Behavioral Responses of the Parasitoid Wasp *Lytopylus rufipes* to Herbivore-Induced Plant Volatiles Released from Pear Leaves

August 2019

Chia Ming LIU

Behavioral Responses of the Parasitoid Wasp *Lytopylus rufipes* to Herbivore-Induced Plant Volatiles Released from Pear Leaves

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Agricultural Science

(Doctoral Program in Biosphere Resource Science and Technology)

Chia Ming LIU

Contents

List of abbreviations

H: (*Z*)-3-hexenyl acetate

O: (*E*)-β-ocimene

L: linalool

D: (*E*)-4,8-dimethyl-1,3,7-nonatriene

F: (*E*,*E*)-α-farnesene

GLVs: green leaf volatiles

HIPVs: herbivore-induced plant volatiles

RIOs: resource-indicating odors

Summary

This study focuses on how the volatiles of pear shoots affect the tri-trophic interaction system among *Pyrus pyrifolia* var. Kosui (pear), *Grapholita molesta* (herbivore), and *Lytopylus rufipes* (parasitic wasp) pear. Since there was no practical method to rear *L. rufipes*, a transitional diet system was first created to establish a *L. rufipes* colony in our laboratory. In this diet system, a sliced apple fruit is infested with neonate larvae of *G. molesta* and then used for triggering the oviposition behavior of *L. rufipes*. After parasitism, the apple slices are placed on artificial diet (Silkmate 2M) for further rearing.

Herbivore-induced plant volatiles (HIPVs) are usually used by natural enemies to search for their hosts. However, there is no information on how pear HIPVs affect insect communities in pear orchards. A 4-arm olfactometer was used to observe the behavioral responses of *L. rufipes* to different odor sources. By comparing odors of intact and host-infested shoots of pear, *L. rufipes* females were found to prefer the host-infested shoots to intact ones. This result indicates that *L. rufipes* females can evaluate infestations through volatile cues.

To clarify the HIPVs that attract *L. rufipes* females, odors of both intact and host-infested shoots were collected and identified by gas chromatography-mass spectrometry (GC-MS). Totally, 5 major components were identified, including (*Z*)-3-

hexenyl acetate (H), (*E*)-β-ocimene (O), linalool (L), (*E*)-4,8-dimethyl-1,3,7-nonatriene (D), and (*E*, *E*)-β-farnesene (F). When I compared individual components were compared against solvent controls, only O and D were preferred by *L. rufipes* females. Further bioassays were conducted to understand the perception of *L. rufipes* females for different combinations of these volatiles. Results showed that *L. rufipes* females responded differently to volatile blends. Female wasps recognized the induced volatile F when other components were present. Furthermore, the quinary blend (HOLDF) was most preferred by *L. rufipes* females than other volatile blends. These results indicate that *L. rufipes* females need the whole volatile blend rather than blends of fewer components or individual chemicals.

In order to understand if the pear HIPVs affected the behavior of *G. molesta* in the field, the oviposition preference bioassays were performed with an acrylic cylinder with a dual choice design. As a result, *G. molesta* females preferred to lay eggs near the pear shoots rather than the leaves. The volatile emissions from shoots was higher than leaves, indicating that female moths can recognize different part of host plant via volatile cues. Moreover, *G. molesta* females laid more eggs near host-infested shoots than intact shoots, suggesting that female moths may be attracted by HIPVs released from infested shoots. Subsequent bioassays between the quinary blend and solvent control demonstrated that female moths preferred to lay eggs in volatile treated areas rather than control areas.

This research provides basic information on the tri-trophic interaction system in pear orchards. However, some questions still remain such as the seasonal fluctuation of volatile emission from pear plants and the attraction of female moths to HIPVs from infested plant that should be considered for practical applications. Also, the quinary volatile blend should be tested in a pear orchard.

Acknowledgements

First of all, I'd like to say thank you to my family especially my wife, Sharon. I couldn't finish my thesis without her supports. Also, my lovely son gives me the motivation to go forward when I feel too much stress. I also appreciate the scholarship provided by the Iwatani Naoji Fundation that allows me to concentrate on my research.

This study started with establishing a rearing method for *Lytopylus rufipes*. To achieve the rearing, I received much help for the moth colony provided by Mr. Masao Kaneko (Nagano Fruit Tree Experiment Station), the *L. rufipes* was collected by Mr. Masataka Sasaki (Fukushima Plant Protection Association), the thinned apples for insect rearing were prepared by Dr. Masahiro Arakawa and Mr. Kouki Yoshida (Fruit Tree Research Center, Fukushima Prefecture), and the pear trees were provided by Prof. Junko Sugaya (Faculty of Life and Environmental Sciences, University of Tsukuba). I am thankful for all of your help that allowed me to start my research project.

In addition, I am really happy to have had a chance to work with all the members in this Applied Entomology and Zoology Laboratory regardless the time you spent here. Ms. Yoshimura took good care of every member especially the international students; Dr. Piyasaengthong was very kind and full of wisdom that always make me feel calm; Dr. Kuramitsu helped me deal with many complex situtions and gave me many comments on research and living in Japan; Mr. Ihsan did some research projects together with me, and his cheerful attitude helped me relax; Ms. Bew was a good listener, and her ability in research and presentation impressed me; Ms. Ayumi, Nee, and Amrul helped me with my experiments; and other members enriched my life in Tsukuba. Thank you for your company.

Finally, I am grateful to all committee members. Prof. Kainoh tell me “not to be emotional” is the way to be a good researcher, but I can still feel your expectations of me. I learned a lot from you not only for my career but also gained other knowledge about many things; Dr. Matsuyama helped me a lot with chemical analyses and synthesizing chemicals for my study, and I really like the manual work when doing experiments. Prof. Taylor asked me “what is your purpose” and “will you stay here for 10 years” in my first presentation. I was stunned by these questions, but they did help me in making a reasonable plan for my research; Dr. Furukawa taught me how to consider a question from other points of view that provides many possible answers that I never thought about; Dr. Kinoshita showed me how important creating a network with not only researchers in Japan but also international cooperation. I am really lucky to have a great committee like this. Thank you very much.