Jurnal Perlindungan Tanaman Indonesia,Vol. 5, No.1, 1 - 6, 1999

DETERMINATION OF THE FACTORS IN DIAPAUSE INITIATION OF WHITE RICE STEM BORER

DETERMINASI FAKTOR-FAKTOR DALAM INISIASI DIAPAUSE PENGGEREK BATANG PADI PUTIH

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INTISARI

Tujuan penelitian ini adalah mendeterminasi faktor-faktor penting yang digunakan penggerek batang padi putih, Scirpophaga innotata *Walker, dalam memasuki diapause. Hasilnya menunjukkan bahwa larva penggerek batang padi putih (PBPP) dengan pakan tanaman padi IR64 yang tumbuh pada tanah yang berpengairan terus menerus yang tersinari hari pendek (11,76 jam pada 21 Juni 1994) tidak memasuki diapause. Namun, penghentian pemberian air pada 75 hari setelah tanam atau 15 hari setelah pembungaan dapat menyebabkan larva PBPP memasuki diapause. Penghentian pemberian air menyebabkan penurunan kelengasan tanah secara progresif, yang mempunyai pengaruh langsung terhadap penurunan kandungan air batang padi, dan kemudian berpengaruh nyata pada peningkatan asam absisat (ABA). Metabolit primer (protein, lemak dan metionin) tidak mempengaruhi kejadian diapause PBPP. Senyawa sekunder ABA telah diketahui mempunyai aktivitas JH (Juvenile Hormone) di dalam larva serangga, peningkatan kandungannya secara progresif di dalam batang padi menyebabkan peningkatan persentase diapause larva PBPP. Peranan ABA dalam inisiasi PBPP perlu diteliti lebih lanjut.*

Kata kunci : Penggerek batang padi putih, asam absisat, hormon yuwana.

ABSTRACT

The objective of this research was to determine the important factors in white rice stem borer larvae, *Scirpophaga innotata* Walker, entering diapause. The results indicates that white rice stem borer (WRSB) larvae feed on rice plants IR64 grown on soils with continual watering which exposed at short day length (11.76 hours at June 21, 1994) will not be in diapause. However, terminated watering 75 days after transplanting or 15 days after flowering on the host growth media caused diapause. These terminated watering caused declining of soil moisture progressively which had direct effect on rice stem water content, and so affected significantly the increase of abscisic acid (ABA). A changes of primary metabolites (protein, lipid and methionine) did not cause diapause. The secondary metabolite ABA had juvenile hormone activity in the insects larvae, progressively increased in its content in the rice stem caused the percentage of larval diapause of WRSB to increase. The role of ABA in diapause initiation of WRSB needs more study.

Keyword : White rice stem borer, abscisic acid, juvenile hormone

INTRODUCTION

The white rice stem borer, *Scirpophaga innotata* Walker, feeds only on rice (Goot, 1925; Triwidodo, 1993). They survive the off-rice season as a diapausing larvae. Diapause was first reported by Dammerman (1915) in Java, when rice was planted once a year during rainy seasons. Dammerman (1915) observed that after harvest, larvae remained in the rice stubbles in a resting condition as long as the dry monsoon lasted (May to

October). At the beginning of the following rainy monsoon, the larvae pupated, and the adults that subsequently emerged laid eggs on seedbeds of the next rice crop. The cause of the diapause was attributed to the dryness of the surroundings after harvest when water irrigation is stopped and rains cease at the beginning of the dry monsoon.

From 1900 to 1940, white rice stem borer occurred in paddy fields along the north coast of Java. From 1940 to 1988, populations of this insect disappeared from the area, and are presently found only in paddy fields along the north coast of West Java. The white rice stem borer outbreaks in the planting season 1989-1990 in West Java caused damage to 75,000 hectare (40%) of the rice crop a yield loss of about US$ 30 million in price (Oka, 1991).

Goot (1925) reported that white rice stem borer diapause was induced when the larva fed on rice plants of reproductive growth stages, and suggested that diapause might be triggered by changes in the nutrient composition of the plants during the grain-ripening process. After the 4th molt, diapausing larvae did not pupate as did nondiapausing larvae, but instead they underwent 3 more molts before residing in the lowest node of the rice stem. The process was irreversible, i.e., these larvae did not pupate when exposed to favorable conditions. And he also reported that rainfall of at least 10 mm was reported to stimulate pupation in diapausing larvae. However, rain would only affect the larvae that had been in the diapause state 3 months or longer.

Renewed interest in diapause was generated by the white rice stem borer outbreak of 1989-1990 in West Java, in which the larvae did not exhibiting diapause even though they fed on maturing rice plants during the wet season (Rauf 1990). However, more than 90% of the larvae were observed to be in diapause at the end of dry growing season (June to July).

Triwidodo (1993) suggested that plant quality plays a minor role in diapause induction, and documented short day length (11.75 hours) at Karawang (located at 6.5°S) as the primary cue for diapause induction, with plant factors (rice growth and variety) possibly having a secondary role. Moisture is required for diapause termination.

For tropical insects, the dominant temperate zone pattern wherein insects use day length to foretell of winter is replaced by a variety of environmental cues tailored to foretell the advent of a specific season (Denlinger, 1986).

Closer to the equator, in a zone extending at least 6.5°S, the signaling capacity of seasonal change in the day length appears to be lost. *Leptopiliana boulardi* L and *Chilo partellus* Swinhoe from 6.5°S do not use day length as an environmental regulator of diapause (Carton & Claret 1982, Scheltes 1978).

Suparno (1996) reported that short day length at 6.6°S was not used as a cue for diapause induction. White rice stem borer larvae that feed on rice plants grown on soils with continual watering at short day length (exposed at 11.76 hours at June 21) did not diapause. The decline in soil water content on rice plants caused an increased percentage of larvae to enter diapause. Soil with saturated water (52% water), field capacity (26%) and half field capacity (13%) caused diapause incidence in 0.4 ± 0.02, 68.7 ± 7.2, and 87.5 ± 12.5% of the larvae, respectively. He concluded that water stress is required for diapause initiation and moistening required for diapause termination.

Water stress was known to affect physiological processes in the rice stem tissues, by increasing the synthesis of the sesquiterpenoid abscisic acid (ABA), the amino acid proline, and lipids, and decreasing methionine and protein content in plant tissues (Millborrow, 1974; Addicott 1983; Suge, 1984). Studies on the phytochemical content in host plants in relation to larval diapause have been conducted by several researchers. In

Trinidad, larval diapause in *Pectinophora gossypiella* Saunders is best correlated with a decline in moisture content and increase in fat content of cotton seeds. In Kenya, larval diapause in *C. zonellus* Swinhoe and *C. argyrolepia* Hamps is correlated with decline in moisture and protein, and increase in fat content of corn stem tissues (Scheltes 1976, Denlinger 1986).

The objective of this research was to determine the important factors in white rice stem borer larvae entering diapause.

MATERIAL AND METHODS

Research were conducted in a green house at the Bogor Research Institute for Food Crops (BORIF), April to October 1994. White rice stem borer adults were collected from rice fields in Karawang, West Java at April 1994, and reared in BORIF.

A split plot experiment was designed to determine phytochemical content caused by watering of the soil media and maturity of host plant in short day length (shortest day length at 11.76 hour), both suspected to play an important role in diapause incidence. Main plot was watering of the soil media included two levels: watering continually until 120 days after transplanting (DAT) or 2 months after flowering and terminating watering 75 DAT. Subplot was maturity of the host plant included five age levels: 62, 69, 76, 83, 90, and 97 DAT. Each treatment was replicated 5 times.

The rice plants (IR64 variety) were planted in pots containing 10 kg of soil (14% water content), with three seedlings per hill in 1 pot. Inoculation of white rice stem borer larvae was conducted at 62, 69, 76, 83, 90, and 97 DAT or 7, 14, 21, 28, and 35 d after flowering. Each pot or hill was inoculated with 20 neonate 1st instars and then caged in plastic millar. Sixty days after inoculation, the infested hills were returned to the laboratory and dissected to determine diapause incidence. The number of diapausing larvae was recorded.

Soil moisture and phytochemical content were measured at a sensitive stage for diapause induction (20 days after inoculation). Phytochemical content in the rice stem samples included water, total fat, crude protein, abscisic acid and methionine content. The rice stem for analysis of the phytochemicals was sampled from the hills uninoculated by white rice stem borer larvae. Crude protein was determined by Micro Kjeldahl. Abscisic acid and methionine content were determined by high performance liquid chromatography (HPLC). Total fat content was determined by Soxhlet. Stem rice samples for abscisic acid and methionine were extracted by methanol. The destruction for methionine conducted in the freezer at minus 20°C. Abscisic acid and methionine standards were obtained from Merck Chemical Industry. Phytochemical content, were counted from dry weight. The data from this experiments was analysed by using general statistical program Irristat 1990.

RESULTS AND DISCUSSION

No diapause incidence of WRSB in the rice stem which was watered continually until 120 days after transplanting (DAT) or 2 months after flowering and exposed to short day length (11,76 hours at June 21). All the larvae developed to adults between 27 and 40 days after inoculation. Mortality rate of the adults in July was more than 90% and may have been caused by high temperatures (30 – 41°C). Rice stem maturation without water content decline did not cause diapause (Table 1). On this condition, stem water content is high, but total fat, protein, methionine and sesquiterpenoid abscisic acid (ABA) content in the rice stem tissues are low. Protein and methionine content indicates a significant difference ($P<0.05$) in different ages. Thus result was very different to Goot (1925), who reported that WRSB diapause was induced when the larva fed on rice plants of reproductive growth stages. Rauf (1990) reported that the WRSB larvae did not exhibiting diapause even though they fed on maturing rice plants during the wet season, while more than 90% of the larvae were observed to be in diapause at the end of dry growing season (June to July). The present data indicates that the larvae were not to be in diapause at the end of dry growing season by watering continually.

Diapause incidence of WRSB were observed in the rice stem with terminated watering at 75 DAT or 15 days after flowering. Neonate larvae which were inoculated to the rice plant at 62, 69, 76, 83, and 90 days after transplanting with terminated watering 75 DAT experience 46.2, 69.0, 98.3, 100.0 and 100.0%, diapause respectively (Table 2). All first instars inoculated at 98 DAT did not survive. The high mortality caused by very low water content of rice stem and high temperature in surroundings. Diapausing larvae could be differentiated from the fifth instar by its larger body sizes, a more intense yellow color, and more visible dorsal blood vessel. Also, diapausing larvae were invariably found with their heads oriented in a downward position, whereas the fifth instar were invariably found with their heads oriented in an upward position.

Terminated watering caused declining soil moisture that had direct effect on decreasing rice stem water content, and so significantly affected in increasing abscisic acid (ABA) content. However, protein and methionine content are low and total fat is very low and showed that non significant difference (Table 2).

Thus results were in accord with Usua (1973), who reported that reducing the water content in the maize stem during maturation were important to diapause induction. Diapause incidence in the maize stem borer, *Busseola fusca*, increase with the age of maize: 24% diapause on 6-9 wk old maize and 91% diapause on 12-15 wk old maize. Fifth instars (penultimate instar) of this borer the most sensitive to diapause induction. After the rains cease on the Kenyan coast, diapause can be observed in maize of different ages and maturity, suggesting that water content is the primary factor for diapause initiation. That diapause is not observed in irrigated fields (Scheltes 1976, 1978a, b). They did not obseve the effect of decreasing water content to phytochemicals content.

Declining of water content was known had affect physiological processes in the rice stem tissues, by decreasing the primary metabolites methionine and protein content, and increasing the synthesis of secondary metabolite abscisic acid (ABA) in plant tissues (Table 2). Quantitative changes of primary metabolites (protein and methionine) in the rice stem in this experiment did not caused diapause of WRSB larvae. Thus data were different with previous study. Goot (1925) reported that diapause might be triggered by changes in the nutrient composition of the plants during the grain-ripening process. Larval diapause in *P. gossypiella* was best correlated with a decline in moisture content and increase in fat content of cotton seeds, and larval diapause in *C. zonellus* and *C. argyrolepia* is correlated with decline in moisture and protein, and increase in fat content of corn stem tissues (Scheltes 1976, Denlinger 1986).

The changes of ABA content suggested very important in diapause initiation of WRSB (Table 2). The role of ABA in the insects was studied by some previous researchers. Abscisic acid injected into the haemolymph of *Sarcophaga bullata* Meigen larvae had an inhibition effect on vitellogenin and ecdysone synthesis (de Man *et al.* 1981). Vitellogenin is a yolk protein synthesized in the fat body before pupation, secreted into the haemolymph, and taken up by oocyte in the adult female, and ecdysone is a molting hormone. Pytel *et al.* (1993) proved that the insect hormones which inhibited the function of ecdyson both in vitellogenin and ecdysone synthesis is the juvenile hormones (JH). From this result could be concluded that this ABA have JH activity. Rockstein (1978) reported that the most important juvenile hormone in initiation of larval diapause is JH I.

Table 1. Diapause incidence of WRSB in the rice stem with watering on growth soil media continually until 120 days after transplanting and phytochemicals content.

Larvae Inoculation (DAT)	Soil Moisture (%)[a]	Phytochemical in rice stem[a] Water (%)	Fat (%)	Methionine (ppm)	Protein (%)	ABA (ppm)	Survival[b]	Diapause Incidence (%)[b]
62	52	87 a	0.63 a	8.8 a	6.3 a	0.05 a	9.2	0.0 a
69	52	88 a	0.62 a	8.0 a	6.2 a	0.25 a	11.2	0.0 a
77	53	85 a	0.63 a	7.8 ab	5.6 ab	0.32 a	10.4	0.0 a
83	52	86 a	0.61 a	7.1 b	5.4 b	0.48 a	12.3	0.0 a
90	51	84 a	0.62 a	6.3 c	5.1 b	0.54 a	10.3	0.0 a

DAT, days after transplanting; [a] 20 days after inoculation; [b] 60 days after inoculation
Number the same column followed by the same letters are not significantly different based on Tukey's multiple comparison test with 0.05 error rate

Table 2. Diapause incidence of white rice stem borer in the rice stem with stopped watering on growth soil media 75 days after transplanting and phytochemicals content

Larvae Inoculation (DAT)	Soil Moisture (%)[a]	Phytochemical in rice stem[a] Water (%)	Fat (%)	Methionine (ppm)	Protein (%)	ABA (ppm)	Survival[c]	Diapause Incidence (%)[b]
62	42	67 a	0.63 a	13.3 a	6.0 a	4.75 a	12 a	42.6 a
69	26	63 a	0.64 a	15.0 a	6.0 a	7.65 b	11 a	68.0 b
77	20	57 b	0.66 a	13.8 a	5.6 a	11.62 c	13 a	97.2 c
83	16	47 c	0.64 a	12.1 a	5.6 a	26.98 d	10 b	100.0 d
90	13	40 c	0.64 a	10.3 a	5.3 a	32.54 e	7 c	100.0 d

DAT, days after transplanting, [a] 20 days after inoculation, [b] 60 days after inoculation.
Numbers the same column followed by the same letters are not significantly different based on Tukey's multiple comparison test with 0.05 error rate

Yagi & Fukaya (1974) reported that JH is a very important factor in larval diapause of the rice stem borer, *Chilo suppressalis* Walker. JH I titer is high at initiation of diapause, drops to intermediate levels for the remainder of diapause, and then drops sharply at diapause termination. The elevated JH I titer is essential for maintaining diapause and for dictating that the occasional molts occurring during diapause will be stationary (larva to larva) rather than progressive (larva to pupa). High ABA content in the rice stem may be useful for precursor of JH I and so making high JH titer at initiation of diapause of WRSB, so that the percentage of WRSB larvae entering diapause are high. Addicott (1983) reported that an increase the percentage of larval diapause of *P. gossypiella* was best correlated with an increase in ABA content of cotton seeds.

It is suggested that ABA is an important plant growth inhibitor and provides both rice plant and WRSB with a chemical means through which environmental factors such as temperature, water stress and solar radiation can be translated into internal regulation to limit growth and reproduction at diapause incidence. By altering rice plant metabolites and directing its effect upon insect metabolism, ABA may correlate these events in WRSB with the physiological state of host plants.

CONCLUSION

White rice stem borer (WRSB) larvae feeds on rice plants IR64 grown on soils with continual watering which exposed at short day length (11.76 hours at June 21) did not enter diapause. However, terminated watering 75 days after transplanting or 15 days after flowering on the host growth media caused diapause. These terminated watering resulted in declining soil moisture and had direct effect in decreasing rice stem water content, and affected significantly in increasing abscisic acid (ABA). A changes of primary metabolites (protein, lipid and methionine) did not cause diapause. The secondary metabolite ABA was known to have juvenile hormone activity in the insect larva. Increasing ABA content progressively in the rice stem increased the percentage of larval diapause of WRSB. The role of ABA in diapause initiation of white rice stem borer needs more study.

ACKNOWLEDGEMENT

I thank M. Iman of the Department of Entomology, Bogor Research Institute for Food Crops, in sharing of his green house and his support some materials in phytochemical analyses by HPLC. This research was supported, in part, by Directorate General of Higher Education, Ministry of Education and Culture, Indonesia.

REFERENCES CITED

Addicot, F.T. 1983. *Abscisic Acid. Praeger*. New York, 607 pp.

Carton, D.J., & J. Claret. 1982. Adaptive significance of a temperature induced diapause in a cosmopolitan parasitoid of Drosophila. *Ecol. Entomol*. 7: 239-247.

Dammerman, K. N. 1915. The white rice stem borer in Java. *Med. Lab. Plantenziekten*. No. 16.

De Man, W., A. de Loof, T. Briers, & Huybrechts. 1981. Effect of ABA on vitellogenin in *Sarcophaga bullata*. *Entomol exp. appl*. 12: 714-720.

Denlinger, D.L. 1986. Dormancy in tropical insect. *Annu. Rev. Entomol*. 31: 239-264.

Goot, Van der. 1925. Levenswijze en bestrijding van den witten rijsboorder op Java. *Med. Inst. Plantenziekten*. No. 66, 308 + append.

Millborrow, B.V. 1974. The chemistry and physiology of abscisic acid. *Ann. Plant. Physiol*. 25: 259-307.

Oka, I. N. 1991. *An overview on rice stem borer central program based on integrated management*. Indonesia IPM National Program. Jakarta (In Indonesian).

Pytel, M. M,, R. Trzcinska, M. Aubry, M. A. Pszczolkowski, & B. Cymborowski. 1993. Regulation of prothoracic gland activity in diapausing larvae of the wax moth, *Galleria mellonella* L. (Lepidoptera). *Insect Biochem. Mol. Biol*. 23: 33-41.

Rauf, A. 1990. *Epidemic analysis of white rice stem borer in Jalur pantura*. Paper presented in the seminar on White Rice Stem Borer Integrated Pest Management. Bogor Agricultural University, Bogor (In Indonesian).

Rockstein, M. 1978. *Biochemistry of Insects*. Academic Press, Inc. New York.

Scheltes, P. 1978a. *Ecological and physiological aspects of aestivation-diapause in the larvae of two pyralid stalk borers of maize in Kenya*. PhD thesis. Landbouwhogeschool. Wageningen, 110 pp.

__________. 1978b. The condition of the lost plant during aestivation-diapause of stalk *Chilo partellus* Swinhoe and *Chilo orichalcociliella* (Lepidoptera: Pyralidae) in Kenya. *Entomol. Exp. appl*. 24: 479-488.

Suge, H. 1984. Hormonal control of growth and development, pp. 133-155. In Tsunoda, S. and N. Takahashi (eds.), *Biology of Rice*. Japan Sci. Press, Tokyo.

Suparno, T. 1996. Pengaruh kelengasan tanah dan hari pendek terhadap kejadian diapause *Scirpophaga innotata* Walker. *J. Penel. Univ. Bengkulu*. 2(7): 15-18.

Triwidodo, H. 1993. *The biology of white rice stem borer in West Java, Indonesia*. PhD Dissertation, Graduate School, University of Wisconsin. Madison.

Yagi, S., & M. Fukaya. 1974. JH as a key factor larval diapause of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). *Appl. Entomol. Zool*. 9: 247-255.