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Disruption of Phloem Transport by the two South African Biotypes of the Russian Wheat Aphid [*Diuraphisnoxia* Kurdjumov (Homoptera: Aphididae)] Feeding on Barley (*Hordeumvulgare* L.)

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Abstract
We investigated the comparative effects of the feeding of two South African biotypes of the Russian wheat aphid (RWA, *Diuraphisnoxia*Kurdjumov), RWASA1 and RWASA2, on the phloem transport functionality of three selected exotic RWA-resistant barley (*Hordeumvulgare* L.) lines. RWASA2 is known to breed faster and as a more aggressive feeder, causing more severe damage than RWASA1 on host plants. We examined the phloem transport capacity of the barley lines, using the phloem-mobile fluorophore, 5,6-carboxyfluorescein diacetate (5,6-CFDA).Feeding by the newly-emerged more aggressive RWASA2 biotype caused a more marked decrease in phloem transport capacity, compared to RWASA1, during both short- and long-term feeding exposure. The extensive phloem damage was mirrored in a significant reduction in transport capacity in non-resistant PUMA, but not to the same extent in the USDA lines, indicative of reduced resistance when these lines were subjected to the South African RWA biotypes. The resistant lines should therefore be explored in breeding programmes for development of RWA-resistant barley lines in South Africa.

Key words: Aphid biotypes, aphid feeding, barley, phloem transport capacity, resistance, Russian wheat aphid.

Introduction

Transport of assimilates in plants from regions of synthesis to regions of utilization and storage is an essential process without which growth and development would be impossible [1,2]. Effective translocation of assimilates has been reported to be the basis of improved plant performance and agricultural yield [3]. This process has been extensively studied in the vascular bundles of several monocots [4-7].The major constituent of the solutes translocated in phloem (phloem sap) is sucrose which is sought after by aphids as a source of nutrients [8]. Aphids' stylets inflict damage on the pressurized sieve tubes of the phloem, breaching the sieve tube walls in order to ingest the sap [9]. Because this sap is low in proteins, aphids ingest large quantities of it [10], so as to acquire sufficient amino acids needed for their survival, they egests excess in form of 'honey dew' [11]. Aphids therefore become secondary sinks, by diverting assimilates primarily meant for distribution to growing plant tissues [12].

Apart from diverting assimilates; the damage to sieve tubes by the aphids must affect the transport capacity of the phloem [9, 13-15]. This implies that aphids, during feeding, become a strong secondary sink which results in pressure loss in the phloem pathway below the points where aphids insert their stylets. Evert et al. [16] demonstrated that aphids' stylets puncturing parenchyma cells and elements of the phloem during feeding caused turgor loss and reduced phloem transport capacity. Botha and Matsiliza [14] suggested that the RWA feeding on a susceptible wheat cultivar might have resulted in feeding-related pressure loss, when they observed reduced phloem capacity to transport assimilates. In addition, probing and feeding on plants cause the formation and deposition of wound callose in sieve tube elements of the phloem. These effects

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contribute to substantial decrease in the rate of transport across the vascular parenchyma to the companion cell-sieve tube complexes.

De Wet and Botha [15] reported that the grass aphid (*Sitobionyakini*) feeding on susceptible *Betta* wheat cultivar, caused extensive callose deposition in phloem tissues, which was manifested in a reduction of phloem transport capacity. Interestingly, the emergence of a second RWA biotype, RWASA2, was reported in South Africa [17]. To our knowledge, only the study by Walton and Botha [18] has so far examined the feeding damage caused by this second biotype and the old biotype, RWASA1, on wheat hosts. It was therefore necessary to investigate the effects of the feeding damage caused by these biotypes on transport functionality of their host plants. This is because in a previous study, we have shown that RWASA2 breeds faster than RWASA1 on resistant and non-resistant barley hosts [19]. We demonstrated that the two biotypes inflict diverse damage symptoms on their host plants and that RWASA2 caused more severe damage than RWASA1. The underlining mechanism behind these diverse damage symptoms inflicted on variety of host plants need to be understood.

In the present study, we focus on the effects of the feeding-related damage caused by RWASA1 and RWASA2 on phloem transport capacity of three selected RWA-resistant barley lines developed by United States Department of Agriculture (USDA), using a susceptible PUMA cultivar as control. We used the phloem-mobile fluorophore, 5,6-carbofluorescein diacetate (5,6-CFDA), which has proved to be reliable in studying phloem transport capacity of wheat and barley [see 14; 15; 9], to examine the effects which feeding by the two RWA biotypes would have on the phloem tissues of the four barley lines to transport photoassimilates. This is in an attempt to relate possible differences in the phloem transport capacities of the three resistant plants to their respective resistance against the two RWA biotypes. We hypothesised that RWASA2, which is the more aggressive feeder [18], would cause greater reduction in phloem transport capacity of the four barley plants than RWASA1 and that the non-resistant PUMA cultivar would show a greater reduction in the rate of phloem transport than any of the three resistant lines. We set out to show that the results demonstrate possible differential trends of phloem transport capacities among the three resistant plants, with the view of identifying the most vigorous in relation to infestation by the two RWA biotypes.

Materials and Methods

Plant Materials, Aphid Colonies and Maintenance

Four barley lines were used: PUMA, a South African commercially cultivated cultivar, non-resistant to RWA [Tolmay, pers. comm.] and three selected RWA-resistant barley lines namely STARS-0502B (PI 47541), STARS-9301B (PI 573080) and STARS-9577B (PI 591617), all developed by the United States Department of Agriculture, Agricultural Research Station (USDA – ARS), Stillwater, Oklahoma [20-23].

The barley seeds were pre-germinated in petri dishes and sown in potting soil (50:25:25; garden soil: compost: vermiculite mixture), in a greenhouse maintained at 20 - 30°C and 65% RH for one week. They were then removed to controlled environment growth chamber (Conviron S10H, Controlled Environment Ltd., Winnipeg, Manitoba, Canada) maintained at a day time maximum of 24°C and 66% relative humidity (RH) and at 22°C, 60% RH (night), with a 14-h photo-period. The light source was a combination of fluorescent tubes (F48T12. CW/VHO 1500, Sylvania, Danvers, MA) and frosted incandescent 60W bulbs (Phillips, Eindhoven, The Netherlands), with a photosynthetic active radiation (PAR) level of 250 μmol^{-2} s^{-1} 30cm below the light source. The plants were watered three times a week with half strength Long Ashton nutrient solution and were grown in the Conviron for two weeks to reach 2 – 3 secondary leaf stage before they were manually infested with the aphids.

Colonies of the two biotypes of the RWA (i.e. RWASA1 and RWASA2) were obtained from the ARC. They were maintained on susceptible young barley cv. Clipper feeder plants [24] and thereafter kept in insect cages in separate growth chambers. Adult apterous aphids were used in all experiments.

Experimental Procedure

Clipcages (25) were used to enclose a 5-cm long leaf segment at the mid-region of either the second or the third leaf above the coleoptile of a fully expanded leaf of each test plant. Control plants were also fitted with clipcages but were not infested with aphids. A leaf segment from the feeder plant containing 10 apterous aphids was carefully introduced into each clipcage. The aphids were allowed 24h to transfer and settle on the confined area before commencing the four treatments which consisted of 24h, 72h (short-term), 7d and 14d (long-term) feeding periods. Ten replicates of each treatment (2 aphid types, each infesting 4 different barley lines) and control (uninfested) plants were set up. These consisted of 90 plants per treatment with a total of 360 plants in all. Experimental procedures were repeated twice.

Preparation of 5, 6-carbofluorescein Diacetate (5,6-CFDA)

The stock solution of 5,6-CFDA (C-195, Molecular Probes, Eugen, Oregon, USA) was made by adding 1ml of 0.2% dimethyl-sulphoxide (DMSO) to 100mg of 5,6-CFDA. This was foil-wrapped and stored at -5°C until needed. A working solution, 217µM in distilled water, was prepared by adding 1µL aliquots of the stock solution to 1ml distilled water in propylene centrifuge tubes, foil-wrapped to prevent cleavage of the 5,6-CFDA by light, frozen at -5°C, defrosted when needed and each tube used immediately.

Leaf Material Treatment

Intact plants were used for all the treatments, using the flap feeding method [9]. After every feeding treatment, the experimental leaf was first rinsed with Ca^{2+}-free buffer (10mM 2-[morpholino] ethanesulfonic acid (MES); 0.5mM $MgCl_2$, 0.5mM KCl and 125mM mannitol, adjusted to pH 7.2), and gently abraded on the abaxial surface with a sterilized needle. 100µL working solution of 5,6-CFDA was applied to the abraded area and covered with transparent polythene film (Housebrand, Brackenfell, South Africa) to prevent evaporation of the solution. In conformity with the classical pattern of assimilates' acropetal and basipetal movements [26], source leaves were always abraded on the part above the clipcage, while sink leaves were abraded below the clipcage.

The fluorophore was allowed 3h to be taken up and transported through the abraded area. At the end of the 3h allowed for the loading and transportation of the dye, the caged region of the leaf where the aphids were confined for feeding was marked with a soft tip marker. The clipcage was gently dismantled, experimental leaf excised at the base and placed on a glass slide. The fluorescent front and the distance transported from the point of application of the dye in control (uninfested) as well as aphid-infested leaves were observed under UV light using Olympus BX61 wide-field fluorescence microscope fitted with U-YFP filter set (10C/Topaz 41028, Chroma Technologies, Battlebro, USA) with excitation of 513nm and an emission of 527nm. Immediately after recording the distance which 5,6-CF moved in the phloem, the portion of the leaf within the clipcage was cut and its abaxial surface was gently scraped under silicone oil on a glass plate, using a sharp single-edge carbon steel razor blade (Agar Scientific, USA), in order to remove the cuticle and expose the underlying vascular tissues. The scraped leaf tissue was promptly mounted on a glass slide in silicone oil and observed under the microscope. Images taken were saved in a database using the programme analySIS (Soft Imaging System GmHb, Germany) and imported as bitmaps to Corel Draw 12 (Corel Corporation, Ottawa, Canada 2003) for presentation.

Data Analysis of distance moved by 5,6-CF

Relative rates of phloem transport in leaves of the experimental plants fed upon by RWASA1 and RWASA2 during the four feeding treatments were simulated, using the distance moved by the cleaved 5,6-CF from the point of application to the fluorochrome front in three hours. Data recorded was expressed as percentage of measurement for control (uninfested) for each treatment day. The resulting data was subjected to arcsine transformation [27]. Homogeneity of variances and normality of the data were examined using Levene's and Shapiro-Wilk's tests respectively (28). A three-way factorial ANOVA was used to examine the differences of the effects of the feeding activities of RWASA1 and RWASA2 on the four barley lines during the four feeding exposures on Statistical version 8. Homogenous groups were further identified using Tukey*posthoc* test at 5% level of significance.

Results

Transport of 5,6-CF in control (uninfested) Leaves

Three hours after the application of 5,6-CFDA, the cleaved product (5,6-CF) moved into the leaf mesophyll, loaded into the bundle sheath cells and thereafter into the vascular bundles (Fig. 1). The dye front was observed in unscraped leaves at approximately 5cm from the point of application of 5,6-CFDA. Similar results were obtained in experiments involving all control (uninfested) leaves of the four lines carried out after 24-h, 72-h, 7-day and 14-day treatment periods. Movement of 5,6-CF in uninfested control as well as in infested leaves always took place from the site of application of the fluorochrome towards the leaf base (basipetal) in source leaves, and towards the lamina tip (acropetal) in sink leaves (data not shown). Figure 1 (A-D) illustrates movement of 5,6-CF in longitudinal and cross veins of leaf blade material of uninfested PUMA. Continuous band of bright fluorescence was observed in longitudinal as well as cross veins.

Transport of 5, 6-CF in Infested Leaves

Figures 2 – 3 illustrate typical wide–field fluorescence images which show movement of 5,6-CF in vascular bundles of infested leaves of non–resistant and resistant barley lines (treated separately) after short-term (24h and 72h) and long-term (7d and 14d) feeding exposures.

Infested Non–resistant Leaves

After 24h of feeding by both RWASA1 and RWASA2, there was slight reduction in the flow of 5, 6-CF (Figs. 2A and B) when compared to its movement in control uninfested leaves (Figs. 1A and C). During longer feeding periods of 72h, 7d and 14d (Figs. 2C-H), there was progressive reduction in the intensity and distance moved by 5,6-CF. Discontinuous bands of fluorescence were observed after 72h of RWASA2 feeding (Fig. 2D). Prolonged feeding for 7 and 14 days by both biotypes showed patchy and uneven distribution of the fluorophore in longitudinal veins (Figs. 2E and G). These also illustrate a reduction in the intensity of fluorescence in files of sieve tubes (Fig. 2H), which becomes more obvious when compared to that obtained in control leaves (Fig. 1B).

Infested Resistant Leaves

Figures 3A-H show typical images of movement and distribution of 5,6-CF in longitudinal veins of infested resistant lines. Contrary to our expectations, the three resistant lines infested with either RWASA1 or RWASA2 generally showed reduction in the transport of the fluorophore in phloem tissues in a manner similar to results obtained in the non–resistant PUMA. Distribution of 5,6-CF is progressively reduced as days of aphid feeding increases. The patchy appear-ance and uneven distribution of cleaved 5,6-CF in the phloem tissues of these resistant lines suggest that both biotypes caused structural damage to the vascular tissues, which adversely affected phloem functional capacity to transport assimilates.

Comparison of the distance moved by 5,6-CF in Infested Leaves of Non–resistant and Resistant Barley Lines

Figures 4 and 5 show effects of feeding by RWASA1 and RWASA2 on phloem transport capacity of the four barley lines, during the short- and long-term feeding treatments respectively. A three-way factorial analysis of variance (ANOVA) of the differences in the means of the transformed data on movement of 5,6-CF, measured as a percentage of the uninfested control treatments, was carried out and subsequently confirmed with Tukey*posthoc* tests at 95% level of confidence.

Table 1 shows results of ANOVA at various levels of interactions for the treatments. In the two treatments, we established from ANOVA that the two aphids, the four barley lines and days of feeding exposures were significantly different ($p<0.01$). We also established that interactions between the aphids and the barley lines and days of feeding exposures and barley lines were significantly different ($p<0.01$). However, interactions between the aphids and their respective days of feeding as well as the three-way interaction of the aphids, barley lines and days of feeding were not significantly different ($p>0.01$) (see Table 1).

Table 1: General Linear Model (GLM) Results of Comparison of Various Levels of Interactions on Movement of 5, 6-CF measured as Percentage of Control during Short – and Long – term Treatments†

Interaction	Short – term	Long – term
Aphid	$F_{1, 144}= 29.53^{*}$	$F_{1, 144}= 78.59^{*}$
Line	$F_{3, 144}= 48.41^{*}$	$F_{3, 144}= 841.97^{*}$
Day	$F_{1, 144}= 442.64^{*}$	$F_{1, 144}= 185.05^{*}$
Aphid X Line	$F_{3, 144}= 12.83^{*}$	$F_{3, 144}= 5.47^{*}$
Aphid X Day	$F_{1, 144}= 1.51$ n.s.	$F_{1, 144}= 0.80$ n.s.
Line X Day	$F_{3, 144}= 3.04^{*}$	$F_{3, 144}= 3.37^{*}$
Aphid X Line X Day	$F_{3, 144}= 2.32$ n.s.	$F_{3, 144}= 0.99$ n.s.

† Separate analyses were conducted for short – and long – term feeding treatments.
Levels of significance are indicated as: n.s. (not significant) $p>0.01$ and * $p<0.01$.

A relatively short time of RWASA1 and RWASA2 feeding significantly reduced distance moved by the fluorochrome in the non-resistant PUMA compared to the three resistant lines (Fig. 4). It was obvious that RWASA2 feeding on PUMA for 24h significantly reduced 5,6-CF movement than RWASA1 feeding. There is no significant difference in the distance moved by the fluorophore due to feeding by the two biotypes in the three resistant lines ($p<0.01$). During longer 72-h feeding period, further reduction in distance moved by the fluorochrome was recorded compared to data at 24h feeding period (Fig. 1). There was no significant difference in the distance the 5,6-CF moved in RWASA1-infested PUMA and STARS-9577B. Movement of the fluorophore was significantly greater in STARS-0502B and STARS-9301B than in both PUMA and STARS-9577B under RWASA1 infestation for 72h. With the exception of STARS-9577B, feeding by RWASA2 for 72 hours caused reduced transport of 5,6-CF in all barley lines, compared to RWASA1 feeding. It was clearly shown that transport of 5,6-CF during short-term treatments of 24h and 72h by the two RWA biotypes is above

60% of control (uninfested) plant. It was evident that among the four lines, STARS-9301B was the least affected by both feeding aphids. This plant recorded more than 80% fluorochrome movement.

Infestation by the two aphids for 7 and 14 days greatly reduced transport of 5,6-CF in non-resistant PUMA compared to the three resistant plants (Fig. 2). During the two feeding treatments, RWASA2 feeding reduced movement of the fluorochrome, more than RWASA1 did. Although there was variation in the reactions of the three resistant plants infested by the two aphids (Fig. 5), it was obvious that RWASA2 feeding reduced fluorochrome movement more than feeding by RWASA1. Similar to the situation during short-term feeding, movement of 5,6-CF in leaves of STARS-9301B was least affected by both aphids during long-term feeding.

Discussions

In this study, we show how feeding by the two RWA biotypes disrupt transport of assimilates in the phloem of resistant and non-resistant barley. The results give a broad view of longitudinal transport taking place in phloem tissues, and illustrate symplasmic movement of the fluorochrome, its subsequent loading into the phloem through bundle sheath-vascular parenchyma and companion cell-sieve tube complexes as described in previous reports (29; 30; 31; 32). In similar investigations Botha and Matsiliza [14] and Saheed et al. [9] used 5,6-CFDA to demonstrate phloem loading through many symplasmic connections between the mesophyll and vascular tissues. They explained that once in the phloem, movement of the cleaved 5,6-CF follows the classical source-sink relationship in transport of assimilates, which is acropetal in sink leaves and basipetal in source leaves, as put forward by Turgeon (26). Continuous movement of assimilates, when not under abiotic or biotic influence (such as aphid feeding in the present study), is unrestricted but confined within the transporting veins as shown in the longitudinal veins of the uninfested control tissues (Figs.1A-D).

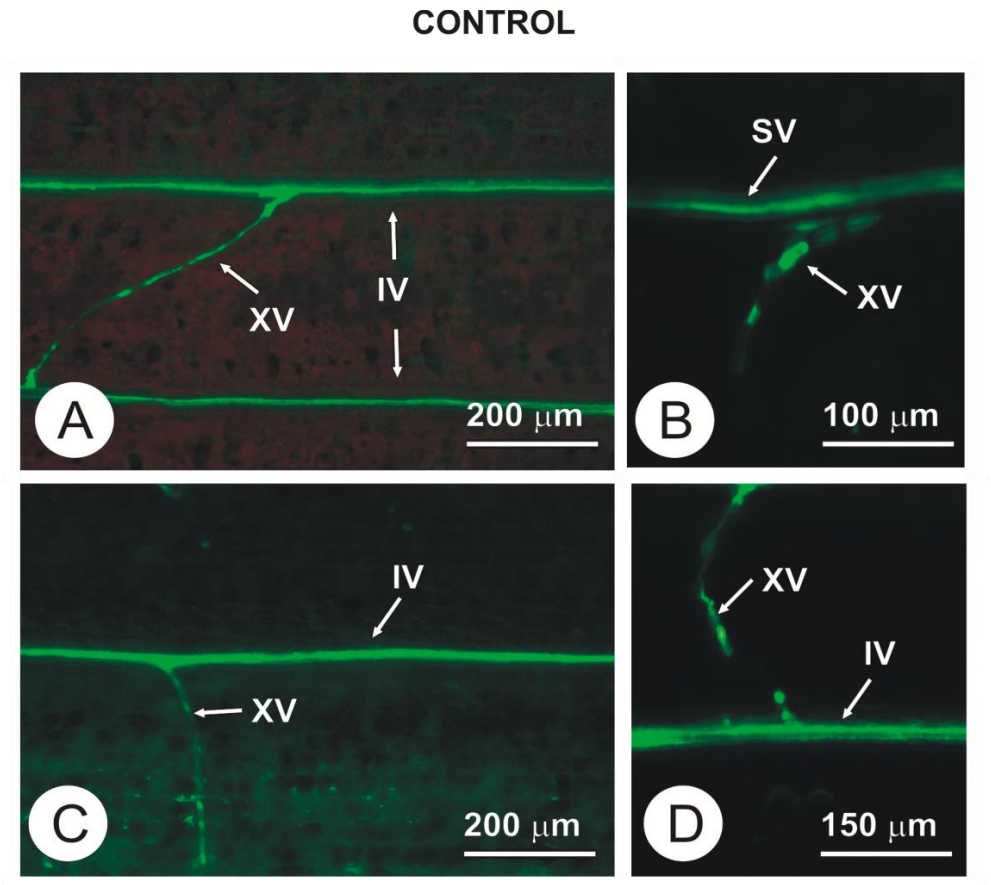

Fig. 1A-D: Transport of 5,6-CF in control (uninfested) leaves of barley. A and Cshow uninterrupted flow of the fluorochrome along intermediate veins (IV) and cross vein (XV). B and D show fluorescence in the cross veins indicating movement of the fluorochrome in sieve tubes.

Our results show that the rate of phloem transport in uninfested control barley tissues is about 2cm per hour, similar to that measured by Botha and Matsiliza [14] and Saheed et al. [9]. The rate was reduced in infested plants, depending on the feeding aphid biotype (RWASA1 or RWASA2), host plant genotype (whether resistant or non-resistant) and duration of infestation as shown in Figures 4 and 5 (short- or long-term). For instance, RWASA2 feeding reduced fluorochrome movement than RWASA1 feeding did, which indicates that RWASA2 must have slowed down assimilate flow than RWASA1. This is statistically confirmed by the ANOVA summary contained in Table 1, which showed that the two aphid biotypes are significantly different from one another. Results of this study is similar to earlier report by Saheed et al. [9] that feeding by two different aphid species, RWA and BCA, caused different rates of reduction in the transport of assimilates in the phloem of a susceptible barley cultivar. In addition, our results indicate that the rate of assimilate flow, through movement of the fluorochrome, reduces as the number of days of infestation by the aphids increases. This agrees with the work by Saheed et al. [9]. The study further showed that phloem transport is significantly reduced in aphid-infested non-resistant PUMA, when compared to the data of the infested resistant lines (Figs. 4 and 5). This is the first study which compares and quantifies disruption of phloem transport in resistant and non-resistant host plants exposed to feeding by biotypes of the same aphid.

Feeding by either RWASA1 or RWASA2 on non-resistant as well as resistant lines affected movement of 5,6-CF (Figs. 2 and 3). During short-term feeding (24h or 72h), the two aphids caused reduction in fluorochrome transported in the assimilate stream of non–resistant (Figs. 2A-D) and resistant (Figs. 3A-D) lines. The disruption to 5,6-CF movement was noticeable after 24h of aphid feeding (non-resistant, Figs. 2A and B; resistant, Figs. 3A and B). This became worse with longer feeding for 72h (non-resistant, Figs.2C and D; resistant, Figs. 3C and D), both showing slightly patchy patterns, implying a disturbance in the flow pathway. This may be due to deposition of wound callose as a result of wounding response induced by the feeding aphids [25].

A clear description of this development is better understood in Figures 4 and 5. Movement of 5,6-CF in non-resistant PUMA infested with RWASA2 for 24h was about 70% of control compared to about 90% with RWASA1; the corresponding values in the resistant lines is approximately 95% of uninfested control (Fig. 4). The values were further reduced during longer feeding for 72h. Sustained feeding exposures (7d and 14d) resulted in greater reduction in phloem transport capacity, the trend of which are RWASA2 > RWASA1 and non-resistant line > resistant lines (Fig. 5). More patchy distribution pattern and reduced intensity of the fluorochrome were observed in non-resistant (Figs. 2E and G) and resistant (Figs. 3E-H) lines. Severe feeding by RWASA2 on non-resistant PUMA for 14 days might have caused complete cessation of transport (Fig. 2H). These results lend support to findings by Botha and Matsiliza [14] that infested leaves of a susceptible wheat cultivar showed little longitudinal or transverse trafficking of 5,6-CF. The feeding aphids in this case have massively damaged vascular tissues and re-routed assimilates containing the fluoro-chrome to themselves, as demonstrated by Saheed et al. [9].

This study shows that feeding by the two RWA biotypes alter the phloem transport functionality of resistant and non-resistant barley lines. Once their stylets penetrate functional phloem, aphids constitute them-selves into local sinks, redirecting and ingesting products of photosynthesis into their guts. Hill [33] showed that if draining of photoassimilates by feeding aphids is sufficiently strong and localised, host plant reacts to it in such a way as if the feeding aphid were its bud. In this manner, aphids compete directly with the primary sink organs of the plant, denying them of essential nutrients normally supplied during assimilates transport through the phloem. This position is again illustrated by Saheed et al. [9] in which 5,6-CF sucked by RWA and BCA were visibly present in honeydew excreted after feeding. Similar phloem feeding insects have been shown to disrupt and redirect assimilates in their respective host plants [13,34]. The alteration to the transport system leads to a decline in flow rate, quality and availability of assimilates, loaded and transported in source leaves and eventually unloaded in sink leaves or organs.

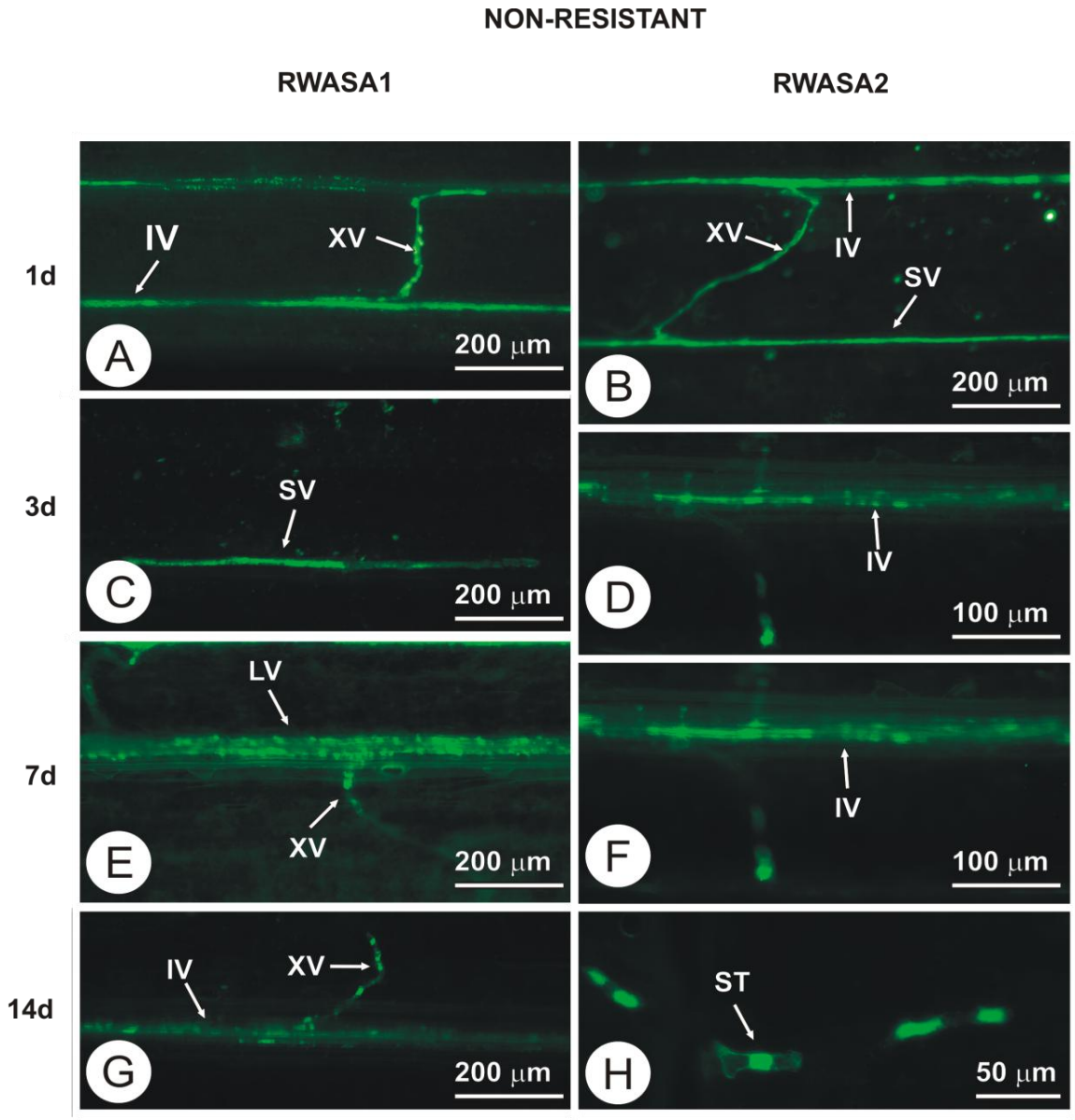

Fig. 2A-H: Transport of cleaved 5,6-CF in veins of non-resistant PUMA line infested with RWASA1 (left hand side) and RWASA2 (right hand side) after short- and long-term feeding exposures. **A** and **B** illustrate intermediate and small veins after 24h of RWASA1 and RWASA2 feeding respectively. The fluorochrome is not as continuous as in the control. **C** shows small vein (SV) after 72h feeding by RWASA1. **D** shows intermediate vein from which RWASA2 fed. **E** illustrates patchy distribution of the fluorochrome in the large vein (LV) after7d of RWASA1 sustained feeding. **F** shows detail from **D.** When compared to RWASA1, RWASA2 feeding caused more reduced rate of fluorochrome movement. **G.** An intermediate vein which appears to have been blocked after a long-term 14d feeding by RWASA1. **H.** Detail of a cross vein showing reduction in fluorescence brightness of sieve tubes from a leaf blade material fed upon by RWASA2.

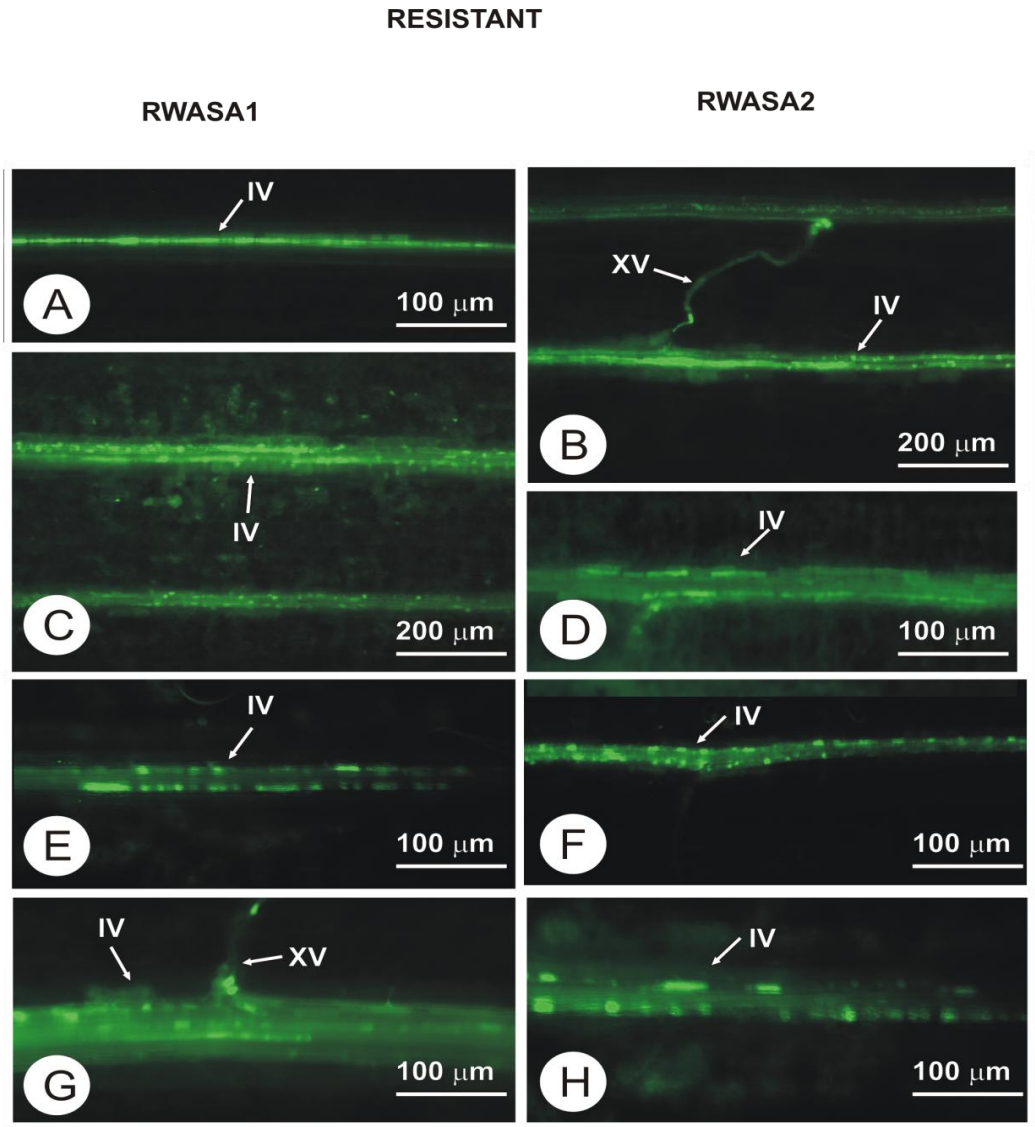

Fig. 3A-H Distribution and movement of 5,6-CF among resistant lines infested with RWASA1 (left hand side) and RWASA2 (right hand side) after short- and long- term feeding periods. **A.** Short- term (24h) feeding by RWASA1 shows marginal disruption to flow in an intermediate vein. **B** shows a more discontinuous flow of the fluorochrome due to RWASA2.**C.**More patchy distribution in intermediate vein upon RWASA1 feeding for 3d. **D** shows detail of RWASA2 3d feeding indicating discontinuous flow of the fluorophore in the intermediate vein. **E** shows aspects of reduced transport during long-term 7d feeding by RWASA1. **F** detail illustrating RWASA2 feeding for 7d which caused uneven distribution of the fluorochrome in the intermediate vein. **G** and **H.** Details of large intermediate veins with greatly reduced transport in the phloem after sustained long-term 14d feeding by RWASA1 and RWASA2 respectively.

The study also shows that RWASA2 feeding caused greater disruption to phloem transport functionality than RWASA1. This suggests that RWASA2 must have inflicted more damage than RWASA1 feeding [18,19]. This observation confirms the position of earlier studies which showed that RWASA2 is not only a resistance-breaking biotype but a more aggressive feeder than RWASA1 [17,18]. As callose deposition is promoted by aphid infestation [see 25 and literature cited], the two biotypes may have induced differential levels of callose deposition as shown by Walton and Botha [18] in wheat, thereby producing various effects on movement of 5,6-CF as illustrated in Figures 4-5. We surmise that components of the saliva of the two aphids differ, which might have resulted in differences in phloem transport capacities of the plants they infest. Further investigations into the inherent qualities of the saliva of the two RWA biotypes is necessary, which we hypothesize might have made RWASA2 to be more devastating than the existing RWASA1 biotype.

The study presented here indicates disparity in levels of damage suffered by non-resistant and resistant lines of barley, in-line with the notion that resistant cultivars of plants are able to cope with aphid infestation and show less physical damage than non-resistant cultivars [35]. Walton and Botha [18] demonstrated differences in the severity of damage sustained by feeding RWASA1 and RWASA2 on susceptible *Tugela* wheat and its resistant *Tugela-Dn* counterpart. Also, De Wet and Botha [15] showed that there was reduction in the transport of assimilates in the phloem of non-resistant Betta wheat, whereas the near-isogenic resistant *Betta-Dn* did not suffer such reduction at all.

In conclusion, the study demonstrated the mechanism behind movement of phloem sap in resistant and non-resistant barley hosts infested with two biotypes of RWA currently known in South Africa. Disruption to phloem transport functionality by the feeding aphids could result into reduction in carbohydrate translocation capacity of the phloem. This can adversely affect plant development and productivity. Ultimate total cessation of phloem transport through severe and sustained aphid feeding will negatively affect barley yield, which loss in production has been reported to be 30 – 60% due to RWASA1 infestation [36]. Reduction in damage to phloem tissues recorded on the exotic resistant lines (STARS-0502B, STARS-9301B and STARS-9577B) shows they are promising in the development of barley lines that can be used to control RWA infestation in South Africa.

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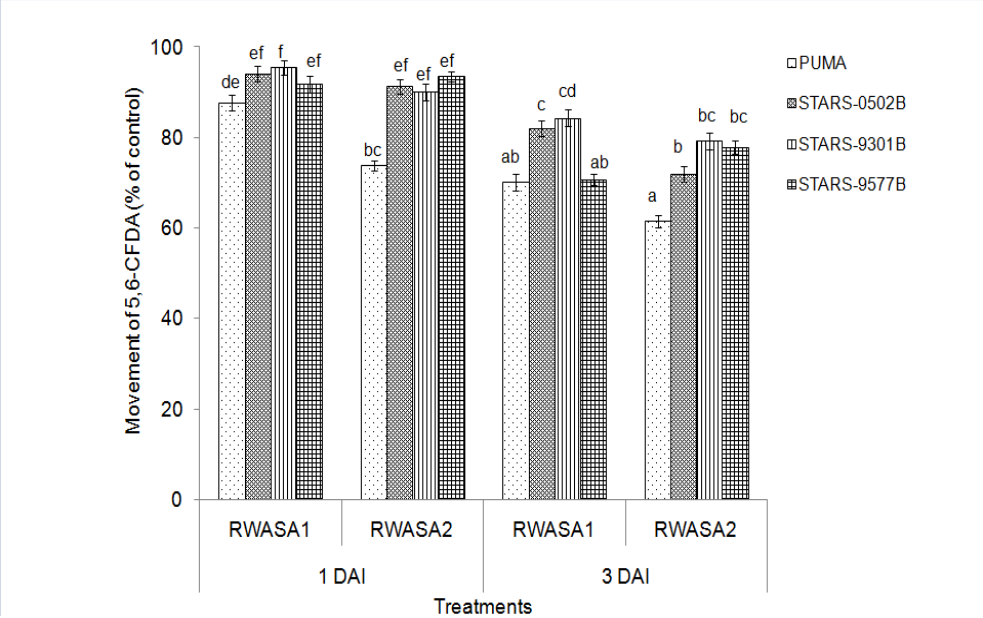

Fig. 4 Comparison of the distance moved by 5, 6-CF from point of application measured as percentage of control in leaves of the four barley lines infested with RWASA1 and RWASA2 during short – term treatments. Letters above each bar indicate results of Tukey*posthoc* test at 5% level of significance to identify homogenous groups. n=10.

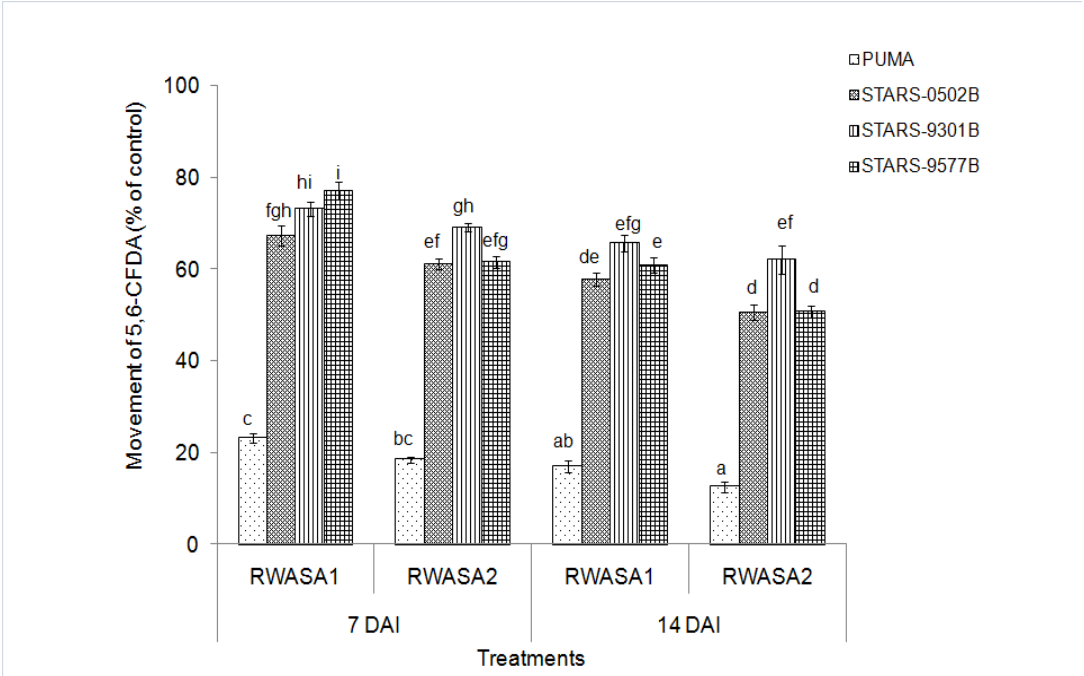

Fig. 5 Comparison of the distance moved by 5, 6-CF from point of application measured as percentage of control in leaves of the four barley lines infested with RWASA1 and RWASA2 during long – term treatments. Letters above each bar indicate results of Tukey*posthoc* test at 5% level of significance to identify homogenous groups. n=10.

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References

(1) Yeo, A. 2007. General Introduction. In: Yeo, A. and Flowers, T. (Eds.) Plant solute transport. Oxford, Blackwell Publishing, pp 1-14.
(2) Pritchard, J. 2007. Solute transport in the phloem. In: Yeo, A. and Flower, T. (Eds.) Plant solute transport. Oxford, Blackwell Publishing, pp 234-274.
(3) Komor, E. 2000. Source physiology and assimilate transport: The interaction of transport metabolism, starch storage and phloem export in source leaves and effects on sugar status in phloem. *Aust. J. Plant Physiol.* 27: 497-505.
(4) Evert, R.F., Eschrich, W. and Heyser, W. 1978. Leaf structure in relation to solute transport and phloem loading in *Zea mays* L. *Planta* 138: 279-294.
(5) Altus, D.P. and Canny, M.J. 1982. Loading of assimilates in wheat leaves: the specialisation of vein types for separate activities. *Aust J Plant Physiol* 9: 571 – 581.
(6) Fritz, E., Evert, R.F. and Nasse, H. 1989. Loading and transport of assimilates in different maize leaf bundles: Digital image analysis of ^{14}C-microautoradiographs. *Planta* 178: 1-9.
(7) Botha, C.E.J. and Van Bel, A.J.E. 1992. Quantification of symplasmic continuity as visualized by plasmodesmograms: Diagnostic value for phloem-loading pathways. *Planta* 187: 359-366.
(8) Turgeon, R. and Wolf, S. 2009. Phloem transport: Cellular pathways and molecular trafficking. *Annu Rev Plant Biol* 60: 207-221.
(9) Saheed, S.A., Jonsson, L.M.V. and Botha, C.E.J. 2010. Russian wheat aphid causes greater reduction in phloem transport capacity of barley leaves than bird cherry-oat aphid. *Acta Bot Croat* 69: 7-18.
(10) Kennedy, J.S. and Fosbrooke, I.H.M. 1971.The plant in the life of an aphid. Symposia of the Royal Entomological Society of London. *Insect-Plant Relationship*, Vol. 6 pp. 129-140.
(11) Douglas, A.E. 1993. The nutritional quality of phloem sap utilized by natural aphid populations. *Ecol. Entomol.* 18: 31-38.
(12) Girousse, C., Faucher, M., Kleinpeter, C. and Bonemain, J. 2003. Dissection of the effects of the aphid *Acyrthosiphonpisum* feeding on assimilate partitioning in *Medicagosativa*. *New Phytol.* 157: 83-92.
(13) Nielsen, G.R., Lamp, W.O. and Stutte, G.W. 1990. Potato leafhopper (Homoptera: Cicadellidae) feeding disruption of phloem translocation in alfalfa. *J. Econ. Entomol.* 83: 807-813.
(14) Botha, C.E.J. and Matsiliza, B. 2004. Reduction in transport in wheat (*Triticumaestivum*) is caused by sustained phloem feeding by the Russian wheat aphid (*Diuraphisnoxia*). *S. Afr. J. Bot.* 70: 249-254.
(15) De Wet, L.R. and Botha, C.E.J. 2007. Resistance or tolerance: An examination of aphid (*Sitobionyakini*) phloem feeding on Betta and Betta-Dn wheat (*Triticumaestivum*). *S. Afr. J. Bot.* 73: 35-39.
(16) Evert, R.F., Eschrich, W., Medler, J.T. and Alfeiri, F.J. 1968. Observation of the penetration of linden branches by the stylets of the aphid *Longistigmacaryae* (Ham.). *Am J Bot* 55: 860-874.
(17) Tolmay, V.L., Lindeque, R.C. and Prinsloo, G.J. 2007. Preliminary evidence of a resistance-breaking biotype of the Russian wheat aphid, *Diuraphisnoxia* (Kurdjumov) (Homoptera: Aphididae), in South Africa. *Afr.Entomol.* 15: 228-230.
(18) Walton, S.M. and Botha, C.E.J. 2008. The effects of two different biotypes (SA-1 and SA-2) of Russian wheat aphid, *Diuraphisnoxia* (Kurdjumov) (Homoptera: Aphididae) on non-resistant and resistant wheat cultivars Tugela and Tugela-*Dn* (*Triticumaestivum*). Proceedings of the 23rd International Congress on Entomology (ICE), pp. 2029.

(19) Jimoh, M.A., Botha, C.E.J., Edwards, O. and Bradley, G. 2011. Population growth rate and relative virulence of the two South African biotypes of Russian wheat aphid, *Diuraphisnoxia* and bird cherry-oat aphid, *Rhopalosiphumpadi*, on resistant and non-resistant barley. *Entomol ExpAppl* 138: 12-20.

(20) Webster, J.A., Porter, D.R., Baker, C.A. and Mornhinweg, D.W. 1993. Resistance to Russian wheat aphid (Homoptera: Aphididae) in barley: Effects on aphid feeding. *J Econ Entomol* 86: 1603-1608.

(21) Mornhinweg, D.W., Porter, D.R. and Webster, J.A. 1995. Registration of STARS-9301B Russian wheat aphid resistant barley germplasm. *Crop Sci* 35: 602.

(22) Mornhinweg, D.W., Porter, D.R. and Webster, J.A. 1999. Registration of STARS-9577B Russian wheat aphid resistant barley germplasm. *Crop Sci* 39: 882-883.

(23) Puterka, G.J., Burd, J.D., Mornhinweg, D.W., Haley, S.D. and Peairs, F.B. 2006. Response of resistant and susceptible barley to infestations of five *Diuraphisnoxia* (Homoptera: Aphididae) biotypes. *J Econ Entomol* 99: 2151-2155.

(24) Saheed, S.A., Botha, C.E.J., Liu, L. and Jonsson, L. 2007. Comparison of structural damage caused by Russian wheat aphid (*Diuraphisnoxia*) and Bird cherry-oat aphid (*Rhopalosiphumpadi*) in a susceptible barley cultivar, *Hordeumvulgare*cv. clipper. *Physiol Plant* 129: 429-435

(25) Saheed, S.A., Cierlik, I., Larsson, K.A.E., Delp, G., Bradley, G., Jonsson, L.M.V. and Botha, C.E.J. 2009. Stronger induction of callose deposition in barley by Russian wheat aphid than bird cherry-oat aphid is not associated with differences in callose synthase or β- 1,3-glucanase transcript abundance. *Physiol Plant* 135: 150-161.

(26) Turgeon, R. 1989. The sink-source transition in leaves. *Annu Rev Plant Physiol* 40: 119-138.

(27) Sokal, R.R. and Rohlf, F.J. 1995. Biometry.3rd Edition. New York, Freeman & Co. 887pp

(28) Johnson, R.A. and Wichern, D.W. 2002. Applied Multivariate Statistical Analysis. Fifth edition. Prentice Hall, Upper Saddle River, New Jersey.

(29) Turgeon, R. and Beebe, D.U. 1991. The evidence for symplasmic phloem loading. *Plant Physiol* 96: 349-354.

(30) Evert, R.F., Russin, W.A. and Botha, C.E.J. 1996. Distribution and frequency of plasmodesmata in relation to photoassimilate pathways and phloem loading in the barley leaf. *Planta* 198: 572-579.

(31) Botha, C.E.J. and Cross, R.H.M. 2001. Regulations within the supracellular highway – plasmodesmata are the key. *S. Afr. J. Bot.* 67: 1-9.

(32) Botha, C.E.J. 2005. Interaction of phloem and xylem during phloem loading – Functional symplasmic roles for thin- and thick-walled sieve tubes in monocotyledons? In: Holbrook, N.M., Zweiniecki, M.A. (Eds.), Vascular Transport in Plants. Elsevier, London, pp. 115-130.

(33) Hill, G.P. 1962. Exudation from aphid stylets during the period of dormancy to bud break in *Tiliamericana* (I). *J. Exp. Bot*. 13: 144-151.

(34) Watanabe, T. and Kitagawa, H. 2000. Photosynthesis and translocation of assimilates in rice following phloem feeding by planthopper *Nilaparvatalugens* (Homoptera: Delphacidae). *J. Econ. Entomol*.93: 1192-1198.

(35) Nkongolo, K.K., Quick, J.S., Limin, A.E., Fowler, D.B., Peairs, F.B. and Meyer, W.L. 1990. Russian wheat aphid (*Diuraphisnoxia*) resistance in wheat and related species. *Can. J. Plant Sci.* 70: 691-698.

(36) Du Toit, F. and Walters, M.C. 1984. Damage assessment and economic threshold values for the chemical control of the Russian wheat aphid, *Diuraphisnoxia* (Mordvilko) on winter wheat. In: Walters MC (Ed.) Progress in Russian wheat aphid (*Diuraphisnoxia*, Mordvilko). Research in the Republic of South Africa. Technical Communi-cation, No. 191. Department of Agriculture, Division of Agricultural Information, Pretoria, South Africa, pp 58-62.