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Research Article

Survival and dispersal of the stem-boring weevil, *Listronotus setosipennis*, and the leaf-feeding beetle, *Zygogramma bicolorata,* one year after their release for the control of the invasive weed, *Parthenium hysterophorus*, at two locations in Ethiopia

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ABSTRACT: The presence and spread of *Listronotus setosipennis* (Coleoptera: Curculionidae) and *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) released in 2017 for the control of *Parthenium hysterophorus* (L.) (Asteraceae: Heliantheae) at two locations in Ethiopia (Arba Minch and Mojo) were assessed twice (July, September) in 2018. At Arba Minch, no *L. setosipennis* was found, only *Z. bicolorata* established and the number of eggs varied from 1.4±0.71/plant (July) to 7.41±4.52/plant (September) at the release site and from 0.52±0.18/plant (July) and 0.68±0.24/plant (September) at the dispersal site. At Mojo, only *L. setosipennis* established with 3.87±1.18 eggs/plant (July) at the release spot and 10.76±1.64 eggs/plant in September whereas after dispersal the eggs numbered 8.69±3.2/plant (July) and 14.98±1.73/plant (September). After one year, *L. setosipennis* and *Z. bicolorata* were found 51.75±3.95 m and 94.15±13.66 m away from the release spots, respectively. The results show that the biocontrol agents have started to establish and have dispersed at the two locations in Ethiopia.

KEY WORDS: Beetle, biological control, Ethiopia, parthenium, weevil

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INTRODUCTION

The noxious weed, parthenium (*Parthenium hysterophorus* L., Asteraceae: tribe Heliantheae), has spread from its origin in tropical and sub-tropical America to many countries in Africa, Asia, and Australia (Evans 1997; Navie *et al*., 1996). Currently, it is found in 14 African countries but has a potential to invade much of sub-Saharan Africa according to a CLIMEX modeling program (McConnachie *et al.,* 2010). In Africa, it is found in pasture lands, crop fields, overgrazed lands, national parks, protected lands, and around rural homesteads resulting in daily exposure of people to its pollen (McConnachie, 2015; Wabuyele *et al.,* 2014). It is able to dominate the landscape it invades because it can produce large number of seeds (up 28,000 per plant), can germinate and grow anytime of the year if soil moisture is adequate and temperature is ideal, has the ability to flower and set seed within four to six weeks of germination, and can tolerate different environmental conditions. Parthenium leaves and pollen can also produce and release a toxic sesquiterpene lactone allelochemical known as parthenin to its surrounding environment (Kanchan and Chandra, 1989). This toxic allelochemical can inhibit the germination and growth of other plants (Evans, 1997; Mersie and Singh, 1987; Navie *et al.,* 1996) thus allowing parthenium to form monoculture in once disturbed and overgrazed fields. People also develop skin problem such as dermatitis when they came in contact to parts of the parthenium plant and especially, the pollen causes respiratory problems such as asthma and bronchitis (Evans, 1997).

Parthenium entered Ethiopia in late 1970's and quickly spread to all regions of the country (McConnachie *et al.,* 2010) and farmers consider it one of the top damaging weeds both in arable and grazing lands (Tamado and Milberg, 2000). In Ethiopia, it reduces yield of food crops such as sorghum ranging from 40% to 95% depending on plant density (Tamado *et al.,* 2002).

Parthenium is found in crop fields such as in teff (*Eragrostis tef L.*) until harvest thereby competing with this important food crop for moisture and nutrients throughout the season. Parthenium displaces valuable pasture species

(Nigatu, 2010) thereby severely affecting livestock production. If livestock graze on a parthenium plant it taints their meat and milk (Tudor *et al.,* 1982) thereby reducing their market value.

Current control measures against parthenium in Ethiopia include hand weeding and slashing mature stands. However, both hand weeding and slashing are mostly ineffective because the weed germinates throughout the rainy season in large number requiring repeated removal from a single field. Furthermore, labor during the peak season may not also be readily available to do hand weeding. In addition, workers occasionally refuse to come back because the weed makes them sick.

Presently, the most sustainable, economical, and effective method of managing parthenium is by using biological control agents. In its native range, parthenium is kept under control by natural enemies. Several countries including Australia, India, and South Africa (Dhileepan and McFadyen, 2012; McConnachie, 2015; Strathie *et al.,* 2011) have introduced natural enemies to control parthenium. One of these natural enemies used against parthenium is *Zygogramma bicolorata* (Coleoptera: Chrysomelidae). Adult *Z. bicolorata* lays eggs usually on the underside of young parthenium leaves as a group or singly. The eggs hatch within four or six days and the larva as well as the adult feed on the leaves of parthenium. Fully developed larva enters the soil and pupates in chambers before it emerges as an adult. The larva and pupal stage take 23 to 28 days depending on the environmental temperature. The adult and the larva feeding on parthenium can cause complete defoliation of the weed.

Australia and India have tested and released *Z. bicolorata* in the field over thirty years ago (Dhileepan and Strathie, 2009). South Africa released the leaf-feeding beetle in 2013 (McConnachie, 2015) after conducting an extensive host range test. At high densities, the adults and larvae of *Z. bicolorata* can entirely defoliate stands of parthenium. Such severe defoliation will weaken parthenium thereby allowing the native vegetation to recover.

The other natural enemy used to control parthenium is the stem-boring weevil, *Listronotus setosipennis* (Coleoptera: Curculionidae). *L. setosipennis* is a nocturnal weevil that hides in leaf-litter leaves during the day. The adult *L. setosipennis* digs holes on newly opened parthenium flower, lays its eggs and covers it with black frass. Thus, the oviposition spots on parthenium flowers appear as black marks. It can also lay egg on petiole of leaves on non-flowering parthenium plants. The eggs hatch within 3 to 5 days and the first instar larva bores into the flower base or leaf base, and enters into the stem. The larva feeds inside the stem for 18 to 30 days and exits at the bottom to enter into soil. The larva undergoes up to five instar changes before it matures and exits the stem to enter into soil. Within four days of entering the soil the larva pupates and the adult ecloses after five days (Wild *et al.,* 1992). Four to five larvae feeding inside a flowering parthenium stem and as low two at rosette stage can kill parthenium (Dhileepan, 2003). *Listronotus setosipennis* has been field released in Australia (Dhileepan and McFadyen, 2012) and South Africa (Strathie *et al.,* 2016) to control parthenium.

Ethiopia has also recently introduced the leaf-feeding beetle, *Z. bicolorata* and the stem-boring weevil, *L. setosipennis,* against parthenium. The host-specificity of *Z. bicolorata* and *L. setosipennis* was assessed against 29 and 31 non-target plant species, respectively (unpublished data). The results showed that *Z. bicolorata* and *L. setosipennis* can only complete their life cycles on parthenium and would not attack other species. Based on these host range test results, the Ethiopian Government gave permission for the field releases of *Z. bicolorata* and *L. setosipennis*.

Releases of both biocontrol agents were carried out during the summer months (June to September) of 2017 in different parts of the country with high parthenium infestations. Some of these sites were visited in July and September 2018 to see if the biocontrol agents survived the preceding dry period. The objective of this study was to determine the presence and spread of *Z. bicolorata* and *L. setosipennis* one year after they were released at two regions of Ethiopia (central, Mojo and southwestern, Arba Minch).

MATERIALS AND METHODS

Culturing of *Zygogramma bicolorata* and *Listronotus setosipennis*

Starter cultures of *Z. bicolorata* and *L. setosipennis* were originally obtained from laboratory-reared stock at the Agricultural Research Council, Plant Protection Research Institute (ARC-PPRI) Hilton, South Africa. Rearing of *Z. bicolorata* and *L. setosipennis* in Ethiopia for release was undertaken in Wollenchiti (N= 08° 39.116' E= 039° 25.214' elevation = 1446 m) in central Ethiopia. Biocontrol agents were reared in sealed metal frames (0.5 m x 0.5 m x 1 m) covered with nylon mesh on the sides and foam between the frame and the door. All corners and joints are sealed with silicone to prevent escape of the biocontrol agents through gaps. Four holes are cut on the floor of the cages to exact fit of a pot with 20 cm diameter. The bottom of the pot protrudes through the whole allowing free water to drain from the soil outside of the cage. The parthenium seedlings were grown in plastic pots (25 cm high x 20 cm in diameter) filled with soil,

sand and compost at 2:1:1 ratio after being transplanted from seedbed (grown from seed) in plant nursery or directly from field. Pots were fertilized with 46% Nitrogen (N) solution. Plants were watered daily as necessary. The breeding cages were placed on benches located in walk-in tunnels (5 m x 7 m x 5 m) with metal frames covered with nylon mesh. The inside of the walk-in tunnels during the rearing period (May to August) had mean minimum temperature of 18.4°C, mean temperature of 19.5°C and mean maximum of 31.7°C. The mean minimum relative humidity was 40.5%, mean relative humidity of 64.7% while mean maximum humidity was 83.3%.

Rearing of *Zygogramma bicolorata*

Four pots containing leafy parthenium plants about 50 cm tall were transferred to the breeding cages. Before transfer to cages, plants were inspected for ants, spiders, other predators and washed with water. All parts of the plant and the surface of the soil in pots were regularly sprayed with 2% solution of sodium hypochlorite to reduce disease and contamination incidences. Spraying the leaves also provides free water for the beetle. On each plant, 25 adult beetles were placed and allowed to feed, mate, and ovipositing removed and transferred to oviposition cages for breeding.

Release

Two-week old adults of *Zygogramma bicolorata* and *Listronotus setosipennis* were collected from the breeding cages and placed in plastic boxes (30 cm x 20 cm x 13 cm) with lids fitted with mesh for aeration that have washed parthenium leaves and flowers (for the weevil) for transport to release sites. The washed parthenium leaves lie on absorbent paper towel were sprayed with 2% sodium hypochlorite solution. The number of adults placed in each plastic container were counted. Fresh parthenium leaves and flowers washed and sprayed with 2% sodium hypochlorite solution were transported in separate plastic containers to be used as replacement during long trips. Containers that have the beetles and the weevils were placed in a shade during transport and during release to minimize heat stress to the weevil and to the beetle. Adults ranging from 1000 to 3000 for the beetle and 500 to 600for the weevil were released at Arba Minch and Mojo sites on August 18, 2017 and July 12, 2017, respectively (Table 1).

Data collection and analysis

Sites that received *Zygogramma bicolorata* and *Listronotus setosipennis* during the 2017 rainy season (July and August) at Arba Minch and Mojo were examined for the weevil in July and August of 2018. Original release spots were identified based on latitude and longitude taken in 2017. To determine establishment, a quadrat of 50 cm x 50 cm within the release spot was randomly selected and the four corners were marked with red flags. In this quadrat the number of parthenium plants, eggs, larvae and adults were recorded for *Z. bicolorata* whereas for *L. setosipennis* the number of rosette and flowering parthenium plants, and oviposition marks on the flowers were recorded. For *L. setosipennis*, larvae were detected by splitting all stems of flowering parthenium plants as well as those at the rosette stage found in quadrat. The quadrats were replicated three times. The spread of *Z. bicolorata* and *L. setosipennis* from the initial release spots was measured by recording the furthest spot where eggs, larvae and adults of each biocontrol agent was found. As a nocturnal weevil, *L. setosipennis* adults were not found during the day so they were not recorded. The larvae of *L. setosipennis* were recorded by splitting the stem of three randomly selected parthenium plants in four directions from the initial release spot.

The numbers of parthenium plants, eggs, larvae and adults (for *Z. bicolorata*) of each biocontrol agents were separately subjected to generalized linear model (GLM) procedure of the SAS software Version 9.4, 2014 (SAS Institute Inc Cary, NC, USA). Means followed by standard error of triplicate measurements are presented.

RESULTS AND DISCUSSION

Arba minch release site

Eggs, larvae and adults of *Zygogramma bicolorata* were recorded in large numbers during July 19, 2018 and September 16, 2018 monitoring periods (Table 2). But

Table 1. Number and dates of *Listronotus setosipennis* and *Zygogramma bicolorata* release and monitoring at Arba Minch and Mojo, locations in Ethiopia

Location	Biocontrol agent	No. of adults released	Release date	Monitoring dates	
Arba Minch	*L. setosipennis*	600	8/18/2017	7/19/2018	9/16/2018
	Z. bicolorata	3000	8/18/2017	7/19/2018	9/16/2018
Mojo	*L. setosipennis*	500	7/12/2017	7/25/2018	9/06/2018
	Z. bicolorata	1000	7/12/2017	7/25/2018	9/06/2018

Listronotus *setosipennis* eggs or larvae were not found at the release site or around it. There was a good stand of parthenium at the release and dispersal sites (Table 2). The number of plants found were 22.67±6.17/quadrat (0.25 m^{-2}) (July) and 18.06±3.03/quadrat in September at the dispersal site and at original release site they ranged from 9.67±2.96to 3.67±0.88 plants/quadrat at both dates of monitoring.

At the release site, there were more eggs (7.41±4.52/ plant) during the second monitoring in September than at the first (1.4±0.71/plant) monitoring in July. Similar data was recorded at the dispersal site. For the larvae it was the opposite with more detection on July than on September monitoring date. No larva was found during the September monitoring at the release site. The number of adult *Z. bicolorata* at the release spot in September was 0.70±0.14 adult/plant and in July the number was 0.04±0.002 adult/plant. However, at the dispersal spot in July, 0.60±0.18 adult/plant *Z. bicolorata* were found while the number in September was 0.27±0.02 adult/plant. *Zygogramma bicolorata* was detected 96.6±15.19 m and 91.7±12.14 m on July, 2018 and September 16, 2018, respectively away from where it was released one year ago.

Mojo release site

One year after release, *L. setosipennis* was thriving whereas *Z. bicolorata* was not detected at the release spot or around it. The site at Mojo during monitoring contained parthenium plants at the rosette and the flowering stages. Parthenium at both growth stages was recorded because larvae reported on (Table 3) were detected from parthenium plants at the rosette stage as well as the flowering stage. At both release and dispersal spots, there were more parthenium plants at the rosette stage than at the flowering stage during the first monitoring in July (Table 3). However, more parthenium at the flowering stage were recorded during the second monitoring in September (6.67±0.88/quadrat and 3.75±1.04/ quadrat at the release and spread sites, respectively) than in July (3.67±0.44/quadrat and 1.25±0.14/quadrat at the release and the spread sites, respectively). At the release site, there were more eggs on parthenium in September (10.76±1.64 eggs/plant) than in July monitoring occasions (3.87±1.18 eggs/plant).

After dispersal, similar results were observed as more eggs/plant was detected in September (14.98±1.73) than in July (8.69±3.2). However, larva counts were inverse as the larvae detected in July were 1.86±0.16 larvae/plant and in September it was 0.34±0.14 larva/plant at the release sites. Similarly, the larvae recorded at the dispersal sites in July was 1.25±0.19 larvae/plant as compared to that in September

Table 2. Presence and spread of *Zygogramma bicolorata* released on August 18, 2017 and assessed on July 19, 2018 and September 16, 2018 at Arba Minch in southwestern Ethiopia for the control of the invasive weed, *Parthenium hysterophorus*

Date of assess.*	Site of assess.	Plants 0.25 m^{-2}**	Eggs $plant^{-1}$	Larvae $plant^{-1}$	Adult $plant^{-1}$	Distance from release (m)
July 19	Release	9.67±2.96	1.4±0.71	8.39±4.1	0.04±0.002	
	Spread	22.67±6.17	0.52±0.18	2.04±0.43	0.60±0.18	96.6±15.19
Sept 16	Release	3.67±0.88	7.41±4.52	0.00±0	0.70±0.14	
	Spread	18.06±3.03	0.68±0.24	0.28±0.08	0.27±0.02	91.7±12.14

Data (mean±SE) is from three replications, assess* - assessment, **Number of parthenium plants in 0.25 m^{-2} quadrat

Table 3. Assessment for the presence and spread of *Listronotus setosipennis* released on July 12, 2017 and assessed on July 25, 2018 and September 6, 2018 at Mojo in central Ethiopia for the control of the invasive weed, *Parthenium hysterophorus*

Date of assess.*	Site of assess.	Rosette 0.25 m^{-2}**	Flowering 0.25 m^{-2}***	Eggs $plant^{-1}$	Larvae $plant^{-1}$****	Distance from release site (m)
July	Release	13.67±0.33	3.67±1.18	3.87±0.16	1.86±0.16	
	Spread	4.25±1.32	1.25±0.14	8.69±3.2	1.25±0.19	56.25±4.54
Sept	Release	20.27±0.82	6.67±0.88	10.76±1.64	0.34±0.14	
	Spread	4.25±0.82	3.75±1.04	14.98±1.73	0.28±0.13	47.25±3.37

Data (mean±SE) is from three replications; asses* - assessment, **number of parthenium plants at rosette stage in 0.25 m^{-2} quadrat, *** number of parthenium plants at flowering stage in 0.25 m^{-2} quadrat, number of larvae/plant was recorded from parthenium plants at both rosette and flowering stages

(0.28±0.05 larva/plant). *Listronotus setosipennis* was recorded 56.25±4.54 m and 47.25±3.37 m away from the release spot in July and September, respectively.

Parthenium is now a major weed in eastern and southern Africa. It is having an adverse impact on crop yield, pasture lands, human health and national parks where it has a negative effect on wildlife. It is now realized that biological control is the only viable and sustainable method to abate its damage.

Large numbers of *Z. bicolorata* eggs, larvae, and adults were recorded at Arba Minch one year after the release of the biocontrol agent. However, *L. setosipennis* was not found in nearby release sites. It is not clear why the weevil was not detected one year after release despite a thorough search at two different times (July and September). Currently there is limited published information on the survival and dispersal of *L. setosipennis* outside of its native range in South America. *Listronotus setosipennis* needs tall parthenium plants with freshly opened flowers to reproduce and spread at the early stage. It is possible that the parthenium plants at the time of release may not have been ideal for the weevil.

However, the Arba Minch area in southwestern Ethiopia is ideal for *Z. bicolorata* development and diapausing. The area is highly infested with parthenium making it ideal for the beetle to rapidly reproduce and spread. The area also receives average monthly rainfall of 209 mm for June, July, August and September with temperatures averaging 24.4°C for the same period (Fig. 1). The relatively high rainfall and temperature favors lush vegetative growth of parthenium which is conducive for the reproduction and spread of *Z. bicolorata*. The beetle prefers parthenium plants with large green leaves for the adult and larva to feed on. Under these conditions, the female adult can lay large number of eggs that hatch into healthy larvae that have good source of food. Production of large numbers of eggs, larvae and adults of *Z. bicolorata* compensates any potential losses to predation and any subsequent unfavorable weather conditions.

The relatively high rainfall for an extended period is also ideal for the larvae to enter soil and the pupae to eclose. Under moist soil conditions, the larva can easily penetrate the soil and form chambers where the pupa develops and the adult can readily emerge. The moist conditions also facilitate the entry of the adult into the soil to diapause during the dry period, which lasts between November and May in many parts of Ethiopia. After diapausing the adult can readily emerge from moist soil than dry soil (Jayanth and Bali, 1993).

Eggs and adults of *Z. bicolorata* were recorded at the release and at the dispersal spots during both monitoring

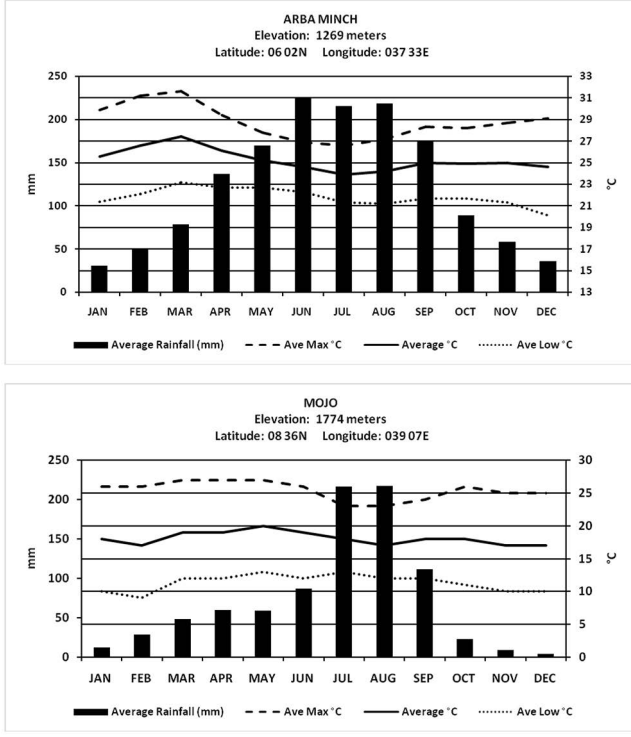

Fig 1. Nine-year average mean maximum, mean minimum and mean temperatures as well as mean monthly rainfall for Arba Minch and Mojo in Ethiopia.

occasions. Larva was also detected at all times except during the September monitoring at the release spot. There were no consistent trends except the number of eggs detected was greater in July than in September at both the release and at the dispersal spots. Staff at Arba Minch indicated that there was a two-week dry spell during the month of August in 2017 and that may have slowed down oviposition by *Z. bicolorata*.

The number of *Z. bicolorata* adults/plant varied from 0.04 on July at the release site to 0.6 in July at the spread site (Table 2). Adult *Z. bicolorata* are not easy to detect during the hot summer days as they hide under leaves and stay inactive. It is possible that some may have escaped detection during monitoring. Even the low adult/plant translates to substantial numbers when expressed per quadrat (0.25 m^{-2}). For example, 0.6 adults/plant in July 19 monitoring translate into 13.5 adults/0.25 m^{-2} area. This is a relatively large number of adults for such a small area.

Zygogramma bicolorata feeds on parthenium plants at the release spot and moves to another area in search of new plants. It is, therefore, expected that there will not be adults at the release spots at the end of the season. However, it is reported that *Z. bicolorata* diapauses throughout the season and some may do it at the release site to emerge after the rains (Jayanth and Bali, 1993).

At Mojo, *L. setosipennis* thrived while *Z. bicolorata* was not detected one year after release. The harsh and dry conditions at Mojo were better tolerated by the weevil than by the beetle. The lack of good parthenium stock did not allow the beetle to reproduce in large number to overcome predation. In addition, the dry soil condition was not ideal for the larva to enter the soil to pupate or for the adult to diapause during the dry season. Average low temperatures at Mojo were below 15°C year round, which is not ideal for *Z. bicolorata* as compared to Arba Minch where temperatures were above 20 °C throughout the year (Fig. 1). The harsh condition at Mojo, therefore, explains the lack of establishment by *Z. bicolorata*.

It took three years for *Z. bicolorata* to establish in India (Jayanth and Geetha Bali, 1994) whereas in Australia twelve years passed before it started to show widespread damage to parthenium (Dhileepan *et al.,* 1996). It is reported that the variation in time taken for a biocontrol agent to establish at release sites depends on the difference in the climate between at its origin and new home (Dhileepan *et al.,* 1996). In addition to climate, soil type at the release site, the level of natural enemies, and abundance and quality of the host plant can also affect the establishment of *Z. bicolorata* (Dhileepan and Strathie, 2009).

Listronotus setosipennis was field released in Australia in 1982 (Wild *et al*., 1992) and in South Africa in 2013 (Strathie *et al.,* 2016). In Australia it readily established at several sites and appears to be well suited for areas with prolonged dry periods and erratic rainfall (Strathie *et al.,* 2011). Similarly, it is quickly establishing in South Africa (50%+ of sites) (L. Strathie pers. comm.). Feeding by *L. setosipennis* could be damaging to parthenium if the damage is initiated at the rosette stage (Dhileepan, 2003). However, to have a significant impact on flower production, a minimum of five *L. setosipennis* larvae/plant are required (Dhileepan, 2003). Despite its slow spread and the need to have more than two (rosette) and five (flowering) larvae per parthenium plant to cause significant damage, the weevil could play an important role in abating the damage caused by parthenium in Ethiopia by augmenting the impact of the leaf-feeding *Z. bicolorata*. In addition, it could be the only biocontrol agent available to control parthenium in the dry regions of Ethiopia.

We observed that the two biocontrol agents responded differently to conditions at Arba Minch and Mojo, Ethiopia. This led to different establishment and performance of the two biocontrol agents at the two places. It is unlikely that one or two biocontrol agents can effectively control parthenium in different ecological zones of Ethiopia. So, effective management of parthenium in all regions of Ethiopia may require the deployment of a suite of biocontrol agents.

We also observed that *L. setosipennis* moved about 50 m from its release spots. The weevil is known to be a slow spreader and its long-distance dispersal possibly occurred through pieces of parthenium stems that were transported by wind or water. At the Mojo site, the most likely mode of dispersal is by wind with pieces of parthenium stems. The rate of spread of the weevil and its population increase in Australia has been very slow (Wild *et al.,* 1992). After sixteen years the weevil was found in less than half of the parthenium-infested sites sampled indicating the rate of dispersal has been very slow.

Zygogramma bicolorata has spread almost 100 m one year after it was released at Arba Minch. The beetle readily disperses in large areas as evidenced by its spread from India to Nepal and other neighboring countries. The detection of eggs and larvae of *L. setosipennis* and *Z. bicolorata* one year after their release and their presence several meters from the release spots indicates that the biocontrol agents are starting to establish and are dispersing from the original sites at two different regions of Ethiopia.

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