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Impact of deltamethrin aerial sprays on adult *Cyrtobagous salviniae* in Botswana

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The risk and effect of the insecticide deltamethrin, applied aerially in Botswana to control and eradicate tsetse fly, was studied on the non-target weevil *Cyrtobagous salviniae,* a biocontrol agent of the aquatic weed *Salvinia molesta*. Environmentally-simulated short-term toxicity bioassay used open iron cages and closed plastic basins containing weevils and salvinia placed in riverine water bodies, where they were exposed to the aerial applications of deltamethrin over the Kwando–Linyanti system in 2006. Control samples were placed 40 km outside the sprayed area. Weevil mortality, determined at 12, 36 and 60 hours after the aerial application, reached a maximum of 27%. No significant difference in mortalities was observed between the closed basins and the open cages. The amount of deltamethrin deposited at ground level was between 1.2 and 6.4 µg m^{-2} and the insecticide toxicity was related to the weevil mortality. Simultaneous field monitoring through five spray applications showed that weevil abundance declined in late winter. Deltamethrin had a negligible impact on the weevil's ability to control salvinia under field conditions, probably due to the weevils' protective mechanism and because vegetation could act as a barrier, preventing the insecticide from reaching the weevils. Minimum impacts of deltamethrin on the weevils in the present study and their recovery in the field are consistent with those of earlier spray applications in the Okavango Delta, Botswana.

Keywords: aerial spraying, deltamethrin, insecticide, nagana, *Salvinia*, tsetse fly

Introduction

Herbicides and insecticides are commonly used aerially to target weeds and insect pathogens in the management of various habitat environments. The presence of tsetse fly has been recognised as a major constraint to development in Africa. In view of the problem, the African Heads of States decided at an Organization of African Union summit held in Lome, Togo, in July 2000 that tsetse fly eradication should be made a collective responsibility of all countries affected (Kgori et al. 2006). Consequently, a Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) was launched with the aim of eradicating tsetse and trypanosomiasis within the shortest possible time (Kgori et al. 2006). Botswana is one of the African countries that embraced the idea of eradicating tsetse flies, to the extent that the government committed a significant amount of funds to tsetse eradication.

Prior to the 1980s, tsetse fly *Glossinia morsitans centralis* Machado (Diptera: Glossinidae) was limited to an area of 30 000 km^2 over the Okavango Delta and the Kwando–Linyanti River system (Davies 1980). It was reported (Sharma et al. 2001, Kurugundla and Serumola 2007) that several head of cattle died in the Okavango Delta in 2000 due to nagana, a parasitic disease caused by *Trypanosoma rhodesiense*, of which tsetse fly is the vector. Realising their impact, the Government of Botswana sprayed deltamethrin to eradicate tsetse flies in the north-west of the country, including the Okavango Delta, in 2001 and 2002 (Perkins and Ramberg 2004). With the successful elimination of tsetse flies in the Delta (Kgori et al. 2006), the government of Botswana embarked upon yet another exercise to eliminate the flies in the Kwando–Linyanti rivers region in the north-eastern part of the country in 2006. The spray block totalled 10 000 km^2, covering Kwando–Linyanti in north-east, Caprivi in Namibia and parts of Zambia and Angola, making it the largest single area ever treated by aerial spraying to control tsetse flies. The entire spray block was divided into seven sub-blocks, each of which was sprayed sequentially from day one to day seven in every cycle on the specified dates.

Deltamethrin, a synthetic pyrethroid, is a broad spectrum insecticide. Pyrethroids are relatively stable, with a high toxicity to a wide spectrum of insects, and are relatively non-toxic to mammals (Elliott 1976), and hence have considerable agricultural potential (Harris and Turnbill 1978). Pyrethroids are much less persistent than organochlorine insecticides, such as DDT and dieldrin, do not produce significant effects on non-target agents, and apparently do not accumulate in the environment (Smith and Stratton 1986, Grant and Crick 1987). Sequential drift sprays of deltamethrin at 0.1 to 0.25 g a.i. ha^{-1} killed a wide range of arthropods in large numbers (Games 1981).

The beneficial host-specific biological control agent *Cyrtobagous salviniae* Calder and Sands was introduced in 1983 to control Kariba weed *Salvinia molesta* Mitchell in the Kwando–Linyanti River system (Schlettwein 1985). The effects of deltamethrin were assessed on non-target organisms, including *C. salviniae*, for the first time in the 2002 spray applications in the Okavango Delta which resulted in 17–40% weevil mortality (Perkins and Ramberg 2004, Kurugundla and Serumola 2007). The present study aimed to determine whether the impact of deltamethrin on *C. salviniae* would differ from that in the 2002 spraying. Salvinia was fairly common in the Kwando and Linyanti rivers until 2002, but by 2003 nearly 80% weed control had been achieved due to the continuous release of the weevil on the weed infestations (Kurugundla 2003). Pyrethroids are especially advantageous for use in cold climate conditions since their effect on the target agents are temperature dependent (Harris and Kinoshita 1977).

Materials and methods

Site selection

Three methods of assessment, viz. toxicity bioassay performed *in situ*, a whole plant method and a standard plant method, were used to determine the impacts of deltamethrin aerial spray on the weevils. For the bioassay method, seven sites on a 60 km stretch of the Kwando River were selected based on water regimes and accessibility. From upstream, these were Kwando pool 1, Kwando pool 2, James Camp, Wetland pool, Lebala pool, Side stream and Selinda canal (Figure 1). Selinda canal and Side stream had flowing water but the other five sites were stagnant water bodies. The whole plant method was conducted at Hyena camp, and at Shummamori and Dumatau channels, whereas the sites for the standard plant method included Selinda canal, Lebala pool, Shummamorei and Hamokata. Similar field assessments were also conducted using the standard plant method at six sites in Caprivi viz., Sitwa 1, Sitwa 2, Namushasha, Lianshulu, Nakatwa and Barerwa (Figure 1), after the spray in the 2nd, 3rd and 5th cycles.

Insecticide application

The ultra low volume formulation of deltamethrin, Deltanex, used for the 2006 aerial spray operation was exactly the same as that used in 2001 and 2002 in the Okavango Delta. Spraying of deltamethrin using fixed-wing aircraft was carried out in five cycles in the entire block, and the Kwando River sub-block received five sequential sprays between 18:00 and 20:00 on 31 May, 20 June, 11 July, 31 July and 16 August 2006 (Figure 1). The application rate for cycles 1 and 2 was 0.3 g ha^{-1}, and 0.26 g ha^{-1} for cycles 3, 4 and 5. The carrier solvent for the insecticide used in the spray was kerosene.

Toxicity bioassay method

The distribution of salvinia and weevil populations varies widely under field conditions. Therefore, field and static short-term toxicity bioassay methods were established (Reish and Cshida 1987) using cages and plastic basins deployed *in situ*. In the 2002 spray program similar studies were conducted using plastic basins. But in 2006 cages were used in the place of basins, because cages were more appropriate as they have continuity with the field water conditions. Nevertheless, the deployment of a single basin at each site in 2006 was done to identify whether toxicity remained the same between cages and basins. Cages of 0.5 m^2 were made of 1.0 cm diameter iron rod and wrapped with chicken mesh on four sides to avoid salvinia from being carried away by flowing water. Twenty-one cages (three per site) were placed in the water bodies at seven randomly selected sites in the Kwando River. One plastic basin (50 cm diameter, 20 cm deep) was placed near the cages at each site. Half a kilogram green salvinia devoid of weevils was placed in each cage and basin. Fifty adult weevils were released onto the salvinia mat in each cage and basin two days before aerial spraying to facilitate insect acclimatisation and establishment. Water in the cages had continuity with field water, while the basins represented closed systems. The cages and basins were directly exposed to the aerial spraying of deltamethrin. Four replicates of representative reference controls, using cages and basins, were maintained for every cycle in a water body about 40 km outside the spray block (Figure 1).

Sampling

Replicate samples of salvinia with weevils were recovered from all seven cages at each site. The first sampling was conducted 12 hours after aerial application of deltamethrin on the following morning between 06:00 and 08:00. Subsequent samples were recovered 36 and 60 hours after spraying. The weevils found floating in the cages at the time of each sampling event, as the result of insecticide spray drift, were picked up by hand and released onto a small fragment of unsprayed salvinia in water in small plastic cups for mortality testing. The other weevils from the salvinia mat were completely separated by extraction using Berlese funnels (Boland and Room 1983) after collecting them from the cages. All seven basins were recovered 12 hours after the insecticide application and weevil data was collected as described above. The same methods were followed for sampling salvinia, collecting weevils and determination of mortality from the control cages. Surface water temperatures were measured at 6 cm depth at 08:00 and 18:00 in the cages as well as in open waters.

Mortality determination

Mortality was determined by exposing the weevils on the host plant to sunlight, under which conditions live weevils would respond actively. Only those weevils that remained alive for more than 12 hours after collection were considered to have survived the spray treatment (Schlettwein and Giliomee 1990). Controls were also subjected to the same methods.

Whole plant method

The whole plant method is normally applied to determine the weevil populations in a unit fresh weight of salvinia in the field, that are required to effect control of the weed (Boland and Room 1983). Salvinia was common at Shummomorei and Dumatau in the fringes among *Cyperus papyrus* L.,

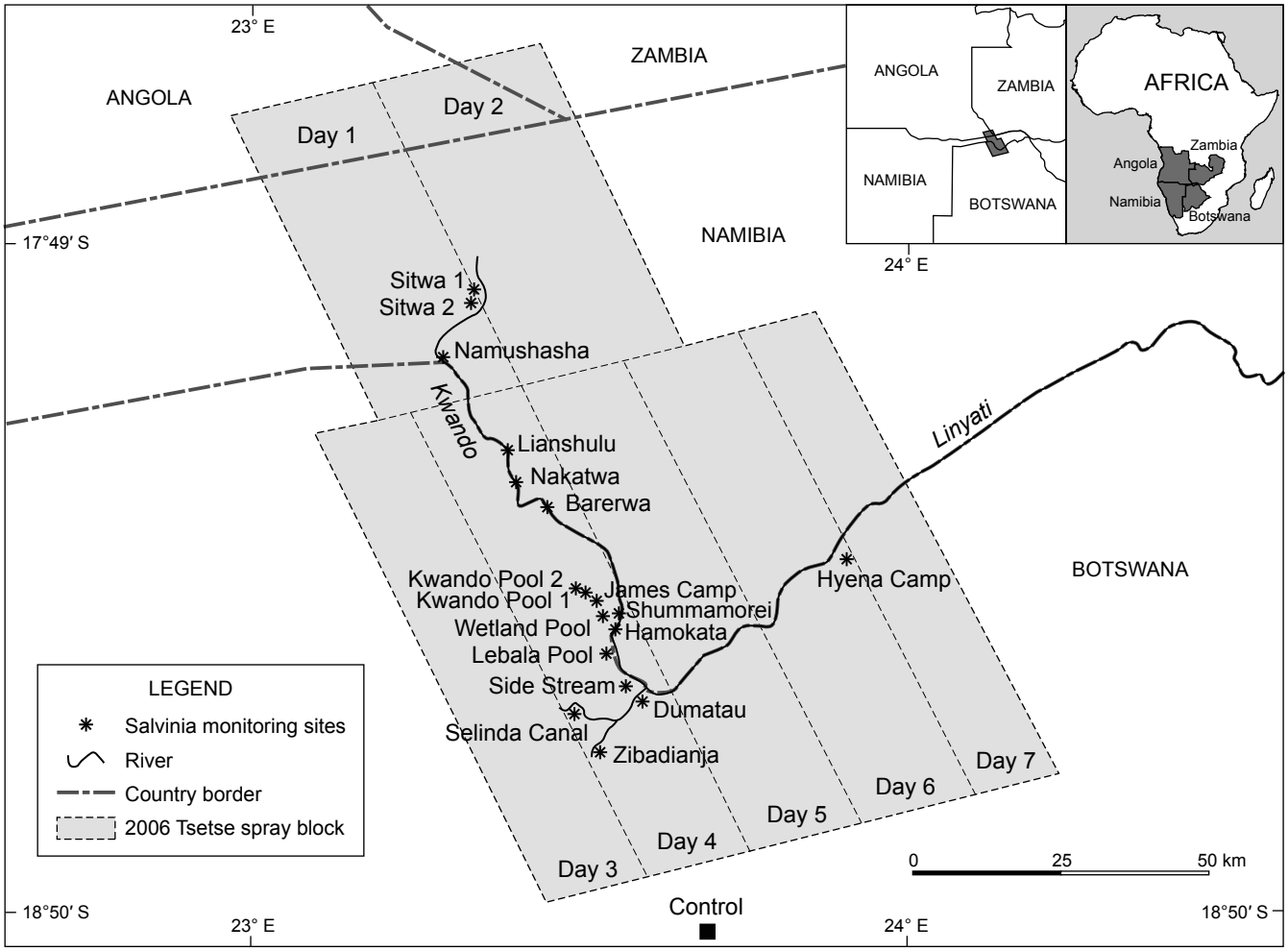

Figure 1: Map of the study area, showing bioassay experimental and field monitoring sites to assess the impacts of deltamethrin spray on salvinia weevils in the Kwando–Linyanti River system (data source: Okavango Delta Information System ODIS, University of Botswana)

Vossia cuspidata (Roxb.) Griff and *Phragmites australis* (Cav.), whereas the stagnant pool at Hyena Camp had dense, open salvinia infestations before the start of deltamethrin spraying. Seven replicate samples were collected 12 hours before the spraying and seven replicate samples the following morning, 12 hours after the spraying, in every cycle to determine the number of weevils per kilogram of salvinia (Boland and Room 1983) at Hyena Camp and at Shummomori, while at Dumatau channels the number of weevils per kilogram of salvinia was determined in every cycle 12 hours after the insecticide spray.

Standard plant method

A standard salvinia plant is defined as one composed of four pairs of leaves, from the terminal bud on the main branch including all side branches within that unit (Room 1983). Twenty standard plants were collected at random in seven replicates in every cycle 12 hours before the spraying, and the same number of replicates 12 hours after spraying at the sites described above, and the numbers of weevils extracted were expressed as number per 20 standard plants. All sites had small groups of salvinia in the gaps amongst vegetation and in hippo paths along the edges of water bodies. Similar assessments were also conducted at six sites in Caprivi.

Preparation and collection of target aluminium foils

Seven replicate 42 × 42 cm sheets of aluminium foil were spread across the bottom of empty plastic basins placed near salvinia containers in all five applications to collect insecticide spray drift. Three replicated sheets of aluminium foil of similar size were prepared at control sites. The targeted foils were collected and folded in the early hours of the day, 10–12 hours after spray application, and immediately placed in a plastic box over ice to preserve the samples, as recommended (IF Grant, Natural Resource Institute, Greenwich, UK, pers. comm.).

Extraction and analysis

Deltamethrin deposits collected on the aluminium target foil were determined at the Natural Resource Institute, Greenwich, UK (NRI 1995). The samples were extracted

for four hours in 250 ml acetone, using a Soxhlet apparatus. Samples were evaporated down to about 20 ml and 500 nanograms of cypermethrin was added as an internal standard. Samples were assayed on a DB5 column (30 m × 0.25 mm × 250 µm), using helium carrier gas and an Electron Capture Detector, model HP 6890. The lowest limit of detection on each sample was c. 8 picograms.

Data analysis

Student's *t*-test was applied to compare the experimental data and the reference controls obtained from the bioassay methods, using the method of Snedecor and Cochran (1989). The survival of weevils in the treatment areas was corrected with respect to that of weevils in the controls to obtain corrected percent mortality. Analysis of variance (ANOVA) was performed using SPSS version 14.0 using corrected percent mortalities in the cages to determine the weevil mortality between the five cycles.

Results

Air temperatures varied during the period of five applications, with maximum daily temperatures ranging between 21 °C and 32 °C and minimum temperatures varying between 1 °C and 14 °C. Colder conditions prevailed during the second part of cycles 1, 3, 4 and 5. Wind from the north, north-east and south-east occurred during the five spray cycles (Bonyongo and Mazwimavi 2007). The mean minimum water temperature in cages for the five cycles at 08:00 was 10.3 °C (±0.6) and mean maximum temperature at 18:00 was 24.8 °C (±0.5). Field water temperatures were between 11.3 °C (±0.8) and 25.3 °C (±0.7) at the same periods.

Mean survival of the 50 adult salvinia weevils in the controls was generally in the range of 45.5 (91.0%) to 47.0 (94.0%) in number, while mean survival in basins and cages exposed to deltamethrin varied from 33.7 (67.4%) to 39.6 (79.2%) (Table 1). The percentage deltamethrin collected on the aluminium foil was in the range of 1.2% to 6.4% (Table 2) of the applied rate, and this amount was responsible for weevil mortality in the range of 15.7% to 27.2% with reference to controls (Tables 1 and 2). Nevertheless, the percent mortality after 60 hours was found to be between 22.0% and 26.0% (Table 2). However, in the 4th cycle on 3 July 2006 the formation of ice crystals and the cold conditions in the weevil extraction cups (<5 °C) increased the death rate of weevils appreciably (live weevils = 22.0 ± 1.8, mortality = 52.5%) ($p < 0.05$) (Table 1). ANOVA and Tukey's analysis for the corrected percent mortalities of weevils were not significant between cycles 1, 2, 3 and 5 (Figure 2) but were significant ($p < 0.05$) only in the 4th cycle. The density of weevils in the salvinia was 102 ± 5.7 per kg (fresh weight) of salvinia in Hyena Camp before the spraying started on 28 May 2006, and the weed was controlled completely by the 4th cycle on 2 July 2006 (Figure 3). Weevil density on salvinia at Shummamorei did not change before and after the spray in the 1st cycle, although it declined after the 2nd cycle (Figure 4), whereas in Dumatau channels the weevil abundance between the cycles seemed to be marginal 12 hours after the spray through the five cycles (Figure 5). In most cases, the number of adult weevils on 20 standard plants was greater than one at all sites monitored during the five spray cycles (Figure 6).

Discussion

The important features in the aerial spraying program for tsetse fly control are the formulation of insecticide and the droplet size (30–40 µm volume median diameter) that kill tsetse flies significantly. Since there were no significant differences in mortality between the closed basin and open cage conditions 12 hours after spraying (Table 1), closed basin habitats did not increase the deltamethrin toxicity, as had been suspected during the 2002 spray programme (Kurugundla and Serumola 2007). Maximum weevil mortality in the present study was 27.2% (Table 1), as compared to 40.0% previously (Kurugundla and Serumola 2007). Cesida (1980) found that pyrethroid toxicity increased at lower temperatures. Therefore the results of the 4th cycle are explained by the cold conditions in the weevil extraction cups increasing the weevil mortality significantly ($p < 0.05\%$) (Table 2 and Figure 2), but not necessarily as a result of deltamethrin spray drift.

Table 2: Percent mortality of weevils per cycle (60 hours) in relation to the deposition of deltamethrin drift (SE = sd$\sqrt{n}$). * = mortality significantly higher than normal ($p < 0.05$), probably because of colder conditions (see text and Figure 2)

Cycle	Deposition (% m^{-2})	Mortality (%)
1	1.2 ± 0.5	24.0
2	6.4 ± 2.2	23.0
3	4.9 ± 0.8	23.1
4	3.1 ± 0.6	52.5*
5	5.0 ± 0.9	26.0
Average	4.1 ± 0.8	29.7 ± 5 .1

Table 1: Mean survival of 50 weevils (SE = sd$\sqrt{n}$) in closed basins and open cages in response to deltamethrin spray drift. Figures in parentheses indicate corrected percent mortality. * $p < 0.05$ with reference to controls

Cycle	Control	Basins	Cages		
		12 hours	12 hours	36 hours	60 hours
1	47.0 ± 1.6 (6.0%)	39.6 ± 3.3 (15.7%)	39.3 ± 2.4 (16.4%)	37.4 ± 4.5 (20.4%)	35.7 ± 1.8 (24.0%)
2	46.5 ± 0.6 (7.0%)	*36.4 ± 3.1 (21.7%)	39.1 ± 2.5 (16.0%)	*35.2 ± 3.3 (24.3%)	*35.8 ± 2.0 (23.0%)
3	45.5 ± 0.6 (9.0%)	35.7 ± 3.3 (21.5%)	37.7 ± 3.3 (17.2%)	*34.4 ± 1.4 (24.6%)	*35.0 ± 1.9 (23.1%)
4	46.3 ± 0.9 (7.4%)	*34.4 ± 2.0 (25.7%)	*35.9 ± 3.1 (22.5%)	*33.7 ± 1.7 (27.2%)	*22.0 ± 1.8 (52.5%)
5	46.3 ± 1.3 (7.4%)	37.0 ± 2.1 (20.1%)	*36.1 ± 1.7 (22.0%)	36.7 ± 1.8 (20.7%)	*34.3 ± 3.1 (26.0%)

No significant difference in weevil mortality was observed between cycles in the range of 4.9% to 6.4% deltamethrin deposition (Table 1), which agrees with the observations of Kurugundla and Serumola (2007). In the present study the 1.2% level deltamethrin drift did not have a significant impact on weevil mortality, whereas Kurugundla and Serumola (2007) reported insignificant weevil mortality at <2.3% level deposition. Schlettwein and Giliomee (1990) found that adult weevil mortality occurred at concentrations of 10 µg m^{-2} of alpha-cypermethrin in aerial applications, whereas in laboratory experiments Semple and Forno (1990) found that adult weevils were highly susceptible to deltamethrin, reporting LC_{50} of 0.038 µg l^{-1} in water. In Zimbabwe applications of 0.25 g a.i. ha^{-1} deltamethrin increased the mortality rates of a wide range of aquatic invertebrates, but the effects were transient and no population declines resulted (Grant and Crick 1987). The floating behaviour of weevils, observed in basins at the time of sampling, was caused by pyrethroids either rendering the insects temporarily inactive, or killing them (Hill 1985).

At Hyena Camp before the spray the number of weevils was 102 per kg of fresh salvinia, declining in abundance appreciably after the 2nd spray cyle (Figure 3), as was the case in several situations where biocontrol was utilised (Naidu et al. 2000). The achieving of salvinia control at Hyena Camp by the 4th cycle showed that the weevils and larvae established well prior to the spray, and that those adult weevils that survived deltamethrin applications targeted the weed significantly. It is noteworthy that the severe damage to salvinia weed infestations was generally caused by larvae tunnelling into the rhizome, where they were well protected from the insecticide (Schlettwein and Giliomee 1990).

The decline in the abundance of weevils at Shummamorei after the 2nd cycle, with a marginal increase after the 5th cycle (Figure 4), was due to the spatial distribution of weevils and to less breeding during cooler conditions (Forno

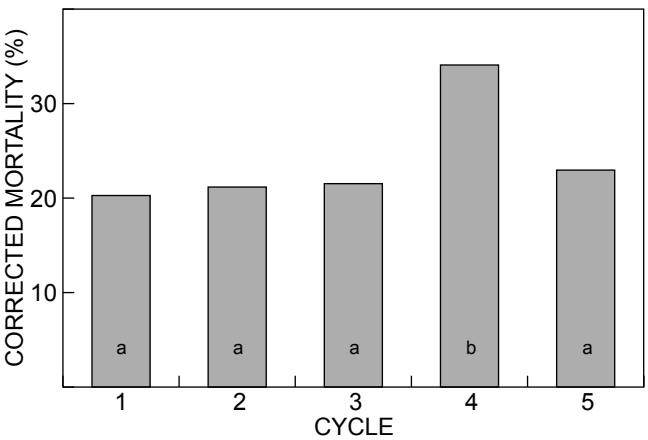

Figure 2: Corrected percent mortalities of salvinia weevils in five spray cycles. Bars with the same letters are not significantly different ($p < 0.05$, ANOVA and Tukey's test)

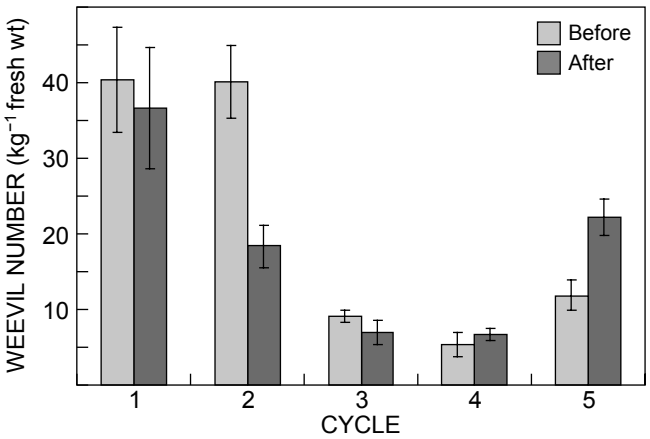

Figure 4: Weevil densities on salvinia before and after each spraying cycle at Shummamorei, Kwando River, as determined by the whole plant method. Error bars denote SE

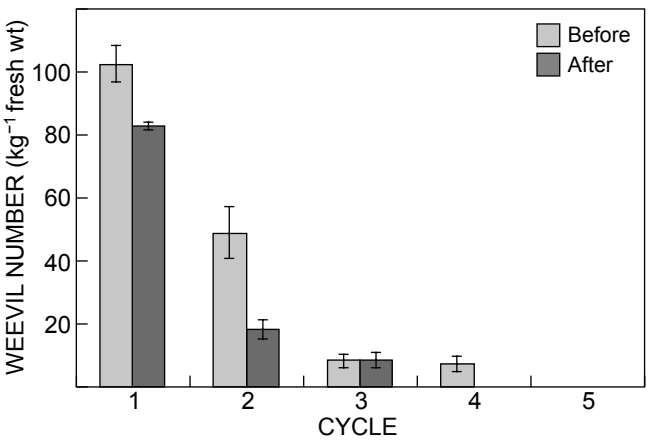

Figure 3: Weevil density on salvinia before and after spray applications at Hyena camp on the Linyanti River, as determined by the whole plant method. Error bars denote SE

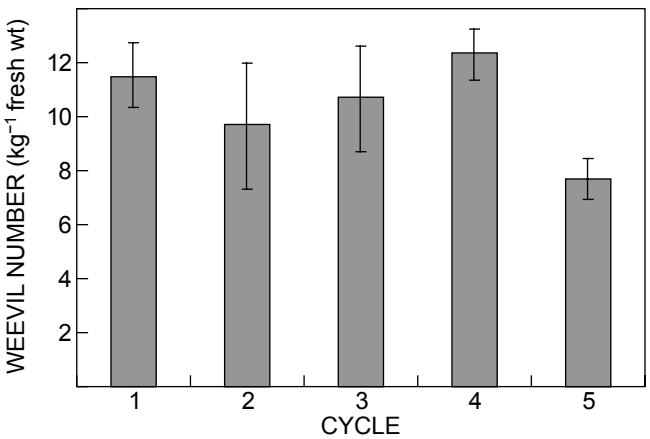

Figure 5: Weevil densities on salvinia plants in the Dumatau area, Kwando River, after each spraying cycle, as determined by the whole plant method. Error bars denote SE

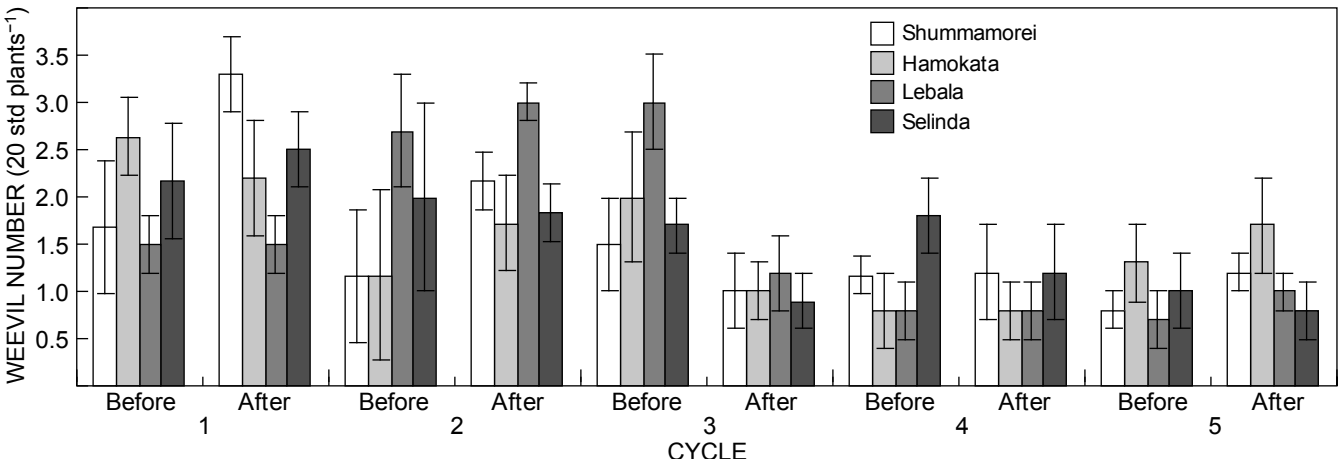

Figure 6: Numbers of weevils on 20 standard salvinia plants at four sites before and after spraying in the five spray cycles. Error bars denote SE

et al. 1983, Naidu et al. 2000), despite some spray effects. At Dumatau channels relatively marginal differences existed in the abundance of weevils between cycles after the spray (Figure 5). In all five cycles the abundance of weevils in 20 standard plants was higher than one (Figure 6) showing that the weevils maintained their equilibrium at Shummonorei, Hamokata, Lebala and Selinda during the spray program. Similar assessments in Caprivi showed that weevil density was more than one in 20 standard plants during the 2nd, 3rd and 5th cycles after the spray (data not presented). There were few plants scattered along the edge of these water bodies showing equilibrium between weevil activity and salvinia growth. At this stage the population density of the weevil should be >1 adult per 20 plants (Forno 1987).

Although deltamethrin deposition caused significant mortalities in the present study's spray cycles (Table 1), the insecticide did not affect the weevils' ability to control salvinia at Hyena Camp (Figure 3), since their density in the field did not vary significantly before and after spraying (Figures 4, 5 and 6). It might be difficult to relate the declines in the abundance of weevils in the field to the deltamethrin toxicity, as toxicity is influenced by factors such as temperature, season of spraying, habitat conditions and a protective mechanism possessed by the life stages of weevils (Schlettwein and Giliomee 1990). In aquatic habitat conditions deltamethrin aerosols could be diluted, partitioned and adsorbed onto various organic sediments (Muir et al. 1985), which would reduce the dose on the weevils in the field infestations. Salvinia was mostly found at the fringes among papyrus and reeds in flowing waters, and in such areas the vegetation might act as a limiting factor for insecticide deposition on the target surfaces, unlike in the open infestations in cages and basins. On cold nights the weevils normally hide in buds, roots and beneath the leaves. They deposit their eggs in buds and underneath leaves, and emerging larvae normally feed inside the rhizome (Forno et al. 1983). Therefore adults and larvae would often be protected from contact with the insecticide (Schlettwein and Giliomee 1990).

Conclusions

With 4.1% m^{-2} deposition of deltamethrin average weevil mortalities are typically 24% (Table 2), and the maximum weevil mortality observed in the 4th cycle in the present study is likely to be the result of cold temperatures that prevailed during the separation of weevils in that particular cycle. Nevertheless, the deltamethrin sprayed aerially did not affect the progress of biological control of salvinia in areas such as Hyena Camp or Shummamorei. The presence of more than 1 adult weevil per 20 plants shows the equilibrium between the host plant and weevil populations, despite some impacts of the insecticide. For example, Lebala pool became completely covered with salvinia two months after the spray period in October 2006, yet by December 2006 the weevils had controlled the weed.

Aerial spraying of deltamethrin for controlling tsetse fly in any given area is not a continuous process. The insecticide is applied only in winter, when the breeding rate of the weevils generally declines. The present study confirms that, although *Cyrtobagous salviniae* was affected negatively by the aerial spraying of deltamethrin, it recovered thereafter as shown by the subsequent effective control of salvinia at Lebala Hippo Pool.

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