

**Comparison of structural damage caused by Russian wheat aphid
(*Diuraphis noxia* Mordvilko) and Bird cherry-oat aphid (*Rhopalosiphum
padi* L.) in a susceptible barley cultivar, *Hordeum vulgare* L. cv Clipper.**

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ABSTRACT

The Russian wheat aphid (RWA, (*Diuraphis noxia* Mordvilko) and the Bird cherry-oat aphid (BCA, (*Rhopalosiphum padi* L.) cause severe damage to grain crops, including barley. An investigation of the effects of these aphids on a susceptible cultivar revealed that BCA-infested barley plants remained healthy-looking for two weeks after feeding commenced. In contrast, signs of stress and damage, including chlorosis and leaf necrosis were evident in RWA infested plants. Our study suggests that damage to the vascular tissue due to sustained feeding by BCA, was not as extensive as that caused by RWA. In addition, there is a marked difference in the salivary secretion pattern within xylem elements punctured by aphids tapping the xylem for water. RWA deposit electron-dense, amorphous to smooth saliva, which completely encases the inner walls of affected elements, and saliva encases pit membranes between xylem elements, and between xylem vessels and xylem parenchyma. Xylem tapped by BCA, contained more granular saliva, which apparently does not occlude vessel wall apertures or the pit membranes to the same extent as was observed with RWA. Damage to phloem tissue, including phloem parenchyma elements, sieve tube-companion cell (CC-ST) complexes as well as thick-walled sieve tubes, was extensive. Plasmodesmata between phloem parenchyma elements as well as pore-plasmodesmata between the CC-ST were occluded by callose. We conclude that severe, perhaps permanent damage to conducting elements in RWA infested leaves may be responsible for the detrimental chlorosis and necrosis symptoms. These symptoms are absent in BCA-infested plants.

KEYWORDS

Russian wheat aphid (RWA), Bird cherry-oat aphid (BCA), ultrastructural damage, barley, aphid saliva.

INTRODUCTION

Aphids are phloem-feeding insects, which may cause little or serious perceptible damage to the plants that serve as their source of food. The Bird cherry-oat aphid, *Rhopalosiphum padi* L (BCA) appears to cause no visible host plant damage as a result of feeding, although it may cause substantial yield loss (Leather et al. 1989 Riedell et al. 1999). In contrast, the Russian wheat aphid, *Diuraphis noxia* Mordvilko (RWA) causes extensive chlorosis and necrosis upon infestation. The visible effects resulting from RWA feeding include distinct white, yellow, purple, or at times reddish-purple longitudinal streaks, severe leaf roll and prevention of unrolling of developing leaves (Walters et al. 1980; Riedell, 1989).

Investigations conducted to date have shown that the RWA and BCA not only elicit different effects on their host plants, but also have a different ecology (Walters et al. 1980; Wikteliuss et al. 1990). The BCA prefers to feed on the lower parts of the plant and in smaller aggregates (Wikteliuss et al. 1990; Pettersson 1994), whereas RWA feeds in dense aggregates, preferentially in rolled leaves (Walters et al. 1980; Burd and Burton 1992).

Studies at the enzyme, protein and transcriptional level have demonstrated that both aphid species induce responses in their host plants. The RWA and the BCA have both been shown to induce pathogenesis-related proteins, such as β -glucanases and chitinases, and it has been reported that the induced responses are stronger in resistant cultivars than in susceptible cultivars (van der Westhuizen et al. 1998a, b; Forslund et al. 2000). There are also marked differences in the induced responses. For instance, the amino acid composition of phloem sap of wheat was found to change upon infestation by RWA, whereas BCA infestation had no such effect in the same wheat cultivars (Sandström et al. 2000). Differences have also been found in oxidative responses. For

example, RWA feeding induces peroxidase (van der Westhuizen et al. 1998a, Ni et al. 2001), but no such induction has been found in BCA-infested plants (Forslund et al. 2000; Ni et al. 2001). These results lend support to the earlier observations of the chlorotic yellow spots seen after RWA infestation, and the absence of these regions after BCA infestation. There is however as yet, no explanation to the curling of the leaves and the yellow streaking in the RWA infested susceptible plant.

In the present study, we hypothesize that leaf curling, necrosis and chlorosis were caused by changes to the transport system, as a result of aphid saliva deposition. Saliva is exuded during probing and feeding activities (Prado and Tjallingii, 1994; Martin et al. 1997; Matsiliza and Botha 2002). The saliva is known to be of two types; that forming the solid stylet sheath which is left behind during probing, and a non-gelling watery saliva (Miles, 1999). The salivary sheath is proteinous, contains active sulphhydryl groups and exhibits enzyme activity, such as polyphenol oxidase, peroxidase as well as glucanase. Likewise, oxidases and pectinases have been reported present in the watery saliva (Miles, 1999).

Past structural and ultrastructural studies primarily examined the pathway of aphid stylets in the plant tissue during feeding activities and used aphids to examine the potential functionality of specific phloem structures (see Evert et al. 1968; 1973; Botha and Mabindisa 1977; Botha et al. 1977; Botha and Evert 1978; Matsiliza and Botha 2002; Botha and Matsiliza 2004). Little attention has been given to the damage occurring at the cellular level which results from aphid feeding and which may explain aphid feeding symptoms such as leaf curling and yellow streaking.

Here we have examined the cell damage inflicted by RWA in a susceptible barley cultivar (cv Clipper) at the ultrastructural level and compared this with the apparently

less damaging BCA. At this time we report our findings using susceptible barley cultivar (cv Clipper) as a basis for future ultrastructural studies on other less susceptible varieties.

MATERIALS AND METHODS

Plant material and aphid colony maintenance

Barley (*Hordeum vulgare* L. var Clipper) seeds were pre-germinated in Petri dishes and sown in potting soil (60:40, peat: vermiculite mixture) in 17cm diameter plastic pots. They were watered twice a week with Long-Aston nutrient solution (Hewit 1966), and grown under controlled environment (Convion S10H Controlled Environments Limited, Winnipeg, Manitoba, Canada) at 24°C, 66% RH (day) and 22°C, 60% RH (night) with a 14h photoperiod. Light sources were a combination of fluorescent tubes (F48T12.CW/VHO1500, Sylvania, USA) and frosted incandescent 60W bulbs (Philips, Eindhoven, The Netherlands) and irradiance levels were 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Russian wheat aphids (*Diuraphis noxia* Mordvilko) and Bird cherry-oat aphids (*Rhopalosiphum padi* L.) were obtained from The ARC–Small Grain Institute, Bethlehem, South Africa. Colonies were maintained separately on young feeder barley plants and kept in insect cages in separate growth cabinets.

Aphid feeding damage studies was carried out at 18°C, 66% RH (day) and 15.5°C, 66% RH (night) for the aphid infested plants experimental cabinets. Five juveniles of each aphid species were transferred to either the second or third leaf above the coleoptiles carefully with a camel hair brush and a small single-leaf aphid cage was used to keep the aphids on leaves of interest. The aphids were allowed to feed and reproduce for two weeks and then leaves were selected and processed for electron microscopy

investigation of feeding-related damage. Aphid population increases from the original five aphids that occurred during the two week time course were determined from ten replicate plants.

Electron microscopy

Leaf material was cut into strips from control, RWA and BCA infested barley plants then carefully trimmed and diced into smaller pieces into cold 6% glutaraldehyde in 0.05M sodium cacodylate buffer using a sharp clean single-edge razor blade. Segments were placed in small vials, subjected to very slight vacuum ($16931.4 \text{ kg/m sec}^2$) for 1h before being placed in the refrigerator at 4°C in a fresh change of fixative. Segments were washed in three changes of cold 0.05M sodium cacodylate buffer pH 7, after which they were transferred immediately to cold 2% osmium tetroxide in 0.05M sodium cacodylate buffer and kept in the refrigerator overnight, then washed in cold buffer and dehydrated in a cold graded ethanol series, followed by two changes in propylene oxide. Embedment was in Spurr's (1969) epoxy resin. Ultrathin sections (silver to gold) were cut using a diamond knife (Drukker, Netherlands) and collected on 300 mesh copper grids (SPI suppliers, Philadelphia, USA). Sections were stained with uranyl acetate followed by lead citrate and viewed and photographed at 80KV using a JEOL JEM 1210 transmission electron microscope (JEOL, Tokyo, Japan).

RESULTS

Figures 1 and 2 show details of the ultrastructure of vascular tissue from barley leaf blades. Figure 3 shows feeding by the two species of aphids and the chlorosis and necrosis elicited by RWA but not by BCA feeding. Barley leaves infested for two weeks with RWA developed chlorosis and necrosis (Fig. 3A) even given their significantly

lower numbers compared with the BCA feeding populations (Table 1). BCA infestations for two weeks did not result in chlorosis or necrosis (Fig 3B).

Figures 1A and B show details of part of an intermediate vascular bundle in control tissue. Two thick walled sieve tubes (solid circles, Fig. 1B) as well as a thin-walled sieve tube (open circle) are visible. In Fig. 1C, pore-plasmodesmal fields (pore-pd, open darts) between a companion cell (above) and sieve tubes (below) are unoccluded.

Probing by RWA is illustrated in Figs 1D-J. The xylem vessels are sealed with electron-dense material (Fig. 1D). Vascular parenchyma cells exhibit signs of severe plasmolysis, with the plasmamembrane and cytoplasm being withdrawn from the cell wall (Fig. 1E). Accretion of salivary material (SS, Fig. 1F) often obliterated the xylem parenchyma cells adjacent to punctured tracheary elements. Mesophyll cells were often damaged during xylem probes as well (Fig. 1F). Salivary material deposited as a result of aphid probing often appeared layered or polylamellate (Fig. 1G) where the common wall between the adjacent parenchymatous cells has been punctured, resulting in the deposition of salivary material on both sides of the wall. Sieve tubes showed various degrees of damage, from little plasmolysis, through to severe plasmolysis in which the plasmamembrane and the underlying cytoplasm when the membrane system was disrupted and pulled away from the cell walls (Fig. 2D). Extensive deposits of callose (C) were associated with punctured and disrupted sieve tubes. In these instances, plasmodesmatal and pore plasmodesmatal connections were occluded. Fig. 1H shows two thin-walled sieve tubes (ST) with evidence of having been punctured. The endoplasmic reticulum in the lower sieve tube however, remains parietally localized (open dart), whilst in the uppermost sieve tube, the ER appears interspersed with deposits of electron-dense material; and amorphous callose (solid dart). Multivesicular

bodies (MVB, Fig. 2C) formed as a result of sudden pressure loss was often associated with the vascular parenchyma associated with damaged sieve tubes. (See box in Fig. 1I and at higher magnification in Fig. 1J).

Figures 2E-K illustrate aspects of the damage caused by sustained BCA feeding. Comparison of these images with those in Figs. 1D-J and 2A-D, leave little doubt that BCA causes or induces less structural damage to the vascular system in barley leaves, compared with that caused by RWA. Partial plasmolysis (Fig. 2E) was often evident, as was obliteration of xylem (Figs. 2E, F). In Fig. 2F, the extent of the damage to the xylem elements is evident. The BCA-probed xylem contained granular salivary material, which occupies much of the lumen of the vessel in this case. Damage here, appears less severe than is the case where RWA has probed the xylem. Note that the adjacent thick-wall sieve tube (closed circle, Fig. 2F) shows partial plasmolysis. Bird cherry-oat aphid probing caused membrane disruption and partial plasmolysis (Fig. 2G, J) to companion cells, as well as to thin-walled sieve tubes (Fig. 2H, K). Here, the sieve area pores connecting the two sieve tubes appear to be occluded by callose. However, damage to vascular parenchyma and companion cells was often more extensive (Fig. 2I). Note that salivary sheath material (SS) is often deposited along the walls of the cells separating the cytoplasm from the wall and the disruption of organelles within the companion cell (Fig. 2I).

DISCUSSION

An observation of interest to us was that the BCA reproduced more quickly than did the RWA over a two week period (Table 1). Our results are consistent with that of Messina et al. (2002) where BCA population growth was higher than that of the RWA at the end

of the primary infestation period on wheat. As such, we expected that more feeding associated damage should be observed with BCA due to the larger number of feeding aphids, since damage to plants is assumed to be caused by the puncturing of cells by aphid stylet and subsequent salivary deposition (Miles, 1999). However, leaves fed on by the larger BCA population, looked visibly healthy (Fig 3B). In stark contrast, RWA infested leaves showed marked signs and symptoms of chlorosis and necrosis (Fig. 3A), despite the smaller feeding populations.

Prado and Tjallingii, (1994) and Martin et al. (1997) demonstrated that puncturing of parenchyma cells by aphids during their probing activities before they eventually reach sieve tubes, involves discharge of watery saliva. Aphid stylet penetration of plant leaves varies from an apparently highly precise process, to one which at times, appears haphazard (Matsiliza and Botha 2002 and references cited therein). Studies conducted by Fouché 1983 (A. Fouché 1983. Thesis, Univ. of Free state, South Africa) and Fouché et al. (1984) demonstrated that changes occurred in the cell organization of wheat tissue, when treated with RWA whole body extracts. Unfortunately, their data cannot be compared directly to the present study in which we have focussed attention on the damage caused by stylet penetration and subsequent aphid feeding. Aphids are known to drink from xylem (Tjallingii, 1994). Puncturing of xylem elements resulted in markedly different damage signatures. RWA appear to eject a massive amount of electron-dense, fluid ('watery') saliva, which in all instances, appears to completely seal the xylem vessel (see Fig. 1D). Bird cherry oat aphids, in contrast seem to deposit a more dense granular saliva in the xylem vessels (see Figs. 2E and F). It is tempting to speculate that the less aggregated deposits resulting from BCA drinking, means less physiological degrading of the apoplasmic transport system, than is the case with RWA

water drinking processes, where xylem elements are ensheathed by an amorphous, electron-dense layer, which seems to completely isolate these elements from surrounding xylem parenchyma.

Our observations further suggest that there are apparent, sometimes striking differences in the pattern of damage produced by probing, feeding and water-drinking aphids. In all cases, the RWA elicits more visible structural damage than the BCA, to comparative tissues. Cell wall probing produces similar results, with a polylamellate salivary deposit commonly found associated with inter as well as intracellular probes. In both cases, RWA and BCA can separate cells along the middle lamella. Our results also confirm that both RWA and BCA puncture parenchyma cells during their search for functional sieve tubes within the phloem tissue and that the cells probed by RWA (Figs 1E, F, I and J) appear to be very badly affected, presumably non-functional. In contrast, similar vascular parenchyma and associated phloem probes performed by BCA seem less severely affected, and plasmolysis generally seems less severe. Presumably, their functionality is not as affected (Figs 2E, G, H, and I).

Probing and clearing (blowing) of stylets as evidenced by deposition of salivary material as observed here both inter- as well as intracellularly, suggests that the aphids, through their actions during penetration, analyse the substrate contained in the cells that they penetrate (see also Klingauf, 1987; Tjallingii, 1995; Miles, 1999 among others). When the aphids eventually reach the sieve tubes, salivary sheath production stops, but ejection of watery saliva continues. The aphids then start to ingest cell sap and maintain a sustained feeding pattern (Tjallingii, 1995; Prado and Tjallingii, 1997; Miles, 1999). Our studies show that the sustained feeding by RWA from sieve elements results in

complete and apparently irreversible damage (Figs 1H and 2D) whereas BCA does not appear to impact such irreversible damage to the sieve elements from where they feed (Fig 2H).

In conclusion, the damage inflicted by the aphids on the transport tissues of barley leaves, is, in the absence of positive identification of virus populations shown to be caused by the physical puncturing of the cells by the aphid stylets and the subsequent deposition of saliva during the feeding process (see also Miles 1999). The results reported here, clearly demonstrate that much of the damage inflicted by RWA in comparison to BCA, may be due to complete physiological destruction of the cells (with a resultant rapid pressure loss) in addition to reported effects associated with saliva deposition itself, of which the most striking and obvious is sealing of xylem vessels by some of the components of the salivary material and damage to sieve tube-companion cells complex of the phloem. It follows that leaf rolling and streaking symptoms shown by plants infested with RWA may in part be due to the apoplastic and symplasmic isolation of both xylem and phloem respectively, thereby limiting nutrient exchange from them to adjacent cells. Chlorosis and necrosis may appear during probing, where puncturing of cells induces oxidative stress. The sealing of xylem and phloem may generate leaf rolling and streaking symptoms.

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REFERENCES

Botha CEJ, Mabindisa SEW (1977) Observations on the penetration of the phloem in young stems of *Nerium oleander* (Linn) by stylets of the aphid, *Aphis nerii* (B. de F.). SA J Sci 73: 276-277

Botha CEJ, Malcom SB, Evert RF (1977) An investigation of preferential feeding habit of four Asclepiadaceae by the aphid, *Aphis nerii* (B. de F.) Protoplasma 92: 1-19

Botha CEJ, Evert RF (1978) Observations of preferential feeding by the aphid, *Rhopalosiphum maidis* on abaxial phloem of *Cucurbita maxima*. Protoplasma 96: 75-80

Botha CEJ, Matsiliza B (2004) Reduction in transport in Wheat (*Triticum aestivum*) is caused by sustained phloem feeding by Russian wheat aphid (*Diuraphis noxia*). SA J Bot 70(2): 249-254

Burd JD, Burton RL (1992) Characterization of plant damage caused by Russian wheat aphid (Homoptera: Aphidae). J Econ Entomol 85: 2017-2022

Evert RF, Eschrich W, Medler JT, Alfeiri FJ (1968) Observation of the penetration of linden branches by the stylets of the aphid *Longistigma caryae* (Ham.) Am J Bot 55: 860-874

Evert RF, Eschrich W, Eichhorn SE, Limbach ST (1973) Observation on penetration of Barley leaves by the Aphid *Rhopalosiphum maidis* (Fitch). Protoplasma 77: 95-110

Fouché A, Verhoeven RL, Hewitt PH, Walters MC, Kriel CF, DeJager J (1984) Russian aphid (*Diuraphis noxia*) feeding damage on wheat related cereals and a Bromus grass species pp 22-33 in MC Walters (ed). Progress in the Russian wheat aphid (*Diuraphis noxia* (Mordvilko) research in the Republic of South Africa. Technical Communication, No 191, Department of Agriculture RSA

Forslund K, Pettersson J, Bryngelsson T, Jonsson L (2000) Aphid infestation induces PR-proteins differently in barley susceptible or resistant to the bird cherry-oat aphid (*Rhopalosiphum padi*) Physiol Plant 110: 496-502

Hewitt EJ (1966) Sand and water culture methods used in the study of plant nutrition. Technical Communications No: 22 (2nd edn). Commonwealth Agricultural Bureau, Farnham England

Klingauf FA (1987) Feeding, adaptation and excretion. In *Aphids: their Biology, Natural enemies and Control*, vol. 2A (ed. A.K. Minks and P. Harrewijn) Elsevier, Amsterdam pp. 225-253

Leather SR, Walters KFA, Dixon AF G (1989) Factors determining the pest status of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) in Europe: a study and review. *Bull Entomol Res* 79: 345-360

Martin B, Collar JL, Tjallingii WF, Fereres A (1997) Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *J Gen Virol* 78: 2701-2705

Matsiliza B, Botha CEJ (2002) Aphid (*Sitobion yakini*, Eastop) investigation shows thin-walled sieve tubes to be more functional than thick-walled sieve tubes. *Physiol Plant* 115 (1): 137-143

Messina FJ, Taylor R, Karren ME (2002) Divergent responses of two cereal aphids to previous infestation of their host plant. *Entomol Exp App* 16: 43-50

Miles PW (1999) Aphid saliva. *Biol Rev* 74: 41-85

Ni X, Quisenberry SS, Heng-Moss T, Markwell J, Sarath G, Klucas R, Baxendale F (2001) Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non-symptomatic cereal aphid (Hemiptera: Aphididae) feeding. *J Eco Entomol* 94: 743-751

Pettersson J (1994) The bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) and Odours. In: SR Leather, A Wyatt, NAC Kidd and KFA Walter (eds.), *Individuals, populations and patterns in ecology*, Intercept Ltd. Andover, pp. 3-12

Prado E, Tjallingii WF (1994) Aphid activities during sieve element punctures. *Entomol Exp Appl* 72: 157-165

Prado E, Tjallingii WF (1997) Effects of previous plant infestation on sieve elements acceptance by two aphids. *Entomol Exp Appl* 82: 189-200

Riedell WE (1989) Effects of Russian wheat aphid infestation on barley plant response to drought stress. *Physiol Plant* 77: 587-592

Riedell WE, Kieckhefer RW, Haley SD, Langham MAC, Evenson PD (1999) Winter wheat responses to bird cherry-oat aphids and barley yellow dwarf. *Crop Sci* 39, 158-163

Sandström J, Telang A, Moran NA (2000). Nutritional enhancement of host plants by aphids – a comparison of three aphid species on grasses. *J Insect Physiol* 46, 33-40

Smith CM, Schotzko D, Zemetra RS, Souza EJ, Schroeder-Teeter S (1991) Identification of Russian wheat aphid (Homoptera: Aphididae) resistance in wheat. *J Econ Entomol* 84: 328-332

Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultra Res* 26: 31-43

Tjallingii WF (1994) Sieve element acceptance by aphids. *Euro J Entomol* 91: 47-52

Tjallingii WF (1995) Regulation of phloem sap feeding by aphids. In *Regulatory Mechanisms in Insect Feeding* (ed. R. F. Chapman and G. ge Beor) Chapman and Hall, New York. pp, 190-209

van der Westhuizen AJ, Qian X-M, Botha A-M (1998a) Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Rep.* 18: 132-137

van der Westhuizen AJ, Qian X-M, Botha A-M (1998b) β -1,3-glucanases in wheat and resistance to the Russian wheat aphid. *Physiol Plant* 103: 125-131

Walters MC, Penn F, Du Toit F, Botha TC, Aalbersberg K, Hewitt PH, Broodryk SW (1980) The Russian wheat aphid. *Farming in South Africa, Leaflet Series, Wheat G3*, 1-6

Wikthelius S, Weibull J, Pettersson J (1990) Aphid host plant ecology: The bird cherry-oat aphid as a model. In: Campbell, R. K. and Eikenbary, R. D. (eds), *Aphids-plant genotype interactions*, Elsevier Science Publishers, B. V. Amsterdam, pp. 21-35

Table 1. Russian wheat aphid (RWA) and Bird cherry-oat aphid (BCA) populations per leaf, two weeks post-infestation with five aphids per leaf from ten replicates.

Aphid	Mean
RWA	34.9 ± 2.6
BCA	69.6 ± 3.7

p-values < 0.05 (<.0001), CI for difference of means = [-44.3, -25.1], ± SE.

LEGEND TO TEXT FIGURES

Fig. 1 (A-C). TEM images of control barley leaves, intermediate vascular bundles are illustrated. Fig. 1A-B. shows close spatial relationship between the thick-walled sieve tubes (solid circles) and the xylem vessels (XV) as well as to a thin-walled sieve tube (open circle). Vascular parenchyma cells (VP), and companion cells (CC) are associated with the thin-walled sieve tubes. Fig. 1C Unoccluded, presumably functional characteristically-branched pore plasmodesmal connection between thin-walled sieve tubes (ST, below) and companion cell (CC) above.

Figs. 1 (D-J). Structural damage caused by RWA feeding. Fig. 1D. Typical punctured xylem vessels, with electron-dense salivary ejecta, encasing the walls of the vessels, occluding the pit membrane between the vessels. Surrounding parenchymatous cells appear unaffected. Part of an undamaged bundle sheath cell (BS) appears unaffected.

Fig.1E. Vascular parenchyma cells (VP) in which the central cell is severely plasmolyzed, presumably after aphid puncture. Fig. 1F. Part of an intermediate vascular bundle in which salivary sheath material (SS) has completely obliterated the xylem parenchyma cells adjacent to xylem vessels. Mesophyll cells to the right have also been punctured and contain salivary material.

Fig. 1G. Multilayered salivary material deposit on either side of the common wall between two vascular parenchyma cells. ML = middle lamella.

Fig. 1H. Two thin-walled sieve tubes from a small intermediate vascular bundle. Damage is extensive, but endoplasmic reticulum remains parietally localized (open dart). In the uppermost sieve tube, the ER is interspersed with extensive electron-dense deposits. Pore connections to the upper sieve tube, are obliterated by electron-dense material. Amorphous callose is deposited at the neck of pores in contrast to open pores between sieve elements in control tissue (Fig. 1C).

Fig. 1I. Part of an intermediate vascular bundle. Multivesicular bodies (box and detailed in Fig. 1J) are present in the companion cell. Fig. 1J. All plasmodesmata appear closed and are associated with distorted plasmamembranes.

Scale bars: A = 5 μ m; B = 5 μ m; C = 1 μ m; D = 5 μ m; E = 5 μ m; F = 5 μ m; G = 2 μ m; H = 3 μ m; I = 5 μ m; J = 2 μ m.

Figs. 2 (A-D). Details of RWA infestation. Fig. 2A. High resolution of a single plasmodesma from Fig. 2B. Fig. 2B. Plasmodesmata between parenchymatous elements appear blocked by electron lucent and electron dense material. Plasmodesmal apertures are associated with callose. Fig. 2C. Multivesicular body adjacent to plasmodesmata between a mestome sheath cell (above) and a vascular parenchyma cell (below). The suberin lamella (SL) forms a compound middle lamella between these cell layers. Plasmodesmata are occluded by electron-dense material. Fig. 2D. Part of a probed thin-walled sieve tube. Electron-dense material, as well as callose (C) is intermixed with extensive salivary deposits associated with the plugged pore-plasmodesma.

Fig. 2 (E-K) Damage caused by the BCA feeding. Fig. 2E. Detail of part of a small vascular bundle. Cells within this vascular bundle show limited aphid-associated damage. Xylem vessels (XV) have been probed for water. Salivary deposits are loosely

granular and electron-dense, but does not encapsulate cell walls as is the case with RWA xylem probes (see Fig.1D for comparison). Fig. 2F detail, showing that vessels are not totally occluded by the loosely granular salivary deposit. The thick-walled sieve tube (closed circle) is partially plasmolysed. Fig. 2G. Detail showing xylem vascular parenchyma (XVP) adjacent to a companion cell (CC). Cells are variously-plasmolyzed. Fig. 2H. Two aphid-probed thin-walled sieve tubes (ST), the uppermost sieve tube shows more evidence of plasmamembrane disruption and lateral sieve areas between these cells are plugged with callose. Plasmodesmata between the lower sieve tube and its associated vascular parenchyma cell appear unoccluded. Fig. 2I. Salivary sheath material (SS) is deposited on either side of the vascular parenchyma-companion cell wall interface. Fig. 2J. Pore-plasmodesmata between a thin-walled sieve tube (below) and a companion cell (above) are occluded. Pore plasmodesmata are partially occluded. Fig. 2K. Plasmodesmata partly-occluded between a vascular parenchyma cell (above) and a companion cell (below).

Scale bars: A = 100nm; B = 250nm; C = 100nm; D = 1 μ m; E = 20 μ m; F = 10 μ m; G = 5 μ m; H = 5 μ m; I = 2 μ m; J = 500nm; K = 800nm.

Figure 3 (A and B) shows feeding aphids and associated symptoms after two weeks. Fig. 3A. RWA-infested leaf in which leaf rolling, chlorosis (arrowheads) and necrotic regions (arrows) is evident. Fig. 3B. BCA infested leaf with no observable symptoms.





