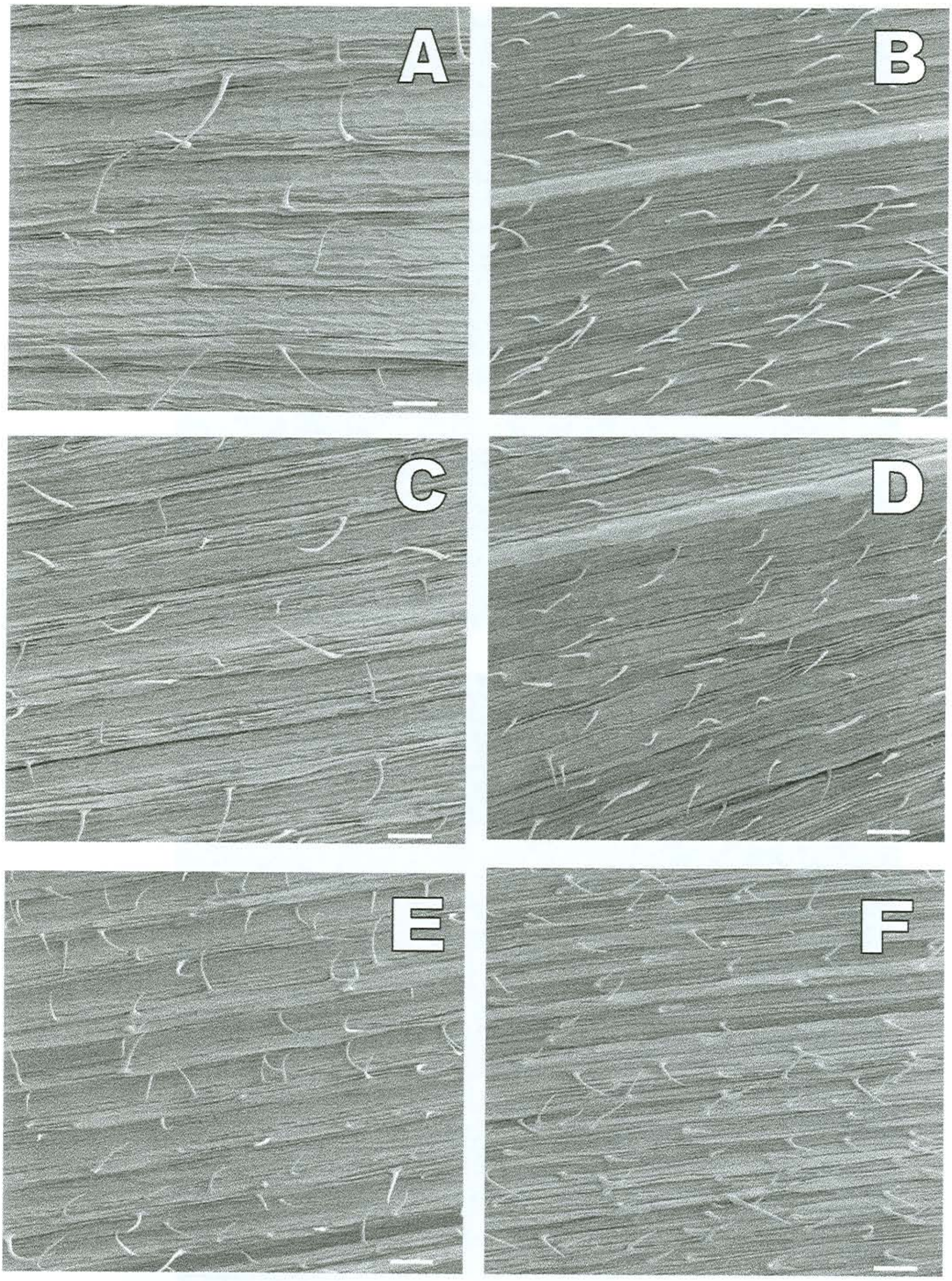

Figure 6.1. Scanning electron micrographs of the position of leaf trichomes on the adaxial and abaxial leaf surfaces of three wheat cultivars (60X; bars = 100 μm). (A) adaxial surface of 'Palmiet'. (B) abaxial surface of 'Palmiet'. (C) adaxial surface of 'Tugela'. (D) abaxial surface of 'Tugela'. (E) adaxial surface of 'Tugela *Dn1*'. (F) abaxial surface of 'Tugela *Dn1*'.

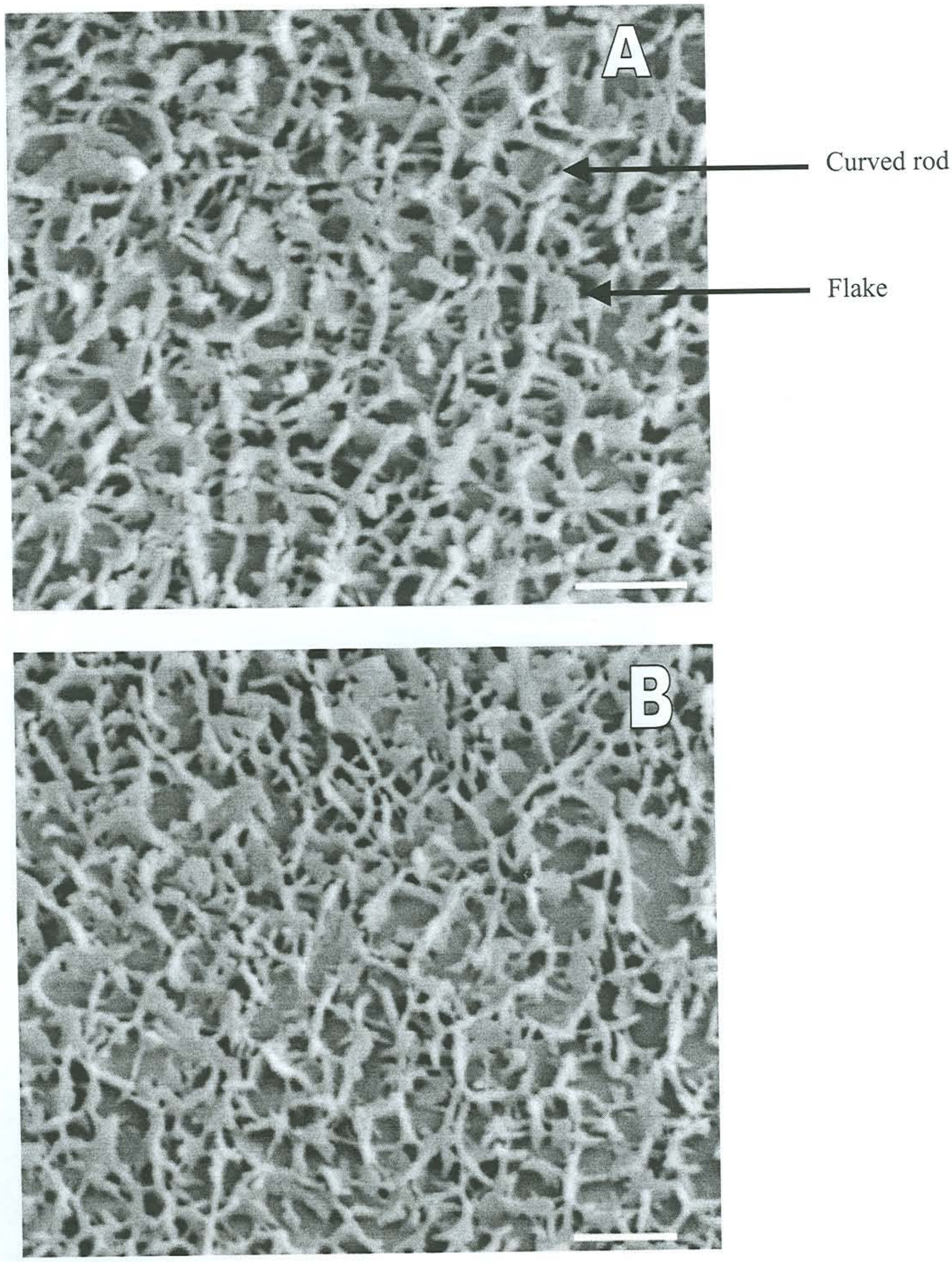

Figure 6.2. Scanning electron micrographs of epicuticular wax ultrastructure on the adaxial and abaxial surface of the 'Palmiet' wheat cultivar (9 500X; bars = 1μm). (A) adaxial surface. (B) abaxial surface.

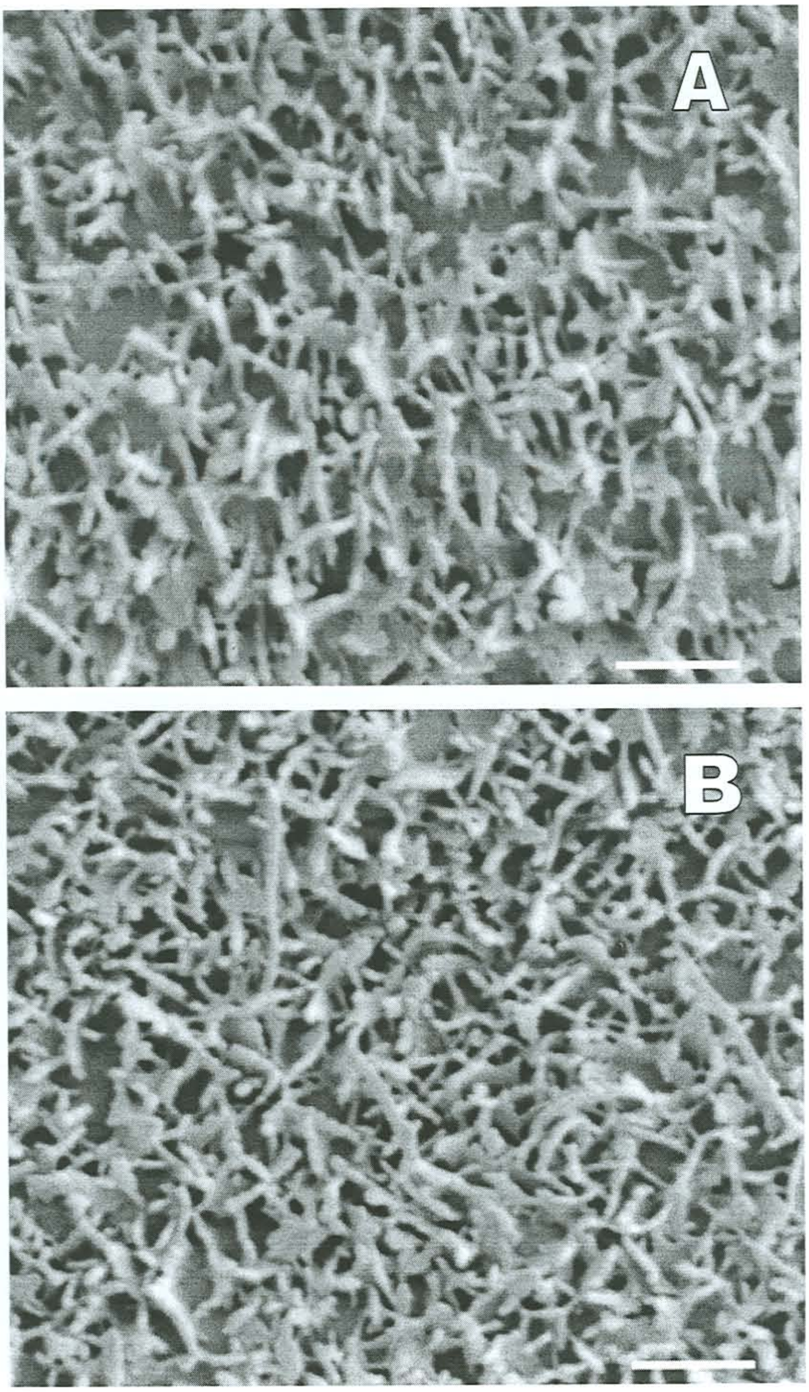

Figure 6.3. Scanning electron micrographs of epicuticular wax ultrastructure on the adaxial and abaxial surface of the 'Tugela' wheat cultivar (9 500X; bars = 1μm). (A) adaxial surface. (B) abaxial surface.

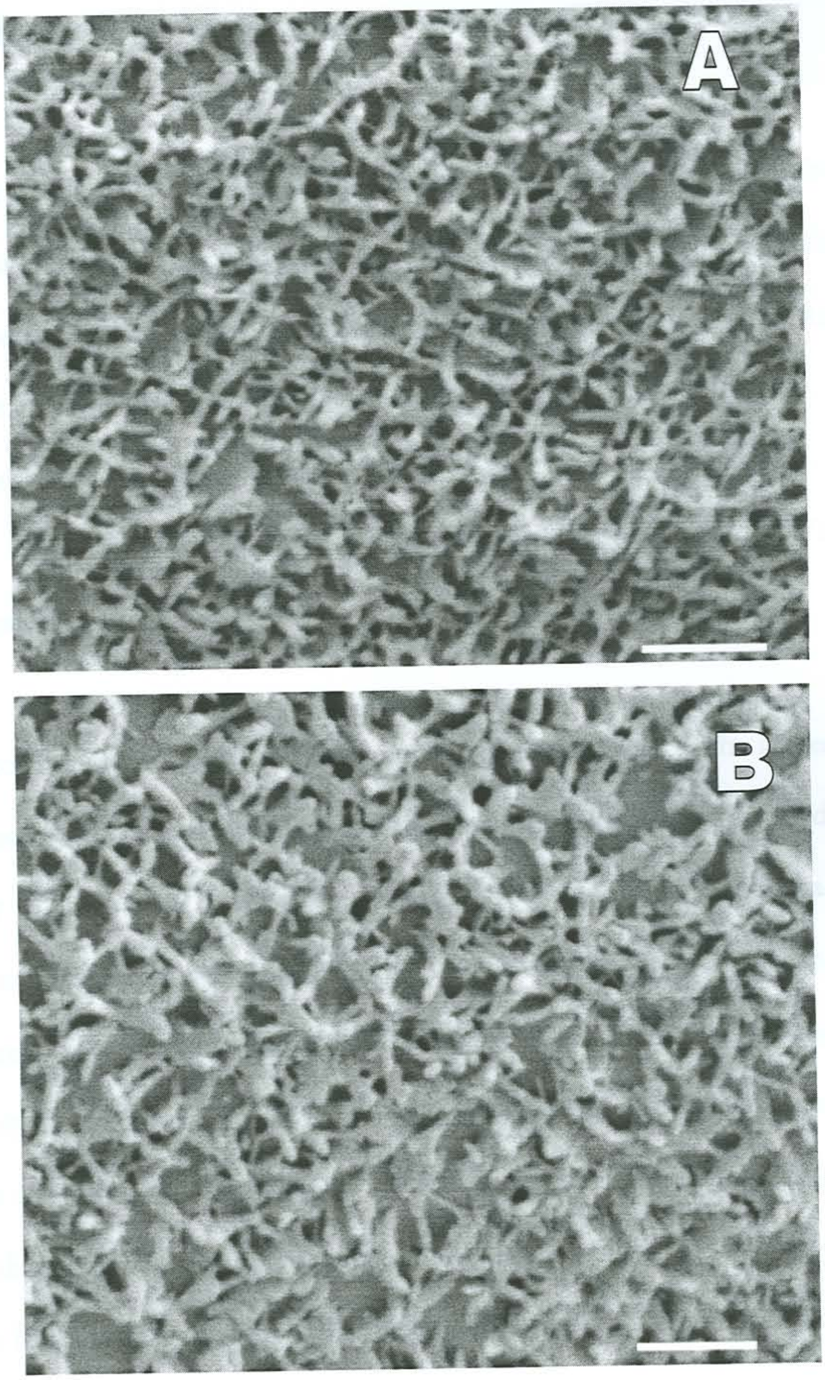

Figure 6.4. Scanning electron micrographs of epicuticular wax ultrastructure on the adaxial and abaxial surface of the 'Tugela *Dn1*' wheat cultivar (9 500X; bars = 1μm). (A) adaxial surface. (B) abaxial surface.

6.5 Discussion

The first surface that the RWA encounters when probing for a potential food source is the epicuticular wax covering the leaf surface. This wax covering is distinctive for each plant species and could play a role in RWA acceptance of the host plant (Dillworth & Berberet, 1990). Leaf trichomes could offer a physical obstacle to RWA feeding as these trichomes are found on or near leaf veins, where the RWA feeds (Ni & Quisenberry, 1997). The effects of these two leaf anatomical structures on RWA feeding were investigated.

Leaf trichome density was examined on the adaxial surfaces of the leaves and significant differences were found amongst the three wheat cultivars investigated. The resistant cultivar ('Tugela *Dn1*') had the highest trichome density. 'Tugela' had a significantly lower trichome density than 'Palmiet', when comparing the two susceptible wheat cultivars. There were no significant differences for the trichome lengths of the three cultivars investigated. Subsequently, only the density of the trichomes seems to play a role as an obstacle to feeding by the RWA. This is contrary to that found by Ni & Quisenberry (1997), that the antixenotic resistance of PI 137739 (SA 1684) was caused, in part if not totally, by long leaf trichomes and that trichome density did not contribute to the resistance of this cultivar when compared to a susceptible cultivar 'Arapahoe'. The cultivar PI 137739 had a lower trichome density compared to the susceptible wheat cultivar, 'Arapahoe' (Ni & Quisenberry, 1997).

The position of the trichomes showed that there were differences for the adaxial and abaxial surfaces. Trichomes on the three wheat cultivars were found to occur mostly on the leaf veins of the adaxial surfaces whereas they were found to occur on the leaf veins as well as between them on the abaxial surfaces. Similarly, Ni & Quisenberry (1997) found that trichomes were mainly found on the leaf veins on the adaxial side of the wheat leaves studied. The position and density of the trichomes could act as a physical impediment to the RWA gaining access to the leaf veins, where feeding occurs.

Cultivars PI 137739 and 'Tugela' are the parental lines of 'Tugela *Dn1*' (Du Toit, 1989). The trichome density of PI 137739 was approximately 14 trichomes per mm^2 (Ni & Quisenberry, 1997) which is similar to that of 'Tugela', but about half that of 'Tugela *Dn1*' (28 trichomes per mm^2). The trichome length of PI 137739 was much greater (473 µm) than that of 'Tugela' and 'Tugela *Dn1*' (216 and 198µm, respectively). PI 137739 displays high levels of antibiosis as well as some antixenotic resistance (Du Toit, 1989). Antixenosis is defined as the nonpreference of plants for insect oviposition, shelter or food (Painter, 1958). The nonpreference of 'Tugela *Dn1*' has manifested itself as a high trichome density. The RWA is less than 2mm in length (Walters *et al.*, 1980) and feeds preferentially on the adaxial surfaces of leaves (Ni & Quisenberry, 1997). As can be seen from Figure 6.4 (A) the high trichome density that occurrs mostly on the adaxial leaf veins (where the aphid feeds), would act as a physical impediment to RWA feeding. 'Tugela *Dn1*' would subsequently be nonpreferred by the RWA for feeding because of difficulties associated with reaching the leaf veins. RWA on other resistant plants have been shown to be restless; they require more time to initiate feeding activities (Kindler *et al.*, 1992; Webster *et al.*, 1993).

A visual comparison of the SEM photos showed that the epicuticular wax structure was found to be similar for both the adaxial and abaxial surfaces amongst the three wheat cultivars. As the wax structure was similar on the RWA resistant and susceptible cultivars, the structure of the wax does not seem to play a role in RWA feeding. Lowe *et al.* (1985) found that on wheat cultivars that were resistant to *S. avenae,* the wax surface was relatively smooth and postulated that the insects had difficulty clinging to and probing these leaves. The findings of the leaf epicuticular wax ultrastructure agree with other studies on the influence of epicuticular wax on RWA feeding (Ni & Quisenberry, 1997; Ni *et al.*, 1998). Subsequently, leaf epicuticular wax ultrastructure does not appear to play important in RWA feeding.

The RWA feeds on leaf veins of the adaxial surfaces of leaves (Ni & Quisenberry, 1997). On the resistant wheat cultivar ('Tugela *Dn1*'), trichomes with a higher density than those of the susceptible cultivars ('Palmiet' and 'Tugela'), were found to occur mostly on the leaf veins of the adaxial surfaces. Subsequently, it was postulated that the density of the leaf trichomes plays a role in the antixenotic resistance that the RWA encounters when feeding on the resistant cultivar. The high density of trichomes acts as a physical impediment to the RWA reaching and feeding from the leaf veins on the adaxial leaf surfaces. The epicuticular wax structure and length of trichomes do not appear to be important in RWA feeding on the three wheat cultivars examined.

6.6 References

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