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Control of grazing by light availability via light-dependent, wound-induced metabolites: The role of reactive oxygen species

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ABSTRACT

Light may influence grazing through its effect on grazers or by influencing the physiology and biochemistry of primary producers. It has recently been established that reactive oxygen species (ROS) produced by a wounded macroalga depend on light via photosynthetic electron transport. Since ROS can act as both chemical messengers and toxins, it is hypothesized that the light-dependence of wound-induced ROS can have ecological consequences for plant–herbivore interactions. Through spin trapping and electron paramagnetic resonance, this study shows that wounded *Ascoseira mirabilis* produced ROS that were correlated with *A. mirabilis* wounding in light but not dark conditions. In feeding assays where sympatric amphipods consumed palatable macroalgae in the presence of wounded *A. mirabilis*, amphipod consumption was correlated with *A. mirabilis* wounding in light but not dark conditions. The nonlinear effect of ROS on grazing suggests that in ecology, as in physiology, low levels of ROS may act as messengers while high levels are toxic.

1. Introduction

The effect of light on plant–herbivore interactions is complex. Grazing strategies can be shaped by numerous factors including resource competition, predator avoidance, and the physiology of the food, each of which may be affected idiosyncratically by light. Yet the effect of light on grazing strategies is often viewed as a consequence solely of the ecology or physiology of herbivores as opposed to the physiology of the primary producers they consume (see Roberts and Paul, 2006). Although these are by no means mutually exclusive, it is important to remember that exposure to light produces unavoidable effects on the physiological state of primary producers, and that these effects may impact grazing. One such effect is the generation of reactive oxygen species (ROS) during photosynthetic electron transport (Asada, 2006). It is not known whether the generation of ROS during photosynthetic electron transport – a simple consequence of light-exposure –may have repercussions on plant–herbivore interactions. If so, a purely physiological effect of light on primary producers may contribute to the evolution of feeding strategies in small primary consumers.

Reactive oxygen species are the partial reduction products of molecular oxygen (O_2) – superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) – and reactive derivatives such as peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) among many others (Halliwell and Gutteridge, 2007). While O_2 is potentially toxic but mostly unreactive and its full reduction product (H_2O) is nontoxic and unreactive, ROS are potentially toxic and many are quite reactive. In spite of and in fact owing to these attributes, ROS play important roles in the physiology and ecology of aerobic organisms (Halliwell, 2006). For example, the production of ROS in an "oxidative burst" is shared among algae, plants, and animals (Suzuki and Mittler, 2012; Potin, 2008; Lamb and Dixon, 1997). The oxidative burst of animals, plants, and algae can be elicited upon wounding or herbivory

Abbreviations: EPR, electron paramagnetic resonance; H_2O_2, hydrogen peroxide; O_2, molecular oxygen; O_2, superoxide anion; OH, hydroxyl radical; ROS, reactive oxygen species.

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(McDowell et al., 2014b; Suzuki and Mittler, 2012) and its role in mediating infection by microbial parasites and pathogens is well established (Yoo et al., 2012; Peng and Kuc, 1992).

Although far less well-studied than the mediation of infection, the wound-induced oxidative burst may also contribute to the mediation of grazing on sessile organisms. The incorporation of H_2O_2 into food or its elevated presence in seawater can decrease the grazing rate of small herbivores in both terrestrial and marine systems (McDowell et al., 2014a; Ramputh et al., 2002). Felton et al. (1994) found that insects that consumed previously wounded soybean plants had greater markers of oxidative damage in their gut and decreased growth compared to insects that consumed unwounded plants. Expressing a wheat oxalate oxidase gene in corn plants both increased tissue H_2O_2 and decreased consumption by and growth of corn borers (Ramputh et al., 2002). Similarly, silencing a germin-like protein in *Nicotiana attenuata* both decreased tissue H_2O_2 and increased growth of larval insects on the plant (Lou and Baldwin, 2006). Deleterious effects of ROS on grazers may be due to direct toxicity and/or may arise downstream of ROS signaling from alteration in gene expression or metabolic pathways (e.g. Mao et al., 2007).

The source of the oxidative burst is often a dedicated enzyme like an NADPH-oxidase (NOX; Bedard et al., 2007). Although it is well known that NOX and NOX homologs are very important in the wound response of both animals and plants (e.g. Miller et al., 2009; Niethammer et al., 2009), it has recently been shown in the kelp *Saccharina latissima* that wound-induced ROS ultimately depend on photosynthetic electron transport (McDowell et al., 2015). The potential involvement of a NOX homolog downstream of photosynthetic electron transport was not explored in McDowell et al., 2015 or the present study as the most common method of inhibiting NOX enzymes – diphenylene iodonium, or DPI – also inhibits photosynthetic electron transport (Chang et al., 2004; Ohtsuka et al., 2004; Corneille et al., 1998). Qualitative data indicate that disrupted photosynthetic electron transport may also contribute to the oxidative burst in Antarctic macroalgae (McDowell et al., 2015) and *Arabidopsis thaliana* (Morker and Roberts, 2011). If wound-induced ROS are light-dependent, can light availability control plant–herbivore interactions in the presence of wound-induced ROS?

Here, organisms from a well-studied ecosystem on the Western Antarctic Peninsula (Amsler et al., 2014) were used to investigate the hypothesis that plant–herbivore interactions in the presence of wound-induced ROS may be controlled by light. The omnivorous amphipod *Gondogeneia antarctica* readily consumes the palatable red macroalga *Palmaria decipiens* while it does not consume the unpalatable brown macroalga *Ascoseira mirabilis* (Amsler et al., 2009). Neither macroalga contains secondary metabolites shown to be unpalatable to *G. antarctica* (Amsler et al., 2005), but both release ROS upon wounding (McDowell et al., 2014b), and preliminary evidence suggests that these ROS are light-dependent (McDowell et al., 2015). Finally, the presence of wounded *A. mirabilis* decreases grazing of *G. antarctica* on *P. decipiens* compared to the presence of intact *A. mirabilis* (experiment conducted under light conditions; McDowell et al., 2014a).

In this study, spin trapping and electron paramagnetic resonance (EPR) were used to show that wound-induced ROS from *A. mirabilis* are light-dependent. Wounded *A. mirabilis* produced less ROS in dark vs. light conditions, and wounding correlated with ROS in the light but not in the dark. Feeding assays were then conducted in which both macroalgal wounding and light were manipulated in order to manipulate wound-induced ROS. Amphipod consumption of the food alga *P. decipiens* in the presence of *A. mirabilis* was correlated with *A. mirabilis* wounding in light but not in dark conditions. These results suggest that light-dependence of plant physiology can translate into algal–herbivore interactions that are controlled by light. Therefore, light may play a more complex role in shaping the grazing strategies of small primary herbivores than previously recognized.

2. Materials and methods

2.1. Study area and species collection

Experiments were conducted at U.S. Palmer Station (64° 46′ S, 64° 03′ W) on Anvers Island off the west coast of the Antarctic Peninsula in April and May, 2013. All organisms were collected by hand within 3.5 km of Palmer Station using SCUBA from a depth of 5–20 m. The amphipod *G. antarctica* was collected subtidally from the brown macroalga *Desmarestia menziesii* by collecting entire *D. menziesii* individuals in a mesh bag (cf. Huang et al., 2007). Amphipods were removed from the algae, sorted by species, and maintained in 2 L plastic bottles with mesh sides inside flowing ambient seawater tables. They were used for feeding assays within 7 days of collection and held without food for 2–3 days prior to the experiments. Individuals of the red macroalga *P. decipiens* and blades from individuals of the brown macroalga *A. mirabilis* were maintained in flowing ambient seawater tables and sampled within 7 days of collection. Samples of macroalgal thallus were excised 1–3 days before use in experiments.

2.2. Electron paramagnetic resonance

The spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was purchased from Enzo Life Sciences (ALX-430-090; Farmingdale, NY). Stock solution of DMPO (1 M) was made in ice cold DI water and stored at −80 °C until use. Samples of *A mirabilis* (2.5 cm^2) were wounded using a razor blade to create a variable number of 2 cm-long parallel slices through the thallus, placed in 2 mL sterile filtered seawater (SFSW; 0.44 μm) containing 100 μM DMPO, and incubated at 1.5 °C. After 20 min, seawater samples were immediately frozen in liquid nitrogen. All EPR sampling was conducted on May 4, 2013. Flash frozen samples were shipped on dry ice to the University of Alabama at Birmingham and stored at −80 °C. Room temperature EPR analysis was conducted within four months of sampling. All samples were thawed at room temperature for 10 min and injected into a Bruker Elexys E500 Aqua-X sample cell (Billerica, MA). The timing for instrumental tuning was kept the same for all samples. Instrument settings were 20 mW power, 1 G modulation amplitude, 160 ms time constant, 320 ms conversion time, one scan. Spectra were analyzed by quantifying the intensity of the third peak (peak minus trough) in the quartet characteristic of DMPO/•OH.

2.3. Feeding assays

Fifteen *G. antarctica* were placed in 100 mL filtered seawater inside each of 20 Nalgene bottles inside a temperature-controlled room held at 1 °C. Replicates of *A. mirabilis* (3 cm^2, n = 20), each from a different individual, were wounded by slicing through the thallus with a clean razor blade and one was placed in each bottle. Each *A. mirabilis* replicate received a variable number of 1 cm-long wounds, between 0 and 24. No number of wounds was repeated, and the order of wounding was randomized. Each *A. mirabilis* replicate had a paired sample from the same individual that was wounded an equal number of times and used as an autogenic control. Autogenic control samples were placed in bottles lacking amphipods and used to control for natural changes in food mass over the course of the feeding assay.

Using alginate-based artificial food in feeding assays with *G. antarctica* greatly increases weighing error compared with using fresh macroalgae (R. McDowell, personal observation). Therefore, one pre-weighed, 1 cm diameter disc of the palatable macroalga *P. decipiens* was placed as food in each bottle. *P. decipiens* food discs were not in contact with *A. mirabilis* samples in containers. *P. decipiens* discs for each experiment came from a single large individual in order to minimize any possible intra-individual differences in their relative palatability. Experiments were conducted using one "food alga" (*P. decipiens*) and a different, wounded alga (*A. mirabilis*)