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Russian wheat aphid causes greater reduction in phloem transport capacity of barley leaves than bird cherry-oat aphid.

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The effects of feeding by the Russian wheat aphid (RWA), *Diuraphis noxia* Mordvilko and the Bird cherry-oat aphid (BCA), *Rhopalosiphum padi* L on the transport capacity of barley *Hordeum vulgare* L leaves were investigated and compared with a view to relating these effects to the visible symptoms shown by the respective infested plants. RWA causes extensive chlorosis and necrosis on an infested plant whereas BCA causes no observable symptoms. Our results using the xenobiotic, phloem mobile fluorophore, 5, 6 carboxyfluorescein diacetate (5, 6-CFDA) revealed striking differences in damage to the transport of assimilates through the phloem by these two aphids. The result clearly suggests that short-term feeding by RWA causes a reduction in transport of assimilates and a more severe reduction or perhaps even permanent cessation of transport during long-term feeding. In contrast, feeding by BCA does not lead to a marked decrease in transport during short-term feeding period, however, a reduction in the transport was recorded during long-term feeding activities. These results perhaps suggest that damage to transport capacities of the barley leaves appears to be partly responsible for the observed symptoms in RWA-infested plants and the lack of them during BCA infestations, symptoms such as reduction or cessation in transport of assimilates to growing tissues may lead to such observable symptoms.

Keywords: Aphid, feeding, *Hordeum vulgare*, phloem transport, *Diuraphis noxia*, *Rhopalosiphum padi*

Abbreviations: BCA – Bird cherry-oat aphid, **RWA** – Russian wheat aphid, **CFDA** – carboxyfluorescein diacetate, **CF** – carboxyfluorescein**.**

Introduction

Transport of synthesised assimilates by the phloem in the vascular bundles of monocots has been extensively studied (see EVERT et al. 1977, 1978, 1988; FRITZ et al. 1989; BOTHA and VAN BEL 1992). Barley leaves, like other typical monocots, contain three different vein

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orders of vascular bundles, which are interconnected by cross veins (EVERT et al. 1996, BOTHA and CROSS 1997). These vascular bundles in the leaf blades are known to serve either as loading bundles (in cross, small and intermediate veins) or in the longitudinal transport of assimilates (in the large bundle) across different plant tissues (LUSH 1976, ALTUS and CANNY 1982, FRITZ et al. 1989, BOTHA and CROSS 1997).

The major constituent of the transported assimilate is sucrose, which in barley leaves is compartmentalised into transport (mesophyll and vascular tissues) and vacuolar pools (FARRAR and FARRAR 1986). Phloem-feeding aphids derive their nutrient supply from these pools, and they aggregate on the parts of the plant where food is of high quality (KENNEDY and BOOTH 1951). Because phloem sap is low in protein, aphids need to ingest large quantities of the sap in order acquire enough amino acids, necessary for their survival, while excess water and sugars are later excreted as 'honeydew' (DOUGLAS 1993). Aphids feed specifically from the sieve tubes of the vascular bundles and preferentially from the thin-walled sieve tubes (MATSILIZA and BOTHA 2002). This shows that they select their feeding sites according to the quality and quantity of the food that is yielded by the feeding site. It is obvious therefore, that aphid feeding activities will adversely affect the transport capacity of the infested plants.

A number of authors have reported that aphids become strong secondary sinks, due to the diversion of assimilates meant for other growing plant tissues. Assimilate diversion may therefore result in morphological symptoms in infested plants (CAGAMPANG et al. 1974, HICKS et al. 1984, NIELSEN et al. 1990, BOTHA and MATSILIZA 2004). Nevertheless, an interesting case occurs when two phloem-feeding aphids – Russian wheat aphid, *Diuraphis noxia* Mordvilko (RWA) and Bird cherry-oat aphid, *Rhopalosiphum padi* L (BCA) infest barley leaves. In this case, RWA-infested leaves express feeding symptoms with a much lower feeding populations over a two week period while BCA-infested leaves do not, despite a much higher BCA feeding population (SAHEED et al. 2007a).

We have recently focused our attention on the study of the feeding mechanisms of aphids during the infestation of plants (see SAHEED et al. 2007a, b; 2009). This feeding mechanism has been further investigated with respect to the effects that RWA and BCA feeding have on the transport capacity of the phloem in barley leaves. We used the phloem mobile fluorophore, 5, 6 carboxyfluorescein (5, 6-CF), to investigate the potential differences in the damage to the phloem transport as caused by BCA and RWA. The diacetate 5, 6-CFDA and some other fluorescein compounds used *in situ* to study phloem transport in plants provide reliable information (TURGEON and BEEBEE 1991, FARRAR et al. 1992, BOTHA et al. 2000). 5, 6-CFDA is non-polar compound, and when applied to damaged cells, it is taken up by plant cells and then moved across cell walls and membranes. Once in physiologically intact tissues, the diacetate is cleaved, resulting in polar free 5, 6-CF which is non-permeable to cell membranes. It fluoresces while moving symplasmically within the contiguous cell of the phloem. 5, 6-CF has been used to study phloem transport during RWA infestation in wheat (BOTHA and MATSILIZA 2004), and during infestation of wheat cultivars by *Sitobion yakini* (DE WET and BOTHA 2007). These two studies provide background information concerning the reduction of phloem transport capacity during aphid feeding. However, literature on the effect of feeding by BCA on the transport capacity of the phloem is non-existent or rare.

The experiment reported in this paper was designed to examine the effects the RWA and BCA had on the transport capacity of the phloem in barley leaf and to possibly relate it to

the diverse morphological symptoms expressed by infested plants (SAHEED et al. 2007a). We hypothesized that RWA causes a greater reduction in phloem transport capacities of the infested plants, leading to the visible symptoms observed and that a much lesser reduction (if any) is caused by the BCA, perhaps, which is why there are no morphological symptoms in BCA-infested plants. These results, we hope, will further elucidate the effects of aphid feeding on the transport capacities of the phloem as it relates it to visible symptoms shown by infested plants.

Materials and methods

Plant material, aphid colony maintenance and treatments

Barley (*Hordeum vulgare* L. cv Clipper) seeds were pre-germinated in Petri dishes and sown in plastic pots containing 60:40; peat: vermiculite mixture potting soil. They were watered twice a week with Long-Aston nutrient solution (HEWITT 1966), and grown under controlled environment (Conviron S10H Controlled Environments Limited, Winnipeg, Manitoba, Canada) at 24 °C, 66% RH day and 22 °C, 60% RH night, 14 h photoperiod. Illumination in the cabinets was provided using a combination of fluorescent tubes (F48T12. CW/VHO1500, Sylvania, Danvers, MA) and frosted incandescent 60W bulbs (Philips, Eindhoven, The Netherlands) and the irradiation level was 250 μmol m^{-2} s^{-1}. The colonies of the RWA and BCA were from the ARC-Small Grain Institute, Bethlehem, South Africa. The raising and maintenance of the aphid colonies was on young barley plants over at least three successive generations, to avoid any effects from previous hosts (SHUFRAN et al. 1992). Insect cages were used to cover the aphids in separate growth cabinets. The maintenance of the colonies was at 18 °C, 66% RH day and 15.5 °C, 66% RH night, 14 h photoperiod. In all cases, five adult aphids were confined using clipcages, to feed on either sink or source leaves for 72 h (short-term) and 14 days (long-term). The leaves used in these experiments were about 12 cm long, while the clipcages, placed at the mid-region of the fully expanded leaves, were about 5 cm in length. Source and sink leaves were used during short-term feeding experiments while only source leaves were used for long-term experiments, with 20 replicate plants established for each treatments.

Leaf material treatment

Intact plants were used for all the treatments. Immediately after the infestation period, the leaves were gently abraded (to allow access to the fluorophore) on the abaxial surface with a sterilized needle. Source leaves were abraded on the part above the clipcage; while the part below the clipcage was used in sink leaves. This is taking into consideration the classical pattern of assimilates movement, which is known to be basipetal (lamina tip to base) in source leaves and acropetal (lamina base to tip) in sink leaves (TURGEON 1989). The abraded portion was rinsed with distilled water before treatment with 100 μL of 5,6-CFDA (carboxyfluorescein diacetate, C-195 Molecular Probes, Eugen, Oregon USA, 217 μM in distilled water, kept foil-wrapped at –5 °C until needed) was added and covered with transparent polythene film (Housebrand, Brackenfell, South Africa) to prevent evaporation. The 5, 6-CFDA was allowed to be taken up through the abrasion and transported for 3h. At the end of the loading and transportation time, the leaves were detached, placed on a glass slide and covered with silicone oil to prevent 5, 6-CFDA from leaking from the leaf.

The fluorescence front, amount (intensity) as well as the distribution was observed under UV light in both control and aphid-infested leaves. This was done with the use of an Olympus BX61 wide-field fluorescence microscope with a U-YFP filter set (10C/Topaz 41028, Chroma technologies, Battlebro USA) with excitation of 513 nm and an emission of 527 nm. Images were saved in a database using the program analySIS (Soft Imaging System GmHb, Germany), and imported as bitmaps to Corel Draw 12 (Corel Corporation Ottawa, Canada 2003) for presentation. The rate of transport (measured by the distance moved by the cleaved 5, 6-CF from the point of application to the fluorochrome front in three hours) was recorded and statistical analysis (ANOVA) was used to compare the differences in the treatments. The results presented are based on acropetal and basipetal movements of the cleaved 5, 6-CF along the assimilate stream in the longitudinal bundles.

Results

Transport of 5, 6-CF in control barley leaves

The movement of the dissociation product of 5,6-CFDA (5, 6-CF) occurs from the site of application in the control, source, as well as sink leaves and it shows that the fluorochrome moved acropetally in sink leaves and basipetally in source leaves. Repeated experiments revealed that the 5, 6-CF uptake starts with the mesophyll, then moves through the bundle sheath before loading into the vascular bundles (Fig. 1A). Loading was seen to start with small and intermediate bundles after which the fluorochrome moves towards the large exporting bundles. The transport of 5, 6-CF appears continuous and undisturbed (Figs. 1B and C) up to the fluorochrome front (Fig. 1D). The unloading sequence of the fluoro-

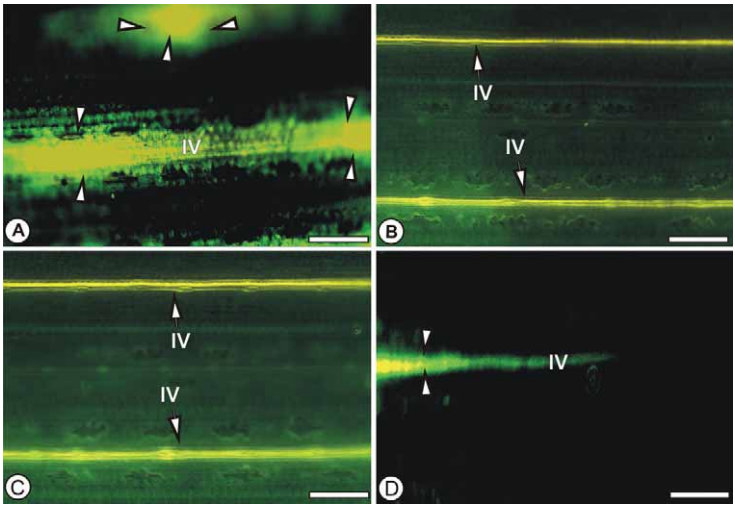

Fig. 1. Transport of cleaved 5, 6-CF in control leaves of barley, **A** – the point of application of the fluorochrome (arrowheads), where the 5, 6-CF is been taken up and transported in the intermediate vein. **B**, **C** – undisrupted transport of the cleaved 5, 6-CF along intermediate veins, where the transport is smooth, even and continuous. **D** – the fluorochrome front (arrowheads) in an intermediate vein. Scale bars: A = 100 μm; B, C = 200 μm; D = 150 μm.

chrome as expected shows a movement from the sieve tubes into the mesophyll tissue via the vascular parenchyma and bundle sheath (data not included). All these movements are purely symplasmic. Three hours after the application of the 5, 6-CFDA, the fluorochrome had moved approximately 5 cm from the point of application.

Transport of 5, 6-CF in BCA-infested leaves

After 72h (short-term) of feeding by BCA, distribution of the 5, 6-CF was patchy (Fig. 2A). Despite this patchiness, there are no apparent reductions in the amount (intensity) or the distance moved by 5, 6-CF from the point of application when compared to the control tissue. However, after 14d (long-term) of continuous feeding, there is an apparent reduction in the intensity and distance moved by the fluorochrome from the point of application in the infested leaves when compared to the control leaves. We present the observed gradual reduction in the intensity of the transported 5, 6-CF (Figs. 2 C–E), and the points of stylet penetrations and an increased fluorochrome concentration (Figs. 2 C–E). Interestingly, traces of ingested 5, 6-CF can be seen in the honeydew excreted by the aphid (Fig. 2B).

Transport of 5, 6-CF in RWA-infested leaves

Transport of 5, 6-CF in RWA-infested barley leaves shows a dramatic reduction in the distance moved as well as the intensity of the fluorochrome after short-term feeding (72h). The intensity of the color of the fluorochrome 5, 6-CF before the point of aphid feeding (arrows) is much higher than after 72h of feeding point (arrowheads)(Figs. 2F–G). Prolonged feeding (long-term) by RWA results in a greater reduction in the intensity and also the distance moved by 5, 6-CF and in most cases, cessation of the transport ensues. We present transport of 5, 6-CF after 14d (long-term) feeding (Figs. 2 H–J), and apparent leakages of the cleaved 5, 6-CF through many points of stylet penetrations (arrowheads; Fig. 2H). We observed fluorochrome in the abdomen of an aphid (Fig. 2I), and the total cessation in the movement of 5, 6-CF which occurred on many occasions (Fig. 2J).

Comparison of the distance moved by 5, 6-CF in control and infested leaves

Analysis of variance (ANOVA) of the difference of means of the distance moved by 5, 6-CF in control, BCA and RWA infested leaves from 20 replicate samples was calculated and confirmed with Tukey's post hoc test (95%). The short-term feeding experiments showed no significant difference (p-values <0.0001) between the distance moved by 5, 6-CF in control and BCA of the infested leaves (Fig. 3). A significant difference was however found between the distances moved by 5, 6-CF in control and BCA infested leaves on one hand and the RWA infested leaves on the other (Fig. 3). In this case, RWA infestation led to a significant reduction in the distance moved by the fluorochrome after 72 h of feeding when compared to the distance moved in both control and BCA infested tissue. However, after 14 d of feeding by the aphids (long-term), the result shows that there is significant difference ($p < 0.0001$) between the distances moved by the 5, 6-CF in the control, BCA and RWA infested leaves. The movement of 5, 6-CF in the leaves infested with BCA was reduced when compared to what was observed in the control leaves (Fig. 4). On the

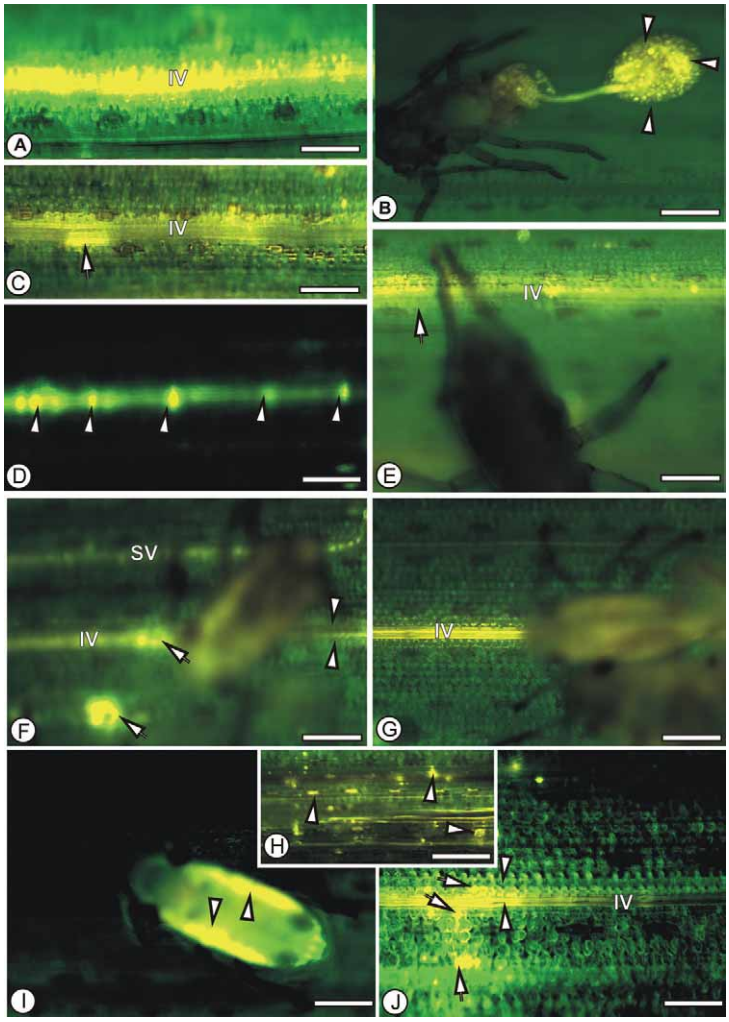

Fig. 2. Transport of cleaved 5, 6-CF in BCA and RWA-infested barley leaves. **A**–**E** – the pattern of transport and distribution of 5, 6-CF in BCA-infested barley leaves. **A** – patchy transport pattern of the fluorochrome during short-term feeding by the aphids in intermediate vein (IV). **B** – the fluorochrome in the honeydew (arrowheads) ejected by the aphid. **C**–**E** – a gradual reduction in the transported 5, 6-CF during long-term feeding by BCA. Stylet points (arrowheads) show a higher concentration of the fluorochrome. **F**–**J** – the pattern of transport and distribution of 5, 6-CF in RWA-infested barley leaves. **F** – reduction in the transported 5, 6-CF after short-term feeding (72h) by RWA. Note the feeding points (arrows) and reduced transport after the points (arrowheads). **G** – feeding in an intermediate vein, reduced transport of 5, 6-CF was observed after the point of aphid feeding. **H** – several stylet points (arrowheads) after long-term feeding. **I** – cleaved 5, 6-CF in the abdomen of an aphid. **J** – complete cessation of further transport of the fluorochrome after severe, long-term feeding by the aphids. Note the leakages of the 5, 6-CF through many stylet tracks (arrows) out of the intermediate vein and stoppage of further movement (arrowheads) of the 5, 6-CF from the feeding points. Scale bars: A = 100 μm; B = 150 μm; C = 100 μm; D = 150 μm; E = 200 μm; F = 150 μm; G = 200 μm; H = 50 μm; I = 150 μm; J = 150 μm.

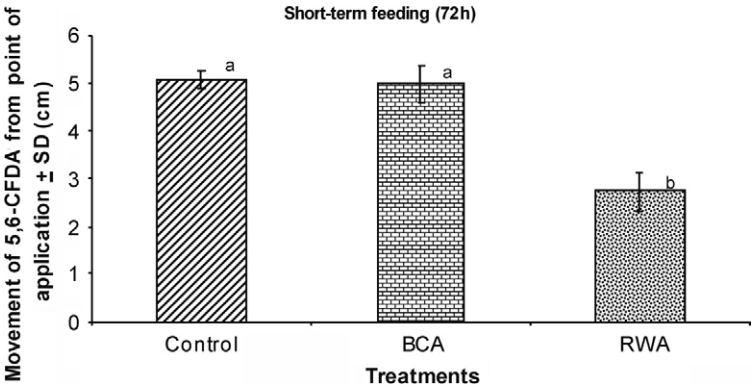

Fig. 3. Comparison of the means of distance moved by 5, 6-CF from point of application in control, BCA- and RWA-infested leaves after short-term (72h) feeding by the aphids (± standard deviation); ANOVA: $F_{2, 57} = 300.98$; $p < 0.0001$

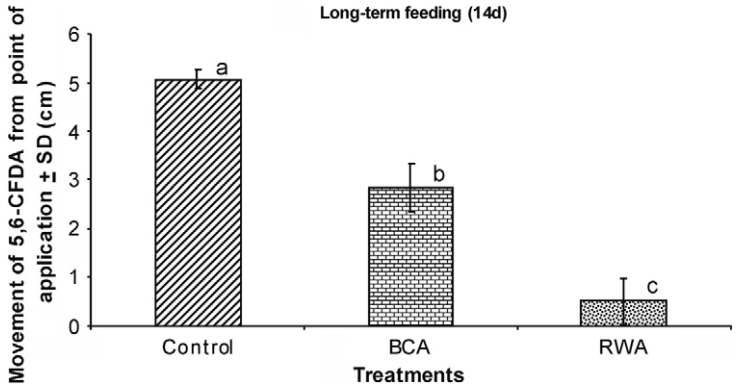

Fig. 4. Comparison of the means of distance moved by 5, 6-CF from point of application in control, BCA- and RWA-infested leaves after long-term (14d) feeding by the aphids (± standard deviation); ANOVA: $F_{2, 57} = 621.94$; $p < 0.0001$

other hand, transport of 5, 6-CF in RWA-infested leaves is further reduced (Fig. 4) when compared to the movement in control and BCA infested leaves, and on many occasions no transport or total cessation of transport occurs.

Discussion

The results presented here only relate to longitudinal phloem transport as visualised by 5, 6-CF transport and its fluorescence. Transport is generally acropetal in sink leaves and basipetal in source leaves. Longitudinal transport of assimilates (visualised by transport of 5, 6-CF) in control barley leaves shows that the rate (the distance moved from the point of application of 5, 6-CFDA to the 5, 6-CF front in 3 h) of phloem transport is at an average of 5 cm over a 3h period in all classes of veins. This observation is true in both source and sink

leaves. The movement of the phloem-mobile 5, 6-CF follows classical source-sink transport patterns. The data presented here thus lend strong support to an earlier report that demonstrated similar movement of the fluorochrome in wheat leaves (BOTHA and MATSILIZA 2004). Once applied, the symplasm of the mesophyll tissues takes up the fluorochrome (Fig. 1A) and subsequently loads it into the phloem through many symplasmic connections that exist between bundle sheath-vascular parenchyma and companion cells-sieve tube complexes (EVERT et al. 1996, BOTHA and CROSS 2001, BOTHA 2005) and then moved longitudinally. This transport is unrestricted and confined within the transporting veins of the control tissues (Figs. 1 B–C).

However, this movement pattern ends up in severe disruption upon feeding by the aphids. The focus of this work was on the impact on the longitudinal transport of assimilates when RWA and BCA feed on barley leaves. The observed differences in the damage caused by the two aphid species to the leaf ultrastructures (SAHEED et al. 2007a) and corresponding deposition of wound callous (SAHEED et al. 2009) are further confirmed with the current phloem transport results. In short-term (72 h) feeding experiments, infestation by BCA does not result in obvious reduction in the capacity of the phloem to transport assimilate (Fig. 2A), as there was no significant difference in the transport of 5, 6-CF in the assimilate stream of control and BCA-infested leaves (Fig. 3). By contrast, RWA infestation led to a significant reduction in the 5, 6-CF transported in the assimilate stream and by implication, the phloem transport capacity within 72 h of feeding (Figs. 2F-G, 3). However, reduction in the 5, 6-CF transported which resulted in a significant reduction in the phloem transport capacity was found when BCA fed for 14 d (long-term) in comparison to the control plant (Figs. 2C–E, 4). Obviously, RWA infestation results in a pronounced reduction in the 5, 6-CF transported, and in most cases complete cessation of transport ensues (Fig. 2J). This infestation causes a greater reduction in phloem transport capacity (Fig. 4) when compared to observations in both control and BCA infested leaves. Interestingly, the ingested 5, 6-CF in theassimilates were visible in the egested honeydew of BCA (Fig. 2B) and also in the abdomen of RWA (Fig. 2I). The data presented here show that feeding by RWA resulted in patchiness in 5, 6-CF distribution (Figs. 2H, J) to a much greater extent than observed in BCA infested leaves (Figs. 2C–E).

These results clearly support the position that aphids redirected assimilates that are normally transported in the veins of the leaves, thereby depressing the sink strength in growing regions of the plant. Diversion and disruption of transported assimilates have been reported in different species of phloem feeding insects. NIELSEN et al. (1990) have reported the disruption of assimilate transport when the Potato leafhopper (*Empoasca fabae*) feeds on *Medicago sativa* (alfalfa). Feeding by the planthopper (*Nilaparvata lugens*) on rice has also been shown to result in removal of assimilates and reduction in photosynthesis (WATANABE and KITAGAWA 2000), this feeding eventually causeing a reduction in rice growth and yield. HILL (1962) had earlier shown that if the redirection of assimilates by the phloem feeding insects is strong and localized, the plant will react to it in some respect, as if a bud were involved.

This work revealed that diversion and disruption of the assimilate transport pathway in barley leaves is more apparent in response to RWA than to BCA feeding. This suggests that the damage inflicted by RWA is greater than that caused by BCA. Therefore, we can say that RWA is a more aggressive feeder than BCA. RWA disrupts the phloem system thereby

reducing its transport capacity; this is evident with the reduction in the transport of the 5, 6-CF – a phloem-mobile xenophore. The RWA apparently redirects more assimilate to itself than the BCA and this position finds support in our earlier papers where we have shown that damage to leaf ultrastructures was more severe and callous formation (wounding effects) was more intense when RWA infested plants than when BCA infested them (SAHEED et al. 2007a, 2009).

In terms of yield losses, we suggest that the greater disruption and diversion of assimilates in RWA infested barley leaves appears to be responsible for the higher yield losses (30% to 60%) reported during RWA-infestation (DU TOIT and WALTERS 1984). On the other hand a reduced disruption and diversion of assimilates results in a reduced (21%) yield loss that occurs during BCA-infestation (RIEDELL et al. 1999). This suggestion finds supports in earlier observations in rice, where disruption and diversion of assimilates causes yield losses in infested plants (WATANABE and KITAGAWA 2000). The results thus presented further elucidate the mechanism behind aphid feeding and responses of the infested plants, by revealing more reasons why RWA-infested leaves show symptoms of chlorosis, necrosis and leaf roll and leaves infested by BCA do not.

In conclusion, the symptoms shown by RWA infested plants, which eventually lead to the subsequent losses in yield greater than in BCA-infested plants, are suggested to be partly due to the aphid's ability to inflict severe damage on the phloem transport of the infested plant. This damage leads to noticeable reduction in the transport of assimilates within 72h of RWA infestation and a significant reduction during prolonged feeding which, in most cases, results in total cessation of phloem transport. BCA feeding, conversely, does not appear to cause as much serious damage to the transport capacities of the phloem, with only reduced assimilate transport occurring during a prolonged infestation.

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