



Application of predator-associated cues to control small brown planthoppers: non-consumptive effects of predators suppress the pest population

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Abstract Predator non-consumptive effects (NCEs) have been well studied in many ecosystems and NCEs can alter the behavior, morphology and life history of prey, producing strong trait-mediated indirect effects (TMIEs) on host plants. However, studies involving the application of NCEs to control pests in the field, and instances of combined laboratory bioassay and field practice are rare. Here, we examine the development, reproduction and behavior of small brown planthoppers, *Laodelphax striatellus* (Fallén), when exposed to predator cues from caged predators (*Paederus fuscipes* Curtis), or predator body extracts (in solvents with different polarities) in the laboratory. Field foliage sprays of these extracts were also used to test their effects on the *L. striatellus* population and rice plant biomass. Nymph development and egg hatch rate in *L. striatellus* were not influenced, but adult longevity was shorter, and fecundity and weight gain were lower, when nymphs were exposed to the predator cues. Adults exposed to predator cues also gained less weight and laid fewer eggs. The

poorer developmental and reproductive performances might result from lower activity levels observed in threatened *L. striatellus*. The field foliage sprays of predator cues decreased *L. striatellus* abundance and increased rice plant biomass, suggesting their possible application for pest control. Predator cues extracted using chloroform increased stronger NCEs and TMIEs, indicating their non-polar characteristics. Our studies advance the understanding of how NCEs shape the life history and behavior of *L. striatellus* and improve rice growth, laying new foundations for future research on novel pest control materials and methods.

Keywords Rice pest · *Laodelphax striatellus* · Generalist predator · *Paederus fuscipes* · Predation risks · Cascading effects

Introduction

Field releases of natural enemies of agricultural pests for biological control and thus to mitigate plant damage have been greatly applauded (van Driesche and Heinz 2004). That natural enemies can not only reduce herbivore abundance by direct consumptive effects (CEs), but can also reduce herbivore fitness by indirect non-consumptive effects (NCEs), has long been of interest in pest management (Hermann and

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Landis 2017; Culshaw-Maurer et al. 2020). Most studies have reported NCEs of natural enemies altering the behavior (Freitas and Oliveira 1996; Kersch-Becker and Thaler 2015), morphology (Weisser et al. 1999; Dixon and Agarwala 1999), and life history (Fischer et al. 2012; Wineland et al. 2015; Xiong et al. 2015) of insect pests.

Predator-induced phenotypic plasticity often incurs a cost to prey fitness (Peacor et al. 2013), and results in substantially reduced prey populations (Sheriff et al. 2020; Culshaw-Maurer et al. 2020). In turn, NCEs can lead to strong trait-mediated indirect effects (TMIEs) on plants (Schmitz et al. 2004; Thaler and Griffin 2008; Bestion et al. 2015; Wineland et al. 2015). For example, in an old-field system, chelicerae-glued spiders shifted the feeding-time budgets of grasshoppers, slightly increasing their mortality, which cascaded to increase plant biomass (Schmitz et al. 1997). The TMIEs, along with the NCEs, demonstrate the influence of predators on their prey and on ecosystems (Werner and Peacor 2003; Peckarsky et al. 2008), providing a foundation for developing NCEs in biological control programs (Culshaw-Maurer et al. 2020).

However, successful pest control practices with NCEs require an understanding of the mechanisms by which prey detect and respond to predation risk (Hermann and Landis 2017). Many prior laboratory experiments have been designed to clarify the mediating mechanisms (Hoeffler et al. 2012; Ninkovic et al. 2013; Hermann and Thaler 2014). For example, Khudr et al. (2017) found the combination of visual and odor risk cues (predator cadavers), or a combination of odor and tactile cues (predator cues sprayed on foliage), could hinder aphid reproductive success. Alongside laboratory investigations, field applications of predator-associated cues have also been conducted (Silberbush et al. 2010; Aflitto and Thaler 2020). For example, the application of an aggregation pheromone to a potato field reduced the abundance of Colorado potato beetle larvae and associated plant damage (Aflitto and Thaler 2020). These existing works demonstrate NCE application is possible and can provide novel pest control strategies (Op de Beeck et al. 2016). Nevertheless, few studies currently examine the application of predator-associated cues for biological control compared to the application of natural enemies and pesticides due to the poor understanding of the underlying mechanisms. Thus,

the exploitation of the cues mediating NCEs, and the capacity of NCEs in pest control remain under-studied (Hermann and Landis 2017; Culshaw-Maurer et al. 2020).

The small brown planthopper (SBPH), *Laodelphax striatellus* (Fallén) (Hemiptera: Delphacidae), is a rice pest of economic importance because of its sap-feeding behavior and transmission of plant viruses (Otuka et al. 2008, 2012; Zheng et al. 2017). Although pesticides can control the SBPH, major problems arise from the emergence of insecticide resistance (Ma et al. 2007), their adverse effects on the diversity of natural enemies, and food safety. Environment-friendly methods to control the SBPH are needed urgently, and biological control using natural enemies is one such method.

The rove beetle *Paederus fuscipes* Curtis (Coleoptera: Staphylinidae) is one of the most abundant natural enemies of SBPH. We found nymph SBPH exposing to predation risks from *P. fuscipes* tend to develop into winged adults (Wen and Ueno 2021). Thus, we assumed SBPH should also alter its life history or behavior. However, until now no such phenomena in SBPH, or other rice planthoppers, has been detected. Here, we combined laboratory and field studies to reveal the NCEs of *P. fuscipes* on the development, reproduction and behavior of SBPH, and how NCEs may produce TMIEs on rice plants. We highlight the NCEs of *P. fuscipes* on the life-history/behavioral plasticity of SBPH, forming a basis for understanding the mechanism underlying NCEs, and pointing to new pest management methods.

Methods and materials

Insects

We used SBPH that was reared on rice seedlings (*Oryza sativa* L. ssp. *japonica*) in a stock culture in a transparent square cage (30 × 25 × 35 cm, with meshed windows). The SBPH was obtained from rice fields (Liji, West Ward, Fukuoka, Japan) in 2018. The adult rove beetles (*P. fuscipes*), collected from rice paddies (Kuwabara, West Ward, Fukuoka, Japan), were reared in a transparent cylindrical cage (d = 12 cm, h = 20 cm, with meshed windows). We used wild-caught rove beetles in these experiments because we could not maintain them over multiple

generations in laboratory conditions. They were reared on mixed diets with *Heteromurus* spp. (Collembola: Entomobryidae) and the fungus gnat, *Bradysia* sp. (Diptera: Sciaridae), for three weeks before being used in our tests, in order to eliminate the possibility of disturbances from alarm pheromone. Both SBPH and rove beetles were maintained under a L:D 16:8 photoperiod at 25 °C and 60% RH (kept separately in different incubators).

The development and reproduction of SBPH when exposing nymphs to risk

Two 10-day-old rice seedlings were raised in the tube (Supplementary Fig. S1) ten days before the tests started on cotton soaked in 5 ml of an 800-times diluted nutrition solution (HYPONeX, HYPONeX Japan corp. Ltd., Osaka, Japan). A transparent bottle was attached to the tube, separated by a mesh (mesh hole < 1 mm).

For the caged rove beetle treatments, one pair of rove beetles (1♀ + 1♂) was used as the visual and odor risk cues. For the rove beetle-associated cues treatments, rove beetle body extracts in solvents with different polarities were used as the odor risk cues. We assumed these extracts might differ in the quantity and/or quality of rove beetle cues and lead to different levels of SBPH responses. One pair of rove beetles were frozen at - 24 °C for 10 min, and submerged in 100 µl chloroform/100 µl ethanol/20 ml distilled water. The time for chloroform and ethanol extraction was 10 min, whereas that for water extraction was 24 h, because, in preliminary tests, extracts with extraction time shorter than 12 h in water elicited a weak SBPH response (Supplementary Fig. S2). After extraction, the rove beetles were removed, and the extract volume was adjusted to 20 ml by adding distilled water. All extracts were stored at 4 °C.

One first instar nymph was introduced into the tube (Supplementary Fig. S1). The nymph was allowed to acclimate within the tube for 5 min. The paired rove beetles, or cotton soaked onto 1 ml chloroform extract (CE)/ethanol extract (EE)/water extract (WE), were put into the bottle to produce risk cues. We supplied the rove beetles with water (wet cotton). The bottle along with rove beetles and wet cotton, or “risk cotton”, was replaced every two days. In the control treatments, cotton soaked with distilled water (WC) or diluted chloroform solution (CC, 100 µl/20 ml) or

diluted ethanol solution (EC, 100 µl/20 ml) was used. Sixty replicates were set for each treatment.

In all treatments, we replaced the rice seedlings every five days. After the nymphs developed into adults, the winged adults were randomly numbered and paired (wingless adults were rare), and reared in their respective experimental conditions until death. During this period, we removed the rice seedlings bearing eggs to a new tube without rove beetles every three days, and supplied two new rice seedlings to paired adults for laying eggs continually. The nymph duration, adult longevity, fecundity per female, adult weight gain, and egg hatch rate were measured. Fecundity per female was measured by noting the number of hatched eggs (newly-hatched first instar nymphs) and unhatched eggs (rice seedlings that did not yield newly hatched nymph after three days were dissected under a microscope (HOZAN, Tool Ind. Cd. Ltd., Osaka, Japan) to count the unhatched eggs). The weight gain of adults (female and male) was calculated using the formula: weight gain = body weight at death - body weight at molt. Where body weight at molt was measured by weighing the newly molted adult, and the body weight at death was measured by weighing the naturally dead adult (death time < 6 h).

The development and reproduction of SBPH when exposing adults to risk

We reared one pair of newly molted adults per tube (Supplementary Fig. S1). We aimed to determine if SBPH had different life-history traits when they did not experience predation risks or predation in the nymph stages. The treatments and the measured variables (except nymph duration) were similar to those described above. Sixty replicates were set for each treatment.

Behavioral plasticity when exposing SBPH to risk

We assume the poor development and reproduction of SBPH were caused by the behavioral changes when exposed to predation risks. A behavioral bioassay was conducted to examine this hypothesis. The paired rove beetles or “risk cotton” (see above) were put in the bottle (Supplementary Fig. S1). Then one 4th or 5th instar nymph SBPH, or one female or male SBPH (nymphs and adults were starved for 12 h) was introduced into the tube. The SBPH was allowed to

acclimate within the tube for 1 min. We recorded the time SBPH spent feeding, walking, non-feeding quiescence, or was on plants within 16 h (light period) using a video camera (Panasonic, HC-VX992M-T, Osaka, Japan). The video was analyzed to count the time spent for each behavior. Ten replicates were set for each treatment. We did not use 1st, 2nd, or 3rd instar nymphs because they were too small (< 1.5 mm) to observe their feeding behavior.

Foliage sprays of predator cues in the field

We sprayed rove beetle body extracts on rice plants in field mesh cages, to test their efficiencies in controlling SBPH. Field experiments were conducted in the organic arable rice paddies at Kuwabara, West Ward, Fukuoka, Japan from 12 May to 1 August 2021. Pesticides have not been applied in these paddies in recent decades, eliminating the possibility of disturbances from pesticides on the test SBPH.

On 12 May 2021, 40 meshed cages (70 × 50 × 50 cm, with meshed windows) were placed on an unused rice paddy. All cages were placed 1 m apart in a rectangular pattern. Previous studies conducted in our laboratory showed that cages 0.5 m apart did not influence each other when treated with foliar sprays of rove beetle extracts (in chloroform). One thousand rice seedlings raised from seeds in the laboratory for ten days were planted in each cage. The rice seedlings were allowed to develop for ten days, and 30 pairs of winged SBPH (50% females) were introduced into the cage. Foliar sprays or predator release occurred 2 h after SBPH introduction.

We prepared the body extracts (CE, EE and WE) by taking 90 rove beetles (50% female), previously starved for 24 h, and freezing them at -24 °C for 10 min, and dividing them equally into three groups of treatments (50% female in each group). These beetles were submerged in 10 ml of chloroform, ethanol or distilled water. The extraction time was 8 min, 8 min and 24 h, respectively. After extraction, we adjusted the volume of the extracts to 10 ml by adding their respective solvents, followed by diluting them at a rate of 1:1000 by adding distilled water. Hence, we obtained 10 l of body extracts for each group of rove beetles. Each extract was divided into five volumes of 2 l extracts (one for each of five replicates). We used diluted chloroform (CC) or ethanol (EC) (1:1000 by adding distilled water), or distilled water (WC) as the control.

The 40 cages were randomly numbered and evenly divided into eight groups. Four groups were treated with the foliage sprays of CE, EE, WE and rove beetle releases (RB, five paired), respectively. The remaining groups were treated with three control solutions (CC, EC, WC) and RC (without any foliar sprays), respectively. Each 2 l body extract or control solution was sprayed evenly on the rice plants using an 800 ml spray can. Because it rained heavily in the evening of 23 May, we sprayed again in the morning of 24 May (the number of rove beetles in the cages were checked, but it did not change). The rice plants were watered every two days. We did not set up experiments with caged predators because foliage sprays of predator-associated cues should be more practical in any “real world” application than the release of caged predators.

We recorded the number of 1st-3rd instar nymphs (young nymphs), 4th-5th instar nymphs (elder nymphs), and adult SBPH after 70 days. The SBPH were collected and placed in an oven (ETTAS, ASONE, Osaka, Japan) set to 50 °C for 24 h, cooled and weighed on a scale (Mettler-Toledo International Inc., Tokyo, Japan). Five hundred live rice plants were removed from the soil, washed and blotted. We then separated the roots from the shoots and dried them in an oven at 40 °C for 48 h. Dry plants were cooled and weighed.

Data analysis

All data analyses were conducted with R 4.0.3 software (R Core Team 2020). The nymph and adult development periods, female fecundity, weight gain, time SBPH spent feeding, non-feeding quiescence, walking on plants, and the activity index (time allocated to walking divided by time spent on non-feeding quiescence walking) were compared by using Kruskal–Wallis tests, followed by Tukey’s tests for multiple comparisons. The generalized linear models (GLM, predictor variable: risk treatments) with the family of Poisson distribution (number of SBPH, log link function) or Binomial distribution (proportion of young/elder SBPH nymphs/adult SBPH, logit link function) or Gamma distribution (dry weight of SBPH/above-ground dry weight of rice plants, inverse link function) were used to analyze the differences among treatments, followed by Tukey’s post-hoc analysis. Because on 9 July 2021 six cages (one CE, one CC,

two RC, two EC) were destroyed by heavy rain, these cages were excluded from the data analysis.

Results

The development and fecundity of SBPH under predation risks

Predation risks significantly affected adult longevity, fecundity and weight gain of SBPH, and these effects were also influenced by when they were exposed to predation risk. When nymphs were exposed to predation risks, their nymph duration were not affected ($df = 6$, $\chi^2 = 5.08$, $p = 0.533$; Table 1), but the adult longevity were significantly decreased when exposed to caged rove beetles (female: $df = 6$, $\chi^2 = 17.47$, $p = 0.008$; male: $df = 6$, $\chi^2 = 21.03$, $p = 0.002$; Table 1). We noted that the body weight at molt in all treatments was not significantly different (female: $df = 6$, $\chi^2 = 7.26$, $p = 0.297$; male: $df = 6$, $\chi^2 = 1.52$, $p = 0.959$), but the weight of females at death differed significantly (female: $df = 6$, $\chi^2 = 36.58$, $p < 0.001$;

male: $df = 6$, $\chi^2 = 3.86$, $p = 0.695$). Thus, their weight gain was measured. Female weight gain and fecundity were affected (weight gain: $df = 6$, $\chi^2 = 25.60$, $p < 0.001$; fecundity: $df = 6$, $\chi^2 = 28.47$, $p < 0.001$; Fig. 1a, Table 1). The caged rove beetles, chloroform extract, ethanol extract and water extract treatments significantly reduced female fecundity and weight gain, whereas male weight gains were not affected ($df = 6$, $\chi^2 = 1.40$, $p = 0.966$; Fig. 1b). The egg hatch rates were not influenced ($df = 6$, $\chi^2 = 1.83$, $p = 0.934$; Table 1).

When SBPH adults were exposed to predation risks, adult longevity did not differ among treatments (female: $df = 6$, $\chi^2 = 3.19$, $p = 0.785$; male: $df = 6$, $\chi^2 = 6.67$, $p = 0.352$; Table 2). However, female fecundity and weight gain were significantly lower when exposed to caged rove beetles or chloroform extract (fecundity: $df = 6$, $\chi^2 = 20.55$, $p = 0.002$; weight gain: $df = 6$, $\chi^2 = 26.71$, $p < 0.001$; Table 2; Fig. 1c). Male weight gain and egg hatch rate were not affected (weight gain: $df = 6$, $\chi^2 = 3.23$, $p = 0.779$; egg hatch rate: $df = 6$, $\chi^2 = 1.51$, $p = 0.959$; Table 2; Fig. 2d).

Table 1 Development durations, fecundity and egg hatch rate when nymph SBPH (first instar) were exposed to predation risks (mean \pm SE, N = total number of SBPH monitored)

Treatments	Nymph duration (days)	Female longevity (days)	Male longevity (days)	Fecundity (eggs)	Hatch rate (%)
Caged rove beetles	13.59 \pm 0.22a (N = 41)	16.27 \pm 0.59b (N = 21)	11.84 \pm 0.62b (N = 19)	125.37 \pm 6.41b (N = 19)	90.52 \pm 0.02a (N = 19)
Chloroform extract	13.61 \pm 0.20a (N = 44)	17.16 \pm 0.89ab (N = 24)	15.16 \pm 0.96ab (N = 19)	127.79 \pm 5.67b (N = 19)	92.69 \pm 0.01a (N = 19)
Ethanol extract	13.28 \pm 0.23a (N = 46)	18.08 \pm 1.13ab (N = 22)	14.76 \pm 0.78ab (N = 21)	126.33 \pm 7.14b (N = 21)	91.44 \pm 0.02a (N = 21)
Water extract	13.73 \pm 0.24a (N = 45)	17.71 \pm 0.83ab (N = 23)	15.00 \pm 0.86ab (N = 21)	128.76 \pm 6.82b (N = 21)	90.92 \pm 0.02a (N = 21)
Chloroform control	13.19 \pm 0.16a (N = 47)	20.07 \pm 0.89a (N = 26)	16.20 \pm 0.79a (N = 20)	163.05 \pm 10.25a (N = 20)	91.12 \pm 0.02a (N = 20)
Ethanol control	13.24 \pm 0.20a (N = 45)	20.14 \pm 1.19a (N = 21)	15.25 \pm 0.74ab (N = 21)	158.05 \pm 7.41a (N = 21)	93.18 \pm 0.02a (N = 21)
Water control	13.15 \pm 0.24a (N = 46)	19.91 \pm 0.91a (N = 23)	16.35 \pm 0.60a (N = 20)	164.70 \pm 7.73a (N = 20)	92.23 \pm 0.02a (N = 20)

The chloroform extract, ethanol extract and water extract indicate the rove beetle body extracts in chloroform, ethanol and water, respectively. The chloroform control, ethanol control and water control indicate the control solutions without predator cues. Different lowercase letters within the same column denote significant differences among treatments (Tukey's test, $p < 0.05$)

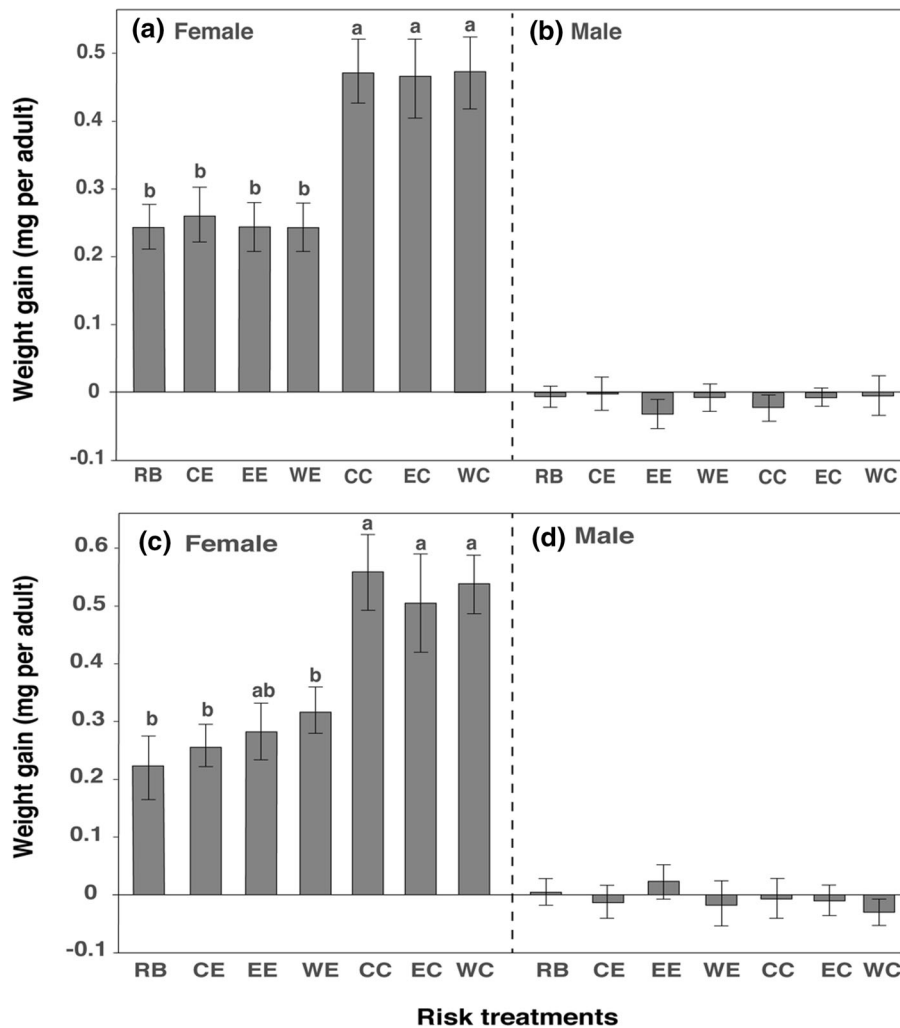


Fig. 1 The weight gains of adults (mean \pm SE) when SBPH nymphs (a, b) or adults (c, d) were exposed to predation risks. Risk treatments: RB (rove beetles), CE (chloroform extract), EE (ethanol extract) and WE (water extract). Control treatments:

CC (chloroform control), EC (ethanol control) and WC (water control). Different lowercase letters above the bars denote significant differences among treatments (Tukey's test, $p < 0.05$)

The behavior of SBPH under predation risk

The feeding time and time spent on plants did not differ significantly among treatments (feeding: $df = 6$, $\chi^2 = 3.40$, $p = 0.757$; on plants: $df = 6$, $\chi^2 = 11.08$, $p = 0.086$). However, significant differences were found in the time SBPH spent on non-feeding quiescence and walking (non-feeding quiescence: $df = 6$, $\chi^2 = 43.41$, $p < 0.001$; walking: $df = 6$, $\chi^2 = 39.74$, $p < 0.001$). The activity indexes of 4th and 5th instar nymphs were not affected (4th: $df = 6$, $\chi^2 = 6.79$, $p = 0.341$; 5th: $df = 6$, $\chi^2 = 2.50$, $p = 0.868$; Fig. 2a, b), but those of females and males

were significantly lower in the caged rove beetle, chloroform extract, ethanol extract and water extract treatments (female: $df = 6$, $\chi^2 = 40.32$, $p < 0.001$; male: $df = 6$, $\chi^2 = 36.22$, $p < 0.001$; Fig. 2c, d).

The efficacy of rove beetle-body extracts in controlling SBPH

The body extracts or rove beetles significantly influenced the numbers (Supplementary Table S1, Fig. 3a) and dry weights (Supplementary Table S1, Fig. 3b) of SBPH, and the above-ground dry weight of rice plants (Supplementary Table S1, Fig. 3c). The proportions of

Table 2 Development durations, fecundity and egg hatch rate of SBPH when adult SBPH were exposed to predation risks (mean \pm SE, N = total number of SBPH monitored)

Treatments	Female longevity (days)	Male longevity (days)	Fecundity (eggs)	Hatch rate (%)
Caged rove beetles	17.54 \pm 1.06a (N = 13)	14.21 \pm 0.59a (N = 14)	116.08 \pm 8.83b (N = 13)	91.68 \pm 0.02a (N = 13)
Chloroform extract	17.40 \pm 0.87a (N = 15)	14.60 \pm 0.91a (N = 15)	120.40 \pm 9.92b (N = 15)	90.24 \pm 0.02a (N = 15)
Ethanol extract	18.67 \pm 1.16a (N = 12)	14.23 \pm 0.71a (N = 13)	136.67 \pm 10.91ab (N = 12)	92.24 \pm 0.02a (N = 12)
Water extract	17.71 \pm 0.83a (N = 14)	15.46 \pm 0.81a (N = 13)	137.84 \pm 11.02ab (N = 13)	92.39 \pm 0.02a (N = 13)
Chloroform control	19.77 \pm 1.40a (N = 13)	15.93 \pm 1.32a (N = 14)	162.23 \pm 9.70a (N = 13)	92.03 \pm 0.02a (N = 13)
Ethanol control	18.79 \pm 1.00a (N = 14)	16.07 \pm 0.68a (N = 14)	157.07 \pm 7.30a (N = 14)	92.80 \pm 0.03a (N = 14)
Water control	19.21 \pm 1.11a (N = 14)	15.40 \pm 0.65a (N = 14)	157.71 \pm 7.62a (N = 14)	91.93 \pm 0.03a (N = 14)

The chloroform extract, ethanol extract and water extract indicate the rove beetle body extracts in chloroform, ethanol and water, respectively. The chloroform control, ethanol control and water control indicate the control solutions without predator cues. Different lowercase letters within the same column denote significant differences among treatments (Tukey's test, $p < 0.05$)

elder nymphs and adult SBPH were also affected (Supplementary Table S1, Fig. 3a). Rice plants treated with rove beetles or chloroform extract had significantly lower numbers of SBPHs, but had a higher proportion of adults (Fig. 3a), and higher above-ground dry weights (Fig. 3c). Surprisingly, the dry weight of surviving SBPH in rove beetle and chloroform extract treatments were higher than in other treatments (Fig. 3b).

Discussion

Exposure to predation risks greatly suppressed SBPH development and reproduction. The poorer performances may result from development/predation risk tradeoffs (Rawlings 1994; Nelson 2007; Elliott et al. 2015). Increased anti-predation resource or energy investment may detract from growth and reproduction (Lima and Dill 1990; Werner and Anholt 1993; McPeck et al. 2001). Exposing nymphs, but not adults, to the risk cues reduced adult longevity, suggesting the

timing of risk exposure can affect the expression of life-history plasticity (Wirsing et al. 2021).

Egg production number is strongly dependent upon female body size (Kozłowski 1992). The smaller female SBPH (less weight gain) had lower fecundity, reflecting severe reproductive costs. The average longevity of female SBPH in caged rove beetle treatments was less than 17 days, but the oviposition duration of SBPH was 17–19 days at 25 °C in the laboratory. The shorter oviposition duration may also cause lower fecundity. Also, prey may retain eggs inside their ovaries until reaching safe oviposition sites (Montserrat et al. 2007), resulting in a lower egg output.

The reduction of activity levels in SBPH is probably an anti-predator behavior. Staying inactive reduces the encounter rate with predators and increases survival success (Beleznaï et al. 2015; Hermann and Landis 2017). However, the quality and/or quantity of food intake suffers, as inactive SBPH only feed in a small area of rice plants (inactive), leading to heavier developmental and reproductive costs as discussed above.

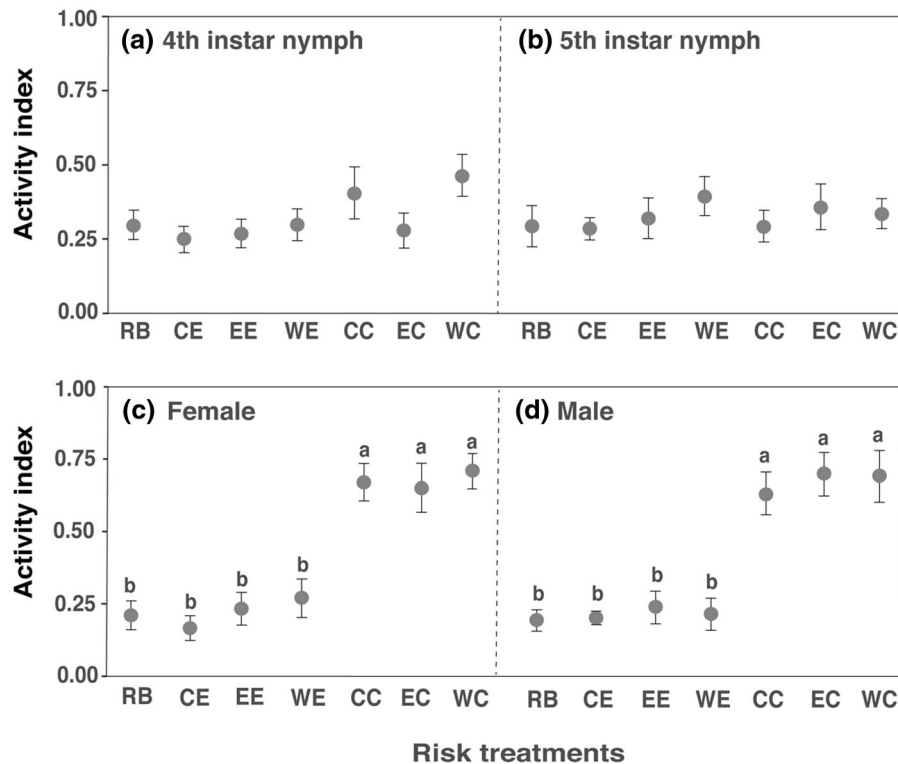


Fig. 2 The activity index (mean \pm SE) of SBPH when 4th (a) and 5th (b) instar nymph, and female (c) and male (d) SBPH were exposed to predation risks. Risk treatments: RB (rove beetles), CE (chloroform extract), EE (ethanol extract) and WE

(water extract). Control treatments: CC (chloroform control), EC (ethanol control) and WC (water control). Different lowercase letters above the bars denote significant differences among treatments (Tukey's test, $p < 0.05$)

However, lower activity levels do not always lead to reduced development and fecundity in SBPH. For example, females SBPH were inactive in most of the treatments, but adult lower fecundity and weight gain were only found in caged rove beetle and chloroform extract treatments. It is possible SBPH may not exhibit continued anti-predator behavior after it noticed that the ethanol and water extracts were less risky (the risk levels of body extracts are discussed later). It is widely reported that prey can adjust their behavior to match the risk levels of predator cues, and optimize their development (Beauchamp and Ruxton 2011).

SBPH nymphs were less affected by predation risks, possibly due to their immature sensory system (Zacharuk and Shields 1991), and the lag phases between predator perception and the expression of plasticity (Weiss and Tollrian 2018; Graeve et al. 2021). The lag explanation was supported by the fact that when nymphs (not adults) were exposed to predation risks, their adult longevities were shortened. Further, the weight gain of male SBPH was not

affected by predation risk, differing from that of female SBPH. Females SBPH suffer greater physiological costs than males under predation risk (Lima and Dill 1990; Post and Götmark 2006), as they need to stay and deposit eggs in the bare rice stem, increasing the time window for exposure to predators.

The field release of rove beetles, or foliage sprays of their body extracts, reduces SBPH abundance, demonstrating the capability of rove beetles or predator cues as a SBPH control. For the rove beetle treatment, the reduced SBPH abundance may be largely because of the CEs, but for body extract treatments, it may be due to the numerous negative effects caused by NCEs, for example, weaker immunity (Ramirez and Snyder 2009; Duffy et al. 2011), physiological stress (Werner and Peacor 2003) or production of the less-fecund winged forms (Wen and Ueno 2021).

Plant biomass increased both in treatments with rove beetles and chloroform extract. The TMIEs of rove beetles were driven by direct consumption of victim SBPH (CEs), and by inducing chronic stress on

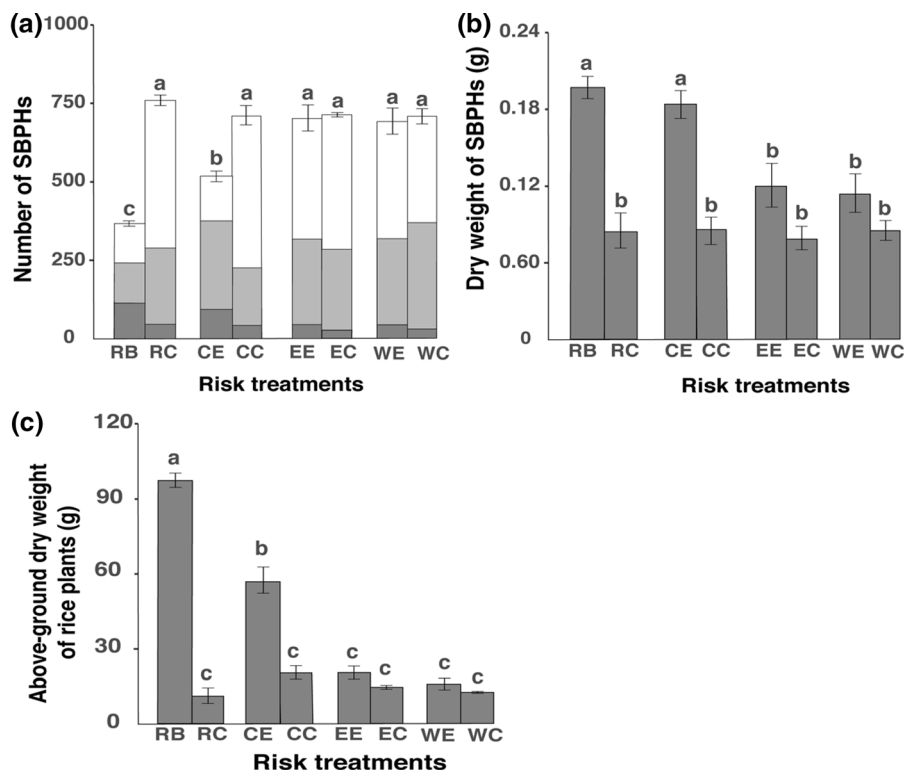


Fig. 3 Effects of predator release and foliage sprays of predator cues on the number of SBPH (a, mean \pm SE, the white, grey and black colors in the bar indicate the proportion of young nymphs, elder nymphs and adults, respectively), the dry weight of SBPH (b, mean \pm SE) and the above-ground dry weight of rice plants (c, mean \pm SE) after 70 days. Risk treatments: RB (rove

beetles); CE (chloroform extract), EE (ethanol extract) and WE (water extract). Control treatments: CC (chloroform control), EC (ethanol control), WC (water control) and RC (without rove beetle or foliage spray). Different lowercase letters above the bars denote significant differences among treatments (Tukey's test, $p < 0.05$)

the surviving SBPH (NCEs). Those of chloroform extracts, however, were produced only by NCEs. We noticed the NCEs accounted for almost 60% of the CE + NCEs on the plant biomass, suggesting stronger NCEs than CEs (Beckerman et al. 1997; Werner and Peacor 2003). Further, plant biomass was negatively correlated with SBPH abundance, and positively correlated with SBPH biomass. The reduced biomass of rice plants, due to the greater abundance of SBPH, adversely affects SBPH weight gain (less food), explaining why the higher SBPH biomass unexpectedly occurs in these well-controlled treatments.

Higher proportions of adult SBPH occurred in rove beetle and chloroform extract treatments. For the former treatment, the size-selective rove beetles prefer to consume 2nd-4th instar SBPH nymphs, leaving SBPH adults surviving in the cage. For the later treatments, the development of SBPH accelerated in

the field cages (Supplementary Table S2). The first occurrence of SBPH adults (F3 generation) in this treatment was 2–3 days earlier than those in other treatments, resulting in more adults at the end of the experiments, probably due to the lower intraspecific competition and predation risks.

The non-polar solvent (chloroform) extract elicited NCEs on SBPH, and TMIEs on rice plants more efficiently than using polar solvents (ethanol and water). We assumed the non-polar solvent may extract more quality or/and quantity of predator cues. Many arthropod natural enemies have been shown to be more responsive to compounds extracted by non-polar solvents than polar solvents (Würf et al. 2020; Peschke and Metzler 1987; Wen et al. 2017; Lo Pinto et al. 2013).

Rove beetles usually communicate via chemical cues (Dettner and Liepert 1994; Laurent et al. 2005). Extracts of female cuticular hydrocarbons (*Aleochara*

curtula (Goeze)) can induce a strong male grasping response (Peschke and Metzler 1987), serving as a sex pheromone. Their abdominal gland secretions function as both sex pheromones and defensive compounds (Peschke and Metzler 1982; Peschke 1983), serving as intraspecific communications, but can serve additionally as interspecific cues (Stökl and Steiger 2017; Hemptinne et al. 2001; Nakashima et al. 2006). Thus, the predator cues mediating NCEs on SBPH (kair-omone) may also have a secondly pheromonal function. Accordingly, stronger positive effects on the *P. fuscipes* population and stronger CE and NCEs on the SBPH population, can be expected, because an additional function as a pheromone (for example, sex-pheromone) will benefit the development of *P. fuscipes*, and will, in turn, adversely affect SBPH population growth. Furthermore, spraying body extracts before the introduction of SBPH, will impede settlement of SBPH on the plants because SBPH tended to avoid *P. fuscipes* body extracts (Supplementary Fig. S1), resulting in stronger NCEs and TMIEs.

In summary, studies presented here, combining laboratory and field experiments, confirm the impact of NCEs of rove beetles and their cues on the development, reproduction and behavior of SBPH, and demonstrate the effect of TMIEs on plant biomass. These studies also suggest a novel method to control SBPH without the application of natural enemies or pesticides.

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Data availability All data available from the figshare. Dataset (<https://doi.org/10.6084/m9.figshare.14178341.v1>).

Code availability All the data analysis were conducted in R version 4.0.3.

Declarations

Conflict of interest We declare we have no competing interests.

Ethical approval This article does not contain any studies with human participants or vertebrates performed by any of the authors.

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