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# *Rhopalosiphum padi* Feeding – Attempted Symptomatic Defence Mechanisms in Barley Leaves Include Wound Callose Deposition?

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**Abstract**

The deposition of callose and the damage-related symptoms subsequently expressed by infested plants were investigated after feeding on barley leaves by bird cherry-oat aphid (BCA), *Rhopalosiphum padi* L. Feeding by this aphid does not result in appearance of visible damage to the plants, provided the feeding population is small. Using aniline blue fluorochrome, we confirmed that whilst low feeding density (5 aphids) results in appearance of wound callose in sieve tubes, this only occurs after 14d of feeding, when the feeding population had increased. Continued feeding results in progressively more callose deposition and by 21d, severe damage has been caused. In contrast, feeding by larger populations (50 adult aphids), results in the appearance of wound callose within 72h, in longitudinal and cross veins. We suggest that this wounding response appears to play a role in the appearance of golden yellow streak symptoms reported to occur in leaves where BCA feeding density was high.

***Keywords:*** aphid feeding density, barley, Bird cherry-oat aphid, callose, phloem, wound callose

## Introduction

The formation and deposition of callose by plants in response to cell damage is reported to occur within a minute (Gunning and Steer, 1996; Radford *et al.*, 1998; Nakashima *et al.*, 2003). Feeding by some aphid species usually results in wound callose formation-especially in susceptible plants (Botha and Matsiliza, 2004; de Wet and Botha, 2007). Rapid deposition of callose in sieve plate pores of the phloem is considered to be an effective defence response to aphid feeding. Wounding-induced callose seals sieve plates and lateral sieve area pores, thus preventing assimilate loss (Sjölund, 1997). Callose deposition as a result of wounding by phloem feeding aphids might reduce phloem transport (Botha and Matsiliza, 2004), which may subsequently result in series of morphological symptoms in infested plants (Cagampang, 1974; Hicks *et al.*, 1984; Nielsen, *et al.*, 1990). *Diuraphis noxia* (Russian wheat aphid, RWA) induce rapid wounding and severe symptoms such as chlorosis, necrosis and leaf rolling, which may appear on its host even with a single aphid infestation (Walters *et al.*, 1980; Saheed *et al.*, 2007a, b). In contrast, small populations of *Rhopalosiphum padi* (bird cherry-oat aphid, BCA) do not induce rapid visible damage symptoms or wound callose (Messina *et al.*, 2002; Saheed *et al.*, 2007a; 2009). However, an increase in the BCA population size has been shown to results in the development of golden yellow streak symptoms in host plants (Agronomy guide 2002; UCIPM 2007).

In this paper, we explore the effect of BCA feeding population size specifically to address the question — does wound callose formation and deposition form part of the process resulting in the expression of known morphological symptoms after heavy infestation by BCA?

## Materials and methods

### *Plant material, aphid colony maintenance and treatments*

The cultivation of barley seeds (*Hordeum vulgare* L. cv Clipper), maintenance and illuminations of growth cabinets are as previously described (Saheed *et al.*, 2007a). The colonies of the bird cherry-oat aphid (*Rhopalosiphum padi* L.) were obtained from the ARC-Small Grain Institute, Bethlehem, South Africa. The maintenance of the colony and conditions of the growth cabinets where they were raised are as described (Saheed *et al.*, 2007a). Mature barley leaves (second leaf above coleoptile) and clip-cages, previously described by Noble (1958), of 2 cm in diameter were used to confine aphids to preselected feeding sites on mature barley leaves (two-weeks old). Two experimental approaches were adopted here. In the first approach, five adult aphids per clip-cage were positioned midway between the base and tip of the leaf. The enclosed aphids were allowed to feed for 14 d after which averages of 70 aphids were present in each clip-cage (see Saheed *et al.*, 2007a). Thereafter, leaves were selected at 24h intervals to 21d for the study of wound callose deposition. In the second series of experiments, 50 adult aphids were confined in clip-cages midway between the base and tip of mature leaves. These colonies were allowed to settle and feed for 72 h after which the leaves were used to examine wound callose distribution. Leaves of the same age, with attached

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empty clip-cages, served as controls. Ten replicates (one mature leaf per plant) were set up for each treatment.

*Fluorescence microscopy*

Aniline blue (427 µM in distilled water) was prepared and the stock solution was kept foil-wrapped at 4°C until needed). Whole leaves, including controls, which had not been subjected to aphid feeding, were used. After marking the position of the clip-cages (where aphids had been confined), these leaf segments were severed and immediately transferred to $Ca^{2+}$-free MES 10 mM buffer containing 0.5 mM $MgCl_2$, 0,5 mM KCl and 125 mM mannitol pH 7.2 (Saheed *et al*., 2009). The abaxial surface was gently scraped on a glass plate using a single edge razor blade to remove the cuticle and epidermis, and to expose the underlying mesophyll and vascular tissues. Aniline blue fluorochrome was applied to the leaf strips on glass slides and covered with cover slips. After a 30 min dark incubation period, the sections were gently washed in $Ca^{2+}$-free buffer to remove excess fluorochrome. Leaf tissue was examined to determine the distribution and extent of callose using an Olympus BX61 wide-field fluorescence microscope (Olympus Tokyo Japan, Wirsam Scientific Johannesburg, South Africa) using a yellow GFP BP (with additional 10C/Topaz) filter cube (Chroma Technology Corp. Rockingham, USA). Images were saved using analySIS (Soft Imaging System GmHb, Germany), and selected images were exported to Corel Draw 12 (Corel Corporation Canada).

**Results**

*Morphological symptoms observation*

Small colonies (five aphids) induced yellow streaking in infested barley leaves after 14d. Feeding-related damage symptoms became more noticeable up to the 21d infestation period. Interestingly, no visible damage was observed in leaves exposed to caged colonies containing 50 aphids after 72h.

*Callose in control leaves*

Damage caused by gently scraping the abaxial leaf surfaces in $Ca^{2+}$-free MES buffer, was minimal. Little wound callose was evident in control leaf segments (Fig. 1 A, B), with some callose associated with sieve plates (SP) and pore-plasmodesmal units (PPUs), in small, intermediate (IV) vascular bundles and in cross veins in both experimental procedures.

*Callose deposition in BCA-infested leaf tissues*

Wound callose was formed in all aphid-infested longitudinal vascular bundles as well as cross veins. In addition to phloem-associated callose, callose was also associated with stylet tracks. Where the starting aphid colony was small (five aphids), callose was not observed within the vascular tissue until after 14d of feeding. Callose formation and deposition became more evident and increased from 14 to 21d (Fig. 1 C, D), by which time large areas of the vascular system had been covered with callose extensively. Most of the callose was associated with sieve tubes and associated parenchymatous elements. Where 50 aphids were allowed to feed, by 72h, severe damage to the vascular system was evident and a great deal of the vascular tissue gave a positive response to aniline blue staining (Fig. 1 E-F). Here, all vein classes were probed extensively. Stylet tracks (Fig. 1D, E) stained positively for callose.

**Discussion**

Callose formation and deposition was expected to be a rapid response (Gunning and Steer, 1996; Radford *et al*., 1998; Nakashima *et al*., 2003). However, we report here an observation that appears to suggest the contrary. This is because deposition of wound callose becomes noticeable within longitudinal veins, only after 14 d of feeding when the starting small colony (5 aphids) had grown to about 70 aphids (Saheed *et al*., 2007a). This was in agreement with our earlier observation even when the starting aphid population was ten (Saheed *et al*., 2009). The callose deposition thereafter increases until the end of the experiments-21d (Fig. 1 C, D). Of interest however, is the feeding for 72h by a larger (50) BCA population, which results in rapid appearance of wound callose within longitudinal veins (Fig. 1 E, F), suggesting that the signal for callose induction and appearance appears rapidly but in this case, only if BCA populations were increased. This strongly suggest that population size of the feeding aphids have a crucial role to play in the response of the plant to such infestation. Similarly, stylet tracks were positively stained for callose, which also supports the notion that aphid saliva may have a role to play in callose induction, and may be an indicator of critical changes in plant's morphological, physiological as well as genetic changes (see reviews by Tjallingii 1995; Miles, 1999).

The responses of plants to aphid infestation have been suggested to be species-specific (Gill and Metcalf, 1977) as signals that initiate these responses are aphid-derived. Among the responses, wound callose formation is the most visible early effect, caused by depressurization of sieve tubes. Increase in the influx of cellular $Ca^{2+}$ is known to cause increase callose formation through calmodulin pathway (Botha and Cross, 2001; Will and van Bel, 2006; Will *et al*., 2007). Thus, differences in response to feeding may be induced by differences in saliva-associated signals, as a result of the injection of proteins with $Ca^{2+}$-biding capacity into sieve tubes during the pre-feeding stage (Will and van Bel, 2006; Will *et al*., 2007). Interestingly, when RWA feeds upon the same barley cultivar, we have observed that

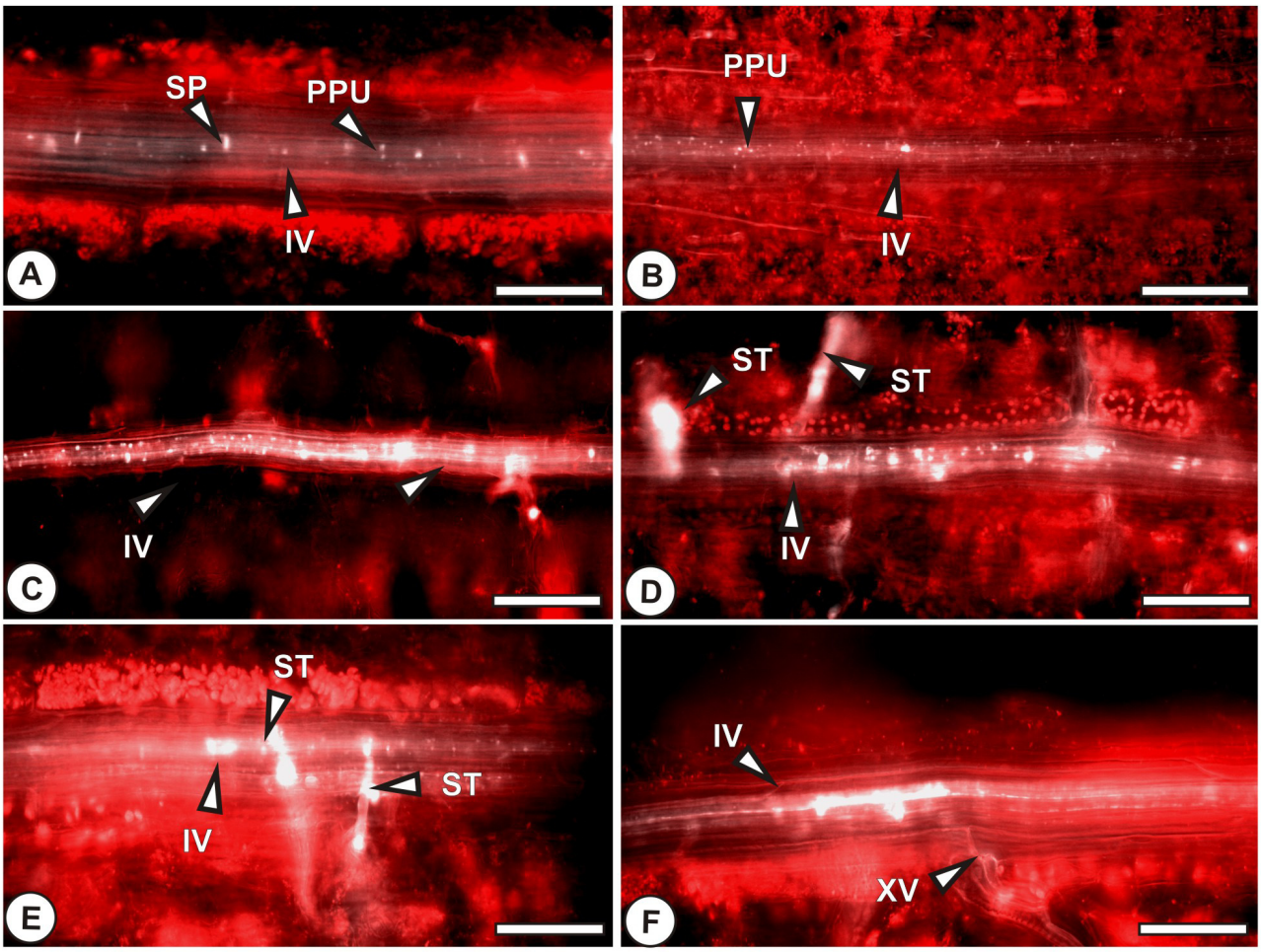


Fig. 1 (A, B): Show distribution of callose formed as a result of scrapping in control barley leaves. The region where the aphid cages were placed, is shown in Fig. A and B. No wound callose formation occurs in the intermediate vein (IV) illustrated here except for limited callose associated with sieve plates (SP) and pore plasmodesma units (PPUs)

Fig. 1 (C, F): Wound callose formation pattern after prolonged feeding (21d) by small BCA colonies and after 72h feeding by large BCA populations

Fig. 1 C, D: Shows extensive formation of aphid-induced wound callose in longitudinal intermediate veins (IV) after 21d of feeding.

Fig. 1 E, F: Wound callose has been deposited in longitudinal intermediate veins and stylet tracks after 72h of feeding by a large (50 aphid) colony. Sieve plates and pore-plasmodesmal units are associated with wound callose; stylet tracks (ST) are extensively callosed. Scale bars: A – D = 200 μm; E – F = 100 μm

aphid-induced wound callose deposition occurs as a result of feeding by small aphid populations, within 24 h of commencement of feeding (Saheed *et al.*, 2009), which contrasts with the current observations using BCA.

We have previously reported that RWA-infested barley leaves developed symptoms of chlorosis and necrosis, while BCA-infested leaves were still healthy, even after two weeks of infestation by low aphid populations (Saheed *et al.*, 2007a; Saheed *et al.*, 2009). We speculated that the differences observed in ultrastructural damage to the vascular tissue caused by the two aphid species, might be responsible for the differences in the observed symptoms (Saheed *et al.*, 2007a). It is known that wound callose deposition effectively seals punctured phloem sieve tubes (Sjölund, 1997). Extensive deposition will however, result in disruption and decrease in the volume of the transported assimilates through the phloem tissues (Hicks *et al.*, 1984; Nielsen *et al.*, 1990; Botha and Matsiliza 2004), due to the blocking of sieve plate pores, plasmodesmata and pore plasmodesmal units. A damage response via wound callose deposition is therefore expected to result in series of cascading symptomatic expressions such as leaf streaking, cessation of transport, followed by the eventual death of the plant.

The data presented here confirms that wound callose deposition only appears when higher feeding population of BCA is involved, we therefore suggest that wound callose deposition appears to play a role in the appearance of golden yellow streaking symptoms reported during heavy population infestation of the aphid (Agronomy guide, 2002; UCIPM, 2007). Whilst callose formation may possibly be linked to the appearance of the golden yellow streaking symptoms reported during heavy population infestation by BCA there is another, equally plausible and more probable reason. A recent study by Botha *et al*. (2008) has demonstrated that solute exchange takes place from xylem vessels, to parenchymatous elements within leaf blade tissues of rice. Furthermore, it has been demonstrated that molecules which, under normal circumstances, cannot cross the plasmamembrane, do so at the pit membrane interface endocytotically. Thus, any process, involving blockage of the critical interface between the xylem and the phloem in leaves, will have a marked effect on transport and exchange efficacy that will eventually translate to morphological symptoms.

In conclusion, it appears that the knowledge of salivary composition, relative population density of the feeding aphids, duration of infestation and most importantly, a good knowledge of the ultrastructural implications of damage, are crucial in understanding the mechanism of interaction between aphids and their host plants. It follows that high aphid density leads to high levels of vascular damage (evidenced as apoplasmic isolation in the xylem; symplasmic isolation in the phloem) and that this, in turn signals different visible symptomatic effects and the beginning of the eventual death of the plant. We suggest that deposition of aphid induced wound callose is involved in the reported morphological symptoms shown by infested plants during heavy BCA infestations.

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