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# Biological control of *Salvinia molesta* in South Africa revisited

G.D. Martin[a,*], J.A Coetzee[b], P.S.R. Weyl[a,1], M.C. Parkinson[c,d], M.P. Hill[a]

[a] Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa
[b] Centre for Biological Control, Botany Department, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa
[c] South African Institute for Aquatic Biodiversity, Private Bag 1015, Grahamstown 6140, South Africa
[d] Department of Ichthyology and Fisheries Science, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

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ABSTRACT

The aquatic weed *Salvinia molesta* D.S. Mitch. (Salviniaceae) was first recorded in South Africa in the early 1900s, and by the 1960s was regarded as one of South Africa's worst aquatic weeds. Following the release of the weevil, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) in 1985, the weed is now considered under successful biological control. However, the post-release evaluation of this biological control programme has been *ad hoc*, therefore, to assess the efficacy of the agent, annual quantitative surveys of South African freshwater systems have been undertaken since 2008. Over the last ten years, of the 57 *S. molesta* sites visited annually in South Africa, the weevil has established at all of them. Eighteen sites are under successful biological control, where the weed no longer poses a threat to the system and 19 are under substantial biological control, where biological control has reduced the impact of the weed. Since 2008, the average percentage weed cover at sites has declined significantly from 51–100% cover to 0–5% cover in 2017 ($R^2 = 0.78$; $P < 0.05$). Observations of site-specific characteristics suggest that biological control is most effective at small sites and more difficult at larger and shaded sites. Our findings show that *S. molesta* remains under good biological control in South Africa, however, some sites require intermittent strategic management, such as augmentative releases of *C. salviniae.*

## 1. Introduction

*Salvinia molesta* D.S. Mitch. (Salviniaceae) is a free-floating aquatic fern originating from South America. In many tropical and subtropical regions outside of its native range, *S. molesta* grows rapidly and is regarded as a major aquatic weed (Cilliers et al., 2003). Unmanaged infestations of *S. molesta* negatively affect water quality, increase evapotranspiration and result in economic loss (McFarland et al., 2004; Van Wilgen and Lange, 2011). In South Africa, the primary impacts of *S. molesta* derive from its ability to form dense mats, which cover aquatic ecosystems, resulting in reductions in aquatic biodiversity associated with floating species (e.g. Coetzee et al., 2014). In unmanaged sites, the percentage cover of *S. molesta* remains constant even when plant quality changes. This is because even in subtropical climates, the plant can find favourable growing conditions throughout the year (van Oosterhout et al., 2006), and if adverse conditions do occur, *S. molesta* can regenerate from the brown weed mat that may appear dead (van Oosterhout et al., 2006). For example, frost may kill exposed leaves and buds, but leaves and buds within the mat survive and there is no resulting reduction in plant cover.

Throughout *S. molesta's* invaded range, where it has become problematic, control measures implemented include chemical, mechanical and biological control (Mitchell, 1979; Nelson et al., 2001; CRC, 2003; Sullivan et al., 2011). However, in South Africa, only biological control is implemented for the management of *S. molesta*.

Biological control, using the host-specific weevil *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), has proved to be successful in the species' management, wherever it has been introduced (Room et al., 1981; Forno and Bourne, 1985; Room and Thomas, 1985; Julien et al., 2009), including South Africa (Cilliers, 1991; Cilliers et al., 2008; Coetzee et al., 2011). However, Schooler et al. (2011) using the infestation of *S. molesta* in Kakadu National Park, Australia, as a case study, showed that control by *C. salviniae* depended on a number of environmental factors, including flooding and periodic drying out of the waterbody. They also showed that when the biological control agent was present at a site, a reduction in weed cover was not always achieved. Additionally, systems where the biological control agent had resulted in a reduction of cover could return to a covered state as a result of a stochastic event. Therefore, understanding the drivers of *S. molesta* and *C. salviniae* populations, and the effect of stochastic events

* Corresponding author.
*E-mail address:* g.martin@ru.ac.za (G.D. Martin).
[1] Current address: CABI, Rue des Grillons 1, Delémont 2800, Switzerland.

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over an extended period, in South Africa, could improve the biological control programme, allowing for effective management of the weed.

*Salvinia molesta* has been present in South Africa since the early 1900s (Cilliers, 1991; Cilliers et al., 2008) and is most troublesome in Mpumalanga, KwaZulu-Natal, Western Cape and Eastern Cape provinces (Coetzee et al., 2011). During the 1980s, it was considered second only to *Eichhornia crassipes* Mart. Solms-Laub. (Pontederiaceae) (water hyacinth) as the most problematic aquatic weed in South Africa, resulting in the South African Department of Water Affairs initiating a biological control programme against the weed (Cilliers, 1990). *Cyrtobagous salviniae* was subsequently collected in 1985 from Eastern Caprivi, Namibia, where a biological control programme against *S. molesta* had already been implemented, and released in South Africa onto three impoundments in the north eastern Limpopo Province (Cilliers, 1991). Following its release, the weevil rapidly controlled *S. molesta* infestations throughout its invaded range. By the late 1990s, control of *S. molesta* in South Africa by *C. salviniae* was considered complete (Hoffmann, 1995), with no other management interventions necessary, except for redistribution of the weevil to newly infested sites (Coetzee et al., 2011), because even though the weevil has been shown to disperse from infested sites (Micinski et al., 2016), our experience is that the weevil is a poor disperser between sites and often requires introduction into newly invaded sites and /or re-introduction following stochastic events.

The success of biological control programmes are rarely quantified (Thomas and Reid, 2007; Carson et al., 2008; Morin et al., 2009), and on only a few occasions have the impacts of biological control programmes been evaluated continuously over several years (Carson et al., 2008). Generally, post-release evaluations are neglected or omitted altogether owing to inadequate resources and funding (Morin et al., 2009), and if they do occur, are often once-off or short-term investigations. Of the post-release evaluations that do occur, many are poorly planned and lack the experimental rigour that will provide meaningful results (Carson et al., 2008). Over the last 20 years, a limited number of studies have investigated the efficacy of a biological control programme on host plants in the field, and highlighted the significant economic and ecological benefits of biological control, and the benefits of conducting quantitative post-release evaluations (e.g. Hoffmann and Moran, 1998; Hoffmann et al., 1998; McConnachie et al., 2003; Wood and Morris 2007; Carson et al., 2008; Turner et al.; 2008; Morin et al., 2009; Post et al., 2010).

Long-term post-release evaluations have many benefits; they can identify effects of a released agent over many years where short-term or once-off evaluation of weed populations may not provide reliable data; they can highlight situations where the current biological control is ineffective and further control measures, such as additional releases or in some cases, additional agent species or other control measures, might be needed; and they can provide an economic evaluation of the benefit of biological control (Morin et al., 2009; Hayes et al., 2013; Hinz et al., 2014; Suckling, 2014).

Despite the reported success of the *S. molesta* biological control programme in South Africa, there have been no quantitative, long-term, assessments of the success since the release of *C. salviniae* in 1985. Furthermore, new records of *S. molesta* infestations have been recorded in the SAPIA (South Africa Plant Invaders Atlas) database between 2003 and 2007, prompting the need for the initiation of a long-term, post-release evaluation of this control programme. Here we analyse ten years of data from a long-term, post-release evaluation of *S. molesta* in South Africa, and we examine the influence of certain environmental variables contributing to the success of the programme.

## 2. Methods

Annual surveys of South African freshwater systems invaded by aquatic weeds have been conducted since 2008 by the Centre for Biological Control, Rhodes University. The intention of the surveys was to collect quantitative long-term data on the status of infestations by the five worst floating aquatic weed species; *E. crassipes*, *Pistia stratiotes* L. (Araceae) (water lettuce), *S. molesta*, *Myriophyllum aquaticum* (Vell. Conc.) Verd. (parrot's feather) and *Azolla filiculoides* Lam. (Azollaceae), and document any new invader species in South African freshwater systems. The majority of sites were surveyed in the summer of each year. In 2010, 2011 and 2012, high rainfall and flooding events influenced the timing of surveys. On average, 20 ± 2 *S. molesta* sites were visited annually.

The data recorded at each site included: site characteristics (longitude and latitude, province, altitude and water body type lentic (still or calm water) or lotic (flowing water) and size of the water body (calculated from satellite imagery), years surveyed, and the percentage of the water body under weed cover. Percentage cover was determined by visually inspecting the site from a set point (a GPS co-ordinate) and photographs were taken to allow comparisons over time. A reduction in percentage cover and not plant density, quality, or insect damage parameters, was used as an indicator of successful biological control. At all *S. molesta* sites visited, 100 plants were randomly selected and scored for the presence of *C. salviniae* adults, larvae and feeding damage. Initial release date of control agents was also noted, as well as the number of *C. salviniae* that were released at each site, this was calculated by amalgamating the release data from: mass-rearing facilities around the country, government appointed biological control implementation officers from each province, and land owners who may have had *C. salviniae* release data. Some data were incomplete as numerous unrecorded releases have taken place over the years and, although *C. salviniae* is a poor disperser, it could move locally between sites.

Some additional information was added for each site, including the amount of disturbance (e.g. wind & flow action) experienced at each site. This was adapted from Sullivan et al. (2011) who classified *S. molesta* sites in Australia.

The amount of shading and the water quality for each site was estimated based on visual observations and data from field surveys. Shading was determined as high if the site was shaded by dense surrounding trees or dense marginal vegetation, compared to low shaded sites where the majority of the water surface received continuous sunlight with minimal surrounding tall trees and bushes. As the majority of sampled sites were not on major drainage systems, no long-term water quality data exist for the sampled sites. Additionally, once-off water sampling would not provide useful water quality data, therefore, the nutrient status of the site was deemed high if the sites were known to have high nutrient inputs from intensive surrounding agriculture, industry and direct nutrient inputs from sewage or drainage pipes. Low nutrient sites had no obvious signs of nutrient inputs or were found in undisturbed environments such as game farms or uninhabited catchments. Finally, the number of months where the mean daily atmospheric temperature exceeded 19 °C was estimated for each site, as *C. salviniae* only oviposits when the temperature is in excess of 19 °C (Hennecke and Postle, 2006), even though it can survive at colder temperatures (Allen et al., 2014). The daily maximum and minimum temperature dataset used for this analysis was derived from the South African Atlas of Climatology and Agrohydrology (Schulze, 1997).

For each site, the level of biological control was assigned a qualitative measure (from Hoffmann, 1995; McFadyen, 1998; Klein, 2011): complete control, where no other control measures are needed at the site; substantial control, where biological control agents need to be re-released after stochastic events; negligible control, where, in spite of damage inflicted by the agents, further management is still required; and control undetermined, where either the release of the agents has been too recent for meaningful evaluation, or the level of control has fluctuated between the control levels and a stable state has not yet been achieved. It is not uncommon for *S. molesta* cover to fluctuate over time even if the biological control agents are present (Schooler et al., 2011). Distribution records and the level of biological control at each *S. molesta* site from the annual surveys were overlaid onto a map of mean annual temperature in South Africa (Schulze, 1997) using ArcView v.9. (ESRI, 2009) to visualise the distribution of *S. molesta* in South Africa, and determine whether temperature or geographic location may affect the distribution and/or the control achieved at sites.

The SAPIA data were interrogated for information on *S. molesta* distribution prior to 2008. To determine whether the number of *S. molesta* sites identified in South Africa increased over time, a linear regression was conducted to determine the relationship between time in years and the number of sites recorded every year, from the commencement of the annual surveys in 2008. Additionally, a linear regression between time and percentage cover at each site over the surveyed years was conducted, to determine if there was a reduction in overall cover over time. The control achieved at sites was compared between lentic and lotic systems using a Chi-square test of independence. All regression analyses were conducted in Microsoft Excel (Microsoft Office 2015).

A Principal Component Analysis (PCA) in PAST: Paleontological Statistics package ver. 1.81 (Hammer et al., 2001) determined which characteristics from the 57 *S. molesta* sites in South Africa influenced the level of control achieved. Site characteristics included latitude, longitude, altitude, size of water body, % weed cover, nutrients and temperature (mean daily temperature). The two-dimensional (2D) PCA plot indicated the similarity of sites to each other. A bi-plot was constructed to investigate the relationship of the sites to those variables.

To visualise *S. molesta* cover throughout South Africa over the surveyed years, for each year surveyed, we grouped the sites into one of four "% cover" categories (0–5; 6–25; 26–50; 51–100%), where 0–5% is regarded as very low weed cover and acceptable from a management perspective, 6–25% as low cover, 26–50% cover as medium cover and 51–100% as high cover and unacceptable from a management perspective. These were then plotted against each other for each year. Additionally, the percent of sites falling into each of those categories was compared to the percent of sites surveyed in that particular year and to the other years surveyed. We analysed the change in number of sites per cover category over the surveyed years using a linear regression.

## 3. Results

### 3.1. Salvinia molesta in South Africa

A total of 57 independent *S. molesta* sites were surveyed between 2008 and 2016. These sites were located in six provinces, with Limpopo (16 sites), Mpumalanga (12 sites) and the Western Cape (12 sites) provinces having the most sites (Fig. 1). There were no recorded *S. molesta* infestations in the Free State, North West and Northern Cape provinces. *Salvinia molesta* was limited to the warmer north and east coast of South Africa. It was also prevalent in the Mediterranean climate of the Western Cape and the coastal Eastern Cape provinces (Fig. 1). It was not present in the colder, drier interior of South Africa (Fig. 1).

The combined SAPIA and survey distribution data show that the number of recorded *S. molesta* infestations increased by approximately one to two sites per year between 1995 and 2006, however, in 2008, following the first national survey, the number of known records of *S. molesta* in South Africa increased from 14 to 39 and subsequently increased, albeit at a slower rate thereafter ($R^2 = 0.93$; $P < 0.001$) (Fig. 2). Although the mean percent cover of *S. molesta* decreased from 65 ± 15% (mean ± SE) in 2008, to 15 ± 5% in 2015, and 12% ± 2.4% (mean ± SE) in 2017 ($R^2 = 0.14$; $P < 0.001$), the large variation in percent cover at the sites contributes to the reason for the low value of the correlation co-efficient.

### 3.2. Biological control

At the 57 *S. molesta* sites in South Africa, 18 were under complete control, 19 were under substantial control and only one site was currently regarded as under negligible control (Fig. 1). There was not enough information available to determine the current level of control at 19 sites. Lentic systems were proportionately more frequently under complete control than lotic systems ($\chi^2 = 12.837$, df = 3, P = 0.0050) (Fig. 3). Sixteen lotic sites, as opposed to only three lentic sites, were classified as control undetermined (Fig. 3).

Neither the distribution, nor the level of control, of *S. molesta* in South Africa was determined by altitude or temperature. Complete, as well as substantial control, can be achieved in the more-temperate north of South Africa as well as in the Mediterranean region of the country, suggesting that achieving complete control is due to individual site characteristics (Fig. 1).

### 3.3. Site characteristics

Site specific characteristics were analysed using a PCA to determine whether location or environmental drivers could explain the level of biological control success of *C. salvinae* on *S. molesta* in South Africa. The first two principal components (PC1 eigenvalue = 36.9; PC2 eigenvalue = 15.7) explained only 52.6% of the variation between the sites. The PCA indicated that latitude, longitude and disturbance were the three main variables explaining the variability at sites where control was undetermined or substantial. This is because the level of control of a number of the perennial river systems (high disturbance) was classified as control undetermined during the surveys. This was supported by Fig. 2, which separated lentic and lotic sites into different levels of control; 52% of lentic systems were under complete control, compared to only 15% of lotic systems. The PCA indicated that shade and nutrients influenced the level of control (Fig. 4). The distribution of the sites according to the PCA showed that complete control and substantial control can be achieved under very different conditions, and that a single site characteristic does not explain the level of success (Fig. 4). Sites on the left side of the central axis are primarily influenced by shade and nutrients.

### 3.4. Current status of S. Molesta in South Africa

Since the initiation of national surveys, there has been a significant reduction in percentage cover of *S. molesta* at infested sites in South Africa, from an average of 65 ± 15% in 2008 to and 12% ± 2.4% (mean ± SE) in 2017 (Fig. 1). Lentic systems experienced better control than lotic systems. In 2008, the majority of sites had cover of between 51 and 100% of *S. molesta*. By 2016 there was a dramatic reduction in the number of sites with between 51 and 100% cover ($R^2 = 0.78$; $P < 0.05$ (Fig. 5). Conversely, in 2008 very few sites were considered under acceptable control, with between 0 and 5% weed cover, but by 2016 there was a significant increase in the number of sites visited where weed cover was between 0 and 5% ($R^2 = 0.8$; $P < 0.05$). The number of sites with 6–25% cover and 26–50% cover remained constant over the years, and remained in the minority of sites (Fig. 5).

## 4. Discussion

Post-release evaluations that continuously monitor a weed's status within a country, as well as the effectiveness of the associated biological control agent(s), should be an integral component of any biological control programme (Carson et al., 2008). Morin et al. (2009) suggest that continuous, regular monitoring of the effects of a released agent over many years is one of the best monitoring tools as short-term responses of weed populations to the agent are not always consistent with long-term trends. Long-term monitoring also accounts for possible confounding factors affecting the perceived level of control, and may provide evidence that the agent is responsible for the observed changes in the weed. This study is one of the first long-term post-release

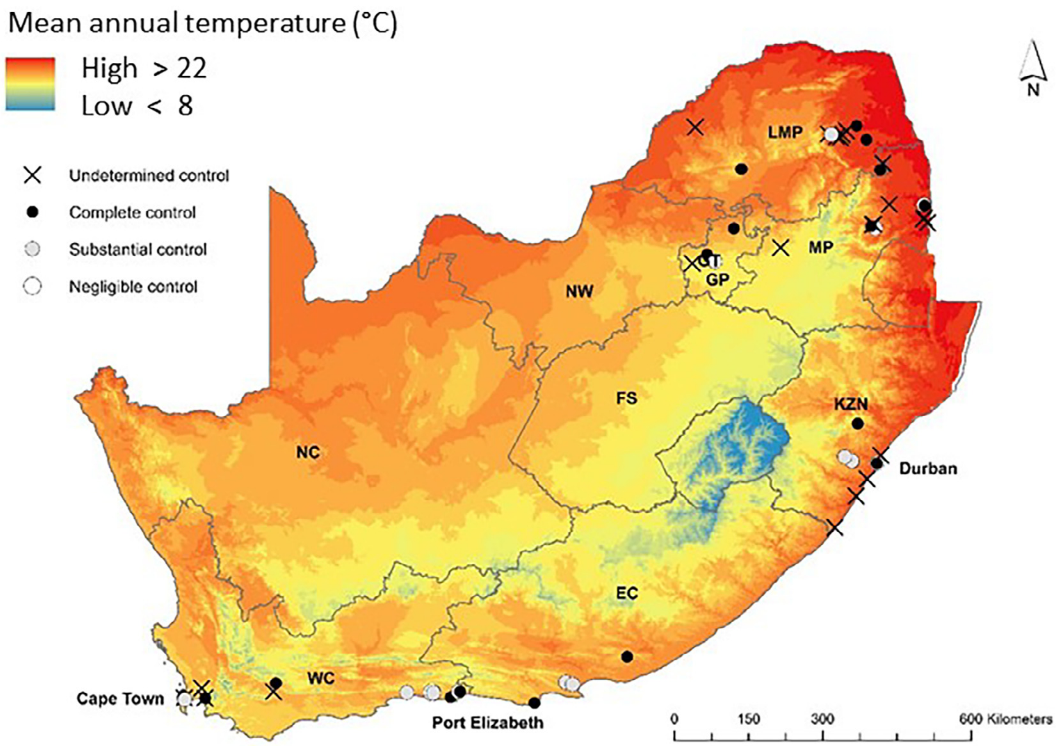


**Fig. 1.** Distribution of *Salvinia molesta* in South Africa, in relation to the mean daily temperatures, High ≥22 °C – Low ≤8 °C. Black circles represent sites under complete control, grey circles those under substantial control, X represent sites with undetermined control, and the single white circle, just to the left of Port Elizabeth, a site under negligible control. Climate data generated from the South African Atlas of Agrohydrology and Climatology (Schulze 1997). Gauteng (GP), Mpumalanga (MP), Limpopo (LMP), North West (NW), KwaZulu-Natal (KZN), Eastern Cape (EC), Western Cape (WC), Northern Cape (NC), Free State (FS) provinces.

evaluations of an aquatic weed biological control programme in South Africa, and provides valuable insights into the complexity of the control of *S. molesta* and the importance of long-term monitoring (Hill et al., 2008; Schooler et al., 2011; Moore and Hill, 2012). We have shown clear, country wide, significant reduction in percent cover of the weed over the last decade, as a result of the release of *C. salviniae*, even though weed populations still fluctuate at some sites. These fluctuations, however, are difficult to predict as they are often a result of flooding events and may change the level of control at a site in either direction (see Schooler et al., 2011).

*Salvinia molesta* still has a wide distribution in South Africa and new populations continue to be discovered. *Cyrtobagous salviniae* is, however, present at every recorded site, often leading to reductions in weed cover within a few years, supporting the conclusion made by Cilliers (1991) and Coetzee et al. (2011) that *S. molesta* is under complete control in South Africa, even in the more-temperate climates. The successful biological control of S. *molesta* by *C. salviniae* has been shown in many countries around the world, including a number in Africa (Room, 1986; Cilliers, 1987; Cilliers, 1991; Julien and Griffiths, 1998). Success, however, is more-readily reported in tropical and subtropical regions (Julien et al., 2009), whereas success in temperate regions has been less frequent (Cilliers, 1991; Sullivan and Postle, 2010;

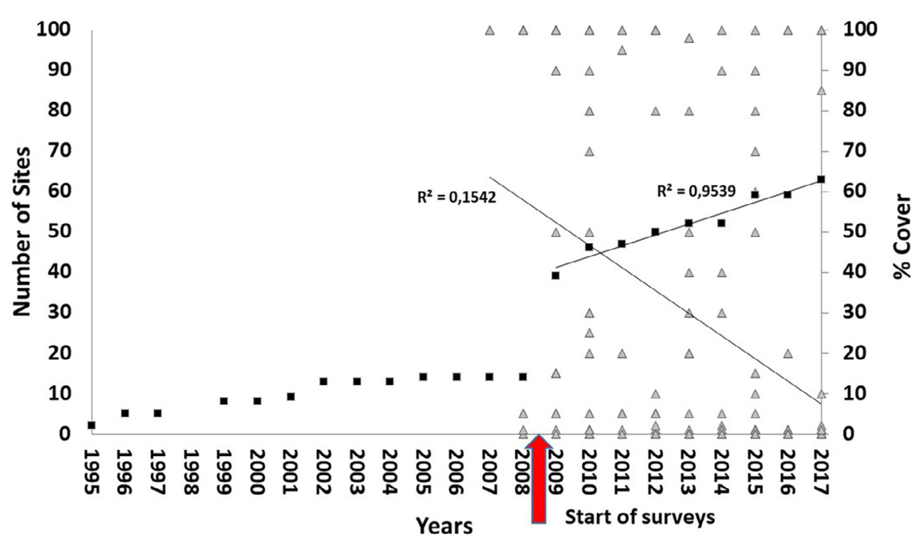


**Fig. 2.** Cumulative number of *Salvinia molesta* sites recorded per year in South Africa (Y = 2.64x + 41.25) ($R^2$ = 0.93; $P < 0.001$) and the % cover of sites surveyed per year throughout the country (Y = −6.15x + 65.6) ($R^2$ = 0.14; $P < 0.001$).

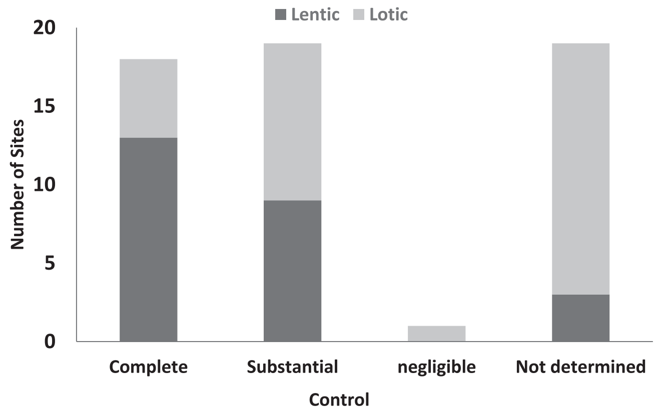


**Fig. 3.** The relationship between lentic and lotic sites and the level of control achieved across all surveyed sites ($\chi2 = 12.837$, df = 3, P = 0.0050).

Obeysekara et al., 2015).

Long-term analyses, in some countries, have highlighted scenarios where expected control has not been achieved, for example, in the Northern Territory, Australia, where high water temperatures have been associated with the failure of *C. salviniae* to control *S. molesta* (Oliver, 1993). Conversely, in New South Wales, Australia, and Louisiana and Texas in the USA, acceptable control has not been achieved because the cooler climate did not support the growth of *C. salviniae* (Sullivan and Postle, 2010; Grodowitz, 2011). There is also the example in Australia where *S. molesta*, growing in low nutrient waters, did not provide adequate nutrients for weevil populations to increase to affect control (Room et al., 1989). Finally, the importance of flooding events in upsetting the *S. molesta* and *C. salviniae* relationship has been highlighted (Schooler et al., 2011). This study has shown that in South Africa, site-specific characteristics also influence the level of control achieved, where geographic location and climate alone do not determine control, while characteristics such as temperature, shade from surrounding tall and/or overhanging vegetation, nutrient concentration, waterbody size and type can potentially contribute to the control achieved at a site.

This post-release evaluation suggests that dense surrounding vegetation (shade) may be limiting control. This is in support of Sullivan et al. (2011) who showed that in Australia, *S. molesta* in shaded sites experienced lower temperatures and had less open water available, resulting in reduced control by *C. salviniae*. Additionally, since *S. molesta* is a sterile pentaploid hybrid fern (Mitchell, 1972), eradication, especially in small systems, should be possible as long as small populations are removed from surrounding vegetation.

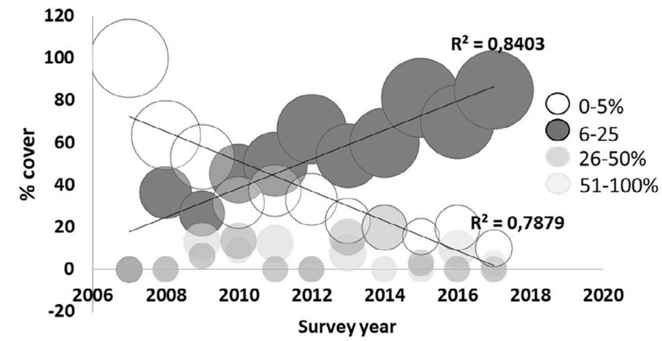


**Fig. 5.** The percentage of sites visited annually grouped into a percent cover category (0–5; 6–25; 26–50; 50–100), illustrating a significant decline in the percentage of sites with high cover over time (Y = 6.99x + 17.59 ($R^2 = 0.80$, $P = 0.0004$), and a corresponding significant increase in the percentage of sites with no cover (Y = −7.60x + 74.01) ($R^2 = 0.78$, $P = 0.0007$). The size of the circle represents the percentage of sites visited annually that fall into that % cover category.

The role of nutrient inputs may influence the level of control. Although it has been shown that increased nitrogen concentration in *S. molesta* promotes herbivorous weevil population development (Room and Thomas, 1985), excessive nutrients allow the plants to remain healthy while supporting high numbers of weevils, which remain ineffective in reducing the weed population. Inferences regarding the water quality, shade and disturbance at sites in this study relied on the field observations of researchers, landowners and government biodiversity officers and not empirical data, as no continuous, quick and reliable sampling method for these environmental parameters are available given the extent and duration of the study.

Flooding, herbicide application and manual extraction can remove

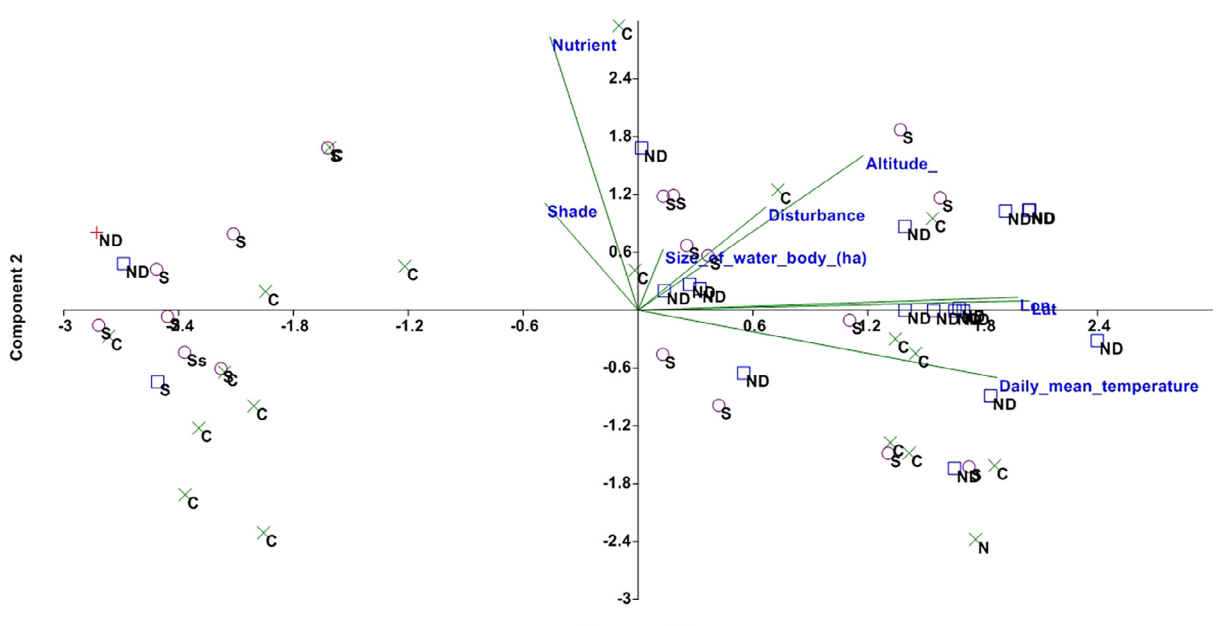


**Fig. 4.** Principal Component Analyses (PCA) of site specific influences that contribute to the management of *Salvinia molesta*. C – complete control, S – substantial control, N – negligible control, ND – control not determined. The first two principal components accounted for only 52.6% of the variation in the data.

*S. molesta* and *C. salviniae* from water bodies (Knutson and Mukherjee, 2012). After these events, rapid regrowth of *S. molesta* frequently occurs, from small trapped populations, often devoid of *C. salviniae*.

This does not mean biological control was unsuccessful at these sites, but that even complete biological control sites require continued management and monitoring to ensure that agents are re-introduced when necessary. A number of South African lotic systems are under this kind of management, where weed populations can fluctuate because of external factors. Some of South Africa's largest and most important rivers e.g. The Swartkops River, Eastern Cape Province, and the Letaba River, Limpopo Province, have proven difficult to manage as flooding events and herbicide applications on other floating invasive species within the systems, remove the insects from the systems. This is evident from the fluctuation in levels of *S. molesta* cover and *C. salvinia* damage in many of the lotic systems which are more susceptible to flooding compared to the lentic systems in South Africa.

Because *C. salviniae* is not a particularly good disperser (Forno and Julien, 2000), mass-rearing centres ensure a continuous supply of healthy *C. salviniae* for distribution around the country to newly identified or reinvaded sites (Coetzee et al., 2011). This requires a small, continuous investment to ensure healthy insects are available when required.

The significant environmental and economic losses caused by infestations of *S. molesta* in numerous tropical and sub-tropical countries have been documented (Doelman, 1989; Pieterse et al., 2003). The economic implications stem from the high cost of physical and chemical control, which is often short-term and unsuccessful in the long-term (Doelman, 1989; Chikwenhere and Keswani, 1997). The value and return on investment of *S. molesta* biological control has also been determined in a few countries (Doelman, 1989; Chikwenhere and Keswani, 1997; Page and Lacey, 2006). In South Africa, the direct impact and costs of *S. molesta* infestations are difficult to quantify as many of the invaded sites are on private land, and within larger invaded systems and rivers that have no direct influence on any industry. Biological control has resulted in the long-term reduction in *S. molesta* infestations around South Africa, which would undoubtedly, although not measured, have improved aquatic biodiversity and decreased the amount of water lost through evapotranspiration. It has also reduced the need to use herbicides and manual labour to remove the weed.

Long-term monitoring of weed infestations and assessing the state of biological control provides valuable information to inform biological control programmes, and aid in developing adaptive management strategies to best deal with weed infestations. Post-release evaluation mechanisms should thus be included in all weed biological control programmes, and sufficient resources and funding should be ring-fenced for this at the start of biological control programmes.

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