An effector from cotton bollworm oral secretion impairs host plant defense signaling

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Insects have evolved effectors to conquer plant defense. Most known insect effectors are isolated from sucking insects, and examples from chewing insects are limited. Moreover, the targets of insect effectors in host plants remain unknown. Here, we address a chewing insect effector and its working mechanism. Cotton bollworm (Helicoverpa armigera) is a lepidopteran insect widely existing in nature and severely affecting crop productivity. We isolated an effector named HARP1 from H. armigera oral secretion (OS). HARP1 was released from larvae to plant leaves during feeding and entered into the plant cells through wounding sites. Expression of HARP1 in Arabidopsis mitigated the global expression of wounding and jasmonate (JA) responsive genes and rendered the plants more susceptible to insect feeding. HARP1 directly interacted with JASMONATE-ZIM-domain (JAZ) repressors to prevent the COI1-mediated JAZ degradation, thus blocking JA signaling transduction. HARP1-like proteins have conserved function as effectors in noctuidae, and these types of effectors might contribute to insect adaptation to host plants during coevolution.

Insect effector | Plant defense | Jasmonate signaling | Coevolution

Plants and insects have developed sophisticated mechanisms to adapt to each other during coevolution. About 50% of the insect species feed on plants. To escape or survive from attacks by herbivorous insects, plants are not only equipped with physical barriers (such as cuticles, trichomes, and thorns) and toxic compounds, but also initiate an intricate network of signal recognition and transduction upon insect challenge (1). In plants, the initial signal perception and transduction are essential for an appropriate defense against biotic stress. Plants can recognize herbivore-associated molecular patterns (HAMPs) and trigger various defense signal transduction (2, 3).

The phytohormone jasmonate (JA) plays an important role in activating defense against biotic attacks including chewing insects (4). CORONATINE INSENSITIVE1 (COI1), a component of the ubiquitin E3 ligase SCFCOI1 is the first reported jasmonoyl-isoleucine (JA-Ile) receptor (5–7). JASMONATE-ZIM-domain (JAZ) proteins bind to transcription factors such as MYC2 to restrict JA signal output (8). The contents of JA and JA-Ile in plant cells are maintained at a low level in the absence of stress and rise rapidly upon external stimuli, such as wounding or insect herbivory (1). JA-Ile promotes COI1-JAZ interaction and triggers JAZs degradation, releasing transcription factors to activate downstream defense genes (6, 9–11).

To adapt to their host plants, insects have developed multilayered mechanisms, including a highly specialized oral cavity for feeding and complex digestive systems for enzymatic processing of toxin-laden diets (12–15). In addition, herbivorous insects contain active molecules in their oral secretion (OS), which either trigger or interfere with plant defense during herbivory (16, 17). For example, certain fatty acid conjugates (FACs) and lipases in the OS of caterpillars can be recognized by plants and act as elicitors to induce plant defense response (18, 19). Even the proteolytic fragments of chloroplastic ATP synthase γ-subunit from Fabaceae plants in insect OS are able to elicit plant defense (20). Besides elicitors, the insect-released molecules that disturb host–plant defense response are defined as insect effectors (3). The first reported effector protein in a herbivorous insect is glucos oxide (GOX) from Helicoverpa zea, which inhibits nicotine accumulation in tobacco (21). The presence of insect effectors was further supported by the observation that the Spodoptera littoralis larvae fed on leaves pretreated with the OS gained more weight increase than the larvae fed on control leaves (22). Analysis of secretory proteins in aphids revealed multiple effector proteins in saliva of sucking insects (23–27). A set of salivary glands secreted proteins from green peach aphid (Myzus persicae) have distinct locations in its infected plants, indicating that these proteins may be directly involved in plant–aphid interaction (28, 29). The well-studied effectors in saliva, AcpC002 from the pea aphid (Acrystiphon pisum) and its

Significance

Plants recognize insect-derived molecules and make the accurate defense response during herbivory. However, insects release effectors that disturb host plant defense responses for fitness. Effectors are crucial components in biotic interactions. We identified a caterpillar-derived effector (HARP1) from oral secretion of cotton bollworm, a devastating agricultural pest. HARP1 is released from larvae to plant leaves during feeding and is able to migrate from wounding site into plant cells automatically. HARP1 interacts with JASMONATE-ZIM-domain (JAZ) proteins, the suppressor of Jasmonate (JA) pathway, and blocks signaling transduction by preventing JAZ degradation. HARP1-like proteins are widely distributed and have conserved function in noctuidae, and they may contribute to insect adaptation to host plants during coevolution.

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homolog in the green peach aphid (*M. persicae*, MpC002), were helpful for aphid fitness when reared on plants (23, 27). Recently, a new effector Bt56 from whitefly (*Bemisia tabaci*) was identified, which helped whitefly become more adapted to host plants (30). However, most known insect effectors are isolated from sucking insects and very few effectors other than GOX from chewing insects have been reported. Furthermore, the targets of insect effectors and the working mechanism of how insect effectors counteract host plant defense remains largely enigmatic.

Cotton bollworm (*Helicoverpa armigera*) is a chewing insect and one of the most devastating pests in agriculture. In this study, we identified an effector, HARP1, from *H. armigera* OS. HARP1 shows similarity to venom R-like proteins from the parasitoid wasp (*Nasonia vitripennis*) venom glands, which were proposed to interfere with the animal host immune system (31). Our investigation demonstrates that HARP1 is able to migrate into host plant cells and interact with multiple JAZ repressors. HARP1–JAZ interaction stabilizes JAZ degradation and blocks JA signaling transduction. REPAT38, a homolog in *Spodoptera exigua*, acts similarly as HARP1, which, together with the wide distribution of HARP1 homologs in insects, suggests a relation between the evolution of HARP1-like proteins and host adaptation in herbivorous insects.

**Results**

**HARP1 Is an Effector in Cotton Bollworm OS Impairing Plant Wounding Response.** OS of a lepidopteran larva contains the mixture of saliva and regurgitant from the insect gut (32). When insects were fed on plants, the OS sticks to the leaf wounding sites and this made it possible for OS to directly interact with plant signals and modulate plant defense. To test the activity

![Image of Fig. 1 showing HARP1 in H. armigera OS migrates into the leaf cells through the wounding damage sites.](image-url)
of insect OS on plant defense, we collected OS from cotton bollworm and painted it on the mechanically wounded sites of *Arabidopsis* leaves. As the JA alarm signal is rapidly triggered by wounding, we first examined the expressions of JA-responsive genes, including *LOX2, MYC2*, and *VSP2*. We found that the induction of JA-regulated genes by wounding was attenuated in the OS-treated leaves (*SI Appendix, Fig. S1*). We then performed proteomic analyses of the OS collected from the fourth-instar *H. armigera* larvae fed on artificial diet or *Arabidopsis* leaves by liquid chromatography-mass spectrometry (LC-MS). In total, 149 proteins were identified (*Dataset S1*). The accumulation of 65 proteins in the OS sample from the larvae fed on *Arabidopsis* leaves was increased (>1.5 fold) compared with that fed on artificial diet, among which 49 proteins were digestion related (*Dataset S1*). We examined the remaining 16 proteins (*SI Appendix, Table S1*) and found that one is similar to a probable venom R-like protein 1 (*HARP1*). *HARP1* is 122 amino acids in length and contains a predicted signal peptide at the N terminus (analyzed by “SignalP 4.1 Server”, http://www.cbs.dtu.dk/services/SignalP). As venom proteins in the carnivorous insects often target to host immune system during predation (31) and the *HARP1* protein accumulation in OS increases upon herbivory, we wondered whether this class of proteins in herbivorous insects also affect the host plant defense.

**HARP1** transcripts were abundant in foregut and midgut tissues but near the limit of detection in salivary glands (*Fig. L4*). Interestingly, the **HARP1** protein level was much higher in OS than in midgut and gut fluid, and notably, its accumulation was increased in OS when the larvae fed on the artificial diet supplemented with 0.1% gossypol, the major defense compound in cotton plants (33) (*Fig. L4*), which also stimulates the expression of defensive P450 genes in cotton bollworm midgut (12, 13). The **HARP1** accumulation in OS also varied with host plants, as the HARP1 was enriched in OS from *Arabidopsis* that HARP1 can be induced when the larvae fed on a rather toxic or unfavorable food. The HARP1 was enriched in OS from

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**Fig. 2.** HARP1 reduces plant response to wounding. (A and B) Genes were less induced upon wounding in the presence of HARP1. *Arabidopsis* (A) and cotton (B) leaves were mechanically wounded and painted with the prokaryotically expressed HARP1 (W+HARP1) or Venus (W+Venus) solutions (1 mg/mL) on the wounded sites. Samples were collected 4 h later, and the gene expressions were detected by qRT-PCR. The expression in unwounded plants (CK) was set to 1. Data were analyzed by Student’s t test. *P < 0.05, **P < 0.01. Error bars represent ± SD (n = 3 biological replicates in A, n = 5 biological replicates in B). All of the experiments were repeated three times, and the results were consistent. (C–E) The impacts of HARP1 reduction in OS on the larval adaptation to plants. (C) HARP1 accumulation was reduced in OS from the larvae fed on 35S:dsHARP1 plants. Third-instar larvae were fed with wild-type (WT) and 35S:dsHARP1-4 plant leaves for 4 d. HARP1 level in OS samples from the larvae fed on the WT (OS*WT*) and the 35S:dsHARP1-4 (OS*dsHARP1-4*) *Arabidopsis* leaves were detected by immunoblot. The amount of total proteins in each loading was quantified by Bradford assay and visualized by CBB staining. (D) The growth of *H. armigera* larvae fed with 35S:dsHARP1-4 plants was inhibited compared with those fed with wild type. Weight increases of third-instar larvae fed on *Arabidopsis* leaves of WT and 35S:dsHARP1-4 (dsHARP1-4*) for 4 d were recorded. Data were analyzed by Student’s t test. *P < 0.05. Error bars represent ± SEM (n = 32). (E) Gene expressions in plant leaves after the treatment with the OS samples as described in C. *Arabidopsis* leaves were wounded and painted with OS*WT* and OS*dsHARP1-4*, respectively; samples were collected 2 h after treatment. qRT-PCR was used to detect gene expressions. The gene expression in the unwounded plants (CK) was set to 1. *P < 0.05, **P < 0.01. Error bars represent ± SD (n = 5 biological replicates).
Band intensity was quantified by ImageJ and was shown under each blot. The time after wounding. Anti-HA antibody was used to detect JAZ3-HA (JAZ3).

| Loading was quantified by Bradford assay and visualized by CBB staining. COI1 ratios were listed in the bottom. The amount of total proteins in each intensity of the untreated than in HARP1. JAZ3 accumulation. The JAZ3-HA level is more stable in HARP1 reduces COI1-JAZ3 coprecipitation. Recombinant proteins of HIS-JAZ3 (Pull down) immunoprecipitation. Anti-MYC antibody was used to detect the proteins of 35S:6MYC-HARP1, which grew normally in our greenhouse. Two lines with high (line 1 and line 7) and one line with low (line 2) HARP1 expression levels (SI Appendix, Fig. S2B) were selected for further analyses. H. armigera larvae fed on the plants with high-level HARP1 gained significantly more weight than those fed on the wild type, whereas the larval growth was not obviously affected when fed on the plants with low-level HARP1 (SI Appendix, Fig. S3 A and B). Consistent with the HARP1 painting assay, the JA-responsive genes of TAT1, MYC2, and VSP2 were less induced in 35S:6MYC-HARP1-1 plants but not in the low-level HARP1 plants 35S:6MYC-HARP1-2 by mechanical wounding than that in wild-type plants (SI Appendix, Fig. S3 C and D). To investigate the global effects of HARP1 on plant wounding response, we performed RNA-sequencing (RNA-seq) using the wild-type and the 35S:6MYC-HARP1 (line 1) plant leaves. In the wild type, mechanical wounding led to a significant transcriptional reprogramming: 1,224 wound-induced genes (WIGs) and 1,395 wound-repressed genes (WRGs) were detected. However, the up- and down-regulated genes were reduced to 512 and 259, respectively, in the 35S:6MYC-HARP1 (SI Appendix, Fig. S4A and Dataset S2). This indicated that the plant wounding response was largely affected by the presence of HARP1. We then analyzed the expression level of 1,224 WIGs in the 35S:6MYC-HARP1 plant and found that 418 of them showed less or no induction after wounding (SI Appendix, Fig. S4B). Gene Ontology (GO) enrichment analysis revealed that these 418 HARP1-aFFECTed genes were enriched in several exogenous stimulus-related pathways, including response to JA stimulus (P = 5.5e-9) (SI Appendix, Fig. S4C). In wild-type plants, 34 genes clustered in the GO term, response to JA stimulus, were obviously induced by wounding, whereas 16 of these genes, about 47% (16/34), showed less or no induction in 35S:6MYC-HARP1 plant (SI Appendix, Fig. S4D). Further analysis by qRT-PCR confirmed 9 of the 13 selected HARP1-aFFECTed genes were obviously less induced by wounding in 35S:6MYC-HARP1 (SI Appendix, Table S3 and Fig. S5). Furthermore, the induction was JA-dependent as the wounding induction of these nine genes was largely suppressed in the JA-insensitive mutant coil-2 (5) (SI Appendix, Fig. S6). These data demonstrate that expression of HARP1 in transgenic plants hampered the JA-Ile signal-mediated wounding response.
HARP1 expressing plants under WT and weight increase when fed on 35S:6MYC-HARP1 jazQ upon wounding. The prokaryotically expressed HARP1 (W) were set to 1. Data were analyzed by Student’s t test. *P < 0.05, **P < 0.01, ***P < 0.001. Error bars represent ± SD (n = 3 biological replicates). (B) The gene inductions were not affected by HARP1 in the jazQ mutant upon wounding. The prokaryotically expressed HARP1 (W+HARP1) and Venus (W+Venus) was painted on the wounding sites of WT and jazQ plant leaves. The unwounded plants were used as control (CK). Gene expressions were detected by qRT-PCR 4 h after treatment. The gene expressions in unwounded WT were set to 1. Data were analyzed by two-way ANOVA followed by multiple comparisons (Tukey test). (*P < 0.05, **P < 0.01). All of the experiments were repeated at least two times, and the results were consistent.

**Fig. 4.** Wounding responses and insect resistance were not obviously affected by HARP1 in the jaz quintuple mutant jazQ. (A) Wounding responses were higher in jazQ. The wild-type (WT) and jazQ leaves were treated with mechanical wounding; gene expressions in leaves of the unwounded and the wounded (W) plants were detected by qRT-PCR 4 h after treatment. The gene expressions in unwounded WT were set to 1. Data were analyzed by Student’s t test. *P < 0.05, **P < 0.01, ***P < 0.001. Error bars represent ± SD (n = 3 biological replicates). (B) The gene inductions were not affected by HARP1 in the jazQ mutant upon wounding. The prokaryotically expressed HARP1 (W+HARP1) and Venus (W+Venus) was painted on the wounding sites of WT and jazQ plant leaves. The unwounded plants were used as control (CK). Gene expressions were detected by qRT-PCR 4 h after treatment. The gene expressions in unwounded WT were set to 1. Data were analyzed by Student’s t test. *P < 0.05, **P < 0.01. Error bars represent ± SD (n = 5 biological replicates). (C) H. armigera larvae gained similar weight increase when fed on 35S:6MYC-HARP1 jazQ (HARP1 jazQ) and jazQ leaves. The third-instar larvae were fed with plant leaves for indicated days, and the weight increases were measured (n = 24). (D) 35S:6MYC-HARP1 jazQ and jazQ plants exhibited similar gene inductions upon wounding. The HARP1 expressing plants under WT and jazQ background were treated with wounding (W) and samples of WT, 35S:6MYC-HARP1 (HARP1), jazQ, and 35S:6MYC-HARP1 jazQ (HARP1 jazQ) were collected 4 h after treatment. Gene expressions were detected by qRT-PCR. The expression in the unwounded WT was set to 1. Data were analyzed by two-way ANOVA followed by multiple comparisons (Tukey test). (*P < 0.05, **P < 0.01). All of the experiments were repeated at least two times, and the results were consistent.

**HARP1 Directly Interacts with JAZ Proteins from Arabidopsis, Cotton, and Tobacco.** Transient expression of a GFP-HARP1 fusion protein in tobacco (Nicotiana benthamiana) leaves showed that the protein was mainly localized in nucleus (SI Appendix, Fig. S7). This finding, combined with the observation that the HARP1 protein was mainly localized in nucleus (Fig. 1S), suggests that HARP1 may affect the JA signal transduction pathway within the nucleus. As JAZ proteins are nucleus-localized and the master negative regulators of JA signal transduction pathway within the nucleus. As JAZ protein in tobacco (Fig. 1S), as well as from the nonpreferred hosts of Arabidopsis (A. thaliana) and tobacco (N. benthamiana). We found that HARP1 exhibited a clear binding activity to multiple JAZ proteins, including 5 of the 10 Arabidopsis JAZs and four of the five cotton JAZs tested, in addition to the one tobacco JAZ (Fig. 34 and SI Appendix, Fig. S84). The Arabidopsis JAZ3 (AT3G17860) was then used in subsequent experiments. A JAZ3 variant lacking the C-terminal Jas motif (JAZ36C) retained the ability to bind with HARP1, whereas removal of the ZIM domain (JAZ36ZIM) or ZIM-containing N terminus (JAZ36N) abolished the interaction (SI Appendix, Fig. S9A and B). indicating that ZIM domain is required for JAZ3-HARP1 interaction. 35S:JAZ36N-HA and 35S:JAZ36C-HA plant (36) were then used for pull-down assays. JAZ3N-HA and JAZ3C-HA were both detectable in total protein extractions of 35S:JAZ36N-HA and 35S:JAZ36C-HA plant leaves but only JAZ36C-HA could be communoprecipitated with HIS-HARP1 (SI Appendix, Fig. S9C). These results indicate that HARP1 binds to the N-terminal region of JAZ3.

We next examined the molecular consequences of HARP1–JAZ3 interaction. Independent pull-down assays three times revealed the consistent results that HARP1 reduced the JAZ3–COI1 coprecipitation (Fig. 3B and SI Appendix, Fig. S9D). The JAZ3 C-terminal fragment (JAZ3N) still could interact with COI1 (37) but not HARP1 (SI Appendix, Fig. S9 A–C) and accordingly, the JAZ3N–COI1 interaction was not affected by HARP1 (Fig. 3C). We then generated 35S:6MYC-HARP1 35S:JAZ3-HA and 35S:6MYC-HARP1 35S:JAZ36N-HA plants by crossing. Upon wounding and MeJA treatments, the JAZ3-HA accumulated to a higher level when HARP1 was coexpressed (Fig. 3D and E and SI Appendix, Fig. S10 A and B). However, the level of JAZ36N-HA, which was free of HARP1 binding, was...
not affected by 35S:6MYC-HARP1 (SI Appendix, Fig. S10 C and D). These results indicate that HARP1 stabilizes JAZ3, likely through interfering with the COI1-mediated protein degradation.

The Effector Activity of HARP1 Is Eliminated in the jaz Quintuple Mutant. To provide further evidence that the cotton bollworm effector HARP1 attenuates the JA-mediated plant defense through JAZ proteins, we used a jaz quintuple mutant (jazQ), which is hypersensitive to JA treatment because five JAZ genes (JAZ1/3/4/9/10) were disrupted (38), and these mutated genes largely overlap with the five JAZ proteins (JAZ1/3/4/9/12) interacted with HARP1 in the yeast two-hybrid assay. In general, the larvae fed with jazQ leaves grew more slowly than those fed with wild-type leaves (SI Appendix, Fig. S11), and the JA response genes (VSP2, TAT1, MYC2) were induced to a higher degree in jazQ upon wounding treatment (Fig. 4A), consistent with the previous report (39). In contrast to the wild-type Arabidopsis, application of recombinant HARP1 to the wounded sites of jazQ leaves did not obviously repress the induction of the JA-response genes (Fig. 4B). HARP1 was then overexpressed in jazQ (35S:6MYC-HARP1 jazQ). Two independent lines (line 5 and line 6) with high and similar HARP1 level to that of the 35S:6MYC-HARP1-1 were further analyzed (SI Appendix, Fig. S2C). In the wild-type background, the high level of HARP1 led to a clear attenuation of wounding response and reduced the resistance upon cotton bollworm feeding. By contrast, no obvious difference in larval growth was observed between the jazQ and 35S:6MYC-HARP1 jazQ groups; the jazQ and 35S:6MYC-HARP1 jazQ plants responded to wounding similarly (Fig. 4 C and D). These data demonstrate that JAZ proteins are required by HARP1 to attenuate the plant JA responses and insect resistance.

Conserved Function of HARP1-Like Proteins in Noctuids. Phylogenetic analyses revealed that HARP1-like proteins are widely present in Lepidoptera and highly conserved in noctuid insects (Fig. 5A and SI Appendix, Table S4). REPAT38 (AFH57158.1) from beet armyworm (S. exigua) (40) shares 83% identity with HARP1 (SI Appendix, Table S4). Like HARP1, REPAT38 interacted with multiple JAZs of Arabidopsis (Fig. 5B and SI Appendix, Fig. S8B). Spraying the purified recombinant REPAT38 to the wounded sites of Arabidopsis leaves led to reduced inductions of VSP2, MYC2, and TAT1 (Fig. 5C). The 35S:6MYC-REPAT38 Arabidopsis plants were generated and screened by immune blot assay, and 35S:6MYC-REPAT38-4 was chosen for further assay (SI Appendix, Fig. S3C). We found that, similarly to HARP1, REPAT38 reduced the resistance to insect feeding (SI Appendix, Fig. S12A) and the JA-regulated wounded response (SI Appendix, Fig. S12B) in transgenic Arabidopsis. These results...
suggest that the function of HARP1-like proteins is likely conserved in noctuid insects.

The Oligophagous *Plutella xylostella* Is More Adaptable to HARP1-Expressing Nonhost Plants. Diamondback moth (*P. xylostella*) is a lepidopteran species specifically lived on *Brassica* plants (41). The less conserved HARP1 homolog from diamondback *P. xylostella*, PXH1L (XP_011548876.1), is 41% identical to HARP1 (SI Appendix, Table S4). In yeast two-hybrid assay, PXH1L did not interact with JAZs of *Arabidopsis* except JAZ4 (SI Appendix, Figs. S8C and S13A), which had a relative low transcript level in leaves based on our RNA-seq data (Dataset S2). GFP and 6MYC-HARP1 was transiently expressed in *N. benthamiana* leaves, respectively (SI Appendix, Fig. S13B). In general, the growth was largely inhibited when the *P. xylostella* larvae were fed with the nonhost tobacco (*N. benthamiana*). Interestingly, when fed on the *N. benthamiana* leaves expressing HARP1, the larvae gained more weight than those fed on the GFP-expressing leaves (SI Appendix, Fig. S13C). These results suggest that HARP1 could improve the performance of oligophagous insects to nonhost plants.

Discussion

Various types of insect elicitors are well studied (42), and a recent report reveals that some insect gut microbes release to OS through regurgitation and eliciting plant defense (43). Compared with the extensive studies of elicitors, relatively few studies are focused on insect effectors. In this study, we isolated a chewing insect-derived effector, HARP1, which is released from OS to plant cells and intercept JAZ degradation through interfering with COI1–JAZ3 interaction, thus attenuating JA signaling transduction triggered by insect-wounding damages (Fig. 5D). Although the binding sites of HARP1 and JAZ3 did not overlap with the jas degron, which is required for JAZ3–COI1 interaction, it was possible that the combination of HARP1 and JAZ3 affected the spatial structure of the C-terminal of JAZ3 and, thus, the JAZ3–COI1 interaction. Such regulation of JAZ stabilization was also reported in recent reports (36, 44). Insects have multiple and complex strategies adapting to their host plants. Effectors like HARP1 gives an example that insects can interfere with the plant defense response for better subsistence. Insect OS contains abundant signaling molecules including elicitors and effectors and plays important roles in insect–plant interactions (45, 46); there should be other effectors that contribute to adapting to host plant.

The phytohormone JA plays an important role in regulating the inductive defense against insect herbivores in plants (22). JAZ proteins, the main repressors of JA signaling, also serve as the molecular link in connecting to other signaling pathways (36, 44, 47, 48). In plant biotic interaction, some pathogen-derived effectors target JAZ proteins for successful infections (44, 49). Although the effectors from microbial pathogens have been extensively studied (50–53), examples of the insect effectors, especially from chewing insects, are limited, and HARP1 represents a type of insect effector that hijacks JAZ proteins. Our study reveals that microbe pathogen and insects use the similar strategy to interfere plant defense signaling for fitness.

The fourth and fifth instar larvae of cotton bollworm are large in size and have a big appetite, which causes fast biomass loss of host plants. It seems that the induced plant defense is less effective against such large insects. However, a newly hatched larva is vulnerable to plant defense, ingests less, and causes minor injuries to the host plant. Therefore, the struggle between host plant and the newborn larva is crucial for both sides. JA-regulated defense response is conserved in higher plants. Release the effector proteins like HARP1 to weaken the JA response in the host plant during feeding seems to be an effective way for young larvae to survive at the very early life stage.

Insect herbivores have developed multiple adaptive mechanisms along with their feeding habits. Oligophagous insects evolved specific detoxification systems, which enable insects to successfully live on a defined group of plants accumulating similar specialized metabolites usually toxic to insects. For example, the diamondback moth is specified to live on *Brassica* plants and has developed glucosinolate sulfatases to desulfate glucosinolates, blocking the formation of cyanogenic products in *Brassicas* (54). However, the polyphagous insects are compatible to a broad range of plants. Since HARP1 can bind with JAZ proteins from different groups of plants (Fig. 3A), it may constitute one of the molecular basis for cotton bollworm to use a wide variety of plant species as food resources. Our findings suggest that the HARP1-type effectors may have consequences in the evolution of insect adaptation to their host. Further identification of effectors from herbivorous insects will enrich our knowledge about plant-plant coevolution and help design novel strategies to control the populations of chewing pests.

Materials and Methods

The detailed information about plant materials and treatments, insect culture and feeding test, OS collection and preparation, gene expression, immunoblot, immunohistochemistry and pull-down assays, transcriptome analysis and proteomic analyses are described in SI Appendix, SI Materials and Methods.

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