EVALUATION OF TWO OIL SOLUBLE DYES, CONGO RED AND SUDAN RED 7B, FOR MARKING VARIOUS STAGES OF THE ASIAN CORN BORER, *Ostrinia furnacalis* (Guenee)

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ABSTRACT

Oil soluble dyes, Congo Red and Sudan Red 7B were evaluated as markers for mass reared Asian cornborer (ACB). Aliquot of the oil soluble dye was added to the IPB Modified CIMMYT artificial diet and was fed to F1 ACB neonates wherein growth and development were monitored. The Congo Red was tested at 100, 250, 400 and 600 ppm concentrations, while Sudan Red 7B was tested at 600, 700 and 800 ppm. Based on the results of the test using Congo Red, there were no markings on the egg masses laid by the marked adults. It was also found out that Congo Red dye had an adverse effect to pupal weight and it also exhibited poor marking ability on pupae. Test results using the Sudan Red 7B dye on 600 and 700 ppm concentrations showed no adverse effects on the larval and the pupal development. In addition, Sudan Red 7B was able to mark ACB pupae including egg masses laid by the marked adults. Without altering the duration of the development, 700 ppm Sudan Red 7B was used effectively as marker for mass-reared ACB with enhanced effect on the pupal weight, the larval length, and the larval width. In conclusion, the use of Sudan Red 7B that successfully marked pupae can be used for field studies on adult dispersal and behavior.

Key words: Asian corn borer, Congo Red, marker, oil soluble dyes, Sudan Red 7B

INTRODUCTION

The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenee) (Lepidoptera: Crambidae), is a major insect pest of corn in the Philippines causing as much as 100% yield loss in the corn production (Caasi-Lit et al., 1989). However, despite being a major pest that causes great economic losses, knowledge about its ecology

remains fragmented (Yorobe & Quicoy, 2006; Caasi-Lit, 2015). Information on the behavior of ACB specifically its oviposition and its dispersal could be vital for formulating strategies for Insect Resistance Management (IRM). IRM strategies, in turn, are crucial in all Bt corn growing countries, for farmers to enjoy longer the benefits of this transgenic technology.

Prior to formulation of novel IRM strategies and studying aspects of ACB ecology (i.e., adult dispersal and behavior), efficient and effective methodologies are needed in order to establish standard protocols. Along this line, this study tested possible markers (dyes) as a contribution to the team working on the adult dispersal of ACB.

Oil soluble dyes are considered as effective markers for Lepidoptera and other insects (Hagler & Jackson, 2001). Studies by Ostlie et al. (1984), Hunt et al. (2000), and Vilarinho et al. (2006), all support the successful use of oil soluble dyes for Lepidoptera with certain dyes capable of marking all of developmental stages. Any adverse effect in the organism should be assessed, especially if the dye is incorporated into the diet. Among the dyes used in the previous studies were Congo Red and Sudan Red 7B which are both available in the market. These two dyes are commonly used as indicators and as biological stains and have been reported also to have successfully stained other Lepidoptera. However, these dyes were also reported and considered carcinogenic to humans and may deter larval growth at high concentrations (Kim et al., 2016; Ostlie et al., 1984).

This study generally aimed to evaluate two oil soluble dyes, Congo Red and Sudan Red 7B, for their effectiveness as markers for ACB. Specifically, the study aimed: (1) to prepare ACB artificial diet infused with Congo Red or Sudan Red 7B; (2) to assess the development of the ACB grown under artificial diets infused with the two dyes; and (3) to determine the marking ability of each dye.

MATERIALS AND METHODS

Preparation of artificial diet

The artificial diet was prepared according to the procedure modified by the Entomology Laboratory of the Institute of Plant Breeding (IPB). Details of ingredients and preparation were presented in the paper of Caasi-Lit et al. (2015).

The preparation of dye solutions was adapted from Hunt et al. (2000). The dyes used have the following IUPAC Names: Congo Red: disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalen-2-yl)diazenylphenyl]phenyl]diazenyl-naphthalene-1-sulfonate (Sigma Aldrich Catalogue No: C6277); and Sudan Red 7B: *N*-Ethyl-1-((4-phenyldiazenyl)phenyl)diazenyl)naphthalen-2-amine (Sigma Aldrich Catalogue No: 53373) (Kim et al., 2016). To prepare the stock solution, 1 g of each dye was dissolved in 10 ml corn oil and manually mixed for 10 min. A

desired amount of the stock solution was added to the blending diet prior to the addition of the coagulating agent. To attain the desired final concentration using dilution formula of C1V1=C2V2, the computed stock solution was added to the 800 ml artificial diet.

Rearing procedures

The rearing protocol of the IPB Entomology Laboratory was followed. In a 250-g block slice of the artificial diet, 100 neonates were introduced using a camel-hair brush. For this experiment, three replications were prepared for each treatment. The set ups were frequently checked for molting and growth of larvae. Once the larvae became fifth instars, they were transferred into a pan with dry tissue paper to hasten pupation. Pupae were collected and placed in an acrylic pan in preparation for adult emergence. Upon emergence, adults were placed in an ovipositional cage lined with a sheet of wax paper on top, which served as an egg-laying substrate for female ACB moths. The adults were fed with honey solution which was replaced every day. Each piece of wax paper lining on the oviposition cage was collected every day and checked for the possible markings on the egg masses.

Evaluation of Congo Red at 100, 250, 400 and 600 ppm

Congo Red was evaluated using test concentrations of 100, 250, 400 and 600 ppm based on the work of Vilarinho et al. (2006). Larval and pupal duration, pupal weight, and adult longevity were determined to assess possible effects of the dye on the development of ACB. Laid egg masses were also observed for traces of marking.

Evaluation of Sudan Red 7B at 600, 700 and 800 ppm

Based on the results of tests with Congo Red, the concentrations for Sudan Red were increased to 700 and 800 ppm. Larval and pupal duration and weights, and percent of marked egg masses were also determined.

Evaluation of Sudan Blue (=Sudan Blue II, =Oil Blue 35; IUPAC Name: 1,4-bis(butylamino)anthraquinone) at 600, 700 and 800 ppm was also conducted. However, the dye concentrations were able to mark only a few larvae, and no pupae nor adults. Thus, experiments with Sudan Blue were terminated.

Statistical analysis

One-way analysis of variance in StatPlus by AnalystSoft (2016) was used to determine significant differences among the treatments. Box Plot available in the same software was also used to plot the results using box-and-whisker diagrams.

RESULTS AND DISCUSSION

Evaluation of Congo Red at 100, 250, 400 and 600 ppm

The addition of Congo Red to the diet did not affect the larval and pupal duration, as well as the adult longevity (Table 1). The duration (number of days) of development was almost the same for all concentrations.

Table 1. Development of Asian corn borer reared on Congo Red-infused artificial diet.

Dye Concentration	Larval duration (days)	Pupal duration (days)	Adult longevity (days)
Control	18	5	7
100 ppm	18	5	7
250 ppm	18	5	7
400 ppm	19	5	8
600 ppm	19	5	8

Using one-way ANOVA, differences among the mean weights of pupae ($p= 5.73015E-9$) were found significant at $\alpha=0.05$ (Figure 1). The median pupal weights from Congo Red-infused artificial diets were lower compared to those from the Control diet. The lighter pupal weights could be attributed to the increased concentration of the dye used (Hunt et al., 2000).

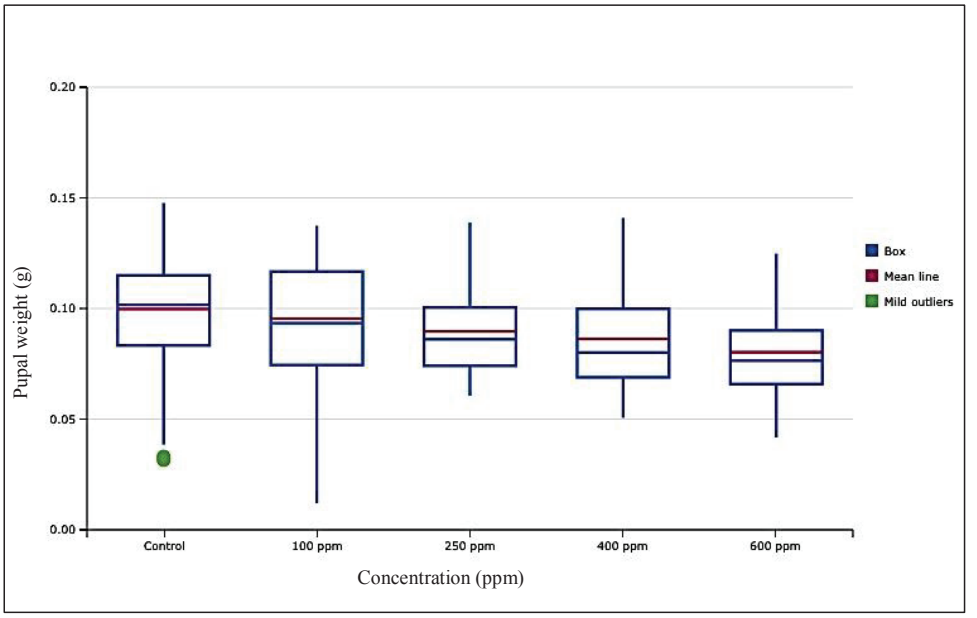

Figure 1. Box-and-whisker plot of weights of ACB pupae reared in artificial diet infused with different concentrations of Congo Red.

Although Congo Red was able to mark ACB larvae, pupae, and adults, wing scale patterns in the latter were more pronounced. There were no markings observed on egg masses laid by female ACB moths. It was also observed that as adults aged and their wing scales fell off, the mark also disappeared or faded. Retained marks on some adults were more likely due to the reduced intake of food which shortened lifespan.

Evaluation of Sudan Red 7B at 600, 700 and 800 ppm

Larval and pupal durations were not significantly different from the Control. Larval period for all treatments and Control lasted for 18-19 days, pupal period lasted for 5-8 days, and adult longevity lasted for 7-10 days.

One-way ANOVA showed that mean pupal weights were significantly different at α=0.05 among the concentrations (p= 0.00193). Box plot graph of the means also revealed that at 800 ppm, weights of pupae were more dispersed, having a wider 50-percentile (Figure 2). In addition, the median pupal weight was lowest at 800 ppm compared with those at other concentrations. Ostlie et al. (1984) observed deleterious effects of dyes on meridic diets with markers. Again, the adverse effect could be attributed to the high concentration of the dye (Hunt et al., 2000).

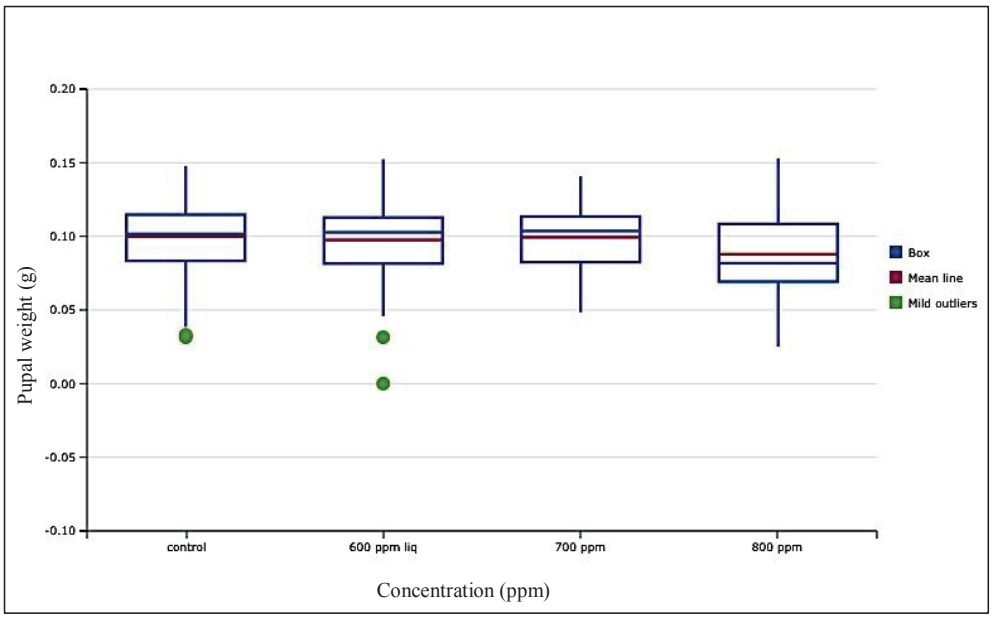

Figure 2. Box-and-whisker plot of ACB pupal weight reared on artificial diet infused with different concentrations of Sudan Red 7B.

The low mean pupal weights observed in this study may be due to the high concentration of Sudan at 800 ppm and of Congo Red at >250 ppm. The increased amount of dyes in the diet could have resulted to more metabolic processing needed, probably consuming more energy that should have been spent for growth and maintenance. The numerical differences between the dye concentrations (i.e., upper limit of 600 ppm for Congo Red and 800 ppm for Sudan Red 7B) found to be tolerable for ACB, may be due to the distinctive compositions of the two dyes.

The mean pupal weights shown in Box-and-whisker plots of the Control, 600 ppm, and 700 ppm were not significantly different, or essentially similar. The median and whisker ranges of the Control were comparable to those of 600 ppm and 700 ppm. In contrast, the mean pupal weight at 600 ppm was variable as shown by the mild outliers. Based on the mean pupal weights from the different treatments, 700 ppm is the suggested concentration for marking ACB due to the similarity of results with those of the Control, indicating unaltered pupal development.

In addition, there were significant differences among the mean widths (p= 0.00059) of the larvae subjected to different concentrations of Sudan Red 7B but none (p=0.31295) among the mean larval lengths. Therefore, Sudan Red 7B at any concentration did not affect the length of the developing larvae.

At 700 ppm, the larval widths were almost uniform, having 50-percentile Box plot occupying a small range (Figure 3). The highest median larval width was recorded at 700 ppm. Based on the larval width, the addition of 700 ppm of Sudan Red 7B dye possibly favored the growth and/or fat accumulation of ACB larvae.

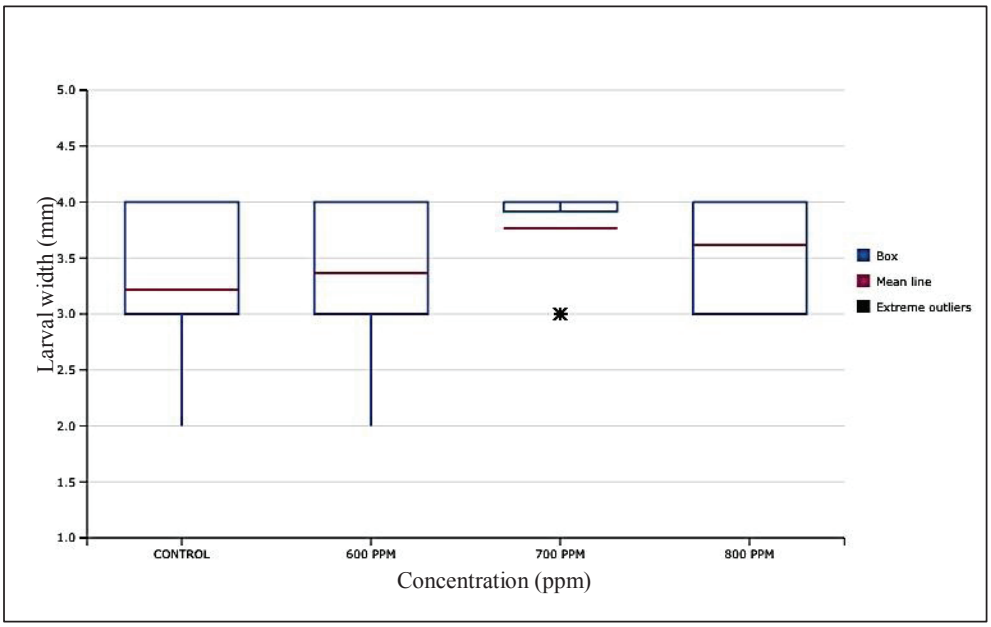

Figure 3. Box-and-whisker plot of larval width of ACB reared on artificial diet infused with different concentrations of Sudan Red 7B.

Sudan Red 7B at 600, 700 and 800 ppm successfully marked ACB in all the developmental stages. Larvae and day-old pupae were pink, while adults had more striking wing scale marks similar to Congo Red-marked ACBs. Sudan Red 7B also successfully stained the egg masses laid by the marked female adults, which were slightly pink rather than the usual white or creamy egg masses. From the 50 pairs of marked adults placed in an ovipositional cage, there were 105 out of 170 egg masses that manifested the pink markings. Sudan Red 7B at 700 ppm was able to mark 61.67% of the total egg masses laid. Figure 4 shows the life cycle of ACB with the stages showing marked and unmarked samples.

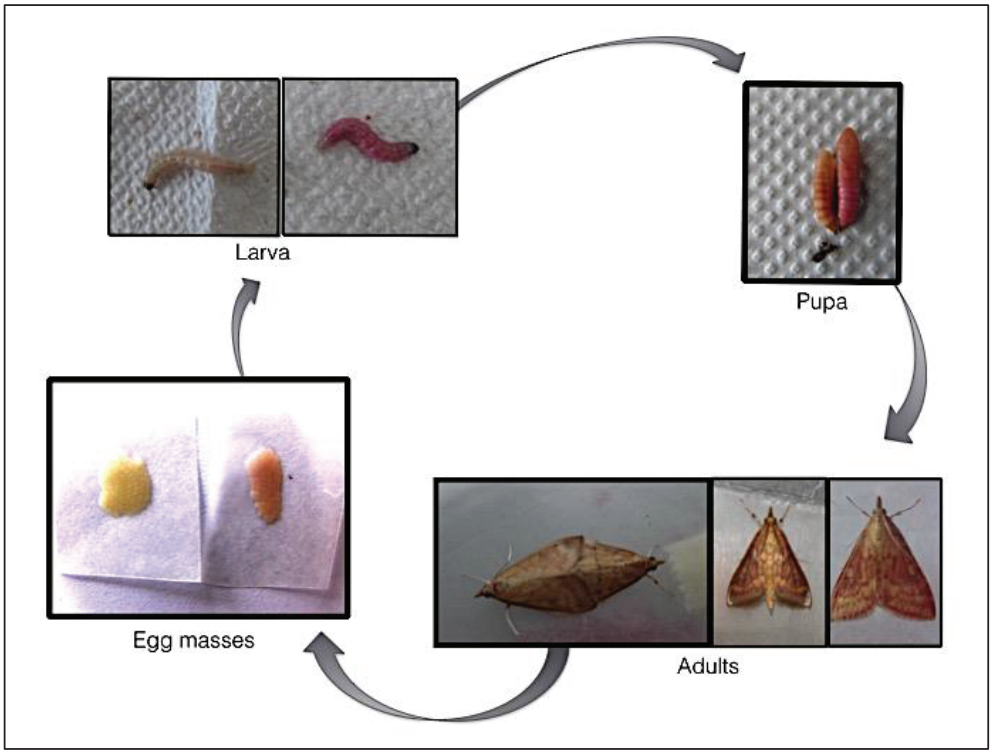

Figure 4. Comparative appearances of various stages of ACB reared on artificial diets: Pictures on the left-hand side are typical egg mass, larva, pupa, and female and male adults reared on IPB Modified CIMMYT artificial diet without dye; Those on the right-hand side are similar ACB stages when reared on diet with 700 ppm Sudan Red 7B added.

A suitable marker should primarily not alter the growth and development of the test insects and must not cause any adverse effect on the specimen or individual test insects. Based on the results, Sudan Red 7B did not cause adverse effects on ACB except at higher concentration (800 ppm). Sudan Red 7B was also able to stain all the developmental stages of ACB including egg masses laid by the marked female adults. The use of oil soluble dyes (i.e., Sudan Red 7B) in marking is considered useful because the dye is inexpensive, requires minimal labor, reduces handling errors, and can be easily seen by the naked eye (Hagler & Jackson, 2001). Moreover, for the dispersal or ovipositional studies, the data could still be generated without the adult being recaptured since Sudan Red 7B is able to stain laid egg masses.

CONCLUSION

Congo Red and Sudan Red 7B dyes were evaluated for their marking ability and were observed for any adverse effects on ACB. Although Congo Red was able to mark larvae, pupae, and adults, there were still no marks observed on the egg masses laid by the marked female adults. It was also observed that the Congo Red can affect pupal development. In contrast, Sudan Red 7B did not alter the duration of development and the larval length of ACB. Also, Sudan Red 7B at 700 ppm was observed to favor the development of ACB, with larvae wider and pupae heavier than the others. Sudan Red 7B marking was visible in all the developmental stages of the ACB including the laid egg masses by the marked female adults. Comparing the two oil soluble dyes, Sudan Red 7B at 700 ppm gave significant results and is here recommended for marking ACB.

RECOMMENDATIONS

This study presents evidences that Sudan Red 7B at 700 ppm is a suitable and efficient marker for ACB. However, there is still a need for further studies to look on the effects of the dyes on the fecundity of ACB. Continuous feeding for more than one generation should also be considered to determine whether there would be inter-generational marking ability of the dye.

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