

1 **Cooperative herbivory between two important**
2 **pests of rice**

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20

21 **Abstract**

22 Normally, when different species of herbivorous arthropods feed on the same
23 plant this leads to fitness reducing competition. We found this to be uniquely
24 different for two of Asia's most destructive rice pests, the brown planthopper
25 and the rice striped stem borer. Both insects directly and indirectly benefit from
26 jointly attacking the same host plant. Double infestation improved plant quality,
27 particularly for the stemborer because the planthopper fully suppresses
28 caterpillar-induced production of proteinase inhibitors. It also drastically
29 reduced the risk of egg parasitism, due to diminished parasitoid attraction.
30 Females of both pests have adapted their oviposition behaviour accordingly.
31 Their strong preference for plants infested by the other species even overrides
32 their avoidance of plants already attacked by conspecifics. This uncovered
33 cooperation between herbivores is telling of the exceptional adaptations
34 resulting from the evolution of plant-insect interactions, and points out
35 mechanistic vulnerabilities that can be targeted to control two major pests.

36

37 **Keywords:** *Nilaparvata lugens*, *Chilo suppressalis*, rice defense suppression,
38 conventional interspecific competition theory, collaborative interaction

39

40

41 **Introduction**

42 Species that feed on the same resource are commonly regarded to be
43 competitors¹⁻³. In the great majority of cases, species that share a food
44 resource negatively affect each other's performance, and the conventional
45 interspecific competition theory is widely recognized for a diverse range of
46 taxonomic groups including plants, birds, reptiles, marine invertebrates,
47 insects and microbes^{1,4,5}. Yet, there are instances of mutually beneficial
48 interactions between species that feed on the same food source. This is mostly
49 known for microbes that can assist other organisms in various ways to help
50 reach, convert and digest food⁶⁻⁸. In very rare cases, higher organisms have
51 also evolved cooperative interactions to be better exploit and share resources,
52 as, for instance, has been shown for predators with complementary hunting
53 tactics⁹⁻¹¹.

54 Amongst arthropods, mutually beneficial interactions have been reported
55 mainly for social insects with food providing partners¹². The classic example is
56 the symbiotic relationship between ants and aphids, in which aphids produce
57 sugar-rich honeydew that is collected by the ants, and in exchange ants care
58 for and protect the aphids from predators and parasitoids^{13,14}. In none of these
59 associations the arthropods share the same food sources, and we are not
60 aware of any example of mutually beneficial exploitation of the same resources
61 by arthropods.

62 The most commonly food sources shared among arthropods are plants, with
63 virtually all plants being attacked by a number of different species. This has so
64 far assumed to always lead to competition^{1,3}. The extent of this competition
65 can vary and the consequences can be highly asymmetrical, but in all reported
66 examples the effects of feeding on the same plant are negative for at least one
67 of the herbivores, independently of whether they are phylogenetically close,
68 have the same mode of feeding, or feed on the same tissues^{1,15-18}. In certain
69 cases, one herbivore species can benefit from the presence of another
70 herbivore species; for instance, by causing physiological changes in the plants

71 that make these plants less toxic and/or more nutritious^{16,17,19-22} or by masking
72 the (volatile) foraging cues used by natural enemies²³⁻²⁷. To our knowledge,
73 however, there are no known examples of two species of herbivores both
74 consistently benefitting from presence of each other on the same host plants.

75 It has been postulated that mutually beneficial interactions among species of
76 insect herbivores must exist, but no specific examples have been uncovered
77 yet^{20,28}. It is one thing to demonstrate that two herbivores benefit from jointly
78 feeding on the same plant, it is another to conclude that they actively pursue
79 the plant mediated-benefits. Here we propose such a coevolved partnership
80 between the brown planthopper (BPH), *Nilaparvata lugens* and the rice striped
81 stem borer (SSB), *Chilo suppressalis*, two of the most devastating pests of rice
82²⁹. Our hypothesis that both insects benefit from attacking the same plant was
83 prompted by our recent finding that BPH can escape parasitism of its eggs by
84 preferentially ovipositing on rice plants that are already infested by SSB²⁵. The
85 apparent reason for this oviposition strategy is that, *Anagrus nilaparvatae*, the
86 most common egg parasitoid of BPH, uses volatiles emitted by BPH-infested
87 plants to locate plants with eggs. Plants that are co-infested by SSB release a
88 different blend of volatiles that is not attractive to the parasitoid²⁵. Previous
89 work also indicates that SSB larvae perform poorly on rice plants that are
90 already infested by conspecifics due to induced plant resistance, and that
91 females show a strong oviposition preference for uninfested rice plants relative
92 to SSB-infested rice plants, in accordance with the 'mother knows best'
93 principle³⁰. BPH has been shown to suppress certain defenses in rice^{31,32}.
94 This raises the question whether sharing host plants with BPH can help SSB to
95 counter the direct defenses of rice plants and possibly mitigate the defense
96 responses to SSB infestation, and, if so, whether it also has adapted its
97 oviposition behavior accordingly.

98 To answer these questions, we tested the performance of SSB larvae on
99 uninfested rice plants, and plants infested either by BPH only, by SSB only, or
100 by both species. We further tested if the oviposition preferences of SSB moths

101 matched the measured performances on the differently pre-infested plants.
102 The results prompted us to investigate the potential molecular and biochemical
103 mechanisms that may be involved, with a focus on protease inhibitors. To also
104 address how BPH may affect the indirect defenses of rice plants against SSB,
105 we further tested the odor preferences of *Trichogramma japonicum*, an
106 important egg parasitoid of SSB, for differently treated plants. With these
107 olfactometer assays and additional cage experiments we tested whether the
108 presence of BPH can also reduce the risk of SSB moth eggs to be parasitized.
109 The combined results support our hypothesis of a co-evolve cooperation
110 between two intimately interacting herbivorous species that feed on the same
111 plants, which exceptionally goes against conventional competition theory of
112 interspecific interactions among phytophagous insects¹. The elucidation of the
113 underlying plant-mediated mechanisms that facilitate this cooperation can be
114 the basis for plant breeding strategies to control the two exceedingly important
115 rice pests.

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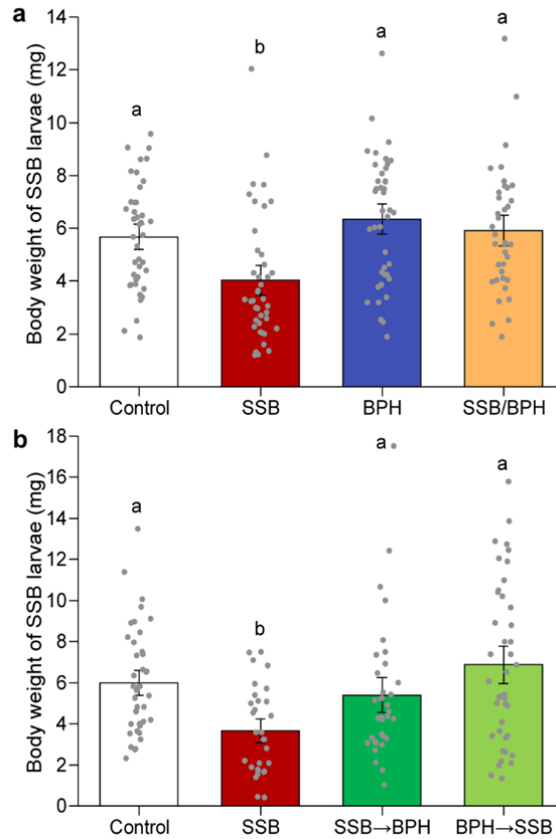
117 **Results**

118 **Performance of SSB caterpillars on herbivore-infested rice plants**

119 When *C. suppressalis* larvae were allowed to feed for 7 days on rice plants
120 that were either uninfested (control), infested by SSB larvae alone (SSB), BPH
121 nymphs alone (BPH), or both SSB larvae and BPH nymphs (SSB/BPH), the
122 body weight of SSB caterpillars was significantly different among the
123 treatments ($F_{3,165} = 8.462$, $P < 0.001$) (Fig. 1a). The body weight of SSB larvae
124 was significantly lower when fed on plants that had been infested by SSB
125 larvae than on all other plant treatments (all $P < 0.01$). Importantly, there was
126 no difference in larval weight among the other three treatments (uninfested
127 plants, feeding on BPH-infested plants or SSB/BPH-infested plants; $P > 0.05$).
128 These results imply that additional infestation by BPH fully eliminated the
129 negative effects of SSB-infestation on successively feeding conspecifics. And
130 this effect seems to be independent of infestation sequence, as weight gain

131 was only marginally different between SSB larval that were placed on dual
132 infested plants, infested, with SSB treatment occurring either before or after
133 BPH infestation ($P = 0.051$) (Fig. 1b).

134



135

136 **Figure 1. Weight of SSB larvae after seven days of feeding on**
137 **differentially infested rice plants.**

138 **a** Neonates of SSB were individually placed on rice plants that were either
139 uninfested (Control), infested by SSB larvae only (SSB), BPH only (BPH), or
140 both SSB and BPH (SSB/BPH, two species were simultaneously introduced to
141 the plants); **b** Neonates of SSB were individually placed on rice plants that
142 were either uninfested (Control), infested by SSB only, or both SSB and BPH
143 in sequencing order (SSB→BPH, plants were infested with SSB larvae for 24 h
144 then BPH were added for another 24 h; BPH→SSB, plants were infested with
145 BPH for 24 h then SSB larvae were added for another 24 h). Bars indicate
146 mean \pm SE. Data was analyzed using Two-way analysis of variance followed
147 by LSD post hoc test. Small letters indicate significant differences between
148 treatments ($P < 0.05$) (N = 30–46).

149

150

151 **Oviposition preferences of SSB females**

152 ***Oviposition preference in greenhouse***

153 When given a choice between uninfested and SSB-infested plants, SSB
154 females laid significantly fewer eggs on SSB-infested plants than on
155 uninfested plants (RT-test applied to a GLMM, Poisson distribution error; $P <$
156 0.001) (Fig. 2a). However, compared to uninfested plants, the females strongly
157 preferred to lay eggs on BPH-infested plants (RT-test applied to a GLMM,
158 Poisson distribution error; $P = 0.007$) (Fig. 2b) or on SSB/BPH-infested plants
159 (RT-test applied to a GLMM, Poisson distribution error; $P = 0.03$) (Fig. 2c). As
160 expected, SSB females also laid significantly more eggs on BPH-infested or
161 SSB/BPH-infested plants (RT-test applied to a GLMM, Poisson distribution
162 error; both $P < 0.001$) relative to SSB-infested plants (Fig. 2e, f), while they laid
163 similar numbers of eggs on BPH-infested and SSB/BPH-infested plants (Fig.
164 2g).

165 When SSB females were offered the 4 types of rice plants simultaneously,
166 SSB females laid significantly different numbers of eggs among treatments
167 (Fig. 2h; RT-test applied to a GLMM, Poisson distribution error; $P < 0.001$),
168 with the most eggs on plants infested by both SSB and BPH, and slightly less
169 on BPH-infested plants and uninfested plants. Importantly, SSB females laid
170 only very few eggs on SSB-infested plants, significantly less than on the other
171 three treatment (all $P < 0.001$) (Fig. 2h).

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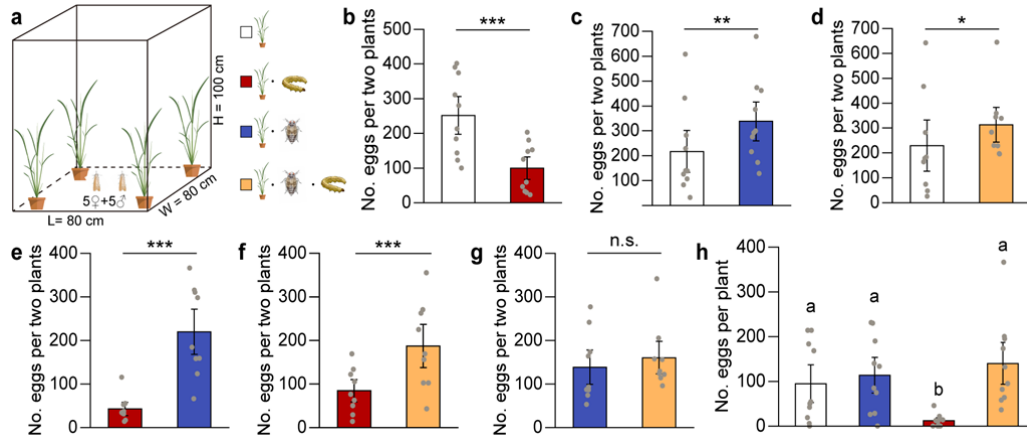
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182 **Figure 2. Oviposition preference of SSB female moths in greenhouse**
183 **experiments.**

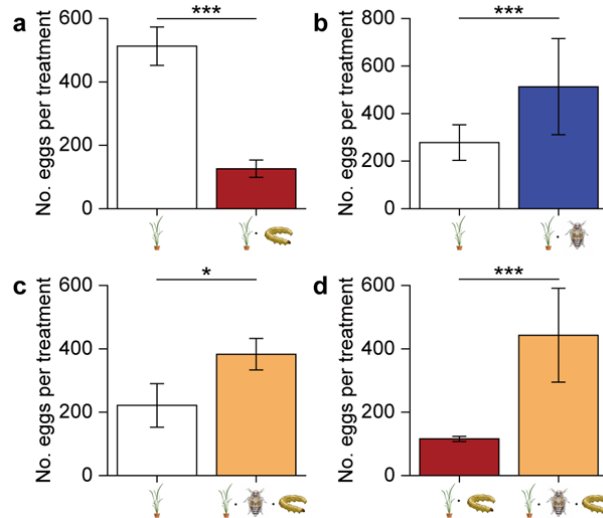
184 Number of eggs laid by female *C. suppressalis* on rice plants that were either
185 uninfested (Control), infested by SSB larvae only (SSB), BPH only (BPH), or
186 by both SSB and BPH (SSB/BPH). **a** Schematic drawing of the oviposition
187 experiments. **b** Control versus SSB larva-infested plants. **c** Control versus
188 BPH-infested plants. **d** Control versus dually infested plants. **e** SSB
189 larva-infested plants versus BPH-infested plants. **f** SSB larva-infested plants
190 versus dually infested plants. **g** BPH-infested plants versus dually infested
191 plants. **h** SSB female moths were exposed to 4 types of rice plants together. LR
192 tests applied to a GLMM were conducted for the number of eggs (Poisson
193 distribution error). Each bar represents the mean \pm SE. Data columns with
194 asterisks (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$, or with small letters ($P < 0.05$)
195 indicate significant differences between treatments; n.s. indicates a
196 non-significant difference ($P > 0.05$) (N = 9–11).

197

198 ***Oviposition preferences under field conditions***

199 Consistent with the results from the greenhouse experiments, far more eggs
200 were laid on uninfested plants than on SSB-infested plants (RT-test applied to
201 a GLMM, Poisson distribution error; $P < 0.001$) (Fig. 3a). Compared to
202 uninfested rice plants, the females preferred to lay eggs on BPH-infested
203 plants ($P < 0.001$) or SSB/BPH-infested plants ($P = 0.03$) (Fig. 3b, c). When
204 given a choice between SSB-infested plants and SSB/BPH-infested plants, the
205 females preferred to lay eggs on the co-infested plants, as expected (RT-test
206 applied to a GLMM, Poisson distribution error; $P < 0.001$) (Fig. 3d).

207



208

209 **Figure 3. Oviposition preference of SSB female moths in field cage**
210 **experiments.**

211 Number of eggs laid by female *C. suppressalis* on rice plants that were either
212 uninfested (Control), infested by SSB larvae only (SSB), BPH only (BPH), or
213 both SSB and BPH (SSB/BPH). **a** Control versus SSB larva-infested plants. **b**
214 Control versus BPH-infested plants. **c** Control versus dually infested plants. **d**
215 SSB larva-infested plants versus dually infested plants. LR tests applied to a
216 GLMM were conducted for the number of eggs (Poisson distribution error).
217 Each bar represents the mean \pm SE. Data columns with asterisks (***) $P < 0.001$,
218 * $P < 0.05$) indicate significant differences between treatments (N = 3–4).

219

220 Rice plant defense responses to herbivore infestation

221 Gene expression changes

222 RNA-seq analysis was carried out to assess gene expression changes in
223 response to infestation by SSB, BPH or both species. A partial least squares
224 discriminant analysis on all 12 transcriptomic datasets provided a global view
225 of the total gene expression across the four treatments. As shown in Fig. 4a,
226 the first two principal components (PCs) explained 39.5% (PC1), and 13.3%
227 (PC2) of the total variance, respectively. PC1 revealed a clear separation of
228 samples with SSB infestation (SSB and SSB/BPH) from others (BPH and
229 control). And PC2 separated samples with BPH infestation (BPH and
230 SSB/BPH) from others (SSB and control). Taken together, these results
231 suggest that each infestation regime had distinctly different effects on the gene
232 expression of rice plants.

233 The gene expression analyses showed that feeding by SSB resulted in the
234 differential expression of 12512 genes (absolute $\log_2(\text{fold change}) > 0$ and $P <$
235 0.05), of which 6533 genes were up-regulated and 5979 genes
236 down-regulated. Infestation by BPH alone induced a relative weak response of
237 rice plant, that is, 2523 differentially expressed genes including 1292
238 upregulated genes and 1231 downregulated genes. Co-infestation by the two
239 species induced the upregulation of 3640 genes and the downregulation of
240 4082 genes (Fig. 4b). Interestingly, compared to SSB-infested plants, 992
241 genes were downregulated when plants were co-infested with BPH and SSB,
242 and the gene ontology (GO) enrichment analysis showed that these repressed
243 genes were largely associated with defense responses, such as JA-related
244 process, and enzyme inhibitor activity (Supplementary Data 1).

245

246 ***JA and SA associated gene expression and their accumulation***

247 A total of 10392 *Arabidopsis* orthologs of rice genes were included in
248 phytohormone-related gene expression signatures (Supplementary Data 2).
249 The results showed that, when compared to uninfested plants, genes
250 associated with JA and SA pathways were generally upregulated in plants
251 infested by SSB larvae, BPH nymphs, or both species (Fig. 4c). However, dual
252 infestation by SSB and BPH resulted in an apparent downregulation of both JA
253 and SA pathways in rice, as when compared to SSB-infested plants.
254 Specifically, four genes involved in JA biosynthesis, *OsLOX9*, *OsJAR1;2*,
255 *OsDAD1;3*, and *OsAOC*, were significantly induced upon SSB infestation but
256 were not induced when plants were co-infested by both BPH and SSB
257 (Supplementary Data 3). More surprisingly, two genes involved in JA pathway,
258 *OsLOXL-2* and *OsAOS3*, and nine SA-responsive genes (*OsPR2*, *OsPR4*,
259 *OsPR4B*, *OsPR4C*, *OsPR4D*, *OsPR6*, *OsPR10*, *OsPR10A*, and *OsPR10B*)
260 were activated by SSB infestation, but were suppressed by dual infestation
261 (Supplementary Data 4).

262 Consistent with the phytohormone-related gene expression results, SSB

263 infestation induced a significant increase in the levels of both JA (12 hr vs. 0 hr,
264 $P < 0.001$; 24 hr vs 0 hr, $P < 0.001$) and SA (12 hr vs. 0 hr, $P < 0.001$; 24 hr vs 0
265 hr, $P = 0.004$; Fig. 4d, e). However, when BPH nymphs were added after 12 hr
266 infestation by SSB alone, the levels of JA and SA in rice plants significantly
267 decreased (JA: $P = 0.02$; SA: $P < 0.001$; Fig. 4d, e).

268

269 ***Protease inhibitor-associated gene expression and their accumulation***

270 When determining GO terms that were significantly enriched ($P_{adj} < 0.05$) in
271 the set of 992 downregulated DEGs comparing dual infestation samples and
272 SSB infestation samples, we identified several molecular function terms that
273 were associated with protease inhibitor activity including serine-type
274 endopeptidase inhibitor activity (GO:0004867), enzyme inhibitor activity
275 (GO:0004857), endopeptidase inhibitor activity (GO:0004866), peptidase
276 inhibitor activity (GO:0030414) (Supplementary Data 1). After screening for
277 genes involved in these categories, we found 11 genes related to protease
278 inhibitors that were highly induced by SSB infestation, but were significantly
279 attenuated by the additional infestation of BPH nymphs (Fig. 4f). The
280 expression of nine genes was validated by qRT-PCR, showing similar
281 expression patterns among the four treatments as obtained with RNA-seq
282 (Supplementary Fig. 1), confirming the reliability of the RNA-seq data.

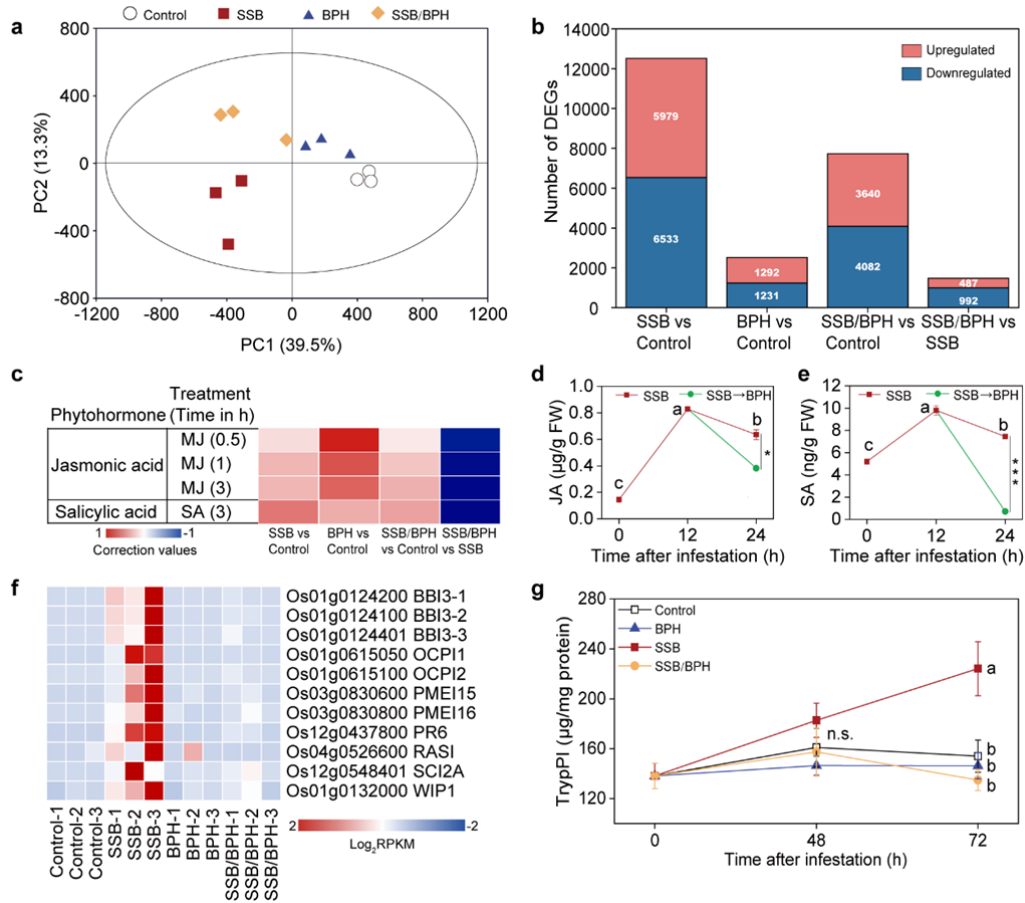
283 Prompted by the observed changes in protease inhibitor-associated gene
284 expression, we further measured the contents of TPIs in rice plants responding
285 to different herbivore infestations. The results showed a significant increase in
286 TPIs content after 72 hr SSB infestation compared to uninfested plants ($P =$
287 0.03). This TPIs content increase was not observed for BPH-infested plants or
288 SSB/BPH-infested plants (Fig. 4g).

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294 **Figure 4. Rice plant responses to herbivore infestation.**

295 **a** Partial least squares discriminant analysis (PLS-DA) of all detected genes in
 296 rice plants that were either untreated (control), infested by SSB, BPH, or both
 297 herbivores (SSB+BPH) for 48 h (three biological replicates per treatment). The
 298 percentage of variation of the data explained by principal component 1 (PC1)
 299 and PC2 is in parentheses (39.5 and 13.3%, respectively). The score plot
 300 displays the grouping pattern according to the first two PCs and the ellipse
 301 defines the Hotelling's T2 confidence interval (95%) for the observations. **b**
 302 Differentially expressed genes among differently treated rice plants. **c**
 303 Hormonometer analyses for JA and SA signatures based on transcriptomic
 304 responses of rice to herbivory. The colors indicate similarity between herbivore
 305 infestation and a particular hormone response (blue and red for negative and
 306 positive correlations, respectively, see bottom). MJ, methyl jasmonate; SA:
 307 salicylic acid. The contents of endogenous JA (**d**) and SA (**e**) in rice plants
 308 subjected to SSB infestation or SSB plus BPH in sequence. Letters indicate
 309 significant differences among SSB treatment with different time points;
 310 asterisks indicate significant differences between SSB and SSB→BPH
 311 treatments ($P < 0.01$; $N = 3$). Bars indicate mean \pm SE. **f** Heatmap of the
 312 expression of 10 enzyme inhibitors genes. Log₂-transformed RPKM values are
 313 plotted. BBI 3-1, Bowman-birk inhibitor 3-1; BBI 3-2, Bowman-birk inhibitor 3-2;

314 BBI3-3, Bowman-Birk inhibitor 3-3; OCPI1, *Oryza sativa* chymotrypsin
315 inhibitor-like 1; OCPI2, *Oryza sativa* chymotrypsin inhibitor-like 2; PME1 15,
316 pectin methylesterase inhibitors 15; PME1 16, pectin methylesterase inhibitors
317 16; PR6, pathogenesis-related proteins 6; RASI, rice alpha-amylase/subtilisin
318 inhibitor; SCI2A, subtilisin-chymotrypsin inhibitor-2A; WIP1, wound-induced
319 protease inhibitor. FPKM, fragments per kilobase of transcript per million
320 fragments mapped. **g** Time course of the contents of trypPI in rice plants that
321 were either uninfested (Control), infested by SSB, BPH, or both (N=3). Bars
322 indicate mean \pm SE. Data was analyzed using one-way ANOVA followed by
323 LSD post hoc test. Letters indicate significant differences between treatments
324 ($P < 0.05$). n.s., not significant ($P > 0.05$).

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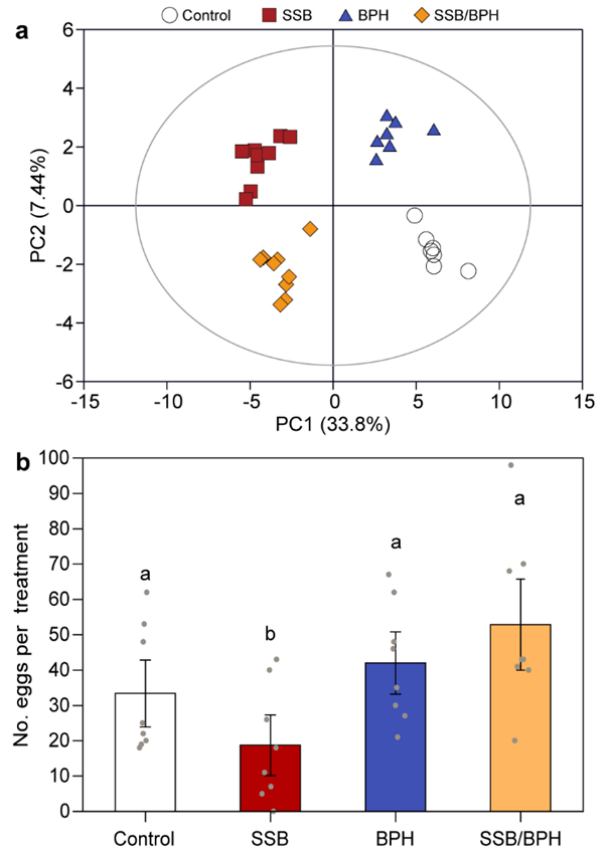
326 **Volatile profiles of rice plants and their effects on the oviposition** 327 **behavior of SSB females**

328 A total of 61 compounds were detected in the headspace of the four plant
329 treatments (Supplementary Data 5). Plants infested by both SSB and BPH
330 emitted the highest amounts of volatiles, followed by plants infested by SSB,
331 plants infested by BPH, whereas control plants emitted the lowest amounts of
332 volatiles. A projection to partial least squares-discriminant analysis (PLS-DA)
333 using the contents of all detected volatiles showed a clear separation among
334 the four treatments (Fig. 5a). The first two significant principle components
335 (PCs) explained 33.8% and 7.44% of the total variance, respectively.
336 Consistent with gene expression data, the first PC showed a clear separation
337 between plant infested with SSB (SSB and SSB/BPH) from others (BPH and
338 control), and the second PC separated BPH-infested samples and
339 SSB-infested samples (BPH and SSB) from others (SSB/BPH and control).

340 Using volatiles that had been collected from plants subjected to the four
341 types of treatments as odor sources (applied on filter paper strips), we tested
342 the oviposition preference of SSB females. They differently distributed their
343 eggs among the four treatments (Fig. 5b, RT-test applied to a GLMM, Poisson
344 distribution error; $P < 0.001$), with highest number of eggs observed on or near
345 filter paper that had been treated with volatiles collected from
346 SSB/BPH-infested plants, which was statistically similar to the numbers of

347 eggs laid on BPH-infested plants and uninfested plants. However, SSB
348 females laid significantly lower numbers of eggs on filter paper treated with
349 volatiles collected from SSB-infested plants compared to any of the other
350 treatment (all $P < 0.001$) (Fig. 5b).

351



352

353 **Figure 5. Volatiles released by rice plants and effect on oviposition**
354 **behavior of *C. suppressalis* female.**

355 **a** Projection to latent structures-discriminant analysis (PLS-DA) of volatile
356 emissions produced by rice plants that were either untreated (Control),
357 infested by SSB, BPH, or both herbivores (SSB/BPH) for 48 h. The score plot
358 display the grouping pattern according to the first two components and the
359 ellipse defines the Hotelling's T2 confidence interval (95%) for the
360 observations. **b** Number of eggs laid by female *C. suppressalis* on filter paper
361 that had been treated with volatiles collected from 4 types of differently treated
362 rice plants. LR tests applied to a GLMM were conducted for the number of
363 eggs (Poisson distribution error). Each bar represents the mean \pm SE. Letters
364 above bars indicate significant differences between treatments (RT-test
365 applied to a GLM, Poisson distribution error; $P < 0.05$; N = 8).

366

367

368 **Responses of *T. japonicum* wasps to herbivore-infested rice plants**

369 In a dual-choice assay, the *T. japonicum* wasps showed a strong preference for
370 plants infested by SSB eggs ($P = 0.004$) over uninfested intact plants (Fig. 6a).
371 When offered plants carrying SSB eggs in both sides of the olfactometer, the
372 wasps exhibited a significant preference for plants that were additionally
373 infested by SSB larvae relative to plants infested by SSB eggs alone ($P =$
374 0.002). In contrast, the wasps were significantly more attracted to plants with
375 eggs alone than plants that were additional infested by BPH nymphs ($P <$
376 0.001) or both SSB larvae and BPH nymphs ($P = 0.001$). We further tested the
377 preferences of wasps that were offered plants with combinations of herbivore
378 and egg infestation. The results (Fig. 6a) showed that *T. japonicum* females
379 significantly preferred to rice plants with SSB and eggs over plants coinfeasted
380 by BPH and eggs ($P < 0.001$) or plants with SSB, BPH, and eggs ($P = 0.01$).
381 Finally, as expected, the wasps showed a significant preference for the odor of
382 plants with the combination of SSB, BPH, and eggs over the odor of plants
383 with BPH and eggs ($P = 0.009$). These results imply that additional infestation
384 by BPH resulted in the repellence of *T. japonicum* wasps.

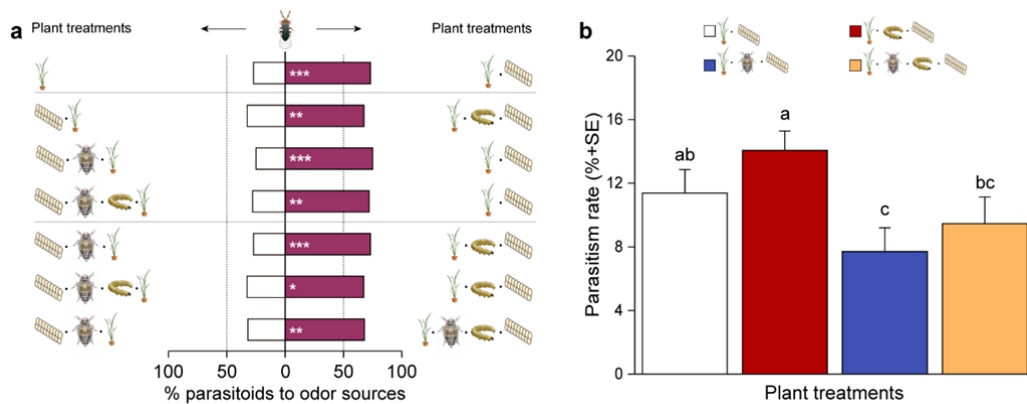
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386 **Parasitism rates of SSB eggs by *T. japonicum* wasps**

387 In the greenhouse cages with the four plant treatments, the rate of parasitism
388 of SSB eggs by *T. japonicum* wasps were highest on plants infested with SSB
389 larvae only (Fig. 6b), which was significantly higher than on plants infested with
390 BPH only ($P < 0.001$) or plants infested with both herbivore species ($P = 0.016$).
391 The lowest parasitism rate of SSB eggs was observed on plants infested by
392 BPH only. Although parasitism was lower on plants infested by both species,
393 this was not significant different from parasitism on control plants ($P = 0.28$).
394 The parasitism rates of SSB eggs in the cages nicely reflected the trends of
395 responses of the parasitoids in the olfactometer (Fig. 6b).

396

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398

399 **Figure 6. Preferences of females of the egg parasitoid *Trichogramma***
400 ***japonicum*.**

401 **a** Choice of *T. japonicum* wasps when offered the odor of differently treated
402 plants in a Y-tube olfactometer. Bars represent the percentages of wasps
403 choosing either of the odor sources. Asterisks indicate significant differences
404 from a 50:50 distribution (binomial test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; N
405 =58-80). **b** Parasitism rates of SSB eggs by *T. japonicum* in the greenhouse
406 experiments. The proportions data was subjected to an arcsin square-root
407 transformed before analyses. Each bar represents the mean \pm SE. Letters
408 above bars indicate significant differences between treatments (likelihood ratio
409 test applied to a generalized lineal model; $P < 0.05$; N = 12).

410

411 Discussion

412 Defining *cooperation* as “any interaction in which an actor confers a fitness
413 benefit to another individual and receives an (inclusive) fitness benefit in
414 return”³³, we may conclude that the observed mutually beneficial interactions
415 between SSB and BPH indeed represent a unique example of cooperation
416 between two herbivores. We found that not only do the herbivores directly
417 (mitigated plant toxicity) and indirectly (reduced exposure to parasitoids)
418 benefit from jointly attacking plant, we also found that both have adapted their
419 host plant selection and oviposition behavior to optimize the benefits that they
420 derive from each other.

421 Our previous work shows that BPH prefers to feed and lay eggs on
422 SSB-infested rice plants, which are more nutritious and on which their eggs
423 escape parasitism^{25,34}. The current study demonstrates similar, even stronger

424 benefits for SSB when infesting plants that are already under attack by BPH.
425 The feeding assays show that SSB infestation induces rice defense responses
426 that cause significantly reduced fitness in SSB larvae that subsequently feed
427 on the same plants (Fig. 1). Remarkably, additional infestation by BPH
428 completely neutralized the negative effects of SSB defense induction; SSB
429 caterpillars placed on plants already infested by conspecifics grew
430 considerably better on plants that were also attacked by BPH, independent of
431 order of infestation. On BPH-infested plants the caterpillars performed just as
432 well as on previously uninfested control plant (Fig. 1)

433 RNA-seq and biochemical analyses showed that infestation by BPH
434 suppresses a broad spectrum of defense related genes (Fig. 4f,
435 Supplementary Data 1), with one of the main consequences being a significant
436 reduction in SSB-induced production of proteinase inhibitors (Fig. 4g). These
437 are key defensive compounds of rice plants that are particularly effective
438 against chewing herbivores, including SSB^{35,36}. We also found that
439 co-infestation with BPH suppresses the expression of SA- and JA-associated
440 genes that are normally upregulated by SSB infestation (Fig. 4c,
441 Supplementary Data 4). This suppression led to reduced levels of JA and SA in
442 the plants (Fig. 4d, e). The JA signal transduction pathway is responsible for
443 the production of TPIs in rice plant, and it is known that SSB performs better on
444 JA-deficient mutant rice lines mainly due to reduced TPIs levels^{36,37}.
445 Collectively, this insight explains why BPH feeding neutralizes the negative
446 effects of SSB defense induction.

447 Insect attack typically induces defense responses in plants³⁸⁻⁴¹, but it is
448 also increasingly evident that various insect herbivores have the ability to, at
449 least partially, suppress plant defenses by interfering with JA or SA
450 biosynthesis and that this can enhance their own performance and that of
451 conspecifics^{28,42-45}. This can also benefit certain other species that feed on the
452 same plants. For example, the silverleaf whitefly (*Bemisia tabaci*) activates SA
453 signaling and represses JA-regulated defenses, leading to enhanced nymphal

454 development of this insect²⁸, which also benefits spider mites²⁶. None of
455 these studies appear to show reciprocal benefits for species feeding on the
456 same plant.

457 Consistent with ‘mother knows best’ hypothesis⁴⁶, SSB females avoid to lay
458 eggs on rice plants that are already infested by conspecifics, thus ensuring
459 that their offspring evade the negative effects that SSB-induced defenses³⁰.
460 Here we show that SSB females have adapted their oviposition behavior to
461 preferentially oviposit on BPH-infested rice plants, independent of whether
462 SSB larvae are already present or not, as compared to healthy plants (Figs. 2
463 and 3). By doing so, they benefit from the BPH-mediated suppression of rice
464 defense responses (Fig. 1a). However, the performance of SSB larvae was not
465 any better on rice plants that were already co-infested by SSB plus BPH
466 compared to their performance on healthy plants. So why did SSB females
467 prefer to lay eggs on dual-infested plants rather than on healthy plants (Fig.
468 2d)? The experiments with the egg parasitoid *T. japonicun* provide a plausible
469 explanation, as they showed that the presence of BPH significantly reduced
470 the attractiveness of rice plants to the wasp (Fig. 6a). The cage experiment
471 confirmed that the presence of BPH decreased the risk for SSB eggs to be
472 parasitized, implying that the oviposition strategy of SSB females is highly
473 adaptive (Fig. 6b).

474 It appears that certain well-adapted herbivores can also manipulate the
475 emission of volatiles of their host plants⁴⁷. In most such cases, herbivore
476 infestation suppresses certain key volatile compounds and thereby possibly
477 reduce the attractiveness of plants to certain natural enemies^{26,27,42,48,49}. Other
478 herbivores can benefit from this as well. Simultaneous feeding by slugs, for
479 instance, suppress caterpillar-induced volatiles in cabbage plants, thereby
480 reducing the attractiveness of the plants to parasitoids²⁷. Similarly, when
481 spider mite-infested Lima bean plants are also infested by whiteflies this leads
482 to a reduced emission of the volatile (E)- β -ocimene compared to plants
483 infested by spider mites only, resulting in a reduced attraction of predatory

484 mites²⁶. In other cases, double infestation may actually lead to higher
485 quantities of volatiles being emitted, but the blend is altered in a way that it is
486 no longer attractive to parasitoids²³. This is also the case for our study system.
487 We previously showed that BPH preferentially oviposits on SSB-infested rice
488 plants, thereby avoiding the attraction of the egg parasitoid *Anagrus*
489 *nilaparvatae*²⁵. These various examples confirm that insect herbivores not
490 only can evolve the ability to use volatiles to identify host plants of better
491 nutritional quality, but also plants where their offspring can escape natural
492 enemies⁵⁰.

493 Our combined results, including the additional tests showing that SSB
494 infestation of rice plants significantly increases the performance of BPH
495 nymphs (Supplementary Fig.2) strongly supported the conclusion of mutually
496 beneficial interaction between SSB and BPH. This goes against the ingrained
497 notion of competition between phytophagous insects that share a common
498 host plant, and how this competition shapes insect assemblages^{1,15,17,51-53}.
499 The resource-based competition theory has been challenged before, but the
500 examples involve specific asymmetric beneficial plant-mediated interactions,
501 meaning that only one of the herbivore species benefits from the presence of
502 another^{20,21,54-58}. In these cases, the benefit is never reciprocal, nor do they
503 seem to represent tightly coevolved interactions with specific behavioral
504 adaptations as found for BPH and SSB in the current study and certain
505 vertebrates⁹⁻¹¹.

506 The interaction between SSB and BPH reported here seems to represent a
507 highly evolved collaboration to cope with and exploit the direct and indirect
508 defense responses of rice plants. The two species share the same host plants
509 and have a similar spatial and temporal distribution throughout Asia's rice
510 paddy area^(25; <https://www.cabi.org/isc/>). Why has their interaction evolved
511 into collaboration rather than competition? It is likely because of their
512 differential feeding strategies. Although the two insects occur side-by-side on
513 rice plants, SSB is a stemborer insect that feeds inside the rice plants, while

514 BPH is a phloem-sucking insect that feeds at the surface on leaf blades and
515 leaf sheaths ^{25,59}. Hence, there is usually no direct physical interaction
516 between the two species. During the coevolutionary arms race between
517 herbivores and host plants, both sides may evolve multiple defense
518 mechanisms against the other. We speculate that the cooperative relationship
519 between SSB and BPH may be the result of two opposing coevolutionary arms
520 races that in combination benefit both herbivores.

521 Although the mutually beneficial interaction between the stem borer and
522 planthopper bears no resemblance to any of the known interactions between
523 other herbivore species attacking a same plant ^{1,15}, the reported type of
524 cooperation is unlikely to be unique. We postulate that agricultural pests are
525 especially prone to rapid evolutionary changes that allow such cooperation to
526 emerge. As pest populations build up in vast monocultures their main
527 challenges revolve around coping with plant defenses and avoiding and
528 resisting their specific natural enemies, whereas finding host plants is no
529 longer a challenge. In such scenarios, different insect species will encounter
530 each other frequently. Unlike the cultivated plants, the insects are subject to
531 natural selection and can evolve traits to jointly overcome plant defenses,
532 without the cultivated plants being able to coevolve to resist these traits. The
533 plants are at the mercy of human selection, which is focused at traits that favor
534 yield and nutritional value, often at the cost of reduced resistance against
535 pests and diseases ^{60,61}. Yet, as we discover and unravel the intricate
536 adaptations in the insects we can start steering this human selection in favor of
537 potent pest resistance traits. For the specific example uncovered in our study,
538 interfering with the ability of BPH to suppress the biosynthesis of proteinase
539 inhibitors could be a highly effective and sustainable strategy to control two of
540 rice's most common and most harmful pests.

541 In summary, the current study reveals a highly adaptive, mutually beneficial
542 relationship between rice planthoppers and stem borers that is mediated by
543 opposing rice plant defense responses. The findings represent a unique

544 example of a cooperative interaction that challenges traditional interspecific
545 competition theory. The two insect species take advantage of the rice defense
546 responses induced by each other in a manner that suggests that together they
547 are the tentative winners in the arms race with rice plants. The results are also
548 illustrative of the complexity and intricate dynamics of the interaction between
549 plants and insects, and challenge the conventional paradigms of interspecific
550 competition. Future work should further unravel more details about the
551 molecular mechanisms underlying the insect-controlled interactions, which
552 might lead the development of rice varieties that disrupt the cooperative
553 interaction as potential strategy to control the two pests.

554

555 **Methods**

556 **Plants and insects**

557 Rice plants (*Oryza sativa*, cultivar Minghui63) were grown in a greenhouse at
558 27 ± 3 °C with $75 \pm 10\%$ RH (relative humidity) and a photoperiod of 16:8 hr
559 L:D (light:dark). The cultivation of rice plants followed the same procedure as
560 described previously²⁵. Plants were used for experiments when they were at
561 the tillering stage, which occurred about 44–49 days after sowing.

562 *C. suppressalis* larvae were reared on an artificial diet as described⁶². Ten
563 percent honey water solution was provided to supply nutrition for the adults. *N.*
564 *lugens* were maintained on a BPH-susceptible rice variety Taichung Native 1
565 (TN1)³⁴. *T. japonicun* were obtained from Keyun Industry Co., Ltd (Jiyuan,
566 China). Newly emerged adult wasps were maintained in glass tubes (3.5 cm
567 diameter, 20 cm height) and supplied with 10% honey water solution as a food
568 source and were maintained for at least 6 hr to ensure freely mating, before
569 females were used for the following experiments. All three species were
570 maintained in climatic chambers at 27 ± 1 °C, $75 \pm 5\%$ RH, and a photoperiod
571 of 16:8 hr L:D.

572

573 **Performance of caterpillars on insect-infested rice plants**

574 Multiple types of rice plants were prepared: i) uninfested plants, meaning that
575 potted rice plants remained intact without insect infestation; (ii) SSB-infested
576 plants, each potted rice plant was artificially infested with one 3rd instar SSB
577 larva that had been starved for > 3 hr for 48 hr; iii) BPH-infested plants, each
578 potted rice plant was artificially infested with a mix of fifteen 3rd and 4th instars
579 BPH nymphs for 48 hr; iv) SSB/BPH-infested plants, each potted rice plant
580 was simultaneously infested with one SSB larva and 15 BPH nymphs for 48 hr;
581 (v) SSB→BPH-infested plants, each potted rice plant was artificially infested
582 with one SSB larvae alone for the first 24 hr, then 15 BPH nymphs were
583 additionally introduced for another 24 hr; vi) BPH→SSB-infested plants,
584 namely each potted rice plant was artificially infested with 15 BPH nymphs for
585 the first 24 hr, then one SSB larvae were additionally introduced for another 24
586 hr. Plant treatments were conducted as described in detail in our previous
587 study ²⁵. During herbivory treatment, the uninfested plants were placed in a
588 separate room to avoid possible volatile-mediated interference. During the
589 subsequent bioassays, both SSB caterpillar and BPH nymphs remained in or
590 on the rice plants.

591 Two bioassays were conducted to test the performance of *C. suppressalis*
592 larvae feeding on differently treated rice plants. The first bioassay included the
593 plants treatments i, ii, iii, and vi, and the second bioassay included the plants
594 treatments i, ii, v and vi. Three 2-day-old larvae of *C. suppressalis* were gently
595 introduced onto the middle stem of each rice plant using a soft brush. The
596 infested rice plants were then placed in climatic chambers at $27 \pm 1^\circ\text{C}$, $75 \pm 5\%$
597 relative humidity, and a photoperiod of 16:8 hr L:D. The *C. suppressalis* larvae
598 were retrieved from the rice plants after 7 days feeding, and they were
599 weighed on a precision balance (CPA2250, Sartorius AG, Germany;
600 readability = 0.01 mg). The mean weight of the three caterpillars on each plant
601 was considered as one biological replicate. The experiment was repeated four
602 times using different batches of plants and herbivores, resulting in a total of
603 30–46 biological replicates for each treatment.

604

605 **Oviposition-preferences of *C. suppressalis* females choosing among**
606 **differently infested rice plants**

607 ***Greenhouse experiment***

608 In the greenhouse, seven choice tests were conducted with *C. suppressalis*
609 females including i) SSB-infested plants versus uninfested plants; ii)
610 BPH-infested plants versus uninfested plants; iii) SSB/BPH-infested plants
611 versus uninfested plants; iv) SSB-infested plants versus BPH-infested plants; v)
612 SSB-infested plants versus SSB/BPH-infested plants; vi) BPH-infested plants
613 versus SSB/BPH-infested plants; and vii) the test in which *C. suppressalis*
614 females were exposed to all four types of rice plants. The experiments were
615 performed as described in detail by ³⁰. In brief, four potted plants were
616 positioned in the 4 corners of a cage (80 cm x 80 cm x 100 cm) made of
617 80-mesh nylon nets for each test. For paired comparisons, two potted plants
618 belonging to the same treatment were placed in opposite corners of each cage,
619 and in the test with 4 types of rice plants, each type of plant was positioned in
620 one of the four corners of each cage. Five pairs of freshly emerged moths (less
621 than 1 day) were released in each cage, and a clean Petri dish (9 cm diameter)
622 containing a cotton ball soaked with a 10% honey solution was placed in the
623 center of the cage as food source. After 72 h, the number of individual eggs on
624 each plant were determined. The experiment was conducted in a greenhouse
625 at $27 \pm 3^{\circ}\text{C}$, $65 \pm 10\%$ RH, and a photoperiod of 16:8 hr L:D. Each choice test
626 was repeated with 9–11 times (replicates).

627 ***Field cage experiment***

628 The oviposition preference of SSB females was further assessed in a field
629 near Langfang City (39.58° N, 116.48° E), China. Four choice tests were
630 conducted: i) SSB-infested plants versus uninfested plants; ii) BPH-infested
631 plants versus uninfested plants; iii) SSB/BPH-infested plants versus uninfested
632 plants; and iv) SSB/BPH-infested plants versus SSB-infested plants. The
633 treated rice plants were prepared as described above and were transplanted

634 into experimental plots (1.5 m × 1.5 m). For each pairwise comparison, six
635 plots of rice plants were covered with a screened cage (8 m × 5 m × 2.5 m)
636 made of 80-mesh nylon net to prevent moths from entering or escaping. Each
637 of the six plots contained 9 rice plants of a particular treatment, with 3 plots per
638 cage representing the same treatment. Plots were separated by a 1-m buffer
639 and they were alternately distributed in a 3 × 2 grid arrangement in each cage
640 (Supplementary Fig. 1). Approximately 50 mating pairs of newly emerged *C.*
641 *suppressalis* adults (< 24 hr) were released into each cage. After 72 hr, the
642 number of individual eggs on each plant were determined. The total number of
643 eggs of three plots in each cage was regarded as one replicate, 3–4 replicates
644 were conducted for each pairwise comparisons.

645

646 **Rice plant response to herbivore infestation**

647 ***RNA-seq and data analysis***

648 To explore the molecular mechanisms underlying the rice plant-mediated
649 interaction between BPH and SSB, gene expression changes in rice response
650 to infestation by SSB, BPH or both were analyzed by RNA-seq. The rice plants,
651 uninfested (control) or infested, were prepared as described above. After 48h,
652 the stems of the plants were harvested and frozen in liquid nitrogen. Samples
653 from 5 individual plants of the same treatment were pooled together as one
654 biological replicate, and three replicates were collected for each treatment.

655 RNA-seq analyses were performed as described previously⁶³. In brief, total
656 RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA)
657 and treated with RNase-free DNase I (NEB, Ipswich, MA, USA) to remove any
658 genomic DNA. Library preparation and RNA-seq were performed by Novogene
659 (Beijing, China) using an Illumina Hiseq 4000 system, resulting in ~45–55
660 million raw reads per sample. Raw reads were subjected to quality checking
661 and trimming to remove adapters, poly-N sequences, and low-quality bases
662 (Phred quality score Q < 20). The yield clean data of each sample were
663 aligned to the rice reference genome IRGSP-1.0 (<https://rapdb.dna.affrc.go.jp>)

664 using HISAT2 (v2.09) ⁶⁴, and the number of reads mapped to each gene was
665 counted with featureCounts (v1.5.0-p3) ⁶⁵. The expression level of each gene
666 was calculated as FPKM (fragments per kilobase of transcript per million per
667 million fragments mapped) according to an established protocol ⁶⁶. Expression
668 differentiation analyses were conducted DESeq2 R package (v. 1.18.0) ⁶⁷.
669 Genes with absolute value of $\log_2(\text{fold change}) > 0$ and $P\text{-value} < 0.05$ were
670 defined as differentially expressed genes (DEGs). The enriched functions of
671 DEGs in RNA-seq data sets were annotated with the Gene Ontology (GO)
672 function using the clusterProfiler R package ⁶⁸, and GO terms with Benjamini–
673 Hochberg false discovery rate (FDR) adjusted $P\text{-value} (Padj) < 0.05$ were
674 considered significantly enriched. The transcriptional signatures of hormonal
675 responses of rice plant to herbivory relative to gene expression in *Arabidopsis*
676 induced by diverse phytohormones was analyzed using Hormonometer
677 program ⁶⁹. Since trypsin protease inhibitors (TPIs) serve as indicators of
678 induced resistance in rice plants, especially against chewing herbivores such
679 as SSB ^{35,36}, the analyses focused on expression profiles of TPIs-related
680 genes among the four plant treatments. The expression of nine selected TPIs
681 genes were validated by quantitative real-time PCR (qRT-PCR) analyses as
682 previously described ⁷⁰. qRT-PCR was conducted on a Bio-RadCFX96 Touch
683 Real-time PCR Detection System instrument (Bio-Rad, Hercules, CA, USA)
684 using TransStart[®] Top Green qPCR SuperMix (TransGen Biotech, Beijing,
685 China). The rice *ubiquitin 5* gene was used as the internal standard to
686 normalize the variations in gene expression. The primers used are listed in
687 Supplementary Table 1.

688

689 ***Quantification of endogenous jasmonic and salicylic acid***

690 Our RNA-seq results suggested that additional infestation by BPH significantly
691 suppressed the expressions of genes related to the defense hormones
692 jasmonic acid (JA) and salicylic acid (SA). Both types of genes are highly
693 upregulated in response to SSB infestation ⁷¹. To confirm this, we quantified

694 the JA and SA levels in rice plants with two treatments: i) rice plants that were
695 infested with one third-instar SSB larva alone for 24 hr; ii) rice plants that were
696 first infested with one third-instar SSB larva for 12 hr and then also with 15
697 BPH female adults for another 12 hr. Rice stems were harvested at three time
698 points: 0 hr (uninfested control plants), as well as 12 hr and 24 hr after
699 infestation. For each treatment, stems from 5 individual plants were harvested
700 and pooled together as one biological replicate, and three replicates were
701 collected for each time point.

702 Endogenous measurements of JA and SA were performed by the plant
703 hormone platform at the Institute of Genetics and Developmental Biology,
704 Chinese Academy of Sciences as previously described⁷².

705 ***Quantification of TPIs***

706 We further measured the accumulation of TPIs in rice plants subjected to
707 insect infestation. These experiments were prompted by the RNA-seq results
708 indicating that the upregulation of TPIs-related genes in response to SSB
709 infestation is significantly suppressed after co-infestation with BPH. The same
710 plant treatments were included as used for RNA-seq but with new batches of
711 plants. Samples were collected at 48 hr and 72 hr of insect infestation. Intact
712 rice plants that served as controls were also sampled at the same time points.
713 Samples from five individual plants were pooled together as one biological
714 replicate, and three replicates were collected for each treatment. All samples
715 were immediately frozen in liquid nitrogen and stored at -80°C until further
716 analyses.

717 TPIs contents was determined using enzyme linked immunosorbent assay
718 (ELISA) kits (J&L Biological, Shanghai, China). The stem samples were
719 ground into a fine power in liquid nitrogen using a mortar and pestle, and each
720 sample (0.1 g) was homogenized in 0.01 M PBS (Phosphate Buffered Saline)
721 buffer (pH = 7.4) (Sigma-Aldrich, St. Louis, MO, USA) with a sample–PBS
722 proportion of 1:9 (1 g plant sample/9 ml of PBS). Samples were centrifuged at
723 4000 g for 15 min at 4°C and the supernatant was collected. The ELISA

724 experiments were performed following the protocols provided with the kits. The
725 optical density values were recorded at 450 nm using a microplate
726 spectrophotometer (PowerWave XS2, BioTek, Winooski, VT, USA). The
727 protein concentrations in plant samples were measured using a bicinchoninic
728 acid (BCA) protein assay kit (Aidlab Biotechnologies Co., Ltd., Beijing, China)
729 according to the manufacturer's instructions. The amount of protease inhibitor
730 was calculated based on a standard curve, and results were expressed as μg
731 protease inhibitor per mg protein.

732

733 **Effect of insect-induced volatiles on the oviposition behavior of SSB** 734 **moths**

735 ***Collection and analysis of rice plant volatiles***

736 Individual rice plants were either uninfested or infested with SSB larvae alone,
737 BPH nymphs alone, or both species simultaneously for 48 hr using the method
738 described above. The emitted volatiles were trapped for 3 hr (21:00–24:00, the
739 time period that SSB lay their eggs), and then analyzed and identified as
740 described²⁵. The compounds were quantified as a percentage of peak areas
741 relative to the internal standard (nonyl acetate) per 3 hr of trapping for one
742 plant. For each treatment, collections and analyses were repeated 7–9 times.

743 ***Odor preferences of SSB females***

744 The response of SSB females to volatiles released from differently treated rice
745 plants were investigated to better understand the mechanism underlying the
746 moth's oviposition preferences. The total volatiles emitted from uninfested
747 plants, SSB-infested plants, BPH-infested plants and SSB/BPH-infested plants
748 were collected for this experiment. Plant treatments and volatiles collections
749 were the same as described above but without the addition of the internal
750 standard. The collected volatiles were diluted in paraffin oil (purity 99%;
751 Sigma-Aldrich, St. Louis, MO, USA) at 1:4 (v/v) and were stored at $-80\text{ }^{\circ}\text{C}$
752 before use.

753 One ml of each of the four types of volatile solutions were separately

754 pipetted on the center of a filter paper strip (4 cm × 21 cm), which were then
755 hung from the four corners of a cage (45 cm × 45 cm × 45 cm) made of
756 80-mesh nylon net. Five pairs of freshly emerged SSB moths (< 24 hr) were
757 released in each cage. After 72 hr, the number of eggs deposited on the filter
758 paper strips and the surface of the nylon nets near each paper strip were
759 determined. This oviposition choice test was repeated 8 times.

760

761 **Response of the egg parasitoid *T. japonicum* to herbivore-infested rice**
762 **plants**

763 Multiple types of herbivore-infested rice plants were prepared: i) uninfested
764 plants (control); ii) SSB-infested plants; iii) BPH-infested plants; iv)
765 SSB/BPH-infested plants; v) plants infested with SSB eggs (referred to as
766 egg-infested plants); vi) plant infested with SSB larvae and their eggs (referred
767 to as SSB/egg-infested plants); vii) plants infested with BPH nymphs and SSB
768 eggs (BPH/egg-infested plants); and viii) plants infested with both SSB larvae,
769 BPH nymphs and SSB eggs (referred to as SSB/BPH/egg-infested plants). To
770 prepare these treatments, plants were first artificially infested with herbivores
771 for 48 hr as described above, then some of them were subjected to SSB eggs
772 deposition. For that, two potted rice plants of the same type were placed in a
773 cage (45 cm × 45 cm × 45 cm) made of 80-mesh nylon nets, then 30 pairs of
774 freshly emerged moths (< 24 hr) were released in each cage to mate and lay
775 eggs. After 24 hr, the plants were removed from the cage and those that
776 carried 200-250 eggs were used as odor sources. During the period of egg
777 deposition and the subsequent olfactometer experiments with the parasitoid,
778 all insects remained in or on the rice plants.

779 To test the behavioral responses of *T. japonicum* to differently treated rice
780 plants, they were offered the following pairs of odor sources: i) uninfested
781 plants versus egg-infested plants; ii) uninfested plants versus SSB-infested
782 plants; iii) uninfested plants versus BPH-infested plants; iv) egg-infested plants
783 versus SSB/egg-infested plants; v) egg-infested plants versus BPH/eggs

784 infested plants; vi) SSB/egg-infested plants versus BPH/egg-infested plants;
785 vii) egg-infested plants versus SSB/BPH/egg-infested plants; viii)
786 SSB/egg-infested plants versus SSB/BPH/egg-infested plants; and ix)
787 BPH/egg-infested plants versus SSB/BPH/egg-infested plants.

788 Responses of *T. japonicum* females to these odor sources were investigate
789 in a Y-tube olfactometer as described ²⁵. Newly emerged adult wasps were
790 maintained in glass tubes (3.5 cm diameter, 20 cm height) for at least 6 hr to
791 ensure that they would mate, before females were used for the experiments.
792 Two rice plants of the same treatment were enclosed in a glass bottle and
793 used as one odor source, and each pair of odor sources was replaced after ten
794 parasitic wasps were tested. For each treatment, a total of 64–88 female
795 wasps were tested. The experiments were conducted between 10:00 and
796 16:00 on several consecutive days.

797

798 **Parasitism rates of *C. suppressalis* eggs by *T. japonicum* wasps**

799 In a cage experiment, we further tested if the differences in parasitoid
800 attraction observed in the olfactometer for the differentially infested plants can
801 result in differences in parasitism rates of SSB eggs under realistic conditions.
802 The following herbivore-treated plants were prepared as described above:
803 SSB eggs on uninfested, SSB-infested, BPH-infested and SSB/BPH-infested
804 plants. The four types of plants were placed in the four corners of a cage
805 (60cm × 60 cm × 60 cm) made of 80-mesh nylon nets, respectively.
806 Subsequently, 40 pairs of newly emerged wasps (<1 day old) were released
807 into the cage. After 48 hr, the rice leaves with SSB eggs were collected, and
808 the total number of SSB eggs on each plant was counted and their
809 parasitization status was determined under a microscope two days later; the
810 eggs turned black after being parasitized for 3 days. The experiment was
811 replicated 12 times. The experiment was performed in a greenhouse at 27 ±
812 3°C and with 75 ± 10% RH and a photoperiod of 16:8 hr L:D.

813

814 **Statistical analyses**

815 All data were checked for normality and equality of variances prior to statistical
816 analysis. Likelihood ratio test (LR test) applied to a generalized lineal mixed
817 model (GLMM) for overdispersion and grouped design were conducted to
818 compare the number of eggs laid by SSB females on rice plants (Poisson error
819 structure with log link function). Likelihood ratio test (LR test) applied to a
820 generalized lineal model (GLM) were conducted to compare the parasitism
821 rates of SSB eggs by *T. japonicun* (normal distribution error) with the cage
822 treated as a random factor; the percentage data of parasitism rates were
823 arcsin square-root transformed prior to analyses. Two-way analysis of variance
824 (ANOVA) followed by least significant difference (LSD) test was used to
825 compare the body weight increases of the SSB larvae on different plant
826 treatments. The contents of JA and SA in different samples was analyzed
827 using one-way ANOVA followed by Tukey honest significant difference (HSD)
828 test or two-sided Student's *t*-test. Behavioral responses of *T. japonicun* in
829 Y-tube assays were analyzed using binomial test with an expected response of
830 50% for either olfactometer arm; parasitoids that did not make a choice were
831 excluded from the analysis. Differences in volatile emission and in gene
832 expression were analyzed by partial least squares-discriminant analysis
833 (PLS-DA)^{25,55} using SIMCA 14.1 software (Umetrics, Umeå, Sweden). The
834 omics data were normalized by medians, log-transformed, and then
835 auto-scaled (mean centered and divided by the standard deviation of each
836 variable) using Metaboanalyst 4.0 software⁷³ before they were subjected to
837 PLS-DA. All statistical analyses were conducted with SPSS 22.0 (IBM SPSS,
838 Somers, NY, USA), except for the PLS-DA were performed using SIMCA 14.1
839 software.

840

841 **Data availability**

842 RNA-seq raw data have been deposited to the Gene Expression Omnibus
843 (GEO) database in the National Center for Biotechnology Information (NCBI)

844 (note: accession number is not open yet). All other relevant data are available
845 from the corresponding author upon reasonable request.

846

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852 **Author contributions**

853 Y.Li conceived and directed the project. Y.Li, Q.L., X.H., and T.C.J.T. designed
854 the study. X.H. and S.S. performed the experiments. Q.L., X.H., S.S., Y.Li, and
855 T.C.J.T analyzed the data. Q.L., X.H., S.S., Y.P., G.Y., Y.Lou, T.C.J.T., and Y.Li
856 wrote the manuscript. All authors have read and approved the manuscript for
857 publication.

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859 **Competing interests**

860 The authors declare no competing financial interests.

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862 **Additional information**

863 **Supplementary information** The online version contains supplementary
864 material available.

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