Analysis of volatile components in host plants of *Xylotrechus quadripes*

Zijun Luo1, Yingzhi Chen1, Liping Liu1, Genyun Pan1 and Ruifang Wang1, 2

1Pu’er University, Pu’er Yunnan 665000

2Corresponding author’s email: peurdr_wang@foxmail.com

Abstract. *Xylotrechus quadripes* has a wide range of host plants. As a drillstem pest, it erodes the xylem of the trunk, resulting in the reduction of coffee production, and the control process is also facing difficulties. In this study, solid phase microextraction and gas chromatography were used to analysis the volatile components of host plants of *X. quadripes*. The results showed that 36, 23, 46 and 40 kinds of volatiles were identified from the four plants, mainly terpenes, some esters, alcohols and ketones. The terpenes were mainly monoterpenes and sesquiterpenes. The same volatiles of four plants were (+)-limonene, styrene, β-pinene, (-)-α-muurolene, (-)-α-cubebene and β-caryophyllene, which provides a basis for the prevention and control of *X. quadripes* using plant volatiles as attractants.

1. Introduction

Yunnan coffee has a unique natural environment. After more than 60 years of development, Yunnan Province has become a major coffee producing area in China. There are more than 100 varieties of coffee planted in Yunnan Province. *Acalolepta cervina* and *Xylotrechus quadripes* are the main drillstem pest of coffee in Yunnan Province, the latter is difficult to control. Longicorn is very active in bright sunlight and likes to lay its eggs on the main stem of the exposed coffee tree. Therefore, in the process of planting coffee in Yunnan, intercropping with other crops or planting shade plants are often used to provide shade for coffee trees, reduce the harm of longicorn pests and ensure stable and high yield of coffee. The main shade tree species are *Ricinus communis* L., *Aristolochia scholaris* (L.) R. Br., *Artocarpus heterophyllus* Lam., *Adina pilulifera* (Lam.) Franch. ex Drake and *Psidium guajava* Linn. et al.

Plant volatiles, called herbivore induced plant volatiles (HIPVs), play an important role in attracting natural enemies. The main components of HIPVs are monoterpenes or sesquiterpenes. Plant volatile substances, also known as plant aromatic substances, mainly include alkenes, alcohols, aldehydes, terpenes and other organic components containing benzene or aromatic rings. Studies have shown that plants release specific volatile compounds to attract longicorn, terpenes and alkanes are the main components. The control methods of longicorn include intercropping shade trees, scraping egg marks, chemical control, catching adults artificially, cleaning coffee garden and so on. It is urgent to study the volatile compounds of host plants and secondary host plants, which has an important prospect in the development of green agriculture in the future. By studying the types of volatile compounds among plants, we can expand the application of plant source pheromones in pest control. One method is to control the localization of the host plant volatiles by insects, and the other is to apply exogenous plant volatiles to attract pests. In order to achieve the goal of green pest control.
2. Research materials and methods

2.1. Research materials

2.1.1. Materials select C. arabica, A. heterophyllus, A. scholaris and P. guajava branches with good growth. Before sunrise, cut the branches with a clean shears and taken it back to the laboratory immediately soak them wounds in deionized water and collect volatile compounds by SPME. The sampling site was Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

2.1.2. Research instruments. Agilent Technologies GC-MS 5977B gas chromatography mass spectrometry, Manual solid phase microextraction injector, 65μm PDMS/DVB Solid phase microextraction head.

2.2. Research method

2.2.1. Collection of volatile substances. The volatile compounds were collected by solid-phase microextraction. For volatile components, the equilibrium time of headspace extraction is shorter under the same sample mixing conditions [1].The specific operation method is as follows: cover the branch with transparent polyethylene bag, tie up and fix the upper and lower ends tightly, then insert the manual SPME injector with 65 μm PDMS/DVB solid-phase microextraction head and extract for 30 minutes. At the end of sampling, the sample was injected into the chromatograph for 0.5 min, and the injection time was 0.5min.

2.2.2. GC-MS Analysis conditions. The operation time of GC was 50.5 min, the initial temperature of column box was 50 ℃ and the holding time was 2 min. Firstly, the temperature was raised to 230 ℃ every minute at 4 ℃ for 0 min, and then to 280 ℃ every minute at 20 ℃ for 1min.Equilibrium time is 0.25 min, max temperature is 325 ℃. Front SS inlet He non-shunt injection.Heater is 250 ℃, pressure 7.6522 psi, total flow 64 mL/min, spacer purge flow 3 mL/min, split outlet purge flow 60 mL/min maintain 1 min. GSV quantitative tube volume 1 mL, sample loading 0.5 min, injection 0.5 min. Chromatographic column flow rate 1mL/min, Chromatographic outlet pressure 0 psi. MSD initial temperature is 50 ℃ pressure 0.5735 psi flow rate is 1 mL/min Average linear velocity is 36.445 cm/sec, residence time is 1.3719 min.MSD output and MSD transmission line is 250 ℃. MS ion source is 230 ℃, four lever is 150 ℃.

2.3. Volatile matter analysis and screening

The mass spectrum data were obtained by GC-MS analysis and searched in NIST08. L library to determine the volatile components. The relative content was calculated by peak area normalization method with the all peak area as the index. The siloxanes and a small amount of impurity peaks produced by the heating of the solid phase microextraction head, and the components with similarity less than 90 were eliminated.

3. Discussion and conclusion

3.1. Discussion

Ding Jiawen et al. and others used GC-MS technology to analyze 47 kinds of volatile components in the flower of Elaeagnus angustifolia. Among them, dimethyl phthalate, isopropyl myristate, ethyl cinnamate, decanoate and linalool had better attractant activity to longicorn beetle under appropriate ratio [2]. Wang Baixin et al. studied the volatile components of healthy Pinus massoniana Lamb. By using gas chromatography-mass spectrometry, seven components were screened out, including (1R) - (+) - α - pinene, β - pinene, myrcene, Terpinene, isohexene, myrcene, (1R) - (+) - α - longleaf pinene and caryophyllene. Monochamus alternatus had strong reaction to (1R) - (+) - α - pinene in healthy P.
Among the attractants prepared by Wei Dan [4] and others, the attractant containing *Elaeagnus angustifolia*, (E)-2-hexenal, (z) - 3-Hexen-1-ol was the best. Ma Yan et al. used dynamic headspace method to investigate the effect of *Juglans regia* on the behavioral response of *Apriona germari*. The results showed that terpenes and aromatic compounds were the most of the volatiles released from *Juglans mandshurica* under different damage degrees, and there were significant differences between them. *A. germari* had physiological and electrical responses to pinene, Folin acetate, nonanal, α - terpineol, 2-ethylhexyl acrylate and n-hexadecane released from *Carya cathayensis* under the damage state [5].

### 3.2. Conclusion

Four kinds of plant volatile compounds were collected under SPME conditions, and the total ion flow chromatogram of volatile compounds was obtained by GC-MS analysis. The volatile compounds were identified by GC-MS and NIST08. L library. Table 1 show the results of volatiles from experimental plants. A total of 79 volatile compounds were identified, including 37 terpenes, 20 esters, 6 alcohols, 5 olefins, 3 ketones and 8 alkanes. There are 8 kinds of the same volatiles in the four species (+)-Limonene, β-Caryophyllene, (-)-α-cubebeine, (-)-α-muurolene, (-)α-Copaene, β-pinene, Styrene, and Methyl 2-ethylhexanoate.

Plants attract insects by volatilizing specific volatiles. At the same time, insects also identify and locate plants by volatiles, thus forming a symbiotic relationship. The volatiles of plants are mainly composed of some small molecular weight substances, such as alcohols, esters, terpenes, etc., which constitute their special marker volatiles in a certain proportion. The results showed that the relative content and species of terpenes were very rich in the five species. Terpenes were important products in plant secondary metabolism. They were mainly composed of isoprene, which were divided into chain and ring structures. According to the number of isoprene, they were divided into monoterpenes, diterpenes, triterpenes, sesquiterpenes and so on. The terpenes in the species are monoterpenes and sesquiterpenes. Under the coffee cultivation mode, the volatile compounds of plants in the long-term growth process are similar, which has a certain impact on the selective feeding of insects. Further field experiments are needed to identify whether the selected volatile compounds are attractive to insects.

#### Table 1. Summary of volatile compounds in experimental plants.

| retention time(min) | Name               | Molecular formula | Type            | Relative content (%) |
|---------------------|--------------------|-------------------|-----------------|----------------------|
|                     |                    |                   |                 | C.a  | A.h  | P.g  | A.s  |
| 13.69               | Limonene           | C_{10}H_{16}      | Terpenes        | --   | 28.3 | 4    | 2.13 | 9    |
| 13.83               | (+)-Limonene       | C_{10}H_{16}      | Terpenes        | 29.3 | 58.3 | 6.02 | 6    |
| 10.65               | S(-)-Limonene      | C_{10}H_{16}      | Terpenes        | --   | 0.61 | --   | --   |
| 28.28               | β-Caryophyllene    | C_{15}H_{24}      | Terpenes        | 21.1 | 30.3 | 2.40 | 4    |
| 27.06               | α-Panasinsanene    | C_{15}H_{24}      | Terpenes        | --   | 0.25 | 4    | 6    |
| 22.35               | (+)-Cyclosativene  | C_{15}H_{24}      | Terpenes        | --   | 0.10 | --   | --   |
| 33.05               | Caryophyllene      | C_{15}H_{24}      | Terpenes        | --   | 0.54 | 4    | 0.41 |
| 25.24               | (-)-α-Cubebeine    | C_{10}H_{16}      | Terpenes        | 0.62 | 0.63 | 0.24 | 4    |
| 27.02               | (-)-α-Gurjunene    | C_{15}H_{24}      | Terpenes        | --   | 0.85 | 0.36 |    |
| 30.11               | (-)-α-Muurolene    | C_{15}H_{24}      | Terpenes        | 0.34 | 0.24 | 0.32 | 0.40 |
| 8.37                | (1R)-(+)α-Pinepine | C_{10}H_{16}      | Terpenes        | 0.84 | 0.69 | 0.06 | --   |
| 25.56               | (-)-α-Copaene      | C_{15}H_{24}      | Terpenes        | 1.11 | 0.84 | 8.41 | 1.92 |
| 15.23               | dimethylolcta-1,3,6-triene | C_{10}H_{16} | Terpenes        | --   | 0.27 | --   | --   |
| Compound                  | Formula | Type      | Percentage 1 | Percentage 2 | Percentage 3 | Percentage 4 |
|---------------------------|---------|-----------|--------------|--------------|--------------|--------------|
| β-Bisabolene              | C₁₅H₂₄  | Terpenes  | --           | 0.17         | 0.09         |              |
| (Z)-β-Ocimene             | C₁₀H₁₆  | Terpenes  | --           | 2.01         | 0.48         | --           |
| (Z)-α-Bisabolene          | C₁₅H₂₄  | Terpenes  | 0.21         | 1.12         | 0.73         |              |
| (-)-Isocaryophyllene      | C₁₅H₂₄  | Terpenes  | --           | 0.60         | --           |              |
| (−)-allo-Aromadendrene    | C₁₅H₂₄  | Terpenes  | --           | 0.71         | 0.51         |              |
| α-Selinene                | C₁₅H₂₄  | Terpenes  | 3.72         | --           | 8.22         | 4.01         |
| (α-Caryophyllene          | C₁₅H₂₄  | Terpenes  | 1.28         | --           | 3.34         | 1.23         |
| α-Piene                   | C₁₀H₁₆  | Terpenes  | --           | 1.16         | 0.11         | 0.30         |
| Azulene                   | C₁₀H₁₄  | Olefins   | 1.71         | 0.75         | 0.99         | --           |
| γ-Terpinene               | C₁₀H₁₆  | Terpenes  | --           | 0.16         | 0.06         |              |
| α-Terpinene               | C₁₀H₁₆  | Terpenes  | --           | --           | 0.29         |              |
| Cyclooctatetraene         | Cs₈H₈   | Olefins   | 1.33         | --           | 0.65         |              |
| Aromadendrene             | C₁₅H₂₄  | Terpenes  | 1.64         | --           | 3            |              |
| α-Longipinene             | C₁₅H₂₄  | Terpenes  | --           | --           | --           | 0.08         |
| (+)-Camphene              | C₁₀H₁₆  | Terpenes  | 0.25         | --           | --           | --           |
| Styrene                   | C₈H₈    | Olefins   | 0.63         | 0.76         | 0.22         | 0.38         |
| (-)-Camphene              | C₁₀H₁₆  | Terpenes  | 0.31         | --           | --           |              |
| (-)-Calamenene            | C₁₅H₂₂  | Olefins   | 0.35         | 0.16         | 0.56         |              |
| Triene neo-alloocimene    | C₁₀H₁₆  | Terpenes  | --           | 0.24         | 0.10         | --           |
| (-)-β-Bourbonene          | C₁₅H₂₄  | Terpenes  | --           | --           | 0.28         | --           |
| Dodecane                  | C₁₂H₂₆  | Alkenes   | 1.08         | --           | --           | --           |
| Tridecane                 | C₁₃H₂₈  | Alkenes   | 0.42         | --           | --           | --           |
| Tetradecane               | C₁₄H₃₀  | Alkenes   | 1.88         | --           | --           | 0.15         |
| Pentadecane               | C₁₅H₃₂  | Alkenes   | 1.31         | 0.35         | --           | 0.30         |
| Hexadecane                | C₁₆H₃₄  | Alkenes   | 0.59         | --           | --           | --           |
| N-heptadecane             | C₁₇H₃₆  | Alkenes   | 0.34         | --           | --           | 0.30         |
| Icosane                   | C₂₀H₄₂  | Alkenes   | 0.06         | --           | --           | 0.05         |
| β-Cubebene                | C₁₅H₂₄  | Terpenes  | --           | --           | 0.21         | 0.23         |
| β-Myrcene                 | C₁₀H₁₆  | Terpenes  | --           | --           | 0.65         | 0.15         |
| 1,5-dimethyl-1,5-cyclooctadiene | C₁₀H₁₆ | Terpenes  | --           | --           | 6.44         | --           |
| m-cymene                  | C₁₀H₁₄  | Olefins   | --           | 0.23         | --           | --           |
| Diisobutyl phthalate      | C₁₆H₂₂O₄ | Esters    | 4.45         | --           | 0.60         | 2.30         |
| Methyl 2-ethylhexanoate   | C₈H₁₈O₂ | Esters    | 2.72         | 0.21         | 0.06         | 0.22         |
| Methyl 2-ethylheptanoate  | C₁₀H₂₀O₂ | Esters    | 0.45         | --           | --           | 0.23         |
| Ethyl benzoate            | C₈H₁₀O₂ | Esters    | 5.16         | --           | --           | --           |
| Ethyl phenylacetate       | C₁₀H₁₀O₂ | Esters    | 3.15         | --           | --           | --           |
| Ethyl palmitate           | C₁₈H₃₆O₂ | Esters    | 2.80         | --           | --           | --           |
| Ethyl tetradecanoate      | C₁₆H₃₂O₂ | Esters    | 2.46         | --           | --           | --           |
| Ethyl laurate             | C₁₄H₂₈O₂ | Esters    | 0.71         | --           | --           | --           |
| No.  | Compound Description                  | Molecular Formula | Type    | Relative Response |
|------|--------------------------------------|-------------------|---------|-------------------|
| 16.38| Methyl salicylate                     | C₈H₈O₃            | Esters  | 0.60              |
| 7.45 | Methyl 2,4-dimethylpentanoate         | C₉H₁₆O₂            | Esters  | --                |
| 21.72| Ethyl 3-phenylpropanoate              | C₁₁H₁₄O₂           | Esters  | 0.85              |
| 23.25| Ethyl caprate                         | C₁₂H₂₆O₂           | Esters  | 0.31              |
| 19.99| Ethyl nonanoate                       | C₁₁H₂₂O₂           | Esters  | 0.28              |
| 13.46| Phenylethyl alcohol                   | C₈H₁₀O             | Esters  | 1.03              |
| 13.45| Oct-1-en-3-ol                        | C₈H₁₀O             | Esters  | --                |
| 9.56 | Decane                               | C₁₀H₁₈O            | Alkenes | --                |
| 3.4- | Dimethylacetophenone                 | C₁₀H₁₂O            | Ketones | 0.62              |
| 48.91| 2-Hexyl-1-decanol                    | C₁₆H₁₄O            | Esters  | 0.07              |
| 28.77| Linalool                             | C₁₀H₁₄O            | Esters  | 0.98              |
| 11.56| (-)-β-Pinene                         | C₁₀H₁₆              | Terpenes| --                |
| 10.99| β-Pinene                             | C₁₀H₁₆              | Terpenes| 2.10              |
| 32.71| (+)-Calarene                         | C₁₅H₂₄              | Terpenes| --                |
| 26.93| 2-Ethylhexanol                       | C₈H₁₈O             | Esters  | --                |
| 11.75| Acetophenone                         | C₈H₁₄O             | Ketones | 0.78              |
| 35.12| (+)-Carvone                          | C₁₀H₁₄O            | Ketones | 0.51              |

Note: C.a: Coffea arabica L.; A.h: Artocarpus heterophyllus Lam.; P.g: Psidium guajava Linn.; A.s: Alstonia scholaris (L.) R. Br.; --: means the volatile compounds has not been detected.

References

[1] Xiao Dan 2015 Application and Prospect of headspace solid phase microextraction [J]. China Health Engineering 14(01) 88-92
[2] Ding Jiawen, Liu Qiang, Zhu Gengping, et al. 2014, EAG and behavioral responses of Cerambycidae hongyuanensis to eight compounds in Elaeagnus angustifolia [J]. Journal of Tianjin Normal University (NATURAL SCIENCE EDITION) 34(02) 71-75
[3] Wang Baoxin, Xu Xiulan, Xiao Qiangang, et al. 2019 EAG response of Monochamus alternatus to volatiles from healthy Pinus massoniana [J]. Agricultural staff (19) 103-104
[4] Wei Dan, Qin Qin, Xun Feng, et al. 2012 EAG and behavioral response of longicorn beetle to different attractants and field trapping effect [J]. Journal of Tianjin Normal University (NATURAL SCIENCE EDITION) 32(03) 71-74 + 95
[5] Ma Yan, Shi Liyang, Zhao Yi, et al. 2018 Comparison of volatile components of Carya cathayensis under different damage conditions and GC-EAD and behavioral response of Apriona germari to its components [J]. Acta Entomologica Sinica 61(05) 574-584