THE SIGNIFICANCE OF FREE AIR CO_2 ENRICHMENT AND OPEN ROOF VENTILATIO GREENHOUSE SYSTEMS IN A STUDY OF MEALWORM BEETLE, *Tenebrio molitor* L. (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT

Tenebrio molitor L. (Coleoptera: Tenebrionidae) is an insect storage pest that has been used as a subject in Integrated Pest Management (IPM) research. The aim of this study is to determine the importance of conducting insect-related studies, especially on *T. molitor* under a Free Air CO_2 Enrichment (FACE) System and Open Roof Ventilation Greenhouse System (ORVS). FACE system provides a natural microclimate and biotic interactions, while ORVS is an artificial environment with regulation of its environmental parameters. More accurate comparisons can be made to the results obtained under the similar environmental factors including elevated CO_2 concentration. Based on the results, the mortality time of *T. molitor* adults in ORVS (5-6 weeks) is the fastest, followed by FACE (9-10 weeks) and RR as a control (11-12 weeks). The highest significant time difference shows by the last adult individual dead is between ORVS versus RR is 6 weeks. Therefore, mortality rate of *T. molitor* adult and their life span are directly proportional to the elevated CO_2 concentration. It is shows that the higher concentration of CO_2, with faster mortality rate and shorter the life span of the adults. Since the study of insects using both systems is still limited, the data from this preliminary study can be used as reference for future research.

Keywords: *Tenebrio molitor*, mortality, CO_2, FACE, ORVS, life span

ABSTRAK

Tenebrio molitor L. (Coleoptera: Tenebrionidae) adalah serangga perosak simpanan gandum, di mana serangga tersebut telah digunakan sebagai subjek dalam kajian Pengurusan Perosak Bersepadu (IPM). Tujuan kajian ini adalah untuk mengenal pasti kepentingan dalam menjalankan kajian berkaitan serangga, terutamanya *T. molitor* di dalam *Free Air CO_2 Enrichment (FACE) System* dan *Open Roof Ventilation Greenhouse System (ORVS).* Sistem FACE menyediakan mikro iklim semulajadi dan interaksi-interaksi biotik, manakala ORVS ialah persekitaran buatan dimana parameter-parameter persekitarannya telah diregulasi. Perbandingan yang lebih tepat boleh dibuat daripada hasil yang diperolehi di bawah faktor-

faktor persekitaran yang sama, termasuk peningkatan kepekatan CO_2. Berdasarkan hasil, kadar kematian *T. molitor* dewasa di dalam ORVS (5-6 minggu) adalah terpantas, diikuti oleh FACE (9-10 minggu) dan RR sebagai kawalan (11-12 minggu). Perbezaan bererti ($p < 0.05$) antara masa terpanjang dihitung berdasarkan individu dewasa terakhir yang mati, antara ORVS berbanding RR iaitu selama 6 minggu. Oleh itu, kadar kematian/jangka hayat *T. molitor* dewasa adalah berkadar terus dengan peningkatan kepekatan CO_2. Ini menunjukkan bahawa semakin tinggi kepekatan CO_2, semakin cepat kadar kematian dan semakin pendek jangka hayat *T. molitor* dewasa. Oleh kerana kajian terhadap serangga menggunakan kedua-dua sistem tersebut masih kurang, data-data daripada kajian awal ini boleh digunakan sebagai sumber rujukan untuk kajian-kajian pada masa hadapan.

Kata kunci: *Tenebrio molitor*, kematian, CO_2, FACE, ORVS, jangka hayat

INTRODUCTION

Open Roof Ventilation Greenhouse System (ORVS) is a closed system that has been created and built to provide an environment with high atmospheric CO_2 concentrations (Albright 2002). Other environmental factors such as inside and outside temperature and humidity were measured and controlled using psychrometers (Boulard & Draoui 1995). Generally, this system has been developed to provide a suitable environment by supplying adequate and consistent CO_2 concentration for plant growth and development inside the greenhouse. ORVS is a closed system with movable shade which requires computer control and a good PPFD sensor (Albright et al. 2000). According to Vanaja et al. (2006), the circular tube serves to release or disperse the CO_2-enriched air and has been assisted by air blowers to ensure the CO_2 gas is evenly distributed within the chamber. Nowadays, a normal practice for cooling the greenhouse atmosphere is by opening the vent (Kittas et al. 1997). Because of that, it is important to minimize the CO_2 loss by maintaining the same level of CO_2 in and CO_2 out (Ohyama et al. 2005).

FACE is an open system which is built to provide an ambient environment with high CO_2 concentrations to conduct research on vegetation and other ecosystem components (Hendrey et al. 1993). The field conditions with natural microenvironment and biotic interactions become the most important factor in the construction of this system (Machacova 2010). Atmospheric CO_2 concentration at daytime was elevated by~130 ppm in a FACE (Miglietta et al. 2001). Pure CO_2 concentrations about 510 ppm has been distributed to the six CO_2 elevated octagons from a central CO_2 tank (Scherber et al. 2013). A ringshaped pipe surrounding the plot works in CO_2 delivery in the FACE system and is disseminated by vertically oriented pipes. CO_2-enrich emissions are also controlled by the vertical pipes valves where it can be opened and closed depending on wind direction changes (von Felten et al. 2007). To increase the CO_2 mixing rate with air, the blowers (Pinter et al. 2000) or injecting CO_2 at high pressure through small orifices (Miglietta et al. 2001) were used and conducted in the FACE system.

To compare the results obtained from the ORVS treatment (closed system) with the FACE system (open system) is the significance of this study to be carried out. FACE system provides a natural microclimate such as changing weather conditions and biotic interactions among individual plants (Machacova 2010) and animals. The large-scale FACE plots also displaying the most realistic future environmental conditions due to the increment of CO_2 levels (Ainsworth & Long 2005). Otherwise, ORVS is an artificial environment (Kellomaki et al. 2000), where certain parameters such as humidity, CO_2 concentration and temperature has been controlled or manipulated (Albright 2002; Sanchez-Guerrero 2005), with adequate airflow rates between internal and external greenhouse environment (Harmanto 2006).

Other than that, only side-by-side tests of ORVS and FACE technology, where the studies conducted under the same enrich CO_2 levels and on the same environmental factors can be compared more accurately and reduce the bias (Ainsworth & Long 2005). According to Kimball et al. (1997), impacts of elevated CO_2 studies are often conducted using the FACE approach as it can demonstrate more accurate and definitive results. In this study, the samples of *T. molitor* were used to determine the direct effects of high CO_2 level on their development, survivability, morphology and genetics.

Then, it is important to identify the direct impacts of climate change, especially due to the increment of atmospheric CO_2 concentration towards animals and plants. Most of the previous studies that have been conducted using FACE and ORVS have focused on plants. Based on observations, there are still less publications related to the study of insects conducted in both systems, especially ORVS. In general, one of the ORVS functions is to provide a closed environment with insect screen to prevent pests from approaching crops (Kittas et al. 2005). Therefore, a study on *T. molitor* was carried out on both systems to enhance knowledge of the high CO_2 concentration effects on its morphology, biology and genetics, which subsequently became an indicator for other insects. The objective of this study is to determine the significance of *T. molitor* study in two different systems with enriched CO_2 concentrations, including FACE and ORVS.

MATERIALS & METHODS

Rearing Process of Mealworm Beetle, *Tenebrio molitor*

The larvae of mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) was obtained from a local supplier located at Bandar Baru Bangi, Selangor. To ensure that the larvae are *T. molitor*, the species key by Bousquet (1990) was referred during the identification process of species based on its morphology. The larvae samples were reared in 40 × 28 × 24 cm plastic aquariums and were placed in the Cytogenetic 2 Laboratory. To ensure that the *T. molitor* larvae get enough food and water sources, oats and cucumbers have been supplied throughout the rearing activity (Siemianowska et al. 2013). Certain tools such as CO_2 Meter Version 8802-EN-00 (CO_2 concentration in ppm unit) and Hygrometer Digital (temperature (°C) and humidity (%)) were used to measure specific parameters and were recorded. The samples of *T. molitor* larvae were monitored until the emergence of their adults.

Isolation, Observation, Collection, Preservation and Analysis of *Tenebrio molitor* Adults

Aquarium size 19 × 14 × 12 cm has been set up by 30 units. Each aquarium was filled with 4 cm height of sawdust at the bottom and 4 cm height of sifted soil. After that, forty individuals of adults were picked and put in each aquarium. Every ten sets of aquarium were placed in Free Air CO_2 Enrichment System (FACE) and Open Roof Ventilation System (ORVS) with an increment of CO_2 gas and Rearing Room (RR) as control. CO_2 concentrations (in ppm), humidity (%), temperature (°C), no of valve and wind speed (m/s) were observed and documented at every sampling time. The dead body of *T. molitor* adults were picked using forcep, stored in vile and preserved in 70% alcohol. The mortality rate/life span of adults was recorded, measured and analyzed using Microsoft Excel version 2013.

Table 1 No of dead adults of *Tenebrio molitor* from RR (control), FACE and ORVS system

Week	System / CO_2 (ppm)		
	RR	FACE	ORVS
	441-553	400-550	300-950
1-2	41	173	288
3-4	159	66	109
5-6	72	81	3
7-8	83	44	
9-10	17	36	
11-12	28		

CO_2= carbon dioxide; S= during sampling activity; RR= rearing room; FACE= Free Air CO_2 Enrichment system; ORVS= Open Roof Ventilation Greenhouse system.

RESULT

Table 1 shows the no of dead adults of *T. molitor* from both systems and control which has been collected at every sampling activity. The dead adult samples of *T. molitor* from ten sets of aquarium were collected from both systems (FACE and ORVS) and RR. As results, the mortality rate of *T. molitor* adults in ORVS (5-6 weeks) is fastest with the highest no of dead individuals in the first two weeks (288 individuals; 72 percents), compared to FACE (9-10 weeks) and RR (11-12 weeks). The significant time difference shows by the last dead adult individual; ORVS vs RR (6 weeks), FACE vs RR (2 weeks), and ORVS vs FACE (4 weeks).

DISCUSSION

The release of high CO_2 gas due to anthropogenic activities has increased its concentration in the atmosphere. The presence of the CO_2 gas at high concentration levels can affect the growth rate such as prolong development times (Goverde & Erhardt 2003), abundance, richness and diversity of a herbivore (Kopper & Lindroth 2003) compared with ambient CO_2 levels. There are many studies related to the effect of CO_2 concentrations, especially on insects that have been carried out. The results show that different insects, with different maturities showing different responses to different CO_2 concentrations. Based on Spratt et al. (1985), the *Trogoderma granarium* larvae which is a destructive pest of grain products, showing the reaction effect at 60% of CO_2 after 17 days at 30°C of temperature. Other than that, the adult stage shows the lowest tolerance to CO_2 than one immature life stage (Annis 1987).

Based on the results of this study (Table 1), mortality time of *T. molitor* adults in ambient conditions, with normal CO_2 concentration are slower than FACE and ORVS. Higher CO_2 concentrations that have been released on both systems of 800-950 ppm, which is 400 ppm higher than ambient levels, have been shown to accelerate the mortality time of *T. molitor* adults.

The reduction in CO_2 concentrations reading during sampling is due to the absorptions of the CO_2 gas by the plants or relatively high photosynthetic activity (Sanchez-Guerrero et al. 2005) found in both systems especially FACE, wherein CO_2 is the most important source of photosynthesis. ORVS is a closed system which able to maintain a higher CO_2 rate longer than FACE (open system). Therefore, the duration of *T. molitor* exposed to higher CO_2

concentration levels is longer and indirectly increases the CO_2 absorption rate through its body surface. Because of that, it has resulted in an increase in the effects of elevated CO_2 on it and thereby accelerates the mortality time and reduce life span of *T. molitor* adults in ORVS. In fact, the location of the FACE system itself in the forest ecosystem also affects the CO_2 loss rate due to the presence of large trees.

Other studies were done on *T. molitor* which focusing on the larval development and genetic changes in FACE and ORVS. It is shown that there were no significant changes on the development of larvae samples under control condition. But, slight and moderate changes were observed under FACE and ORVS with parallel changes in their genetic data (Nur Hasyimah et al. 2018). According to Nur Hasyimah and Yaakop (2018), prolonged CO_2 exposure towards parent and the first generation of *T. molitor* affect their development by decreased their development pattern.

According to Mbata et al. (2000), pupae of *Callosobruchus subinnotatus* Pic (Coleoptera: Bruchidae) and adults of pharate suffer from complete mortality at a slower rate after being treated by hypoxic than hypercarbic atmosphere. Under numerous elevated atmospheric CO_2 concentrations, the *Platynota stultana* (Lepidoptera: Tortricidae) pupae shows a different pattern of development and mortality response than under low O_2 atmosphere. The pupae had a greater energy scarcity under increment of CO_2 concentrations than under decreased O_2, although a similar reduction in the rate of metabolism (Zhou et al. 2001). Similar results shown by adults of *Tribolium castaneum* death is due to the depletion of triglyceride reserves caused by high CO_2 level (Ofuya & Reichmuth 2002).

CONCLUSION

FACE and ORVS have been built to assist in studies related to elevated CO_2 in the ecosystem. The effects of elevated CO_2 concentrations above the ambient level on insects, especially *T. molitor* can be compared more accurately by controlling the other abiotic factors in the environment. Besides, both FACE and ORVS conditions with enriched-CO_2 concentration are mimic to the expected future ecosystem that are currently experiencing an increase in CO_2 level in the atmosphere, where it is one of the greenhouse gases that contributes to global warming. Most of the previous studies have focused on the direct effects of atmospheric CO_2 enhancement on plants have been carried out on both systems. However, the use of FACE and ORVS for the study of insects is still very limited. Therefore, more studies on *T. molitor* using FACE and ORVS should be intensified to increase human understanding of elevated CO_2 effects towards their biology, physiology, genetics, morphology, etc. which subsequently became an indicator for other insects. As a summary, elevated CO_2 concentration accelerates the mortality time of *T. molitor* adults compared to ambient level (~ 450 ppm).

ACKNOWLEDGEMENT

This project was fully supported by Climate Change Institute (IPI) using UKM-YSD Chair in Climate Change Grant (ZF2015-025) and GUP-2016-022. Special thanks and gratitude to Universiti Kebangsaan Malaysia (UKM) and Universiti Teknologi Mara (UiTM) for providing study facilities and scholarship.

REFERENCES

Ainsworth, E.A. & Long, S.P. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A metaanalytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* 165: 351–372.

Albright, L., Both, A.J. & Chiu, A.J. 2000. Controlling greenhouse light to consistent daily integral. *Transactions of the ASAE* 43(2): 421–431.

Albright, L.D. 2002. Controlling greenhouse environments. *Proceedings IS on Tropical and Subtropical Greenhouses*, pp. 47-54.

Annis, P.C. 1987. Towards rational controlled atmosphere dosage schedules: a review of current knowledge. *Proceeding 4th International Working Conference of Stored Product Protection*, pp. 128–48.

Boulard, T. & Draoui, B. 1995. Natural ventilation of a greenhouse with continuous roof vents: measurements and data analysis. *Journal of Agricultural and Engineering Research* 61: 27-36.

Bousquet, Y. 1990. *Beetles Associated with Stored Products in Canada: An Identification Guide.* Ottawa: Research Branch Agriculture Canada.

Cannon, R.J.C. 1998. The implications of predicted climate change for insect pests in the UK with emphasis on nonindigenous species. *Global Change Biology* 4: 785– 796.

Denman, K.L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P.M., Dickinson, R.E., Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandran, S., da Silva Dias, P.L., Wofsy, S.C., & Zhang, X. 2007. Couplings between changes in the climate system and biogeochemistry. In Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. & Miller, H.L. (Eds). "*Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*", pp. 511-539. Cambridge: Cambridge University Press.

Goverde, M. & Erhardt, A. 2003. Effects of elevated CO_2 on development and larval food-preference in the butterfly *Coenonympha pamphius* (Lepidoptera, Satyridae). *Global Change Biology* 9: 74–83.

Guerenstein, P.G. & Hildebrand, J.G. 2008. Roles and effects of environmental carbon dioxide in insect life. *Annual Review of Entomology* 53: 161–178.

Harmanto, Tantau, H.J. & Salokhe, V.M. 2006. Influence of insect screens with different mesh sizes on ventilation rate and microclimate of greenhouses in the humid tropics. *Agricultural Engineering International: The CIGR Ejournal* 8: 1–18.

Hendrey, G.R., Ellsworth, D.S., Lewin, K.F. & Nagy, J. 1993. A free air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* 5: 293–309.

Houghton, J.T., Callander, B.A. & Varney, S.K. 1992. Climate *Change 1992. The Supplementary Report to the IPCC Scientific Assessment*. Cambridge: Cambridge University Press.

IPCC. 2007. *IPCC Summary for Policymakers, Climate Change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Intergovernmental Panel on Climate Change.

Kellomaki, S., Wang, K.Y. & Lemettinen, M. 2000. Controlled environment chambers for investigating tree response to elevated CO_2 and temperature under boreal conditions. *Photosynthetica* 38(1): 69-81.

Kimball, B.A., Pinter, P.J., Wall, G.W., Garcia, R.L., LaMorte, R.L., Jak, P.M.C., Frumau, K.F.A. & Vugts, H.F. 1997. Comparisons of responses of vegetation to elevated carbon dioxide in free-air and open-top chamber facilities. In: Allen, L.H., Kirkham, M.B., Olszyk, D.M., & Whitman, C.E. (Eds.). *Advances in Carbon Dioxide Effects Research*, p. 113-130. Madison, WI, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Kittas, C., Katsoulas, N. & Bartzanas, T. 2005. Effect of vents' opening and insect screen on greenhouse ventilation. *International Conference "Passive and Low Energy Cooling for the Built Environment*", pp. 59-64.

Kopper, B.J. & Lindroth, R.L. 2003. Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia* 134: 95–103.

Machacova, K. 2010. Open top chamber and free air CO_2 enrichment - approaches to investigate tree responses to elevated CO_2. *iForest* 3: 102-105.

Mbata, G.N., Hetz, S.K., Reichmuth, C. & Adler, C. 2000. Tolerance of pupae and pharate adults of *Callosobruchus subinnotatus* Pic (Coleoptera: Bruchidae) to modified atmospheres: a function of metabolic rate. *Journal of Insect Physiology* 46: 145–151.

Miglietta, F., Peressotti, A., Vaccari, F.P., Zaldei, A., Deangelis, G.P. & Scarascia-Mugnozza. 2001. Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* 150: 465–476.

Nur Hasyimah, R. & Yaakop, S. 2018. Morphological changes on development of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) in Rearing Room System, Free Air CO2 Enrichment System and Open Roof Ventilation System. *AIP Conference Proceedings* Vol. 1940, pp. 20048.

Nur Hasyimah, R., Nor Atikah, A.R., Halim, M., Muhaimin, A.M.D., Nizam, M.S., Hanafiah, M.M. & Yaakop, S. 2018. CO_2 effects on larval development and genetics of mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) in two different CO_2 systems. *Applied Ecology and Environmental Research* 16(2): 1749–1766.

Ohyama, K., Kozai, T., Ishigami, Y., Ohno, Y., Ochi, Y. & Toida, H. 2005. A CO_2 Control System for a Greenhouse with a High Ventilation Rate. *Proceeding IC on Greensys*, pp. 649-654.

Ofuya, T. I. & Reichmuth, C. 2002. Effect of relative humidity on susceptibility of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) to two modified atmospheres. *Journal of Stored Products Research* 38: 139–146.

Pinter, P.J., Kimball, B.A., Wall, G.W., LaMorte, R.L., Hunsaker, D.J., Adamsen, F.J., Frumau, K.F.A., Vugts, H.R., Hendrey, G.R., Lewin, K.F., Nagy, J., Johnson, H.B., Wechsung, F., Leavitt, S.W., Thompson, T.L., Matthias, A.D. & Brooks, T.J. 2000. Free-air CO_2 enrichment (FACE): Blower effects on wheat canopy microclimate and plant development. *Agricultural Forest Meteorology* 103: 319–333.

Sanchez-Guerrero, M.C., Lorenzo, P., Medrano, E., Castilla, N., Soriano, T. & Baille, A. 2005. Effect of variable CO_2 enrichment on greenhouse production in mild winter climates. *Agricultural and Forest Meteorology* 132: 244252.

Scherber, C., David, J.G., Karen, S., Rune, J.K., Inger, K.S., Anders, M., Kristian, R.A., Klaus, S.L., Teis, N.M., Claus, B. & Soren, C. 2013. Multi-factor climate change effects on insect herbivore performance. *Ecology and Evolution* 3(6): 1449-1460.

Siemianowska, E., Kosewska, A., Aljewicz, M., Skibniewska, K.A., Polak-Juszczak, L., Jarocki, A. & Jedras, M. 2013. Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. *Agricultural Science* 4(6): 287-291.

Spahni, R., Chappellaz, J., Stocker, T.F., Loulergue, L., Hausammann, G., Kawamura, K., Flückiger, J., Schwander, J., Raynaud, D., Masson-Delmotte, V. & Jouzel, J. 2005. Atmospheric methane and nitrous oxide of the late Pleistocene from Antarctic ice cores. *Science* 310: 1317-1321.

Spratt, E., Dignan, G. & Banks, H.J. 1985. The effects of high concentrations of carbon dioxide in air on *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae). *Journal of Stored Product Research* 21:41– 46.

Vanaja, M., Maheswari, M., Ratnakumar, P. & Ramakrishna, Y.S. 2006. Monitoring and controlling of CO_2 concentrations in open top chambers for understanding of plants response to elevated CO_2 levels. *Indian Journal of Radio and Space Physics* 35: 193-197.

Von Felten, S., Hättenschwiler, S., Saurer, M. & Siegwolf, R. 2007. Carbon allocation in shoots of alpine treeline conifers in a CO_2 enriched environment. *Trees* 21: 283294.

Zhou, S., Criddle, R.S. & Mitcham, E.J. 2001. Metabolic response of *Platynota stultana* pupae under and after extended treatment with elevated CO_2 and reduced O_2 concentrations. *Journal of Insect Physiology* 47: 401– 409.