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Feeding Activity and Host Preference of the Milfoil Midge, *Cricotopus myriophylli* Oliver (Diptera: Chironomidae)

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ABSTRACT

By the early 1980's several well-established weed beds of *Myriophyllum spicatum* L. in the Okanagan Valley lakes system of British Columbia, Canada failed to surface and flower as they had previously. This failure was found to be the result of feeding damage by the larvae of a chironomid, *Cricotopus myriophylli* Oliver. Larvae of *C. myriophylli* become established on the apical portions of *M. spicatum* stems, construct cases and feed on the meristematic tissue, suppressing the plant's growth. One larva is capable of cropping one meristem of *M. spicatum*, suppressing growth within one week of its introduction. Additional trials indicated the larvae are very host-specific to *Myriophyllum* species.

Key words. Myriophyllum spicatum, British Columbia, Okanagan Valley lakes, biocontrol, natural predators.

INTRODUCTION

Eurasian watermilfoil, *Myriophyllum spicatum* L., was first introduced into British Columbia in the Vernon Arm of Lake Okanagan about 1970 (Aiken *et al.* 1979). When first noted it occupied approximately 20 ha and by 1987 approximately 2000 ha of littoral zone were infested (Newroth pers. comm.) In the late 1970's and 1980, several well-established weed beds of *M. spicatum* in the Okanagan Valley lakes system failed to surface and flower. Researchers found this failure to be the result of feeding damage by larvae of a chironomid (B.C. Ministry of Environment 1979, Kangasniemi 1983, Kangasniemi and Oliver 1983) which was later named *Cricotopus myriophylli* (Oliver 1984). Larvae of *C. myriophylli* become established on the apical portions of *M. spicatum* stems, construct cases and feed on meristematic tissue located there (B.C. Ministry of Environment 1981, Kangasniemi 1983, Kangasniemi and Oliver 1983, Oliver 1984). Denuding the plant of growing tissue in this manner could result in suppressing *M. spicatum* growth to an economically acceptable level.

Laboratory trials were conducted to ascertain the number of *C. myriophylli* larvae per meristem necessary to suppress *M. spicatum* growth, how quickly growth could be suppressed, and the host preferences of the insect.

METHODS AND MATERIALS

Feeding damage trials. Experiments to estimate the optimum number of larvae per meristem necessary to suppress plant growth were conducted in four 76 liter aquaria, each separated into 4 cells with a measured stem of *M. spicatum* planted per cell. Randomly into each cell were placed 1, 2, or 3 larvae, with the remaining cell left devoid of larvae as a control. Field observations indicated that more than two larvae rarely become established on the same meristem. Larvae used in the trial were early- to mid-third instars and were field collected. After one week the stems were removed and new growth was measured. This process was repeated with fresh plants and insects to provide for 8 replications of each treatment and control. A one-way ANOVA was used to determine if there was a significant difference among groups and a Newman-Keuls test was conducted to test which groups differed (Zar 1984).

Viability of those tips fed-upon by larvae were assessed by clipping all meristems (n = 32) from their stems, simulating natural abscission. These were planted (16 into the substrate, and 16 placed onto the substrate to simulate sinking after abscission) in a 37 liter aquarium prepared with 0.5-1 cm potting soil, covered with 2-4 cm of lake sediment, and the aquarium filled with lake water. This aquarium was aerated, maintained at 20 C and checked at weekly intervals for 2 months.

Growth suppression trials. The amount of growth which was suppressed by the feeding of *C. myriophylli* was assessed using a 2-way ANOVA design which allowed for replication and complete randomization. Ten 76 liter aquarium were each separated into 8 cells, all of the 80 cells were planted with a stem of *M. spicatum* standardized to 12 nodes in length with approximately the same amount of meristematic material. Four cells in each aquarium were then chosen at random and a larva of *C. myriophilli* was placed on the plant in each of those cells. The larvae were field collected mid- to late-second instars. All the plants and insects from 2 aquaria (8 treated and 8 untreated cells) were removed at 2 day intervals for 10 days and the new growth of the plant was removed. New growth was assessed as any increase in length over the 12-node starting length or any newly-developed meristematic tissue including axillary tips. The new growth was dried at 70 C for 48 hours and weighed with a Mettler HP10 analytical balance. The aquaria then were replanted with standardized plants and fresh second instar larvae; the entire procedure was re-

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peated 5 times providing for 40 replications of both treatments and controls at each 2-day interval.

Feeding preference trials. The feeding of *C. myriophylli* on 12 species of native aquatic macrophytes was tested. The plant species tested were recommended by biologists with the B.C. Ministries of Environment, and Agriculture and Fisheries as those thought to be important in the rearing of sportsfish (Table 1). The suitability of these species as potential food sources for *C. myriophylli* was first tested in isolation (starvation) trials. If any significant feeding activity was noted (which included completion of development by the larvae to pupation and successful emergence as adults) then the plant was subjected to a choice trial against *M. spicatum* to see which species was preferred as a food source.

Starvation trials were conducted in four 38 liter aquaria separated into 10 cells and each of the 40 cells was planted with healthy growing stems of the plant to be tested. All plants contained meristematic tissue and were allowed to grow for 2 days to become established in the tank. Field-collected second instar *C. myriophylli* larvae were removed from their cases and placed on the plants, one per cell. Each cell was monitored at 2 day intervals until the larva had pupated or died. Evidence of feeding activity was apparent both from structural damage to the plant, typical of the feeding patterns of *C. myriophylli*, as well as from larval gut contents. It was possible to evaluate larval gut contents non-invasively since the larvae are translucent when alive and their gut contents, including color and some structural details, are visible.

Choice trials were conducted in aquaria similar to those used in feeding suppression trials. All 8 cells in each of four aquaria were planted with stems of the two plants to be compared. As only *M. exalbescens* showed feeding damage in the starvation trial, it was the only native species compared with *M. myriophylli* in the choice trials. The stems of both plants were in good growing condition and were standardized to 12 nodes in length. Larvae were removed from their cases and placed into the water column, one larva per cell. After 2 days, both species of plants were examined for feeding damage. Leaving the larvae for longer than 2 days could result in their transferral to the other plant after cropping the first of available meristematic tissue. Results of the isolation/starvation tests were analyzed by a 1-tailed binomial test. A two-tailed binomial test was used to analyze the results of the choice trials between *M. spicatum* and *M. exalbescens* (Zar 1984).

TABLE 1. NATIVE AQUATIC MACROPHYTE SPECIES TESTED IN ISOLATION (STARVATION) AS SUITABLE FOOD SOURCES FOR *CRICOTOPUS MYRIOPHYLLI* (– INDICATES NO FEEDING ACTIVITY, –+ INDICATES ISOLATED FEEDING ACTIVITY, + INDICATES PRESENCE OF FEEDING ACTIVITY, + + INDICATES STRONG FEEDING ACTIVITY).

Species Tested	Case Building	Feeding
Elodea canadensis	–	–
Ranunculus aquatilis	–+	–
Ceratophyllum demersum	–	–
Potamogeton crispus	–	–
P. pectinatus	–	–
P. zosteriformis	–	–
P. natans	–	–+
P. amplifolius	–	–
Nuphar polysepalum	–	–
Nmphaea odorata	–	–
Lemna minor (floating)	–	–
Myriophyllum exalbescens	+	+ +

RESULTS

Feeding damage trials. Mean growth rate of plants with 0, 1, 2, or 3 *C. myriophylli* larvae feeding on them were charted (Figure 1). A one-way ANOVA indicated there was a highly significant difference in the growth rates of *M. spicatum* plants ($P < 0.0005$). A Newman-Keuls multiple comparison indicated a significant difference in the growth rate of control and treatment plants but no significant difference among the treatment groups. Observations on the feeding larvae indicated that one individual could crop a meristem in 3 to 5 days. In addition, all of the larvae had completed one instar within the week and some were nearing the prepupal stage.

Usually only one larva became established on each apical tip, although this did not always preclude a second or even a third from becoming established on the same stem. Additional larvae were located on either an axillary meristem or on the stem itself just below the meristem.

None of the browsed meristems which were planted grew or developed to establish a new plant. The meristems of the control tips planted in the same aquarium sub-

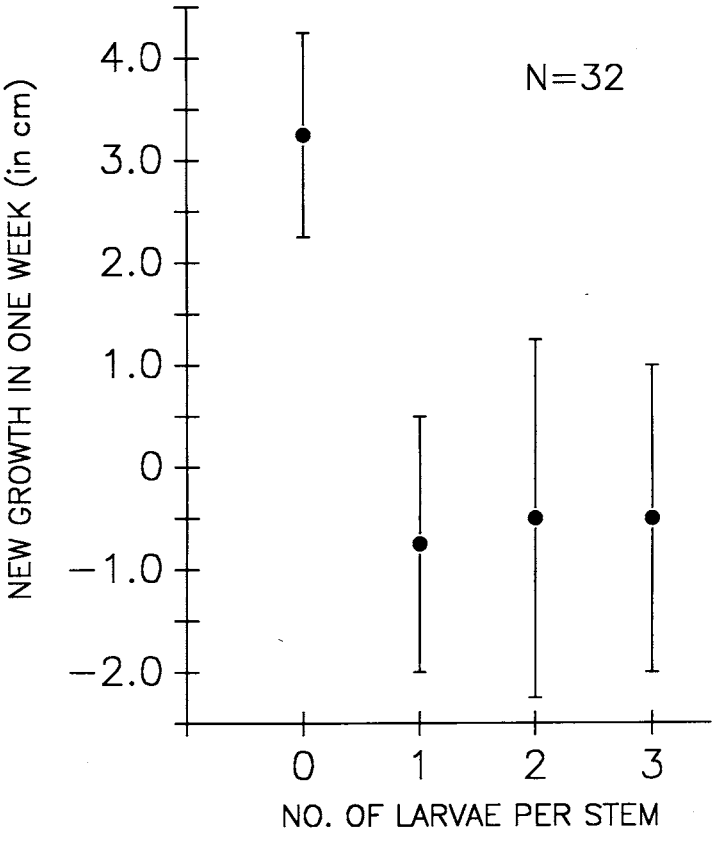

Figure 1. New growth in one week of *M. spicatum* meristems with 0, 1, 2, or 3 larvae of *C. myriophylli* feeding on them (bars are 95% Confidence Intervals).

sequently grew well, indicating that conditions for growth were suitable in the test aquaria.

Growth suppression trials. A two-way ANOVA indicated a significant difference ($P < 0.0005$) in the growth rates between treated and control individuals of *M. spicatum*, the control plants growing while the fed-upon plants did not develop any new growth (Figure 2). The effect of time was also highly significant ($P < 0.0005$) with the control plants growing continuously through the trial. The interaction effect was also found to be significant ($P < 0.025$) probably indicating that growth rate or treatment effect varies over time. There was no significant difference in the amount of new growth in all treated groups through time. While there was no significant difference in the amount of new growth of control groups at day 2 and 4, there was a significant difference in new growth of control plants between day 2 and all other time intervals.

Most meristematic tissue of the apical tip was totally destroyed by the larvae within 2 to 3 days. Unless axillary meristems developed, there was no growing tissue present after this. By the end of the trial (10 days) most larvae were in the prepupal stage.

Feeding preference trials. Of the 12 native plants tested as potential food sources for *C. myriophylli*, the only species fed upon was *Myriophyllum exalbescens*, a native milfoil (Table 1). There was limited feeding on *Potamogeton natans* by 2 larvae, but gut contents were brown, not green, indicating that they had been feeding on the dead tissue at the leaf edges and not the healthy meristem tissue. Although the larvae were found on the leaf of *P. natans*, no larval cases were present on the plant. A limited amount of case-building activity by three larvae on *Ranunculus aquatilis* was observed, but the guts of all individuals were empty and no feeding damage was observed on the plant. The cases were constructed entirely of silk without incorporation of any plant material. None of the larvae on native plants other than *M. exalbescens* survived to pupation. A two-tailed binomial test did indicate a preference for feeding on *M. spicatum* over *M. exalbescens* (22 of 32 larvae fed on *M. spicatum*, $P = 0.035$). There was, however, a preference for pupating on *M. exalbescens* over *M. spicatum* ($P = 0.035$).

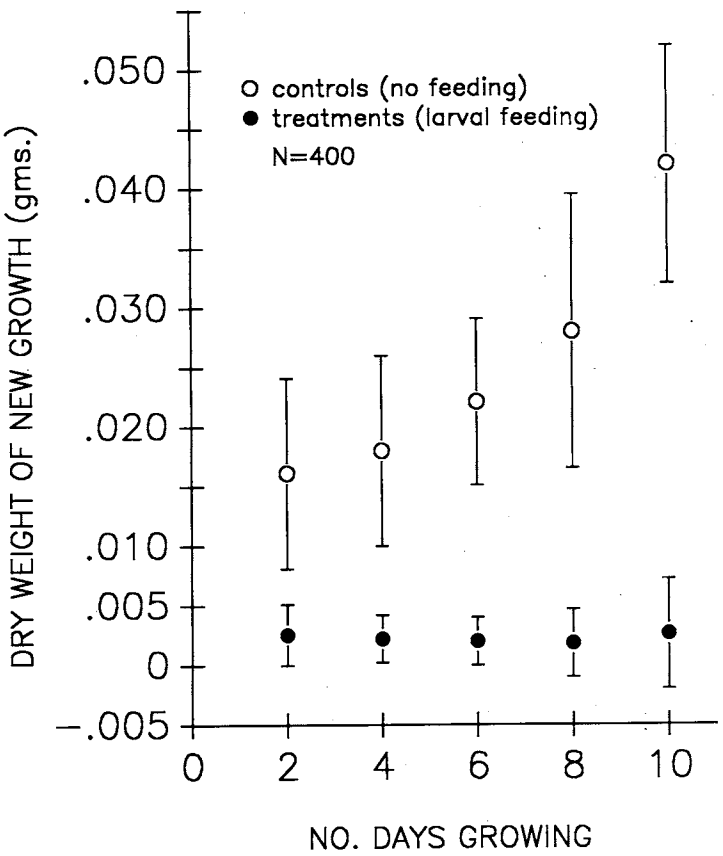

Figure 2. New growth over time of *M. spicatum* meristems with 0 or 1 *C. myriophylli* larva feeding on them (bars are 95% Confidence Intervals).

DISCUSSION

One larva can eat all the meristematic tissue from an apical tip of a planted milfoil stem, thereby inhibiting growth. This happens so rapidly that there is no significant difference in the new growth of apical tips with 1, 2, or 3 larvae feeding on them. The rapidity with which one larva can completely strip a meristematic region, well within the time period required to complete the second or third larval instar, implies that each larva requires more than one meristem to complete development.

The feeding activity of the insect results in brown, necrotic patches surrounding the feeding area. These necrotic patches failed to produce new growth either while still attached to the plant or after fragmentation when the detached fragment had become established on the bottom of the aquarium. Since all fragments which were used in the feeding trials contained nodes, and planted fragments from control plants grew well, it must be concluded that those fragments fed upon by *C. myriophylli* larvae don't readily establish new plants. This is surprising considering *M. spicatum* can produce a new plant from a 1 cm long fragment if it contains a node (B.C. Ministry of Environment 1981).

C. myriophylli larvae can suppress the growth of *M. spicatum* under laboratory test conditions. The suppression of growth and flowering seen in the Okanagan weedbeds in the early 1980's and the localized control still seen in some of the weedbeds is good evidence that this also is possible in the field. After becoming established on the plant, larvae construct cases and begin to feed. Once all meristematic tissue has been removed from the plant, no new growth occurs. The suppression of growth in this manner indicates that insects can prevent growth and it may be possible to restrict the plant's growth to levels below the water surface. In Vernon Arm of Lake Okanagan, many of the weed beds on the windward shore show feeding damage to as high as 80% of the plants. Some of these more heavily damaged areas have not surfaced in a number of years, although the level of control varies from year to year as would be expected.

The strong evidence for *C. myriophylli's* feeding preference for *M. spicatum* over native aquatic plant species, and the actual inability of the insect to feed on most of these, leads to the conclusion that the larvae are feeding-dependent on *Myriophyllum* species. As the feeding stage of *C. myriophylli* is an obligate aquatic, no terrestrial plants were

tested for suitability as potential hosts. None of the native aquatic plant species tested in isolation showed any evidence of feeding damage. This is even more convincing considering insects will often feed on plants in starvation trials that would not normally be suitable as food sources. The demonstration of feeding-dependence on *Myriophyllum* species under these conditions is strong evidence for the safety of introducing *C. myriophylli* into areas where it is not already present. Although it is unknown whether *C. myriophylli* is a native species, it has not been reported from areas where *M. spicatum* is not present and its strong preference for the introduced milfoil suggests it may be introduced. It is not surprising that *M. exalbescens* proved suitable as a potential food source for *C. myriophylli*, since it is very closely related to *M. spicatum* and the two plants are so morphologically similar that they often can be separated taxonomically only by using phytochemical techniques (Ceska 1977, Ceska and Ceska 1986).

Oliver (1984) has stated that *C. myriophylli* belongs in the *Cricotopus sylvestris* group. In many rice producing areas of the world, *C. sylvestris* is considered a pest, often referred to as the 'Rice Midge' or 'Rice Seed Midge' (Berczic 1979, Gigarick 1984). The propensity for attacking rice also may extend to its close relative *C. myriophylli*. Therefore, before *C. myriophylli* can be exported as a biocontrol agent to any rice growing region its feeding activities on rice must be tested.

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