First publ. in: Principles and Practices in Plant Ecology / ed. by Inderjit ...
Boca Raton, Fla., CRC Press, 1999, pp. 179-199

Allelopathy in Benthic and Littoral Areas: Case Studies on Allelochemicals from Benthic Cyanobacteria and Submersed Macrophytes

Elisabeth M. Gross

CONTENTS

12.1 ABSTRACT

Photosynthetic organisms in littoral and benthic habitats are in general situated in close proximity to their competitors. Benthic algae and cyanobacteria compete for space and grow adjacent to each

other. Submersed macrophytes can be overgrown by epiphytic algae and are surrounded by other macrophytes and phytoplankton. The particular spatial setup in these habitats makes allelopathy a powerful strategy. Released compounds can more or less directly reach and act on target organisms. Allelopathic compounds may either be released into the water or transferred by direct cell–cell contact from donor to recipient.

This chapter is not intended as a review on allelopathy in benthic and littoral areas. Two case studies of allelopathic interactions are discussed: 1) photosynthesis inhibitors in the benthic cyanobacteria *Fischerella muscicola* and *F. ambigua*, and 2) algicidal hydrolyzable polyphenols from the submersed macrophyte *Myriophyllum spicatum.*

12.2 INTRODUCTION

Benthic cyanobacteria and submersed macrophytes face a similar challenge; they both have to compete for light and, therefore, for space with other photosynthetically active organisms. Direct competition for nutrients is generally less pronounced between submersed macrophytes and algae. Macrophytes obtain their major nutrients from the sediment, whereas algae obtain nutrients from the open water (Carignan and Kalff, 1980, 1982; Phillips et al., 1978). Nutrient competition can play a role in cyanobacterial mats (Fong et al., 1993). In both the benthic and the littoral habitat, the spatial distance between organisms is relatively small compared with the situation in the open water. Nevertheless, both systems have specific characteristics that influence the interaction with other competing primary producers. In the benthic system, cyanobacterial mats often consist of only one species. Light and oxygen gradients are very steep (Revsbech et al., 1989). Near-damaging light intensities at the surface of such mats are only millimeters away from zones of light limitation lower in the cyanobacterial mat, generating a highly stratified environment. Potential hazards arise from other cyanobacteria that would overgrow the mat and deprive it of sufficient light. Possible counterbalancing strategies would be to grow faster or to release growth inhibitors. Cell–cell contact between competing species is highly possible, thus facilitating the direct transfer of allelochemicals.

In the littoral system, thick epiphyte cover, scums of filamentous algae, and high phytoplankton densities can deprive submersed macrophytes of sufficient light. In addition, with increasing depth, light intensity decreases and the light spectrum changes. Counterbalancing strategies used by submersed macrophytes include the formation of finely dissected leaves, low light and CO_2-compensation points, fast growth, canopy building (Wetzel, 1983), and the excretion of algal growth inhibitors (Wium-Andersen, 1987; Gopal and Goel, 1993). Epiphytes covering submersed macrophytes would be directly subjected to allelochemicals released by the host macrophyte. But even phytoplankton in littoral zones can be affected since the water exchange in dense macrophyte beds is very limited (Losee and Wetzel, 1993). Because the competing species in both systems are in close physical proximity, potential allelochemicals can act more efficiently and are not diluted as in the open water. Therefore, allelopathy in these environments would be a forceful strategy.

Since Molisch (1937) coined the term, allelopathy has been used often in conflicting ways. Molisch (1937) used allelopathy only to describe biochemical interactions among higher plants and between higher plants and microorganisms. However, other authors use the term allelopathy for biochemical interactions between plants and animals and among animals (Seitz, 1984; Rizvi and Rizvi, 1992). Whittaker and Feeny (1971) proposed the use of *allelochemics.* They wrote: "We review here a class of interactions termed *allelochemic* (. . .) involving chemicals by which organisms of one species affect the growth, health, behavior, or population biology of organisms of another species (excluding substances used only as foods by the second species)." Thus, the term *allelopathy* should only be used for biochemical interactions among plants and between plants and microorganisms. *Allelochemistry* would be the overall concept and would include biochemical interactions of any two different species, including animals. The same plant-derived compound may affect other primary producers as well as animals, so that we should use the precise terms to describe each specific interaction.

The original definition of allelopathy by Molisch (1937) includes both stimulatory and inhibitory actions. However, the majority of allelopathic studies focuses on negative effects exerted by one species upon another. This is reflected in a too restrictive translation of the term allelopathy. Allelopathy is derived from the Greek words *allelon* meaning 'one another,' 'mutually,' or 'in turn' and *pathos* meaning 'suffering,' but also 'accident,' 'experience,' 'feeling.' Most definitions of allelopathy only use the translation 'suffering' for *pathos. Pathos,* as used for example in "sympathetic" or "pathetic" clearly translates not as 'suffering' but rather as 'feeling' or 'sensitive.' Rice (1974) concentrated exclusively on inhibitory allelopathic interactions in his first volume, but corrected this perception in the second volume (1984). It is now commonly accepted that certain allelochemicals may stimulate target organisms at very low concentrations but inhibit at higher concentrations (Rice, 1984).

All proposed new definitions for allelopathic interactions (see Willis, 1994) do not seem to improve the original meaning but only to cause more confusion. Staying closer to the original definition by Molisch (1937) will allow most of the processes observed in this field to be covered (see also discussion in Rice, 1974, 1984).

Whereas allelopathy still seems to be a mechanism that has not received its appropriate attention, more and more scientists show interest in chemical ecology. In the field of limnology this is reflected by recent reviews on planktonic (Larsson and Dodson, 1993) and benthic organisms (Dodson et al., 1993). Chemical ecology tries to close the gap between natural product chemistry and ecology. In the past, natural product chemists would often describe new compounds from (aquatic) organisms without paying attention to their ecological function. On the other side, ecologist and limnologists investigate chemical cues responsible for observed patterns without knowing about the molecular structure of the compounds involved. The increased use of analytical techniques in ecology and limnology and more frequent cooperations of ecologists and natural product chemists have greatly improved our understanding of allelochemicals.

Allelochemicals found in cyanobacteria and macrophytes are mainly secondary metabolites. The wide array of secondary metabolites in cyanobacteria attracts natural product chemists because many of these compounds show pharmacological activity. Few of these studies cover ecological aspects of these compounds. Still, we can hypothesize that these compounds are biosynthesized not for pharmaceutical reasons but for a purpose beneficial to the producing organism. Every year, new compounds and even new classes of compounds are elucidated from cyanobacteria (Patterson et al., 1994; Borowitzka, 1995). The situation is totally different for macrophytes. Submersed macrophytes are thought to be inferior in secondary metabolism (McClure, 1970) compared with emerged macrophytes or even terrestrial vegetation. Macrophytes similar to other angiosperms will most likely produce well-known compounds or derivatives of known compounds as allelochemicals. The most recent review on macrophyte secondary metabolites can be found in the volumes of "Chemotaxonomie der Pflanzen" (translation, Chemotaxonomy of Plants) (Hegnauer, 1962–1995). Only a couple of other surveys cover this field (Bate-Smith, 1962; McClure, 1970; Su and Staba, 1973; Hutchinson, 1975; Ostrofsky and Zettler, 1986; Pip, 1992). However, without more convincing data the conclusion that (submersed) macrophytes are less active producers of secondary metabolites seems premature. The increasing number of publications (Della Greca et al., 1995; Yoshikawa et al., 1993) rather indicates that this has been due to a lack of research in this field.

Two examples of allelochemical interactions will be presented here that focus on the benthic cyanobacterium *Fischerella* and the submersed macrophyte *Myriophyllum.* Both studies involve a threefold approach: 1) to isolate and identify the major allelochemical(s), 2) to study possible release mechanisms for the allelochemicals in order to evaluate how they reach target organisms, and 3) to reveal the mode of action of the active compounds to its target organism. Identifying the active compounds present in the producing organisms is essential for investigating whether or not this compound is released into the environment. Controlled studies can provide insight into mechanisms ruling allelopathy. Both studies would have been impossible without axenic cultures. Non-axenic

cultures always have accompanying organisms that interfere with the identification of released compounds. It is nearly impossible to separate the organic compounds excreted by accompanying organisms from those of the allelopathically active organism. Additionally, the accompanying organisms can metabolize allelochemicals and change their original structure. In both cases presented here, the structure of the algicides hints at the possible mode of action, or *vice versa*. Overall, this threefold approach covers a wide range of possible interactions between producer and target organism and permits the evaluation of ecological implications, even when substantial methodical problems prevent field studies.

12.3 LIPOPHILIC ALLELOCHEMICALS FROM BENTHIC CYANOBACTERIA: PHOTOSYNTHETIC INHIBITORS FROM *FISCHERELLA*

Allelopathic interactions of algae, although not referred to as such, were observed as early as 1917 by Harder. He found that aging cultures of the cyanobacterium *Nostoc* showed a decreased growth-rate and explained this by the accumulation of autotoxic organic compounds in the culture medium. Biochemical interactions as a cause for phytoplankton succession were first proposed by Akehurst (1931). Further information was gained with cultures of algae and cyanobacteria by Rice, 1954; Proctor, 1957; Pratt et al., 1944; Pratt, 1966; and Harris, 1971. None of these studies allowed an estimation of the importance of allelopathy under natural conditions. Reviews on algal allelopathy can be found in articles by Rice (1984), and Inderjit and Dakshini (1994). The most convincing experiments for the involvement of allelopathy in phytoplankton succession were conducted by Keating (1977, 1978). Two thirds of her mostly axenic algal isolates from Linslay Pond exhibited allelopathic activity. The direction of the allelopathic effects followed the natural phytoplankton succession: the culture filtrate of one algae or a certain cyanobacterium showed neutral or inhibitory activity toward the predecessor but neutral or stimulatory activity towards the following algae.

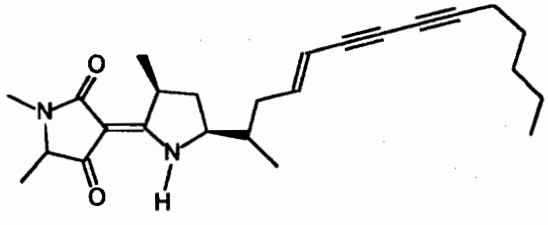
Allelochemicals released by planktonic algae and cyanobacteria must be effective at very low concentrations considering high dilution effects. For this reason, Lewis (1986) questioned if allelopathy is an important factor in phytoplankton succession, since individual cells or colonies are separated from each other by large distances—dozens to hundreds of cell diameters—even in dense populations. However, in benthic habitats allelopathy should be a powerful competitive strategy because of the proximity of adjacent species. In order to avoid light limitation, benthic cyanobacteria have to compete for space with other cyanobacteria and benthic algae. Cyanobacterial mats consist very often of only one species, suggesting that this species has out-competed others, either by fast growth or due to allelopathically active compounds. Strong evidence for the involvement of allelochemistry in this habitat is provided by the fact that most of the yet chemically characterized allelochemicals from cyanobacteria have been isolated from benthic species. For example, *Scytonema hoffmanni* produces the low molecular lipophilic compound cyanobacterin (Pignatello et al., 1983) that inhibits photosynthetic electron flow in other algae (Gleason and Paulson, 1984), but also in chloroplasts isolated from angiosperms (Gleason and Case, 1986). Algicidal indole-derivatives have been isolated from *Hapalosiphon fontinalis* (Moore et al., 1984).

Fischerella muscicola turned out to be the most active species in a screening of 65 filamentous, nitrogen-fixing cyanobacteria for the production of cyanophages or cyanobactericidal compounds (Flores and Wolk, 1986). None of the investigated strains contained cyanophages but seven produced cyanobactericidal compounds. Our studies used a combined effort to isolate and identify the main allelochemicals from *Fischerella* and consequently study possible release mechanisms and the mode of action (Gross et al., 1991, 1994; Papke et al., 1997). The following sections focus especially on particular features of the *Fischerella* allelochemicals: 1) the structure elucidation, 2) the transfer to target organisms, 3) their herbicidal action, and 4) environmental factors influencing their production.

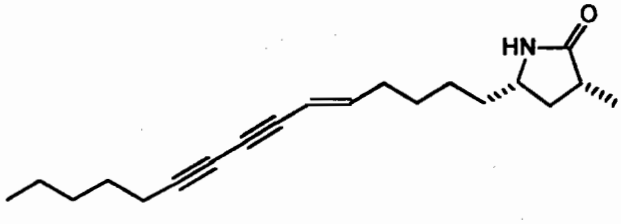
12.3.1 Structure Elucidation of *Fischerella* Allelochemicals

Fischerella muscicola and *F. ambigua* produce allelopathically active compounds against other cyanobacteria, chlorophytes, and diatoms. The allelopathic activity mainly is based on one major allelochemical; some minor, less active compounds are also involved (Gross et al., 1991; Papke et al., 1997). The major allelochemical is lipophilic, protease-insensitive, and of low molecular weight (Gross et al., 1991). The isolation of this compound, which we named fischerellin (Gross et al., 1991) was guided by a special agar diffusion assay (Flores and Wolk, 1986; Gross et al., 1991). C18-HPLC and chemical derivatizations were used to isolate and characterize this allelochemical (Gross, 1990; Gross et al., 1991). The structure of the main allelochemical, now renamed more precisely fischerellin A (Figure 12.1), has been elucidated recently (Hagmann and Jüttner, 1996). The isolation yield is reported with approximately 0.005 percent of the dry weight.

Photodiode-array analysis of crude extracts from *F. muscicola* and *F. ambigua* show at least three to four compounds with ultraviolet (UV)-spectra very similar to fischerellin A (maxima at 240, 252, 267, and 283 nm). We isolated and identified one of these compounds (Papke et al., 1997) and named it fischerellin B. Fischerellin B inhibits cyanobacteria in the agar diffusion assay (Papke et al., 1997) and inhibits photosynthesis of *Trichormus variabilis* by 40 percent at a concentration of 10 μM (Gross, unpublished results). Purified fischerellin B (0.55 mg) was obtained from 70 g of freeze-dried cyanobacteria, giving a yield of less than 0.0008 percent of the dry weight. The structure was elucidated by UV-, NMR-, and mass-spectroscopy. In this case, a derivatization of the natural product and stereocontrolled synthesis of the derivative allowed the determination of the absolute configuration by means of chiral gas-chromatography. The compound was identified as (3*R*,5*S*)-3-methyl-5-((5*E*)-pentadec-5-ene-7,9-diynyl)-pyrrolidin-2-one (Figure 12.1). To our knowledge, fischerellins A and B are the first fully characterized enediyne metabolites from cyanobacteria (Hagmann and Jüttner, 1996; Papke et al., 1997).



a) Fischerellin A from *Fischerella muscicola* (Hagmann and Jüttner, 1996)



b) Fischerellin B from *Fischerella ambigua* and *F. muscicola* (Papke et al., 1997)

FIGURE 12.1 Structures of fischerellins A and B from *Fischerella muscicola* and *F. ambigua*.

These two enediyne allelochemicals seem also to be the first cyanobacterial metabolites in which the ecological function was described before their potential pharmaceutical value. Fischerellins A and B contain an enediyne sidechain; other compounds with similar structural features are used as antibiotic and anticancer drugs (Nicolaou and Dai, 1991; Smith and Nicolaou, 1996). Furthermore, structural features of the N-containing heterocyclus of fischerellins A and B indicate their activity as potent inhibitors of photosystem II electron transport (see Section 12.3.3).

12.3.2 Transfer of Allelochemicals in Benthic Cyanobacteria

How do *Fischerella* allelochemicals come in contact with target algae and cyanobacteria? Intact cell filaments of *Fischerella* cause clearing areas in the agar diffusion assay; thus, it is obvious that somehow the allelochemical reaches other cyanobacteria and algae. Consequently, we looked for fischerellin A in the culture medium. The *Fischerella* culture medium was subjected to solid phase extraction (SPE) with C18-cartridges. The C18-adsorbent would trap lipophilic compounds released into the mineral medium. But the HPLC separation of the SPE-eluate did not exhibit any trace of fischerellin A (Gross, 1990). Furthermore, this fraction did not show allelopathic activity in our bioassay. In these experiments we found no evidence for an inhibition of indicator cells when undiluted, cell-free culture supernatant fluids from *Fischerella musicola* UTEX 1829 were used, as has been reported in the original work by Flores and Wolk (1986). The authors do not report how dense the producing strain was grown to cause such an effect. We rather hypothesize that the active compound(s) are transmitted by direct contact of cells. For this purpose the lipophilic nature and low molecular weight of fischerellins A and B would be very suitable. Lipophilic compounds of low molecular weight (MW $<$ 600 Da) can easily pass cell membranes.

To mimic direct cell contact as it would happen in adjacently growing cyanobacteria, we used XAD beads in coculture with a *Fischerella* culture (Gross et al., 1994). The XAD-16 beads had a mean diameter of 1.6 mm and a lipophilic surface. All cyanobacteria adherent to cell surfaces, benthic as well as epilithic and symbiotic organisms, have a hydrophobic cell surface; in contrast, all planktonic cyanobacteria have a cell surface that is highly hydrophilic (Shilo, 1989). The experiments were run for one or six weeks. Fischerellin A could be detected in the methanolic extract of the XAD-beads but not in the SPE-eluate of the culture medium. The allelochemical must have bound to the surface of the XAD beads. This proved our hypothesis that *Fischerella* allelochemicals are not released into the culture medium but directly transferred to target organisms by cell–cell contact.

Further support for this hypothesis comes from the work by Flores and Wolk (1986). When producing cells were placed on a piece of dialysis membrane and actively grown for three to four days, the inhibitor diffused through the dialysis membrane and into the agar. After the membrane and cells were removed, the agar was overlaid with indicator cells in soft agar. A clearing zone could be observed where the *Fischerella* allelochemicals had passed the dialysis membrane. However, this test does not prove that fischerellins or other allelochemicals from *Fischerella* can pass dialysis membranes if they are placed into culture medium.

12.3.3 Fischerellins, Biological Herbicides

Fischerella cells and isolated fischerellins coming in close contact with other cyanobacteria and algae lead to cell death in these organisms indicating that vital functions in these photosynthetic organisms are interrupted. In contrast, various Eubacteria are not inhibited by *Fischerella* or isolated fischerellin (Gross et al., 1991). The respiratory electron transport of the chlorophyte *Nannochloris* is not affected by the addition of fischerellin A, but the photosynthetic electron transport is severely inhibited. Further studies with the filamentous cyanobacterium *Trichormus (Anabaena) variabilis* ATCC 29413, strain P-9 (for renaming see Komarek and Anagnostidis, 1989) elucidated photosystem II (PS II) as the target site of fischerellin A, similar to many synthetic herbicides such as atrazine

or DCMU. Structural features of the fischerellins meet those of urea and triazine herbicides. All members of the serine family of herbicides contain a lipophilic group in close association with an sp^2 hybrid and an essential positive charge (Trebst et al., 1984). Further experiments are warranted to reveal the exact mode of action of fischerellins on PS II. Photosynthesis in *Fischerella muscicola* itself was not affected by fischerellin A (Gross, 1990; Gross et al., 1991). It is not clear yet how *Fischerella* protects itself from the deleterious effect of fischerellins. Since neither whole cells nor cell fragments, which were obtained by french-press treatment, responded to the addition of fischerellin A, a structural rather than a metabolic protection is likely.

Inhibition of photosynthesis seems to be a widespread mode of action for aquatic allelochemicals, especially from benthic cyanobacteria. The cyclic sulfur compounds dithiane and trithiane from *Chara* sp. (Wium-Anderson et al., 1982) inhibit photosynthesis of diatoms and samples of natural phytoplankton; the target site of these biological herbicides is not given. Similar to fischerellin A, cyanobacterin from *Scytonema hofmanni* (Gleason and Paulson, 1984) and an allelochemical from *Oscillatoria* (Bagchi, 1995) inhibit PS II electron transport. All these cyanobacterial PS II inhibitors are lipophilic and of low molecular weight. It is striking that all these different species produce chemically different compounds with the same biological activity. Since light is the most important factor for the survival of benthic cyanobacteria, inhibition of photosynthesis of competing species would be especially effective. The widespread use of this defense strategy indicates a convergent evolution of this pattern among benthic cyanobacteria.

12.3.4 Environmental Factors Affecting the Production of Allelochemicals in *Fischerella*

Allelochemicals should be especially effective and useful to the producing organism if certain essential resources are limiting. On the other hand, the biosynthesis of allelochemicals certainly involves metabolic costs, so that under limiting conditions, their production could be decreased. Examples from literature provide evidence for both cases. The content of secondary metabolites in cyanobacteria depends on the culture conditions, but is generally highest under nitrogen (N) or phosphorus (P) limitation (Moore et al., 1988). The content of the cyclic didepsipeptide cryptophycin from the cyanobacterium *Nostoc* ATCC 53789 increases under P-limitation (Schwartz et al., 1990). The same response happens with the intracellular toxin content in the dinoflagellate *Prymnesium parvum*, but toxin content stays constant during N-, thiamin, and vitamin B_{12} limitation (Shilo, 1971). P-limitation also increases toxin content in the dinoflagellate *Protogonyaulax tamarensis,* but in this case toxin levels decrease under N-limitation (Boyer et al., 1987). *Trichormus doliolum* excretes 30 times more of an allelochemical under P-limitation than in full strength medium (von Elert, 1994; von Elert and Jüttner, 1997). Since nitrogen, phosphorus, and light limitation may affect the production of allelochemicals, their impact on fischerellin production in *Fischerella muscicola* was investigated (Gross et al., 1994).

Phosphorus is often the most limiting resource for freshwater cyanobacteria (Schindler, 1977; Sommer, 1989). Assuming that the release of allelochemicals is of competitive advantage to the producing organism, this should be especially effective during peak population growth in summer when phosphorus is limiting. But P-limitation (1 μM instead of 40 μM $P\text{-}PO_4$) of *F. muscicola* did not change the content of fischerellin A per unit biomass. P-limited cultures reached only 40 percent of the biomass of control cultures and had 60 percent less chlorophyll content per unit biomass. This indicates that *Fischerella* invests the same amount of energy per unit biomass for the production of fischerellin, irrespective of P-availability.

Fischerella is a nitrogen fixing species, so nitrogen deprivation should not necessarily influence the production of fischerellins. Nevertheless, nitrogen depletion (1/100 of normal $N\text{-}NO_3$ supply, 10 μM instead of 1 M) significantly decreased fischerellin content by 75 percent. In the planktonic environment, this pattern would not be a disadvantage for *Fischerella*, since chlorophytes, diatoms, and

other algae are not capable of N-fixation and would be already inhibited by N-depletion. But in benthic areas, the main competitors are other N-fixing cyanobacteria. Cyanobacteria are adapted to low light conditions. Since competition for light is a major factor for benthic cyanobacteria, it would be advantageous for *Fischerella* to maintain or even increase the production of fischerellin A under low light conditions. However, light limitation (10 compared with 60 μmol photons PAR m^{-2} sec^{-1} under normal light conditions) led to a significant decrease of fischerellin A production by more than 90 percent. It appears that both nitrogen and light depletion cause an energy shortage in the cyanobacterial cells, which then are no longer capable of synthesizing the same amount of this allelochemical. N-fixation is very costly in terms of ATP for cyanobacteria. Furthermore, fischerellin is an N-containing metabolite, and under nitrogen shortage, N rather might be used for protein biosynthesis. Light shortage reduces the ATP yield gained by photophosphorylation. These results provide evidence for the metabolic or energetic costs of the production of such allelochemicals. However, considering the high algicidal activity of fischerellins, even light- and nitrogen-depleted cells may produce sufficient quantities to inhibit other competing species.

12.4 ALLELOPATHY AND INTERFERENCE IN SUBMERSED MACROPHYTES: INSIGHTS FROM *MYRIOPHYLLUM SPICATUM*

Shallow eutrophic lakes can have two alternative equilibria, either turbid, dominated by algal blooms or clear with a dominance of submersed macrophytes (Phillips et al., 1978; Blindow et al., 1993; Scheffer et al., 1993). Shifts between one state and the other occur rather quickly without prolonged intermittent phases. Observations in shallow eutrophic lakes show that a dense cover of submersed macrophytes can keep the water clear despite high enough nutrient concentrations to support phytoplankton growth (Blindow et al., 1992; Ozimek et al., 1993; Scheffer et al., 1993). Many ecological mechanisms are probably involved in this process. The release of allelochemicals by submersed macrophytes to suppress algal growth seems to be one strategy (Phillips et al., 1978; Wium-Anderson, 1987; Scheffer et al., 1993). Further evidence for allelopathic interactions of macrophytes comes from observations of monospecific stands of some macrophytes (*Chara*: Wium-Anderson et al., 1982; *Myriophyllum spicatum*: Grace and Wetzel, 1978; Smith and Barko, 1990) and low epiphyte densities on certain macrophytes (*Chara*: Wium-Anderson et al., 1982; *Ceratophyllum demersum*: Gough and Woelkerling, 1976). A certain degree of host-specificity of epiphytes (Gough and Woelkerling, 1976; Eminson and Moss, 1980; Burkholder and Wetzel, 1990) indicates biochemical interactions between epiphytes and their macrophyte.

Proving allelopathy in aquatic systems under field conditions is very difficult, therefore, most studies on macrophyte allelopathy have been performed under controlled laboratory conditions. This may be considered a drawback. However, this work with *Myriophyllum* shows that laboratory studies can provide insights in ecological mechanisms that would be nearly impossible to obtain from field observations alone. This section focuses on five issues that arose from work on allelopathy of submersed macrophytes, especially members of the Haloragaceae as follows:

1. A high percentage of submersed macrophytes from northern German lakes produce allelochemicals. Although the focus will be on inhibitory biochemical interactions, possible stimulatory allelopathic relationships will be discussed.
2. Phenolic compounds, especially hydrolyzable polyphenols, appear to be common allelochemicals in the family of the Haloragaceae. Such allelochemicals may have certain advantages for aquatic plants with respect to concentration, solubility in the aquatic medium, and cost of biosynthesis and turn-over.

3. Algicidal hydrolyzable polyphenols released by ***Myriophyllum*** will end up in the large pool of humic-like compounds in lake water. Possible release mechanisms, metabolic features, and effects on target organisms will be given.
4. Structure-activity relationships define the main algicidal hydrolyzable polyphenol from *M. spicatum* not only as a very potent inhibitor of algal exoenzymes, but also of other modes of action.
5. According to the original definition, allelopathy does not imply competition (Molisch 1937, Rice 1984). The hypothesis will be tested that interference, meaning coupled allelopathic and competitive interactions (Muller, 1969), may be an effective strategy for some submersed macrophytes.

12.4.1 Widespread Occurrence of Allelopathic Interactions in Submersed Macrophytes

In agreement with Molisch's (1937) definition of allelopathy, both stimulatory and inhibitory biochemical interactions will be considered. However, negative effects may be predominant because submersed macrophytes face a severe competition especially for light with epiphytes and phytoplankton, and also with other macrophytes for space and nutrients. Inhibitory allelochemicals seem to be rather broad in their specificity toward certain target algae or cyanobacteria (Gross et al., 1991; Gross, 1995). This might be of advantage for the producing organism in order to affect most epiphytes and phytoplankton with only one or a few inhibitors. On the other side, stimulating allelopathic interactions should be very specific. Positive effects on all algae and excessive epiphyte or phytoplankton growth could easily threaten the survival of the macrophyte. It might be this difference in specificity of action that has biased research on allelopathy toward inhibitory interactions, overlooking stimulatory effects.

12.4.1.1 Stimulatory Allelopathic Interactions

Observations on freshwater (Wetzel, 1983) and marine macrophytes (Harlin, 1973) indicate that they rely in part on organic compounds obtained from accompanying microorganisms and algae. Some macrophytes may no longer be capable of a sufficient synthesis of certain vitamins, phytohormones, or enzymes (Godmaire and Nalewajko, 1989) and, therefore, obtain those, perhaps in exchange for extracellular organic compounds (EOC), from bacteria or microalgae. A couple of studies with axenic cultures of macrophytes and algae provide further evidence for stimulatory biochemical interactions. It seems that macrophytes are not at all a neutral substrate for epiphytes (Cattaneo and Amireault, 1992).

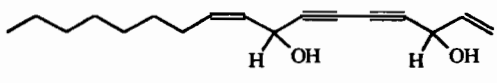
Axenic cultures of macrophytes grow better when vitamins were added to the culture medium (Vitamin B_{12} to *Chara*: Wetzel and McGregor, 1968; Thiamin-HCl to *Myriophyllum spicatum*: Gross, 1995). In carbonate-poor culture medium, some macrophytes, capable of using bicarbonate (Grace and Wetzel, 1978; Wetzel, 1983), grow better when bacteria are present compared with axenic cultures (*Potamogeton pectinatus*: Ailstock et al., 1991; *Myriophyllum spicatum*: Godmaire and Nalewajko, 1989; Gross, 1995). It might be easier for those macrophytes to use respiratory CO_2 from the bacteria than to rely on their own carboanhydrase activity. Such biochemical interactions between submersed macrophytes and their epiphytes can be described as stimulatory allelopathic relationships. No final conclusions can be drawn at this point since vigorous studies on isolated stimulatory allelochemicals are still unavailable. Some studies (Gough and Woelkerling, 1976; Eminson and Moss, 1980; Burkholder and Wetzel, 1990) indicate a certain host-specificity of epiphytes on macrophytes, suggesting the involvment of stimulatory allelopathic interactions. There are indications that certain epiphytes are only found on certain submersed macrophyte species; but no comprehensive study has been published thus far.

12.4.1.2 Inhibitory Allelopathic Interactions

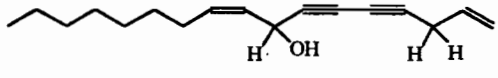
The allelopathic potential of submersed macrophytes seems to be high, as described above. This is reflected in the reviews by Wium-Andersen (1987), Gopal and Goel (1993), and Elakovich and Wooten (1995). The identified allelochemicals belong to rather different chemical classes such as sulfur compounds, polyacetylenes, and oxygenated fatty acids (see Figure 12.2). A screening of 17 different, mainly submersed, macrophytes of northern German lakes revealed that 8 species show a significant cyanobactericidal and algicidal activity of the crude methanolic extract (Gross, 1995). Very active macrophyte species are *Ceratophyllum* sp., *Myriophyllum* sp., and *Hottonia palustris* as well as the aquatic moss *Fontinalis antipyretica*. No published data exist thus far on the allelopathic activity of *Fontinalis* and *Hottonia*, but allelopathy has been described for *Ceratophyllum demersum* (Kogan and Chinnova, 1972; Wium-Anderson et al., 1983), *Myriophyllum spicatum* (Fitzgerald, 1969; Planas et al., 1981; Agami and Waisel, 1985), and *M. verticillatum* (Aliotta et al., 1992). Wium-Anderson et al. (1983) postulated elemental sulfur as the active allelochemical in

Trithiane Dithiane

a) Algicidal cyclic sulfur compounds from *Chara globularis* (Wium-Andersen et al., 1982)

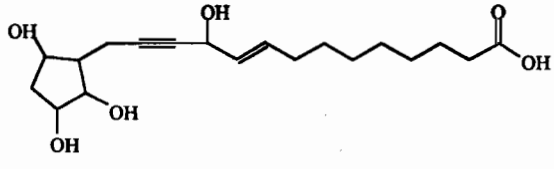


Falcarindiol



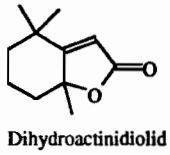
Falcarindol

b) Algicidal polyacetylenes from *Berula erecta (Sium erectum)* (Wium-Andersen et al., 1987)



Trihydroxycyclopentenyl-fatty acid

c) Algicidal fatty acid derivative from *Eleocharis microcarpa* (van Aller et al., 1983)



Dihydroactinidiolid

d) Allelochemical against macrophytes from *Eleocharis coloradensis* (Stevens and Merril, 1980)

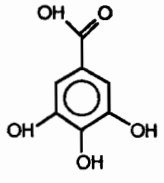
FIGURE 12.2 Structures of allelochemicals in macrophytes.

Ceratophyllum demersum. Gas-chromatography/Mass spectroscopy (GC/MS) studies of volatile organic compounds released into the incubation water by *C. demersum* indicate that low molecular weight sulfur compounds might be responsible for the release of algicidal elemental sulfur (Gross, 1995). *C. demersum* also seems to have some allelopthic activity against other macrophytes (Elakovich and Wooten, 1995). Two further species of the Haloragaceae from different locations also exhibited a strong algicidal activity, namely *Myriophyllum heterophyllum*, a neophyte from North America that invaded a small lake in western Germany (Heider Bergsee) and *Proserpinaca palustris* from the Talladega wetland, Alabama, USA. This screening shows that there is an overall high probability of finding inhibitory allelopathic activity of submersed macrophytes.

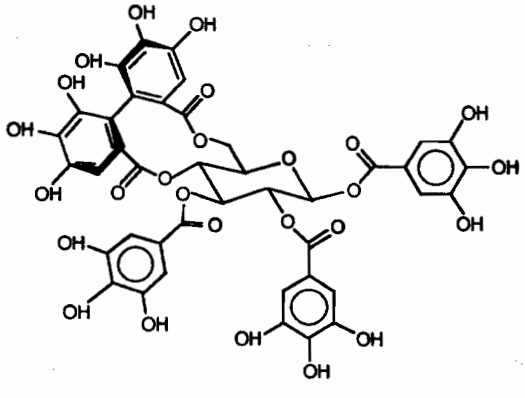
12.4.2 Hydrolyzable Polyphenols: A New Class of Allelochemicals in Haloragaceae

Hydrolyzable polyphenols (synonymous for tannins) are known as herbivore deterrents or as antibiotics (Haslam, 1989). Only recently it became obvious that they also have potent algicidal properties (Saito et al., 1989; Aliotta et al., 1992; Gross et al., 1996). Phenolic compounds and especially hydrolyzable polyphenols are well suited as allelochemicals in the littoral zone, since their molecular structure has lipophilic and hydrophilic sites, allowing both attachment to plant surfaces and dissolution in water.

A simple assay can reveal the presence of phenolic allelochemicals. Treating a crude extract of any allelochemical-producing macrophyte with insoluble polyvinylpyrrolidone (PVP) removes over 95 percent of the phenolic compounds (Loomis and Battaile, 1966; Gross et al., 1996). Phenolic allelochemicals are presumed responsible for the allelopathic activity when an extract is active prior to PVP-treatment and is inactive afterward. Most of the crude extracts of macrophytes used in the above-mentioned screening were subjected to the PVP-assay. *Fontinalis antipyretica* as well as all members of the Haloragaceae produce phenolic compounds as allelochemicals. For more precise information on the structure of the phenolic compounds, enzymatic and acid hydrolysis of all



gallic acid



Tellimagrandin II

FIGURE 12.3 Structure of the *Myriophyllum spicatum* allelochemical Tellimagrandin II and its precursor gallic acid.

haloragacean crude extracts were performed. Both tests revealed that all haloragacean species produce hydrolyzable polyphenols since the enzymatic as well as the acid hydrolysis showed the presence of gallic and ellagic acid (Gross, 1995; Gross et al., 1996). All members of the Haloragaceae contain polyphenols, according to a literature survey (Mole, 1993). Tellimagrandin II (Figure 12.3) is the main allelochemical in *Myriophyllum spicatum* (Gross et al., 1996). None of the other haloragacean species produces this allelochemical. Their biological activity is based on other, yet unidentified, hydrolyzable polyphenols.

The polyphenolic content in all haloragacean species, measured with the Folin-Ciocalteau assay (Box, 1983; Gross et al., 1996), ranges between 6 and 15 percent of the dry weight, which is much higher than in other submersed macrophytes (less than 1 percent of the dry weight; Gross, unpublished results). Other macrophytes producing hydrolyzable polyphenols are *Trapa japonica* (Nonaka et al., 1981), *Trapa bicornis* (Yoshida et al., 1989), *Nuphar japonica* (Ishimatsu et al., 1989), *Nuphar variegatum* (Nishizawa et al., 1990), and *Nymphaea tetragona* (Kurihara et al., 1993). All of these species have floating leaves; nothing is known about algicidal activities of their polyphenols. However, *Trapa bicornis* contains tellimagrandin I and II (Yoshida et al., 1989). A thorough screening of (submersed) macrophytes for the production of (poly)phenolic allelochemicals could provide valuable information.

Contents of polyphenols in angiosperms are generally higher than other secondary metabolites, such as alkaloids, terpenoids, and cyanogenic compounds. Nonphenolic macrophyte-derived allelochemicals, as shown in Figure 12.2, are present in very low concentrations (Stevens and Merril, 1980; Wium-Anderson et al., 1982, 1983) with extraction yields usually much lower than 1 percent of the dry weight. Only very few nitrogen-containing allelochemicals (e.g., alkaloids) are known from submerged macrophytes. *Chara globularis,* for example, produces the antibiotically active charamin, a quarternary amine (Anthoni et al., 1987). Nitrogen-containing secondary metabolites are generally considered to be costly for the producing plant (Bryant et al., 1983). Therefore, the production of phenolic compounds for defense against other organisms may be a less costly method and allows use of superfluous assimilated carbon.

12.4.3 Excretion of Algicidal Hydrolyzable Polyphenols

The study of the release of allelochemicals by aquatic organisms faces extreme difficulties due to abiotic and biotic interferences. Nevertheless, this study is necessary to verify the release of allelochemicals and their effect on target organisms. *Myriophyllum spicatum* (Eurasian watermilfoil, or short milfoil) may release polyphenols by several mechanisms, two of which are exudation from intact, living tissue and leaching from decaying shoots. Both processes happen simultaneously in a macrophyte bed (Wetzel, 1983). Further leaching can occur due to injury by herbivory, mechanical damage (wave action) or during autofragmentation of shoots. Milfoil has secretory trichomes (Mayr, 1915; Godmaire and Nalewajko, 1990), which contain polyphenol-like substances, according to histochemical studies (Tunman, 1913; Janson, 1918). Tannins (polyphenols) have been considered responsible for the low epiphyte density on the green algae *Spirogyra* (Pankow, 1961). These algicidal compounds must be actively released, since only living cells showed this pattern and dead *Spirogyra* filaments were covered with epiphytes. Hydrolyzable polyphenols have been identified from *Spirogyra* sp., which contains 2.0 to 6.4 percent of the dry weight as hydrolyzable polyphenols, among them tetra- to undeca-galloylglucose derivatives (Nishizawa et al., 1985). *Spirogyra varians* produces the α-glucosidase-inhibitor 1,2,6-tri-*O*-galloyl-3-digalloyl-β-glucose (Cannel et al., 1988).

Axenic cultures are the method of choice for undisturbed studies on the release of allelochemicals (Keating, 1977; 1978; Gross et al., 1991; Gross, 1995). Some scientists may argue that nonaxenic specimens reflect the more natural state. However, using such material makes it much more difficult to distinguish compounds originating from the producing organism itself from those released by accompanying algae and microorganisms. For example, macrophyte-derived com-

pounds are metabolized by epiphytes. Furthermore, many bacterial contaminants in lab cultures probably arrived long after isolation of the donor species and did not play any role in the original field relationship. An axenic culture of *M. spicatum* has been established (Gross, 1995; Gross et al., 1996) to study the release of polyphenolic allelochemicals under controlled conditions. In the incubation medium of axenic cultures, single phenolic compounds have been identified. Among them are traces of the main allelochemical tellimagrandin II as well as ellagic acid and several other, yet unidentified low molecular hydrolyzable polyphenols (Gross and Sütfeld, 1994; Gross, 1995). Non-axenic shoots exhibit different patterns of released compounds indicating that they are rapidly metabolized by bacteria. Only short-term incubations of non-axenic milfoil shoots in sterile mineral medium reveal similar release patterns to axenic cultures. Considering the high phenolic content (approximately 10 percent of the dry weight) of milfoil and the often dense stands of this macrophyte, an estimate of the amount of released phenolic allelochemicals can be made. Given a maximum biomass of milfoil of 280 to 1150 g m^{-2} (Grace and Wetzel, 1978) and the content of Tellimagrandin II ranging from 10 to 15 $\mu g\ mg^{-1}$ dry weight of *M. spicatum*, dense stands could contain 1 to 6 mg/l (1.1 to 6.4 μM) of this highly algicidal hydrolyzable polyphenol. Further, given that 5 to 50 μg of tellimagrandin II led to a distinct inhibition of all tested algae and cyanobacteria in the agar diffusion assay, a release of only 1 percent of the main inhibitor would be sufficient to severely affect epiphytic or phytoplanktic organisms. Other hydrolyzable polyphenols found in and released by milfoil exert further but less inhibitory activity. Substantial improvements of methods are necessary to confirm such interactions *in situ*. It is not impossible that microbially degraded hydrolyzable polyphenols from milfoil are also allelopathically active. Phenolic compounds may be active before and after microbial degradation (Inderjit, 1996). A couple of studies indicate that milfoil releases allelochemicals. Agami and Waisel (1985) have shown that culture water from milfoil inhibits the growth of *Najas* sp. This appears to be caused by allelopathic interactions since nutrient effects could be ruled out. Organic compounds released by milfoil inhibited phytoplankton growth in a western German lake (G. Friedrich, unpublished results; Gross, 1995).

12.4.4 Hydrolyzable Polyphenols: Inhibitors of Algal Exoenzymes And More

A predominant biological action of polyphenols is their strong interaction with proteins (Haslam, 1989; Appel, 1993). Polyphenols may attenuate digestive enzymes of herbivores (Feeny, 1976; Appel, 1993). In aquatic systems, humic material includes allochthonous (leaf-litter) and autochthonous (macrophyte- and algal-derived) polyphenols. Allochthonous polyphenols decrease phytoplankton densities and change community structure of the phytoplankton in freshwater systems in the Doñana-National Park, southern Spain (Serrano and Guisande, 1990). Wetzel (1990, 1991, 1992, 1993) showed that humic compounds and simple phenolic acids inhibit algal exoenzymes, such as alkaline phosphatase activity (APA).

A special bioassay system has been developed to test whether tellimagrandin II and other milfoil polyphenols inhibit APA. Inhibition of APA is measured fluorescence-spectroscopically with MUF-P (Methylumbelliferyl-Phosphate) as a substrate (Gross, 1995; Gross et al., 1996). Polyphenols released by milfoil into the surrounding medium, as well as polyphenols extracted from plant tissue, strongly inhibit APA of selected cyanobacteria, chlorophytes, diatoms, and a sample of natural epiphytes (Gross, 1995; Gross et al., 1996). Polyphenols extracted from the culture medium by means of solid phase extraction (Gross and Sütfeld, 1994; Gross et al., 1996) led to a 60 percent inhibition of APA at concentrations as low as 1.6 mg l^{-1}. Higher concentrations in the range of 5 to 15 mg l^{-1} of humic acid or monophenols are needed for a comparable degree of inhibition (Wetzel, 1991, 1993). An exponential relationship exists between the concentration of tellimagrandin II used and the effective inhibition of APA in the cyanobacterium *Trichormus variabilis* P-9 (Gross et al., 1996). As little as 0.2 mg l^{-1} (0.2μM) tellimagrandin lead to a 10 percent inhibition, 3.4 mg l^{-1}

(3.6 μM) to a 40 percent inhibition. Hydrolyzable polyphenols are unspecific inhibitors of enzymes; the type of inhibition should therefore be noncompetitive (Haslam, 1989). Preliminary data suggest that the interaction between milfoil polyphenols and APA follows the kinetics of a noncompetitive inhibition (Gross, 1995).

Previous studies on hydrolyzable polyphenols have shown that the biological activity is correlated with the size of the molecule, the degree of oxidation, and the number of hydroxyl groups and aromatic systems (Zucker, 1983; Haslam, 1989; Appel, 1993). For example, β-1,2,3,4,6-penta-*O*-galloyl-D-glucose exerts a tenfold stronger inhibition on β-glucosidase than β-1,2,3-Tri-*O*-galloyl-D-glucose (Haslam, 1989). In agreement with this, the large and complex hydrolyzable polyphenol tellimagrandin II is a stronger inhibitor of APA of the cyanobacterium *Trichormus variabilis* P-9 and the chlorophyte *Scenedesmus falcatus* than the simple phenolic compound gallic acid even when the latter is used in a fivefold concentration to provide the same amount of hydroxy groups and aromatic systems than tellimagrandin II (Gross, 1995; Gross et al., 1996).

The strong algicidal activity observed with milfoil polyphenols cannot only be explained by interactions with membrane-bound and extracellular enzymes of algae, but also is probably a result of further, yet unknown, interference with essential metabolic processes of target cells. Polyphenols with a molecular weight of 800 to 3000 Da are considered small enough to pass through bacterial cell membranes (Field et al., 1990). Most of the milfoil polyphenols have molecular weights lower than 1000 Da (Gross, 1995; Gross et al., 1996) and would fall into this category. Lemke et al. (1995) have shown that humic acids can change membrane permeability in bacteria with a hydrophilic surface structure. Tellimagrandin II and other milfoil polyphenols probably can pass through both algal and bacterial cell membranes and exert further deleterious action on metabolic processes inside the target cell.

12.4.5 Allelopathy and Interference

Complex interactions in lakes do not permit easy distinction between allelopathic and competitive interactions. Evidence for coupled allelopathic and competitive interactions have been described from many shallow eutrophic lakes (Phillips et al., 1978; Scheffer et al., 1993; van Donk et al., 1993). Competition for resources implies the uptake or removal of resources (exploitative competition), whereas allelopathy involves the addition of biochemically active compounds (interference competition) (e.g., Willis, 1994). Muller (1969) used interference in a slightly different way to describe combined allelopathic and competitive interactions. The hypothesis is raised that allelopathy is especially effective under high competition for nutrients and light. This hypothesis was tested with Eurasian watermilfoil. The influence of phosphorus, nitrogen, and light on the production of algicidal hydrolyzable polyphenols in milfoil will be discussed.

Milfoil derives most of its phosphorus from the sediment (Carignan and Kalff, 1980). It never seems to be limited by this element (Gerloff and Krombholz, 1966; Anderson and Kalff, 1986). On the other hand, tellimagrandin II and other released or plant-bound polyphenols inhibit alkaline phosphatase of target organisms as shown above. Milfoil biomass peaks in mid-summer when phytoplankton in freshwater lakes is severely P-limited (Schindler, 1977; Sommer, 1989). Epiphytes may also be affected since they obtain most of their phosphorus from the water and not from their milfoil host (Carignan and Kalff, 1982). Inhibiting algal extracellular alkaline phosphatase would then be especially deleterious.

Nitrogen and light can both influence the biosynthesis of phenolic compounds (Haslam, 1986). The activity of phenylalanin-ammoniumlyase, a crucial enzyme in the biosynthesis of phenolic compounds, is regulated by light (Hahlbrock et al., 1976; Kuhn et al., 1984). Low light decreased the total phenolic content in milfoil, but the tellimagrandin II content stayed constant (Gross, unpublished results). Nitrogen limitation has frequently been shown to increase the phenolic content in

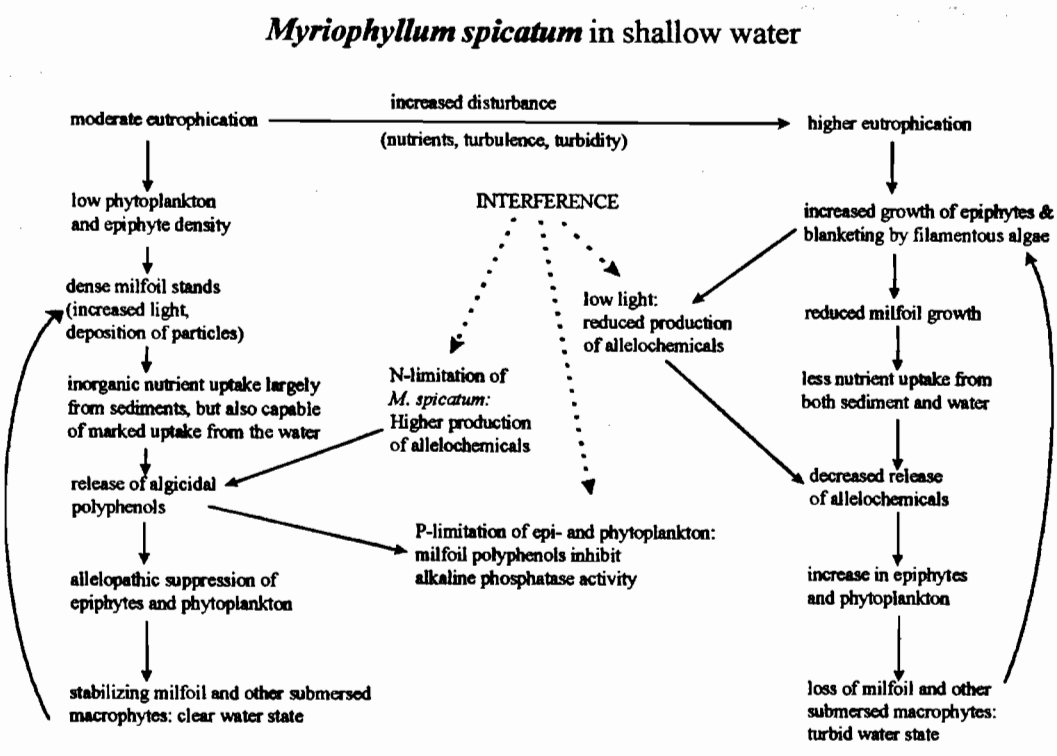


FIGURE 12.4 Coupled interaction of allelopathy and competition (interference) in *Myriophyllum spicatum* stands.

angiosperms (Hall et al., 1982; Haslam, 1986; Estiarte et al., 1994; Arnold et al., 1995). Milfoil growth is often N-limited (Gerloff and Krombholz, 1966; Sytsma and Anderson, 1993), which could influence the polyphenol content. Non-axenic milfoil shoots from Schöhsee that were obviously N-limited (tissue C:N ratio of 24:1, see Duarte, 1992), had significantly higher total amounts of total phenolics and tellimagrandin II than axenic milfoil grown in N-rich culture medium (Gross, 1995; Gross et al., 1996). Nitrogen limitation led to a higher content of tellimagrandin II in milfoil but did not affect the total phenolic content (Gross, unpublished results). This indicates a strong interaction between resource availability and the production of phenolic allelochemicals. Moreover, the main allelochemical found in milfoil exhibits different regulation patterns than the total phenolic compounds.

Figure 12.4 illustrates possible interactions of competition and allelopathy in the relationship between milfoil and algae. It describes how some major nutrients affect the production and effectiveness of milfoil allelochemicals and how this may influence the dominance of either milfoil or algae. Phillips et al. (1978) have described alternating stable states of submersed macrophytes and phytoplankton and considered the excretion of organic suppressors of algal growth by macrophytes to be an important mechanism in this relationship. Figure 12.4 extends the model of Phillips and coworkers (1978) and adjusts it for the interactions associated with milfoil. Overall, milfoil has been shown to be a highly competitive species (Grace and Wetzel, 1978; Smith and Barko, 1990). Furthermore, according to this and other studies (Fitzgerald, 1969; Planas et al., 1981; Agami and Waisel, 1985; Gross and Sütfeld, 1994; Gross et al., 1996) allelopathic interactions have to be included as a potent defensive strategy.

12.5 CONCLUSIONS

Limited spatial distance between competing organisms in benthic and littoral habitats makes allelopathy a powerful strategy. However, only detailed investigations can reveal how and in what form allelochemicals reach target organisms. Lipophilic allelochemicals of low molecular weight from the benthic cyanobacteria *Fischerella* sp. are transmitted by direct cell–cell contact. More polar hydrolyzable polyphenols from *Myriophyllum spicatum* and other Haloragaceae are released into the surrounding water, where they are rapidly metabolized. Still, also metabolized phenolic compounds exert a deleterious effect on membrane-bound and extracellular enzymes of algae. Valuable information about the ecological impact of allelochemicals was gained in these two systems by applying a threefold approach: 1) isolating active compounds, 2) studying possible release mechanisms, and 3) evaluating the mode of action in target organisms. Additionally, both systems revealed that the production and potency of allelochemicals is influenced by the availability of certain resources, such as phosphorus, nitrogen, and light. The production of allelochemicals can incur metabolic costs for the producing organism as has been shown when *Fischerella muscicola* was grown under nutrient and light-limiting conditions. Interference, meaning coupled competitive and allelopathic interactions, seems to be a powerful strategy in littoral areas, and may well be the driving force in the successful establishment of *M. spicatum.*

ACKNOWLEDGMENTS

Appreciation goes to Colleen Kearns and Kathleen I. Keating for improved spelling, grammar, and syntax of the manuscript. I thank K. I. Keating, Inderjit, and two anonymous reviewers for helpful comments and discussion. This work was supported in part by the Volkswagen Foundation Germany and the Max-Planck Society Germany.

REFERENCES

Agami, M. and Waisel, Y., Inter-relationship between *Najas marina* L. and three other species of aquatic macrophytes, *Hydrobiologia,* 126, 169, 1985.

Ailstock, M. S., Fleming, W. J., and Cooke, T. J., The characterization of axenic culture systems suitable for plant-propagation and experimental studies of the submersed aquatic angiosperm *Potamogeton pectinatus* (Sago pondweed), *Estuaries,* 14, 57, 1991.

Akehurst, S. C., Observations on pond life, *J. R. Microsc. Soc. London,* 51, 236, 1931.

Aliotta, G., Molinaro, A., Monaco, P., Pinto, G., and Previtera, L., Three biologically-active phenylpropanoid glucosides from *Myriophyllum verticillatum, Phytochemistry,* 31, 109, 1992.

Anderson, M. R. and Kalff, J., Nutrient limitation of *Myriophyllum spicatum* growth *in situ, Freshw. Biol.,* 16, 735, 1986.

Anthoni, U., Nielsen, P. H., Smith-Hansen, L., Wium-Andersen, S., and Christophersen, C., Charamin, a quaternery ammonium ion antibiotic from the green alga *Chara globularis, J. Org. Chem.,* 52, 694, 1987.

Appel, H. M., Phenolics in ecological interactions: the importance of oxidation, *J. Chem. Ecol.,* 19, 1521, 1993.

Arnold, T. M., Tanner, C. E., and Hatch, W. I., Phenotypic variation in polyphenolic content of the tropical brown alga *Lobophora variegata* as a function of nitrogen availability, *Mar. Ecol. Progr. Ser.,* 123, 177, 1995.

Bagchi, S. N., Structure and site of action of an algicide from a cyanobacterium, *Oscillatoria late-virens, J. Plant Physiol.,* 146, 372, 1995.

Bate-Smith, E. C., The phenolic constituents of plants and their taxonomic significance, I. Dicotyledones, *J. Linn. Soc. London (Bot.),* 58, 95, 1962.

Blindow, I., Long-term and short-term dynamics of submerged macrophytes in two shallow eutrophic lakes, *Freshw. Biol.,* 28, 15, 1992.

Blindow, I., Andersson, G., Hargeby, A., and Johansson, S., Long-term pattern of alternative stable states in two shallow eutrophic lakes, *Freshw. Biol.,* 30, 159, 1993.

Borowitzka, M. A., Microalgae as sources of pharmaceuticals and other biologically active compounds, *J. Appl. Phycol.*, 7, 3, 1995.

Box, J. D., Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters, *Water Res.*, 17, 511, 1983.

Boyer, G. L., Sullivan, J. J., Andersen, R. J., and Taylor, J. R., Effects of nutrient limitation on toxin production and composition in the marine dinoflagellate *Protogonyaulax tamarensis, Mar. Biol.*, 96, 123, 1987.

Bryant, J. P., Chapin, F. S., III, and Klein, D. R., Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory, *Oikos*, 40, 357, 1983.

Burkholder, J. M. and Wetzel, R. G., Epiphytic alkaline-phosphatase on natural and artificial plants in an oligotrophic lake—reevaluation of the role of macrophytes as a phosphorus source for epiphytes, *Limnol. Oceanogr.*, 35, 736, 1990.

Cannell, R. J. P., Farmer, P., and Walker, J. M., Purification and characterization of pentagalloylglucose, an a-glucosidase inhibitor antibiotic from the freshwater green alga *Spirogyra varians, Biochem. J.*, 255, 937, 1988.

Carignan, R. and Kalff, J., Phosphorus sources for aquatic weeds: water or sediments?, *Science*, 207, 987, 1980.

Carignan, R. and Kalff, J., Phosphorus release by submerged macrophytes: significance to epiphyton and phytoplankton, *Limnol. Oceanogr.*, 27, 419, 1982.

Cattaneo, A. and Amireault, M. C., How artificial are artificial substrata for periphyton, *J. North Am. Benthol. Soc.*, 11, 244, 1992.

Della Greca, M., Fiorentino, A., Monaco, P., Previtera, L., and Zarelli, A., Effusides I–V: 9,10-dihydrophenanthrene glucosides from *Juncus effusus, Phytochemistry*, 40, 533, 1995.

Dodson, S. I., Crowl, T. A., Peckarsky, B. L., Kats, L. B., Covich, A. P., and Culp, J. M., Non-visual communication in freshwater benthos: an overview, *J. North Am. Benthol. Soc.*, 13, 268, 1994.

Duarte, C. M., Nutrient concentration of aquatic plants—patterns across species, *Limnol. Oceanogr.*, 37, 882, 1992.

Elakovich, S. D. and Wooten, J. W., Allelopathic, herbaceous, vascular hydrophytes, *in Allelopathy: Organisms, Processes, and Applications*, Inderjit, Dakshini, K. M. M., and Einhellig, F. A., Eds., American Chemical Society, Washington, D.C., 1995, 58.

Eminson, D. and Moss, B., The composition and ecology of periphyton communities in freshwaters. I. The influence of host type and external environment on community composition, *Brit. Phycol. J.*, 15, 429, 1980.

Estiarte, M., Filella, I., Serra, J., and Penuelas, J., Effects of nutrient and water-stress on leaf phenolic content of peppers and susceptibility to generalist herbivore *Helicoverpa armigera* (Hubner), *Oecologia*, 99, 387, 1994.

Feeny, P. P., Plant apparency and chemical defense, *Rec. Adv. Phytochem.*, 10, 1, 1976.

Field, J. A. and Lettinga, G., Biodegradation of tannins, *Metal Ions Biol. Sys.*, 28, 61, 1992.

Field, J. A., Lettinga, G., and Habets, L. H. A., Measurement of low molecular weight tannins: indicators of methanogenic toxic tannins, *J. Ferment. Bioeng.*, 69, 148, 1990.

Fitzgerald, G. P., Some factors in the competition or antagonism among bacteria, algae and aquatic weeds, *J. Phycol.*, 5, 351, 1969.

Flores, E. and Wolk, C. P., Production, by filamentous, nitrogen-fixing cyanobacteria, of a bacteriocin and of other antibiotics that kill related strains, *Arch. Microbiol.*, 145, 215, 1986.

Fong, P., Donohoe, R. M., and Zedler, J. B., Competition with macroalgae and benthic cyanobacterial mats limits phytoplankton abundance in experimental microcosms, *Mar. Ecol. Progr. Ser.*, 100, 98, 1993.

Gerloff, G. C. and Krombholz, P. H., Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants, *Limnol. Oceanogr.*, 11, 529, 1966.

Gleason, F. K. and Case, D. E., Activity of the natural algicide cyanobacterin on angiosperms, *Plant Physiol.*, 80, 834, 1986.

Gleason, F. K. and Paulson, J. L., Site of action of the natural algicide, cyanobacterin in the blue-green alga, *Synechococcus* sp., *Arch. Microbiol.*, 138, 273, 1984.

Godmaire, H. and Nalewajko, C., Growth, photosynthesis, and extracellular organic release in colonized and axenic *Myriophyllum spicatum*, *Can. J. Bot.*, 67, 3429, 1989.

Godmaire, H. and Nalewajko, C., Structure and development of secretory trichomes on *Myriophyllum spicatum* L., *Aquat. Bot.*, 37, 99, 1990.

Gopal, B. and Goel, U., Competition and allelopathy in aquatic plant-communities, *Bot. Rev.*, 59, 155, 1993.

Gough, S. B. and Woelkerling, W. J., Wisconsin Desmids. II. Aufwuchs and plankton communities of selected soft water lakes, hard water lakes and calcareous spring ponds, *Hydrobiologia*, 49, 3, 1976.

Grace, J. B. and Wetzel, R. G., The production biology of Eurasian watermilfoil (*Myriophyllum spicatum* L.): a review, *J. Aquat. Plant Manage.*, 16, 1, 1978.

Gross, E. M., Isolation, chemical and physiological characterization of cyanobacteriocidal compounds from *Fischerella* (Cyanobacteria) (in german), Master thesis, Eberhard-Karls-Universität, Tübingen, Germany, 1990.

Gross, E. M., Allelopathische Interaktionen zwischen Makrophyten und Epiphyten: Die Rolle hydrolysierbarer Polyphenole aus *Myriophyllum spicatum*, Dissertation, Christian-Albrechts-Universität, Kiel, Germany, 1995.

Gross, E. M., Wolk, C. P., and Jüttner, F., Fischerellin, a new allelochemical from the fresh-water cyanobacterium *Fischerella muscicola*, *J. Phycol.*, 27, 686, 1991.

Gross, E. M. and Sütfeld, R., Polyphenols with algicidal activity in the submerged macrophyte *Myriophyllum spicatum* L., *Acta Hortic.*, 381, 710, 1994.

Gross, E. M., von Elert, E., and Jüttner, F., Production of allelochemicals in *Fischerella muscicola* under different environmental conditions, *Verh. Internat. Verein. Limnol.*, 25, 2231, 1994.

Gross, E. M., Meyer, H., and Schilling, G., Release and ecological impact of algicidal hydrolyzable polyphenols in *Myriophyllum spicatum*, *Phytochemistry*, 41, 133, 1996.

Hagmann, L. and Jüttner, F., Fischerellin A, a novel photosystem-II inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity, *Tetrahed. Lett.*, 37, 6539, 1996.

Hahlbrock, K., Knobloch, K. H., Kreuzaler, F., Potts, J. R. M., and Wellmann, E., Coordinated induction and subsequent activity changes of two groups of metabolically interrelated enzymes, *Eur. J. Biochem.*, 61, 199, 1976.

Hall, A. B., Blum, U., and Fites, R. C., Stress modification of allelopathy of *Helianthus annuus* L. debris on seed germination, *Am. J. Bot.*, 69, 776, 1982.

Harder, R., Ernaehrungsphysiologische Untersuchungen an Cyanophyceen, hauptsaechlich dem endophytischen *Nostoc punctiforme*, *Zeitschr. Bot.*, 9, 145, 1917.

Harlin, M. M., Transfer of products between epiphytic marine algae and host plants, *J. Phycol.*, 9, 243, 1973.

Harris, D. O., Growth inhibitors produced by the green algae (Volvocaceae), *Arch. Microbiol.*, 76, 47, 1971.

Haslam, E., Secondary metabolism—fact and fiction, *Nat. Prod. Rep.*, 3, 217, 1986.

Haslam, E., Plant Polyphenols: Vegetable Tannins Revisited, Cambridge University Press, Cambridge, NY, 1989.

Hegnauer, R., Chemotaxonomie der Pflanzen, Birkhäuser Verlag, Basel, 1962–1995.

Hutchinson, G. E., A Treatise in Limnology, Vol. III, Limnological Botany, John Wiley & Sons, New York, 1975.

Inderjit, Plant phenolics in allelopathy, *Bot. Rev.*, 62, 186, 1996.

Inderjit and Dakshini, K. M. M., Algal allelopathy, *Bot. Rev.*, 60, 182, 1994.

Ishimatsu, M., Tanaka, T., Nonaka, G. I., Nishioka, I., Nishizawa, M., and Yamagishi, T., Tannins and related compounds. LXXXV. Isolation and characterization of novel diastereomeric ellagitannins, nupharins *a* and *b*, and their homologues from *Nuphar japonicum* DC, *Chem. Pharm. Bull.*, 37, 129, 1989.

Janson, E., Über die Inhaltskörper der *Myriophyllum*-Trichome, *Flora (Germ.)*, 110, 265, 1918.

Kane, M. E. and Gilman, E. F., *In vitro* propagation and bioassay systems for evaluating growth regulator effects on *Myriophyllum* species, *J. Aquat. Plant Manage.*, 29, 29, 1991.

Keating, K. I., Allelopathic influence on blue-green bloom sequence in a eutrophic lake, *Science*, 196, 885, 1977.

Keating, K. I., Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure, *Science*, 199, 971, 1978.

Kogan, S. I. and Chinnova, G. A., Relations between *Ceratophyllum demersum* (L.) and some blue-green algae, *Gidrobiol. Zh.*, 8, 21, 1972.

Komarek, J. and Anagnostidis, K., Modern approach to the classification system of Cyanophytes 4—Nostocales, *Arch. Hydrobiol.*, 82, 247, 1989.

Kuhn, D. N., Chappel, J., Boudet, A., and Hahlbrock, K., Induction of phenylalanin-ammonia-lyase and 4-coumarat: CoA ligase mRNAs in cultured plant cells by UV light or fungal elicitors, *Proc. Natl. Acad. Sci. USA*, 81, 1102, 1984.

Kurihara, H., Kawabata, J., and Hatano, M., Geraniin, a hydrolyzable tannin from *Nymphaea tetragona* Georgi (Nymphaeaceae), *Biosci. Biotech. Biochem.*, 57, 1570, 1993.

Larsson, P. and Dodson, S., Invited review—Chemical communication in planktonic animals, *Arch. Hydrobiol.*, 129, 129, 1993.

Lemke, M. J., Churchill, P. F., and Wetzel, R. G., Effect of substrate and cell-surface hydrophobicity on phosphate utilization in bacteria, *Appl. Env. Microbiol.*, 61, 913, 1995.

Lewis, W. M. and Lewis, J., Jr., Evolutionary interpretation of allelochemical interactions in phytoplankton algae, *Am. Nat.*, 127, 184, 1986.

Loomis, W. D. and Battaile, J., Plant phenolic compounds and the isolation of plant enzymes, *Phytochemistry*, 5, 423, 1966.

Losee, R. F. and Wetzel, R. G., Littoral flow-rates within and around submersed macrophyte communities, *Freshw. Biol.*, 29, 7, 1993.

Mayr, F., Hydropoten an Wasser-und Sumpfpflanzen, *Beih. Bot. Centralbl. Abt.* I, 32, 278, 1915.

McClure, J. W., Secondary constituents of aquatic angiosperms, *in Phytochemical Phylogeny*, Harborne, J. B., Ed., Academic Press, London, 1970, 233.

Mole, S., The systematic distribution of tannins in the leaves of angiosperms—A tool for ecological-studies, *Biochem. Syst. Ecol.*, 21, 833, 1993.

Molisch, H., Der Einfluß einer Pflanze auf die Andere—Allelopathie, Fischer, Jena, Germany, 1937.

Moore, R. E., Cheuk, C., and Patterson, G. M. L., Hapalindoles: new alkaloids from the blue-green algae *Hapalosiphon fontinalis*, *J. Am. Chem. Soc.*, 106, 6456, 1984.

Moore, R. E., Patterson, G. M. L., and Carmichael, W. W., New pharmaceuticals from cultured blue-green algae, *in Biomedical Importance of Marine Organisms*, Fautin, D. G., Ed., 1988, 143.

Muller, C. H., Allelopathy as a factor in ecological process, *Vegetatio*, 18, 348, 1969.

Nicolaou, K. C. and Dai, W. M., Chemistry and biology of the enedyine anticancer antibiotics, *Angew. Chem.* (Int. ed.), 30, 1387, 1991.

Nishizawa, M., Yamagishi, T., Nonaka, G. I., Nishioka, I., and Ragan, M. A., Tannins and related compounds, Part 34: Gallotannins of the freshwater green algae *Spirogyra* sp, *Phytochemistry*, 24, 2411, 1985.

Nishizawa, K., Nakata, I., Kishida, A., Ayer, W. A., and Browne, L. M., Some biologically active tannins of *Nuphar variegatum*, *Phytochemistry*, 29, 2491, 1990.

Nonaka, G., Harada, M., and Nishioka, I., Eugeniin, a new ellagitannin from cloves, *Chem. Pharm. Bull.*, 28, 685, 1980.

Nonaka, G., Matsumoto, Y., and Nishioka, I., Trapain, a new hydrolyzable tannin from *Trapa japonica* Flerov, *Chem. Pharm. Bull.*, 29, 1184, 1981.

Ostrofsky, M. L. and Zettler, E. R., Chemical defences in aquatic plants, *J. Ecol.*, 74, 279, 1986.

Ozimek, T., Van Donk, E., and Gulati, R. D., Growth and nutrient-uptake by two species of *Elodea* in experimental conditions and their role in nutrient accumulation in a macrophyte-dominated lake, *Hydrobiologia*, 251, 13, 1993.

Pankow, H., Über die Ursachen des Fehlens von Epiphyten auf Zygnemalen, *Arch. Protistenk.*, 105, 417, 1961.

Papke, U., Gross, E. M., and Francke, W., Isolation, identification and determination of the absolute configuration of fischerellin B. A new algicide from the freshwater cyanobacterium *Fischerella muscicola* (Thuret.), *Tetrah. Lett.*, 38, 379, 1997.

Patterson, G. M. L., Larsen, L. K., and Moore, R. E., Bioactive natural products from blue-green algae, *J. Appl. Phycol.*, 6, 151, 1994.

Phillips, G. L., Eminson, D., and Moss, B., A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters, *Aquat. Bot.*, 4, 103, 1978.

Pignatello, J. J., Porwoll, J., Carlson, R. E., Xavier, A., Gleason, F. C., and Wood, J. M., Structure of the antibiotic cyanobacterin, a chlorine-containing Y-lactone from the freshwater cyanobacterium *Scytonema hofmanni*, *J. Org. Chem.*, 48, 4035, 1983.

Pip, E., Phenolic compounds in macrophytes from the lower Nelson River System, Canada, *Aquat. Bot.*, 42, 273, 1992.

Planas, D., Sarhan, F., Dube, L., Godmaire, H., and Cadieux, C., Ecological significance of phenolic compounds of *Myriophyllum spicatum*, Verh. *Internat. Verein. Limnol.*, 21, 1492, 1981.

Pratt, D. M., Competition between *Skeletonema costatum* and *Olistodiscus luteus* in Narragansett Bay and in culture, *Limnol. Oceanogr.*, 11, 447, 1966.

Pratt, R., Daniels, R. C., Eiler, J. J., Gunnison, J. B., and Kumler, W. D., Chlorellin, an antibacterial substance from *Chlorella*, *Science*, 99, 351, 1944.

Proctor, V. W., Studies on algal antibiosis using *Haematococcus* and *Chlamydomonas*, *Limnol. Oceanogr.*, 2, 125, 1957.

Revsbech, N. P., Christensen, P. B., and Nielsen, L. P., Microelectrode analysis of photosynthetic and respiratory processes in microbial mats, *in Microbial Mats—Physiological Ecology of Benthic Microbial Communities*, Cohen, Y. and Rosenberg, E., Eds., American Society for Microbiology, Washington, D.C., 1989, 153.

Rice, T. R., Biotic influences affecting growth of planktonic algae, *Fish. Bull. USA,* 54, 227, 1954.
Rice, E. L., Allelopathy, 1st ed., Academic Press, New York, 1974.
Rice, E. L., Allelopathy, 2nd ed., Academic Press, Orlando, FL, 1984.
Rizvi, S. J. H. and Rizvi, V., Allelopathy—Basic and Applied Aspects, Chapman and Hall, London, 1992.
Saito, K., Matsumoto, M., Sekine, T., Murakoshi, I., Morisaki, N., and Iwasaki, S., Inhibitory substances from *Myriophyllum brasiliense* on growth of blue-green algae, *J. Nat. Prod.,* 52, 1221, 1989.
Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., and Jeppesen, E., Alternative equilibria in shallow lakes, *TREE,* 8, 275, 1993.
Schindler, D. W., Evolution of phosphorus limitation in lakes, *Science,* 195, 260, 1977.
Schwartz, R. E., Hirsch, C. F., Sesin, D. F., Flor, J. E., Chartrain, M., Fromtling, R. E., Harris, G. H., Salvatore, M. J., Liesch, J. M., and Yudin, K., Pharmaceuticals from cultured algae, *J. Ind. Microbiol.,* 5, 113, 1990.
Seitz, A., Are there allelopathic interactions in zooplankton? Laboratory experiments with *Daphnia, Oecologia,* 62, 94, 1984.
Serrano, L. and Guisande, C., Effects of polyphenolic compounds on phytoplankton, *Verh. Intern. Verein. Limnol.,* 24, 282, 1990.
Shilo, M., Toxins of Chrysophyceae, *in Microbial Toxins,* Kadis, S., Ciegler, A., and Ajl, S. J., Eds., Academic Press, New York, 1971, 67.
Shilo, M., The unique characteristics of benthic cyanobacteria, *in Microbial Mats—Physiological Ecology of Benthic Microbial Communities,* Cohen, Y. and Rosenberg, E., Eds., American Society for Microbiology, Washington, D.C., 1989, 207.
Smith, A. L. and Nicolaou, K. C., The enedyine antibiotics, *J. Med. Chem.,* 39, 2103, 1996.
Smith, C. S. and Barko, J. W., Ecology of Eurasian watermilfoil, *J. Aquat. Plant Manage.,* 28, 55, 1990.
Sommer, U., The role of competition for resources in phytoplankton succession, *in Plankton Ecology, Succession in Plankton Communities,* Sommer, U., Ed., Springer Verlag, Berlin, 1989, 57.
Stevens, K. L. and Merril, G. B., Growth inhibitors from Spikerush, *J. Agric. Food Chem.,* 28, 644, 1980.
Su, K. L. and Staba, E. J., Preliminary chemical studies on aquatic plants form Minnesota, *LLoydia,* 36, 72, 1973.
Sytsma, M. D. and Anderson, L. W. J., Nutrient limitation in *Myriophyllum aquaticum, J. Freshw. Ecol.,* 8, 165, 1993.
Trebst, A., Donner, W., and Draber, W., Structure activity correlation of herbicides affecting plastoquinone reduction by photosystem II: electron density distribution in inhibitors and plastoquinone species, *Z. Naturf.,* 39C, 405, 1984.
Tunmann, O., *Pflanzenmikrochemie.* Bornträger, Berlin, Germany, 1913.
van Aller, R. T., Pessoney, G. F., Rogers, V. A., Watkins, E. J., and Leggett, H. G., Oxygenated fatty acids: a class of allelochemicals from aquatic plants, *in The Chemistry of Allelopathy,* Thompson, A. C., Ed., American Chemical Society, Washington, D.C., 1985, 387.
van Donk, E., Gulati, R. D., Iedema, A., and Meulemans, J. T., Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake, *Hydrobiologia,* 251, 19, 1993.
von Elert, E., Analytical investigation of allelopathic interactions between cyanobacteria and phytoplankton in eutrophic Ostholsteinische Seen (in German), Dissertation thesis, Eberhard-Karls-Universität, Tübingen, Germany, 1994.
von Elert, E. and Jüttner, F., Factors influencing the allelopathic activity of the planktonic cyanobacterium *Trichormus doliolum, Phycologia,* 35, 68, 1997.
Wetzel, R. G., Limnology, 2nd ed., W. B. Saunders, Fort Worth, TX, 1983.
Wetzel, R. G., Edgardo Baldi Memorial Lecture. Land-water interphases, metabolic and limnological regulators, *Verh. Internat. Verein. Limnol.,* 24, 6, 1990.
Wetzel, R. G., Extracellular enzymatic interactions: storage, redistribution and interspecific communication, *in Microbial Enzymes in Aquatic Environments,* Chrost, R. J., Ed., Springer Verlag, New York, 1991, 6.
Wetzel, R. G., Gradient-dominated ecosystems—sources and regulatory functions of dissolved organic matter in fresh-water ecosystems, *Hydrobiologia,* 229, 181, 1992.
Wetzel, R. G., Humic compounds from wetlands: complexation, inactivation, and reactivation of surface-bound and extracellular enzymes, *Verh. Internat. Verein. Limnol.,* 25, 122, 1993.
Wetzel, R. G. and McGregor, D. L., Axenic culture and nutritional studies of aquatic macrophytes, *Am. Midl. Nat.,* 80, 52, 1968.

Whittaker, R. H. and Feeny, P. P., Allelochemics: chemical interactions between species, *Science,* 171, 757, 1971.

Willis, R. J., Terminology and trends in allelopathy, *Allelop. J.,* 1, 6, 1994.

Wium-Andersen, S., Allelopathy among aquatic plants, *Arch. Hydrobiol., Beih. Ergebn. Limnol.,* 27, 167, 1987.

Wium-Andersen, S., Anthoni, U., and Houen, G., Elemental sulphur, a possible allelopathic compound from *Ceratophyllum demersum, Phytochemistry,* 22, 2613, 1983.

Wium-Andersen, S., Anthoni, U., Christophersen, C., and Houen, G., Allelopathic effects on phytoplankton by substances isolated from aquatic macrophytes (*Charales*), *Oikos,* 39, 187, 1982.

Wium-Andersen, S., Jorgensen, K. H., Christophersen, C., and Anthoni, U., Algal growth inhibitors in *Sium erectum* Huds, *Arch. Hydrobiol.,* 111, 317, 1987.

Yoshida, T., Yazaki, K., Usman Memon, M., Maruyama, I., Kurokowa, K., and Okuda, T., Bicornin, a new hydrolyzable tannin from *Trapa bicornis* and revised structure of alnusiin, *Heterocycles,* 29, 86, 1989.

Yoshikawa, M., Hatakeyama, S., Tanaka, N., Matsuoka, T., Yamahara, J., and Murakami, N., Crude drugs from aquatic plants. 2. On the constituents of the rhizome of *Alisma orientale* Juzep originating from Japan, Taiwan, and China—absolute stereostructures of 11-deoxyalisols-b and b-23-acetate, *Chem. Pharm. Bull.,* 41, 2109, 1993.

Zucker, W. V., Tannins: does structure determine function? An ecological perspective. *Am. Nat.* 121, 335, 1983.