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Review

The biological control of *Parthenium hysterophorus* L. in Pakistan: status quo and future prospects

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Abstract

Parthenium, *Parthenium hysterophorus* L. poses a serious environmental and socio-economic threat in Pakistan. It was identified as a priority for control in Pakistan and an integrated control programme has been launched against this invasive weed. In 2009 the biological control agent, *Zygogramma bicolorata* Pallister, was documented in Pakistan and thought to have naturally dispersed into the region. Despite the presence of this biological control agent for the past decade in Pakistan, the population level impact on parthenium weed has been limited by several factors, including climate, diapause and limited dispersal. A redistribution programme was initiated to promote this biological control agent in Pakistan. In addition to this, the parthenium winter rust, *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parmelee, was reported in Pakistan for the first time in 2019. Despite this rust being widespread there appears to be limited impact, most likely linked to environmental conditions and the timing of infection. Monitoring programmes for this rust have been initiated for the winter of 2020. CABI, through its Action on Invasives programme, has established a quarantine facility for the screening of complementary biological control agents not yet in Pakistan against parthenium and other invasive weeds in the country. With this quarantine facility the importation of the stem boring weevil, *Listronotus setosipennis* (Hustache) was possible and host range testing is underway. The biological control of parthenium in Pakistan is still at an early stage, and considerable effort is required to fill the management toolbox for this invasive weed.

Key words: Action on Invasives, Asia, invasive weed, redistribution, inadvertent introduction, *Listronotus setosipennis, Puccinia abrupta* var. *partheniicola, Zygogramma bicolorata*

Introduction

Invasive species undermine the livelihoods of vulnerable rural communities since they are heavily dependent upon natural resources and healthy ecosystems for their livelihoods (Khan et al. 2013; Shackleton et al. 2007;

Shameem et al. 2014). Such invasive pests—including pathogens, weeds, invertebrates and vertebrates—have a negative impact on food, pasture for livestock, human and animal health, trade, vital natural resources and ecosystem services (water, fisheries, fodder, fuel, medicinal plants and biodiversity) (Pimental et al. 2000, Pimental 2002; Pratt et al. 2017).

Parthenium hysterophorus L. ("parthenium") is a highly invasive ruderal annual weed which is spreading rapidly through vast areas of the tropics and sub-tropics, with highly deleterious effects on native biodiversity, human and animal health, and productivity of both crops and grasslands (Shabbir et al. 2019). Several factors contribute to the invasiveness of parthenium. It can thrive under a very wide range of climatic conditions, germinating at temperatures ranging from 12 °C to 27 °C, tolerating saline conditions, and with a deep tap root which enables it to survive in low-moisture areas (Adkins et al. 2019). It can grow and flower year-round, without a period of dormancy; but in unfavourable conditions it exhibits phenotypic plasticity, forming low-growing rosettes which only bolt and flower when conditions improve (Kohli and Rani 1994; Adkins et al. 2019). Like many invasive plants parthenium it is a prolific seeder, where individual plants can produce over 150,000 seeds in its lifetime, though the majority of plants produced less than 4,000 seeds (Dhileepan 2012), which can remain viable in the soil for more than two years (Tamado et al. 2002b) and are readily dispersed by human and animal movement, and on trucks and farm machinery. Furthermore, it is allelopathic, which enhances its ability to displace native vegetation (Bajwa et al. 2016; Adkins et al. 2019); and this contributes more to crop losses than direct competition for light and nutrients. Finally, and crucially, there is a lack of natural enemies outside its native range (Andonian and Hierro 2011).

Parthenium has an extensive native range in the Americas, occurring in the eastern USA as far north as New York and Ohio, and throughout Mexico and Central America into South America, extending as far as Argentina and Chile (EPPO 2014; Mainali et al. 2015; Adkins et al. 2019). It was first recorded outside its native range in the 19th century (1810 in India, 1880 in South Africa), but most introductions date from the 1950s and 1960s. These are thought to have originated from contaminated consignments of grain from the USA (Boulos and El-Hadidi 1984; Zahran and Willis 1992). Parthenium has since then invaded 48 countries as reported by Shabbir et al. (2019) and at least another two countries since then (A. Shabbir *pers. comm.*). Parthenium is presumed to have entered the Asian sub-continent into India along with food grains imported from the USA where it was identified in Pune (formally Poona), in the Maharashtra district, first observed in 1955 (Vartak 1968), and has since spread to most of the sub-continent. Introduction of this weed in Pakistan is possibly from India. It is thought to have entered Pakistan, Nepal and Bangladesh via road connections (Shabbir et al. 2012): thousands of vehicles cross between India and these countries every day at several places.

In Pakistan, parthenium was first reported from Gujarat district of the Punjab Province in the 1980s (Razaq et al. 1994). For the first 20 years following its introduction it remained restricted to northern Punjab, but since 2000 its range has extended throughout Punjab and into Khyber Pakhtunkhwa (KP), the Federally Administrated Tribal Areas (FATA), Azad Jammu and Kashmir, and Sind Provinces. It has spread along roads, canals and rivers, and the invasion has been further exacerbated by irrigation and floods (Shabbir et al. 2012). Parthenium is rapidly spreading in various parts of Punjab, KP and Kashmir, and is becoming a dominant weed in different terrestrial ecosystems. The weed is highly invasive in nature and is reported as a major weed for agro-ecosystems in Pakistan (Tamado et al. 2002a; Adkins and Navie 2006; Khaliq et al. 2015; Netsere 2015; Safdar et al. 2015, 2016; Bajwa et al. 2019a–d).

As an aggressive coloniser of grassland, open woodland and disturbed ground, and also possessing allelopathic properties, parthenium effectively displaces native flora and forms dense monospecific stands (Qureshi et al. 2018; Adkins et al. 2019). For herbivorous mammals parthenium, which is itself unpalatable and even toxic to grazing animals (Dhileepan and Strathie 2009; Narasimhan et al. 1980), reduces food supply through direct competition with grasses and other palatable plant species. Furthermore, meat and milk are both tainted by consumption of parthenium, and the tainted products may be dangerous for human consumption (Tudor et al. 1982; Ayele 2007). The species' allelopathy is due to a range of toxic compounds produced by the plant, of which the most potent allelochemicals are the sesquiterpene lactones parthenin, hysterin and ambrosin, along with a range of other phytotoxic flavonoids (Patel 2011). Parthenin is also known to be toxic to a number of insect species. This damage to insect populations can in turn affect predatory invertebrates, reptiles, amphibians and birds, for all of which insects are an important component of the diet (Witt and Belgeri 2019).

Parthenium is considered highly allergenic and in some cases poisonous. Prolonged exposure to the plant for 1–10 years (for example working in parthenium-infested fields) results in severe allergic reactions in 10–20% of the population (McFadyen 1998). These include hay fever, asthma, allergic rhinitis and skin allergies such as eczematous contact dermatitis (Evans 1997), and in India this has led to farms being abandoned, as there is currently no treatment for these conditions other than moving away from affected areas (Narasimhan et al. 1980). There is some evidence that parthenium can also increase the incidence of malaria, as it is a species preferred by mosquitoes as a source of nectar, and under favourable climatic conditions can continue to flower throughout the year (Nyasembe et al. 2012; Stone et al. 2018).

CABI's Action on Invasives programme aims to protect and improve the livelihoods of 50 million poor rural households impacted invasive species, using an environmentally sustainable, regional approach to comprehensive management. The purpose of this programme is to strengthen capacity to enable invasive species management practices to prevent, control and manage priority invasive species at local, national and regional level in a gender-responsive manner. While the aim of Action on Invasives is to strengthen overall capacity to tackle invasive species, many of the activities focus on priority species as case studies. Parthenium is one of these. Biological control is key to sustainable management of many invasive species including parthenium: this manuscript aims at outlining the current status of the biological control of parthenium in Pakistan, and future prospects for the region as a whole.

Biological control options for parthenium in Pakistan

It is widely acknowledged that integrated control is the most effective strategy in managing pests. In the case of plants, it involves the use of herbicides, manual or mechanical control, and biological control agents in an integrated way. Although, three main biological control approaches are available for weeds, inundative and augmentative approaches are primarily used in agro-systems targeting crop weeds, either native or exotic, while classical biological control is typically used against exotic invasive weeds (Müller-Schärer and Schaffner 2008). The main benefits of classical biological control are that the agents establish self-perpetuating populations and often establish throughout the range of the target weed, including areas which are not accessible for chemical or mechanical control; control of the weed is permanent; there are no negative impacts on the environment; the cost is low relative to other approaches and usually requires a once-off investment; and benefits can be reaped by many stakeholders regardless of their financial status and whether they contributed to the initial research (Greathead 1995).

Biological control of parthenium will not only reduce its spread, but will also reduce the costs of manual and chemical control, in cases where there is a need to further reduce abundance. Trials done in South Africa revealed that it took 3.25 person days to apply 0.5 L of herbicides to a one-hectare parthenium infestation with two follow-up sprays (Goodall et al. 2010). More follow-up sprays will probably need to be applied. High labour and herbicide costs, including those of protective gear, will drive up management costs which can be reduced significantly by the introduction of biological control agents.

Options for the biological control of parthenium have been explored since the 1970s, and to date a total of nine insect species and two rust fungi have been used as control agents in Africa (South Africa, Uganda, Tanzania, Ethiopia), Asia (India, Sri Lanka, Nepal, Pakistan, China, Papua New Guinea) and Oceania (Australia, Vanuatu) (Table 1; Dhileepan et al. 2019).

Table 1. Introduction history and current status of parthenium biological control agents around the world with insights into the feasibility and potential challenges for Pakistan. (Data from Dhileepan and Strathie 2009; Dhileepan and McFadyen 2012; K. Dhileepan *unpublished data*; L. Strathie *unpublished data*; S. Adkins *unpublished data*; S. Tang *unpublished data*). Table adapted from Dhileepan et al. 2019.

Biological control agents	Country of introduction	Year imported/ reported	Year release approved/commenced	Establishment status	Feasibility and potential challenges for Pakistan
Lepidoptera: Tortricidae					
Epiblema strenuana Walker	Australia	1982	1982	Widespread and abundant	*Epiblema strenuana* although considered impactful and effective under certain conditions (Dhileepan 2009) has been found to feed and develop on the oil seed crop Niger, *Guizotia abyssinica* Cass. which is economically important to the region and grown in India. Therefore, if this species is considered for biological control in Pakistan this needs to be taken into account. Currently this species is not considered for release in Pakistan.
	India	1985	Not released		
	Sri Lanka	2003	2004	Unknown	
	Papua New Guinea	2004	Colony failed	Localised	
	Vanuatu	2014	No deliberate release		
	South Africa	2010 and 2018	Testing in progress		
	China	1990	Released on ragweed	Established on parthenium in Guangxi Province	
Platphalonidia mystica (Razowski & Becker)	Australia	1991	1992	Localised	*Platphalonidia mystica* has not proven to be the most effective agent in Australia and is not currently prioritised for Pakistan.
Lepidoptera: Sessidae					
Carmenta nr *ithacae* (Beutenmüller)	Australia	1996	1998	Widespread and abundant	The root feeding moth, *Carmenta* nr *ithacae,* is wide spread in Australia and is considered impactful and effective. Based on the narrow host range of this species it should be prioritised for release into Pakistan.
	South Africa	2014	Testing in progress		
Lepidoptera: Bucculatricidae					
Bucculatrix parthenica Bradley	Australia	1983	1985	Widespread	
Coleoptera: Chrysomelidae					
Zygogramma bicolorata Pallister	Australia	1980	1981	Widespread and abundant	*Zygogramma bicolorata* is already present in Pakistan and efforts to improve its range and potential efficacy are being investigated and reported in the study.
	India	1983	1984	Widespread and abundant	
	Pakistan	2003	No deliberate release	Widespread	
	Nepal	2009	No deliberate release	Widespread and abundant	
	South Africa	2005	2013	Localised establishment	
	Ethiopia	2007	2013	No establishment	
	Tanzania	2013	2013	No establishment	
	Uganda	2018	2018	Unknown	
Coleoptera: Curculionidae					
Listronotus setosipennis Hustache	Australia	1981	1982	Abundant and widespread	*Listronotus setosipennis* has been imported into quarantine in Pakistan and is currently undergoing host range testing.
	South Africa	2003	2013	Widespread establishment	
	Ethiopia	2007	2013	No large scale releases.	
	Uganda	2018	2018	Unknown	
	Pakistan	2019	Testing in progress		
Smicronyx lutulentus Dietz	Australia	1980	1981	Abundant and widespread	The seed feeding weevil *Smicronyx lutulentus* has proved host specific and can be extremely damaging to the reproductive output of *P. hysterophorus.* This biological control agent should be prioritised for release into Pakistan.
	India	1985 and 2018	Colony failed in 1985.		
	South Africa	2010	Testing in progress	Widespread establishment	
	Ethiopia	2015		Established	
Conotrachelus albocinereus Fiedler	Australia	1992	1995	Localised	*Conotrachelus albocinereus* has not proven to be the most effective agent in Australia and is not currently prioritised for Pakistan.
Homoptera: Delphacidae					
Stobaera concinna (Stål)	Australia	1982	1983	Localised	*Stobaera concinna* has not proven to be the most effective agent in Australia and is not currently prioritised for Pakistan.

Table 1. (continued).

Basidiomycotina: Uredinales					
Puccinia abrupta partheniicola Parmelee	Australia	1991	1991	Localised and abundant	The winter rust *Puccinia abrupta partheniicola,* is already present in Pakistan. The distribution and potential impact of the pathogen is currently under investigation and the information is presented in this study.
	Ethiopia	1997	No deliberate release	Widespread and abundant	
	India	1980	No deliberate release	Localised	
	South Africa	1995	No deliberate release	Localised	
	Nepal	2011	No deliberate release	Localised	
	China	2007	No deliberate release	Localised	
	Pakistan	2019	No deliberate release	Widespread and abundant	
Puccinia xanthii var. *parthenii-hysterophorae* Seier, Evans & Romero	Australia	1999	1999	Widespread	The host range testing of a pathogen for release into Pakistan may prove to be a logistical barrier since there currently is no appropriate quarantine facility for a plant pathogen.
	Sri Lanka	2003	Unknown	Unknown	
	South Africa	2004	2010	Localised	

In most cases, a classical biological control approach has been used, in which natural enemies from the native range have been released as biological control agents after host range testing. In some cases, however, the agent has been found to be already present following an unknown or accidental introduction. In Pakistan, biological control options are limited and there have so far been no deliberate introductions of agents to control parthenium. Nevertheless, *Zygogramma bicolorata* Pallister, known as the Mexican or parthenium leaf beetle, which was deliberately introduced from Mexico to India in 1983, was reported in Pakistan in 2006 and now has a widespread distribution in the country (Javaid and Shabbir 2007). In addition to this, the winter rust, *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parmelee, has also been recorded from Pakistan and is now widely distributed through the range of parthenium in Pakistan (Iqbal et al. 2020). In April 2019, CABI started working with the stem boring weevil, *Listronotus setosipennis* (Hustache) which has been imported to the quarantine facility in Pakistan for host range testing.

Zygogramma bicolorata

Of the three species present in Pakistan, *Z. bicolorata* is the most studied and widely used agent against parthenium worldwide. Both adults and larvae feed on parthenium leaves, and early stage larvae also feed on terminal and auxiliary buds, moving on to leaves as they mature (Kaur et al. 2014). This insect can cause almost complete defoliation of parthenium, resulting in significant reductions not only in biomass, plant height and density but also flower production and seed set. In central Queensland for example, *Z. bicolorata* inflicted 91–100% defoliation, and reduced weed density by 32–93%, plant height by 18–65%, plant biomass by 55–89%, flower production by 75–100%, soil seed bank by 13–86% and seedling emergence in the following season by 73–90% (Dhileepan et al. 2000). In India, *Z. bicolorata* caused 85–100% defoliation, resulting in nearly 100% reduction in parthenium density in the Bangalore area (Jayanth and Bali 1994; Jayanth and Visalakshy 1996). Similar impacts have been observed in other areas in India (Kumar 2000; Dhiman and Bhargava 2005; Jaipal 2008). Host range testing of this leaf-feeding beetle has been extensively carried out in Australia, India, South Africa and Ethiopia, suggesting to be considered completely host specific (Mersie et al. 2019).

Distribution of parthenium and *Zygogramma bicolorata* in Pakistan

Since its inadvertent introduction to Pakistan, recorded by Javaid and Shabbir (2007) in March 2006, *Z. bicolorata* has gradually spread from northern Punjab to other areas where parthenium is invading, including KP and Kashmir; but its spread is slower than that of parthenium. During a recent road survey in June and July 2019, 34 districts selected randomly were visited in KP, Punjab and Sindh in order to characterize the distribution of parthenium and *Z. bicolorata.* We then selected 61 grids (10 km × 10 km) which were distributed randomly across the districts previously selected (Punjab: 30, KP: 25, Sindh: 6). In each grid, we set up 20 circular plots of 20 m radius, following the road network. Due to accessibility problems some grids were under-sampled (N = 1102 plots: five grids with less than 10 plots). The plots were located every 2 km along the left side of the road. In each plot, the habitat type, road type, presence of parthenium, and presence of, and level of defoliation by, *Z. bicolorata* at plot level were recorded. *Zygogramma bicolorata* presence probability across habitat and road type was compared using a logistic generalized linear model. For habitat, the predominant type per plot was considered. To ensure a comprehensive comparison, we discarded habitats found in fewer than ten plots from the analysis (i.e. agroforestry, aquatic and riparian, gardens and orchards). All road types were considered in the analysis.

Parthenium was recorded in 27 of the 34 districts, while *Z. bicolorata* was recorded in only seven in Punjab and KP Provinces, and none in Sindh. This suggests that the agent is not yet present in much of the invaded region of Pakistan (Figure 1). Furthermore, its distribution was extremely sparse as it was only found in 11% of the plots with parthenium in grids with at least one positive identification (Figure 2; 2% across all the plots with parthenium in the whole dataset). All the plots where *Z. bicolorata* was identified showed defoliation symptoms. Most of these plots (80%) showed less than 30% defoliation, while the rest (20%) showed between 30 and 60% defoliation. *Zygogramma bicolorata* did not show a preference for specific habitats or road types (Figure 2; Tukey contrast post-hoc test $p > 0.05$). Roads might promote insect dispersal, but this analysis did not find higher levels beside larger roads.

The redistribution of *Z. bicolorata* is crucial in order to fully exploit this biological control agent's capabilities in Pakistan. CABI, through the Action on Invasives programme, has initiated a redistribution campaign: in October 2018, 950 beetles were released at two sites in Sindh Province, and in 2019, 3500 beetles were released in four districts of Punjab Province.

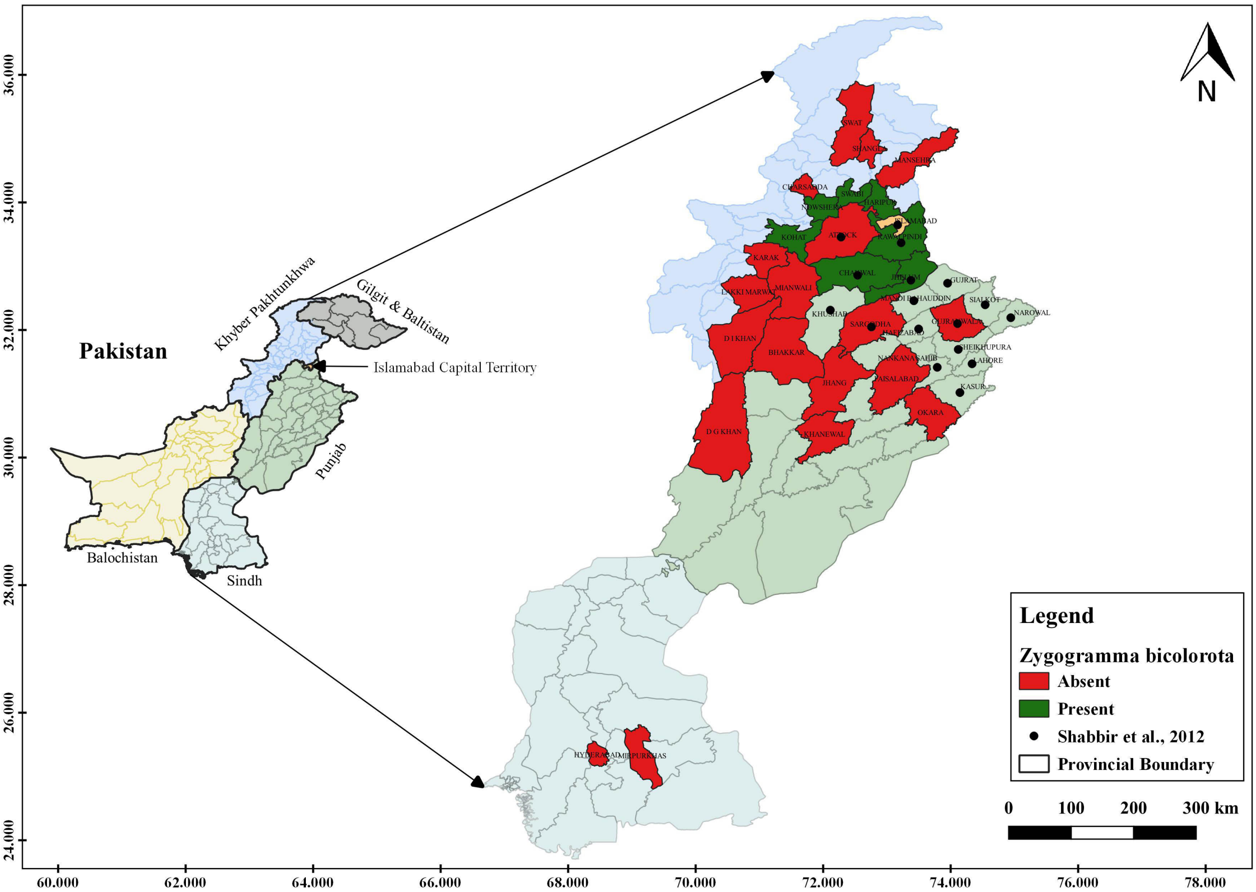

Figure 1. Occurrence (green = presence and red = absence) of *Zygogramma bicolorata* in the 27 districts where *Parthenium hysterophorus* was recorded. The presence of *Z. bicolorata* in the districts as recorded by Shabbir et al. (2012) are represented by black dots..

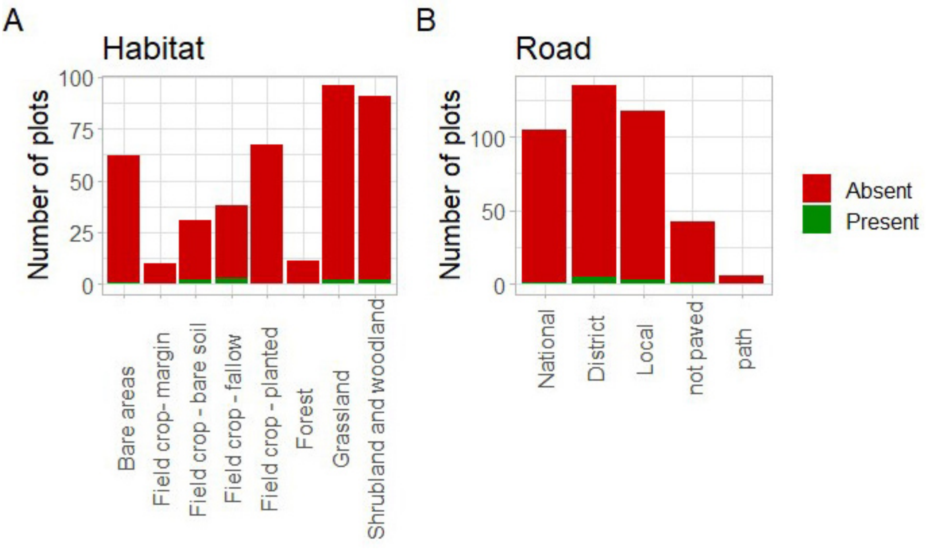

Figure 2. Number of circular plots of 20 m radius with *Parthenium hysterophorus* where the presence (green) or absence (red) of *Zygogramma bicolorata* was identified.

Establishment has been confirmed in the Faisalabad district of Punjab Province, but is yet to be confirmed at the other sites in the Punjab and Sind Province. This should be considered a priority for future studies and surveys.

Diapause in *Zygogramma bicolorata*

A major disadvantage of this species is that it goes into diapause in the soil under cold conditions in Pakistan (November to April), and there is a period in early spring (February–March) especially in northern Pakistan when parthenium is starting to sprout but *Z. bicolorata* has not yet emerged from diapause. According to King (2008), *Z. bicolorata* requires relatively warm temperatures and high humidity to remain active. The thermal physiology suggests that the adults will enter into a chill coma at temperatures as high as 11.8 ± 0.3 °C, however, the lethal lower temperature (LLT_{50}) for the adult beetle is −6.7 °C and the upper lethal temperature (ULT_{50}) is 44–45 °C. The possibility of breaking this diapause artificially, so that the insect becomes active earlier in the growing season (February or March) of parthenium as suggested by Hasan et al. (2018), has been explored by CABI, and this pilot experiment is described below.

On 27 September 2018, 1000 *Z. bicolorata* individuals were collected from the field and divided equally into 20 cages (dimensions 90 × 45 × 45 cm). The experimental setup resembled that of Hasan et al. (2018), where each cage had a tray in the bottom containing a 5 cm layer of sifted sandy loam soil to facilitate burrowing during diapause. The soil was regularly sprinkled with fresh water. The cages were stored outdoors to experience natural conditions, however they were protected from direct sunlight and rain. The insects were fed with cut shoots of parthenium on a daily basis and every fortnight the number of active, dead and missing (burrowed and thus presumed in diapause) adults was counted until all adults had either entered diapause or died. From 11 to 16 March 2019, the trays of soil were moistened and placed in an incubator at 35 °C to artificially break diapause of the adults. Each day the adults were collected and placed into cylinders in the laboratory (24 ± 2 °C: 12 h light/12 h dark cycle) with fresh cut plant material to feed. On the 16 March 2019, all live adults were returned to their original cages, which now contained a fresh rosette parthenium plant. The adult activity (feeding and oviposition) was tracked on a daily basis until 22 March 2019.

By 8 November 2018, all adults had either entered into diapause (98.5 ± 0.5%) or died (1.5 ± 0.5%). No adults were observed to be active between 8 November and 11 March when the trays were moved into the incubator to break diapause. Over the period 11–16 March, diapause was broken in 428 individuals (46.6 ± 1.9%) while the rest remained in the soil and were presumed dead. Of these individuals, when placed onto fresh rosettes under natural environmental conditions on the 16 March, no feeding was observed and 35.8 ± 3.8% mortality was recorded between 16 and 22 March. This could be due to the environmental stress of diapause break and being immediately placed outdoors at relatively cold ambient temperatures (10 °C min and 24 °C max). The remaining individuals went directly back into diapause, burrowing into the soil of the potted plant. Despite being relatively successful at breaking diapause, no adult feeding or oviposition was recorded, the majority of adults went directly back into diapause, and there was high mortality with only around 4% ± 0.9% survival. We had assumed that if diapause could be broken and insects released earlier in the season, the biological control of parthenium in Pakistan could be improved. However, based on these results, the efficacy of making early releases of adults in order to obtain knock-down of the plants before the first flowering period is extremely low and unlikely to be successful. Diapause in insects is an extremely complex process involving a combination of environmental cues, physiology, behaviour and genotype (Hodek 2012). The *Z. bicolorata* individuals where diapause was broken early, may not have been "ready" to perform "normally" under the prevailing environmental conditions. This is in contrast to laboratory populations, where following diapause there was no significant effect on fecundity, and longevity of individuals was actually prolonged (Hasan et al. 2018).

The inadvertent introduction of *Z. bicolorata* has offered Pakistan an opportunity to apply biological control to parthenium with limited investment into host range testing and applications for permissions for release. Although future research into the biology, ecology and impact of this species in Pakistan is warranted, the study has shown that this species alone is unlikely to achieve full control of parthenium. Therefore, strategically, the resources available for biological control should focus on the development of additional agents for release into Pakistan in order to obtain long term sustainable control of parthenium.

***Puccinia abrupta* var. *partheniicola* in Pakistan**

Winter rust (*P. abrupta* var. *partheniicola*) is a pathogen of parthenium which is native to Argentina, Bolivia, Brazil and Central America (Evans 1997), and was introduced to Australia from Mexico in 1991 (Dhileepan and McFadyen 2012). It is also present (from unrecorded or accidental introductions) in China, Ethiopia, India, Kenya, Mauritius, Nepal, South Africa, Tanzania (Dhileepan and Strathie 2009; Shrestha 2012; Winston et al. 2014), and most recently in Pakistan (Iqbal et al. 2020). It is the most widely studied of the fungal pathogens of parthenium. A study in Australia of infection at three different growth stages found the rust to be most effective on young plants at rosette stage with 20 to 48% biomass reduction depending on competition levels (Fauzi 2009). It is most effective against parthenium under cold conditions, especially in Ethiopia (altitude 1500–2500 m), reducing both growth and seed production (Taye et al. 2002; Bekeko et al. 2012).

Winter rust infection was observed at several locations near Lahore and Rawalpindi Districts of Punjab Province between February and May 2019.

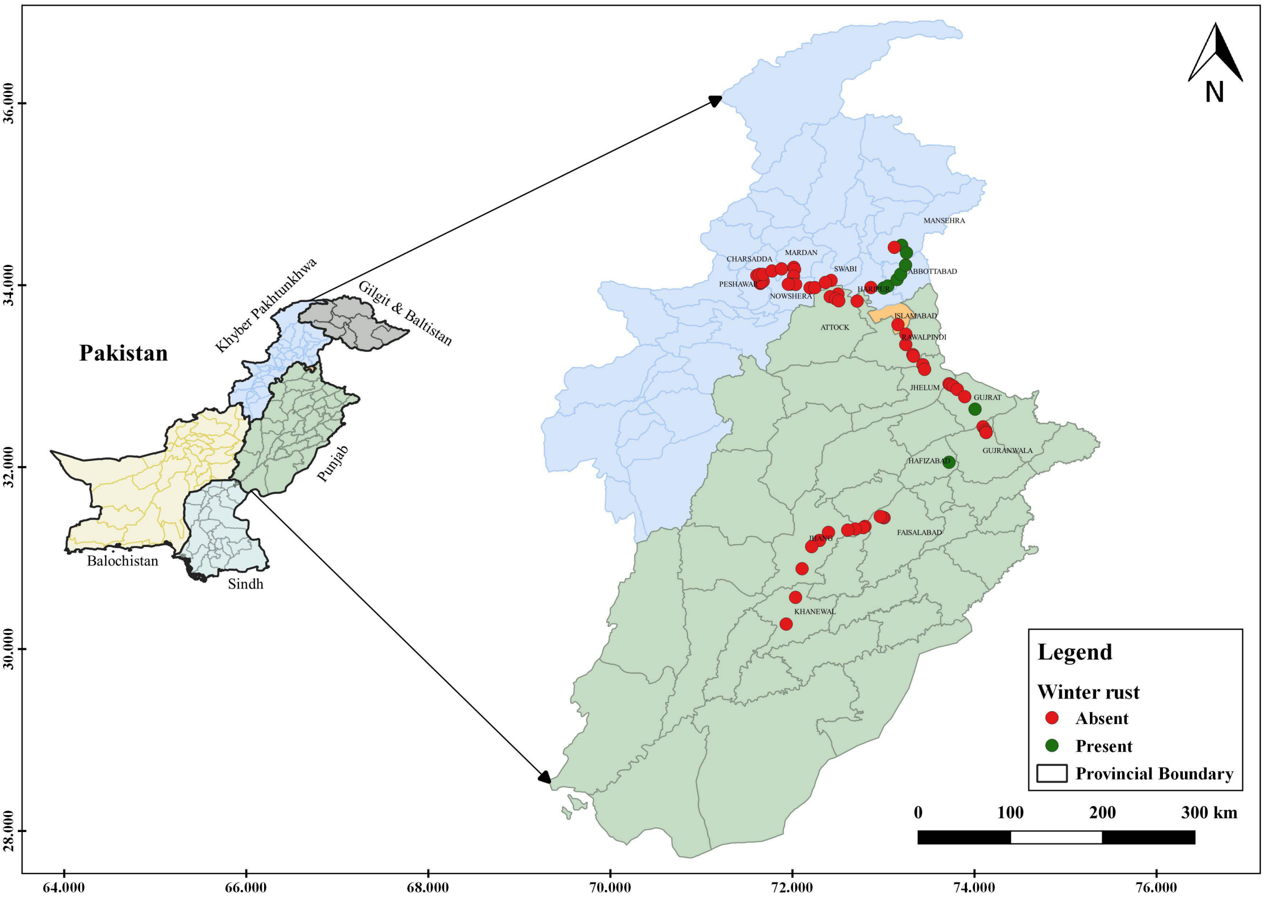

Figure 3. Surveyed sites in Punjab and Khyber Pukhtunkhwa Provinces for the presence (green) or absence (red) of the winter rust *Puccinia abrupta* var. *partheniicola*.

During surveys at 65 sites in 19 of these districts in 2019, winter rust was recorded at 21 sites in eight districts (Iqbal et al. 2020). In a recent survey (January 2020), 64 sites in 16 districts of Punjab and KP Provinces were surveyed. The winter rust was recorded at two sites in Punjab and eight in KP Province (Figure 3). Although the presence of this rust in Pakistan will aid in the management of parthenium, it is likely that its impact will be limited by abiotic factors. Temperature and dewpoint have been shown to be the limiting factors for urediniospore infection and survival, thus limiting impact of this rust in Australia where it was deliberately introduced (Dhileepan et al. 2018). Whilst it would be useful to monitor the distribution of this rust on an annual basis in winter, any redistribution would be unlikely to achieve a better level of control than is already experienced in the field.

Listronotus setosipennis in Pakistan

The stem-boring weevil *L. setosipennis,* another potential biological control agent for parthenium, is native to Brazil and Argentina. It has so far been deliberately introduced in Australia (Dhileepan and McFadyen 2012), South Africa (Dhileepan et al. 2019), Ethiopia (Mersie et al. 2019), and most recently Uganda (R. Molo *pers. comm.*), but has not yet been recorded in South Asia. In a study under greenhouse and field cage conditions in Australia *L. setosipennis* showed promise as a biological control agent for parthenium, particularly on alluvial and black soils, and in areas with a prolonged dry season (Dhileepan 2003). In Australia, three agents, *Z. bicolorata, L. setosipennis* and a stem-galling moth (*Epiblema strenuana* (Walker)), have been found to have a significant impact on parthenium (Dhileepan 2009). Of these, *E. strenuana* has been rejected as a biological control agent for parthenium in India, because it was found during laboratory testing also to attack *Guizotia abyssinica* Cass. (niger), a seed/oil crop of economic importance which is closely related to parthenium (Kaur et al. 2014). However, there was no damage to this species by *L. setosipennis* during host range testing in Ethiopia (Mersie et al. 2019), making this a suitable agent to consider for release in Pakistan.

The adult weevils are nocturnal, feeding mainly on parthenium leaves and flowers. The females oviposit in holes chewed in flowers, leaf bases or stem surfaces and axillary buds and seal the hole with black frass (Strathie et al. 2011). In the field in the native range, ten or more eggs were observed per plant, occasionally with two eggs per flower (Wild et al. 1992). Oviposition can occur throughout the year as long as suitable plants are available (Wild et al. 1992). Larvae emerge after 3–5 days. The first instar larvae are highly mobile and feed in the flowers, while the mature larvae feed within the stem and move to the root where they feed prior to pupation. Emergence of adults from pupae is triggered by rain (Wild et al. 1992). Adults diapause in the soil during the dry season (Dhileepan and McFadyen 2012). The life cycle takes about seven weeks and adult weevils can live up to eight months (Wild et al. 1992).

Host range testing of *Listronotus setosipennis* in Pakistan

With a view to eventual release of *L. setosipennis* as a biological control agent for parthenium in Pakistan, CABI is currently undertaking host range testing in its post entry quarantine facility in Rawalpindi. The national quarantine authorities (Plant Sciences Division of the Pakistan Agricultural Research Council (PSD PARC)) approved the importation of the weevil specifically for this purpose, and 200 adult *L. setosipennis* were imported from South Africa in April 2019. In order to obtain permission for field release, the safety of *L. setosipennis* needs to be demonstrated in a Pakistani context through traditional host range testing methods (McFadyen 1998). In the history of the classical biological control of weeds this has proven to be an effective method to limit any risk, with as little as 13% of all agents released worldwide resulting in any non-target attack, of which the vast majority was predictable (Hinz et al. 2019).

A test plant list was established based on phylogenetic relationships between the target weed and other plant species (Wapshere 1974) in collaboration with National Herbarium Programme, National Agricultural

Research Centre, Pakistan. It is generally accepted that species closely related to the target species are at greater risk of attack than species more distantly related. The Asteraceae is a massive plant family in Pakistan, which is represented by 758 species in 15 tribes. However, the tribe Heliantheae to which parthenium belongs is only represented by 27 species in Pakistan (Ghafoor 2002).

A considerable amount of testing of the host range of *L. setosipennis* has already been done in Australia (68 species and/or varieties) (Wild et al. 1992) and South Africa (52 species and/or varieties) (Strathie et al. 2011; Strathie and McConnachie 2012) and Ethiopia (31 species and/or varieties) (Mersie et al. 2019). Since many species have already been tested in a wide range of tribes, a condensed test plant list is justified for Pakistan focusing on native and crop or ornamental species within the tribe Heliantheae that have overlapping distributions with parthenium and are likely to be at a higher risk of non-target attack. We also put emphasis on ornamental and crop species across multiple tribes within the family Asteraceae. Host range testing of *L. setosipennis* began in October 2019 in Pakistan with the view of submitting an application for field release to PSD PARC and the Ministry of Environment and Climate Change once testing is complete.

Potential future agents to consider

It is accepted that the biological control of parthenium is a long-term commitment and will require several agents (Dhileepan et al. 2019). In Australia, there has been considerable effort in the biological control of parthenium with nine insects and two fungal agents released since the 1970s (Dhileepan et al. 2019). It is now becoming apparent that these releases have had an impact through reduction in plant densities and historical seed banks (Dhileepan 2007). The situation in Pakistan is unlikely to be different, and several agents are going to be required to achieve an acceptable level of control. With the current suite of agents available (Table 1), future research should focus on the seed feeding weevil, *Smicronyx lutulentus* Dietz: this insect is known to be extremely host specific and very damaging to seed production, which is a vital mode of reproduction and spread of parthenium (McClay 1983, 1985). In the longer term, other agents could be considered including the root feeding moth, *Carmenta* sp. nr *ithacae* (Beutenmüller) and the summer rust, *Puccinia xanthii* var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero.

Conclusions

Although the prevention of invasive species establishment through a strong policy framework around quarantine and domestic control is the first step in invasive species management, once a species is established other management strategies are required. Biological control offers a cost effective, safe and sustainable control option for several invasive weeds, including parthenium, although the biological control of weeds is in its infancy in Pakistan (Shabbir et al. 2019), with only a single agent, *Cactoblastis cactorum* [Berg.] being deliberately introduced to the country in the 1990s against *Opuntia* spp. (Zimmermann et al. 2000). The biological control of parthenium in Pakistan has great potential: there are already two agents, *Z. bicolorata* and *P. abrupta* var. *partheniicola* well established in the country, with a third, *L. setosipennis* imported into Pakistan and undergoing host range testing in quarantine.

If a regional approach to parthenium control in Asia is considered, the full responsibility of parthenium biological control should not rest solely on Pakistan. Biological control agents do not respect political boundaries and Pakistan has already benefited from the agents already present in other countries. Both *Z. bicolorata,* which was released into India in the 1980s, and the winter rust already found their way into Pakistan, most likely through Nepal or India. Additional agents need to be strategically considered, for example the release of *L. setosipennis* into Pakistan could potentially be beneficial for the region as a whole (India and Nepal) where parthenium is widely distributed. Considerable time and resources would be saved through regional collaboration and shared responsibility among the countries in Asia where parthenium has invaded and caused considerable losses.

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