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Low Temperature Reproduction of *Cyrtobagous salviniae*: Good News for Biological Control of Salvinia in a Temperate Climate

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ABSTRACT

Biological control of salvinia (*Salvinia molesta* D.S. Mitchell) in temperate regions is problematic because of the cooler climate; however, field trials using salvinia weevil (*Cyrtobagous salviniae* Calder and Sands) in the temperate Hawkesbury-Nepean catchment New South Wales, Australia, show that biological control of salvinia in temperate regions is possible at river, creek, and dam sites. Samples of weevil infested salvinia taken from the field were inspected for immature stages of the weevil. The air and water temperatures preceding the presence of these life stages were examined. Reproduction of salvinia weevil commenced during the first week of spring in the Hawkesbury-Nepean catchment at a mean air temperature as low as 16 C. This has positive implications for the future of biological control of salvinia in other temperate regions.

Key words: Curculionidae, life stages, *Salvinia molesta*, salvinia weevil, Salviniaceae.

INTRODUCTION

Salvinia weevil (*Cyrtobagous salviniae* Calder and Sands) was first introduced for biological control of salvinia (*Salvinia molesta* D.S. Mitchell) in Australia in 1980 (Room et al. 1981). The salvinia weevil controls salvinia in at least 12 countries, mostly in tropical and subtropical areas and also in some temperate climates (Julien et al. 2002). However, biological control of salvinia has not been as successful in temperate regions as it has been in tropical and subtropical regions of the world. In the Hawkesbury-Nepean catchment, New South Wales, Australia, trials in the 1980s showed that salvinia could be controlled biologically at some sites, but because of the unpredictability of results and the length of time to control, the local weed control authorities continued to control salvinia with herbicides (M. Julien, pers. comm.). A 5 year study in the United States concluded that salvinia was controlled biologically at sites in Texas and Louisiana (Tipping and Center 2004) where air temperatures recorded from nearby weather stations fell as low as -9 C in winter (Tipping and Center 2003). Results from a 3 year field trial (Sullivan, Postle and Julien, unpubl. data) in the temperate Hawkesbury-Nepean catchment show that salvinia was often controlled biologically at river sites and sometimes at creek and dam sites.

Growth rates of salvinia at different temperatures and nutrient levels have been established (Room 1986, Room and Thomas 1986, Owens et al. 2004). The optimum temperature for growth of salvinia is 20 to 30 C (Mitchell and Tur 1975). Salvinia growth was found to occur at 12 C (Room 1986), but growth is still very limited at 16 C (Cary and Weerts 1983). Equations for the effects of nitrogen (N) content of salvinia on growth specified a 4-fold increase in growth between 0.8% N and above 5.0% N (Room 1986).

Adult salvinia weevils feed at temperatures above 13 C (Forno et al. 1983), and although adult feeding alone may restrict growth of salvinia (Forno and Bourne 1985), it does not destroy it. Adult weevils primarily feed on buds (Forno et al. 1983), the plant parts with the highest nitrogen content (Room and Thomas 1986). Salvinia is destroyed by the tunnelling of larvae inside the plant (Julien et al. 1987). Therefore, the lowest temperature at which the salvinia weevil can commence reproduction and weevil development can be completed is crucial to the success of biological control.

Forno et al. (1983) examined the life cycle of salvinia weevil at various temperatures related to a tropical or subtropical climate. Weevils were kept at 27 C for 14 d prior to oviposition to ensure that females were gravid. At constant temperatures, the maximum numbers of eggs oviposited occurred at 23 and 27 C (0.590 and 0.580 eggs per female per day, respectively) and negligible oviposition occurred below 21 C (0.041 eggs per female per day at 21 C and 0.019 eggs per female per day at 19 C). These results led to the assumption that salvinia weevils might not be a suitable biological control agent in temperate climates. However, recent studies (Hennecke and Postle 2006) showed that salvinia weevils will oviposit at lower temperatures than previously reported if exposed to colder temperatures prior to oviposition (13 C for 4 d). Significant numbers of eggs (0.25/d) were laid at a constant 19 ± 0.5 C, compared to a control group which laid 0.45 eggs per day at 27 C (Hennecke and Postle 2006).

Laboratory studies on the development of salvinia weevils in relation to temperature provided estimated developmental zeros (the temperature below which no development occurs) of 16.3 ± 0.8 C for larvae and 15.6 ± 1.5 C for pupae (Sands et al. 1983). The minimum threshold for oviposition has not been determined (Forno et al. 1983, Hennecke and Postle 2006) but is likely to be higher than the minimum

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threshold for adult feeding or larval and pupal development; therefore, weevils are likely to complete development if temperatures are high enough for oviposition.

The aim of this field study was to determine when in the spring season the first salvinia weevil eggs, larvae and pupae appeared in salvinia of the Hawkesbury-Nepean catchment and determine the temperatures that preceded these life stages.

MATERIALS AND METHODS

Nine salvinia infested sites were chosen from within the Hawkesbury-Nepean catchment (three each from the river, tributary creeks, and local dams). All sites were located <20 km from latitude 33.680003 S and longitude 150.784998 E. Salvinia weevil populations had been present at five of the sites for 2 years while salvinia weevils had been released at the other four sites in autumn just 5 months prior to the first samples being taken.

Eight salvinia plants from each site were collected every 2 weeks over a period of 3 months commencing on 16 August 2006. Plants were inspected using a binocular microscope (7×) for the presence of salvinia weevil eggs, larvae, and pupae.

Data loggers recorded hourly the air and water temperature (2 cm above and 2 cm below the water surface, respectively) at six of the sites. For those sites where eggs, larvae, or pupae were found and temperature data were available, a comparison was made between the dates when immature stages were first found and the associated preceding temperatures. Minimum, maximum, and mean air and water temperature data were calculated for the week prior to when life stages were found (Table 1).

RESULTS

Salvinia weevil immature stages were found at seven of nine sites, including two where releases had been made only 5 months previously (Table 2). However, at these two sites (Wallacia River and South Creek) only four eggs and one egg were found, respectively.

Eggs were found at all three river sites, and at two of these during the first week of spring on 6 September. At Mamre Creek a single egg was found on 6 September, and at the other two creek sites, eggs were found from 14 September onward. Eggs were found at only one dam site (Pitt Town Dam) on 6 October. Larvae were found at two river sites and one creek from 11 October, and pupae at two river sites on 7 and 8 November. At only two sites were all three stages found, and these were both on the river where salvinia weevil populations had been established for 2 years. The two sites where no eggs, larvae, or pupae were found were dam sites where salvinia weevil releases were made in autumn only 5 months prior to the first samples being collected.

The sites where eggs, larvae, or pupae were found and which had available temperature data were two river sites (Nepean Castlereagh and Hawkesbury Lowlands), a creek site (Mamre Creek), and a dam (Pitt Town; Table 3). Unfortunately the data logger for the Hawkesbury Lowlands site failed mid way through the trial.

DISCUSSION

There was more evidence of salvinia weevil activity on the river than on the creeks and dams, consistent with findings from a 3 year study in the Hawkesbury-Nepean catchment (Sullivan, Postle and Julien, unpubl. data) where it was observed that salvinia was biologically controlled at all river sites but at only some of the creeks and dam sites. There was only one river site (Nepean Castlereagh), one creek site (Mamre) and one dam site (Pitt Town) that had temperature data for the duration of the trial (Table 4). These three sites had increasing trends for both the monthly mean air and water temperatures (2 cm above and 2 cm below the water surface respectively). The monthly mean water temperature was always higher than the monthly mean air temperature for the Nepean Castlereagh River site however; the monthly mean water temperature was similar to the monthly mean air temperature for Mamre Creek and Pitt Town Dam sites. Also, the monthly mean water temperature for the Nepean Castlereagh River site was much higher than the monthly mean water temperatures for Mamre Creek or Pitt Town Dam sites. Larger bodies of water have a larger thermal inertia that dampens temperature fluctuations (Room and Kerr 1983, Whiteman and Room 1991), and this should buffer the temperatures experienced by salvinia and salvinia weevils. This

TABLE 1. AIR AND WATER TEMPERATURES FOR THE WEEK PRIOR TO WHEN SALVINIA WEEVIL EGGS, LARVAE, AND PUPAE WERE FOUND (MEAN ± STANDARD DEVIATION).

		Air temperature (C)			Water temperature (C)		
	Site	Max	Min	Mean	Max	Min	Mean
Eggs							
	Hawkesbury Lowlands	28	9	16 ± 5	25	12	18 ± 3
	Pitt Town	33	5	17 ± 8	23	13	17 ± 3
Larvae							
	Nepean Castlereagh	35	10	18 ± 6	28	20	23 ± 2
	Mamre Creek	34	11	18 ± 4	25	16	18 ± 2
Pupae							
	Nepean Castlereagh	33	14	19 ± 3	28	21	23 ± 2

[1]Hawkesbury Lowlands and Nepean Castlereagh are river sites; Mamre Creek is a creek site; Pitt Town is a dam site.

TABLE 2. TYPE AND LOCATIONS OF SITES, TIME SINCE SALVINIA WEEVILS WERE RELEASED AT EACH SITE, AND PRESENCE OF IMMATURE STAGES.

		Time since salvinia weevils were released	Presence of immature stages
River	Sites		
HL	Hawkesbury Lowlands	2 years	Yes
NC	Nepean Castlereagh	2 years	Yes
WR	Wallacia	5 months	Yes
Creek	Sites		
MC	Mamre Creek	2 years	Yes
CC	Currency Creek	2 years	Yes
SC	South Creek	5 months	Yes
Dam	Sites		
PT	Pitt Town Dam	2 years	Yes
FR	Freemans Reach Dam	5 months	No
WD	Wallacia Dam	5 months	No

buffering of air temperatures from the larger body of water in the river can be seen where the Nepean Castlereagh River mean air temperatures had a smaller standard deviation than Mamre Creek or Pitt Town Dam (Table 4). Mamre Creek and Pitt Town Dam were unshaded, although other creeks and dam sites had more shading from surrounding trees when compared to the wide river that was mostly unshaded.

Salvinia weevil eggs were found at two of the autumn release sites established only 5 months earlier. Although the number of eggs found was low (four eggs at Wallacia River and one at South Creek), observing salvinia weevil reproduction at these sites was encouraging, albeit unexpected, because salvinia weevil populations were low, and only small samples were taken for examination.

Salvinia weevils were laying eggs by 6 September on the Hawkesbury-Nepean River at a mean air temperature of 16 C, and a mean water temperature of 18 C (Table 1). Larvae were present at a mean air and water temperature of 18 C and pupation had commenced at 19 C air temperature and 23 C water temperature. The lower threshold for development in the field found in this study appears to be similar to those estimated by Sands et al. (1983).

Most parts of salvinia in mats are warmer than air temperatures (Room and Kerr 1983), and this warmer microclimate should enable earlier oviposition than would otherwise be expected from looking at air temperatures.

Adult weevils stop feeding below 13 C (Forno et al. 1983), while salvinia growth has been observed at 12 C (Room 1986); therefore, it is likely that buds would always be produced at temperatures where adult weevils feed. Maximum winter air temperatures of 16 to 18 C are common in the Hawkesbury-Nepean catchment, and because salvinia mats are warmer than air temperatures (Room and Kerr 1983), bud production and adult feeding should continue through out the winter. This trial observed successful over-wintering by adult weevils that began ovipositing when temperatures were suitable.

TABLE 3. SITE TYPE, LOCATION AND PRESENCE OF IMMATURE STAGES FOR SITES WHERE TEMPERATURE DATA WERE AVAILABLE.

Date	Eggs	Larvae	Pupae
06 Sep 2006	HL, NC, MC		
19-21 Sep 2006	HL, NC		
06 Oct 2006	PT		
10-11 Oct 2006	HL, NC	NC	
07-08 Nov 2006	HL	HL, NC, MC	HL, NC

[1]HL = Hawkesbury Lowlands River site; NC = Nepean Castlereagh River Site; MC = Mamre Creek Site; PT = Pitt Town Dam Site.

Constant temperatures have been compared with the mean of fluctuating temperatures to try to predict reproductive behavior of aquatic insects in the field (Sweeney 1984). If this comparison is used, the mean of 16 C at which salvinia weevils laid eggs in the field in this study is lower than the constant temperature of 19 C at which only negligible numbers of eggs were laid in the laboratory (Forno et al. 1983). It is evident, however, that the maximum and minimum temperatures are highly relevant, and the day degrees above a given temperature threshold is the most likely trigger for the commencement of oviposition. In the week preceding the finding of eggs at the Hawkesbury Lowlands river site there were 57 h where the air temperature reached 19 C or above. The maximum temperature was 28 C, the minimum 8 C and the mean 16 C. The minimum development threshold for salvinia weevil oviposition has not been determined. Few eggs are laid below 19 C (Forno et al. 1983), although Hennecke and Postle (2006) found significant numbers of eggs (0.25/d) were laid at a constant 19 C. Although the minimum development threshold for oviposition is likely to be lower than 19 C, we have arbitrarily used 19 C as our minimum development threshold for calculating degree days. There were 6.04 day degrees over 19 C for the week preceding the finding of eggs. The week starting 14 days prior to oviposition was cooler and had only 11 hours where the temperature rose above 19 C. This week had a maximum of 21 C, a minimum of 6.4 C, a mean of 13 C and 0.66 degree days over 19 C.

Sample sizes were small and collections were made at 2 week intervals, so oviposition may have commenced prior to 6 September. Larval development and pupation may also have commenced earlier than shown in this study. The minimum developmental thresholds reported by Sands et al. (1983) of 16.3 ± 0.8 C for larvae and 15.6 ± 1.5 C for pupae suggests that reproduction could have commenced earlier in the field and at lower temperatures than we found. More work is needed in

TABLE 4. MONTHLY AIR AND WATER TEMPERATURES (MEAN ± STANDARD DEVIATION).

		Air temperature (C)			Water temperature (C)		
Site	Period	Max	Min	Mean	Max	Min	Mean
Nepean Castlereagh	16/08/2006–15/9/2006	29	5	14 ± 5	27	13	17 ± 2
	16/09/2006–15/10/2006	37	9	18 ± 6	31	17	22 ± 3
	16/10/2006–15/11/2006	36	8	19 ± 5	31	18	23 ± 2
Mamre Creek	16/08/2006–15/9/2006	30	1	12 ± 6	21	6	13 ± 3
	16/09/2006–15/10/2006	39	3	17± 8	20[2]	13[2]	16[2] ± 2
	16/10/2006–15/11/2006	36	3	18 ± 6	28[2]	14[2]	19[2] ± 3
Pitt Town	16/08/2006–15/9/2006	35	-1	12 ± 7	20	6	13 ± 3
	16/09/2006–15/10/2006	40	5	18 ± 8	25	13	17 ± 3
	16/10/2006–15/11/2006	40	5	18 ± 6	25	15	18 ± 2

[1]Nepean Castlereagh = river site; Mamre Creek = creek site; Pitt Town = dam site.
[2]Incomplete dataset.

the field and/or laboratory to accurately determine the minimum developmental thresholds for salvinia weevils. Over time, there will likely be genetic selection for weevils able to tolerate and reproduce at lower temperatures.

Given that the optimum temperature for growth of salvinia is 20 to 30 C (Mitchell and Tur 1975), it seems likely that the salvinia weevil begins its reproductive cycle at the lower end of the optimum temperature range for salvinia growth. Thus, salvinia weevil populations should begin to increase in early spring in temperate regions when salvinia is growing slowly, and this population increase will assist salvinia weevils in their ability to control salvinia. During this trial, salvinia infestations at the two older established river sites were controlled by salvinia weevils (unpublished data) and were not able to increase during spring 2006.

Salvinia weevils began reproducing in early spring in the Hawkesbury-Nepean catchment, and we suggest that early spring would be the optimum time to release and establish salvinia weevils in the field in temperate regions with similar climates. Releases in early spring maximize the duration of warm weather, enabling populations of salvinia weevil to increase to higher levels and leading to more effective salvinia control. Larger numbers of weevils per release and multiple releases would enhance establishment.

The implications of these results for biological control of salvinia in temperate climates are positive. Salvinia does not actively grow during the winter in temperate climates and is killed after 3 h at -3 C (Whiteman and Room 1991). Therefore, salvinia weevils do not need to reproduce at cold temperatures, but do need to survive winter to breed again in spring. The density of salvinia mats on the surface of the water has an insulating effect (Room and Kerr 1983), which helps salvinia weevils survive short periods of freezing temperatures. Salvinia weevil has been shown to survive in areas where the air temperatures are as low as -9 C (Tipping and Center 2003). Salvinia weevil populations in the Hawkesbury-Nepean catchment survived the winters of 2005 to 2008, one of which had below average minimum temperatures and 41 frosts. The coldest air temperature recorded during that period was -4 C (Australian Bureau of Meteorology 2009).

This study confirms observations in the field (Sullivan, Postle and Julien, unpubl. data) that salvinia weevils are an effective biological control agent of salvinia in the Hawkesbury-Nepean catchment, and suggests biological control can be a useful tool in the integrated management of salvinia in temperate regions with similar climates.

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