2022

Does Aphid Feeding Trigger the Production of H202 in Wild Barley?

Richard Justin Holewa

Does aphid feeding trigger the production of H_2O_2 in wild barley?

VAN ECK LABORATORY

Richard Justin Holewa & Leon van Eck, Ph.D.

AUGSBURG UNIVERSITY

Abstract

In an *in vivo* experiment to see if the oxidative burst of reactive oxygen species (ROS) is utilized as a defense mechanism in barley against the bird cherry-oat aphid (*Rhopalosiphum padi*), aphids were pre-starved then allowed to feed on cultivated (*Hordeum vulgare*) and wild barley (*H. spontaneum*) accessions. Hydrogen peroxide (H_2O_2) generated in barley leaves in response to aphid feeding was quantified with DAB (3,3'-diaminobenzidine) staining. Stained leaves were digitized and analyzed using Fiji. Proportions of stained areas were statistically tested for significance (with pooled t-tests and ANOVA at 5%). No significant differences were detected between wild and cultivated barley, nor between aphid-challenged and control tissues. Therefore, ROS are not a notable component of barley's defense machinery when preyed upon by *R. padi*.

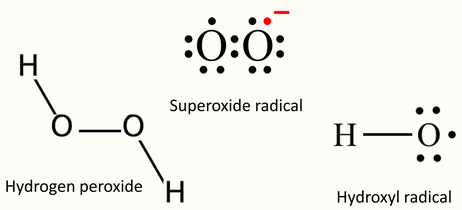


Figure 1: Examples of reactive oxygen species that are released during an oxidative burst response.

Background

Previous lab studies suggested that *R. padi* fertility and fecundity is reduced when feeding on wild barley accessions compared to commercial barley cultivar 'Morex'. The plant defense mechanism(s) responsible are not yet known.

Research Question

Does barley elicit an oxidative burst response? If so, does wild barley have a stronger response compared to cultivated barley?

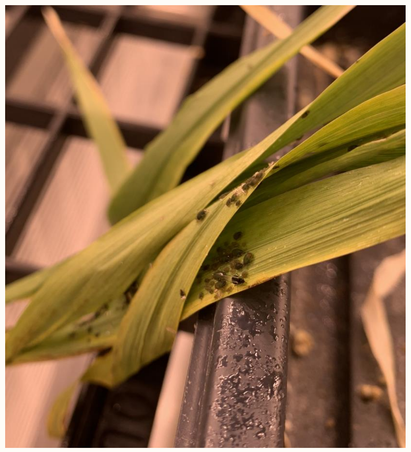


Figure 2: A large group of feeding *R. padi* on barley.

Conclusion

Oxidative burst is likely not a defense mechanism employed by barley to combat feeding by *R. padi*.

Acknowledgements

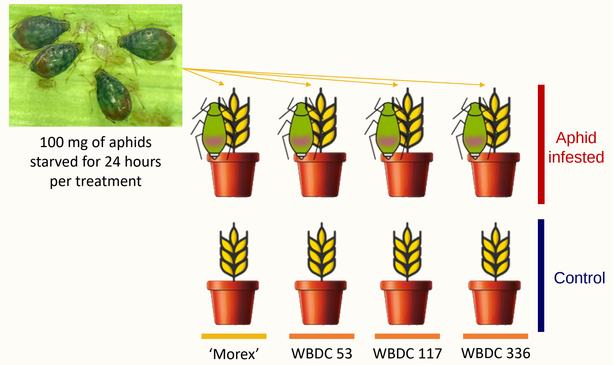
Thank you to Noreen and Steve Thompson for funding this research, Professor Brian Steffenson (UMN) for access to germplasm, and to URGO for creating opportunities to do research at Augsburg.

Scan QR code for relevant references and video presentation

Walkthrough

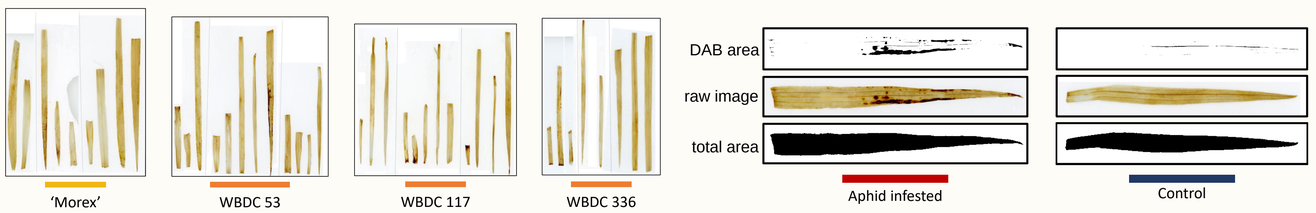
Experimental Design

Barley plants used in aphid challenges included one commercial barley cultivar ('Morex') and three wild barley accessions (Wild Barley Diversity Collection; WBDC53, WBDC117, WBDC336). Aphids were starved for 24 h before infestation. Aphids fed on plants for a subsequent 24 h period before plant tissues were harvested for histological staining.



Workflow

Harvested leaf sheaths and laminae were incubated for 6-9 h in a 0.1% (w/v) 3,3'-diaminobenzidine (DAB) solution, pH 3.83. DAB forms dark brown deposits in the presence of H_2O_2 and plant peroxidases. Chlorophyll was cleared overnight with 75% ethanol and Visikol. Stained and cleared samples were scanned and files imported into image processing software (ImageJ/Fiji). Twelve well-stained and cleared samples were selected for further analysis: two aphid treatments and one non-infested control for each cultivar/accession.



Results & Analysis

Three analyses were done comparing % area difference between stained and total area: all plant parts, only leaves and tillers, and aphid feeding regions. Statistical significance was tested with pooled t-tests and ANOVA at 5% significance. There was no difference between wild and cultivated barley nor between the control and the aphid infested barley. Oxidative burst is likely not a prominent defense mechanism utilized by barley during *R. padi* feeding. Since release of ROS is not a prominent mode of defense, other plant defense mechanisms may be more substantially upregulated during *R. padi* feeding.