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Host-specificity and biology of *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) introduced into India for the biological suppression of *Chromolaena odorata* (Linnaeus) King & Robinson

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ABSTRACT: ***Chromolaena odorata*** **(Linnaeus) King and Robinson is a native of tropical America and has become a serious invasive weed in the wet/dry tropics of Western India.** ***Cecidochares connexa*** **(Macquart) (Diptera: Tephritidae) was introduced from Indonesia into India in 2002 for the biological suppression of the weed. A pure culture of the tephritid was established on** ***C. odorata*** **and the biology studied. Eggs were laid inside unopened new leaf buds. The egg incubation period was 5.65 ± 0.67 days. The gall was visible 15 days after oviposition and in 43.95 ± 4.7 days, formation of windows could be seen indicating complete larval development. One to eight larvae were found in each gall. The total developmental period from egg to adult was 64.85 ± 5.12 days. The adults emerged through the windows and peak emergence (70.4%) was at 1000 hours. Adults were active from 0800 to 1400 hours and mated on the day of emergence. Each female on an average laid 81.12 ± 34.03 eggs and the oviposition period varied from 7 to 12 days. Host-specificity tests carried out under quarantine conditions on 75 host plant species belonging to 29 families revealed that the gall fly is capable of feeding and reproducing only on** ***C. odorata.***

KEY WORDS: Biology, *Cecidochares connexa*, *Chromolaena odorata*, host-specificity tests

INTRODUCTION

Chromolaena odorata (Linnaeus) King & Robinson is an herbaceous shrub native to the tropical America, which has become a serious invasive weed in the wet/dry tropics of Africa and Asia (McFadyen, 1989). Its infestation in 1933-34 in plantations of Buxa and Jalpaiguri divisions of Assam resulted in suppression of *Acacia catechu* and *Dalbergia sissoo* regenerations in high forests (Sen Gupta, 1949). In India, it is now very well distributed in northeastern and southern states. It has occupied pastures, marginal lands and open areas and has become a menace in coconut, rubber, oil palm, tea, teak, coffee, cardamom, citrus and other plantations, orchards and forests. During dry season, it can be a serious fire risk in the forests (Singh, 1998).

Insects that had adapted to feeding on *C. odorata* were surveyed in Karnataka, Tamil Nadu and Kerala, but none of them was found promising as a biocontrol agent (Singh, 1998). Classical biological control attempts were made through introduction of natural enemies from the native range of *C. odorata*. A host-specific hairy defoliator, *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) was imported from Trinidad by the Commonwealth Institute of Biological Control (CIBC), Indian Station, Bangalore in 1970. After the initial biology and host-specificity studies, the agent was field released in 1971 in Kodagu (Karnataka) and Kottayam (Kerala). However, the insect could not establish. In 1984, a Sri Lankan strain of *P. pseudoinsulata* was released in Chickmagalur, Kodagu and Bangalore in Karnataka but establishment was not observed (Anonymous, 1986). Following further releases, the insect was reported to have established at Mallesara near Teerthahalli in Shimoga District and Sullia, Dakshina Kannada District, Karnataka State, but the effect on the weed was negligible (Anonymous, 1985, 1986, 1987). A seed feeding weevil, *Apion brunneonigrum* Beguin-Billecoq (Coleoptera: Apionidae) from Trinidad was introduced in 1982 and supplied to Kerala Agricultural University and Central Horticultural Experiment Station, Chettalli, Kodagu. Field releases were made both at Thrissur (Kerala) and Kodagu (Karnataka) but there was no establishment in the field.

A stem gallfly, *Cecidochares connexa* (Macquart) (Diptera: Tephritidae), which has been promising in Indonesia, Thailand, Papua New Guinea and East Timor (Wilson and Widayanto, 2004; Orapa and Bofeng, 2004), was thought appropriate for introduction. The shipment of *C. connexa* was received during November 2002 from BIOTROP, Bogor, Indonesia and a culture of the insect was established in the quarantine laboratory of the Project Directorate of Biological Control for further testing. Studies were made on its biology, nature of gall formation, host-specificity and impact on *C. odorata*, the results of which are reported in this paper.

MATERIALS AND METHODS

A culture of the gallfly was established in the quarantine at Project Directorate of Biological Control, Bangalore from 28 females and 30 males received from Indonesia (import permit from PPA, GOI No. IP-12/2002 PQD dated 19.9.2002). This colony was used in the experiments described in this paper, and the present laboratory and future field colonies in India shall be constructed to have been derived from this colony.

Establishment of pure culture

Chromolaena odorata seedlings growing in the wild were uprooted, pruned just above collar region and planted in pots filled with FYM and red soil in equal proportion. The new terminal buds were clipped just above the lowest opened leaves to encourage more side shoots till each plant had more than 14 growing tips, providing sufficient growing tips for oviposition by one female. It took 15 days for plant establishment and another 30 days to obtain a minimum of 14 growing shoots. Following this method the plant height could be maintained below 30 cm before exposing to adult flies in a cage (0.5 x 0.5 x 0.75 m, made from a wooden frame covered with nylon cloth on three sides and glass door in the front) for oviposition. Plants higher than 30 cm when enclosed in a cage for 70 days grew tall and their terminal bud, which transforms into a gall, started bending and breaking. Freshly emerged male and female were kept in a glass vial (2.5 x 15 cm) for a day to facilitate mating. A pair of mated flies was enclosed along with the plant (with more than 14 shoots) in a cage. The flies were allowed to die in the cage. The oviposited plant was maintained in the same cage and observation on the development of galls recorded until the emergence of adult flies from the galls.

Biology

Freshly emerged male and female adults were enclosed in a glass vial and were allowed to mate for the first day. Cotton swab dipped in water was provided as food. Time taken for mating was noted. Egg laying pattern was studied by enclosing a pair

of mated male and female adults in a cage along with a Chromolaena plant with five growing shoots for oviposition. Each shoot tip was cut the next day and dissected under a binocular microscope for counting and measuring the eggs. The same flies were enclosed for the second day with a fresh plant with growing shoots. Ten shoot tips with eggs laid on second and third day were kept in a Petri-dish (12cm diam.) with moist cotton for recording the incubation period. The fourth and fifth day oviposited plants were enclosed in a separate cage, their shoots with eggs tagged and the development of the galls on these shoots observed until the emergence of adults. Oviposition studies continued till all the females died. The diameter of the galls was measured using a vernier callipers. When windows were formed 45-50 days after oviposition, ten galls were dissected every day to observe the stage of the larvae and the pupal duration recorded. Adult emergence pattern during the day was studied by collecting adults emerged from 0800 hours at hourly interval. Adult longevity studies were done by enclosing a pair of freshly emerged adults in a glass vial (2.5x15cm) with cotton wad dipped in water and honey (50%), separately.

Host-specificity

Plants were chosen from the earlier list of plants tested at Marihat Research Center, Sumatra, Indonesia and the Indonesian Department of Agriculture (McFadyen *et al.*, 2003). Following the internationally accepted centrifugal method, suitable substitutions were made for plants not cultivated in India, and other plants included while finalizing the list of plants for host-specificity tests. This was done in consultation with botanists at UAS, GKVK, Bangalore. Several of these test plants were raised from seeds or stem cuttings, and few of them procured from scientific nurseries. In all 75 host plants belonging to 29 families were tested for their suitability for oviposition and feeding by the stem gallfly.

Paired choice tests

A pair of flies were introduced into each cage with one *C. odorata* and another test plant and maintained until the death of flies in each treatment. The flies' activity (resting, mating, probing the plant or laying eggs) and number of days the flies were alive was noted in each treatment. All the test plants were maintained and examined for gall formation until such time when galls were formed on the *C. odorata* plants kept along with the test plant. There were three replications for each paired test.

No-choice tests

Two test plants along with two pairs of adult flies were kept in the cage. The activity of the flies was observed until death of the adults. All the test plants were maintained for at least 30 days and carefully examined for any gall formation.

RESULTS AND DISCUSSION

Establishment of pure culture

From a shipment of 30 males and 28 females received from Indonesia, 54 males and 42 females were produced in the first generation and 127 males and 133 females in the second generation. A pure culture of *C. connexa* was thus established for further studies.

Biology

Adult males lived for 10.75 ± 3.45 days and females 9.87 ± 1.8 days when left on the host plant. The emerged adults were active between 0800 and 1400 hours (sunrise at 0600). McFadyen *et al.* (2003) recorded adult activity between 0800 and 1400 hours at Indonesia. Mating took place on the host plant between 0800 and 1100 hours. The mating duration varied from 68 to 100 minutes (mean 84.07 ± 10.09; n=14). Oviposition usually occurred between 1000 and 1400 hours. Females flew from plant to plant, walked over the stems and tips and then probed and oviposited in the unopened buds. Eggs were laid into new unopened terminal or axillary buds. Black dead tissue in unopened leaves and holes on opposite opened leaves indicated the likely presence of eggs in the shoot. Each female laid 28 to 132 eggs (mean 81.12 ± 34.03; n=8). According to McFadyen *et al.* (2003), each female laid 50 to 70 eggs. The oviposition period lasted 7 to 12 days and peak egg laying (12.87 eggs) was

seen on the fourth day after emergence (Fig.1). Eggs are pale white, elongate oval, sculptured with hexagonal cell like structures and measure 0.59 mm in length and 0.20 mm in width. Eggs were laid in groups of 2 to 20 in each bud. The eggs hatched in 5.65 ± 0.67 days and the larvae tunneled into the stem tissue.

According to McFadyen *et al.* (2003), the eggs measured 0.8mm in length and 0.2mm in width and females laid 2 to 16 eggs in packed masses in each bud. The oviposition period and adult emergence pattern were not studied by earlier workers.

The pre-pupal and pupal period lasted 19.5±3.2 days, and the whole life cycle from egg to adult 55 to 74 days, averaging 64.85±5.12 days. A pre-pupal and pupal period of 15 to 25 days and egg to adult developmental period of 47 to 75 days was reported by McFadyen *et al.* (2003). The sex ratio of the progeny from a single female was 1:1.1 (F:M; n=13). The adults emerged between 0900 and 1300 hours and peak emergence (70.4% of emerged adults) was at 1000 hours (Fig. 2). Adult longevity studies revealed that females lived from 7.95 ± 1.7 (n=4) days when fed on water and 10.22 ± 3.1 days (n=8) when fed on 50 percent honey. Similarly

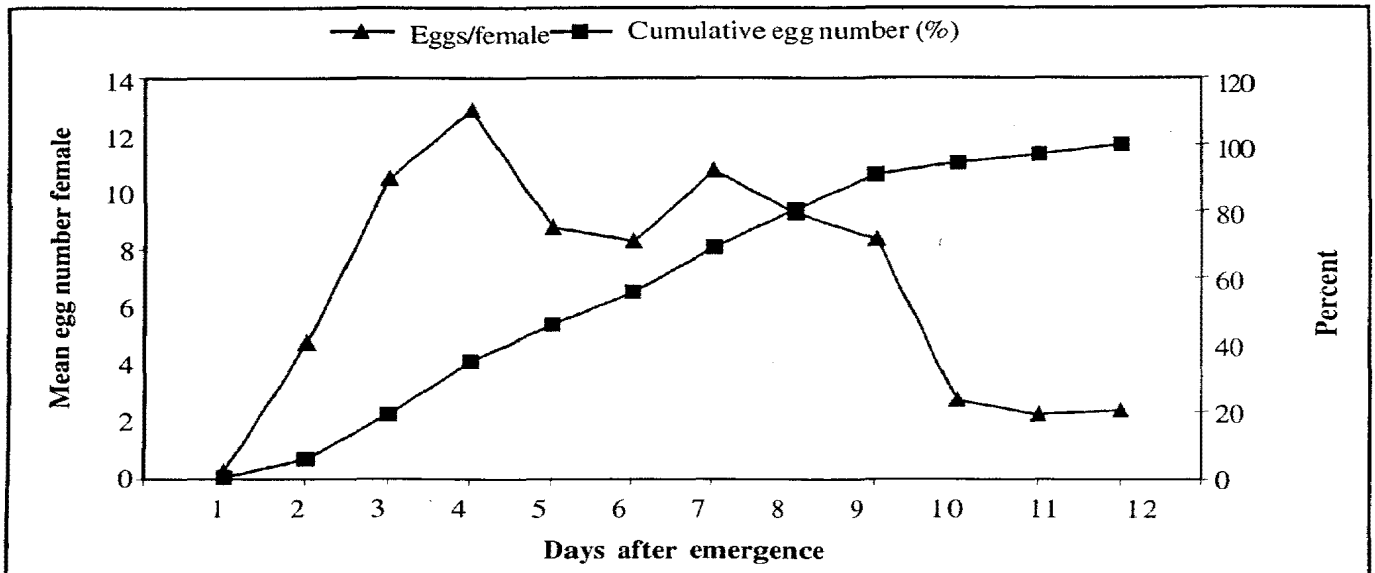

Fig. 1. Egg laying pattern of *Cecidochares connexa* (n=8)

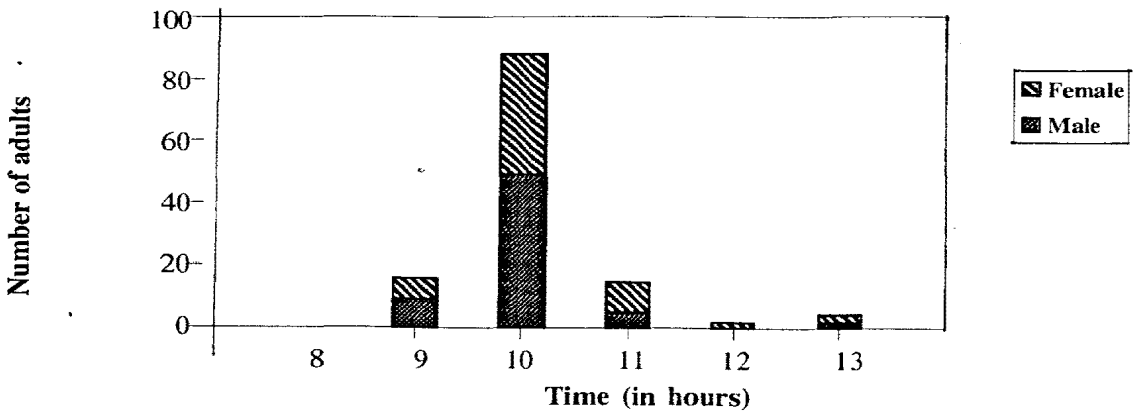

Fig. 2. *Cecidochares connexa* adult emergence pattern

males lived for 9.4 ± 0.5 days when fed on water and 7.18 ± 0.9 days when fed on honey.

The gall generally developed at the node where eggs were laid. Occasionally the gall was inter-nodal or formed at an axillary bud. Initially a small swelling was seen 14 days after oviposition and the gall gradually increased in size and was fully grown in 43.95 ± 4.7 days (Fig. 3.). McFadyen *et al.* (2003) found that the gall steadily developed until the larvae were fully grown in 30-50 days. Mature galls were green but woody, 1.8–2.9cm long and 0.8 - 1.3cm in diameter. One to eight larvae were found in a single gall (mean 2.67 ± 1.95; n=50). McFadyen *et al.* (2003) observed two to four larvae per gall in Indonesia. Mature larvae cut an emergence tunnel to the gall surface, leaving a thin "window" of epidermal tissue, which the adult breaks for emergence. Larvae usually construct separate emergence windows but some times two may use the same one. Gall development pattern was not studied by earlier workers.

Host-specificity

Among the 75 plants belonging to 29 families, oviposition was not observed on 74 plants either in free-choice or in no-choice tests (Tables 1-3) except on *C. odorata*. In host-specificity tests conducted in Indonesia, *C. connexa* did not lay eggs on any of the 55 species of plants in the choice tests. However, in the no-choice tests, females laid eggs on *Austroeupatorium inulaefolium* and *Ageratum conyzoides*, but the maggots did not develop and no galls were formed (Sipayung and Desmier de Chenon, 1994; McFadyen *et al.*, 2003). *Austroeupatorium inulaefolium* does not occur in India and the insect did not lay eggs on *A. conyzoides* during choice and no-choice tests. Eggs were also not laid on *Eupatorium adenophorum*, a very close relative of *C. odorata*. Muniappan and Bamba (2000) did not observe egg laying on 12 plants in the host-specificity tests conducted at Guam. Similarly, at Philippines, Aterrado and Bachiller (2000) did not observe egg laying on eight plants. Host-specificity tests here in India revealed that *C. connexa* could lay eggs and complete its life cycle only on *C. odorata* and proved beyond doubt its safety to other plants as has been proved in other parts of the world.

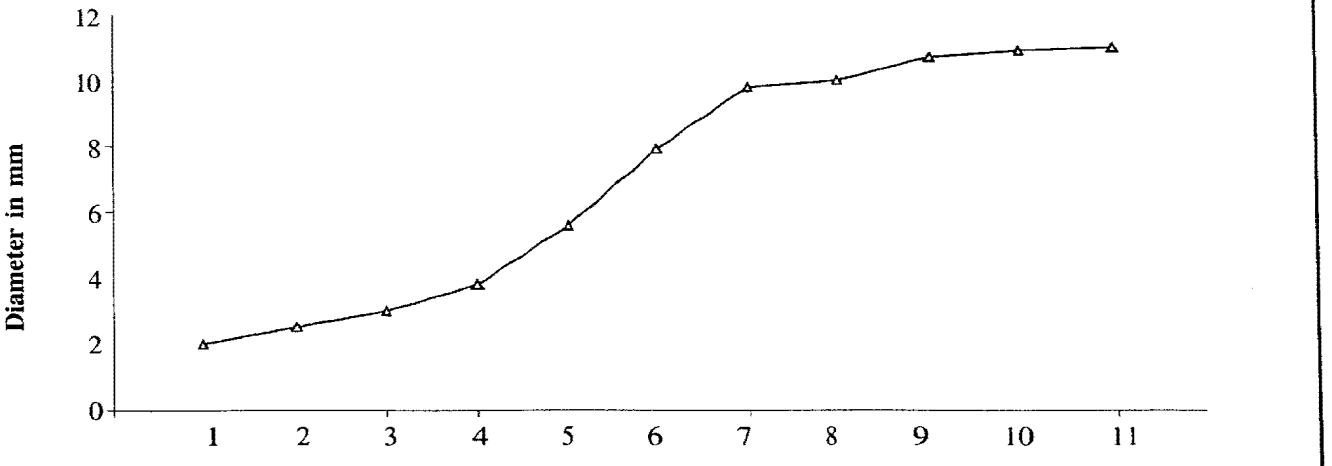

Fig. 3. Growth pattern of *Cecidochares connexa* induced stem gall in *Chromolaena odorata*

Table 1. Host-specificity tests for *C. connexa* on fruit, vegetable and ornamental plants

Sl. no.	Family / Scientific name	Economic importance	Observation results		
			Visits	Eggs	Galls
	Amaranthaceae				
1	*Amaranthus tricolor*	Vegetable	-	-	-
	Asteraceae				
2	*Aster amellus*	Ornamental	-	-	-
3	*Calendula officinalis*	Ornamental	-	-	-
4	*Cosmos bipinnatus*	Ornamental	-	-	-
5	*Dahlia pinnata*	Ornamental	-	-	-
6	*Gerebera jamesonii*	Ornamental	-	-	-
7	*Lactuca sativa*	Vegetable	-	-	-
8	*Solidago canadensis*	Ornamental	-	-	-
9	*Tagetes erecta*	Ornamental	-	-	-
10	*Zinnia elegans*	Ornamental	-	-	-
	Balsaminaceae				
11	*Impatiens balsamina*	Ornamental	-	-	-
	Caesalpinaceae				
12	*Caesalpinia pulcherrima*		-	-	-
	Convolvulaceae				
13	*Ipomoea batatas*	Vegetable	-	-	-
	Cruciferae				
14	*Raphanus sativus*	Vegetable	-	-	-
	Cucurbitaceae				
15	*Cucumis melo*	Fruit	-	-	-
16	*Cucumis moschata*	Vegetable	-	-	-
17	*Cucumis sativus*	Vegetable	-	-	-
	Euphorbiaceae				
18	*Manihot esculenta*	Vegetable	-	-	-
	Leguminosae				
19	*Pisum sativum*	Vegetable	-	-	-
	Malvaceae				
20	*Hibiscus rosasinensis*	Ornamental	-	-	-
	Mimosaceae				
21	*Calliandra haematocephala*	Ornamental	-	-	-
22	*Albizia lebbek*	Avenue	-	-	-
	Myrtaceae				
23	*Eugenia jambolana*	Fruit	-	-	-
24	*Psidium guajava*	Fruit	-	-	-
	Oleaceae				
25	*Jasminum sambac*	Ornamental	-	-	-
	Papilionaceae				
26	*Dolichos lablab*	Vegetable	-	-	-

Sl. no.	Family / Scientific name	Economic importance	Observation results		
			Visits	Eggs	Galls
	Puniccaceae				
27	*Punica granatum*	Fruit	-	-	-
	Rutaceae				
28	*Citrus reticulata*	Fruit	-	-	-
	Sapotaceae				
29	*Achras sapota*	Fruit	-	-	-
	Solanaceae				
30	*Capsicum annuum*	Vegetable	-	-	-
31	*Lycopersicon esculentum*	Vegetable	-	-	-
32	*Solanum melongena*	Vegetable	-	-	-
33	*Solanum tuberosum*	Vegetable	-	-	-

- = no visits, no eggs, or gall.

Table 2. Host-specificity tests for *C. connexa* on cereals and cash crops

Sl. no.	Family / Scientific name	Economic importance	Observation results		
			Visits	Eggs	Galls
	Amaranthaceae				
1	*Anacardium occidentale*	Commercial	-	-	-
	Asteraceae				
2	*Carthamus tinctorius*	Oilseed	-	-	-
3	*Chrysanthemum indicum*	Oilseed	-	-	-
4	*Guizotia abyssinica*	*Oilseed*	-	-	-
5	*Helianthus annus*	Oilseed	-	-	-
	Cruciferae				
6	*Brassica nigra*	Oilseed	-	-	-
	Euphorbiaceae				
7	*Hevea brasiliensis*	Commercial	-	-	-
8	*Ricinus cummunis*	Oilseed	-	-	-
	Labiatae				
9	*Mentha arvensis*	Spice	-	-	-
	Lauraceae				
10	*Cinnamomum zeylanicum*	Spice	-	-	-
	Liliaceae				
11	*Allium sativum*	Spice	-	-	-

Sl.no.	Family / Scientific name	Economic importance	Observation results		
			Visits	Eggs	Galls
	Malvaceae				
12	*Gossypium hirsutum*	Fibre	-	-	-
	Moraceae				
13	*Morus alba*	Commercial	-	-	-
	Papilionaceae				
14	*Arachis hypogea*	Oilseed	-	-	-
15	*Glycine max*	Pulse	-	-	-
16	*Vigna unguiculata*	Pulse	-	-	-
	Piperaceae				
17	*Piper nigrum*	Spice	-	-	-
	Poaceae				
18	*Oryza sativa*	Cereal	-	-	-
19	Zea mays	Cereal	-	-	-
	Rubiaceae				
20	*Coffea arabica*	Beverage	-	-	-
	Rutaceae				
21	*Murraya koenigii*	Spice	-	-	-
	Solanaceae				
22	*Nicotiana tabacum*	Narcotic	-	-	-
	Sterculiaceae				
23	*Camellia sinensis*	Beverage	-	-	-
24	*Theobroma cacao*	Beverage	-	-	-
	Umbelliferae				
25	*Coriandrum sativum*	Spice	-	-	-
	Verbenaceae				
26	*Tectona grandis*	Timber	-	-	-

- = no visits, no eggs, no galls

Table 3. Host-specificity tests for *Cecidochares connexa* on weeds, fodder and green manure plants

Sl.no.	Family / Scientific name	Economic importance	Observation results		
			Visits	Eggs	Galls
	Asteraceae				
1	*Ageratum conyzoides*	Weed	-	-	-
2	*Bidens pilosa*	Weed	-	-	-
3	*Chromolaena odorata**	Weed	+	+	+
4	*Eclipta alba*	Weed	-	-	-
5	*Eupatorium adenophorum*	Weed	-	-	-
6	*Legasca mollis*	Weed	-	-	-
7	*Mikania micrantha*	Weed	-	-	-
8	*Sonchus arvensis*	Weed	-	-	-
9	*Spilanthes acmella*	Weed	-	-	-
10	*Tithonia divaricata*	Weed	-	-	-
11	*Tridax procumbense*	Weed	-	-	-
12	*Xanthium strumarium*	Weed	-	-	-
	Mimosaceae				
13	*Leucaena leucocephala*	Fodder	-	-	-
	Papilionaceae				
14	*Crotalaria juncea*	Green manure	-	-	-
15	*Gliricidia sepium*	Green manure	-	-	-
	Verbenaceae				
16	*Lantana camara*	Weed	-	-	-

* Except *on C. odorata, Cecidichares connexa* neither visited any above mentioned plant species nor oviposited eggs or made galls.

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