α-蒎烯对黄曲条跳甲的拒避作用

Sundufu Abu James[1], 龚恒亮[2], 黄寿山[1*],
(1. 华南农业大学昆虫生态研究室, 广州 510642; 2. 广州甘蔗糖业研究所, 广州 510316)

摘要: 拒避性异种他感物质有可能作为 21 世纪对环境友好的植物保护剂用于农业害虫的防治。采用四臂嗅觉仪、室内笼罩试验和田间试验相结合的方法, 研究了松节油的主要成份 α-蒎烯, 对黄曲条跳甲的拒避作用, 并用干扰作用控制指数方法进行了评价。结果表明: 在实验室, 不同的浓度下, 其拒避作用最大可达 95.45%, 相应的干扰作用控制指数(IIPC)为 0.05; 在笼罩试验条件下, 最大拒避作用的强度为 91.60%, IIPC 为 0.09; 而田间小区试验中, 最大拒避作用下降到 54.48%, IIPC 则为 0.46。拒避作用与浓度正相关。采用上述系列方法有助于开展拒避性植物保护剂的规范化研究。

关键词: 黄曲条跳甲; α-蒎烯; 拒避作用; 四臂嗅觉仪; 干扰作用控制指数

Repellency of α-pinene against stripped flea beetle, *Hyllotreta striolata* (F).

Sundufu Abu James[1], GONG Heng-Liang[2], HUANG Shou-Shan[1*] (1. *Lab. of Insect Ecology, South China Agricultural University, Guangzhou* 510642, *China*; 2. *Sugarcane Research Institute of Guangzhou, Guangzhou* 510316, *China*). ***Acta Ecologica Sinica*, 2003, 23(2): 303~307.**

Abstract: Repellant allelochemicals could be the 21st century environmentally friendly plant protectants applied in agricultural pest control. α-pinene, a monoterpene, was evaluated for repellent activity against *Phyllotreta striolata* in a four-armed olfactometer, flight-chamber and field tests. In the laboratory, repellency reached 95.45% with a corresponding IIPC of 0.05 in olfactometry; in the flight-chamber trial, repellency and IIPC were 91.60% and 0.09, respectiviely. In the field, however, repellency and IIPC were reduced to 54.48% and 0.46, respectively. Overall, dilution-dependent repellency was exhibited in all methods; and increasing dilution elicited decreased repellency and hence protection.

Key words: *Phyllotreta striolat*; α-pinene; repellence, olfactometer; interference index of population control
文章编号: 1000-0933(2003)02-0303-05 **中图分类号**: Q968, S186 **文献标识码**: A

1 Troduction

Volatile phytochemicals apparently play an important role in tritrophic systems that include the host plant, herbivore, and parasitoid (or predator). Knowledge of such systems which demonstrates that allomonic volatiles are important means by which some plants are antixenosic towards potential guests is well known[1]. In particular, repellent activities of essential oils and their derivatives toward various insects such as boll weevils, mosquitoes, red flour beetles, rice weevils, German cockroaches, houseflies and mites have been extensively reported[2~4]. We therefore report on the repellency of α-pinene, a monoterpene, against stripped flea beetle (SFB), *Phyllotreta striolata* (F) (Coleoptera: *Chrysomilidae*),

Foundation item: The research was supported by the National Natural Science Foundation of China (No. 39930120)
* Author for correspondence
Received date: 2002-02-07; **Accepted date**: 2002-08-22
Biography: Sundufu Abu James, Master student. Area of interest: Insect Ecology.
Acknowledgements: We wish to thank all contributors

the result of which could be used as a promising alternative for protecting cruciferous plants from SFB attack.

2 Materials and methods

Insect Adult *Phyllotreta striolata*, used in the laboratory experiments, was collected from cabbage plants in the farm of South China Agricultural University.

Chemical The studied phytochemical, α-pinene [(1R,5R)-2,6,6-trimethylbicyclo (3.1.1) hept-2-ene], which is component of turpentine oil from pine tree (*Pinaceae*), was kindly provided by Dr. Gong Hengliang of the Ginone Agrochemical Application Technical Research Institute of Guangzhou.

Olfactometry The olfactometer used in this study was initially designed by Vet *et al.*[5]. A first series of experiments was conducted to determine beetles repellence of odours from α-pinene (0., 2 E.C) diluted in series 200x, 400x and 800x, a vial with humidified air served as control. With the aid of an air-pressure (sucked at −15mmHg), four air streams of 300ml/min were introduced in the central diaper region through the flow meters into each of the different arms of the olfactometer. Fifty adult beetles were individually introduced into the chamber through the exit hole and could make two types of choice. The first choice was made when a beetle reached the arbitrary transverse diagonal of one of the odour compartment.

Before the first choice was made, a beetle was given 2 min. in the exposure chamber, at the end of which it was regarded as unmotivated and not recorded. The remaining 8 min. was allocated for the second choice that was recorded as the beetle stayed in any of the odour compartments beyond the first choice line. To neutralize any asymmetry in the experimental setup, the vials containing odour sources were orientated after testing 10～15 beetles followed by thorough cleaning of the chamber with 99.7% ethanol absolutely and allowing the solvent to dry for 5～10 min. The experimental environment was at 25℃ and 60%～70% *RH*.

Olfactory responses were analysed using one-variate *T*-test (SPSS software, version 10); the degree of repellency was calculated based on the following formula[6]:

Degree of repellency = $[1-(A/B)] \times 100\%$

Where: A = number of beetles in treatment; B = number of beetles in control

The Interference Index of Population Control (IIPC) for evaluating the effect of factors repelling insects away from plants was employed[7]. This index is calculated thus:

$IIPC = NTr/NCk$

Where: NTr = number of beetles recorded in treatment; NCk = number of beetles recorded in control.

Flight-chamber experiment A second series of tests was conducted to determine the repellence potential (degree of degree of repellence) by α-pinene diluted serially (200×, 500×, 1000×, 1500×, 2000×) of the Chinese vegetable (*Brassica campestris* L. var. *utilis*). All tests were carried out at room temperature and lasted for 24 hrs. Viable seeds were sown in 9-cm diameter Petri dishes; after germination (unto the two-leaves stage), the seedlings were thinned to two per dish. The treatments were in 4 replicates with 6 Petri dishes in each replication. Treatment with distilled water served as control. In all experiments, groups of 6 dishes were distributed every 10cm in a rectangular design within a white-cloth tent cage. Overall, 72 female beetles were introduced into the seedlings, with each treatment dish receiving 3 beetles. Since adult beetles chew small holes through the under surface of leaves, the number of round shot-holes was chosen as a measure of protection afforded by the chemical. Summary statistics was computed using the SAS package (SAS Inc., version 6.12) and the degree of protection and IIPC calculated as stated above.

Field experiment　The experimental plots were located at the plant protection experimental site, South China Agricultural University, Guangzhou, China.

Three 8m × 1.5m plots were laid out as replication. The treatments consisted of α-pinene in serial dilutions [200 × (2.5ml), 400 × (1.25ml), 600 × (0.625ml)] and water (500ml) as control; each treatment replicated 3 times, assigned in a randomized block (replicates) were separated from one another by 0.5m.

One-quarter of a $33m^2$ quadrat was used as the basic sampling unit through out the study. For population census, each of the 4 sub-plots (2 × 0.5m) of the 3 plots (replicates) was divided into 3 quadrats. Direct counting of beetles was made twice a day (10:00 a.m and 4:00 p.m) for three consecutive days. Data were subjected to ANOVA followed by separation of the means that used the Fisher's LSD test (SAS Inc., version 6.12).

3 Results and discussions

Pesticides developed on the basis of active molecules obtained from natural products are considered to be more biodegradable, non-mutagenic, and non-toxic to homeotherms, and harmless to predators and parasites, than those synthesized directly on the basis of petrochemical derivatives[8]. A few of them are also reducers or inhibitors of insecticide detoxifying enzyme in insects[9]. Natural products such as plant extracts and essential oils have been screened and identified to contain bioactive agents as repellent against insect pests[9]. The present study demonstrated that α-pinene, amonoterpene, possess a strong repellent property against *P. striolata*. Results from the olfactmetry revealed that, at both choices, the beetles significantly showed preference for the compartment with humidified air over those with serial dilutions of the stimulant (table 1). Au understanding of odour dispersion is a requisite for an accurate interpretation of odour-induced behaviours[17]. As odour molecules disperse from a source, the odour concentration in the air declines with increasing distance. With the test stimulant serially diluted (200x, 400x and 800x), the number of beetles expected within the various compartments should positively correlate with an increase in serial dilution. However, such a trend was not observed at the first choice level. The observed trend might be highly correlated with the sector of the entry tube via which the beetle first approached the chamber floor. In contrast, the number of beetle in the odour compartments demonstrated positive trend with increasing dilutions at the second/final choice level; this result is in consonance with the degree of repellency and IIPC calculated. The stimulant diluted 200x elicited 95.45% repellency at the second/final choice level as compared to 59.09% and 40.91% for 400x and 800x respectively. IIPC revealed that at the first choice, with α-pinene diluted 200x, 400x and 800x, the population of SFB was decreased 0.61, 0.78, and 0.39 times respectively, and, at the second choice level, the decrease was 0.05, 0.40, and 0.59. Overall, the interference of α-pinene evidently decreased the population of SFB (0.002) as against the control and the stimulant diluted 200x being relatively the most effective.

Although data collected at the second choice level seemed reliable when using the 4-armed olfactometer in behavioural assays, however, recent studies made used of the cumulative time spent among the 4 arm zones of the olfactomter[10]. The cumulative time spent at the 4 compartments significantly differ; SFB spent more time in the air compartment than in odour sources diluted 800x, 400x and 200x with lesser time spent in the last odour field.

In the flight-chamber set-up, with different sets of dilution, the repellence potential of α-pinene diluted 200x was maintained (table 2). This was also confirmed from results of the field trial (table 3). Here, because of poor germination, the number of seedlings within the subplots (not recorded) was not

Table 1 Response of adult stripped flea beetle, *Phyllotreta striolata*, to odour of α-pinene in an olfactometer

Concentration	*N*	200×	400×	800×	A×	*P*
1st choice	50	11	14	7	18	0.01*
Degree of repellency		38.89	22.22	61.11	—	
IIPC		0.61	0.78	0.39	—	
2nd choice	45	1	9	13	22	0.01*
Degree of repellency		95.45	59.09	40.91	—	
IIPC		0.05	0.40	0.59	—	
Mean % time spent/field	50	6.63	19.91	29.53	43.94	0.12*

N = Total number of beetles that expressed the first choice or both choices. * One-variate *t*-test ($P<0.05$). Degree of repellency and IIPC calculated from the formulae above

evenly distributed. This might be responsible for the non-significant differences amongst some treatments. Comparatively, however, subplots with α-pinene diluted 200x had significantly fewer number of beetles. Within the plots, the number of beetles recorded in plot 3 was lower because following treatment application, most of the beetles made for the near by untreated plot. The degree of repellence and IIPC indicated similar trend with increasing dilution. This result appears to corroborate a recent survey which recorded ecological effectivity of terpenoids that shows them universally to be concentration or dosage dependent[11], either as individual components or with respect to their total quantity.

Table 2 Degree of propection of *B. compestris* by α-pinene in a flight-chamber trial

Treatment	200×	500×	1000×	1500×	2000×	Water(CK)
$\bar{x}$	4.75±2.21c	7.75±1.93c	11.74±1.93	19.00±7.76bc	30.50±7.54b	53.75±2.56a
Degree of repellency	91.6	85.58	78.14	64.65	43.26	—
IIPC	0.088	0.144	0.218	0.353	0.567	—

Mean (±S.E) shot-holes in the leaves, followed by the same letters are not significantly different at $\alpha=0.05$ (LSD). Degree of Protection is computed similarly as the degree of repellency above.

Table 3 Adult stripped flea beetle, *Phyllotreta striolata*, in plots treated with α-pinene

Treatment	200× (2.5ml)	400× (1.25ml)	800× (0.63ml)	Water (500ml)
$\bar{x}$	9.04±2.49b	14.01±2.90ab	19.87±3.85a	19.86±4.81a
Degree of repellency	54.48	29.46	0.05	—
IIPC	0.455	0.705	1.001	—

Means followed by the same letter are not significantly different at $\alpha=0.05$ (LSD).

Apparently, the effect of the monoterpene, α-pinene, on insects is well documented. They affect the behaviour of a variety of insects and act as attractants, oviposition stimulants, or defenses[12,13]. Non-host volatiles nonanal and α-pinene inhibited attraction of male Cherry Bark Tortrix, *Enarmonia formosana*, to traps baited with synthethic pheromone and deterred oviposition by gravid female CBT[14]. Besides its effect on insects, α-pinene and other monoterpenes are potent inhibitors of seed germination and growth of several plant species[16].

Repellent and/or deterrent chemicals may play a major role in the unsuitability of non-host plants as food for insects. The elucidation of these chemicals is important not only for understanding the ecological aspect of insect-plant relationships, but also for their potential in pest control. We believe that the result of this study could contribute towards circumventing the problem of SFB resistance to synthetic insecticides, particularly in the South of China where SFB infestation is a major problem.

References

[1] Vet L E M and Dicke N. Ecology of infochemicals used by natural enemies in a tritrophic context. *Ann. Rev.*

Ent., 1992, **37**: 141～172.

[2] Guardiola V G, De Minguel P, Primo E and De Minguel P. Repellent activity against *Blattella germanica* of components of *Schinus molle* L. *Rev. Agro. Tecno. Alimen.*, 1990, **30**: 341～346.

[3] Kraus B, Koeniger N and Fuchs S. Screening of substances for their effect on *Varroa jabsoni*: Attractiveness, repellency, toxicity and masking effects of ethereal oils. *J. Apicul. Res.*, 1994, **33**: 34～43.

[4] Singh D and Singh A K. Repellent and insecticide properties of essential oils against the housefly, *Musa domestica* L. *Insect Sci. Applic.*, 1991, **12**: 487～491.

[5] Vet L E M, Van Lenteren J C, Heymans M, *et al.* An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiol. Entomol.*, 1983, **8**: 97～106.

[6] Mumcuoglu K Y, Galun R, Bach U, *et al.* Repellency of essential oils and their components on human body louse, *Pediculus humanus humanus*. *Entomol. Exp. Appl.*, 1996, **78**: 309～314.

[7] Pang X F, Liang G W and Zeng L. Evaluation of the effectiveness of natural enemies. *Acta Ecologica Sinica*, 1984, **4**(1): 46～56.

[8] Kulkarni R A. The future of pest control and pesticide industry. *Pure Appl. Chem.*, 1986, **58**: 917～924.

[9] Brattsten L B, Holyoke C W, Leeper J R. Insecticide resistance: Challenge to pest management and basic research. *Science*, 1986, **231**: 1255～1260.

[10] Singh D, Siddiqui M S and Sharma S. Cedarwood oil as a potential insecticidal agent against mosquitoes. *Naturwiss*, 1984, **71**: 265～266.

[11] Manjunatha M, Pickett J A, Wadhams L J, *et al.* Response of flower thrips, *Frankliniella occidentalis* and its predator *Amblyseius cucumeris* to Chrysanthemum volatiles in olfactometer and greenhouse trials. *Insect Sci. Applic.*, 1998, **18**(2): 139～144.

[12] Harborne J B. Recent advances in the ecological chemistry of plant terpenoids. In: Harborne J B and Tomes-Barberan F A, ed. *Ecological Chemistry and Biochemistry of Plant Terpenoids.* Oxford: Clarendon Press, 1991a.

[13] Honda K. Chemical basis of differential oviposition by lepidopterous insects. *Arch. Insect Biochem. Physiol.*, 1995, **30**: 1～23.

[14] Speight M R and Wainhouse D. Ecology and management of forest insects. Clarendom Press, Oxford, 1989. 374.

[15] Crawford M, Gerhard G and Regine G. Cherry Bark Tortrix, Earmonia formosana: Olfactory recognition of and behavioural deterrence by nonhost angio- and gymnosperm volatiles. *J. of Chemical Ecology*, 2000, **26**(4): 809～821.

[16] Langenheim J H. Higher plant terpenoids: A phytocentric overview of their ecological roles. *J. of Chemical Ecology*, 1994, **20**(6): 1223～1270.

[17] Elkinton J S and Carde'R T. Odour dispersion. In: Bell W J and Carde'R T, eds. *Chemical Ecology of Insects.* Massachusetts: Chapman and Hall Ltd, 1984.

参考文献

[7] 庞雄飞，梁广文，曾玲. 昆虫天敌作用的评价. 生态学报，1984，4(1)：46～56.