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Predatory bugs of *Zygogramma bicolorata* Pallister: An exotic beetle for biological suppression of *Parthenium hysterophorus* L.

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Success of any biocontrol agent in a natural system is determined by biotic and abiotic factors over a period of time. We undertook in-depth investigation on the natural enemies associated with *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), an exotic beetle introduced in India for biological suppression of *Parthenium hysterophorus* L. and report its predation by three species of predatory bugs, namely *Andralus spinidens* Fab., *Cantheconidea furcellata* Wolf and *Sycanus pyrrhomelas* Walker. While the former two pentatomid bugs fed upon grubs exclusively, the latter reduviid predated upon both grubs and adults. The appearance of these bugs followed a definite succession pattern coinciding with the availability of host stage in field conditions. A single adult bug of *A. spinidens* and *C. furcellata* consumed on an average of 23 ± 1.3 and 21 ± 1.4 grubs respectively, whereas the assassin bug, *S. pyrrhomelas* consumed 12 ± 0.9 grubs or 7.6 ± 0.4 adults of this beetle per day. Our findings suggest that the foreseen consequences regarding the unlimited outbreak of this exotic beetle in the absence of a host, if completely destroyed, may not hold true, since the native natural enemies have started adopting this exotic beetle as a prey host in the natural ecosystem.

EVER since its introduction in India¹, *Parthenium hysterophorus* L. (Asteraceae) has become a serious weed of pastures, wastelands and agricultural fields in India². The problems posed by the weed to human health, agriculture, livestock production and biodiversity are well documented³. Considering the cultural and chemical strategies in vogue, efforts on biological control of this weed were initiated in 1983 with the introduction of Mexican beetle, *Zygogramma bicolorata*⁴. The beetle got established in and around the released site and showed considerable potential in suppressing the weed in subsequent years⁵. However, optimistic reports regarding the spectacular suppression of this weed by the beetle were tempered by the observation of beetle attack on sunflower in a few locations^{6–8}, which led to the establishment of the Parthenium Fact Finding Committee by Indian Council of Agricultural Research to look into the matter. After thorough investigations, the committee declared the beetle safe to sun-

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flower and recommended its further releases for suppression of this weed, thereby leading to an end to the raging controversy. This, coupled with the promising bio-control potential of this beetle as evidenced by the present and past successes⁹, gave a new momentum to the existing bio-control efforts in the country. Taking into consideration the widespread success of this beetle in other parts of the country, it was introduced in Jammu during 1992. However, its establishment and impact assessment thereafter have remained unexplored, except for some preliminary studies¹⁰.

Since insect host range can be assumed as the result of selection to maximize the production of offspring, it is difficult to assert that an insect species which is currently host-specific will continue to remain so in the future. The extension of host range will theoretically be favoured increasingly by an insect's inability to find its present host¹¹. There have been reports on local and temporary damage to non-host plants by the introduced natural enemies for weed suppression as a result of outbreak and starvation following sudden destruction of target host. This has resulted in reviving apprehension on ecological impact of this beetle on other hosts. Such conflicts on host transference need attention and should be resolved to overcome the foreseen ecological consequences. The current investigations were, therefore, planned to explore the future impact of this beetle as a result of its unlimited outbreak after complete defoliation of target host.

Jammu and Kashmir stretches between 32°17'N–37°6'N lat. and 73°26'E–80°30'E long. and comprises three regions, viz. Jammu, Kashmir and Ladakh. On the basis of geomorphic features, the State is divided into the outer plains, the Shivaliks, the middle Himalayas, the valley of Kashmir, the greater Himalayas and the plateau of Ladakh. While the former three geomorphic regions hold conditions conducive for multiplication of *P. hysterophorus*, in the latter areas the distribution of this weed is almost negligible. Biological control efforts for the suppression of this weed were initiated in 1992. Explorative survey on its establishment and effectiveness thereafter, was conducted to assess the role and impact of this beetle in the former three regions, with special reference to its natural enemies. The predator population and host–predator ratio in field was assessed on 20 randomly selected plants of *P. hysterophorus* L. at weekly intervals in Jammu, India. Apart from this, studies on the biology and predatory potential of these bugs were carried out in the laboratory (30 ± 2°C, 70% RH, 16 : 8 h light : dark cycle). A laboratory culture of each species was maintained separately by field-collected eggs laid on *Parthenium* leaves. The petioles of these leaves were plugged with wet cotton to avoid desiccation till hatching of the eggs. In order to study the biology of the bugs, three separate batches of the newly hatched nymphs ($N = 15$) of each species were placed individually in petri dishes (80 mm × 15 mm). Prey density was always maintained uniform and consisted of twenty-five 2nd instar grubs of *Z. bicolorata* per predator per

day. The nymphs were provided with fresh grubs every day after replenishing the old ones. Total post-embryonic developmental period (covering the entire nymphal and adult duration) of the predators raised on grubs were recorded. For evaluating the predatory potential of different nymphal stages and adults, three separate sets of experiments were conducted for each species as described above, wherein the daily consumption of 2nd instar grub by the predators was assessed. In order to study the fecundity, ten pairs of newly formed adult bugs of each species were kept individually for mating in glass petri dishes (150 mm × 20 mm) for pentatomids and glass jars (200 mm × 150 mm × 100 mm) for assassin bugs. Sufficient number of prey host alongwith cotton swab soaked with 10% sugar was provided daily. Observations were made to ascertain the egg-laying pattern and number of eggs laid. The predation behaviour was recorded on ten individuals of each species on 3rd instar grub, visually as well as with the help of a web camera (Logitech Quick Cam), for more accurate results.

Observations on the distribution of *Parthenium* weed, *Z. bicolorata* and its natural enemies revealed new records on predation of this exotic beetle by three predatory bugs, viz. *Andrallus spinidens* F., *Canthoconidea furcellata* Wolf and *Sycanus pyrrhomelas* Walker. Over the past ten years the beetle has dispersed over an area of 7000 km². However, outbreak population densities for the last three years were recorded in subtropical regions, particularly in the outer plains. Predator activity was also recorded exclusively in the outer plains during these years (Figure 1). The predators followed a definite succession pattern. The pentatomid, *A. spinidens* was the first one to appear on *Parthenium* in the month of July 2002, with a population ranging from 0.22 (second week of July) to 1.83 (third week of August) and an average of 0.77/plant over the period of activity up to the first week of September (Figure 2 a and 3). The host–predator ratio over the period of activity of the bug was observed to be 276 : 1. This was followed by the occurrence of another pentatomid, *C. furcellata* during the third week of August 2002, which lasted up to the last week of October (Figure 2 e and 3). The population density of this bug varied from 0.14 to 1.02 per plant, with an average host–predator ratio of 304 : 1. The reduviid, *S. pyrrhomelas* was the last to appear (Figure 2 i and 3) during the second week of September 2002, when the plant harboured maximum number of old-stage grubs or adults/plant and remained active up to the first week of November. The bugs were found in quite low numbers (0.05–0.37 per plant) with the host–predator ratio of 268 : 1 for grubs and 163 : 1 for adults. While the former two pentatomids predated upon the grub-stage exclusively, the latter reduviid preferred to feed upon adults (Figure 2 l) or old-stage grubs.

Under field conditions, pentatomid bugs deposited clusters of barrel-shaped eggs with elongated chorionic processes that ring the opercula cap on the plant surface.

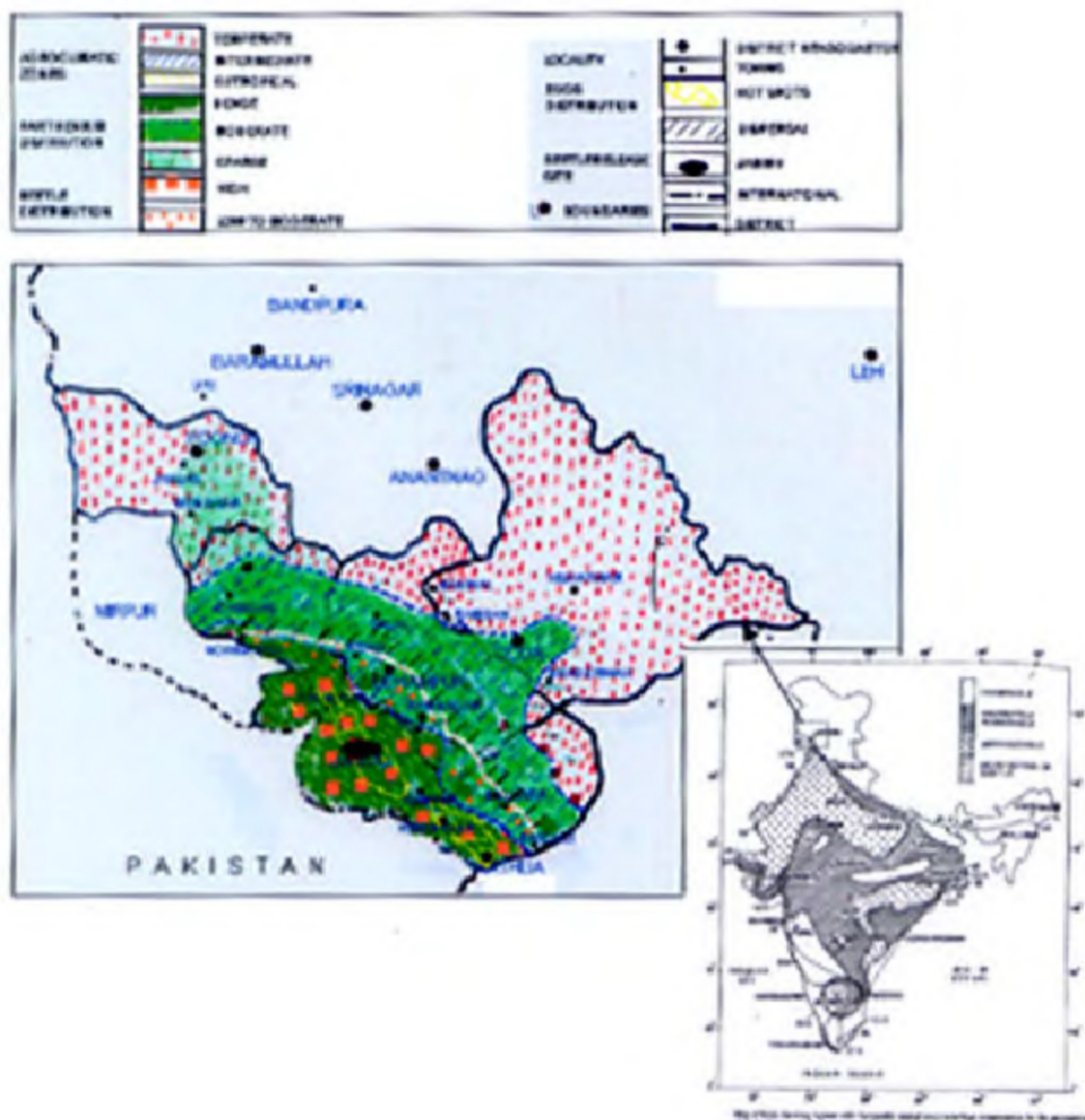


Figure 1. Distribution of *P. hystrophorus*, *Z. bicolorata* and predatory bugs in Jammu & Kashmir, India.

However, in the laboratory both the species oviposited on glass surface, cotton or muslin cloth. The gravid female after a pre-oviposition period of 1–3 days, moved up and down over the object on which the eggs were to be deposited, bent the abdomen and rubbed the object with its tip before egg-deposition. The first pentatomid, *A. spinidens* laid 4–8 egg batches containing 16–71 eggs, with an average of 41.2 eggs per cluster (Figure 2 b). The egg stage lasted for 6–7 days with an average of 6.24 ± 0.38 days (Table 1). The newly hatched nymphs (Figure 2 c) congregated in a circular pattern on *Parthenium* leaf and fed upon the leaf sap. The nymphs passed through five instars generally, but occasionally 4th instar nymphs changed

directly into adults. The duration of first to fifth instar nymphal stages was 2.52 ± 0.20 , 3.16 ± 0.45 , 2.88 ± 0.38 , 2.82 ± 0.27 and 3.92 ± 0.27 days respectively. The total life cycle from egg to adult ranged from 20.6 to 28.0 days, with an average of 24.62 ± 1.40 days. Similarly, the second pentatomid species, *C. furcellata* completed its life cycle in 19.0 to 29.0 days, with an average of 24.12 ± 1.70 days. During its life span of 6.0 to 13.0 (average 10.04 ± 1.26) days, the adult bug laid on an average 432 ± 15.60 eggs in 6 to 10 batches, each containing 20 to 83 eggs (Figure 2 f). The egg hatched in 5.7 to 8.0 days, with a mean incubation period of 6.46 ± 0.51 days. The younger nymphs were reddish-brown in colour (Figure 2 g). The nymphal

period lasted for 12.7 to 15.4 days and passed through five instars, with average duration of 1.46 ± 0.17 , 2.50 ± 0.16 , 2.62 ± 0.13 , 2.56 ± 0.17 and 4.48 ± 0.21 days respectively.

The eggs of assassin bugs were found on the *Parthenium* leaves in field conditions. However, in captivity, they laid most of the eggs on the underside of a muslin cloth tied upon the mouth of a glass jar, with occasional laying of eggs on glass surface. The eggs were laid in groups of 58–93 eggs/cluster, covered with glutinous, white froth-like substance (Figure 2j). Upon hatching, the nymphs remained on their eggshells and even fed upon them and subsequently preferred to remain on leaves of *Parthenium*. The nymph passed through five instars and preferred to feed on the grubs except the final instar nymph, which could predate on adults also. Each female, after a pre-oviposition period of 1–3 days laid 4–7 egg

batches, each containing 71 to 93 eggs, with an average of 80 ± 0.43 eggs per cluster. The egg stage lasted for 12.0 to 15.0 days, with an average of 13.88 ± 0.52 days. While the respective duration of the first four nymphal instars was 10.40 ± 0.54 , 12.96 ± 0.77 , 11.4 ± 0.54 and 29.0 ± 0.32 days, the fifth instar nymph lasted for 58.48 ± 0.76 days before changing into adult, which lived for 64.0 ± 4.62 days. The total life cycle ranged from 165.0 to 205.0 days, with an average of 184.8 ± 6.61 days.

Studies on predation behaviour of pentatomid bugs reflected that when a prey was detected, the adult and nymph of bugs extended their broad rostra directly forward in an attempt to pierce it and inject paralyzing enzymes (Figure 2a, d and e). The time taken for insertion of proboscis into the host in the 3rd instar grub by adults of *A. spinidens* and *C. furcellata* ranged from 18 to 24 and 19 to 26 s respectively. While the former took a total feeding time of

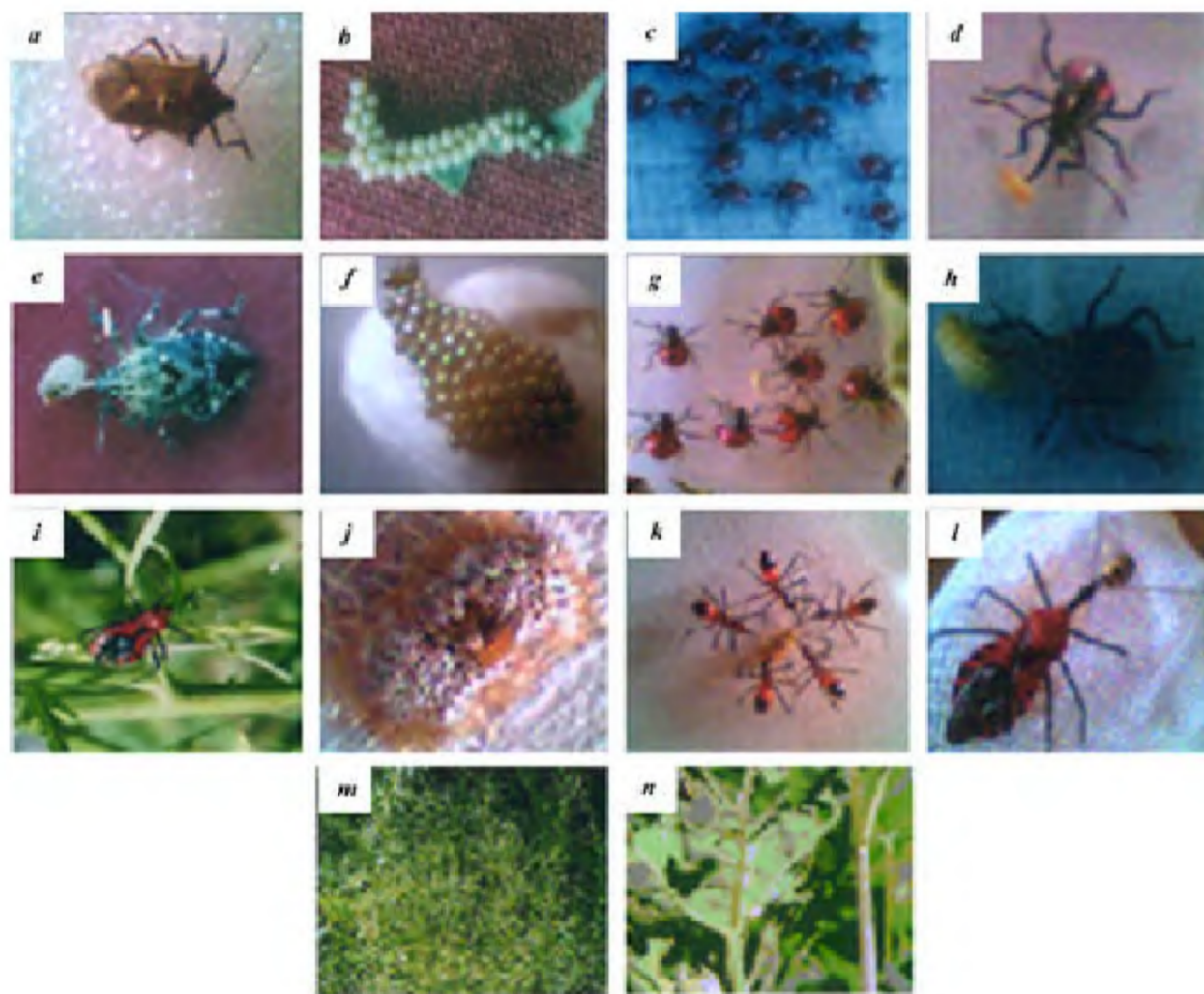


Figure 2. a–d, *Andrellus spinidens*. a, Adult; b, eggs; c, Newly hatched nymphs; d, Nymphal predation on *Z. bicolorata* grub. e–h, *Canthoconidea furcellata*. e, Predating adult; f, Eggs; g, 2nd instar nymphs; h, Nymphal predation on *Z. bicolorata* grub. i–l, *Sycanus pyrrhomelas*. i, Adult; j, Eggs mass; k, Gregarious predation by 2nd instar nymphs; l, Adult predation on *Z. bicolorata* adult. m, Phenotypic appearance of defoliated parthenium at the time of incidence of bugs. n, *Z. bicolorata* adult and grubs feeding on *Xanthium strumarium*.

45–62 min, the latter spent 33–47 min for complete feeding of 3rd stage grub of *Z. bicolorata*. The time taken for discharging/throwing out the host after complete feeding showed wide variation between the two species. In the former species it was as high as 8 min, while in the latter it ranged from 0.10 to 0.45 min only. This was attributed to the peculiar shape of the rostrum of pentatomids, wherein the presence of a thin tube-like stylet inside acted as push–pull mechanism for handling and discharge of host. The former could not use it effectively for discharging; the latter rarely used its forelegs for host discharging, as commonly observed in the former species. The nature of predation in the case of assassin bugs showed that when provided with a host, the nymph (upon detection) stops about 0.5 cm away from it, moves slowly and extends its proboscis for insertion into the host. Generally, the early nymphal instars could not feed upon the older grubs individually, but in the case of food scarcity gregarious predation on the single older grub was noticed (Figure 2 i). As the adult bug reached near the host, the fluttering movement of antennae perceived it and started moving its hind legs over abdominal region. With the help of backward movement of its hind legs, it adjusted its rostrum for piercing into the host, thereby injecting saliva with the stylets and finally practised pre-oral digestion of internal body fluid. The insertion time in adult beetle ranged from 7–12 s, with a feeding and discharge time of 61–120 min and 6–8 s respectively. The discharging of host was done with the help of forelegs.

It was found that the adult stages were more voracious than the nymphal instars. First instar nymphs of pentatomids did not show any predation on the 2nd instar grubs; however, in the case of reduviid, it could consume 8.40 ± 0.50 grubs/day (Figure 4). The second to fifth instar nymphs of *A. spinidens*, *C. fusciflata* and *S. pyrrhomelas* consumed 12.4 ± 0.92 , 24.6 ± 1.02 , 20.20 ± 0.86 and $62.80 \pm$

2.06 ; 14.0 ± 0.54 , 29.8 ± 0.37 , 28.4 ± 0.92 and 75.0 ± 1.00 ; and 23.6 ± 1.02 , 27.4 ± 0.81 , 50.8 ± 0.96 and 200.0 ± 2.23 second instar grubs of *Z. bicolorata* respectively. It was observed that none of the stages, i.e. adult and grub of pentatomid bugs predated upon the adult beetle. However, a single adult bug of *S. pyrrhomelas* during its longevity, consumed a total of 413.40 ± 11.90 adults, with an average daily consumption of 7.6 ± 0.4 adults or 12.0 ± 0.9 grubs. When adults were starved for 24 h, the bug fed on the grubs subsequently without relaxing and the feeding potential increased to the extent of 8 grubs/h. In all the three predatory bugs, females were more voracious than males.

The recently reported feeding by *Z. bicolorata* on related weed *Xanthium strumarium* and sunflower as a result of overpopulation and starvation following sudden destruction/disappearance of target weed was considered as a pointer towards the expansion of its host range. This had resulted in reviving apprehension on the ecological impact of this beetle on other hosts. The current observations

Table 1. Biology of predatory bugs on 2nd instar grub of *Z. bicolorata* reared on *Parthenium* leaves in laboratory ($30 \pm 2^\circ\text{C}$; 70% RH)

Life stage	Period (days \pm SE)		
	<i>A. spinidens</i>	<i>C. fusciflata</i>	<i>S. pyrrhomelas</i>
Egg	6.24 ± 0.38	6.46 ± 0.51	13.88 ± 0.52
First instar	2.52 ± 0.20	1.46 ± 0.17	10.40 ± 0.54
Second instar	3.16 ± 0.45	2.50 ± 0.16	12.96 ± 0.77
Third instar	2.88 ± 0.38	2.62 ± 0.13	11.40 ± 0.54
Fourth instar	2.82 ± 0.27	2.56 ± 0.17	29.00 ± 0.32
Fifth instar	3.92 ± 0.27	4.48 ± 0.21	58.48 ± 0.76
Adult	8.80 ± 1.13	10.0 ± 0.16	64.00 ± 4.62
Total	24.62 ± 1.40	24.12 ± 1.70	184.80 ± 6.61

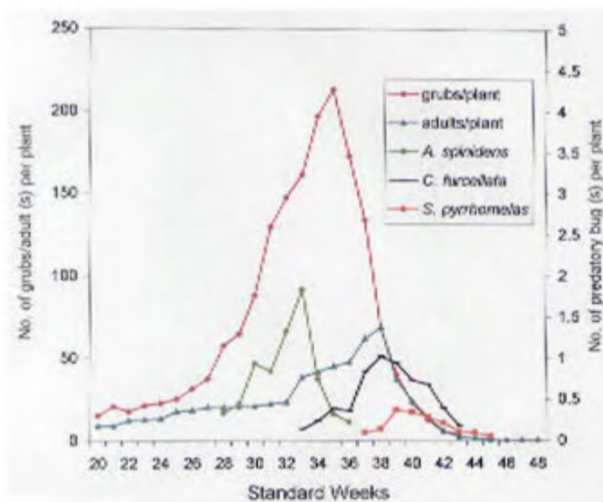


Figure 3. Occurrence of predatory bugs in relation to incidence of *Z. bicolorata*.

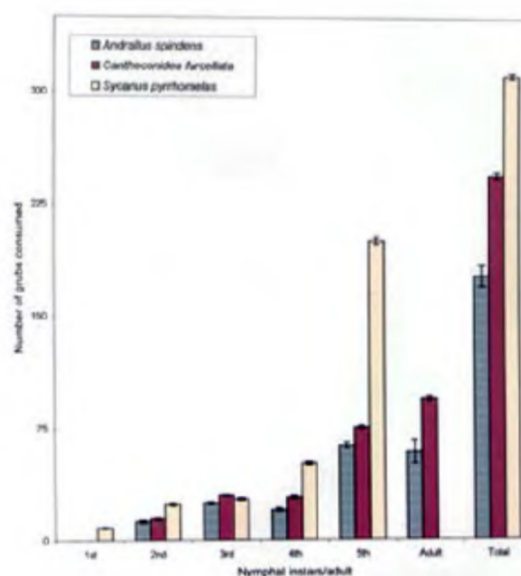


Figure 4. Predatory potential of various nymphal instars and adults of three bug species on 2nd instar grubs of *Z. bicolorata*. Vertical bars represent the standard error.

have revealed that the native natural enemies will be able to check the unlimited outbreak of this beetle. Perusal of available literature does not reveal predation of this beetle by these bugs.

The natural enemies of *Z. bicolorata* have been poorly described, though there are indications that the indigenous parasitoid, *Palexorista* sp. has started adopting this beetle as host in India¹². The occurrence of these predacious bugs, viz. *A. spinidens*, *C. furcellata* and *S. pyrrhomelas* is well established in India¹³⁻¹⁵ and abroad¹⁶. While in India these bug species were found preying commonly upon lepidopteran larvae and less frequently on coleopteran larvae¹⁷, the global scenario suggests that only a few of them are prey-specific and majority may feed upon Lepidoptera, Coleoptera, Hymenoptera, Diptera, Hemiptera and Neuroptera¹⁸.

The appearance of these bugs in succession clearly indicated their preference for a particular host stage for survival and multiplication (Figure 3). In the present investigation, it was revealed that while the former two pentatomids predated upon the grub stage exclusively, the latter reduviid showed best survival and reproduction when it was provided with adult beetles or old-stage grubs. No doubt, the predatory potential of reduviid, *S. pyrrhomelas* was comparatively higher than the pentatomids; the former took long duration for completion of its life cycle. Therefore, pentatomids due to their short life cycle, continuous feeding habits and more number of generations per year, would be equally effective for checking the outbreak of this beetle.

It is interesting to note that these predators were present only in those areas where the beetle population was high and plants were nearly defoliated (Figure 3 m). Further, these predators were not observed in areas where dispersal and establishment of beetles was in progress. Earlier reports also indicated that these predators were of negligible importance at low prey densities, but of much greater value at outbreak densities¹⁹. Negative impact of these predators on establishment of this beetle is currently being over-ruled. Moreover, the limited importance of these bugs in terms of low population, survival, consumption, multiplication and establishment is well documented in applied biological control²⁰. The genus *Sycanus* was introduced into Florida from India for the suppression of soybean looper, and apparently it did not establish²¹. The degree of control by *Sycanus* species in tea plantations against mirid bug was uncertain²². Similarly, the release of *C. furcellata* against Colorado potato beetle and eastern tent caterpillar in 1981 could not yield any recovery²³.

We do not envisage potential threat due to these predatory bugs for the dispersal, establishment and multiplication of *Z. bicolorata*, as their appearance synchronized with the outbreak densities of this beetle. However, it will also be worthwhile to ascertain the impact of these predators as a probable limiting factor for establishment of *Z. bicolorata* in the near future. Our findings also suggest that the foreseen consequences regarding the possible host

transfer due to overpopulation or starvation in the absence of host, if destroyed completely, may not hold true since the native natural enemies are going to act as natural regulating factors for unlimited outbreak of this exotic beetle in the natural ecosystem. We also found that in the case where the host was completely destroyed by this beetle, it did not shift to any other agri-horticultural crops, but heavy feeding upon the related weed, *Xanthium strumarium* was observed in field (Figure 2 n).

It is to be emphasized that since most of these bugs are currently being reared on frozen lepidopteran larvae²⁴, the present findings open new avenues for evolving an alternative, cost-effective rearing technique for mass multiplication of the bugs. Thus we can rear a large number of individuals of each species using *Z. bicolorata* as prey host, in captivity for experimentation. A systematic study on this aspect is needed. Further, studies on these predatory bugs are being reported elsewhere.

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