# Leaf epicuticular wax ultrastructure and trichome presence on Russian wheat aphid (*Diuraphis noxia*) resistant and susceptible leaves

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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 DN'). The lengths of the trichomes showed no significant differences in the three wheat cultivars examined. The resistant cultivar ('Tugela DN') had a significantly greater trichome density compared to 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 a physical obstacle to Russian wheat aphid feeding as the aphid feeds on leaf veins of the adaxial leaf surfaces. The high trichome density on the leaf veins found in the resistant 'Tugela DN' cultivar could prevent the Russian wheat aphid from finding a suitable feeding site. Comparison of the scanning electron micrographs showed that the epicuticular wax structure was similar on both the adaxial and abaxial surfaces amongst the three wheat cultivars, and does not seem to affect Russian wheat aphid feeding.

**Key words**: *Diuraphis noxia*, Russian wheat aphid, trichomes, leaf epicuticular wax ultrastructure.

## 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 the waxes in this layer is distinctive for each plant species and could play a role in RWA acceptance of the host plant (Dillworth & Berberet 1990).

Cuticular waxes of leaves from wheat cultivars that were dark green and glossy (non-glaucous) were implicated in conferring resistance to the aphid *Sitobion avenae.* 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 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 to and probing the relatively smooth surface of the non-glaucous plants.

Ni & Quisenberry (1997) examined the epicuticular wax ultrastructure of two wheat lines, plant introduction (PI) 137739 (RWA resistant) and 'Arapahoe' (RWA susceptible). They found the wax ultrastructure to be similar in the two wheat lines 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 & Quisenberry (1997) postulated that leaf trichomes play a role as a physical obstacle to RWA feeding. RWA that fed on the resistant PI 137739 spent more time probing the leaves before penetration, than on the susceptible 'Arapahoe' cultivar. Feeding duration was also shorter on the resistant line compared to the susceptible cultivar. The resistant line was found to be less preferred of the two. Examination of the

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leaf surface structure revealed that leaf trichomes of PI 137739 were more than six times longer than those of 'Arapahoe', although 'Arapahoe' had a higher trichome density (Ni & Quisenberry 1997). Trichomes are found on or near leaf veins. Long trichomes are likely to be an important physical obstacle to aphids that probe close to leaf veins during phloem feeding.

This study investigated 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 DN'; 'Tugela*5/SA 1684'). 'Tugela' and 'Tugela DN' are near-isogenic lines, differing only by the dominant RWA resistant gene, *Dn1.* The line SA 1684 is also known as PI 137739; PI 137739 and 'Tugela' are the parental lines of 'Tugela DN' (Du Toit 1989).

## MATERIAL AND METHODS

### *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 DN').

### *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 (×5 magnification) attached to an AxioCam. Measurements were done using AxioVision 2.0.5.3 (Carl Zeiss Vision GmbH, Germany, 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 × 2 $mm^2$ area (Ni & Quisenberry 1997). The area examined was in the center of the leaf. The trichomes were counted using a microscope at ×10 magnification. Twenty-one leaves were examined for each of the three cultivars.

### *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 with silicon gel. The leaves were taped to a 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 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 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 × 550 $\mu m^2$ area that was randomly chosen.

### *Statistical analysis*

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

## RESULTS

### Leaf trichome examination

Leaf trichome data is given in Table 1. Trichome density was significantly different in the three cultivars ($F = 40.67$, d.f. = 2,249, $P < 0.05$). The resistant 'Tugela DN' had the most trichomes per $mm^2$ compared to the susceptible cultivars, while 'Tugela' had the lowest trichome density. The susceptible 'Tugela' had 1.7 times fewer trichomes per $mm^2$ in comparison to its near-isogenic line, 'Tugela DN'. 'Palmiet' was found to have 1.37 times fewer trichomes per $mm^2$ than 'Tugela DN'.

No significant differences were found for the length of the trichomes ($F = 2.38$, d.f. = 2,60, $P <$ 0.05) in the three wheat cultivars examined. The

**Table 1**. Trichome length and density in three wheat ('Palmiet' and 'Tugela', RWA susceptible; and Tugela DN, RWA resistant) cultivars.

| Cultivar | No. of trichomes/mm$^2$ | | Trichome length (µm) | |
|---|---|---|---|---|
| | Mean ± S.D. | *n* | Mean ± S.D. | *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 DN | 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.

average length of a trichome on the resistant 'Tugela DN' was 197.67 µm, which was comparable to the two susceptible cultivars (233.19 and 215.57 µm for 'Palmiet' and 'Tugela', respectively).

### Leaf trichome position

SEM examination at ×60 magnification revealed that trichomes on the adaxial surfaces were mostly located on the leaf veins (Fig. 1). Conversely, the trichomes on the abaxial surfaces were located on leaf veins and between veins. This was statistically similar for all three wheat cultivars examined. For 'Palmiet', 'Tugela' and 'Tugela DN' the number of trichomes between the veins on the adaxial surfaces was significantly lower than the number of trichomes between the veins on the abaxial surfaces for 'Palmiet' ($F = 26.55$, d.f. = 1,18, $P < 0.05$), 'Tugela' ($F = 22.46$, d.f. = 1,18, $P < 0.05$) and 'Tugela DN' ($F = 22.62$, d.f. = 1,18, $P < 0.05$) (Table 2). The number of trichomes on the veins of the adaxial and abaxial leaf surfaces were statistically similar for each of 'Palmiet' ($F = 0.86$, d.f. = 1,18, $P < 0.05$), 'Tugela' ($F = 0.01$, d.f. = 1,18, $P < 0.05$) and 'Tugela DN' ($F = 1.95$, d.f. = 1,18, $P < 0.05$).

### Leaf epicuticular wax ultrastructure

Visual examination of the SEM photos of the wheat leaves showed that the epicuticular wax ultrastructure was very similar among the three wheat cultivars on both the adaxial and abaxial surfaces examined (Fig. 2). SEM micrographs 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 structures with few flakes.

## DISCUSSION

The first surface that the RWA encounters when probing 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).

Leaf trichome density was examined on the

**Table 2**. Position of trichomes on the adaxial and abaxial surfaces of three wheat ('Palmiet' and 'Tugela', RWA susceptible; and Tugela DN, RWA resistant) cultivars examined.

| Cultivar | No. of trichomes between veins/253 µm$^2$ | | | No. of trichomes on veins/253 µm$^2$ | | |
|---|---|---|---|---|---|---|
| | Adaxial Mean ± S.D.[1] | Abaxial Mean ± S.D. | *n* | Adaxial Mean ± S.D. | Abaxial Mean ± S.D. | *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 DN | 0.40 ± 0.52$^a$ | 6.20 ± 3.85$^b$ | 10 | 9.70 ± 3.59$^a$ | 6.60 ± 6.04$^a$ | 10 |

Means within same column followed by the same letter are not significantly different ($P < 0.05$).
*n* = the number of wheat leaves examined.

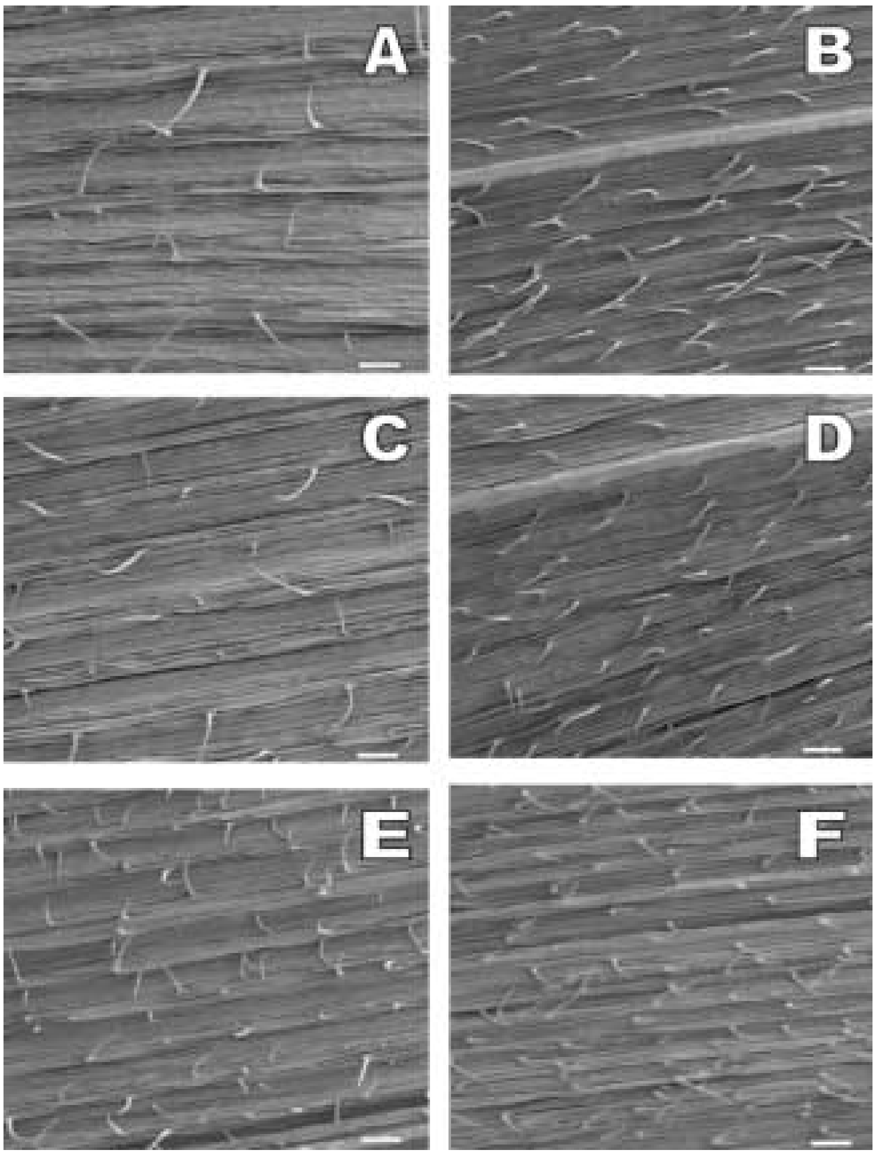


**Fig. 1**. Scanning electron micrographs of the position of leaf trichomes on the adaxial (**A**, **C**, **E**) and abaxial (**B**, **D**, **F**) surfaces of the 'Palmiet' (**A**, **B**), 'Tugela' (**C**, **D**) and 'Tugela DN' (**E**, **F**) wheat cultivars (×60; scale bars = 100 µm).

adaxial surfaces of the leaves and significant differences were found among the three wheat cultivars investigated. The resistant cultivar ('Tugela DN') 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. Consequently, only the density of the trichomes seems likely 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).

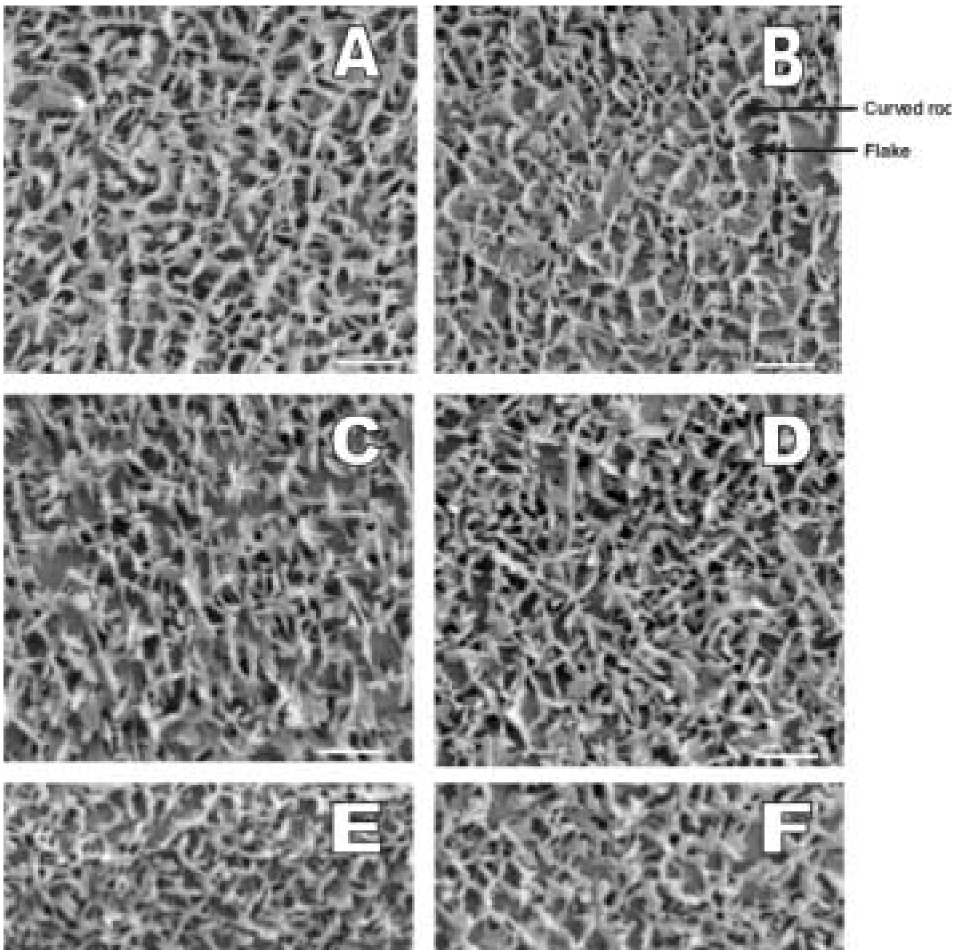


**Fig. 2**. Scanning electron micrographs of the epicuticular wax ultrastructure on the adaxial (**A**, **C**, **E**) and abaxial (**B**, **D**, **F**) surfaces of the 'Palmiet' (**A**, **B**), 'Tugela' (**C**, **D**) and 'Tugela DN' (**E**, **F**) wheat cultivars (×9500; scale bars = 1 µm).

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.

PI 137739 and 'Tugela' are the parental lines of 'Tugela DN' (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 DN' (28 trichomes per mm$^2$). The trichome length of PI 137739 was much greater (473 $\mu$m) than that of the near isogenic 'Tugela' and 'Tugela DN' (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 non-preference of plants for insect oviposition, shelter or food (Painter 1958). The RWA is less than 2 mm in length (Walters *et al.*1980) and feeds preferentially on the adaxial surfaces of leaves (Ni & Quisenberry 1997). As can be seen from Fig. 1E the high trichome density that occurs mostly on the adaxial leaf veins (where the aphid feeds), would act as a physical impediment to RWA feeding. 'Tugela DN' could therefore be less attractive to 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 similar for both the adaxial and abaxial surfaces among 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 this study on 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). Therefore, leaf epicuticular wax ultrastructure does not appear to be 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 DN'), 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 could play a role in the non-preference that the RWA exhibits when feeding on resistant cultivars, while epicuticular wax structure and length of trichomes appear not to be important in RWA feeding (Ni & Quisenberry 1997, Ni *et al.*1998, Bahlmann 2002). The high density of trichomes may act as a physical impediment to the RWA reaching and feeding from the leaf veins on the adaxial leaf surfaces.

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