**What attracts the allies of *Aristolochia contorta*?**

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**Abstract**

1. In the evolutionary arms race between plants and herbivores, sophisticated mechanisms of indirect defense play a pivotal role. This study investigated the intricate ecological dynamics between *Aristolochia contorta, Sericinus montela*, and *Ooencyrtus* spp., with a special focus on the role of volatile organic compounds (VOCs) in plant.
2. This study utilized field surveys, olfactometer experiments, and Gas Chromatography-Mass Spectrometry (GC-MS) analysis to investigate the role of volatile organic compounds.
3. Field surveys showed a 54.6% egg parasitism rate, with quadrats containing *A. contorta* and larvae attracting more *Ooencyrtus* spp. than those with the plant alone. In olfactometer bioassays, *Ooencyrtus* spp. demonstrated a notable preference for leaves damaged by a pattern wheel, attracting 46.8% of *Ooencyrtus* spp. compared to undamaged controls. Moreover, leaves treated with larval saliva were found to be similarly attractive, drawing in 48.7% of Ooencyrtus spp.. In addition, the difference in attraction between leaves with and without larval saliva did not reach statistical significance. GC-MS analysis identified essential VOCs in the damaged leaves, including hexyl acetate, cyclohexene, δ-cadinene, α-pinene, and β-caryophyllene. Additionally, leaves treated with larval saliva revealed the presence of exo-isocitral (0.61%), and β-pinene (0.14%), though in minimal amounts. Despite these complex responses, our analysis suggests that the compounds introduced or increased in concentration by larval saliva do not significantly boost the attraction of *Ooencyrtus* spp.
4. This finding implies that while the VOCs response to damage and saliva application is multifaceted, serving multiple defensive functions, the quantities of these saliva-induced compounds could be insufficient to substantially influence the behavior of *Ooencyrtus* spp. towards the damaged leaves. This research furthers our understanding of the indirect defense strategies of plants, particularly highlighting the vital roles of VOCs in *A. contorta*. Moreover, our findings suggest new avenues for exploring the ecological and evolutionary roles of chemical signals, shedding light on the complex interactions facilitated by these chemical cues in plant defense mechanisms.

**Key words:** *Aristolochia contorta,* *Sericinus montela*, *Ooencyrtus* spp., Damage-induced plant volatiles (DIPVs), Defense strategy, GC-MS, Herbivore, VOCs.

1. **Introduction**

The evolutionary arms race between plants and herbivorous insects has led to the development of sophisticated plant defense strategies, reflecting millions of years of dynamic adaptation and counter-adaptation (Mathur et al., 2024; Whitehill et al., 2023). This ongoing struggle has driven plants to evolve a complex array of mechanisms aimed at deterring insect attacks and minimizing damage (Yactayo-Chang et al., 2020). These strategies evolve in response to the changing tactics of herbivores, which in turn develop adaptations to overcome plant defenses, creating a perpetual cycle of interaction and counteraction (War et al., 2018).

Direct defense mechanisms in plants include physical barriers, like thick leaves or spines, and chemical defenses such as bitter-tasting compounds or toxins (Mahawer et al., 2022; Wari et al., 2022). However, facing herbivores that have developed resistances to these defenses (Wojda et al., 2020), plants have adopted indirect strategies like emitting volatile organic compounds (VOCs), including damage-induced plant volatiles (DIPVs) and herbivore-induced plant volatiles (HIPVs) (Kaplan, 2012; Pearse et al., 2020; Pérez-Hedo et al., 2021). These VOCs are essential in attracting natural predators and parasitoids of herbivores, thus indirectly controlling herbivore populations and reducing herbivory (Davidson-Lowe & Ali, 2021). By alerting neighboring plants to potential threats, this signaling system prompts them to bolster their own defenses, fostering a network of plant communication that contributes to ecological balance (Gebreziher, 2020; Hu et al., 2021; Paudel Timilsena et al., 2020). The interaction between plant-emitted VOCs and parasitoids demonstrates a sophisticated evolutionary strategy that enhances ecosystem stability and diversity by facilitating the control of herbivore populations through the recruitment of their natural enemies (Shivaramu et al., 2017).

*Ooencyrtus* spp., a genus of parasitic wasps, plays a pivotal role in ecological dynamics, highlighting the intricate roles of natural enemies in plant defense strategies (Giovannini et al., 2020). These parasitoids are known for their specialization in parasitizing the eggs of a wide range of herbivorous insects, thereby contributing to the indirect defense strategies of plants (Greenberg et al., 2023). By laying their eggs inside or on the surface of insect eggs (species from the orders Lepidoptera, Hemiptera, Coleoptera, Orthoptera, and Diptera) (Fatouros et al., 2020; Ganjisaffar & Perring, 2020), *Ooencyrtus* spp. larvae consume the host egg content, effectively reducing the population of the next generation of herbivores (Conti et al., 2021; Lesieur & Farinha, 2021). This biological control agent operates within a complex web of interactions, where plants, herbivores, and parasitoids are entwined in a mutualistic relationship (Ferreira & Musumeci, 2021; Power et al., 2021; Rondoni et al., 2021; Sehrawat et al., 2022). The presence of *Ooencyrtus* spp. in an ecosystem can significantly enhance plant survival and fitness by mitigating the impact of herbivorous insects (Power et al., 2021). Their role underscores the importance of biological control in maintaining ecological balance and highlights the intricate strategies evolved by plants to exploit natural enemies in their favor (Bashir et al.; Thompson et al., 2022).

The evolutionary interplay between plants and their herbivorous predators is vividly illustrated in the interaction between the dragon swallowtail butterfly, *Sericinus montela*, a vulnerable species in South Korea, and the Northern pipevine, *Aristolochia contorta* (Park et al., 2023). *A. contorta* produces Aristolochic acid, a potent chemical deterrent against a wide array of herbivores (Park et al., 2023). However, the larvae of *S. montela*, which are exclusively dependent on *A. contorta* for nourishment, have evolved a detoxifying mechanism to neutralize Aristolochic acid, rendering this direct defense strategy ineffective (Palma-Onetto et al., 2024). This remarkable adaptation not only showcases the complex evolutionary dynamics between plants and their herbivorous predators but also highlights the necessity for plants like *A. contorta* to increasingly rely on indirect defenses. Through this specific example, the intricate interplay of adaptation and counter-adaptation in the natural world is underscored (Gardner & Agrawal, 2002; War & Sharma, 2014; War et al., 2018), demonstrating how a single species' evolution can prompt broader ecological shifts towards more nuanced survival strategies (Gardner & Agrawal, 2002).

Despite these insights, the specific interactions between *A. contorta, S. montela*, and *Ooencyrtus* spp., likely mediated by VOCs, remain underexplored. Especially, the majority of research on VOCs has been conducted in controlled laboratory environments (Gebreziher, 2020; Heil, 2004; Poelman et al., 2009; Shivaramu et al., 2017), with only a handful of studies successfully showing an increase in carnivore attack rates on herbivores in the field due to VOCs. To address this gap, we hypothesize that the distribution and behavior of *Ooencyrtus* spp. in environments where *A. contorta* and *S. montela* coexist are influenced by particular VOCs. Our study, employing field surveys and olfactometer experiments, aims to elucidate the role of these VOCs in shaping the interactions within this ecological community. This research will provide valuable insights into the dynamics of plant-insect interactions, highlighting the evolutionary ingenuity of nature in developing survival strategies.

**2. Methods**

1) Field Survey

To investigate the habitats of *A. contorta*, we conducted a comprehensive review of literature and media reports. This review helped us select 7 sites located on roadsides near rivers or rice paddies across South Korea for our study (Figure 1). Field surveys and observations at these sites were initiated in August 2021. We set up total of 64 quadrats in the 7 different sites based on the presence of *A. contorta* alone, *A. contorta* with eggs of *S. montela*, and *A. contorta* with both eggs and larvae of *S. montela*. We conducted specific searches for egg clusters, surveyed the number of eggs per cluster, noted the height at which *S. montela* egg clusters were located on *A. contorta*, confirmed parasitism, and calculated parasitism rates for each region. Additionally, we installed sticky traps (35 cm x 25 cm) at a height of 1.5 meters within the middle of the quadrats, at the height where egg clusters of the vine were most densely distributed, and left them hanging for 48 hours, in order to count the number of *Ooencyrtus* spp. in each condition.

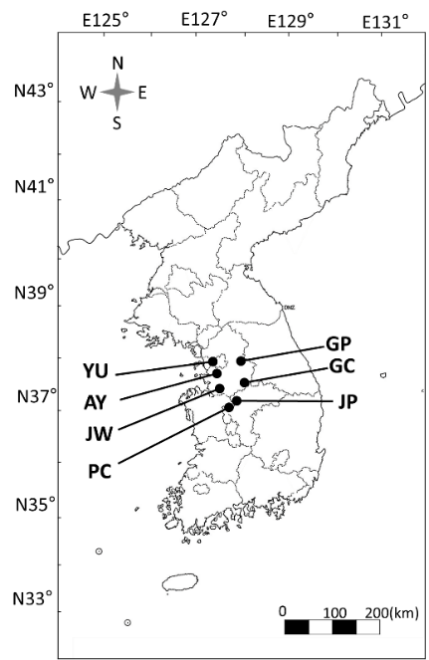


Figure 1 Study sites (filled circles) in South Korea. YU: Yeouido, AY: Anyang, JW: Jinwee, PC: Pyungchon, GP: Gapyeong, GC: Gangcheon, JP: Jeungpyeong.

2) Olfactometer bioassay

We used a T-shaped olfactometer to study the distribution preferences of *Ooencyrtus* spp., offering them different dual choices. The experiments were conducted using a T-shaped olfactometer made of transparent glass, with a diameter of 3 cm, a stem of 30 cm, and arms of 15 cm each. Filtered air, at a rate of 0.3 L/min, was drawn through the olfactometer by a pump connected to a flowmeter. *A. contorta* seeds, collected from AY in November 2021, were cultivated in a greenhouse at Seoul National University in 2022. By August, leaves had been harvested and were subsequently treated under two conditions: controlled and scratched with a pattern wheel. In the first T-shaped olfactometer setup, fresh egg clusters of *S. montela*, which had not been parasitized, were positioned at one end, and fresh eggs of *Paracycnotrachelus chinensis*, known from literature not to be parasitized by *Ooencyrtus* spp. (Fatouros et al., 2020), were placed at the other end, ensuring the same surface area as the *S. montela* clusters. In the second setup, we placed five untreated *A. contorta* leaves with similar area sizes at one end and five leaves that had been scratched with a pattern wheel, also with similar area sizes, at the other end. In the third setup, we placed five untreated *A. contorta* leaves at one end and five pattern-wheel-scratched leaves treated with larval saliva, all of similar size, at the other end. In the last setup, we placed five pattern-wheel-scratched leaves at one end and five pattern-wheel-scratched leaves treated with larval saliva, both groups of leaves having similar sizes, at the other end. 50 - 70 *Ooencyrtus* spp. were then placed at the start of the central arm of the T-shaped and observed for a duration of 30 minutes. A "choice" was determined when an *Ooencyrtus* spp. crossed a predefined threshold line located at the midpoint of each arm and remained at the end of the arm for at least 20 seconds. For the purposes of statistical analysis, only the insects that made a choice towards one arm within the first 30 minutes of observation were included. To avoid bias from possible chemical marking of the tracks, the glass tube was changed after each test. The position of the T-shaped was rotated 180° after each test to counter any directional bias. The T tubes were cleansed in hot water (70°C) and dried in an oven at 60°C before each experiment. This procedure was repeated 10 times, and after this period, the position of each individual was recorded. All experiments were conducted under artificial light at 25 ± 2°C, with white paperboard surrounding the olfactometer to prevent visual perturbations and ensure a homogeneous light environment.

3) GC-MS Analysis

In August 2022, we collected two leaves from each of the total 30 pots of *A. contorta*, with the plant heights about 150 cm (predominant oviposition site). Leaves from 10 of these pots were scratched with a pattern wheel, another 10 had leaves both scratched with the pattern wheel and subsequently applied with larval saliva, while the leaves from the remaining 10 pots were left untreated. The 2 leaves were placed in clean individual 20 ml glass vials and empty vials without any leaves were prepared in the same manner as the leaf samples as controls. Leaf headspace volatiles were collected using polydimethylsiloxane (PDMS) tubing as previously described (Kallenbach et al., 2014). This PDMS tubing exhibits a high affinity for a wide range of volatile compounds, allowing efficient trapping of leaf volatiles from the headspace. Clean strips of PDMS tubing, 1 cm in length, were placed directly inside the vials (including control vials that sampled the surrounding air) and left undisturbed for 24 h. Gas chromatography-mass spectrometry (GC-MS, QP2020, Shimadzu, Kyoto, Japan) connected to a TD-30 thermal desorption unit (Shimadzu, Kyoto, Japan), was used for volatile analysis. The volatile compounds were separated using an Rtx-5MS column (30 m x 0.25 mm ID, 0.25 μm film thickness; Shimadzu, Kyoto, Japan). The sample was injected in split mode with a 40:1 split ratio, and helium was used as the carrier gas with a constant linear velocity of 52.9 cm/s. The gas chromatograph oven temperature program started at 40°C for 5 minutes, then increased to 195°C at a rate of 3°C/min, followed by a further increase to 280°C at a rate of 30°C/min, and held at 280°C for 0.83 minutes. The mass spectrometer operated in electron ionization (EI) mode, and the mass spectra were acquired in scan mode from m/z 33 to 400. The identification of the compounds was confirmed by matching their mass spectra against the NIST 14 mass spectral library. The quantification of the compounds was performed by integrating the peak areas and normalizing them against the leaf area (cm2). From these calculations, the percentage composition of the VOCs was determined.

4) Data Analysis

We employed analysis of variance (ANOVA) after the homogeneity of variance test and post-hoc tests (Duncan’s test) to determine the significance of the number of *Ooencyrtus* spp. among the conditions, with the significance level set at *p* < 0.05. Pearson's correlation coefficient was employed to evaluate the relationship between the number of *Ooencyrtus* spp. and the number of *S. montela* larvae and eggs, with the significance level set at *p* < 0.01. We employed an independent t-test to analyze the relative abundance of VOCs emission from scratched and controlled *A. contorta* leaves. We utilized SPSS software version 23.0 (SPSS, Inc., Chicago, IL, USA) for the statistical analysis.

**3. Results**

1. Field Survey

Our field surveys conducted across the regions revealed significant insights into the oviposition behavior of *S. montela* on *A. contorta*. *Sericinus montela* laid about 58.7 eggs at once (in a single cluster) (Figure 2a). Egg clusters were commonly found at heights between 10 to 200 cm above ground level, with a majority located at approximately 150 cm, indicating a possible preferential height range for oviposition by *S. montela.* The distribution of parasitized clusters is dispersed without preference for the height of *A. contorta* (Figure 2b).

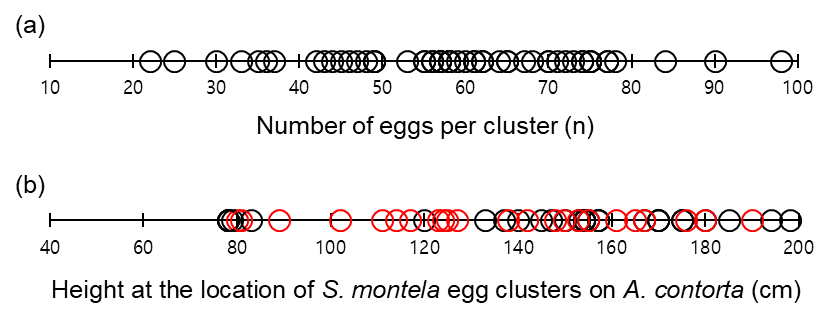


Figure 2 Number of eggs and distribution of *S. montela* egg clusters on *A. contorta*. (a) Number of eggs per cluster, (b) height at the location of *S. montela* egg clusters on *A. contorta* (the red circles are parasitized egg clusters).

Parasitism rates varied by region, with a noteworthy presence of *Ooencyrtus* spp. parasites identified (Figure 3). The overall parasitism rate observed across all regions is around 54.6%. Among the regions, JP exhibits the highest parasitism rate at 66.7%, followed by GP and YU both at 60.0%, JW at 60.0%, GC at 50.0%, PC at 42.9%, and finally, AY with the lowest rate at 40.0%.

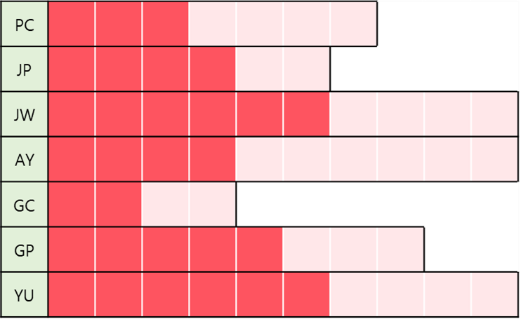


Figure 3 Parasitism in egg clusters (dark red squares for parasitized and light red squares for unparasitized, each square represents one egg cluster).

The sticky traps installed within the quadrats captured an average of 35 *Ooencyrtus* spp. individuals per trap. The highest count of *Ooencyrtus* spp. individuals was associated with *A. contorta* combined with both larvae and eggs, recording an average of 67.2 individuals. This was followed by *A. contorta* with eggs only, with an average of 41.3 individuals. The presence of *A. contorta* with larvae yielded an average of 37.4 individuals. The lowest count was observed in the samples containing only *A. contorta*, with an average of 18.8 individuals (Figure 4). Significant correlations were observed between the number of *Ooencyrtus* spp. and *S. montela* larvae (r = 0.691) and eggs (r = 0.549), both with *p* < 0.01.

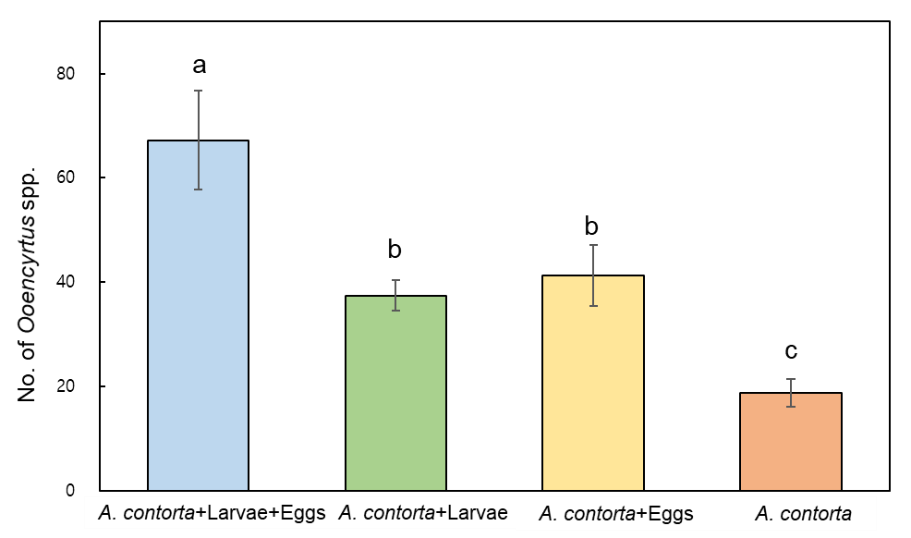


Figure 4 Sticky trap captures of *Ooencyrtus* spp. across different conditions. Letters on the graph indicate significant differences at the 5% level, based on Duncan’s test. Bars indicate standard errors.

1. Olfactometer bioassay

When presented with a choice between non-parasitized *S. montela* egg clusters and *Paracycnotrachelus chinensis* egg clusters in a T-shaped olfactometer, *Ooencyrtus* spp. exhibited no significant preference for *S. montela* eggs. Notably, a considerable proportion of *Ooencyrtus* spp. (46.7%) remained at the base of the olfactometer, opting not to select any of the egg clusters, thereby indicating an absence of preference for the provided options (Figure 5a). Conversely, in separate trials, *Ooencyrtus* spp. demonstrated a distinct preference for *A. contorta* leaves that had been mechanically damaged with a pattern wheel, with these leaves being chosen in 46.8% of the instances (Figure 5b). Moreover, when the leaves had been both scratched with the pattern wheel and treated with larval saliva, 47.6% of *Ooencyrtus* spp. were found on these leaves (Figure 5c), indicating a similar level of attraction to both conditions. Analysis revealed no significant difference in the distribution of *Ooencyrtus* spp. between leaves subjected to mechanical damage and those subjected to mechanical damage with larval saliva (Figure 5d).

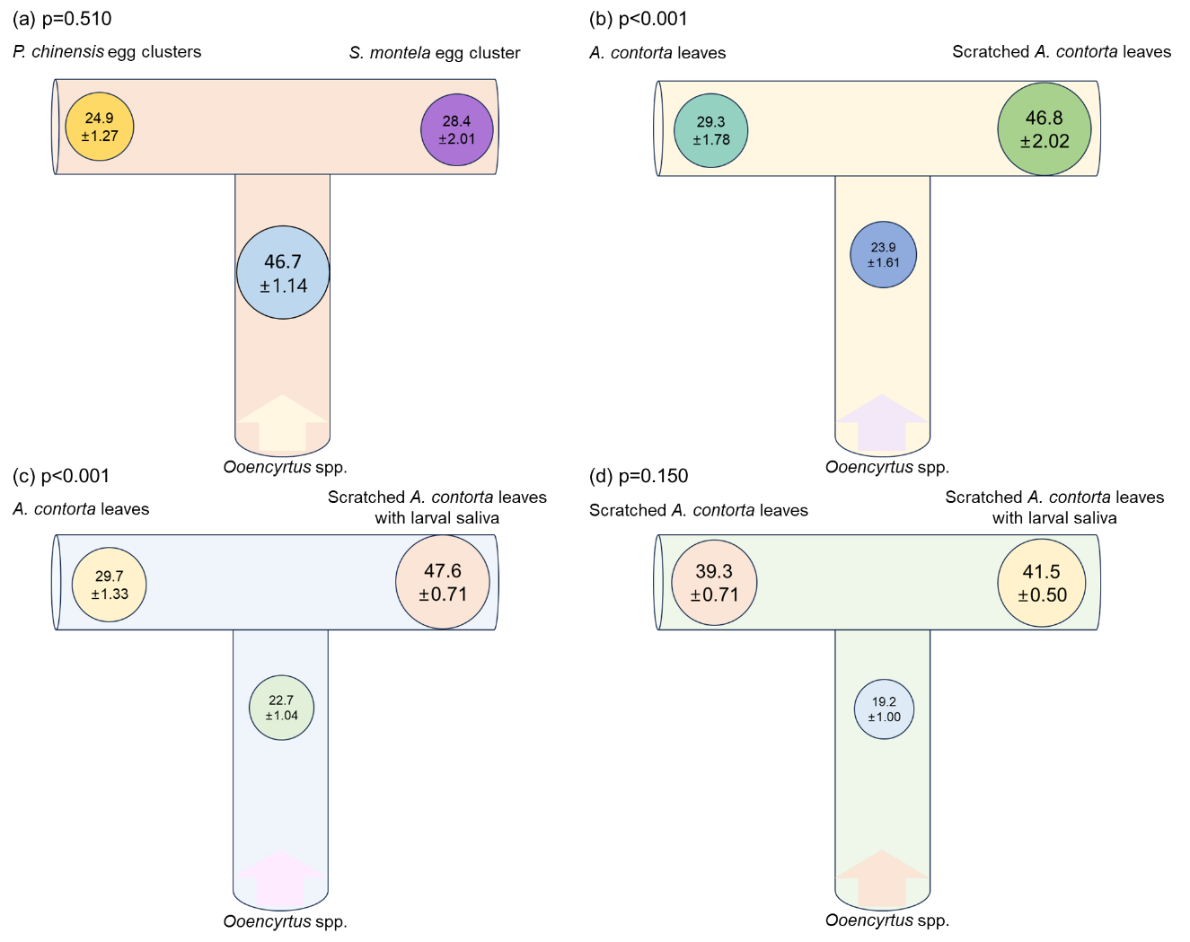


Figure 5 Olfactometer test results revealed the distribution (%) of *Ooencyrtus* spp. choices: (a) between *S. montela* and *P. chinensis* egg clusters, (b) between control and scratched *A. contorta* leaves, (c) between control and *A. contorta* leaves scratched and treated with larval saliva, and (d) between scratched *A. contorta* leaves and leaves scratched and treated with larval saliva.

1. VOCs Analysis

VOCs analysis of *A. contorta* leaves revealed distinct profiles between the controlled leaves, scratched leaves, and scratched leaves with larval saliva (Figure 6). Scratched leaves and those treated with larval saliva emitted a higher diversity of VOCs, with certain compounds showing significant changes in concentration when comparing controlled leaves to those that were scratched. Exo-isocitral was not detected in controlled or scratched leaves but was present at 0.61% after treatment with saliva. β-Pinene also appeared after the application of saliva at 0.14%, despite being absent in both controlled and scratched leaves. δ-Cadinene emerged at a concentration of 1.36% following scratching and increased to 1.81% with saliva application. Cyclohexene appeared at 0.84% after leaf scratching but decreased to 0.52% with saliva treatment. Hexyl acetate, not present in the control, was recorded at 0.21% after scratching and slightly decreased to 0.20% after saliva was added. The concentration of α-pinene rose by 0.19% due to scratching and then decreased by 0.10% with the addition of saliva. β-Caryophyllene concentrations increased by 8.49% after scratching and then decreased by 3.69% after the addition of saliva. The compound (3*E,*7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene continued its rising trend, with an increase of 20.28% after scratching and an additional increase of 1.51% after saliva treatment. Methyl salicylate levels rose by 5.17% with scratching but experienced a significant decrease to 4.68% after saliva application. 3-hexenyl acetate fell by 0.87% due to scratching but rose to 8.24% with saliva, surpassing its initial concentration. trans-α-Bergamotene plummeted by 18.10% post-scratching but saw a slight increase of 0.10% with saliva. Lastly, α-farnesene decreased by 17.01% after the leaves were scratched and then increased by 7.07% after treatment with saliva, compared to its concentration in the scratched leaves.

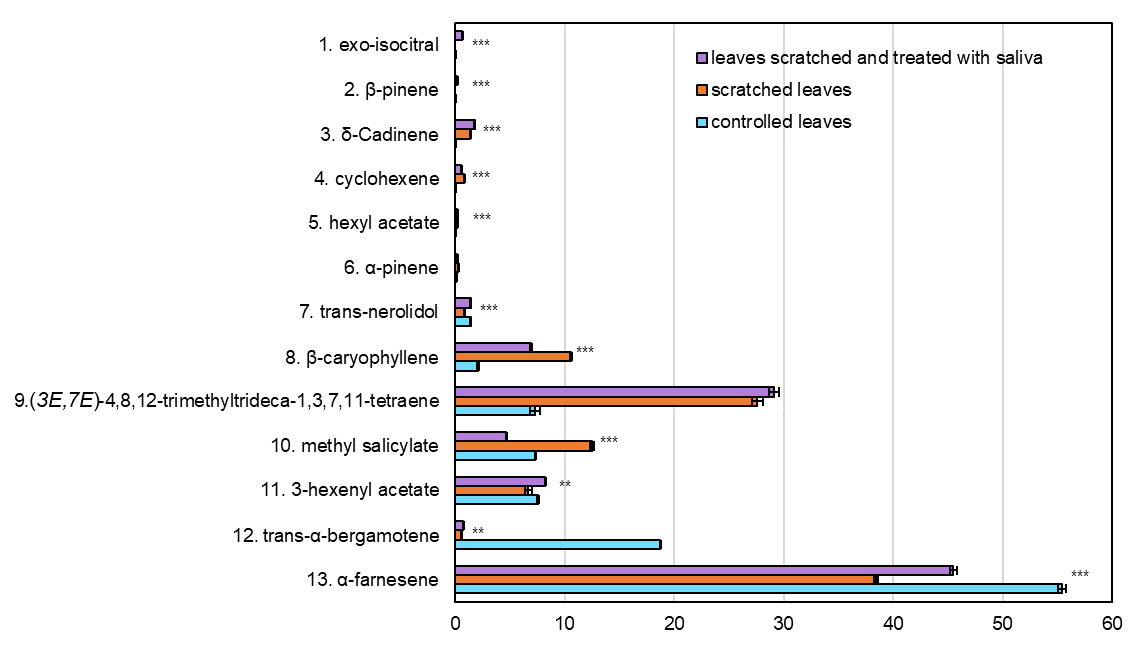


Figure 6 Relative abundance of VOC emissions from *A. contorta* leaves: controlled, mechanically damaged, and damage with saliva treatment. Bars indicate standard errors. \*\*, *p* < 0.01; \*\*\*, *p* < 0.001.

**4. Discussion**

1. Field surveys

The results from our field survey provided a comprehensive overview of the oviposition behavior of *S. montela* on *A. contorta*, revealing that *S. montela* laid an average of 58.7 eggs per cluster, predominantly at heights of 150 cm. This specific height preference for oviposition might be influenced by microclimatic conditions (Braem & Van Dyck, 2023), predation pressure (Obermaier et al., 2006), or the physiological state (Pocius et al., 2022) of *A. contorta* at this height, which could offer optimal conditions for the survival of *S. montela*'s offspring. The dispersed distribution of parasitized egg clusters suggests that *Ooencyrtus* spp., the parasitoid, did not exhibit a height preference in its parasitism behavior, which could imply a broad searching efficiency across different heights of *A. contorta*.

The variability in parasitism rates across regions, with a general observation of 54.6% and as high as 66.7% in some regions, underscored the significant role of *Ooencyrtus* spp. in regulating *S. montela* populations. The high parasitism rates suggest that *Ooencyrtus* spp. could be an effective natural control agent against *S. montela*, highlighting the potential for biological control strategies in managing *S. montela* populations to protect *A. contorta*.

The data from sticky traps installed within the quadrats provided insights into the attraction dynamics of *Ooencyrtus* spp. towards *A. contorta* when associated with *S. montela*. The significant increase in the number of *Ooencyrtus* spp. individuals in the presence of *S. montela* larvae or eggs, compared to the absence of *S. montela*, suggests a compound effect where the combined cues from both plants and larvae create a stronger attractant signal for *Ooencyrtus* spp. This is further supported by the strong correlations between *Ooencyrtus* spp. numbers and the presence of *S. montela* larvae (*r* = 0.691, *p* < 0.01) and eggs (*r* = 0.549, *p* < 0.01). The increase in *Ooencyrtus* spp. in the presence of larvae may be attributed to the VOCs released from leaf damage caused by the feeding larvae, while the higher numbers associated with eggs can be explained by the emergence of new *Ooencyrtus* spp. from these eggs, rather than VOC emissions as indicated by our olfactometer bioassay. Therefore, it was suggested that the volatiles emitted by *A. contorta*, particularly after herbivore damage, might signal *Ooencyrtus* spp., facilitating their role as biological control agents that target the egg stages of *S. montela* (Colazza, 2022). This interaction illustrated the complex ecological relationships within the ecosystem and exemplified how plant volatiles act as indirect defenses by recruiting the natural enemies of herbivores (Pearse et al., 2020).

1. Olfactometer bioassay

Our experiments revealed that *Ooencyrtus* spp. displayed no significant preference between non-parasitized *S. montela* egg clusters and *Paracycnotrachelus chinensis* egg clusters, suggesting a nuanced approach to host selection that may rely on more specific or additional cues than those tested. This observation aligned with previous research indicating that parasitoids are adept at navigating complex environments to locate their hosts, often relying on a combination of stimuli, including host chemical signals and plant volatiles from damage, to make definitive host selection decisions and enhance foraging (Bertoldi et al., 2019; Chesnais et al., 2015; Dicke & Baldwin, 2010; Koutsogeorgiou et al., 2023).

Ooencyrtus spp. significantly prefer mechanically damaged *A. contorta* leaves to undamaged ones, underscoring the critical role of physical damage in attracting these parasitoids. Additionally, the introduction of larval saliva to the damaged leaves did not notably alter their attractiveness; *Ooencyrtus* spp. were drawn to mechanically damaged leaves with and without larval saliva treatment in similar proportions (46.8% and 47.6%, respectively). This suggests that while physical damage is a potent attractor for Ooencyrtus spp., the chemical cues added by larval saliva do not significantly enhance this attraction. This reinforces the role of damage-induced plant volatiles (DIPVs) in attracting parasitoids (Kaplan, 2012), but indicates that saliva-induced volatiles may not significantly influence parasitoid behavior in the context of *Ooencyrtus* spp. and *A. contorta*. This contrasts with some earlier studies that highlighted the importance of herbivore-specific chemicals on parasitoid attraction (Moujahed et al., 2014; Takabayashi, 2022), indicating that physically damaged plant tissue alone can effectively attract these parasitoids. This finding challenges the widely held belief regarding the pivotal role of herbivore-induced plant volatiles (HIPVs) in parasitoid attraction.

1. The role of VOCs in plant defense strategies

The VOCs analysis of *A. contorta* leaves has demonstrated a multifaceted response to physical damage and simulated herbivore activity, showcasing the plant's complex defense mechanisms (Brosset & Blande, 2022). When damaged, plants trigger a defense mechanism where calcium ions and hormones amplify signals, activating genes for VOCs production (Lecourieux et al., 2006; Zhang et al., 2014). This leads to the synthesis of diverse VOCs tailored to enhance defense against herbivory (Divekar et al., 2022; Dudareva et al., 2013). The emission of a greater diversity of VOCs following mechanical damage and the application of larval saliva play crucial roles beyond direct repellence, significantly contributing to the recruitment of natural enemies of herbivores, thereby bolstering the plant's defenses indirectly (Dicke & Baldwin, 2010; Gebreziher, 2018; War et al., 2011). Specifically, exo-isocitral was absent in controlled and scratched leaves but emerged following saliva treatment, indicating a saliva-induced biosynthetic pathway (de Souza Alves et al., 2019). β-Pinene, similarly absent in both controlled and scratched leaves, was detected after saliva was applied, suggesting a unique response to biotic stress (Degenhardt, 2009; Shi et al., 2022). The concentration of α-pinene increased with mechanical damage and then decreased with the addition of saliva, highlighting its role in the immediate damage response, modulated by biological factors in the saliva (Xiao et al., 2012). This pattern was observed alongside a notable change in β-caryophyllene, which suggests its signaling function in recruiting predators and parasitoids (Schröder et al., 2015; Tang et al., 2015).

δ-Cadinene and cyclohexene, not present in controlled leaves, were detected in leaves after scratching, with δ-cadinene increasing and cyclohexene decreasing upon saliva application (Li et al., 2022; Shen et al., 2000; Yang et al., 2011). Hexyl acetate was also not found in control leaves but appeared in scratched leaves, slightly decreasing after saliva treatment, suggesting its role in attracting beneficial insects (Chan et al., 2016; Wang et al., 2013). The concentration of α-pinene increased with mechanical damage and then decreased with the addition of saliva, highlighting its role in the immediate damage response, modulated by biological factors in the saliva (Mofikoya et al., 2020). This pattern was observed alongside a notable change in β-caryophyllene, which suggests its signaling function in recruiting predators and parasitoids (Degenhardt, 2009; Xiao et al., 2012). Trans-nerolidol is worth noting for its potential role in the plant's response to stress, either by attracting parasitoids or acting in other defense-related capacities, highlighting the complexity of VOC-mediated interactions (Chan et al., 2016; Wang et al., 2013). Additionally, (3*E,*7*E*)-4,8,12-trimethyltrideca-1,3,7,11-tetraene showed a significant response to mechanical damage, further augmented with saliva application, indicating its role in defense communication (Schröder et al., 2015; Shi et al., 2022). In contrast, certain compounds decreased in concentration upon damage, suggesting they might be part of the plant's normal metabolic functions that are downregulated in response to stress (Schröder et al., 2015; Shi et al., 2022). Methyl salicylate, whose concentrations changed with damage and saliva treatment, plays a dual role in attracting natural enemies and offering anti-fungal benefits, underscoring its importance in the plant's defense strategy against both herbivores and pathogens (Tang et al., 2015). The interaction with *S. montela* larvae saliva, especially noted in the changes in compounds such as δ-cadinene, points to a complex defense strategy that possibly deters further herbivory or recruits natural enemies (Shen et al., 2000; Yang et al., 2011). Upon treatment with larvae saliva, 3-hexenyl acetate in *A. contorta* leaves increased than its original concentration in control leaves, suggesting a defensive reaction to simulated herbivory (de Souza Alves et al., 2019). Furthermore, the study observed a decrease in concentrations of trans-α-bergamotene and α-farnesene upon mechanical damage, with minor adjustments post-saliva application. These changes suggest that while these compounds are part of the plant's baseline defense, their roles become less pronounced when facing specific herbivore-associated challenges, pointing to a nuanced modulation of defense responses based on the nature of the threat (Krips, 2000; Ozawa et al., 2013; Xiao et al., 2023).

Despite these complex VOC responses to damage and larval saliva application, our analysis indicates that the saliva-induced compounds do not significantly enhance the attraction of *Ooencyrtus* spp., suggesting that not enough of the saliva-induced compounds are released to be sufficiently attractive to *Ooencyrtus* spp.. This underscores that physical damage alone could be already effective in attracting *Ooencyrtus* spp., highlighting the primary role of mechanical damage in eliciting a parasitoid response. Understanding the specific functions of VOCs is vital for ecosystem dynamics and the evolution of plant-herbivore interactions. It informs on how plants communicate distress and interact with both herbivores and protectors, offering insights for ecological resilience (Gish et al., 2015; Mofikoya et al., 2019).

1. Ecological and evolutionary implications

The investigation into *A. contorta*'s VOCs unveiled its adept utilization of sophisticated indirect defense mechanisms through plant signaling, epitomizing the plant's evolutionary acumen. This practice highlights an evolutionary arms race, marking the development of complex plant defense strategies against herbivores (Endara et al., 2023; Kobayashi et al., 2015). *A. contorta*, through the emission of VOCs, particularly VOCs, plays a complex role in the coevolution of plant-insect ecosystems (War et al., 2012). These chemical signals serve dual roles by directly deterring attackers and indirectly benefiting the plant through the summoning of parasitoids like *Ooencyrtus* spp., effectively managing herbivore populations such as *S. montela* (Degenhardt, 2009; Gebreziher, 2018). This strategy not only plays a pivotal role in maintaining ecological balance but also in sculpting community structures, thus underpinning the plant's influence on biodiversity enhancement (Heil, 2004; Pearse et al., 2020). Moreover, the observed mutual adaptations among *A. contorta*, *S. montela*, and *Ooencyrtus* spp. underscore the pivotal role of chemical ecology, demonstrating its contribution to ecosystem stability and resilience.

**5. Conclusion**

This study unveiled the intricate relationship between *A. contorta*, the herbivore *S. montela*, and parasitoids, facilitated by a sophisticated VOC system. Our findings reveal that larval saliva-induced compounds do not significantly increase *A. contorta*'s attractiveness to *Ooencyrtus* spp., suggesting a strategic depth to the plant's defense that doesn't heavily depend on these compounds for altering parasitoid behavior. We explored the evolutionary battle driving complex defense strategies in *A. contorta*, which utilizes indirect methods to counteract herbivore pressure. Our analysis of VOCs like hexyl acetate, cyclohexene, δ-cadinene, α-pinene, and β-caryophyllene uncovered a complex signaling network essential for deterring herbivores and recruiting allies, thus playing a crucial role in the plant's survival and ecological integration. The study also highlights the significance of indirect defense mechanisms, reflecting nature's evolutionary creativity in developing plant strategies against herbivory. This insight into plant-insect interactions and their chemical basis enriches our understanding of ecological interactions and suggests practical applications in sustainable farming and pest control.

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**Conflicts of Interest**

The authors declare no competing interests.

**Author Contributions**

Conceptualization, S.P. and J.G.K.; Methodology, S.P., J.J., H.L., and S.K.; Software, S.P.; Validation, S.K., J.G.K.; Formal Analysis, S.K., J.G.K.; Investigation, S.P. and J.J.; Resources, S.P. and J.J.; Data Curation, S.P., H.L., S.K. and J.G.K; Writing – Original Draft Preparation, S.P.; Writing – Review & Editing, S.K. and J.G.K; Visualization, S.P.; Supervision, J.G.K.; Project Administration, J.G.K.; Funding Acquisition, J.G.K.

**Data Availability Statement**

The article contains all the data that were created and used in this investigation, and the corresponding authors can provide these data upon request.