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Effect of host plant on levels of reactive oxygen species and antioxidants in the cereal aphids *Sitobion avenae* and *Rhopalosiphum padi*

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ABSTRACT

Effects of host plants on levels of reactive oxygen species (ROS) and antioxidant enzymes in tissues of *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.) were studied. Levels of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) increased when aphids were transferred from winter wheat to two cultivars (Witon and Tornado) of winter triticale. ROS increase in triticale depended on the length of time that aphids fed on the triticale. The increase in O_2^- after transfer was greater on the less susceptible cultivar Witon than on the more susceptible cultivar Tornado. The increase in H_2O_2 after transfer was greater in the monophagous *S. avenae* than in the oligophagous *R. padi*. Activities of the ROS-scavenging enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) increased after aphids were transferred from winter wheat to winter triticale; only the increase in CAT was greater in *R. padi* than in *S. avenae*. APX activity in *R. padi* was greater on Witon than on Tornado. The content of the non-enzymatic antioxidant ascorbate (ASA) in aphids decreased when aphids were transferred from winter wheat to Witon, the less susceptible triticale cultivar, but remained unchanged when aphids were transferred to Tornado. The results of these experiments highlight the important role of oxidative stress in interactions between cereal aphids and their host plants.

1. Introduction

Reactive oxygen species (ROS) such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•OH) are generated throughout cells as a result of oxidative metabolism. Herbivores, including phloem-sucking insects, are also subjected to exogenous ROS that are generated by plants to defend against herbivory (Orozco-Cardenas and Ryan, 1999; Krishnan et al., 2007; Sytykiewicz, 2011). Researchers have proposed that the saliva and the injury caused by aphids induce a local and systemic production of ROS in the phloem of the host (Moran and Thompson, 2001; Zhu-Salzman et al., 2004; Divol et al., 2005). Among ROS, H_2O_2 has multiple functions in plant defense against herbivorous insects, including cell-wall reinforcement, direct toxicity, and signaling for the activation of defense genes (Mehdy et al., 1996; Kuźniak and Urbanek, 2000; Morkunas et al., 2011). Kuśnierczyk et al. (2008) showed that ROS are involved in early signaling in *Arabidopsis thaliana* after infestation by *Brevicoryne brassicae* (L.). Argandona et al. (2001) documented the

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involvement of H_2O_2 in the stimulation of cell wall reorganization in barley after infestation with *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (L.).

Plant defense against aphids also includes a variety of allelochemicals that may induce the generation of H_2O_2 and other ROS in herbivores, and these include photodynamically activated furanocoumarins, β-carbonyl alkaloids, thiophenes, and phenolic compounds (Downum and Rodriquez, 1986; Appel, 1993). ROS cause oxidative damage to midgut cells and impair the absorption of nutrients by insect herbivores (Bi and Felton, 1995). ROS react with many intracellular molecules, including proteins, lipids, and DNA, causing cell death. ROS initiate lipid peroxidation that leads to increased permeability of the cell membrane to ions and fluids (Jamieson, 1989). Lipid peroxidation is especially harmful to herbivores because lipids are not only components of membranes but also have unique physiological functions; for example, cuticular hydrocarbons prevent desiccation, and isoprenoid juvenile hormones are involvement in insect development (Downer, 1986).

The balance between the generation and elimination of ROS helps determine the performance of herbivorous insects on plants (Krishnan and Sehnal, 2006). Thus, aphids and other herbivorous insects possess an antioxidant enzyme system to reduce ROS levels. This system is composed of superoxide peroxidase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and ascorbate peroxidase (APX). The SODs convert O_2^- to oxygen and hydrogen peroxide. The CATs and APXs convert hydrogen peroxide to water. Thus, these enzymes work in concert to convert O_2^- and H_2O_2 to H_2O.

The grain aphid *Sitobion avenae* (F.) and the bird cherry-oat aphid *R. padi* (L.) are important pests of cereals in Poland. These aphid species usually coexist on many hosts but occupy different parts of the host plant (Johansson et al., 1997; Gianoli, 2000). *R. padi* prefers the stem and lower leaves, whereas *S. avenae* feeds primarily on the upper leaves and ears (Gianoli, 2000). Both aphid species transmit Barley Yellow Dwarf Virus (BYDV). Our previous studies on cereals aphids have focused mainly on the effect of plant pro-oxidants on antioxidant defense mechanisms of aphids, but little is known about host plant modulation of aphid antioxidant systems. The aim of the present research was to investigate the effect of two cultivars of winter triticale on ROS levels and on the antioxidant system that removes O_2^- and H_2O_2 in tissues of the monophagous *S. avenae* and the oligophagous *R. padi*.

2. Material and methods

2.1. Aphids and plants

The aphids were originally obtained from the aphid stock cultures kept at the University of Natural Sciences and Humanities at Siedlce. The aphids were reared on seedlings of winter wheat cv. Tonacja in an environmental chamber (21 °C, L16:D8 photoperiod, and 70% relative humidity). Two cultivars of winter triticale (× *Triticosecale* Wittm.) that differ in susceptibility to cereal aphids were used, and these were Tornado (more susceptible) and Witon (less susceptible) (Sempruch et al., 2009).

2.2. Experiment

An experiment was conducted with wingless females (apterae) of both aphid species in an environmental chamber at 21 ± 1 °C and 70% relative humidity under a 16-h photoperiod. Aphids were routinely reared on winter wheat as described in the previous section. Seedlings of the two winter triticale cultivars were grown in plastic pots (10 × 10 cm, eight seedlings per pot) filled with medium nutrient fine structure compost with sand. Nine-day-old seedlings of triticale were each infested with 20 aphids obtained from the wheat plants. Aphids that had fed on the triticale plants for 24, 48, 72, and 96 h were collected and assayed for ROS and antioxidant enzymes as described in the following sections. Aphids collected from the wheat plants at time of transfer were subjected to the same assays; these aphids served as time 0 controls. The experiment was conducted in four replicates.

2.3. Preparation of aphid homogenates

Batches of 100 collected aphids were placed in 50 mM potassium phosphate buffer pH 7.0 (for O_2^-, H_2O_2, CAT, ASA, and APX assay) or in 50 mM potassium phosphate buffer pH 7.8 (for SOD assay), and homogenized for 5 min at 0 °C. The homogenates were filtered through two layers of cheesecloth and centrifuged at 3000 *g* for 15 min. The pellets were discarded, and the supernatants were used to assay for the markers of oxidative stress.

2.4. Superoxide assay

Superoxide content was assayed according to the method described by Green and Hill (1984); the method is based on the reduction of nitroblue tetrazolium (NBT). The reaction mixture consisted of 0.5 ml of crude homogenate of aphids and 0.5 ml of 0.4 mM NBT in 0.2 M phosphate buffer (pH 7.8). The increase in absorbance at 490 nm was monitored against the blank contained 0.5 ml of crude homogenate of aphids and 0.5 ml of 0.2 M phosphate buffer (pH 7.8). The reducing activity of NBT by the aphid homogenates was expressed as ΔA_{490}/min/mg of protein.