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# Role of Semiochemicals in Prey-Locating Behavior of a Generalist Predatory Stink Bug, *Eocanthecona Furcellata* (Wolff) (Heteroptera: Pentatomidae)

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**Abstract**

Behavioral responses of a generalist predatory stink bug, *Eocanthecona furcellata* towards larvae of several lepidopteran species were observed in order to analyze the role of semiochemicals in cliciting a prey-locating behavior in this bug. Extracts of *Spodoptera litura* larvae elicited approaching behavior in *E. furcellata*. A hexane-eluted fraction from silica gel chromatography of the larval extracts was also attractive to the bugs. The hexane fraction contained *n*-tetradecane, *n*-pentadecane, n-haptadecane, *n*-heptacosane, *n*-nonacosane, *n*-hentriacontane and 2, 6, 10, 15, 19, 23-hexamethyl-2, 6, 10, 14, 18, 22-tetracosahexaene (squalene). Synthetic *n*-pentadecane was attractive to the bugs. On the other hand, a 15%-ether-in-hexane-eluted fraction from silica gel chromatography of the solvent extracts of S. litura stimulated bugs to display a proboscis-protruding behavior. (*E*) -3, 7, 11, 15-Teramethyl-2-hexadecen-1-ol [(*E*)-phytol] was identified in this fraction, and synthetic (*E*)-phytol showed the same effect on the bugs as the fraction. (*E*)-phytol content of larvae increased depending on the amount of chlorophyll in the diets. These results suggest that (*E*)-phytol in larvae, which is derived from chlorophyll in the prey diet, acts as an important cue in the prey-locating behavior of the generalist predatory stink bug, *E. furcellata*. In conclusion, the 2 different chemicals act as "kairomones" in the prey location for the predatory stink bug, *E. furcellata*.

**Discipline: Insect pest**
**Additional key words:** *n*-pentadecane, (*E*)-phytol, attractant, proboscis-protruding inducer, chlorophyll, kairomone

## Introduction

Many synthetic organic pesticides have contributed to the improvement of mass production of agricultural crops. However, unsuitable use for pest management has caused severe side-effects such as the development of pesticide resistance in pest insects, accumulation of residues in crops and agricultural environments. Under these circumstances, suitable use of natural enemies as one of the technical skills for the integrated pest management (IPM) is attracting worldwide attention.

Natural enemies of herbivorous insects use chemical, visual, acoustic and vibrational cues in foraging behavior. Especially, the importance of semiochemicals in foraging by parasitoids has been well documented[6]. Some studies have suggested the possibility of applying semiochemicals in pest management: by attracting and/or arresting the parasitoids in crop fields, or by enhancing the responses of parasitoids to hosts[6,8,16].

Some investigators have suggested that predators could be applied to pest management against various pest insects[6]. Since predators in general kill the pest insects immediately after catching them, an immediate effect of application of the predators in pest management is expected while parasitoids usually require a long period of time for killing pest insects. A few studies have revealed that chemicals related to prey insects are important cues in foraging for some predators. The eastern yellowjacket, *Vespula maculifrons*[1], the cylindrical bark beetle, *Lasconotus intricatus* Kraus[2], *Enoclerus lecontei* (Wolcott)[9], *Temnochila chlorodia* Mannerheim[15] and *Mederera bistriata* Parent[18] are attracted to pheromone components of their respective preys. Lewis et al.[7] reported that larvae of the green lacewing, *Chrysopa carnea*, use "kairomones" in scales of a moth, *Heliothis zea*. Hislop & Prokopy[4] reported that acarine predators, *Amblyseius fallacis* and *Phytoseiulus macropilis*, respond to silk and feces of a prey mite, *Tetranychus urticae*. However, these studies deal with the relationships

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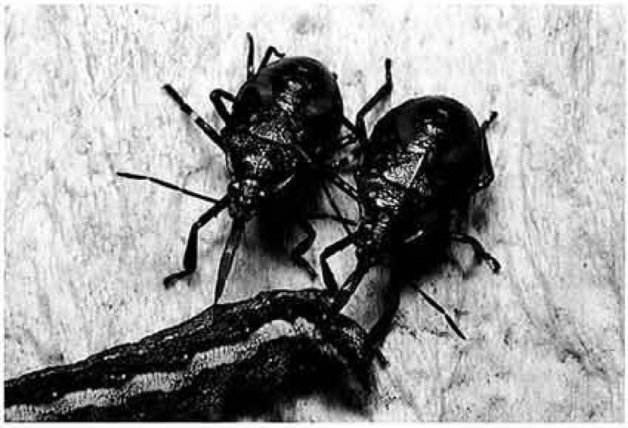

Fig. 1. Nymphs of *Eocanthecona furcellata* feeding on larvae of *Spodoptera litura*

between a predator and its specific prey.

The predatory stink bug, *Eocanthecona furcellata* (Wolff) (Heteroptera: Pentatomidae) (Fig. 1), is a generalist predator that feeds on larvae of Lepidoptera, Coleoptera and Heteroptera and is distributed in India, Southeast Asia, the southern part of China, Taiwan and the Okinawa region of Japan[3,5,10,13]. Takai & Yasuoka[14] demonstrated that *E. furcellata* could be a good biological control agent against the noctuid *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in greenhouses. The bugs can be easily reared on live larvae of *Pieris rapae*[3] and frozen-preserved larvae of *S. litura*[22].

Over the last several years, I conducted a series of studies on the prey-locating behavior of *E. furcellata*, and identified 2 chemical cues eliciting this behavior[19,21,23]. In this report, I summarized the results of these studies.

## Prey-locating behavior of *E. furcellata*

*E. furcellata* bugs were found to approach intact larvae of *S. litura*, and the bugs protruded their proboscis when they were in close vicinity to the larvae of *S. litura* and inserted it through the body surface of preys. This behavior was observed under contact illumination as well as under continuous darkness. A similar behavior was also elicited by dead larvae or solvent extracts of *S. litura* larvae. These findings indicated that odor, or chemical cue(s), from the prey apparently induced the prey-locating behavior of the predatory stink bug.

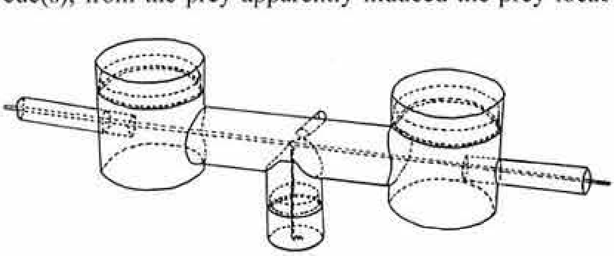

Fig. 2. Linear track olfactometer

### *1) Approaching behavior*

To evaluate the attractiveness of the larval extracts, a linear track olfactometer, which was designed according to Sakuma & Fukami[11] but with minor modifications[23], was used. The olfactomer consisted of a T-shaped wind tunnel and wire structures (Fig. 2). In this bioassay, test insects were made to climb up to a T-junction on the wire and choose a direction at one point. When a small piece of absorbent cotton impregnated with a crude extract of *S. litura* larvae (in 100-μL acetone) was placed at one of the horizontal ends of the T-shaped wind tunnel (sample side) and a small piece of absorbent cotton impregnated with 100 μL of acetone was placed at the other end (control side), 52 bugs chose the sample side and 15 bugs chose the control side. These results indicated that *E. furcellata* nymphs were apparently attracted to the crude extracts of *S. litura* larvae. *E. furcellata* nymphs were also found to be attracted to the ether-soluble layer of *S. litura* larvae (Fig. 3). When the ether-soluble layer of the larval extracts was fractionated on silica gel column chromatography, only the fraction eluted with hexane showed attractiveness.

When the hexane fraction was analyzed using GC-MS, one predominant peak and 6 minor peaks were observed. The predominant one corresponded to *n*-pentadecane ($C_{15}$)(2500 ng/larva). The minor peaks corresponded to *n*-tetradecane ($C_{14}$)(54 ng/larva), *n*-heptadecane ($C_{17}$)(41 ng/larva), *n*-heptacosane ($C_{27}$)(61 ng/larva), *n*-nonacosane ($C_{29}$)(147 ng/larva), *n*-hentriacontane ($C_{31}$)(200 ng/larva) and 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (squalene)(323 ng/larva).

The approaching responses of *E. furcellata* nymphs in the linear olfactometer to the hexane fraction of *S. litura* larvae and synthetic hydrocarbons are shown in Fig. 4. The hexane fraction induced a significant approaching response in bugs at a dose of 0.4 larval equivalents (LE) containing ca. 1 μg of $C_{15}$ in a linear track olfactometer. However, 1 μg of synthetic $C_{15}$ did not induce a comparable response. More than 10 μg of $C_{15}$ was necessary to attract *E. furcellata* nymphs, but no increasing effect was obtained by the addition of the other 6 components, $C_{14}$, $C_{17}$, $C_{27}$, $C_{29}$, $C_{31}$ and squalene, to $C_{15}$.

### *2) Proboscis-protruding behavior*

In a preliminary observation, *E. furcellata* nymphs protruded their proboscis toward the crude extracts of *S. litura* larvae. However they did not protrude their proboscis when they were in close vicinity to a small piece of absorbent cotton impregnated with the hexane fraction or synthetic $C_{15}$. It seemed that any chemical(s) could

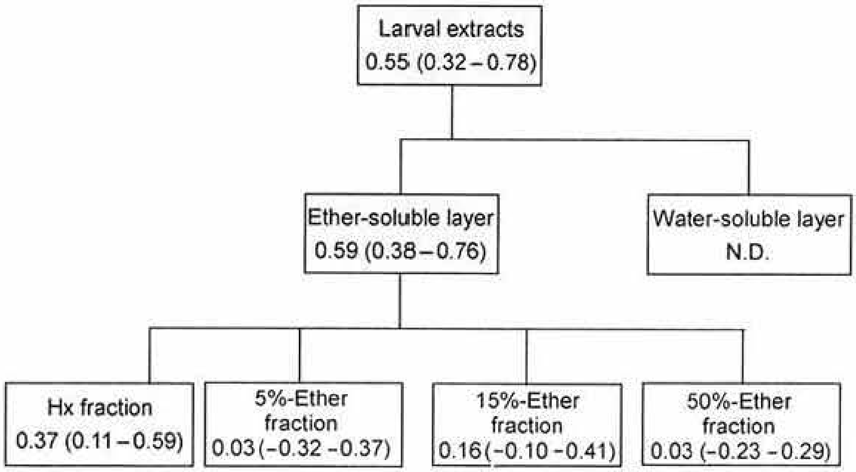


**Fig. 3. Chemical purification processes of the larval extracts of *Spodoptera litura* larvae together with attractiveness to nymphs of *Eocanthecona furcellata* in the linear track olfactometer**

Values represent excess proportion index (EPI)[11]. Positive values indicate a positive approach response. N.D.: not determined. Hx fraction: fraction eluted with hexane from silica gel chromatography. 5%-, 15%-and 50%-Ether fractions: fractions eluted with 5%-, 15%-and 50%-Ether in hexane from silica gel chromatography, respectively.

elicit a proboscis-protruding behavior in the bugs.

A 15%-ether-in-hexane fraction from silica gel chromatography of the solvent extracts of *S. litura* larvae elicited the proboscis-protruding behavior in *E. furcellata*

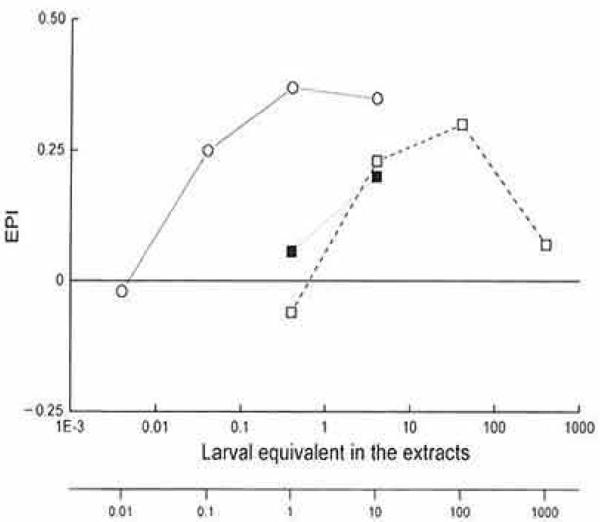


**Fig. 4. The approaching responses of *Eocanthecona furcellata* nymphs in the bioassay using the linear track olfactometer**

-○- the Hx fraction of solvent extracts of *Spodoptera litura* larvae.

-□- synthetic *n*-pentadecane ($C_{15}$).

■ a mixture of $C_{15}$ and 6 other hydrocarbons.

nymphs and a similar response was also observed with a neutral-layer fraction of the 15% fraction (Fig. 5).

Analysis of the neutral-layer fraction using GC-MS showed that it contained (*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol [(*E*)-phytol]. Synthetic (*E*)-phytol elicited a proboscis-protruding response from the predators as well as the 15% fraction (Fig. 6).

## Effect of prey diet on bug's prey-locating behavior

(*E*)-Phytol generally occurs in green plants as an alcoholic moiety of the chlorophyll molecule (Fig. 7)[12] and the amount of free (*E*)-phytol is usually small in plant tissues. However, this compound is obtained by hydrolysis of chlorophyll in the laboratory[17]. (*E*)-Phytol contents in larvae and in their feces were positively correlated with the chlorophyll content in the artificial diets (Fig. 8). Therefore, it is possible that food items for prey insects considerably influence the prey-locating behavior of the predatory stink bug, *E. furcellata*.

The bugs may prefer insects feeding on a chlorophyll-rich diet to those feeding on a chlorophyll-poor diet. When preference responses of *E. furcellata* nymphs to *S. litura* larvae fed with 2 food items, chlorophyll-rich spinach (*Spinacia oleracea*) leaves and bean sprouts (chlorophyll-poor seedlings of *Vigna radiata*), were observed, bugs preferred larvae fed with spinach leaves to those fed with bean sprouts (Fig. 9A): a total of 45

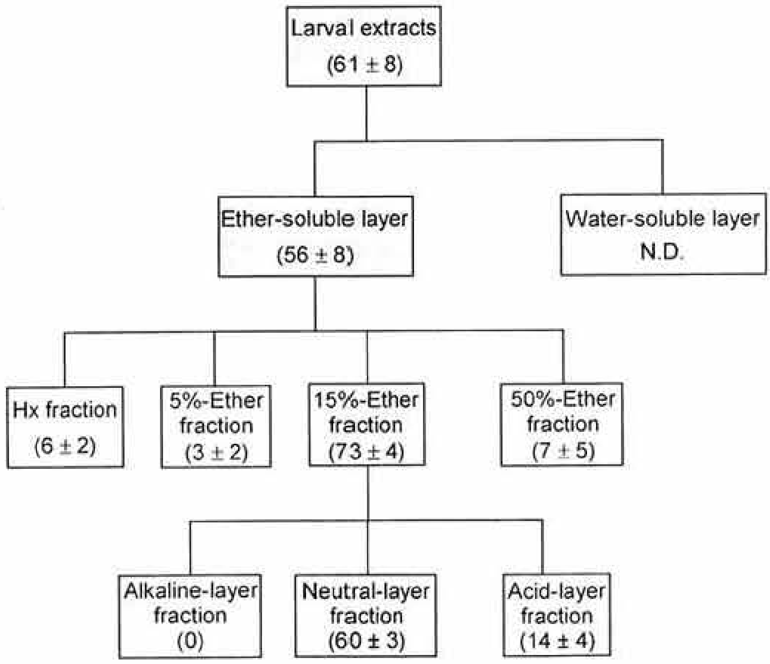


**Fig 5. Chemical purification processes of the larval extracts of *Spodoptera litura* larvae together with proboscis-protruding activity for nymphs of *Eocanthecona furcellata***

Values in the parentheses are mean (±s.e.) percentage of positive response per 10 bugs. N.D.: not determined.

nymphs chose the former and 5 nymphs the latter. Solvent extracts of *S. litura* larvae given spinach leaves contained 9 μg/larva of (*E*)-phytol, but less than 0.001 μg/larva in those fed with bean sprouts. When a *S. litura* larva fed with bean sprouts was coated with 10 μg of (*E*)-phytol and presented to the bugs together with a larva fed with spinach leaves, almost the same number of *E. furcellata* bugs was located on these larvae (Fig. 9B): a total of 24 nymphs chose an (*E*)-phytol-treated larva fed with bean sprouts and 26 nymphs chose a larva fed with spinach leaves. The results indicated that (*E*)-phytol is uti-

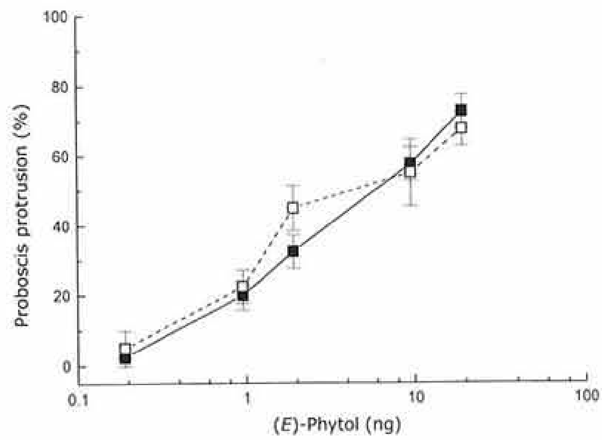


**Fig. 6. Dose-response in proboscis-protruding behavior of *Eocanthecona furcellata* nymphs**

■ the 15% fraction of the larval extracts of *Spodoptera litura*.

□ (*E*)-phytol.

Vertical bars indicated s.e.

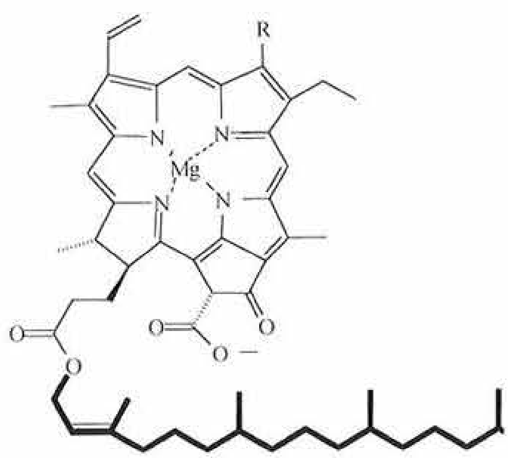


**Fig. 7. Chlorophyll molecule**

Formula presented with a bold line applies to the (*E*)-phytol moiety. R = $CH_3$ or CHO for chlorophyll a or b, respectively.

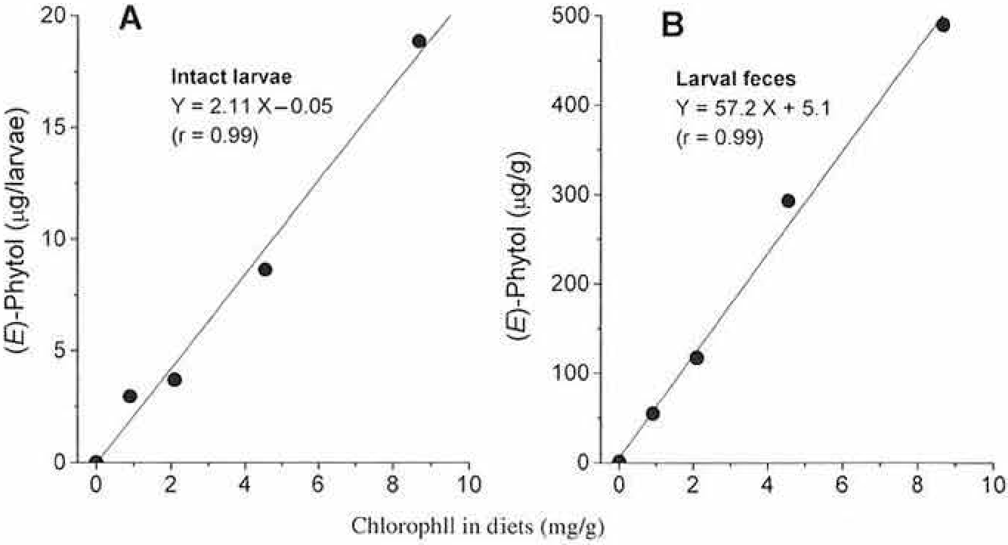


**Fig. 8. Concentration of (*E*)-phytol**
A: in larvae of *Spodoptera litura* fed with artificial diets containing various amounts of chlorophyll.
B: in feces of *Spodoptera litura* fed with artificial diets containing various amounts of chlorophyll.

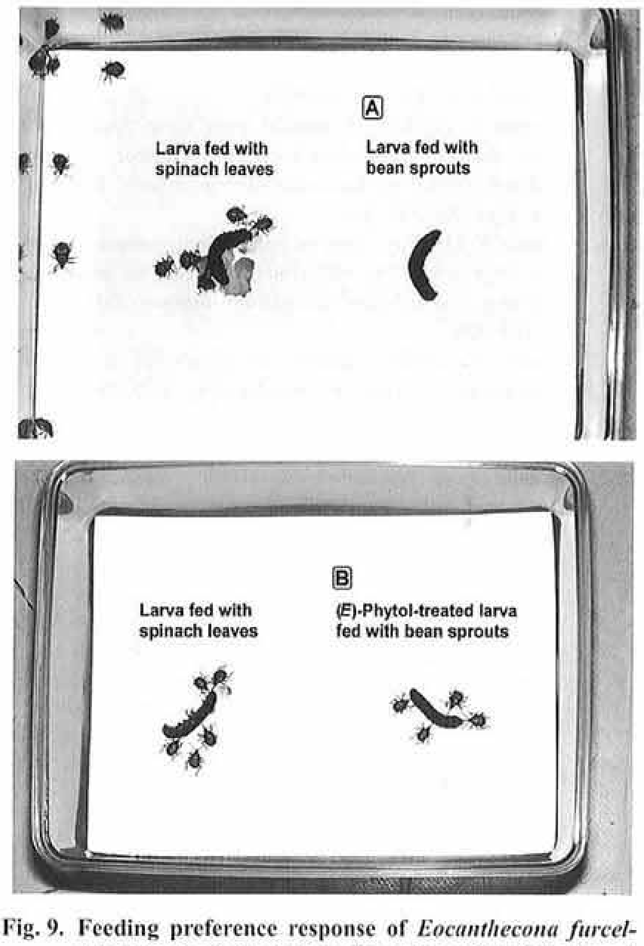


**Fig. 9. Feeding preference response of *Eocanthecona furcellata* nymphs to *Spodoptera litura* larva fed with spinach leaves (left) versus A and B (right)**
A: a larva fed with bean sprouts.
B: an (*E*)-phytol-treated larva fed with bean sprouts.

lized as a "kairomone" for prey-location by *E. furcellata* nymphs.

(*E*)-Phytol was also detected in the extracts of larvae of the pellucid zygaenid, *Pryeria sinica*, a giant silkworm, *Samia cynthia ricini*, an armyworm, *Pseudaletia separata*, the cabbage armyworm, *Mamestra brassicae*, which had been fed on diets containing chlorophyll. These extracts elicited the proboscis-protruding behavior of the bug, and the response depended directly on the (*E*)-phytol concentration in these larvae. Therefore, larvae of lepidopterans feeding on green plants could become potential preys for the generalist predatory stink bug, *E. furcellata*.

## Conclusion

The present studies demonstrated that the generalist predatory stink bug, *E. furcellata*, uses 2 different chemical cues as "kairomones" to locate larvae of several lepidopterans. *n*-Pentadecane ($C_{15}$) acts as a long-range (relatively long) attractant for the bug. On the other hand, (*E*)-phytol induced a proboscis-protruding behavior within a short distance. (*E*)-Phytol could be a key chemical associated with herbivorous lepidopterans for the prey location of this generalist predator, since the chemical originating from chlorophyll in prey diets is found in lepidopteran larvae feeding on green plants and the bugs protruded their proboscis toward an object, even a glass rod, treated with (*E*)-phytol.

The behavioral manipulation for *E. furcellata* with the semiochemicals has not yet been evaluated in the field. However, some applications in pest management have been speculated. $C_{15}$ and (*E*)-phytol could be used as an attractant and an arrestant, respectively, to recruit *E. furcellata* bugs and/or prevent their dispersal from the crop fields. The current studies suggested that (*E*)-phytol may be an indicator of the effects on pest control using *E. furcellata*, if the contents of (*E*)-phytol in pest insects could be measured. (*E*)-Phytol could also be used as a feeding stimulant in artificial diets for mass rearing of *E. furcellata*. Further studies should be carried out for using these compounds for pest management with *E. furcellata* bugs.

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