The source of reactive oxygen species in the wound response of *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders

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Marine Algae
Summer 2012

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Key words: Saccharina latissima, algae, reactive oxygen species (ROS), oxidative burst, woundin

ABSTRACT

Reactive oxygen species (ROS) are commonly produced by cells to defend against invading pathogens. As such, ROS are an important feature of wound responses in organisms as distantly related as algae, vascular plants, and animals. However, despite the fact that most pathogen-elicited oxidative burst are of enzymatic origin, the origin of wound-induced ROS is unknown. In this study, I identified a wound-induced oxidative burst in the kelp *Saccharina latissima*, and this oxidative burst was investigated at different light levels. Both sham wounding and wounding led to ROS production that varied with light level, although the relationship between the magnitude of the oxidative burst and irradiance was not linear. Since the wound-induced burst of *S. latissima* is affected by light level, it probably has its origins in accidental ROS formation due to the breakdown of the photosynthetic electron transport chain.

Abbreviations: DPI, diphenyleneiodonium; H_2O_2, hydrogen peroxide; O_2, molecular oxygen; $OH^{\cdot}$, hydroxyl radical; ROS, reactive oxygen species

INTRODUCTION

Reactive oxygen species (ROS) are the unstable partial reduction products of molecular oxygen (O_2), and the term ROS includes molecules like superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$; Halliwell and Gutteridge 2007). ROS can be produced accidentally, through the nonenzymatic reduction of O_2. Accidental ROS production often occurs through electron leak along sites of electron transport in mitochondria and chloroplasts (reviewed in Turrens 2003, Mehler 1951). ROS can also be produced deliberately, through special ROS-producing enzymes. The enzymatic production of a transient burst of ROS, called the oxidative burst, is a common microbial defense in plants, animals, and algae (Halliwell and Gutteridge 2007). ROS can damage or kill invading pathogens (Radi et al. 1991), cross link the cell-wall (Bradley et al. 1992), and participate in defense signaling cascades (Vandenabeele et al. 2003, Soares et al. 2011).

Although the oxidative burst is most well understood with respect to its role as a defense against pathogens (Lamb 1997, Potin 2008), it can also be elicited by mechanical stress or wounding. Several commercial plant species produce H_2O_2, superoxide, or hydroxyl radicals upon crushing or slicing (Orozco-Cardenas and Ryan 1999, Jih et al. 2003, Angelini et al. 2008, Johnson et al. 2003, Epperlein 1986). Macroalgae can also produce ROS in response to wounding, but this is less well-studied. The rhodophyte *Eucheuma platycladum* produces H_2O_2 upon breakage and stirring (Collén and Pedersén 1994), the siphonous green alga, *Dasycladus vermicularis* produces H_2O_2 and nitric oxide ($NO^{\cdot}$) during wound plug formation (Ross et al. 2006), and several Antarctic macrophytes produce ROS upon wounding (McDowell, unpublished data).

It is not known whether ROS production upon wounding arises from accidental formation due to the breakdown of photosynthetic or mitochondrial electron transport chains or whether they are enzymatically produced. The objective of this study was to determine whether the brown alga *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders produces an oxidative burst upon wounding and whether this burst is dependent on irradiance level. *S. latissima,* an annual species in the kelp family Laminariaceae (Guiry), is known to produce an oxidative burst upon challenge with breakdown products of the cell wall polysaccharide alginate (Küpper 2002). It is common throughout the study region at Friday Harbor Laboratories on San Juan Island, WA, where it is found in low intertidal and high subtidal environments (Mumford 2007). In this study, I first generated a rough photosynthesis-irradiance relationship for *S. latissima* by measuring oxygen production at respiration and five different light levels. From these data I chose four light levels at which to measure the strength of the oxidative burst upon wounding.

METHODS

Macroalgal collection. Experiments were conducted at Friday Harbor Laboratories, located on San Juan Island, WA, USA. Individual *S. latissima* were collected from the exterior of the floating dock. Vegetative samples were excised from macroalgal thalli with a 2 cm diameter cork borer 12-24 hours before use to minimize the effects of wounding. Samples were maintained in flowing ambient seawater (11°C) until use and held in Tupperware containers with mesh sides to allow water flow.

O_2 measurement. Seawater was filtered using a glass fiber prefilter (nominal rating 0.6 µm; Microfiltration Systems, now Advantec MFS, Dublin, CA). O_2 was measured using a Hansatech O_2 electrode system with Oxygraph software (Hansatech Instruments, Norfolk, England). Samples were held in place inside the measurement chamber under constant stirring in 10 mL filtered seawater maintained at 11°C with a water bath system. The measurement chamber had one glass window to allow light from an external 80V light source. Light levels were adjusted by placing window screening between the light source and measurement chamber and were verified before experiments using a Hansatech Quantitherm light meter. Respiration measurements were taken in the dark by wrapping the measurement chamber window with black plastic and covering the chamber with a black plastic-coated box.

O_2 measurement for generation of a photosynthesis-irradiance relationship. Samples of *S. latissima* (n = 3) were placed in the measurement chamber and O_2 production was monitored for 70 minutes; 20 minutes during respiration and 10 minutes at each of the following light levels: 13, 32, 115, 435, and 1165 µmol photons/m^2/s. The rate of oxygen production during the last 5 minutes of respiration and the last 3 minutes of each light level were plotted against irradiance.

O_2 measurement before and after wounding. Samples of *S. latissima* (n = 5) were placed in the measurement chamber and O_2 production was monitored for 10 minutes. At 10 minutes, the sample was removed from the chamber and placed under a darkened screen (transmission: 1 µmol photons/m^2/s) to be wounded. Six parallel wounds were created on each sample with a clean razor blade by cutting the thallus entirely through. Control samples were treated in the same way but no cuts were made. After wounding or

sham wounding, samples were returned to the measurement chamber and O_2 production was monitored for a further 12 minutes. The rate of O_2 production during the 5 minutes before wounding was subtracted from the rate during the 2 minutes following wounding to generate the change in oxygen production rate caused by wounding. This process was repeated for one sample of five *S. latissima* individuals in the dark and at three different light levels: 15, 115, and 1165 µmol photons/m^2/s.

Statistical analyses. The change in O_2 production during wounding was compared at each light level to the change in O_2 production during sham wounding using a paired t-test (Excel). Both the change in O_2 production during wounding and that during sham wounding were compared between different light levels using a one-way ANOVA and a post hoc Tukey HSD test (Vassar stats). The difference between the change in O_2 production during wounding and the change in O_2 production during sham wounding was also analyzed using one-way ANOVA and a post hoc Tukey HSD test (Vassar stats).

RESULTS

Photosynthesis-irradiance relationship. The rate of oxygen evolution increased rapidly between 0 and 32 µmol photons/m^2/s and *S. latissima* reached its maximum rate of photosynthesis somewhere between 115 and 430 µmol photons/m^2/s (Fig. 1).

O_2 production after wounding. At each light level, wounded samples showed a larger change in the rate of O_2 production than sham wounded (control) samples (paired t-test at each light level, $p < 0.05$, Fig. 2). The change in the rate of O_2 production during sham wounding was significantly higher at 115 µmol photons/m^2/s than at 0 (one-way

ANOVA, $p < 0.05$), but other relationships were not significantly different. The change in the rate of O_2 production during wounding was not significantly different between low light levels (0 and 15 µmol photons/m^2/s) or between high light levels (115 and 1165 µmol photons/m^2/s), but low light levels were significantly different from high light levels (one-way ANOVA, $p < 0.05$). These differences are maintained when the change in the rate of O_2 production during sham wounding is subtracted from that during wounding to yield the change in rate of O_2 production attributed solely to wounding (Fig. 3).

DISCUSSION

Since the change in rate of O_2 production was greater at each light level after wounding than after sham wounding, it was possible to identify an oxidative burst upon wounding in *S. latissima* (Fig. 2).This species is known to produce an oxidative burst upon elicitation with oligoguluronates (breakdown products of alginate in the cell wall), and this oxidative burst is sensitive to diphenyliodonium, an inhibitor of flavoenzymes (Küpper 2002). Therefore, we know that the oligoguluronate-induced oxidative burst of *S. latissima* is enzyme-mediated. However, the results of this paper indicate that accidental electron leak during photosynthesis is at least partly responsible for the oxidative burst elicited by wounding.

The change in rate of O_2 production attributed to wounding was not significantly different between low light levels (0 and 15 µmol photons/m^2/s) or between high light levels (115 and 1165 µmol photons/m^2/s), but the change in rate of O_2 production from

wounding at low light levels was significantly different from that at high light levels (Fig. 3). Therefore, the magnitude of the oxidative burst changed with light level, but the relationship was not linear. The oxidative burst in darkness was not different from that at 15 µmol photons/m^2/s, and this was probably due to two factors. First, my set-up made it impossible to keep the sample completely in the dark during the wounding process, so there would have been some electrons in the electron transport chain due to the exposure of light during wounding. Second, even with no contribution from a damaged photosynthetic electron transport chain, damaged mitochondrial electron transport chains may produce some ROS as well, and these will be unaffected by lack of light. In order to fully understand the role of light in wound-elicited ROS, future studies should find a way to wound a sample completely in the dark to see whether there is a baseline ROS release.

The difference in O_2 production attributed to wounding at 15 µmol photons/m^2/s and 115 µmol photons/m^2/s (an order of magnitude change in irradiance level) was clear and significant, yet the difference between 115 µmol photons/m^2/s and 1165 µmol photons/m^2/s was not, even though this is also an order of magnitude change in irradiance level. This suggests that it is not necessarily the amount of photons coming into the alga that dictates ROS production, but the amount of reaction centers capable of using the photons to split water and create a high energy electron. Since *S. latissima* was performing maximal photosynthesis at both 115 and 1165 µmol photons/m^2/s (Fig. 1), the increase in light did not lead to an increase in high energy electrons capable of creating ROS during wounding, and this is probably why the magnitude of the oxidative burst did not change between these light levels.

The sham wounding, or control, treatments also showed some variation with light (Fig. 2). This indicates that ROS created by other types of stress, such as the mild desiccation and temperature stress of being removed from the water, and not just wounding, are affected by incoming irradiance and electron transport chain saturation.

In conclusion, I have shown that the wound-induced oxidative burst of *S. latissima* is affected by light level. The ROS and the electrons that created them are therefore at least partly due to photosynthesis. Future studies should quantify the baseline ROS production (or lack thereof) after wounding in complete darkness. It would also be interesting to investigate whether the wound-induced oxidative burst of *S. latissima* is temperature-dependent. Since most enzymes are strongly affected by temperature, temperature independence would lend support to the idea that the wound-induced burst is not of enzymatic origin.

ACKNOWLEDGEMENTS

I am very grateful to my instructors, Tom Mumford and Bob Waaland, for their ideas, advice, and help with my project. I also thank Dolores Deregibus for assistance with the O_2 measurement system and the entire marine algae class of 2012 for being wonderful. I especially thank Lesley and Ken Nilsson for providing me, through the Adopt-A-Student fund, with the financial aid that allowed me to take this course.

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FIGURE CAPTIONS

Figure 1. O_2 production ± standard error by *S. latissima* at different light levels (n = 3).

Fig. 2. The change in O_2 consumption rate during wounding is greater than that during sham wounding and both vary at different light levels. Bars show the change in O_2 consumption rate during wounding and sham wounding (control) ± standard error. The change in O_2 consumption rate during wounding was significantly greater than that during sham wounding (paired t-test, $p < 0.05$). Letters denote significant differences between light levels among wounded samples and differences between light levels among control samples (one-way ANOVA + Tukey HSD, $p < 0.05$).

Fig. 3. Data are the same as in figure 2. Bars show the change in O_2 consumption rate during wounding minues the change in O_2 consumption rate during sham wounding

(control) ± standard error. Letters denote significant differences between light levels (one-way ANOVA + Tukey HSD, $p < 0.05$).

Figure 1.

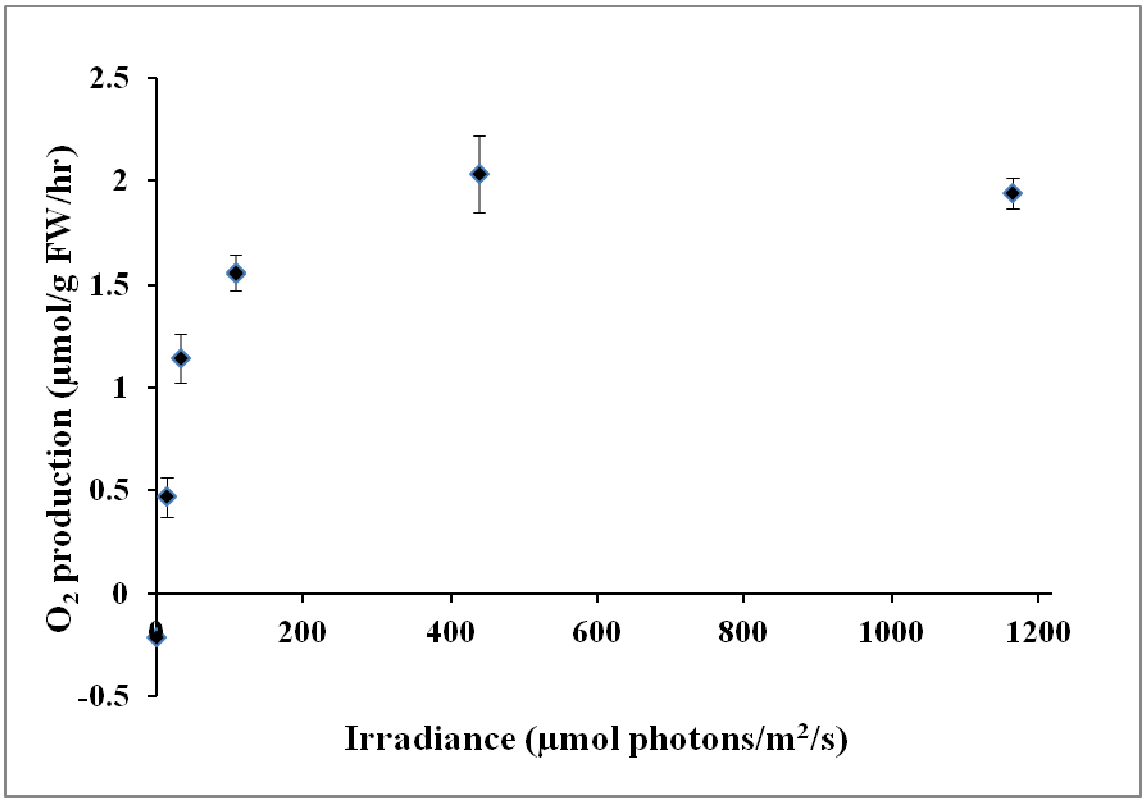

Fig. 1. O_2 production ± standard error by *S. latissima* at different light levels (n = 3).

Figure 2.

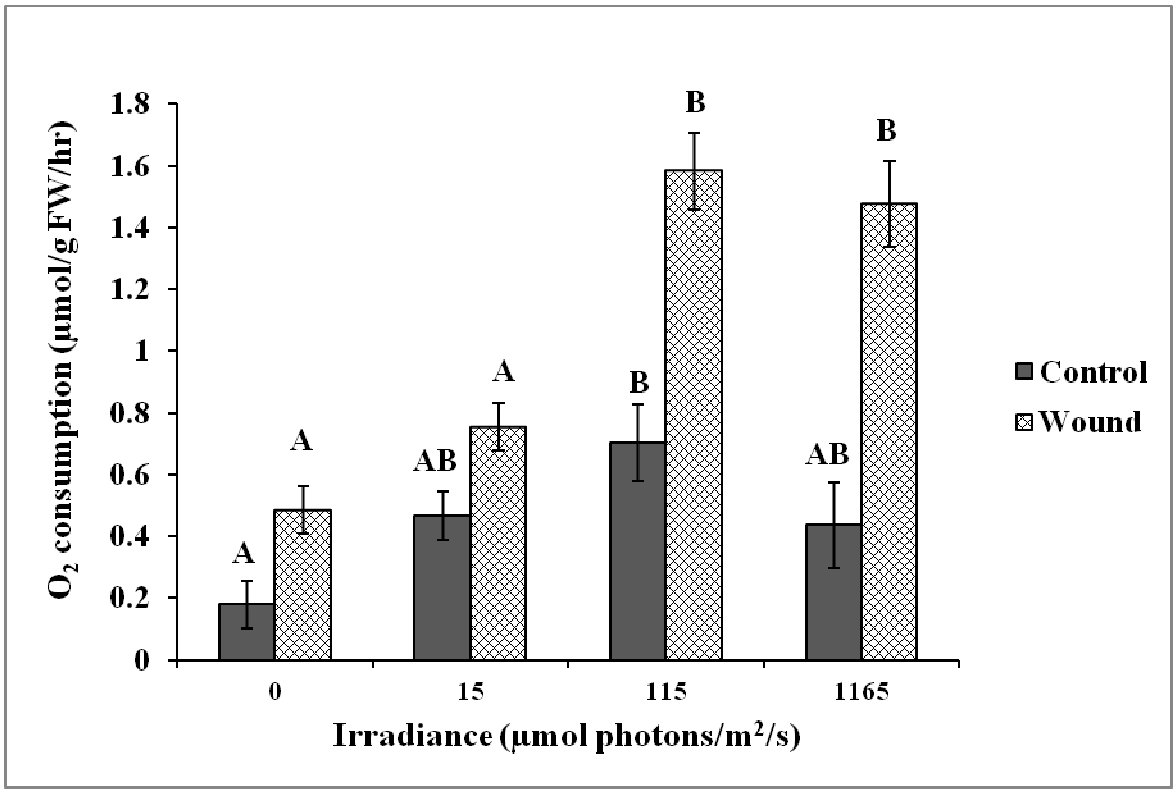

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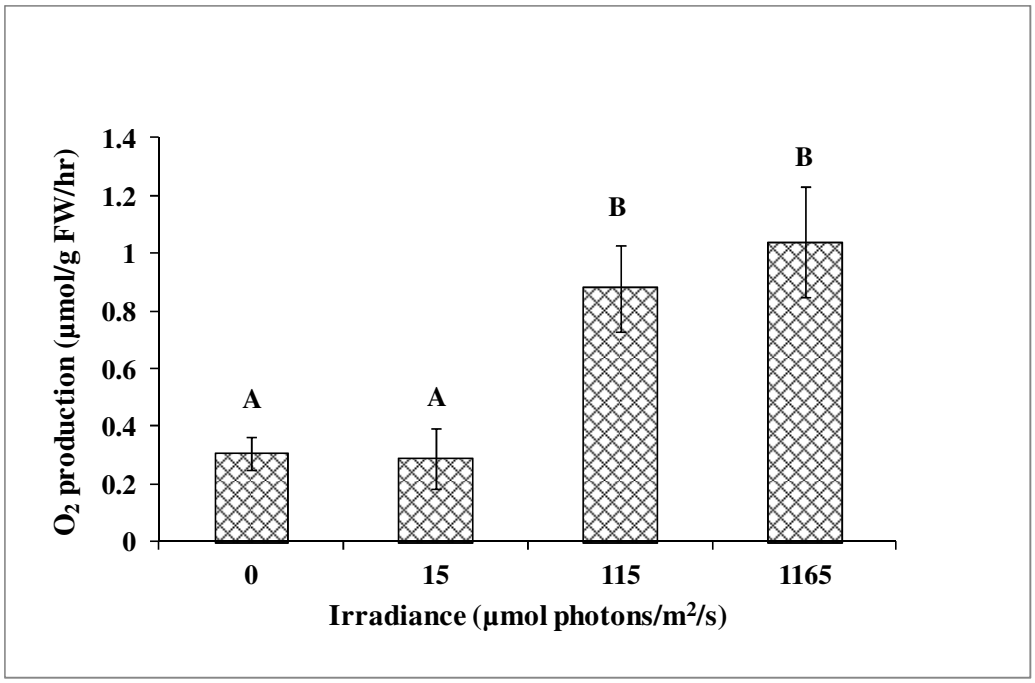
Figure 3.



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