



BACKGROUND

- Sugarcane aphids (*Melanaphis sacchari*) cause severe yield losses in sorghum (*Sorghum bicolor*) every year
- Sugarcane aphids (SCA) use their slender stylets to penetrate plant tissue intercellularly to access the sieve elements for feeding.
- Plants have evolved a wide range of defense mechanisms against insect infestations¹, including callose deposition²
- Callose deposits can serve as phloem blockers as a response to biotic stress, such as aphid and pathogen attack²

RESEARCH OBJECTIVE

To understand the physiological defense responses in sorghum against SCA feeding

MATERIALS & METHODS

- **Plants and Insects:** Sorghum plants were grown in the University of Nebraska-Lincoln greenhouse until reaching their third leaf stage¹. SCA colony was reared in a 12:12hr light:dark cycle, at 26°C
- **Aphid Bioassay:** Ten adult wingless aphids were placed on each plant and total number of aphids were counted 7 days following initial infestation
- **Electrical Penetration Graph (EPG):** The aphid stylet actions below the surface of the plant including probing, various feeding activities and the uptake of phloem sap studied using EPG technique (Fig. 1A). Data was analyzed using the EPG analysis *Stylet+* software
- **Callose Quantification:** Leaves were clip-caged with aphids (Fig. 1B) Harvested leaves were cleared in 100% Ethanol, then stained with 0.01% Aniline Blue [50 mM K₂HPO₄, pH 9.5] for 4 h. Slides were made with 50% glycerol and imaged with a fluorescent microscope (Fig. 2D)

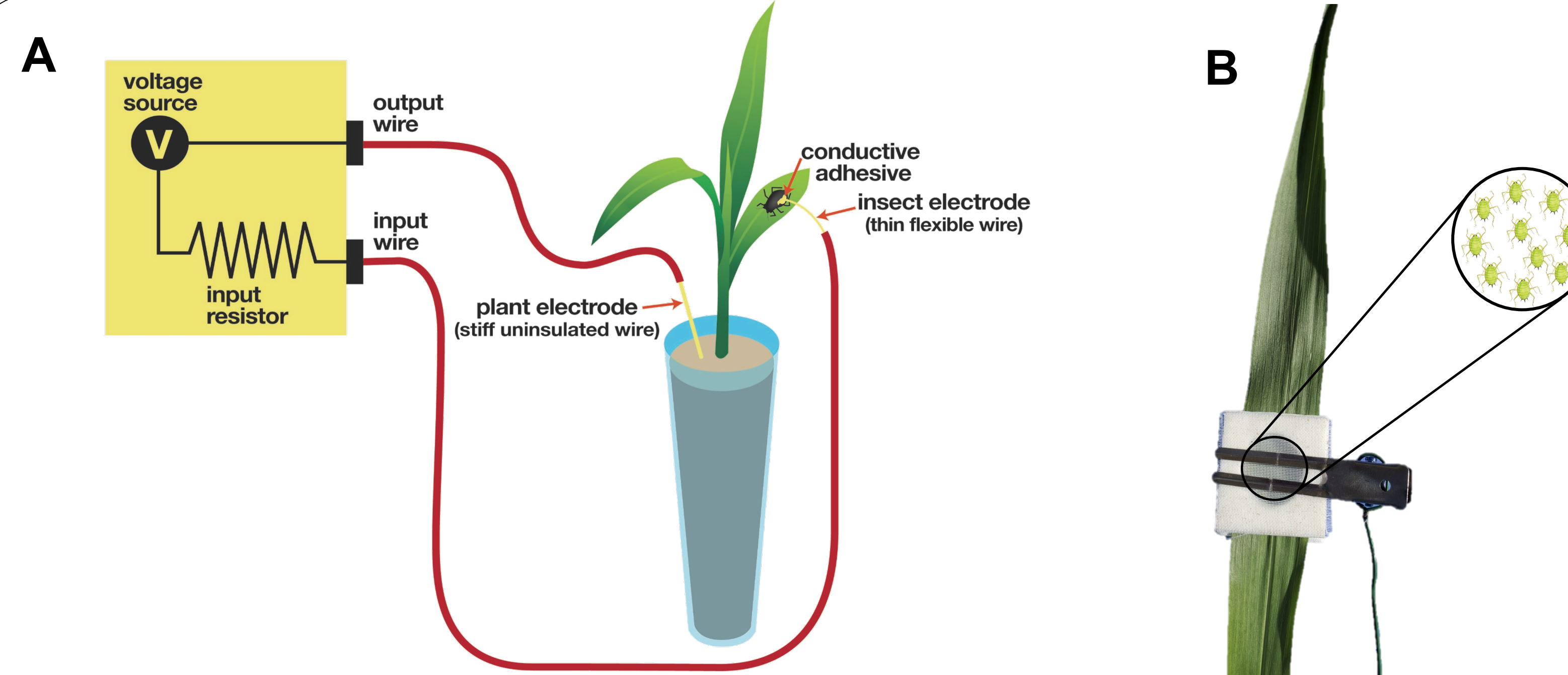


Fig. 1: (A) The circuitry for a typical Electrical Penetration Graph (EPG) setup. (B) Illustration of aphid clip cages used for the callose quantification assay.

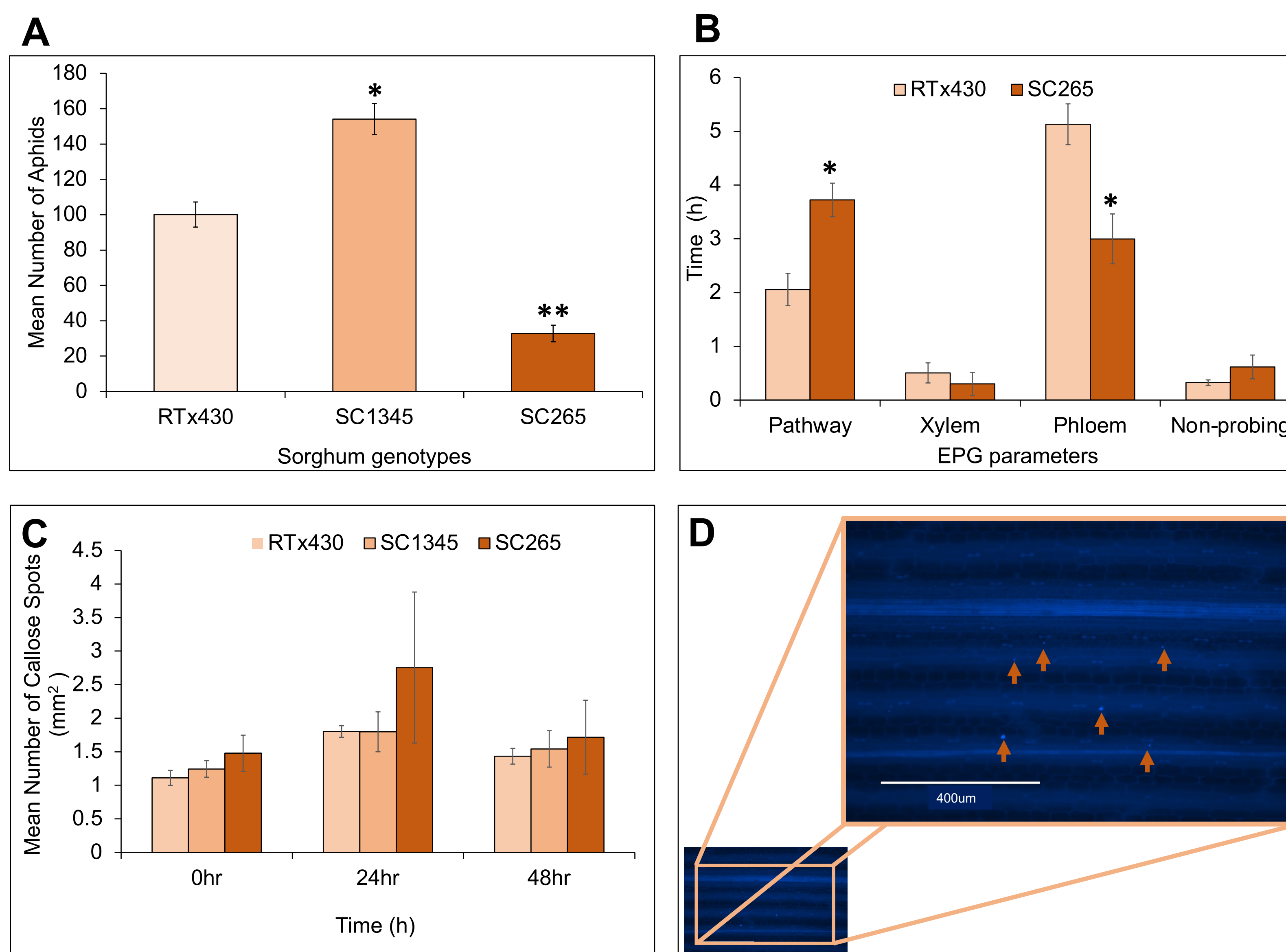


Fig. 2: (A) Mean number of aphids found on sorghum genotypes after 7 days of infestation (n=10). (B) Comparative EPG waveform analysis for genotypes RTx430 (n=17) and SC265 (n=16) after a single, wingless adult aphid was wired and allowed to feed for 8 h. (C) Mean number of callose spots identified under fluorescent microscope per mm² found on different sorghum genotypes (n=3) (D) Arrows pointing to fluorescent Callose deposits in sorghum under 10X magnification. Asterisks above bars represent significant differences relative to RTx430.

RESULTS

- SC265 demonstrates resistance while SC1345 exhibits susceptibility towards SCA infestation (Fig. 2A)
- SCA spent significantly less time in phloem phase while feeding on SC265 as compared to RTx430 (Fig. 2B)
- SC265 had highest callose deposits per mm² at 24 h when compared to RTx430 and SC1345 (Fig. 2C)

FUTURE DIRECTIONS

- Callose quantification at early time points (3, 6 and 12 h)
- Expand studies on other β -glucan polymers used for plant's defense mechanisms
- Understand how other defense mechanisms are modulated with callose accumulation to limit phloem sap-feeding

SIGNIFICANCE

- Baseline study on novel sources of resistance in sorghum against SCA
- Reduction of chemical usage in agriculture

REFERENCES

1. Tetreault, H. M., *et al.* (2019). Global responses of resistant and susceptible sorghum (*Sorghum bicolor*) to sugarcane aphid (*Melanaphis sacchari*). *Frontiers in plant science*, 10, 145.
2. Amsbury, S., Kirk, P. and Benitez-Alfonso, Y. (2017). Emerging models on the regulation of intercellular transport by plasmodesmata-associated callose. *Journal of Experimental Botany*, 69, 105-115.

Additional reading on plant defenses



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