

# A review of aquatic weed biology and management research conducted by the United States Department of Agriculture – Agricultural Research Service<sup>†‡</sup>

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**Abstract:** Ever-increasing demand for water to irrigate crops, support aquaculture, provide domestic water needs and to protect natural aquatic and riparian habitats has necessitated research to reduce impacts from a parallel increase in invasive aquatic weeds. This paper reviews the past 4–5 years of research by USDA-ARS covering such areas as weed biology, ecology, physiology and management strategies, including herbicides, biological control and potential for use of natural products. Research approaches range from field-level studies to highly specific molecular and biochemical work, spanning several disciplines and encompassing the most problematic weeds in these systems. This research has led to new insights into plant competition, host-specificity, and the fate of aquatic herbicides, their modes of action and effects on the environment. Another hallmark of USDA-ARS research has been its many collaborations with other federal, state action and regulatory agencies and private industry to develop new solutions to aquatic weed problems that affect our public natural resources and commercial enterprises. Published in 2003 for SCI by John Wiley & Sons, Ltd.

**Keywords:** aquatic herbicide; water weeds; riparian weeds; invasive weeds; biological control; phenology; management strategy

## 1 INTRODUCTION

The United States Department of Agriculture, Agricultural Research Service (USDA-ARS) has been conducting research to develop and improve control of noxious aquatic vegetation (including algae) since the 1950s when invasive exotic and native plants began to impact on agricultural production by interfering with irrigation storage and delivery systems. From early research located at Ft Lauderdale, FL, additional research needs resulted in establishment of units in Prosser, WA, Davis, CA and Denver, CO and a riparian weed control research unit (primarily Saltcedar control) in Las Cruces, NM. However, from the mid-1980s until the mid-1990s, all but two locations—Davis and Ft Lauderdale—were closed and total scientific staff dwindled to less than half the levels in the late 1970s. (A comprehensive history of aquatic weed control in the USA from its earliest formation to ca 1988 is given in Reference 1.)

Notwithstanding these shifts in ARS research effort, the negative impacts from aquatic vegetation have greatly accelerated in recent years due to: (1) increased demands on water both for irrigation and domestic consumption; (2) continuing introductions of exotic

and invasive aquatic and riparian weeds affecting natural aquatic sites; and (3) increased intensity of aquaculture (primarily fish production) and algae-related impacts. The increasing negative economic and ecological impacts from these pest plants, coupled with a broader awareness of invasive species concerns in general, have resulted in a resurgence in ARS support of related research over the past three to four years. Indeed, exotic species now account for well over 90% of problematic weeds in both irrigation-related uses as well as those impacting natural aquatic and riparian habitats.<sup>2</sup> In addition, native species of pondweed (*Potamogeton* spp) also contribute to serious problems in western irrigation conveyance systems.

Most of the economic and environmental impacts arise from a relatively small number of aquatic and riparian plants (Table 1). Four species have become 'newly' problematic in the last five years. Some plants in this list have been in the USA much longer (eg hydrilla, Eurasian watermilfoil, purple loosestrife), but their increased numbers or dramatic expansions of invaded habitats have warranted new, specific research focus.

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**Table 1.** Important aquatic and riparian weeds in the USA

Species	Impact
<b>Aquatic</b>	
<i>Egeria densa</i> <sup>a,b</sup> (Egeria)	Irrigation, navigation, recreation, flood-control, mosquito habitat, fisheries
<i>Eichhornia crassipes</i> <sup>a</sup> (Waterhyacinth)	Navigation, flood control, irrigation, recreation, fisheries habitat
<i>Salvinia molesta</i> <sup>a,b</sup> (Giant water fern)	Navigation, flood control, irrigation, recreation, fisheries habitat
<i>Hydrilla verticillata</i> <sup>a</sup> (Hydrilla)	Irrigation, storage, recreation, fisheries habitat, species diversity
<i>Ludwigia peploides</i> (Water primrose)	Flood control, mosquito habitat
<i>Myriophyllum spicatum</i> <sup>a</sup> (Eurasian watermilfoil)	Water storage, recreation, irrigation, navigation, species diversity, fisheries
<i>Myriophyllum aquaticum</i> <sup>a</sup> (Parrot feather)	Flood control, mosquito habitat, fisheries habitat, species diversity
<i>Potamogeton</i> spp (Pondweeds)	Irrigation, storage, recreation
<i>P. nodosus</i> (American pondweed)	
<i>P. pectinatus</i> (Sago pondweed)	
<i>P. crispus</i> (Curlyleaf pondweed) <sup>a</sup>	
<i>P. gramineus</i> (Variable leaf pondweed)	
<b>Riparian</b>	
<i>Lythrum salicaria</i> <sup>a</sup> (Purple loosestrife)	Flood control, wetland habitat, species diversity, waterfowl habitat
<i>Typha</i> spp (Cattails)	Flood control, mosquito habitat, irrigation
<i>Arundo donax</i> (Giant reed) <sup>a,b</sup>	Flood control, species diversity, bridge damage, fire hazard
<i>Melaleuca quinquenervia</i> (Melaleuca) <sup>a</sup>	Florida Everglades; flood control; loss of habitat
<i>Spartina alternifolia</i> <sup>a</sup> (Smooth cordgrass)	
<i>Tamarix</i> <sup>a</sup> spp (Saltcedar) <sup>a</sup>	Flood control, groundwater loss, loss of habitat, channelization of rivers, bridge damage
<b>Freshwater algae</b>	
<i>Cladophora</i> spp <i>Rhizoclonium</i> spp, <i>Spirogyra</i> spp	Drip irrigation, mosquito habitat, aquaculture, potable water, recreation
<b>Marine algae</b>	
<i>Caulerpa taxifolia</i> <sup>a,b</sup>	Bay and coastal ecosystems; aquaculture

<sup>a</sup> Exotic species; <sup>b</sup> weeds with impacts in last 5 years.

## 2 THE CONTEXT FOR USDA-ARS RESEARCH

### 2.1 What are past and current Agency relationships

Historically (and continuing today) the US Army Corps of Engineers and the US Bureau of Reclamation conduct federally funded aquatic weed

research/management. At one time, until the late 1980s the Tennessee Valley Authority (TVA) conducted research in this area as well. In addition, there are several state-level research programs and various private and land-grant universities with programs focused on local needs. ARS is a partner in many of these programs providing basic research on biology and chemical control, and in conducting critically important foreign exploration and testing for biological control agents. These partnerships were often formalized into Department-level or Agency-level memoranda of understanding (MOU) which spelled out roles and responsibilities and helped coordinate work. Through these and other less formal collaborations, ARS provides innovative approaches to problems by building on basic and applied research that ranges from molecular and biochemical levels to life-cycle, ecophysiological and population levels.

### 2.2 How do ARS scientists recognize and respond to new problems?

First, the aquatic plant management 'community' is highly interactive and communicative. Private, local, state and federal workers have responsibilities for a large number of aquatic sites from small ponds to lakes, reservoirs, irrigation systems and large natural aquatic and riparian habitats. These managers have access to the research community through an information network consisting of formal Extension Agents, Farm Advisors, regional, national and international society meetings, pertinent websites, e-mails and list servers, newsletters, scientific journals, focused workshops, short-courses and, of course, personal contacts developed over years of successful collaborations and cooperative efforts at solving specific problems. Thus, there is a diverse and dynamic network in place that surfaces new problems and new threats, all of which help identify new research needs.

Second, ARS aquatic weed scientists have a long history of personally spanning the research/technology-transfer/extension 'boundaries'. This approach, creating an effective and flexible continuum across these traditional functions, has served the overall weed-management community well. How else can one properly balance basic and applied research effectively? Thus, new problems are recognized by ARS scientists through their contacts in the field-level, real-world arena, where operational management and control of aquatic and riparian weeds must succeed.

## 3 ARS RESEARCH

This section summarizes research over the past 4–5 that contributes toward solutions to aquatic weed problems. The discussion is primarily focused on truly aquatic species, but touches on some riparian target weeds as well. A brief rationale is also provided for approaches taken. The discussion is organized by the general research focus (eg reproduction), and then by specific target weeds since the same weed may impact

on several different types of aquatic sites or aquatic uses (Table 1). The characteristics of each site or 'use' of the water also delineate both opportunities and limitations for management.

For this reason, the relationship between the target weed impact and the site (and site-use) shapes the type and focus of research. This approach also parallels that taken by several federal and state action-agencies in focusing resources on, and in responding to, pest infestations from both regulatory and management perspectives. Thus, there has to be a balance of basic research (eg plant physiology, plant ecology) and the pragmatic realities dictated by environmental as well as sociopolitical limitations. To help illustrate the complexities of aquatic weed management, Table 2 compares and contrasts weed control in aquatic sites with weed control in cropland sites. Finally, although rice production certainly occurs in aquatic systems, the cultural practices and research foci are more closely aligned with other cropland system weed control and are therefore not included in this paper. (For a review of rice-related weed research, see Reference 3.)

## 4 REPRODUCTION, PERENNATION AND DISPERSAL

### 4.1 Rationale for research

Aquatic weeds generally exhibit a variety of structures for vegetative (asexual) reproduction, many of which serve as overwintering and dispersal mechanisms. ARS research has focused on understanding what external drivers promote the dormancy (perennation) or sprouting and formation of these propagules, their longevity, and what controls their viability and dormancy. This knowledge can lead to new approaches in disrupting life cycles and in assessing the ability for populations of invasive weeds to persist. By examining the environmental variables affecting either the formation or sprouting of these propagules, predictive models can help fine-tune control and eradication methods, particularly with regard to

optimal timing of management actions. In addition, better understanding of reproductive capacities of various plant parts can help in evaluating the short-term and long-term impacts of management practices, especially those relying on cutting, harvesting or dredging. This is important since many invasive aquatic plants can produce viable populations from very small pieces of whole plants.

### 4.2 *Hydrilla verticillata* Presl (hydrilla), *Potamogeton* spp (pondweeds)

Spencer *et al*<sup>4</sup> applied 'degree-day' analysis methods to determine cumulative thermal input required for sprouting of several key aquatic weeds including *H verticillata*, sago pondweed (*Potamogeton pectinatus* L and American pondweed (*P nodosus* Poir) in studies conducted in California and Texas. In addition, this comparison—the first published on these species—showed clearly that species more typically found in northern latitudes had shorter degree-day requirements than those in more southern latitudes. It also showed that the turions (dispersal propagules) and tubers in monoecious hydrilla required fewer degree-days (thus sprouting earlier) than tubers from dioecious hydrilla (Table 3). By comparing field soil temperatures, emergence time and rates of emergence, a predictive model was developed that can be useful in formulating management strategies (eg timing of herbicide use, mechanical removal).

In a related paper, Spencer and Ksander<sup>5</sup> deployed recording thermometers in sediments in Clear Lake, California, and coupled the data from these with (monoecious) hydrilla emergence times based upon surveys conducted by the California Department of Food and Agriculture (CDFA) as part of their Hydrilla Eradication Program. Actual emergence data fit the degree-day models well.

These data also suggest that, in canals that are normally drained ('dewatered') during the winter, it may be possible to encourage 'early' emergence of weeds by flooding of the canals, followed by draining

**Table 2.** Comparison of characteristics between cropland and non-cropland sites (modified from Reference 64)

Characteristic and concerns	Cropland sites	Non-cropland/aquatic sites
Soil environment	Highly controlled (eg types, moisture, nutrients, slopes)	Usually uncontrolled and highly variable; often hypoxic
Non-target plants	Highly manipulated and known (eg specific plants and varieties with selected growth responses). These are crops	Extremely variable, often naturally occurring species and populations, less predictable growth stages. These are native plants which may include ESA 'listed' species (endangered, threatened or species of concern)
Economic impacts	These are specific commodities (yield, quality, market demands)	These are primarily loss of uses for sites, loss of water delivery or flood control capacity; and loss of critical habitat
Regulatory issues	Primary focus on crop residues (human consumption), soil residue ('half-life')	Primary concern on non-target species (especially fish and wildlife), off-site movement, and human contact (in water)
Off-site mobility of herbicides	Unlikely due to predictable cropping patterns and well-delineated target sites; potential movement to ground water	Higher probability of movement due to landscape slopes, runoff, and due to water movement in aquatic sites

**Table 3.** Estimated degree-days required for propagule sprouting in submersed plants (adapted from Reference 4)

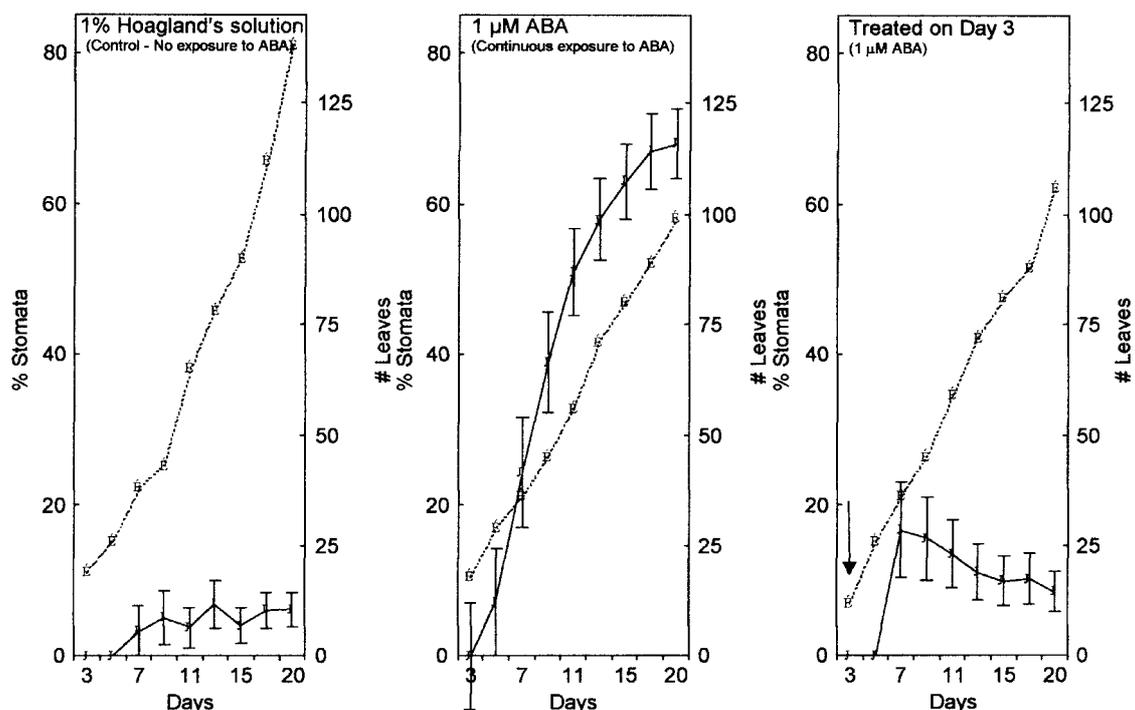
Species	Degree-days to 50% sprouting
<i>Potamogeton nodosus</i>	298
<i>Hydrilla verticillata</i> (monoecious; Turions)	393
<i>Vallisneria americana</i>	445
<i>Hydrilla verticillata</i> (monoecious; Tubers)	777
<i>Hydrilla verticillata</i> (dioecious; Tubers)	1295
<i>Potamogeton pectinatus</i>	1865

or by applications of effective herbicides before crops need to be irrigated. Dewatering or 'drawdown' of canals is a common practice in several western states and may also be an option in some lakes. These results point to the need to monitor both moisture and sediment temperature so that prediction of emergence can be used to properly time herbicide usage. Since soil moisture (and temperature) is a primary 'driver' of propagule emergence, early flooding and 'ponding' (ie damming) of drained canals (eg in late winter) may provide a window of opportunity for the use of herbicides under conditions that eliminate downstream movement of these chemicals and still provide contact with the target weeds. Information on degree-days may also be useful in developing restoration approaches that rely on introductions of various vegetative propagules of native pondweeds. (See also Section 8)

Physiological processes associated with sprouting of vegetative propagules in aquatic weeds are poorly understood. However, in a comparison of American pondweed winter buds and hydrilla

tubers, Spencer *et al*<sup>6</sup> found that, although sucrose was the most abundant soluble sugar in all unsprouted propagules, as sprouting progressed, conversions to glucose and fructose (and to a lesser degree, raffinose and stachyose) occurred as new shoots emerged in all structures over a 28-day period. Clearly, sugar conversions and associated enzyme activities need further examination. Perhaps blocking normal sugar mobilization could reduce annual recruitment; alternatively, changing sediment conditions may favor sprouting and thus provide an opportunity to control newly emerging plants.

Taking another approach, Gee and Anderson<sup>7</sup> followed up prior ARS research at the Davis laboratory on the role of abscisic acid (ABA) in development and heterophylly in *Potamogeton nodosus* Poir. This species, like many other aquatic angiosperms (and some aquatic ferns) modulates leaf development to form 'floating' or 'terrestrial' type leaves, often when emerging in moist sediments. Groups of sprouting winter buds were exposed to ABA in Hoagland's medium for 4 h and the development of individual, tagged leaves was tracked. The study showed that individual leaf responsiveness to ABA is transient and resides in a 'window' of about 3–5 days following emergence of a given leaf (Fig 1.). The mode of action of ABA in this altered gene-expression is not known and perhaps this system can be a model for examining leaf development in general. Furthermore, since several weedy species (eg some *Myriophyllum* spp), exhibit heterophylly, these types of responses may be manipulated to facilitate use of foliar-applied



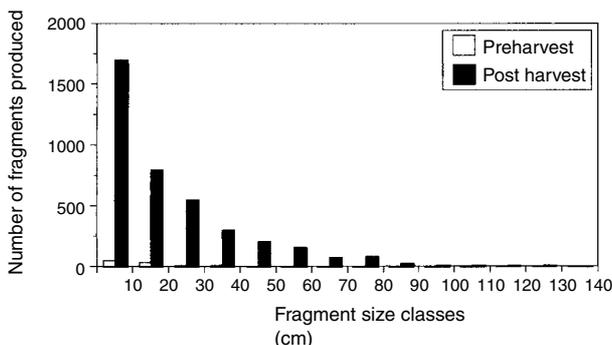
**Figure 1.** Response of *Potamogeton nodosus* to 1  $\mu$ M ABA: (---) number of leaves; (—) % floating-type leaves. Arrow indicates treatment day. Left panel: controls (no ABA); middle panel: continuous exposure to ABA; right panel: 4 h of exposure to ABA on day 3.

herbicides rather than relying on whole-water column exposures.

#### 4.3 *Egeria densa* Planchon (egeria)

As part of cooperative research with the California Department of Boating and Waterways (CDBWW), Anderson<sup>8</sup> examined the production and viability of *Egeria densa* fragments during field-level mechanical control operations in the Sacramento–San Joaquin Delta. In this dynamic, fresh water tidal system, *E densa* has spread to over 6000 acres and impacts several beneficial uses including commercial shipping, recreational uses, irrigation pumping and wildlife habitat. Although there is no doubt that cutting and harvesting approaches can lead to short-term amelioration of the physical blockage from submerged and other types of aquatic weeds, information on the potential for spread and dispersal of ‘escaped’ fragments is nearly absent. Pre- and post-harvesting sampling of fragments, followed by analysis of size-classes and viability showed that within just a few hours, these operations produced several thousand small and large pieces, essentially all of which were viable and thus potentially capable of establishing new populations. Figure 2 shows typical size distribution over a 2-h post-harvest sampling period. Note that the majority of fragments are less than 20 cm and are easily transported via tidal flows and river-flows within the Delta.

*Egeria densa* fragments were maintained in a growth chamber in Delta water. All size classes of fragments examined (9–23 cm) produced many lateral shoots as well as abundant adventitious roots. An interesting finding was that when fragments of different sizes were maintained in common containers they had less root production than when the size classes were kept separate (ie with the same size-class). These results were part of overall decision-matrix inputs to a complex assessment of management options for *E densa*, and clearly showed the risk of increasing the distribution of egeria while achieving a short-term, localized beneficial reduction in biomass through harvesting.



**Figure 2.** Size distribution of *Egeria densa* fragments after mechanical harvesting operations.

## 5 RESPONSES TO NUTRIENTS, SEDIMENTS AND ALLOCATION OF RESOURCES IN AQUATIC WEEDS

### 5.1 Rationale for research

With the exception of truly floating weeds (eg water hyacinth *Eichhornia crassipes* Solms, giant water fern *Salvinia molesta* Mitchell and water lettuce *Pistia stratiotes* L), which must obtain all nutrients from the water column, nearly all of the major nutrients for other, rooted, aquatic weeds (submersed or emergent) are derived from the sediment. Understanding the responses of aquatic weeds to varying sediment nutrients can help in optimizing the timing of control practices and may shed light on ways in which sediment resources may be altered to reduce growth of problematic weeds. Nutrient availability can also affect production of propagules and alter the interaction between target weeds and herbivorous insects that may have potential for biological control. From a preventative (and predictive) perspective, understanding the influences of nutrient availability and native sediments in general can help assess the likelihood for dispersal and successful establishment of newly introduced weeds as well as their ability to compete with native aquatic plants. Moreover, understanding the spatial and temporal allocation of resources within target weeds (eg nitrogen, carbohydrates) during the growing season may point to phases in the life-cycles that are susceptible to disruption by environmental manipulations (eg cutting, altering water and light availability), biological control agents or selective herbicides.

### 5.2 *Myriophyllum spicatum* L (Eurasian watermilfoil)

Anderson and Spencer<sup>9</sup> and Walter<sup>10</sup> documented the spread of this invasive submersed weed in Lake Tahoe and have investigated its potential to become established in various natural sediments within the lake. Lake Tahoe, a high Sierra, oligotrophic lake with famous water clarity, has been the subject of intense national and international limnological research since the late 1960s due to ever-increasing turbidity.<sup>11</sup> Nearly all research, however, had been focused on causes of accelerating phytoplankton production and the role of nutrient inputs and urbanization in this process. The USDA-ARS was the first to bring attention to the ominous dispersal of *M spicatum* from a large population located in a marina in South Lake Tahoe, to other, smaller mooring facilities around the lake proper.<sup>9</sup> Although the original marina population had probably existed since the 1970s (though no concrete documentation of this appears to exist), the continuing spread to other areas is clearly a recent event, since these areas had either been dry during a multi-year drought (prior to 1994), or had been surveyed in 1995–2000 during which time the first populations were encountered. As a result of these findings, graduate work was funded to further delineate populations and to determine if as yet uninfested areas might be

vulnerable. Katey Walter's research<sup>10</sup> demonstrated that transplants of cuttings could indeed survive in these natural sediments, both *in situ* and in small mesocosms. Thus, continued production of fragments from harvesting operations in the main marina and typical boat traffic both have great potential to cause further spread of *M spicatum* to new sites, as long as they are partially protected from high-energy wind and wave action. Furthermore, Walter's work showed that *M spicatum* was capable of shunting sediment-borne phosphorus from roots through the plant shoots and into the water column, a phenomenon that had been shown in other systems, and clearly has serious implications for Tahoe's already excessive external nutrient inputs. This work is continuing with studies in small mesocosms adjacent to Lake Tahoe in an effort to determine best management practices (BMPS).<sup>12</sup>

With the similar concern regarding dispersal of *M spicatum* from Lake Tahoe, Spencer and Ksander<sup>13</sup> examined the levels of N and P in tissues in a range of sites within the Truckee River as it flows from Lake Tahoe toward Nevada and the eastern slopes of the Sierra Nevada where irrigation systems might be threatened. (The Lower Truckee River is the only outflow from Lake Tahoe and thus is a likely route for downstream spread of plants.) They documented that, indeed, populations had become established at several sites extending from the head of Truckee River at least 12 miles downstream. They also showed subsequently that sediment P is low and may be limiting the production of *M spicatum* presently. Furthermore, when levels of N and P in *M spicatum* tissue were compared with published 'critical' N and P levels for this species, it appears that P is limiting growth. However, as populations will surely continue to move downstream, areas with higher available N and P will be encountered, particularly in the irrigation systems at lower elevations. These data, coupled with the strong evidence of continued incursions in Lake Tahoe suggest that effective management is urgently needed. Some approaches are described below in sections covering management research.

Other studies by Spencer and Ksander<sup>13,14</sup> on nutrition and resource allocation focused on constituents in *M spicatum* and other macrophytes, including N levels and phenolic compounds, both of which may influence herbivory. They showed that *M spicatum* grown at Davis, California, has generally slightly higher N levels (2.1%) than plants sampled at the Truckee River (Lake Tahoe) (1.78%), and that the Truckee River plants had much lower phenolic levels, compounds that may discourage herbivorous insects (eg biological control agents). Though these comparisons were not made in any overlapping years, they were made over multiple years and the ranges and levels are consistent within each location.

### 5.3 *Egeria densa*

As part of the ARS cooperative research with CDBWW on the control of *E densa*, the author has been sampling

populations at several sites in the Delta monthly for the past two years. Analysis for allocations of C and N, though still in progress, reveal that this plant behaves as a perennial, with very moderate alterations in tissue nitrogen throughout the season. The study also suggests that adventitious root production is a significant investment in nitrogen, though the relative allocations vary with sample site. Since this plant does not produce a separate vegetative over-wintering propagule (eg turion or tuber), it appears that reserves are sequestered in some shoots and the rhizomes. Only the dioecious male plant is reported in the USA so there is no allocation to seed formation either. More detailed data on phenology and C:N allocations should emerge from the controlled-variable depth studies described below in the section on plant interactions.

### 5.4 *Salvinia molesta*

This invasive floating fern was first documented in the USA 4 years ago, including populations in parts of the Lower Colorado River in California. To assess the risk of further invasion into the Sacramento–San Joaquin Delta, participants in the UC Davis, NFS-sponsored 'Young Scholars Program' and the author inoculated water collected from the Delta and other surface water systems. All waters supported growth of *S molesta*. These types of quick-response study help clarify the need for immediate management and the justification for an eradication program, which is now being conducted by California Department of Food and Agriculture.

## 6 PLANT INTERACTIONS AND COMPETITION

### 6.1 Rationale for research

Whether in natural or artificial environments, aquatic weeds are usually part of interacting populations of plants and thus understanding how they respond to the presence (or absence) of other plants, available resources (nutrients, light, etc) may lead to novel approaches to their management. This research can also provide a basis for successful restoration and re-vegetation using native plants, particularly as part of programs focused on controlling exotic species. These interactions range from physical interference (ie space), to competition for nutrients or light, and even potential allelochemical effects. Though there are also similar interactions that relate to effects of biological control agents, these are discussed below under specific management methods.

### 6.2 *Hydrilla verticillata*, *Potamogeton nodosus*, *Vallisneria americana* Michx

Spencer and Ksander,<sup>15</sup> using statistical approaches that they introduced earlier to aquatic plant interactions (ie reciprocal yield models), examined growth responses when the native *P nodosus* and exotic monoecious *H verticillata* were established from different types and sizes of propagule. In this series of outdoor, shallow container experiments, both intra- and

inter-species competition affected standing crop and production of propagules. *P nodosus* produced longer shoots as well as floating leaves, which reduced light availability to *H verticillata*. However, in some cases, even small *H verticillata* starting propagules (axillary turions) resulted in a larger number of tubers than in winter bud production in *P nodosus*. As the authors point out, one should be cautious in translating these results to field conditions where canopy interferences may not be so static (eg due to water flows and variable depths). However, the data suggest that monoecious *H verticillata* and *P nodosus* may coexist for some period of time. The versatility of propagule formation in *H verticillata* (tubers, axillary turions, fragments) coupled with its dispersal abilities may ultimately allow it to invade and dominate as it has indeed done in several California and other USA habitats.

Van *et al*<sup>16,17</sup> also examined the influence of sediment fertility and the presence of two herbivorous insects (*Hydrellia pakistanae* and *Bagous hydrillae*) on dioecious *H verticillata* grown with the native plant, *Vallisneria americana* Michx. These authors applied Spencer's use of the reciprocal-yield model and showed that higher sediment fertility favored *H verticillata* over *V americana*. Conversely, under 'limiting' nutrients, *Vallisneria* dominated, and also allocated far more biomass to roots than did *Hydrilla*. In the experiments conducted in winter and summer, effects of leaf-mining insects (*H pakistanae*) were sufficient to shift dominance to the native *V americana*, thus removing the interspecific competitive advantage of *H verticillata*. However, this shift was observed with *B hydrillae* only when repeated introductions of this weevil were made during the summer period. These approaches are extremely important in developing models for understanding competitiveness as well as the effects that plant stressors (eg herbivory and nutrient limitation) have on dominance of plant species.

Interactions between aquatic plants can be affected by differences in plant canopy structure and by differential responses to light level and quality. Overall effects of the light environment, or 'light field' (*sensu* Kirk)<sup>18</sup> are poorly understood, in part due to difficulties in manipulating experimental systems that resemble light gradients occurring in natural systems. Anderson<sup>19</sup> developed controlled variable-water-depth culture systems in order to examine changes in plant canopy over time and with depth, as well as light-quality related influences on plant growth and reproduction. The system is comprised of replicated 0.6-m diameter translucent-walled columns with 0.4, 0.8, 1.2 and 1.6 m water depths, containing constantly circulating, temperature-controlled water. Each column is illuminated by a metal-halide lamp. Thus, except for the natural light gradients with depth, conditions among the columns are identical. The native *Ceratophyllum demersum* L was grown (in separate containers) with the exotic *M spicatum* on native Lake Tahoe sediments, and at all depths except

1.2 m, *M spicatum* produced more total biomass. *Myriophyllum spicatum* also allocated more biomass at the mid- to lower depths than did *C demersum*. Plant growth significantly reduced light availability, particularly at depths of 20 cm or more from the surface. Both plants responded to depth by producing long shoots. The culture system is presently being used to examine competitive interactions between *E densa* and *P nodosus* on native Sacramento–San Joaquin sediments.

Spencer and Ksander<sup>20</sup> also examined light and canopy effects between two *Potamogeton* species (*P pectinatus* L and *P gramineus* L), and found that the broader and earlier leaf canopy of *P gramineus* effectively reduced available light to near light compensation levels of *P pectinatus*. These species are both found in western irrigation canals, but usually in separate (though often adjacent) populations. By comparing light levels in a canal beneath *P gramineus* canopies and outside the canopy, and by observing the extremely reduced biomass produced by *P pectinatus* planted in *P gramineus* beds, the authors make a strong argument that shading limits the extent of *P pectinatus* where *P gramineus* is present. However, since this canal, and many others are regularly treated with a general biocide (acrolein), the 'competition clock' gets re-set frequently, which may in part explain why *P gramineus* is not able to completely dominate these systems.

## 7 POPULATION AND DISTRIBUTION STUDIES

### 7.1 Rationale for research

The correct identification of species and the understanding of phylogenetic relationships is essential to understanding population interactions, potential outcrossing and to the pursuit of management of weedy species, particularly when native and exotic species are within the same genus. Furthermore, the use of host-specific biological control (see below also) requires accurate species identification of both host (target plant) and agent.

Madeira *et al*<sup>21,22</sup> used Random Amplified Polymorphic DNA analysis (RAPD) to discern the genetic proximities of several *H verticillata* accessions throughout the world. This procedure, first employed for hydrilla by Dr Fred Ryan at the Davis laboratory<sup>23</sup> provides a direct comparison of genomic DNA pieces and eliminates variables that can be associated with phenotypic plasticity so common in aquatic plants. Madeira's results showed that the California and Florida dioecious strains probably originated from the same Bangalore, India, populations, whereas the monoecious plants may be derived from the Seoul, Korea, populations. In addition to clarifying origins, these data also suggest that any further introductions should be immediately characterized to elucidate potentially new pathways of invasions. Van and Madeira<sup>24</sup> also used RAPDs analysis to examine *Ipomea aquatica* Forsk (water spinach), and were able to discern three

biotypes, but did not find evidence that this cultivated plant, which is a potential weed, has changed genetically, at least within the confidence of the sample size.

Jacono *et al*<sup>25</sup> presented evidence, based upon historic and present distributions, that although considered a native, *Salvinia minima* Baker may have, in fact, been introduced, and that the herbivorous insect *Cryptobagous salviniae* may have similarly been introduced. However, recent genetic analysis<sup>26</sup> suggests that the *C salviniae* found on *S minima* is different from the *C salviniae* originally released in Australia for control of *S molesta*. This emphasizes the need for accurate species identification and for distribution analysis, both of which will increasingly require molecular-level markers and genomic comparisons.

In another approach aimed at analyzing local plant distribution, Spencer and Ksander<sup>27</sup> used video recordings in the clear waters of the Fall River, CA to document species abundance and locations. Using the video recordings, voice recording of notations and a GPS unit, they generated distribution maps for several species over a 2-year period. This approach can reduce expensive field time and provide a readily accessible record of the status of plant populations, and is limited only by water clarity.

ARS scientists at the Southern Regional Research Center have also been developing methods to monitor populations distributions of algae that can affect aquaculture (ie taste, odor) and, using refined optical methods, the broader problem of harmful coastal algae blooms. For example, Dionigi *et al*<sup>28</sup> determined that incidence of off-flavors from algal sources is highly variable, and that current sampling and tasting criteria may not be adequate to identify problem ponds before total harvest. However, chemical analysis alone (ie for geosmin and MIB (2-methylisoborneol; 1-*R*-*exo*-1,2,7,7-tetramethylbicyclo[2,2,1]heptan-2-ol), the major constituents causing bad flavor) may not be sufficient due to patchiness of alga populations within ponds. Other work by Dionigi has suggested that treatments with copper algicides may cause releases of these products as well. A series of papers by Millie and co-workers<sup>29–31</sup> covers the methodologies, limitations and potential applications of pigment-based optimal scanning of natural algal populations. Of particular interest is the potential for tracking blooms of *Gymnodinium breve* Davis, the red tide organism, using absorption spectra derivatives, and the possibility of developing automated systems. This could provide an early warning of incipient blooms by detecting newly developing populations, which might facilitate ameliorative actions. The methods depend upon the presence of the carotenoid gyroxanthin-diester, and the ability to distinguish it from background using a 'liquid waveguide capillary cell' (LWCC), which affords a long optical path and provides the high sensitivity needed. This research sometimes does not get recognized as part of 'weed' control within ARS, yet it provides important tools to solve the very real 'pest' problem of red tide and its associated toxic effects.

## 8 DIRECT MANAGEMENT RESEARCH

### 8.1 Rationale for research

Aquatic weed control strategies rely on incorporating a basic understanding of the target weeds with their relationship to other species in the affected ecosystems, and developing practical methods to minimize (or in some cases eliminate) their impacts. Research at this level focuses on both laboratory and field-level projects and on technology transfer to wide-spread applications of methods.

### 8.2 Biological control and related research

Although this is discussed elsewhere in this issue, I have included some work below as it pertains directly to aquatic or riparian species.

#### 8.2.1 Water hyacinth *Eichhornia spp*

Center *et al*<sup>32,33</sup> have continued to work on the effectiveness of *Neochetina spp* for control of water hyacinth. For example, *N eichhorniae* Warner and *N bruchi* Hust were released at two Florida sites where one, two, three or four thousand weevils were released within *ca* 9 × 9-m PVC frames. The highest augmentations (3000 or 4000 insects) slowed mat growth rate to less than half compared with control (un-inoculated) sites. This effect was attributed to significant reduction in leaf area, thus reducing the photosynthetic capacity of the plants. Center and co-authors also showed that the integration of herbicides (ie 2,4-D) could work if sufficient non-sprayed areas (refuges) are left for weevils to over-winter and to provide sustainable populations of insects that will increase with increase in spring growth of the target plant. It interesting that the data also showed that, in areas where some herbicide maintenance was used, nutritional quality of the host plant was better (eg high N levels in tissues). This approach may be particularly important where very dense mats must be reduced initially through use of herbicides.

#### 8.2.2 Waterlettuce (*Pistia stratiotes L*)

This is another problematic floating weed which has also been the subject of a series of studies on aspects of host-plant quality.<sup>34–36</sup> These authors examined the effects on it of fertilizer (ie N, P, K) levels, and fecundity and larval feeding of the moth, *Spodoptera pectinicornis*. Higher nutritional levels resulted in larger insects and more egg production per adult; also low quality diets resulted in more single eggs compared with more clusters from adults fed better quality host plants. This work also showed that larvae on poor quality plants 'compensated' by increasing consumption rates over three-fold more than those of larvae on highly fertilized plants. Dray *et al*<sup>36</sup> summarize this work in the context of a large number of releases of the moth, and caution that a variety of conditions, not just food quality, may affect successful establishments of agents, particularly highly dispersive species such as moths. Wheeler and Halpern<sup>37</sup> also looked at compensatory feeding on *P stratiotes* by

*Samea multiplicalis*, another Lepidopteran, but noted that when fed on low-quality plants, development was delayed and that nitrogen consumption was also significantly less in these insects than in those fed on *P stratiotes* fertilized at higher rates. Wheeler and Center<sup>38</sup> also examined the effects of host quality and responses of *Hydrellia pakistanae* in several Florida sites where they collected the target weed, *Hydrilla verticillata*. They found that leaf whorls from sites with higher nitrogen and softer tissues (measured by a leaf penetrometer) were associated with lower mortality and shorter developmental times from the neonate larvae to adults. They also noted a preference of the instars in the poorer quality plants for the younger leaves at the tips of shoots. These studies, as well as those on *E crassipes* and *P stratiotes*, clearly shows the importance of examining variation in host-plant quality as part of pre-release field assessments.

### 8.2.3 *Melaleuca quinquenervia* (Cav) ST Blake

The control of *Melaleuca quinquenervia*, an invasive wetland tree native to Australia, has been a major focus of biological control research for ARS,<sup>39</sup> including studies on the biology and reproductive characteristics of the tree,<sup>40</sup> standing crop<sup>41,42</sup> and foreign explorations and evaluations of biological control agents such as the weevil, *Oxyops vitiosa* (snout beetle) and *Lophyrotoma zonalis* (sawfly). Wheeler<sup>43</sup> found that *O vitiosa* larvae reared on leaves from branches that had emerging buds and higher N levels had higher survival rates than those on leaves from branches with dormant buds. Wheeler and Zahniser<sup>44</sup> also developed an artificial diet for this species that out-performed diets of *M quinquenervia* leaves. Montgomery and Wheeler<sup>45</sup> also found that a viscous, orange material coats the larval stage *O vitiosa*, which apparently protects it from some ant predators. Center *et al*<sup>46</sup> reported on the successful establishment of this weevil in South Florida following a 2-year assessment, although dispersal was quite limited. Buckingham<sup>47</sup> reported on the host range of the sawfly within 36 species in the Myrtaceae family as well as 18 species in other families and concluded that the host range is very narrow; eggs were laid on 23 Myrtaceae species, but only three supported development to adults and those adults did not reproduce.

Rayachhetry *et al*<sup>48</sup> also reported on the potential for integrating herbicides (eg imazapyr) with a native fungus, *Botryosphaeria ribis* Grossenb & Duggar and *Puccinia psidii*, a rust fungus.<sup>49</sup> Efficacy was variable, but *B ribis* could cause defoliation in some cases, and, with the application of imazapyr, regrowth was arrested. Testing of the host range of *P psidii* showed that only *M quinquenervia* and two other exotic members of the Myrtaceae were severely affected, though others were susceptible.

### 8.2.4 Microbial products

The use of biological control agents for algae control is, in general, a sparsely studied area. However,

there are 'microbial' products available on the market that purport to improve problems related to algae and to generally improve management of lakes and ponds. Since these products carefully avoid 'algicidal' claims, they have not been subject to the US EPA review required for registration of other algicides such as copper-based products. DuVall and Anderson<sup>50</sup> and DuVall *et al*<sup>51</sup> were the first to examine these products in replicated studies aimed at determining whether, and how, these bacterial and bacterial/enzyme products function. In both small-scale laboratory systems and in larger outdoor in-pond mesocosms, none of these products lowered levels of algae or macrophytes, whereas in the same mesocosm systems, EPA-label algicides did. The stated 'mode of action' of the microbial products is that introduced bacteria multiply rapidly and sequester available nutrients thereby making them unavailable for algal growth. The products examined did produce transient elevations in water column bacteria; however, there was no correlation with macroalgae or phytoplankton production, or macrophyte growth. Since there is an increasing demand for 'non-chemical' alternatives for algae control, product claims need much better substantiation. The next step is larger-scale testing using whole ponds (or small lakes), coupled with analysis of nutrient availability and bioassays that utilize naturally occurring algae.

## 8.3 Chemical control research

Herbicides still constitute a major component of all aquatic weed control. However, there has been only one new active ingredient registered for use in water as a herbicide (triclopyr in 2002) and no new algicide in over 15 years, and a few have not been re-registered during the same period. The ARS research emphasis in this area is on improving efficacy and efficiency of existing materials, investigating potential for new, safer materials and use of natural products, while reducing adverse effects, and integrating methods that overall reduce reliance on chemicals. The main problem for submerged weeds is the difficulty in localizing and maintaining exposures in the target area due to the typically large volumes of water and water movement, even in lakes and ponds. For emergent and floating weeds, the problem is mainly associated with inadequate uptake at the leaf surface not water movement since applications are made aerially to foliage above water.

Anderson<sup>8</sup> investigated methods for providing adequate contact time of herbicides for controlling *E densa* in tidal and flowing waters in the Sacramento–San Joaquin Delta. This work relied upon use of the fluorescent, water-soluble dye, Rhodamine WT, and extensive water and target-plant sampling studies aimed at determining levels of copper following applications. This work was part of an Environmental Impact Report and was used to develop management methods by the responsible state action agency, California Department of Boating and Waterways

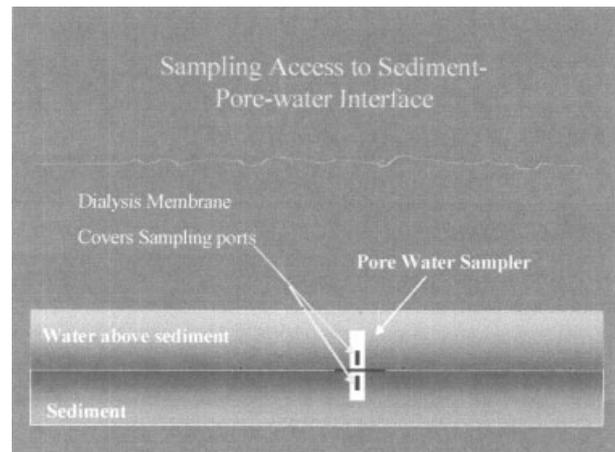
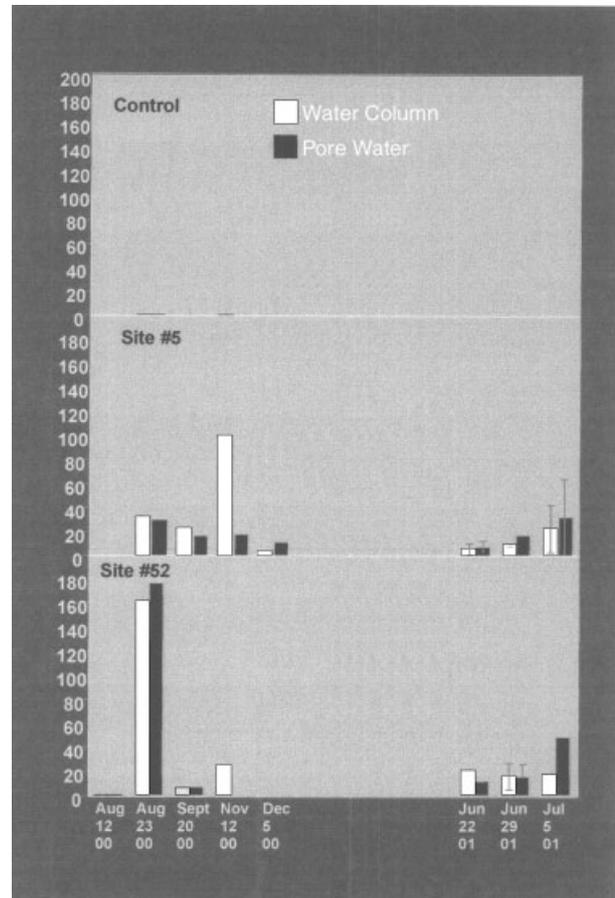
**Table 4.** Dissipation of Rhodamine WT dye in typical egeria-infested sites in the Sacramento–San Joaquin delta (from Reference 52)

Site	Half-life (h)	Estimated 'wash-out' time (h)
White Slough	8	35
Owl Harbor	2–4	12–14
Sandmound Slough	18–20	30–35
Franks Tract	6–7	30–32
Big Break Marina	20–24	40–45
Venice Island	8–10	15–20
Pixley Slough	20–24	90+

(CDBWW) for their *Egeria densa* Control Program.<sup>52</sup> Residence times of the dye (Table 4) were determined in several Delta sites and these data were used to match optimal uses of different, available herbicides (eg chelated copper, fluridone and diquat). Due to the tidal flows, net river flows and the complex hydraulics of the Delta, it is clear that an understanding of the local water movement in and around *E densa* sites is essential for developing strategies to control this plant with herbicides. For example, fluridone requires 6–8 weeks of exposure, while diquat (or copper-containing herbicides) generally require only a few hours. Analysis of copper levels in the water showed that background (pre-treatment) levels were usually attained within 24 h (four tidal cycles) post-application, and that maximum copper uptake in target plants was achieved within 4–6 h after application. The use of dye has been incorporated into ongoing water quality monitoring protocols used by CDBWW in its water hyacinth and *E densa* control programs.

In cooperation with the California Department of Agriculture (CDFA) hydrilla eradication program, Anderson has been using paired-plexiglass dialysis chambers to investigate herbicide levels in sediment pore (interstitial) water and water immediately above the sediment, resulting from repeated applications of pelleted fluridone in Clear Lake, CA. This system relies upon equilibration of dissolved fluridone between the chambers and adjacent interstitial water (Fig 3). Figure 4 shows data from sites in Clear Lake and demonstrates the variability of residues. The results suggest that the pellets may produce a patchy array of herbicide 'release points', and that better distribution may be obtained from lower loading rates, thus providing more pellets per unit area. The data also confirm that bottom applications can produce high enough fluridone residues in the sediment-water to control emerging hydrilla tubers.<sup>53</sup>

In other work aimed at hydrilla eradication, as well as development of alternative natural product herbicides, Spencer and Ksander<sup>54</sup> have followed up their earlier laboratory work showing that very low concentrations of acetic acid (eg 2–5% by volume) can kill hydrilla tubers and winter buds of *P nodosus*. At these concentrations (equal to or less than that of household vinegar), acetic acid

**Figure 3.** Diagram of sampler used to determine levels of herbicides in the pore (interstitial) water. The system is inserted using a telescoping pole and is later retrieved (one to four weeks after deployment) with lines that are attached to floating buoys.**Figure 4.** Fluridone residues in pore water (lower port in Fig 3) and immediately adjacent to the sediments (upper port in Fig 3). Sites are in Clear Lake, CA (from Reference 51).

applied to the bottoms of dewatered (drained) canals can greatly reduce subsequent sprouting. They have shown that hydrilla tuber banks can be reduced in a small irrigation system that is included in CDFA's eradication program. Once methods are developed for sufficient distribution of acetic acid on the canal sides, this approach could be extremely valuable as an

off-irrigation-season method to stop spring/summer weed growth and to reduce propagule 'banks' over time. Since acetic acid is a natural product, and in fact a food, and easily metabolized, residues should not persist and environmental concerns should be minimal.

At the molecular level, very recent work at the ARS Natural Product Utilization Unit in Oxford, Mississippi, has elucidated the genetic basis for tolerance to fluridone in hydrilla.<sup>55,56</sup> This research by ARS scientist Brian Sheffler demonstrated a unique transcript responsible for the decreased efficacy of fluridone in some hydrilla populations and may provide a very useful model for understanding genetic variations in clonal plants. It may also provide a tool for predicting susceptibility of hydrilla infestations prior to herbicide application. If this approach proves reliable, it may add another method to assess the most appropriate types of herbicide (ie modes and sites of action) with other management approaches (eg biological control) to reduce the likelihood of tolerance developing. The research illustrates the effective use of cutting-edge genomic analysis to address very practical problems in developing sustainable, integrated aquatic plant control.

In a tri-state study, Anderson collaborated with colleagues in the Army Corps of Engineers and private industry<sup>57,58</sup> to examine the efficacy, fate and non-target effects of triclopyr, a selective herbicide that has excellent potential for selective control of *M spicatum*, *Eichhornia crassipes* Solms, *Lythrum salicaria* L, and possibly *Lepidium latifolium* L (Perennial pepperweed). This work included analysis of plants, water quality, water residues and uptake into two fish species, and was submitted to the US EPA as part of the overall registration application. With the recent (2002) US EPA registration of 'Renovate' (triclopyr) a new tool for integrated aquatic and riparian weed management will be available for the first time in nearly 20 years. For example, it will enable reduction or management or removal of *M spicatum* populations while not affecting several native pondweeds, algae or other non-susceptible macrophytes.

In another international collaborative research and technology transfer, Anderson and Australian scientists developed an aquatic management program aimed at relieving the A\$40-million (ca US\$75-million) 2000 Olympic water-sports venue (Sydney International Regatta Centre) of massive weed growth.<sup>59</sup> In this system, critical for rowing and kayaking competition, *H verticillata* and *V americana* were controlled with a series of fluridone applications coupled with extensive monitoring. Through meetings with Australian environmental regulators and scientists as well as deployment of equipment and on-site training, this technology provided a solution to potential crisis and enabled the Olympic races to be held on time.

Responding to another recent aquatic 'weed' crisis, ARS has been part of a multi-agency effort to

eradicate the first known introduction of the exotic alga, *Caulerpa taxifolia* (Vahl) C Agard in the northern hemisphere near San Diego, CA.<sup>60,61</sup> This marine alga has decimated nearly 30 000 acres of benthic (sub-tidal) environments in Mediterranean coastal areas over the past 15 years. Its presence threatens, and can potentially spread to, hundreds of miles of Californian coastal habitat and to Mexican waters as well. Anderson, as part of the Southern California Caulerpa Action Team (SCCAT) helped develop eradication methods, and has assessed the efficacy of treatments, which include using containment tarps beneath which chlorine is placed, either as a liquid formulation or solid chlorine-generating tablets. Using sediment cores from treated sites, Anderson showed that no *C taxifolia* survived and that, in fact, seedlings of the native eelgrass emerged in sediment from treated areas.<sup>62</sup> Due to the complex nature of the infested site, and its proximity to the open California coast, successful eradication is essential, even though it will cost at least US \$1 million per year over the next 5 years.

## 9 SUMMARY AND CONCLUSIONS

This review, although encompassing only the past few years, illustrates the wide breadth of expertise and approaches being taken by ARS scientists, even within a few locations. It is clear that emerging research areas will include: host-plant and biological control-agent interactions (host quality in particular); plant-plant interactions (particularly native versus exotic plants); optimizing use of herbicides and exploring potentials for natural-product-based herbicides; and in general, developing and adapting cutting-edge technologies for detection and monitoring invasive species and for building growth models that encompass canopy architecture. Furthermore, the regulatory climate in the USA regarding aquatic pesticide use has changed dramatically following decisions of the Ninth District Court of Appeals in 2001. This appellate court determined (*Tallant Irrigation District vs Headwaters*) that aquatic pesticides used in water constitute a 'waste discharge' under the US EPA's Clean Water Act, thereby necessitating greatly increased monitoring as part of the issuance of permits under NPDES (National Pollution Discharge Elimination System).<sup>63</sup> Therefore, there will be accelerating demands for alternative methods as well as for improved efficiency of current herbicide uses.

ARS scientists have focused their time and resources on the most pressing aquatic plant pest problems. Although research spans basic biology and ecology, the complexities of aquatic systems require a keen awareness of, and appreciation for, practical approaches that are feasible. For this reason, field work and close cooperation and collaboration with federal, state and local action agencies remain an essential component of the research strategies. Through this network, as well as ties with private

industry and university scientists, ARS research projects maintain focus and direction relevant to a wide range of stakeholders. Furthermore, though the many other types of related activities that ARS scientists engage in are not discussed here, these range from outreach and education to active participation in many scientific societies that are concerned with aquatic plant management, wetland ecology and aquatic site restoration. Perhaps one of the hallmarks of this—and other ARS weed programs in general—is flexibility and a proactive culture that encourages scientists to respond quickly to new challenges and new opportunities as they arise.

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