An Ultrastructural and Histochemical Study of the Wound Response in the Coenocytic Green Alga *Caulerpa ashmeadii* (Caulerpales)

R. H. GODDARD* 1 and C. J. DAWES 2

1 Department of Botany, Louisiana State University, Baton Rouge
2 Department of Biology University of South Florida, Tampa

Received February 12, 1982
Accepted in revised form August 16, 1982

Summary

The developmental sequence of events surrounding wound healing in the coenocytic marine green alga *Caulerpa ashmeadii* was investigated. The wound plug was found to consist primarily of polysaccharide, including sulfated forms. Low levels of protein found in the wound plug were interpreted to occur from cytoplasmic leakage during the initial wounding response. An initial retraction of the vegetative cytoplasm from the wound site occurred upon wounding. A cytoplasmic band extended across the vacuole away from the wound plug 1 hour after wounding and migrated to a position adjacent to the wound plug by 2 hours. Cytological changes from 30 minutes to 6 hours resulted in a wound cytoplasm that was similar in appearance to meristematically active areas. New wall formation began at 2 hours following wounding and was complete by 48 hours after wounding.

Keywords: *Caulerpa*; Coenocytic algae; Cell wall regeneration; Siphonous algae; Wound healing.

1. Introduction

The wound healing mechanism in the coenocytic green alga *Caulerpa* is unique because it utilizes no pre-existing mechanism for septa formation, contrary to the mechanism found in *Bryopsis hypnoides*, another siphonous green alga (Burr and Evert 1971). In *Caulerpa* the wound healing mechanism prevents excessive cytoplasmic loss almost immediately by the formation of a gelatinous wound plug and effects permanent repair by new cell wall deposition at the wound site within 24 hours (Lohr 1975). Detailed experimental studies concerning the ultrastructural and chemical events of the wound response in *Caulerpa* are limited (Lohr 1975, Dawes and Goddard 1978, Dreher et al. 1978). Loehr (1975) found that the wound plug in *C. prolifera* was composed primarily of polysaccharide and not protein as found in the wound plug of *Bryopsis* (Burr and Evert 1972). Dreher et al. (1978) proposed that the wound plug in *C. simpliciuscula* was composed of a sulfated glycoprotein or a mixture of protein and sulfated polysaccharide rather than exclusively carbohydrate. The results of Dawes and Goddard (1978) demonstrated the wound plug in seven species of *Caulerpa* to be composed primarily of carbohydrate although the involvement of a glycoprotein or other proteinaceous component in the wound plug was uncertain. More recent studies suggest that the wound plug in *Caulerpa* is composed of a sulfated polysaccharide (Hawthorne et al. 1981) although the involvement of protein as an integral component of the wound plug could not be ruled out (Dreher et al. 1982). The existing evidence concerning the events surrounding the wound response in *Caulerpa* is conflicting. The purpose of the present research is to clarify the developmental and histochemical sequence of the events of the wound response in *Caulerpa ashmeadii*.
and to compare the results obtained with the results known for other members of the genus and other coenocytic green algae.

2. Materials and Methods

*Caulerpa ashmeadii* (Fig. 1) was collected at Indian Bluff Island, on the west coast of Florida (28° 6' 12" N Lat., 82° 47' 0" W Long.) growing in sea grass beds of *Thalassia testudinum* in about 1 m of water. Plants were cleaned of macrophytans, transported in seawater to the laboratory and placed in 37 l aquaria with subsand aeration filters and with four Gro-ux fluorescent bulbs on a 14:10 light: dark cycle for illumination. Unfiltered natural seawater at ambient salinity (ca. 32 ppt) and 25 ± 2.0 C temperature was used as the growth medium with no additional nutrient supplement. Plants selected for chemical analyses and histochemical studies were used immediately.

For chemical analyses, the rhizomes of the plants were wounded under water by cutting with a sharp razor blade and the wound plug was collected after 8 hours. For morphological analyses, ramuli (secondary branches of the blade) were wounded and then fixed for observation at 0, 1, 10, and 30 minutes, and 1, 2, 6, 12, 24, and 48 hours following wounding. Individual ramuli were wounded for light microscopy by severing with a sharp razor blade or by “pressure” wounding (JANSE 1906). Pressure wounds were made by pressing a dulled razor blade across the ramulus. This technique initiated a wound response without breakage of the cell wall (Fig. 2). Only pressure wounded material was used for electron microscopy because of the more localized response. Some ramuli were wounded by cutting with a sharp razor blade for electron microscopy to compare possible cytological differences with pressure wounding.

Chemical analyses of the wound plugs and whole plants were performed as described previously (Dawes and Goddard 1978) using at least 5 replicates from an homogenized sample. Statistical differences between whole plants and wound plugs (p < 0.05) were calculated using Student’s t-test.

For examination with the light microscope, a second pressure wound was made on the ramulus 30 minutes prior to fixation (Fig. 2). This wound was located proximal to the experimental wound and located about 1 cm from the junction of the ramulus with the main axis of the blade. Ramuli were severed from the plant proximally to the second pressure wound for fixation. The second wound effectively isolated the experimental wound and reduced perturbation by eliminating the loss of cytoplasm and vacuolar contents when the ramulus was severed. Specimens were fixed for 4 hours in 5% glutaraldehyde buffered with 0.1 M sodium cacodylate and 50% filtered seawater (pH 8.0). The final osmolality of the fixative vehicle was adjusted to be about 140 mOsm hypertonic to the seawater medium with NaCl. The final osmolality of the fixative vehicle was adjusted to be about 140 mOsm hypertonic to the seawater medium with NaCl. Ambient salinity (ca. 32 ppt) and 25 -- 2.0 C temperature was used as the growth medium with no additional nutrient supplement. Plants selected for chemical analyses and histochemical studies were used immediately.

3. Results

3.1. Chemical Analyses

The results of the comparative general chemical analyses for whole plants and wound plug material of *Caulerpa ashmeadii* are summarized in Table 1. The average ash level of the wound plug material was significantly higher than the ash level of the whole plant. Soluble protein and lipid levels in the wound plug were both significantly less than their respective components in the whole plant by dry weight and by organic weight. In addition, the ratio of the components determined for the wound plugs to the whole plants showed that the relative values for protein and lipid were similar. Soluble carbohydrate levels in the wound plugs were about equal to those in the whole plant on a dry weight basis. On an organic weight basis, however, the wound plugs had significantly higher levels of soluble carbohydrate. The estimate of insoluble carbohydrate in the whole plant was higher on a dry weight basis than in the wound plugs. Insoluble carbohydrate levels were about the same percentage of the organic constituents in the whole plant and the wound plug.

3.2. Histochemical Analyses

Histochemical analyses were strongly positive for polysaccharide and negative for protein in the wound plug. Undisturbed cytoplasm stained lightly with PAS reagent while the wound plug from 1 minute to 48 hours stained intensely, demonstrating the presence of polysaccharides (Fig. 3). Sulfated polysaccharide staining results with DAB and alcian blue showed similar results with only weak staining reactions in normal unwounded material. The most intensely staining regions occurred in the wound plug of specimens fixed from 1 minute to 24 hours after wounding. The wound plug traversed the central vacuole within 10 minutes after wounding as demonstrated with DAB staining.

Alcoholic mercuric bromophenol blue (MAZIA et al. 1953). Alcian dyes were used only on paraffin sections while all other stains were used on both paraffin and epoxy sections. For ultrastructural analyses, individual ramuli were wounded and fixed as previously described or fixed for 4 hours in a modified Karnovsky’s (1965) osmotically adjusted 6% glutaraldehyde-formaldehyde fixative followed by post fixation in buffered 1% osmium tetroxide for 2 hours. Samples were dehydrated gradually to 100% ethanol and transferred to propylene oxide. The tissue was then infiltrated and embedded in Spur’s (1969) low viscosity embedding medium or in an Epon-Araldite mixture (Mollenhauer 1963). Thin sections were cut with glass knives.

**Table 1.** Summary of the comparative general chemical analyses for whole plants and wound plug material of *Caulerpa ashmeadii*. The average ash level of the wound plug material was significantly higher than the ash level of the whole plant. Soluble protein and lipid levels in the wound plug were both significantly less than their respective components in the whole plant by dry weight and by organic weight. In addition, the ratio of the components determined for the wound plugs to the whole plants showed that the relative values for protein and lipid were similar. Soluble carbohydrate levels in the wound plugs were about equal to those in the whole plant on a dry weight basis. On an organic weight basis, however, the wound plugs had significantly higher levels of soluble carbohydrate. The estimate of insoluble carbohydrate in the whole plant was higher on a dry weight basis than in the wound plugs. Insoluble carbohydrate levels were about the same percentage of the organic constituents in the whole plant and the wound plug.