

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

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Contents

List of abbreviations	1
Chapter 1. General Introduction	2
1.1. Oriental Fruit Moth <i>Grapholita molesta</i>	2
1.2. Using Parasitoid Wasps as Biological Control Agents and the Larval Parasitoid Wasp <i>Lytopylus rufipes</i>	3
1.3. Tri-trophic Interactions Mediated by Semiochemicals	7
1.4. Application of Herbivore-Induced Plant Volatiles in Pest Management	8
1.5. Objective of This Study	9
Chapter 2. Establishing Laboratory Colony of <i>L. rufipes</i>	10
2.1. Introduction.....	10
2.2. Materials and Methods.....	13
2.2.1. Rearing Protocol for <i>G. molesta</i>	13
2.2.2. Rearing Protocol for <i>L. rufipes</i>	13
2.2.3. Performance of <i>L. rufipes</i> in the Transitional Diet System	15
2.2.4. Statistical Analysis	16
2.3. Results and Discussion.....	16
2.3.1. Performance of <i>G. molesta</i> and <i>L. rufipes</i> on the Transitional Diet	16
2.3.2. Possible Effects of Thelytokous Reproduction System.....	18
2.3.3. Future Development of Artificial Diets for Mass Rearing	19
Chapter 3. Behavioral Responses of <i>L. rufipes</i> to Headspace Volatiles Released from Pear (<i>Pyrus pyrifolia</i> variety Kosui) Shoots	26
3.1. Introduction.....	26
3.2. Materials and Methods.....	28
3.2.1. Plant Materials.....	28
3.2.2. Collection of Headspace Volatile from Pear Shoots	29
3.2.3. Analyses of Headspace Volatiles	30
3.2.4. Chemicals	31

3.2.5. Bioassays	31
3.2.6. Statistical Analysis	32
3.3. Results.....	32
3.3.1. Behavioral Responses of <i>L. rufipes</i> to Intact and Infested Plant Volatiles.....	32
3.3.2. Volatile Compositions of Pear Shoots.....	33
3.3.3. Behavioral Responses of Female <i>L. rufipes</i> to Each Volatile Component.....	34
3.3.4. Behavioral Responses of Female <i>L. rufipes</i> to Volatile Blends	34
3.4. Discussion	35
Chapter 4. Oviposition Preferences of <i>Grapholita molesta</i> to Host Plant Volatiles	47
4.1 Introduction.....	47
4.2 Materials and Methods.....	48
4.2.1. Daily Fecundity and Oviposition Time of <i>G. molesta</i>	48
4.2.2. Oviposition Preference of <i>G. molesta</i> on Pear Leaves	49
4.2.3. Oviposition Preference of <i>G. molesta</i> between Intact and Infested Pear Shoots...	51
4.2.4. Collection of Headspace Volatile from Pear Shoots and Leaves	51
4.2.5. Oviposition Response to HIPVs released from Pear Shoots	51
4.2.6. Statistical Analysis	52
4.3 Results.....	52
4.3.1. Daily Fecundity and Oviposition Time of <i>G. molesta</i>	52
4.3.2. Oviposition Preference of <i>G. molesta</i> to Pear-Leaves.....	53
4.3.3. Oviposition Preference of <i>G. molesta</i> between Intact and Infested Pear Shoots...	53
4.3.4. Volatile Compositions of Pear Shoots and Leaves.....	54
4.3.5. Oviposition Response to HIPVs Released from Pear Shoots.....	54
4.4 Discussion	54
Chapter 5. General Discussion	67
Summary	74
Acknowledgements.....	77
References	79

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.

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