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Effect of Innoculation Duration and Number of Hole Container to *Cotesia flavipes* (Hymenoptera; Branconidae) Propogation in Laboratory

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ABSTRACT

This research was aimed to study the number of container holes and the inoculation period of *C. flavipes*. This research was conducted in sugarcane Laboratory of Research and Development Sei Semayang Binjai, Sumatera Utara from November 2017 to January 2018. A complete randomized design with two factors and three replications were used. The first factor was the number of holes in container (1 hole d=10 cm, 1 hole d=2.5 cm, 4 hole d=2.5 cm, 7 hole d=2.5 cm and the second factor was inculation period (10, 20, dan 30 minutes). The result showed that the number of holes in each container and the inoculation period was significantly affecting the parasitation period, parasitation presence, number of imago and sex ratio of *C. flavipes.* The highest percentage (33.68%) on the number of container 1 hole d=10 cm while the lowest (7.38%) was in 1 hole d=2.5 cm. In terms of innoculation period, the highest (24.14%) parasitation percentage was in 10 minutes and the lowest (15.81%) was in 30 minutes. The highest number of imago (319.92 *C. flavipes*) based on inoculation treatment = 10 minutes, while the lowest (207.25 *C. flavipes*) = 20 minutes. The highest male (207.78 *C. flavipes*) was in 1 hole d=10 cm and the lowest (48.89 *C. flavipes*) in 1 hole d=2.5cm. The highest female (219.44 *C. flavipes*) was in 1 hole d=10 cm and the lowest (54.00 *C. flavipes*) in 1 hole d=2.5 cm. The highest (168.00 *C. flavipes*) inoculation period = 10 minutes while the lowest (107.5) *C. flavipes* was in inoculation = 20 minutes. The sex ratio male : female = 1 : 1.

Keywords *: Inoculation Period, Number of Container Hole, Cotesia flavipes, Chillo sacchariphagus*

INTRODUCTION

Sugar cane as a producer of sugar is one of the strategic commodities in the Indonesian economy. Sugar is also one of the basic needs of the people and a relatively inexpensive source of calories. Sugar is a basic need hence the dynamics of sugar prices have a direct effect on the rate of inflation (Ministry of Agriculture, 2010).

Sugar needs in Indonesia always increase from year to year and until now it has not been able to be fulfilled by domestic production. Therefore, an effort is needed to increase sugar production maximally. One of the obstacles on sugarcane cultivation is the attack of various types of pests that occur throughout plant growth (Simatupang et al., 2015).

The development of sugar cane in Indonesia in 2014 reached 2,579,173 tons, in 2015 its production increased to 2,623,931 tons, in 2016 sugar cane production reached 2,715,883 tons. PT Perkebunan Nusantara II is one of the state-owned enterprises that has been cultivating sugar cane since 1983. However, in recent years the production and sugar productivity of PTPN II Sumatera Utara has decreased since 1999, reaching 4.6 tons/ha (Disbun Sumut, 2012).

The problem of low productivity of sugarcane and sugar yield can be seen from the side of the farm (*on farm*). One of them is the

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presence of pest attacks, i.e. spotted borer (*Chilo sacchariphagus*) which is one of the important pests and almost always found in sugar cane plantations, especially in Sumatera Utara. Attack symptoms on sugarcane stems are marked by a hole in the stem surface. Every 1% section damages caused by the spotted borer can reduce 0.5% sugarcane weight (Prabowo et al., 2013).

Sugar losses caused by sugar cane pests in Indonesia are estimated to reach 75%, some of which are often destructive and cause considerable losses such as spotted borer (*Chilo sacchariphagus*), gold-fringed stemborer (*Chilo auricilius*), pink borer (*Sesamia inferens*) and by the attack of giant borer (*P. castaneae*) (Febryandi et al., 2015).

The main control of C. sacchariphagus is the parasitoid *Cotesia flavipes* larvae. Although in general, it has a low parasitic level, the parasitoid experienced an increasing and can indirectly be the death factor of the host population. In 1996 it was observed that 5.4% of small larvae were parasitic, 9.4% percentage of parasites in medium-sized larvae and 19.8% of large larvae were parasitized by *C. flavipes* (Oktaviana et al., 2013).

Various biological controls have been carried out by PTPN II research and development dept. by using various parasitoids such as: *Tumidiclava* sp., *S. inferens, Xantocampoplex* sp., *Trichogramma* spp., *C. flavipes.* One of the larval parasitoids that can parasitize *C. sacchariphagus* is *C. flavipes* but has not given satisfactory results. Especially in the effort to multiply *C. flavipes* parasitoid in the Laboratory, there are several obstacles, one of which is the time of inoculation. Information on the time of inoculation is very necessary for the effort to control spotted borer in the laboratory.

Factors that influence parasitoid population density are temperature and humidity. Research related to temperature and humidity on parasitoid is still limited hence research about the number of holes in the container for multiplication of *C. flavipes* in a laboratory is feasible to work on.

MATERIALS AND METHOD

This research was carried out at the Sei-Semayang PTPN II Research and Development Laboratory with an altitude of ± 25 meters above sea level, which was carried out from November 2017 to January 2018.

The materials used in this research were imago of *C. flavipes*, spotted sugarcane borer (*C. sacchariphagus*), young sugar cane, honey and tissue. The tools used in this research were plastic containers with a height of 7 cm and a diameter of 14 cm, solder, wire nets, test tubes, flaps, brushes, black cloth and tweezers.

This research used a Completely Randomized Design (CRD) with 2 factors, namely: Factor I Number of holes in a plastic container with an upper hole diameter of 2.5 cm and a lower hole diameter of 6 cm at 4 levels A0: 1 hole (d = 10 cm), A1: 1 hole, A2: 4 holes, A3: 7 holes. Factor II: inoculation duration of *C. flavipes* parasitoid on *C. sacchariphagus* i.e. B1: 10 minutes, B2: 20 minutes, B3: 30 minutes.

The significant treatment was continued by further analysis, namely by Duncan's Multiple Range Test (DMRT) with a level of 5%.

Observation variable

1. Parasitic percentage

   The percentage of parasitization of *C. flavipes* on *C. sacchariphagus* larvae can be determined by calculating the number of parasitic larvae divided by the total larvae at 100% or by using the formula:

$$\%\text{paratitation} = \frac{\text{parasited larvae}}{\text{All larvae}} \times 100$$

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2. The percentage of *C. flavipes* imago that appears

   The number of imago that appears was calculated by tending *C. flavipes* cocoon in a cage by keeping moisture and spraying water in the hand sprayer and keeping from the ant attack until the new *C. flavipes* imago appears.
3. Sex ratio

To determine the sex ratio of *C. flavipes*, it was done by observing the parasitoid imago that emerged from *C. flavipes* larvae and waited until the parasitoid died. Then the calculation of male and female *C. flavipes* from each treatment was done.

RESULTS AND DISCUSSION

Percentage of Parasitation

The results of average difference test on inoculation duration and number of holes on the percentage of parasitization of Cotesia flavipes in Chilo sacchariphagus larvae were presented in Table 1.

Table 1. Effect of the number of holes on the parasitization percentage of *C. flavipes*

Treatment	Average (%)
A0 (1 hole d = 10 cm)	33,68 **a**
A1 (1 hole d = 2,5 cm)	7,38 **d**
A2 (4 holes d = 2,5 cm)	13,30 **c**
A3 (7 holes d = 2,5 cm)	20,71 **b**

Note: The numbers followed by different letters in the same column were significantly different at the 5% level according to the DMRT test

Based on Table 1, it showed that the highest percentage of parasites was found in treatment A0 (one hole, d= 10cm) of 33.68% with a temperature of 25.4ºC while the lowest was in treatment A1 (one hole, d= 2.5cm) of 7.38% with a temperature of 25ºC. This was because the temperature in the container affects the humidity of the container, the high and low temperatures will cause different levels of humidity and result in different (high or low) parasitic percentages, the larger the hole in the container the higher the percentage of parasitics and the fewer holes in the container, the lower the parasitic percentage. It also depends on the temperature and humidity obtained in each container, according to Hance's (2007) literature which stated that temperature is the main abiotic factor governing insect population dynamics, developmental rates, and seasonal events. Temperature changes will present challenges for parasitoid species and the expected significant impact in trophic interactions.

In addition to the temperature in the container, another factor that affects the percentage of parasites was the *Beuveria besiana* fungus which is thought to originate from *C. sacchariphagus* larvae that have been infected in the field, the fungus cannot produce its own food hence it is parasitic to its host insect. Morphological changes in larvae of *C. sacchariphagus* infected with *B. bassiana* resulted in rigid larvae, slow movements, then harden and die. In the body of the larvae appeared white mycelium. This was in accordance with Wahyudi (2002) research which stated that the toxins produced by *B. bassiana* include beververin which can destroy the fat layer and increase cell permeability which can destroy specific ions hence it can cause abnormal ion transport and then damage the function of cells or larvae cell organelles. On the surface body of the insects that have died and become mummified, white mycelium appears, at first hyphae appear on the soft surface of the body or between segments. Another thing was stated by Putri et al. (2015) who stated that fungi *B. Bassiana* produce toxins (toxic) which can lead to aggressive paralysis in insect larvae and imago. Several types of poisons that have been successfully isolated from B. bassiana include

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beauvericin, beauverolide, isorolide, dyes and oxalic acid.

Table 2. Effect of inoculation duration on the percentage of parasitization of *C. flavipes*

Treatment	Averages (%)
B1 (10 minutes)	24,14 **a**
B2 (20 minutes)	16,35 **b**
B3 (30 minutes)	15,81 **b**

Note: The numbers followed by different letters in the same column were significantly different at the 5% level according to the DMRT test

The highest percentage of parasites on the treatment of inoculation duration was found in treatment B1 (10 minutes) of 24.14% while the lowest was in the treatment B3 (30 minutes) of 15.81%. This was due to the optimal time needed for inoculation was 12 seconds/larvae, the faster the time of inoculation, the greater the success of parasites. The research results of Sagala et al. (2014) on *C. flavipes* parasitoid in *Chillo sacharipagus* larvae stated that the optimal standard time for inoculation was 12 seconds/larva where the longer inoculation time would affect the parasitisation failure, because at the time of inoculation the *C. sacharipagus* larvae were no longer active to parasitize the host.

Number of Imago

In Table 3, it can be seen that the highest treatment was found in treatment A0 (one hole, d= 10 cm) for 423.89 heads and the lowest was A1 (one hole, d= 2.5 cm) for 102.89 heads. This was due to the treatment of A0 as the best treatment with a temperature of 25.4° C. The temperature in the container can affect the number of cocoons of *C. flavipes*, where the *C. flavipes* cocoon will become *C. flavipes* imago. This was in accordance with Smith Jr.'s literature (1993) which stated that the life cycle of *C. flavipes* lasts about 22 days, and about 40 parasitoids develop in each host larvae. The results of development through three larvae instars in the host's body, and then emerge from the host larvae by biting through the integument. The egg period of larvae last around 14 days at 25 °C. After its appearance from the host, the last instar larva rotates and then becomes a cocoon. Pupation takes about six days at 25 °C, after which *C. flavipes* imago appears.

The results of the average difference test of inoculation duration and number of holes on the number of Cotesia flavipes imago were presented in Table 3.

Table 3. Effect of number of holes on the number of *C. flavipes* imago

Treatment	Average
A0 (1 hole, d = 10cm)	423,89 **a**
A1 (1 hole, d = 2,5cm)	102,89 **d**
A2 (4 holes, d = 2,5 cm)	182,22 **c**
A3 (7 holes, d = 2,5 cm)	281,11 **b**

Note: The numbers followed by different letters in the same column were significantly different at the 5% level according to the DMRT test

Table 4. Effect of inoculation duration on the number of *C. flavipes* imago

Treatment	Average
B1 (10 minutes)	319,92 **a**
B2 (20 minutes)	207,25 **c**
B3 (30 minutes)	215,42 **b**

Note: The numbers followed by different letters in the same column were significantly different at the 5% level according to the DMRT test

In Table 4, it showed that the highest value of inoculation duration treatment was found in treatment B1 (10 minutes) of 319.92 heads and the lowest was in treatment B2 (20 minutes) of 207.25 heads. This was caused by

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the size of the cocoon which affected the number of *C. flavipes* imago. The larger the cocoon, the more *C. flavipes* appears and the smaller the cocoon, the less the number of *C. flavipes* imago appears. This happened because of food competition between the larvae of *C. flavipes* in the host's body, which would affect the success of *C. flavipes* into imago. In accordance with Pratiwi (2003) research, which stated that at the time of observation it appears parasitoid infiltrates its ovipositor in each host larva, not all eggs are found in the host's body.

Sex Ratio

The results of average different test on inoculation duration and the number of holes on the sex ratio of *C. flavipes* were presented in Table 5.

Table 5. Effect of number of holes and inoculation duration on the sex ratio of *C. flavipes*

Treatment	Cocoon	Male	Female	Sex Ratio
A0 (1 hole d = 10 cm)	10.11 a	207.78 a	219.44 a	1 : 1
A1 (1 hole d = 2,5 cm)	2.22 d	48.89 d	2.22 d	1 : 1
A2 (4 holes d = 2,5 cm)	4.00 c	86.44 c	4.00 c	1 : 1
A3 (7 holes d = 2,5 cm)	6.22 b	133.56 b	6.22 b	1: 1
B1 (10 minutes)	7.25 a	154.42 a	168.00 a	1 : 1
B2 (20 minutes)	4.92 b	99.75 c	107.50 b	1 : 1
B3 (30 minutes)	4.75 b	103.33 b	112.08 b	1 : 1

Note: The numbers followed by different letters in the same column were significantly different at the 5% level according to the DMRT test

Observation results (Table 5) showed that the highest male sex ratio was found in treatment A0 (one hole, d = 10cm) of 207.78 heads and the lowest wa A1 (one hole, d = 2.5cm) of 48.89 heads. While the highest number of female sex ratios was found in treatment A0 (one hole, d = 10cm) amounting to 219.44 heads and the lowest was A1 (one hole, d = 2.5cm) of 54.00 heads. The highest male sex ratio was found in treatment B1 (10 minutes) of 154.42 heads and the lowest was in B2 (20 minutes) of 99.75 heads. While the highest number of female sex ratios was found in treatment B1 (10 minutes) of 168.00 and the lowest was in B2 (20 minutes) of 107.50.

The smaller the number and diameter of the hole, the lower the temperature in the container. Female parasitoid can adapt to both low and high temperatures. This was in accordance with Budianto et al. (2014) that temperature greatly affects the resistance of parasitoid during the larval phase, because there is a different resistance between male and female parasitoid. Male parasitoid is more vulnerable to extreme low and high temperatures where during the cocoon phase the average temperature in the laboratory is around 28.92ºC so that the appearance of male parasitoid imago becomes longer. Another thing stated by Lv et al. (2011) that parasitic larvae of *C. flavipes* which have copulated will produce various male and female genes, and if larvae are parasitized by female parasitoid which does not copulate it will produce only male offspring.

CONCLUSION

Propagation of *C. flavipes* was carried out in a 1-hole container with a diameter of 10 cm with a temperature of 25.4ºC and duration of 10 minutes inoculation.

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