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**BALANCE OF *CHROMOLAENA ODORATA* AND
CECIDOCHRES CONNEXA POPULATIONS TO ACHIEVE
AN EFFECTIVE BIOLOGICAL CONTROL OUTPUT**

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Table of Contents

Table of Contents	i
List of Tables	ii
List of Figures	ii
ABSTRACT	Error! Bookmark not defined.
1. INTRODUCTION	2
1.1. Background	2
1.2. Objectives	5
1.3. Expected Output	6
2. BENEFIT AND IMPORTANT OF RESEARCH	6
3. METHODOLOGY	6
4. RESULTS AND DISCUSSION	7
4.1. Establishing of Rearing Centers in Indonesia	11
4.2. Coming activities under Laboratory of Weeds and Invasive Plant Species	12
5. CONCLUSSIONS	12
5.1. Conclusions from green house experiments	13
5.2. Recommendation for Rearing Center	13
5.3. Field Observations	13
6. REFERENCES	14

List of Tables

Table 1. The value of parameters of <i>C.connexa</i> life table	7
Table 2. Means number of shoot , galls, flowers, and dry matters of leaves stems, branches, and root, recorded after 5 months growth on the shoot density <i>C.odorata</i> of 5, 10, and 15 shoots	9
Table 3. Means number of shoot , galls, flowers, and dry matters of leaves stems, branches, and root, total DM recorded after 5 months growth when subjected to 0, 1 and 2 pairs of <i>Cecidochares connexa</i> attack.....	10
Table 4. Means number of shoot , galls, flowers, and dry matters of leaves stems, branches, and root, total DM recorded after 5 months growth when imago of <i>C.connexa</i> releases were repeated 2, 4, and 6 weeks	10
Table 5. The humber of plants along 100 line transects crossing each other with quadrats of 2x2 m ²	14

List of Figures

Figure 1 The map of world distribution of <i>Chromolaena odorata</i>	2
Figure 2 Distribution map of <i>Chromolaena odorata</i> in Indonesia	3
Figure 3 The appropriate management technique applied to the invasive alien plant species depending upon the stage of invasion (Groves, 1991).....	4
Figure 4 Diagram of processes in a biological control event.....	5
Figure 5 The prolific growth of <i>C odorata</i> shoot, showing rapid production of shoot despite the present of gall.....	8
Figure 6 The growth of <i>C.odorata</i> shoot toward the end of dry season	8
Figure 7 A poor branch of <i>C.odortat</i> attacked by agrressive <i>C.connexa</i>	9

BALANCE OF *CHROMOLAENA ODORATA* AND *CECIDOCHARES CONNEXA* POPULATIONS TO ACHIEVE AN EFFECTIVE BIOLOGICAL CONTROL OUTPUT

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ABSTRACT

Chromolaena odorata is distributed naturally in North, Central and South America. It spreads and invades the world. CABI formed a working group on the biological control of *C. odorata* and organised its first International Workshop on the biological control of *C. odorata* held in Thailand in 1988. The second workshop was organised at BIOTROP in 1991. Since then the workshop developed into the biological control and management of *C. odorata*. After several international workshop, at 8th international workshop it developed into the biological control and management of *C. odorata* and Eupatoriae. Currently at the 9th international workshop it is developed further into Biological Control and Management of Eupatoriae and other invasive Weeds. A packet of activities including laboratory research, field observation and internships were carried out in 2018 to establish a networking on the biological control of *Chromolaena odorata* using its natural enemy, *Cecidochares connexa* in Indonesia. A green house experiment was organized using factorial design with 3 factors, and randomized in block i.e. factor 1, number of *C. odorata* shoot with 3 levels, 5, 10, and 15 shoot, factor 2, number of *C. connexa* imago released at 3 levels, 0, 1 and 2, and factor 3, repetition of releases, at 3 levels, 2, 4, and 6 weeks repetitions. The increment number of shoot in 5 months in term of monthly relative increment rate the highest was 5 initial shoot giving $r = 0.8058$, while under initial 15 shoot was only 0.6053, the rate of increment seemed to affected by the available nutrient from the growth media in the pot. These relative rate of shoot increment was much higher than that of rate of increment of imago of *Cecidochares connexa*. It is easy to see in the field that the occurrence of galls was much lower than the number of shoot. The release of imago reduced the emergence of new shoot, while repetition of release at 2 weeks interval increased the number of galls more than 4 or 6 weeks interval. *C. connexa* was released in 1995 on *Acacia mangium* forest invaded heavily by *C. odorata*. The forest was logged and replanted in 2013. Line transect sampling revealed a low population of *C. odorata* attacked by *C. connexa*. This condition may represent the balance of populations of *C. odorata* and *C. connexa*. BIOTROP is developing rearing and releasing centres in Indonesia to speed up the distribution of *C. connexa* to control *C. odorata*. These activities will be submitted to the 9th International Workshop in Malaysia next year.

Key words: biological control of *Chromolaena odorata*, *Cecidochares connexa*, frequency of releases

1. INTRODUCTION

1.1. Background

The natural distribution of *Chromolaena odorata* extends from the southern tip of United States of America down to the northern region of Argentina (Fig.1). All species of Chromolaena are in that area, except *C.odorata* which comes out and invades the world except antartics. CABI, Centre for Agriculture and Bioscience International formed an international working group on the Biological Control of *C.odorata* and held the first International Workshop on Biological Control of *C.odorata* in Thailand in 1988. The second International Workshop on Biological Control of *C.odorata* was held at BIOTROP, Indonesia. Since then the events were wider to cover not only on biological control, but also the management of *C.odorata*, such as the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, held in Dursban, South Africa, 23-25 October 2000. At the 8th workshop the event was widen further into Eight International Workshop on Biological Control and Management of *C.odorata* and Other Eupatoriae, held in Nairobi, Kenya 1-2 November 2010. This 8th workshop recognizes that the natural enemies *Pareuchaetes pseudoinsulata* and *Cecidochares connexa* have proven effective against *Chromolaena* in Asia. It is recommended these agents be favourably considered for imtroduction in all countries in Africa and Asia where Asian /West African *Chromolaena* present The 9th International workshop will be eld in Malaysia March 5-8 March 2019.

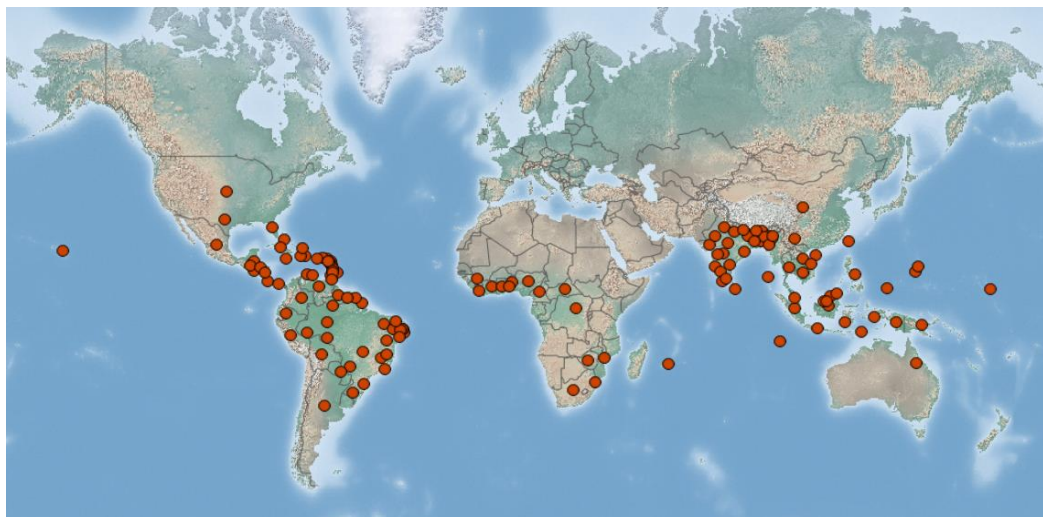


Figure 1 The map of world distribution of *Chromolaena odorata*

The invasion of *C.odorata* in Indonesia represented by a distribution map was also shown in Fig.2. Although the first herbarium of *C .odorata* deposited at Herbarium Bogoriense was collected from Lubuk Pakam only in 1934 (Tjitrosoedirdjo, 1991), currently this species has been found from the west to the east tip and from north to the south tip of Indonesian territory. The invasion was very rapid, inflicting damages to agricultural and forest production systems mainly perennial plantations, and forest plantations such as teak or *Acacia mangium* forest, or natural logged forest.

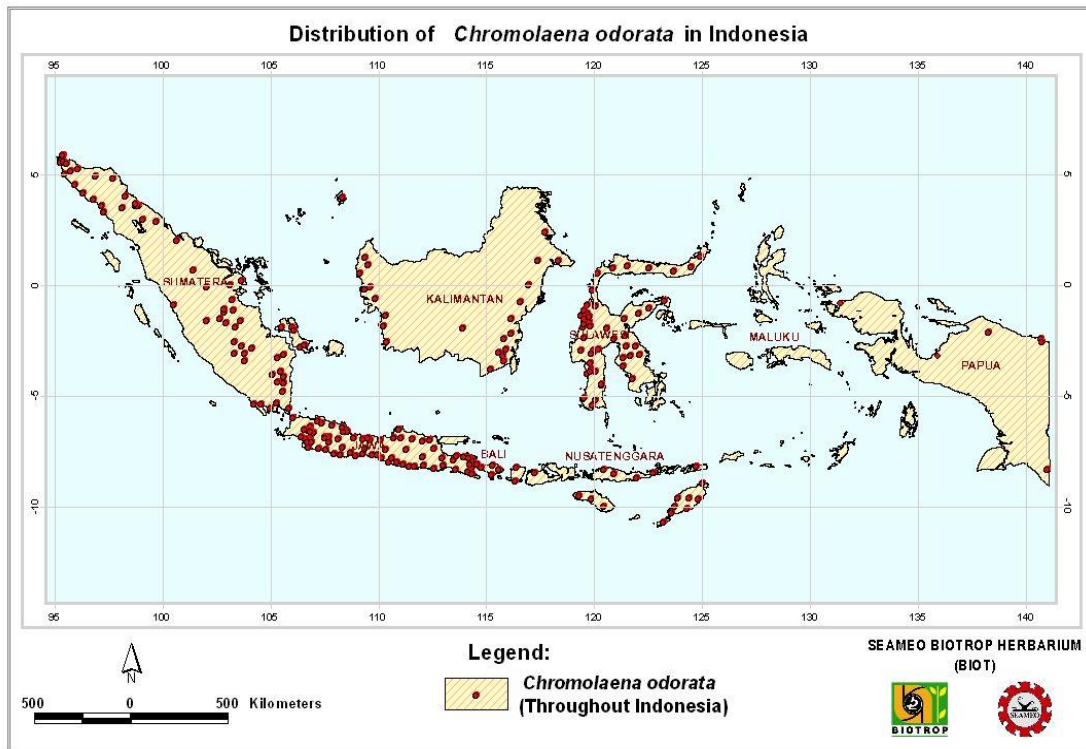


Figure 2 Distribution map of *Chromolaena odorata* in Indonesia

The stage of *Chromolaena odorata* distribution brought BIOTROP capitalizing the biological control technique as shown in Fig.3

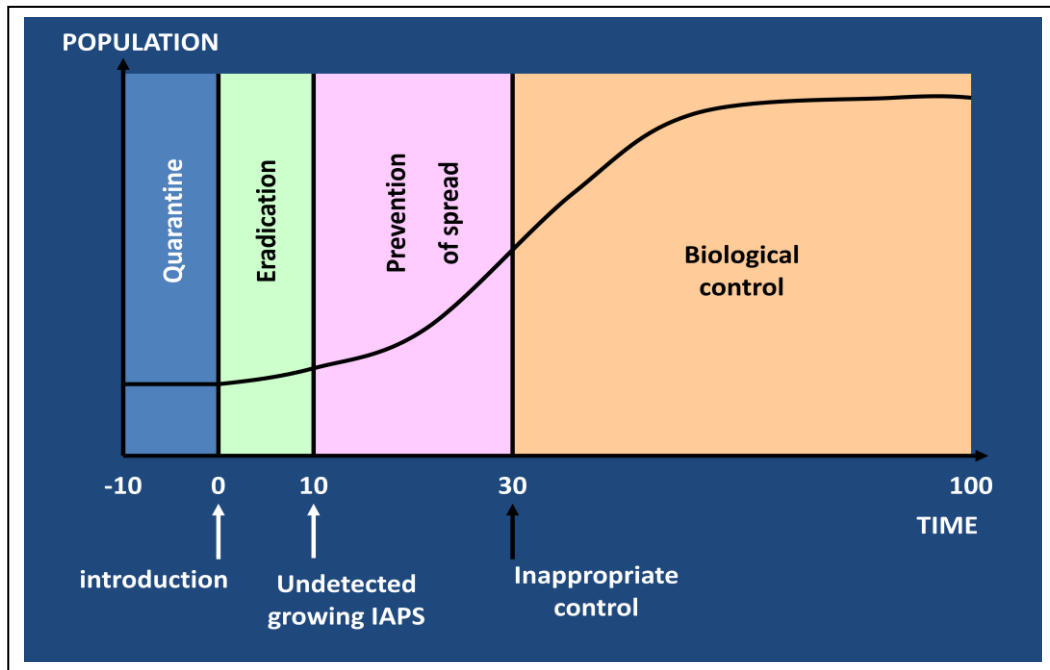


Figure 3 The appropriate management technique applied to the invasive alien plant species depending upon the stage of invasion (Groves, 1991).

When the invasive alien plant species is not in the country yet, the appropriate technical management is installation of strong quarantine regulations preventing the invasive plant from entering the country. When quarantine management fails to intercept the invasive plant species, the invasive plant species is found in the country, the population is still small, it is appropriate to eradicate the invasive plant species. If the invasive species proves to be too big to eradicate it is appropriate to prevent the spread. However when the invasion has been so extensive such as *C.odorata* in Indonesia, wherever you go you find the invasive species the appropriate technical management is biological control using its natural enemies where it may be found in their native distribution

Two natural enemies have been imported to Indonesia, i.e. *Pareuchates pseudoinsulata* dan *Cecidochares connexa* (Tjitrosemito, 1996, 1998, 1999; Tjitrosemito & Kasno, 1991; Widayanti *et al*, 1991). The first species was not established in Indonesia, and it is only *C.connexa* established. The colonies of *C.connexa* have been distributed around in Indonesia, the whole Sumatra provinces, the whole Java provinces, Kalimantan, Sulawesi Bali Lombok Timor etc. Despite the extensive distribution people is always asking you have been releasing *C.connexa*, while *C.odorata* is still around, we can find *C.odorata* in almost any teak plantations, oilpalm plantations, rubber plantations, so what is this biological control, is it effective.

People do not understand that biological control will not eradicate the colony of *C.odorata*, but to reduce the its popolation down into a low populatio level which will not inflict anymore damage to the production systems.The diagramatic piture at Fig.4 may explain the background of biological control technique

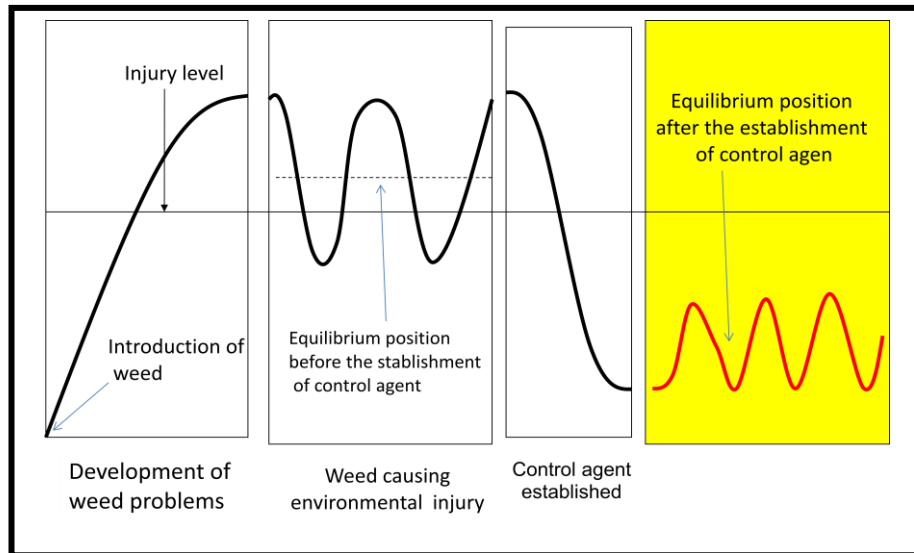


Figure 4 Diagram of processes in a biological control event

A biological control event, i.e. not to eradicate but to shift the population equilibrium of *C.odorata* into a lower population size, that will not inflict anymore damage to the production systems.

This is the main objective of works on the biological control of invasive alien plant species.

1.2. Objectives

- To established centres of *Cecidochares connexa* rearing in Indonesia to provide colonies to release them at an appropriate time
- To provide technical guidance to rear *C.connexa* and to relase in the field
- To reach a population balance between *C.odorata* and *C.connexa* to ensure a low population of *C.odorata*
- To make available for the surrounding community to share the population of *C.odorata*
- To make available for surrounding community to share the population of *C.connexa*
- To monitor the population of galls in *those C.odorata* community and evaluate the status of biological control of *C.odorata*

1.3. Expected Output

To understand a population balance between *C.odorata* and *C.connexa* to ensure a low population of *C.odorata*. Development of *C.connexa* rearing centre to spread the distribution of *C.connexa* more widely in Indonesia.

2. BENEFIT AND IMPORTANT OF RESEARCH

C.odorata is an invasive alien plant species that has been spreading extensively in Indonesia. *C.odorata* is a *long day plant*, flowers in June, entering into the dry season in Java island when the sun is in the southern hemisphere. *C.connexa* a successful biocontrol agent in Papua New Guinea was introduced to Java island in 1995. Despite its well establishment in Java island, indicated by finding a gall in almost any *C.odorata* plant around Java island the population of *C.odorata*, is still found at a varying degree of density.

From the point of view of controlling *C.odorata* it is compulsory to provide *C.connexa* population in the field by releasing again and again to balance the fluctuation of *C.odorata* population. It may not be a classical but more of augmentative biological control.

3. METHODOLOGY

The research works covered activities in the green house, field observations and internship to establish rearing centres in Indonesia.

The works under greenhouse condition was an experimental ones, involving factorial design with 3 factors, i.e. factor 1, was the number of shoot at 3 levels, 5, 10 and 15; factor 2 was the pair number of imago being release at 3 levels, 0, 1 and 2 pairs and factor 3 was the repetition of release, at 3 levels also, 2, 4, and 6 weeks interval. These treatment combinations were randomized in block. The release to have similar time of *C.odorata* growth was worked out to have a number of $2.2.2.3 = 24$ weeks (or about 6 month) of imago released, i.e. 12 release at 2 weeks frequency, 6 releases for 4 weeks frequency, and 4 releases for 6 weeks frequency. The variables collected were the number of shoot, the number of galls, the number of flowers, and dry matter of leaves, stem, and root.

The field observations were carried out in Parung Panjang, at an *Acacia mangium* forest where the first colony of *C.connexa* was released in 1995. Lines transect were drawn from a center followed by transect at 4 cardinal directions each 50 m long. At an interval of 25 m quadrats of $2 \times 2 \text{ m}^2$ were constructed to sample *C.odorata*. The variables collected were the number of stems, galls, and shoots of *C.odorata*.

The internship was organized by inviting prospective persons at universities interested in the development of biological control of invasive alien plant species (weeds). This screening was partly carried out during the Indonesia Weed Science Conference in Lampung last November 2018.

4. RESULTS AND DISCUSSION

The ANOVA of the data from green house experiment no interaction was recorded among the factors. The impact of initial shoot on the number of shoot after 5 months.

The growth increment behaviour was effected by the initial number of shoot which indirectly also reflecting the availability of natural resources. The increment rapidity of shoot not only shown by the existing vegetative but also shown by the flower or generative shoot. This rapid growth in term of shoot was shown a picture from the field Fig 5. While the relative growth rate, r of *Cecidochares connexa* was extremely low Table.1

Table 1 The value of parameters of *C.connexa* life table (Tjitrosemito, 2000)

Cohorts	R_0	T	r	λ
1	13.11	66.96	0.0384	1.039
2	13.90	70.00	0.0376	1.038
3	11.16	73.67	0.0328	1.033
4	18.70	73.67	0.0388	1.039
Means	14.22	71.08	0.0369	1.037

The rapid growth of *C.odorata* shoot was shown by leaving behind the gall with luxuriant green shoot covering the galls. In table 1 the figure representing the performance of female imago of *C.connexa* or a pair of imago, in which under laboratory condition the net reproductive rate was 14.2 female, although in the field mostly likely lower than that figure, In term of galls the growth of *C.connexa* was slow (See Fig,5). At this point it is necessary to have a support from the us to support the increment of *Cecidochares* imago, such as releasing several time the increase the population density of *C.connexa*. In Indonesia (BIOTROP) we are designing the steps by establishing centers of rearing for *C.connexa*. The rearing pays a special attention to provide a good source for *C.connexa* by growing *C.odorata* in pots of fertile soil with sufficient nitrogen fertilizer. The imago at a colony of 100 at least be released into *C.odorata* bush in early morning.



Figure 5 The prolific growth of *C.odorata* shoot, showing rapid production of shoot despite the present of gall

Approaching the dry season, with slow increase of vegetative shoot number this time the galls seemed to restrict the increase of shoot . It may be due to the fact that the vegetative growth changed to generative one (Fig.6),



Figure 6 The growth of *C.odorata* shoot toward the end of dry season

Sometimes a healthy colony of *C.connexa* persisted in a *C.odorata* community which was heavily galled by *C.connexa* during the early growth. The population was so high that the new emerging shoot from galled stem directly oviposited and we have multiplied gall in this branch of *C.odorata* an the end of dry season. This branch will die in the following season. (Fig.7)



Figure 7 A poor branch of *C.odorata* attacked by aggressive *C.connexa*

Table 2 supported the previous finding that in a pot experiment grown with a greater number of shoot when attacked by *C.connexa* showed a shoot increment more than grown with lower number of initial shoot. In general it seemed that galls stimulated the metabolism of *C.odorata* to mobilise its resources , that reduced the stored resources for the following growing season and weaken the *C.odorata* plant. It is important therefore to continuously release the colony of *C.connexa* to weaken the *C.odorata* community

Table 2 Means number of shoot , galls, flowers, and dry matters of leaves stems, branches, and root, recorded after 5 months growth on the shoot density *C.odorata* of 5, 10, and 15 shoots

No.	Means of Variables	Initial Shoot Number		
		5	10	15
1	Shoot	281.00	280.00	309.44
2	Galls	69.22	77.78	132.33
3	Living galls	21.00	18.44	49.44
4	Windowed galls	6.67	7.89	29.00
5	Empty galls	41.56	51.44	54.00
6	Flower	261.00	317.11	540.11
7	Stem D M (g)	20.20	27.00	28.56
8	Brabches DM (g)	24.21	34.55	36.44
9	Leaves D M (g)	23.19	26.86	31.86
10	Root D,M (g)	6.10	13.98	15.41
11	Total Dry matter(g)	93.37	121.26	141.72

Table 3 Means number of shoot , galls, flowers, and dry matters of leaves stems, branches, and root, total DM recorded after 5 months growth when subjected to 0, 1 and 2 pairs of *Cecidochares connexa* attack

No.	Means of Variables	Initial Imago released		
		0	1	2
1	Shoot	400.22	226.89	243.33
2	Galls	0.00	103.22	176.11
3	Living galls	0.00	31.00	57.89
4	Windowed galls	0.00	8.67	34.89
5	Empty galls	0.00	63.56	83.44
6	Flower	225.56	649.44	243.22
7	Stem D M (g)	27.41	26.52	21.84
8	Branches DM (g)	39.24	33.05	22.92
9	Leaves D M (g)	32.05	27.03	22.83
10	Root D,M (g)	13.83	13.57	8.09
11	Total Dry matter(g)	124.04	128.02	104.29

Table 3 revealed an interesting results, the release of imago reduced the vegetative shoot, but does it reduce the shoot increment? It does not because , shoots produced by *C.odorata* in respond to the gall formation, some of them have been converted to generative one in the form of flower. It has a double advantage for the biological control of *C.odorata*, i.e. by forming galls it spends a substancial energy which otherwise should be stored for the following seson to grow. The gall also induces *C.odorata* to produce more shoot, upon which *C.connexa* to oviposit. It is a positive point of *C.connexa* action

Table 4 Means number of shoot , galls, flowers, and dry matters of leaves stems, branches, and root, total DM recorded after 5 months growth when imago of *C.connexa* releases were repeated 2, 4, and 6 weeks

No.	Means of Variables	Repeated releases		
		2	4	6
1	Shoot	274.22	349.56	246.67
2	Total Galls	125.111	65.333	88.889
3	Living galls	40.778	14.222	33.889
4	Windowed galls	27.222	4.222	12.111
5	Empty galls	57.111	46.889	43.000
6	Flower	423.67	185.44	509.11
7	Stem D M (g)	23.226	19.759	32.779
8	Branches DM (g)	24.449	24.732	46.023
9	Leaves D M (g)	22.760	21.163	37.988
10	Root D,M (g)	8.716	7.006	19.768
11	Total Dry Matter(g)	103.839	88.327	164.180

Table 4. showed another important point to observe by the center of rearing is that the production of galls depend upon the dry matter of the supporting *C.odorata*. The repetition of release of *C.connexa* imago when it was done at 2 weeks interval produced more galls, fair enough.

But look at the other treatment that the number of galls was highly affected by the dry matter of *Chromolaena odorata*. It emphasise the important of providing a better food for *C.connexa*.

4.1. Establishing of Rearing Centers in Indonesia

The questions frequently asked by weed managers or even academicians were” why *C.odorata* is still around when stated that biological control has been successful”. Beside the fact that biological control is not eradication, therefore, *C.odorta* will still be around but its population is decreasing and in the next 6 years will be low enough will not inflict anymore damage to the production system, in agriculture, forestry or animal husbandary, the process works slowly unfortunately. It is our assumption that the slow process was mainly due to the population of *C.connexa* was still low, relative to that of *C.odorata*. With this assumption we tried to find a solution by establishing rearing centers so that colonies of *C.connexa* were always available along the year around, and be released in an appropriate time.

Our experience showed that the damage of *C.odorata* by *C.connexa* when *C.odorata* is still at its full swing of growth, i.e. early wet season the damage is minimal, because the colony of *C.connexa* consisted of ony a small population. However if we rerelease the colony of high enouh population the growth of galls may catch up with that of *C.odorata* shoot. On the other hand at the end of wet season where the increment of vegetative shoot is slowing down the release of *C.connexa* colony will inflicted more damage. The first damage comes from gall formation, the second damage comes from the stimulation of galls on *C.odorata* plant to produce more shoot, which will soon be gallified by *C.connexa* . The chain of events will exhaust the *C.odorata* resources, weaken the growth of *C.odorata* in the following season.

These are the emphasizing ideas for establishing rearing centres. Four lectures from 4 agricultural faculties from 4 different universities were invited to BIOTROP to see the actual works on growing *C.odorata* in cages, and observing *C.odorata* in the field and detecting the gall morphology especially to identify different stages of gall such active gall, aborted gall, windowed gall, holed gall. Windowed gall stage is very important it indicates the mature gall, that may be harvested. Aborted galls are unsuccessful gall formation due to predatorship, it is not known if predatorship occurs on newly hatched larve or larvae already inside the gall.

The establishment of Rearing Centers in Indonesia is initiated by BIOTROP by inviting those interested in the biological control of invasive alien plant species, to participate in the internship offered by BIOTROP and led by Dr. Soekisman Tjitrosoedirdjo. The invited persons and participated in the internship were : 1. Dr. M.Husein faculty of Agriculture, Jember University, Jember. 2. Ir. Andi Suryadi , faculty of Agriculture, University of Mulawarman, Samarinda. 3. Dr. Edy Syahputra, faculty of Agriculture, University of Lambung Mangkurat, Pontianak and 4. Ir. Puji Lestari MSi, faculty of Agriculture, University of Lampung, Bandar Lampung. It is expected that the rearing centers will be widened to cover also Sulawesi, Papua and especially eastern part of Indonesia areas mostly invaded by *Chromolaena odorata*

4.2. Coming activities under Laboratory of Weeds and Invasive Plant Species

- The eight International Workshop on Biological Control and Management of *Chromolaena odorata* and Other Eupatorieae recognized that the natural enemies of *Pareuchaetes pseudoinsulata* and *Cecidocharis connexa* have proven effective against *Chromolaena* in Asia. It is recommended these agents be favourably considered for introduction in all countries in Africa and Asia where Asian/West African *Chromolaena* present. This recommendation strengthen the establishment of rearing centers in Indonesia to speed up the control of *Chromolaena* biologically.
- These activities will be reported to the 9th International Workshop on Biological Control and Management of Eupatorieae and other Invasive Weeds , *The Everly Hotel, Putrajaya, Malaysia 5 – 8 March 2019* .

5. CONCLUSIONS

5.1. Conclusions from green house experiments

- Relative Growth Rate of *C.odorata* in term of *shoot increment* is much faster than that of *C.connexa* in term of gall development.
- The production of galls by *C. connexa* was affected by the availability of biomass of *C.odorata*, and possibly also by the quality of biomass especially with the availability of nitrogen
- The release repetition every 2 weeks showed to be beneficial in increasing more galls in the population

5.2. Recommendation for Rearing Center

- Utilize fertile soil to grow of *C.odorata* in pots, to support the growth of *Cecidochares connexa*, avoid the attack of aphids by taking the attacked leaves and burnt, or carefully spray with soft detergent.
- Grow *C. odorata* in closed plastic screen to prevent the predator, parasitoids attack on *C.connexa* colony
- Make sure to have sufficient sunlight to facilitate a good growth of *C.odorata* and ovipositing the female *C.connexa* especially for morning light.
- To execute the repeated release every 2 weeks of imago or galls, a continuous rearing of *C.connexa* is needed.

5.3. Field Observations

The first release of *C.connexa* was in young *Acacia mangium* forest Plantation at Parung Panjang under PERHUTANI (Estate Forest Plantation) in 1995, The forest was invaded heavily by *C.odorata* and the first release was successful in term of controlling the population of *C.odorata* and establishing that *Cecidochares connexa* well established. This special case was also supported by the forest fire rampaging the *Acacia mangium* areas at this early establishment. A great moral support was the fact that *Cecidochares connexa* did not burn to death by forest fire. An accidental founding was discovered in Merapi Mountain Central Java. After eruption in 2010 a substantial trees were burn to death by the running hot gas (wedus gembel). To our surprise in the areas with burnt trees which must be very hot when wedus gembel passed by a view *C.odorata* plants were survived, but more amazing was that *C.odorata* were heavily galled by *C.connexa*. Does this mean that galls prevent the larva ant

pupa from being burnt of those hot gas? Buy it is strenthen our believe that *C.connexa* can control *C.odorata*.

The forest was logged once, and replanted in 2013, and now is considered that *A.mangium* forest will not be replanted again because the trees have been infected by diseases that will make the enterprice will not survive.

During the preparation of replanting shrubs and undergrowth were slashed, stacked and burned. Currently the forest plantation is selectively logged and in the process of conversion into teak forest. The population of *C.odorata* and *C.connexa* were presented in Tbl.5

Table 5 The number of plants along 100 line transects crossing each other with quadrtws of 2x2 m²

Transects	No plants	Dead branch	Holed galls	Active galls	Windowed galls
1	6	12	12	24	1
2	2	0	2	11	3
3	23	2	0	24	2
4	0	0	0	0	0
Total	31	14	14	59	6

The data above recorded the distribution of *C.odorata* in the current *Acacia mangium* forest in Parung Panjang under PERHUTANI (forest estate plantation). That data represented the distribution of *C.odorata* and *C.connexa* in that areas. This area was approximately 1 ha, and the number of individual *C.odorata* was 32 plants. However other shrubs and other weeds were substantial, that requires further action to clear up to planting of teak sapplings. The population of 31 plants in one ha was not much, but the fact that any *C.odorata* plant in this area is always galled by *C.connexa*. Again that *C.connexa* established well in this part of the world.

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