Seasonal Changes in Chemical Composition of Eurasian Watermilfoil (Myriophyllum spicatum L.) and Water Temperature at Two Sites in Northern California: Implications for Herbivory

DAVID F. SPENCER AND GREGORY G. KSANDER

ABSTRACT

We compared seasonal changes in Eurasian watermilfoil (Myriophyllum spicatum L.) characteristics and water temperature for a shallow pond in Davis, CA, and the Truckee River, near Tahoe City, CA. Tissue C and N were 15% lower in plants from the Truckee River than in plants from the Davis pond. Seasonal fluctuations in tissue N were also different. Mean phenolic acid content of Truckee River plants (162 ± 0.012 mg g⁻¹) was less than those from the shallow pond (195 ± 0.012 mg g⁻¹). Phenolic acid content was positively related to tissue C for Truckee River plants and, tissue C:N ratio for Davis pond plants and, tissue C:N ratio for Truckee River plants. Mean monthly water temperature (1990 to 1998) for the Truckee River site was less than 20 C. Water temperatures were warmer in August and September at this site. However, Eurasian watermilfoil collected during these months was characterized by lower levels of tissue N. During a 29-month period beginning January 1994, mean monthly water temperature for the Davis pond exceeded 20 C, only during July to September 1995. Tissue N was generally greater during summer for watermilfoil growing in the pond. These results imply that Eurasian watermilfoil biological control agents may have different developmental rates in these habitats, and thus different impacts on watermilfoil populations.

Key words: aquatic weed, tissue C, tissue N, phenolic acids, Truckee River

INTRODUCTION

Eurasian watermilfoil (Myriophyllum spicatum L.) is an important introduced aquatic weed in North America, where it is found from Florida to Quebec in the east, and California to British Columbia in the west (Couch and Nelson 1985). Its strong competitive abilities allow it to displace native species (Madsen et al. 1991), and its abundant biomass impedes water use for environmental, economic, and recreational purposes (Newroth 1985). Current management techniques include mechanical and chemical methods (Smith and Barko 1990). Recently, a native weevil Eucryptophagus lecontei (Di- eta.), found in the northern U. S. and southern Canada, has been suggested as a possible biological control for this plant (Creed et al. 1992, Creed and Sheldon 1993, Creed and Sheldon 1994a,b, Creed and Sheldon 1995, Creed and Sheldon 1995, Newman et al. 1996, Creed 1998).

A number of factors influence the success of insects, employed as weed biological control agents, by regulating their abundance or the plant’s response to them (Newman et al. 1998). Weather conditions and plant quality are two important factors among others (Newman et al. 1998). Temperature is an important factor in insect development. For example, E. lecontei’s development time decreased as the temperature increased from 19 to 24 C (Newman et al. 1997). Plant quality also affects the herbivore’s ability to persist and reproduce. Previous research with two insects (Hydrilla paki-stanicae and Bagnus hydriella) that feed on the submerged weed hydrilla, (Hydrilla verticillata (L.f.) Royle) showed that plant N content strongly influenced insect growth and development rates (Wheeler and Center 1996, Wheeler and Center 1997). This appears to be true for many additional weevil species as well (Cram 1965a,b, Hillard and Keeley 1984a,b, Sands et al. 1983, Room et al. 1989, Hunt et al. 1995). Another aspect of plant quality that may influence the way herbivores or pathogens interact with a particular plant is the presence of defensive compounds, such as phenolic acids. Harrison and Durance (1989) point out that small increases in phenolic content of leaves can have large effects on potential consumers. For example, Geiselman and McConnell (1981) reported that feeding by Littorina littorea on two species of algae was reduced by more than 50% when the algal material contained 1% dry weight of phenols. When the phenolic content was increased to 2 to 5%, feeding was inhibited completely. There are also several examples of growth inhibition of microbes by phenolic compounds (Waterman and Mole 1984, Gross et al. 1996).

Eurasian watermilfoil occurs in California, but E. lecontei has not been reported from the state (Creed 1998). Therefore, the nature of the potential interaction between E. lecontei and Eurasian watermilfoil under California conditions is unknown. Additionally, most studies on the ecology of Eurasian watermilfoil have been conducted in the eastern U.S. (Grace and Wetzel 1978, Smith and Barko 1990). With the exception of work done in British Columbia (Newroth 1985) and Washington State (Perkins and Sytsma 1987, Getzinger...
et al. 1997), there is little published information on environ-
ment conditions in western lakes and rivers inhabited by
Eurasian watermilfoil. Thus, as a step toward evaluating the
potential of E. lecontei as a biological control agent, we mea-
sured water temperature and water quality parameters during
this study, the following values are typical

\[ \text{Total inorganic P, } 60 \mu g \text{l}^{-1} \]

\[ \text{NH}_4-N, < 50 \mu g \text{l}^{-1} \]

\[ \text{NO}_3-N, 80 \text{ to } 120 \mu g \text{l}^{-1} \]

\[ \text{NH}_3-N, 80 \text{ to } 120 \mu g \text{l}^{-1} \]

\[ \text{NO}_2-N, < 50 \mu g \text{l}^{-1} \]

\[ \text{Total alkalinity} \]

The two sites were two shallow pond located at the USDA-
ARS Exotic and Invasive Weed Research Unit in Davis, CA, and
1.5 m by 10 m. It had a trapezoidal cross section and was 1.5 m

Mean monthly water temperature for the Truckee River
site was less than 20 C for the eight-year data set; warmest wa-
ter temperatures occurred in August and September (Figure
1). Using this data set we estimated accumulated degree-days
for the Truckee River. Given the 309 degree-day requirement
for development from egg to adult for the watermilfoil weevil
(Mazer et al. 1996), the data indicate that if temperature is
assumed to be the limiting factor, then just over two genera-
tions of weevils would be able to develop in the Truckee Riv-
er. During a 26-month period beginning January 1994, mean
monthly water temperature for the Davis pond exceeded 20
C only during July and August (Figure 1). However, because the
water in the pond warmed earlier in the year, the num-
ber of accumulated degree-days was considerably greater in
this habitat. From three to six generations of weevils should
be able to develop at this site depending on year-to-year vari-
ation in temperature. Temperature differences for the pond
and river sites imply that the potential biological control
agent, the watermilfoil weevil, may have different develop-
mental rates in these habitats.

Mean tissue C, over all samples, was higher for plants from
the Davis pond than for plants from the Truckee River (Ta-
ble 1). Tissue C fluctuated measurably through time in both
the pond and river populations and discernible patterns
were difficult to identify. In the pond, Eurasian water-
milfoil tissue C was lowest during late May, late July, and late
September (Figure 2). Tissue C for Truckee River plants was
lowest in December 1997 and May 1998 (Figure 3).

Patterns of seasonal change in tissue C were not as clear as
those reported for total nonstructural carbohydrates in up-
per stems of a southern population of Eurasian watermilfoil
(Madsen 1997). However, Madsen (1997) reported that low
levels of stored carbohydrates occurred in July, April, and
May over the three-year period of his study. He also reported
that a secondary low level of carbohydrates occurred in Octo-
ber for all three years and noted the absence of this second-
ary low point for northern populations of Eurasian

mors for Truckee River water. Fox (1982), using data from
140 samples collected at sites in the upper 22 km of the river
from January, 1968 to December, 1980, estimated alkalinity
as 48.4 mg l\(^{-1}\) in non-drought years. Measurements of pH
from Fox (1982) taken at a site 2 km downstream from its inlet
are site 3 were between 7.0 and 7.8. Flow varies considerably with
season, in a typical water year the mean flow at Tahoe City is
8 m\(^3\) s\(^{-1}\) and legal April. May and July and as low as 5
m\(^3\) s\(^{-1}\) in January, 1968, and 1.9 to 2.6 m\(^3\) s\(^{-1}\) be maintained below the dam at Tahoe City, CA.

Elementary statistics for tissue C, tissue N, and total phe-
nolic acid content were calculated using normal least-squares procedures following Draper and Smith
(1981). We estimated degree-days for both the pond and riv-
er data sets using the averaging method as described in
Zalom et al. (1985). In this method the lower threshold tem-
perature is subtracted from the daily average temperature to
yield the number of degree-days for each day. This method
does not incorporate an upper threshold temperature and
thus may overestimate degree-days on unusually warm days.

RESULTS AND DISCUSSION

Mean monthly water temperature for the Truckee River
site was less than 20 C for the eight-year data set; warmest wa-
ter temperatures occurred in August and September (Figure
1). Using this data set we estimated accumulated degree-days
for the Truckee River. Given the 309 degree-day requirement
for development from egg to adult for the watermilfoil weevil
(Mazer et al. 1996), the data indicate that if temperature is
assumed to be the limiting factor, then just over two genera-
tions of weevils would be able to develop in the Truckee Riv-
er. During a 26-month period beginning January 1994, mean
monthly water temperature for the Davis pond exceeded 20
C only during July and August (Figure 1). However, because the
water in the pond warmed earlier in the year, the num-
ber of accumulated degree-days was considerably greater in
this habitat. From three to six generations of weevils should
be able to develop at this site depending on year-to-year vari-
ation in temperature. Temperature differences for the pond
and river sites imply that the potential biological control
agent, the watermilfoil weevil, may have different develop-
mental rates in these habitats.

Mean tissue C, over all samples, was higher for plants from
the Davis pond than for plants from the Truckee River (Ta-
ble 1). Tissue C fluctuated measurably through time in both
the pond and river populations and discernible patterns
were difficult to identify. In the pond, Eurasian water-
milfoil tissue C was lowest during late May, late July, and late
September (Figure 2). Tissue C for Truckee River plants was
lowest in December 1997 and May 1998 (Figure 3).

Patterns of seasonal change in tissue C were not as clear as
those reported for total nonstructural carbohydrates in up-
per stems of a southern population of Eurasian watermilfoil
(Madsen 1997). However, Madsen (1997) reported that low
levels of stored carbohydrates occurred in July, April, and
May over the three-year period of his study. He also reported
that a secondary low level of carbohydrates occurred in Octo-
ber for all three years and noted the absence of this second-
ary low point for northern populations of Eurasian

watermilfoil. In this respect, the September low points in tissue C observed in the Davis plants suggests that this aspect of the phenology of the Davis plants was similar to that of the southern population studied by Madsen (1997). Conversely, the pattern of tissue C for Truckee River plants more closely resembled patterns reported for northern populations of Eurasian watermilfoil (Madsen 1997).

Overall mean tissue N was 15% lower for plants growing in the Truckee River than for those growing in the Davis pond (Table 1). Seventy-five percent of all tissue N measurements were less than 2.43% for the pond population and less than 2.04% for Truckee River plants. Some tissue N values for plants from the Davis pond and Truckee River were within or below the range reported by Grace and Wetzel (1978), for Eurasian watermilfoil (1.40% to 6.29%).

Patterns of seasonal fluctuation in tissue N were also different for plants from the two sites (Figures 2 and 3). For Davis plants, tissue N was highest from January through March, declined to its lowest point in April through June, and increased slightly during summer (Figure 2). Tissue N for Truckee River plants was greatest in spring/early summer and declined sharply in late summer (Figure 3). Compared to Truckee River plants, tissue N was generally greater during summer for watermilfoil growing in the Davis pond. The pattern of tissue N for plants from the Truckee River was similar to that reported for plants from a Wisconsin lake. Carpenter and Adams (1977) measured tissue N for Eurasian watermilfoil at 15 sites in Lake Wingra between May and September 1975. They reported that tissue N was high in May and subsequently decreased and remained relatively constant during the rest of the growing season. In contrast, the Davis pond plants displayed two peak tissue N concentrations, one January through March, and a second during late summer.

Based on published results with another aquatic weevil, the differences in tissue N for milfoil growing in the pond and river would very likely contribute to different weevil development rates at the two sites. For example, a weevil that feeds on hydrilla increased its relative growth rate by 50%
when fed plant material with 3.5% N versus plant material with 2% N (Wheeler and Center 1997). Since mean tissue N for the Davis plants was only slightly above 2%, weevils might not be expected to develop at the maximum rate in the pond, even though the thermal environment is favorable. Similarly, weevil development in the Truckee River might be expected to be lower than the maximum rate and might even be less due to a combination of low temperatures and low levels of tissue N in resident Eurasian watermilfoil plants.

Phenolic acid content of Truckee River plants was less than for those from the pond (Table 1). For Davis plants, phenolic acid content was greatest during the winter (November through March) and was considerably less during the rest of the year (Figure 2). Phenolic acid content of Davis plants was positively related to tissue C and tissue N, but not tissue C:N ratio (Table 2). Seasonal changes in phenolic acid levels for Truckee River plants were different. Phenolic acid content was lower in December than at other times during the year (Figure 3). Even so, phenolic acid content increased at higher levels of tissue C or at higher tissue C:N ratios, but not tissue N (Table 2).

Phenolic acids may serve as defense against attack by herbivores or microbes. The levels of phenolic acids reported here are less than those reported for Eurasian watermilfoil by Planas et al. (1981). Planas et al. (1981) found a mean phenolic content of 7% and a maximum of 30% for Eurasian watermilfoil grown in laboratory cultures. Gross et al. (1996) reported total phenolic acid content of about 10% for Eurasian watermilfoil collected throughout the summers of 1991 to 1994 from a mesotrophic lake in northern Germany. When converted to percent of dry weight, the mean values reported here were 1.79% and 2.11% for watermilfoil from the Truckee River and Davis pond, respectively. Likewise, the maximum values were 4.26% and 8.99%, on a dry weight basis. During the growing season (April through September) phenolic acid content was similar in plants from both habitats. However, plants from the pond had higher levels during the winter than those in the Truckee River. This pattern differs somewhat from that reported for the marine macrophyte, eelgrass (Zostera marina L). Harrison and Durance (1989) examined seasonal variation in phenolic content of eelgrass shoots. They reported that the concentration of phenolic acids varied seasonally from a low in late winter/early spring of 0.65% dry weight to a high at the end of the active growing season of 1.54% dry weight.

Several factors cause variation in production of plant phenolic acids, among these is mineral nutrition. With respect to N nutrition, it has been generally observed that lower levels of phenolic acids are produced when the N supply is abundant (Waterman and Mole 1994). Thus, we would expect an

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C (%)</th>
<th>N (%)</th>
<th>Phenolic Acid (µM g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Truckee River</td>
<td>Davis Pond</td>
<td>Truckee River</td>
</tr>
<tr>
<td>Number of Samples</td>
<td>140</td>
<td>777</td>
<td>140</td>
</tr>
<tr>
<td>Mean</td>
<td>33.00</td>
<td>38.02</td>
<td>1.78</td>
</tr>
<tr>
<td>Lower 95% CL</td>
<td>32.10</td>
<td>37.81</td>
<td>1.70</td>
</tr>
<tr>
<td>Upper 95% CL</td>
<td>33.90</td>
<td>38.24</td>
<td>1.85</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>5.14</td>
<td>3.02</td>
<td>0.45</td>
</tr>
<tr>
<td>Minimum</td>
<td>16.45</td>
<td>19.54</td>
<td>0.74</td>
</tr>
<tr>
<td>25% Quantile</td>
<td>30.72</td>
<td>39.70</td>
<td>2.04</td>
</tr>
<tr>
<td>Median</td>
<td>34.61</td>
<td>38.20</td>
<td>1.77</td>
</tr>
<tr>
<td>Mode</td>
<td>35.72</td>
<td>36.35</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Figure 2. Phenolic acid content (µM g⁻¹), tissue N (%), and tissue C (%) for Eurasian watermilfoil in the Davis pond during 1994 to 1996. Error bars represent plus or minus 1 standard error, n = 10.
inverse relationship between phenolic acid content and tissue N. This was not the case for Eurasian watermilfoil from either the Davis pond or the Truckee River (Table 2). A second generalization that apparently holds true for Eurasian watermilfoil, at the sites examined here, is that phenolic acid content increases with increasing tissue C. A corollary observation is that at high C:N ratios phenolic acid content should increase (Waterman and Mole 1994). The significant positive relationships between phenolic acid content and tissue C agree with previously reported data from a variety of plants, but to our knowledge this is the first time such a relationship has been reported for Eurasian watermilfoil.

The results of this study imply that the watermilfoil weevil should not be expected to have the same impact on watermilfoil populations at all sites in northern California because of differences in plant quality and water temperature. These results underscore the importance of understanding the relationship between Eurasian watermilfoil quality, water temperature, and development rate for the watermilfoil weevil. The information from this study can be used to design ecologically realistic experiments for assessing this relationship. Additionally, these data indicate that Eurasian watermilfoil growing in high light environments, with abundant photosynthate, may be better able to defend against herbivore or microbial attack due to higher levels of phenolic acids.

ACKNOWLEDGMENTS

We appreciate the comments of Dr. Larry Mitich, Dr. Tom Lanini, and Dr. Robert Blank who read an earlier version of this manuscript. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

LITERATURE CITED


Figure 3. Phenolic acid content (µM g-1), tissue N (%), and tissue C (%) for Eurasian watermilfoil in the Truckee River during 1997 and 1998. Error bars represent plus or minus 1 standard error, n = 15.

Table 3. Regression Equations for Phenolic Acid Content (µM g-1) versus Tissue C (%), Tissue N (%), or Tissue C:N Ratio. The column labeled "P" gives the probability of a greater t-statistic for a test of the null hypothesis that the slope equals zero.

<table>
<thead>
<tr>
<th>Site</th>
<th>Equation</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis Pond</td>
<td>Phenolics = -275.91 + 12.37 C</td>
<td>0.10</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Phenolics = -122.43 + 34.32 N</td>
<td>0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>Davis Pond</td>
<td>Phenolics = 123.58 - 32.93 C</td>
<td>0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>Truckee River</td>
<td>Phenolics = 63.2 + 5.10 C:N</td>
<td>0.06</td>
<td>0.005</td>
</tr>
</tbody>
</table>


Creed, R. P., Jr., S. P. Sheldon and D. M. Cheek. 1992. The effect of herbi-

Madsen, J. D., J. W. Sutherland, J. A. Bloomfield, L. W. Eichler and C. W. Waters.

Harrison, P. G. and C. Durance. 1989. Seasonal variation in phenolic con-


Hilliard, R. A. and L. L. Keeley. 1984b. The effects of dietary nitrogen on

Boylen 1991. The decline of native vegetation under dense Eurasian

35: 15-21.

32: 189-200.


16: 1-11.


Neumann, M. A. and D. M. Bronte. 1987. Harvesting and carbohydrate ac-

Neuweiler, F. R. 1993. A review of Eurasian watermilfoil impact and manage-

New Zealand. Proc. 1st International Symposium on water-


Zalom, F. G., P. B. Goodell, W. W. Barnett and W. J. Bentley. 1985. Degree-days, the calculation use of heat units in pest manage-

Regulated Rivers: Res. & Man-

Zalom, F. G., P. B. Goodell, L. T. Wilson, W. W. Barnett and W. J. Bentley. 1985. Degree-days, the calculation use of heat units in pest manage-


Newfoundland and Labrador. Proc. 1st International Symposium on water-


Ramos, D. F. Buhan, L. Dohe, H. Godmane and C. Carleis. 1981. Ecologi-


Sands, D. P. A., M. Schotz and A. S. Bourne. 1983. The feeding characteris-

Sands, D. P. A., M. Schotz and A. S. Bourne. 1983. The feeding characteris-


Wheeler, G. S. and T. D. Center. 1997. Growth and development of the bio-

Hydrel-

Zalom, F. G., P. B. Goodell, L. T. Wilson, W. W. Barnett and W. J. Bentley. 1985. Degree-days, the calculation use of heat units in pest manage-

University of California, DANS, Leaflet 21575, 10 pp.