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Predatory bugs show a preference for egg-laying sites based on plant topography

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Background. Oviposition site selection is an important factor in determining the success of insect populations. *Orius* spp. are widely used as biological control for a wide range of soft-bodied insect pests such as thrips, aphids, and mites. *Orius strigicollis* (Heteroptera: Anthocoridae), is the dominant *Orius* species in southern China; however, it currently remains unknown what factor drives its selection of an oviposition site after mating.

Methods. Here, kidney bean pods (KBPs) were chosed as the oviposition substrate, and choice and nonchoice experiments were conducted to determine the preference of oviposition sites on the KBPs in *O. strigicollis*, and the mechanism of oviposition behavior was revealed through observation and measuring of oviposition action, egg hatching rate and oviposition time.

Results. We found that *O. strigicollis* preferred the seams of the pods for oviposition, especially the seams at the tip of the KBPs. Choice and nonchoice experiments showed that females did not lay eggs when the KBP tail parts were unavailable. The rates of eggs hatching on different KBP parts were not significantly different, but the time required for females to lay eggs on the tip seam was significantly lower. Decreased oviposition time is achieved on the tip seam as the insect can exploit support points found there and gain leverage for insertion of the ovipositor.

Discussion. The preference of oviposition site for *O. strigicollis* is significantly influenced by the topography of KBPs surface. Revealing such behavior and mechanism will provide an important scientific basis for the future development of oviposition molds for predatory bugs.

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Abstract

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Keywords oviposition behavior, site selection preference, egg hatching, plant topography, *Orius strigicollis*

Introduction

Insects tend to have the ability to select particular egg-laying sites in order to increase the survival rate of their offspring (*Grostal & Dicke, 1999*; *Choh & Takabayashi 2007*; *Barbosa-Andrade et al., 2019*). Several factors can influence this behaviour, for example, the existence of natural enemies or competitors (*Rouault et al., 2007*; *Choh et al., 2015*; *Saitoh & Choh 2018*), and site properties such as food resources availability (*Bond et al., 2005*), illumination intensity (*Yang, 2006*) and temperature (*Notter-Hausmann & Dorn, 2010*). Apart from those common factors, some rare external physical factors such as the site size (*Reich & Downes, 2003*), shape or colour (*Markheiser et al., 2008*) can also play a role in the selection of oviposition sites.

These underlying cues are complex and are less well understood than other aspects of insect behavior (*Lundgen et al., 2007*). However, due to the feeding habits of the phytophagous insects, the plants bring importance to the life histories as well as the agricultural value of the predators that feed on these phytophagous insects (*Lundgen et al., 2007*; *Puysseleyr & Hofte, 2011*). Previous studies have shown that both plant species and variations in plant parts or tissues influence the oviposition behavior of predatory insects (*Isenhour & Yeargen, 1982*; *Coll, 1996*; *Lundgren & Fergen, 2006*). Of the many plant morphological features, the plant physical structure is one of the important factors that is known to significantly affect this reproductive behavior, either positively (*Benedict et al., 1983*; *Griffen & Yeargan, 2002*) or negatively (*Simmons & Gurr, 2004*). The mechanisms that drive female oviposition decisions have evolved such that female insects will choose sites with the optimal plant-based resources for the survival of their offspring (*Malheiro et al., 2018*; *Mitchell et al., 2019*). However, whether there are other factors that influence the choice of oviposition sites by predatory insects remains to be explored.

Orius spp. are widely used in biological control methods to control many pests worldwide, because they exhibit a higher search efficiency for their host than other species and are fast- moving and active (*Minks et al., 1988*). For example, *Orius strigicollis* Poppius (Heteroptera: Anthocoridae), is an important native natural predator of a wide range of soft-bodied insect pests such as thrips, aphids, and mites in several agronomic systems (*Cocuzza et al., 1997*; *Sengona et al., 2008*; *Zhang et al., 2012*; *Bonte & De Clercq, 2011*), and feeds on lepidopteran pest eggs and hatched larvae (*Bonte & De Clercq, 2011*; *Ali et al., 2020*). There are several studies about *O. strigicollis* behavior that focus on its predatory advantages and its influence on agriculture (*Zhou et al., 2006*; *Ali et al., 2020*), but the mechanisms whereby the oviposition behavior of *O. strigicollis* are influenced by plant characteristics are poorly understood. However, studies on another zoophytophagous heteropteran, *Orius insidiosus*, have reported that plants species as well as the variations within each plant significantly influenced their oviposition behavior (*Coll, 1996*; *Lundgren & Fergen, 2006*) and that they prefer to lay eggs on thinner epidermal plant surfaces, where the vesicular and cellular tissues are conducive to the survival and development of nymphs (*Lundgen et al., 2007*). As *O. strigicollis* is a natural enemies of plant pests, studying its oviposition site selection behavior could further expand its agricultural value and lay the foundation for the large-scale production of natural enemy-based products.

Kidney bean pods (hereafter KBPs) are widely used in the indoor rearing of thrips and omnivorous bugs, because of their freshness and convenience (*Bonte & De Clercq, 2010*; *Li et al., 2018*). We observed that *O. strigicollis* preferred KBPs for oviposition. Therefore, we used the KBP as an oviposition substrate to study the mechanism of egg-laying selection preference in *O. strigicollis*. We hypothesized that *O. strigicollis* females lay more eggs on the seams part of the KBPs, especially the bean tips, and that the most likely mechanism driving female oviposition decisions is the physical comfort of the laying position, which is directly related to egg-laying efficiency. Here, we attempted to answer the following questions: 1) Do *O. strigicollis* females exhibit oviposition site selection behavior, and where do females choose to lay eggs? 2) Does the presence of the bean tail influence the oviposition behavior under choice and nonchoice conditions, or is the bean tip is the best place for *O. strigicollis* females to lay eggs? 3) Why do *O. strigicollis* females select a specific location?

Materials & Methods

Insects rearing and experimental preparation

Orius strigicollis adults were collected from open areas and vegetable fields outside of Hangzhou (30.43898ºN, 120.41134ºE), Zhejiang Province, P.R. China, and maintained in a climate control room. The rearing conditions were 26 ± 2 ºC, 70 ± 10 % RH, with a photophase of 14 h. All growth stages of *O. strigicollis* were reared in 4.3 L glass jars (see Supplement 1 for more details) with a circular slant (i.e., the opening of the jar is on the side), capped with plastic screw-on lids. The KBPs (length: 20.6 ± 5.1 cm) were used in experiments as an oviposition substrate for *O. strigicollis*. From the nymph to adult stages, the predatory bugs were fed western flower thrips, *Frankliniella occidentalis*.

Oviposition site selection preferences

A pair of KBPs were laid flat on the filter paper inside the jars, and five mated *O. strigicollis* females were placed into each jar and allowed to oviposit for 48 hours. *F. occidentalis* nymphs were placed in each jar as food. The climatic conditions were the same as those described above. The KBPs were collected 48 hours later for egg counting. The number of eggs per pod and the number of eggs in different positions on the pod (face or seam) were recorded. Finally, the egg numbers on different parts of the pods were counted under a Nikon SMZ1500 zoom stereomicroscope (Nikon, Japan). The KBPs were divided into three parts for this count, i.e., tail, middle, and head. Each treatment was replicated 20 times.

Influence of restricting KBP access on the oviposition site selection in *Orius strigicollis*

The tail of the KBP was wrapped with parafilm to render the preferred oviposition site inaccessible, then nonchoice and choice testing were conducted to determine the oviposition site selection of *O. strigicollis* under different treatments. Egg counts were made as described above. A pair of intact KBPs was used as a control for each jar. The nonchoice testing was replicated 19 times, and the choice testing was replicated 14 times.

Differences in egg number and egg hatching rate on middle and tail of KBP

To better identify the optimal oviposition site of *O. strigicollis*, we refined middle into the left middle (Middle-L) and right middle (Middle-R), and tail into left tail (Tail-L) and right tail (Tail-R). The tail of the KBP was then further categorized into four parts, i.e., the left neck (Neck-L), right neck (Neck-R), left tip (Tip-L), and right tip (Tip-R). Each pair of KBPs in each jar was considered a group, and the experiments were performed again as described above. The egg numbers on each of the further-divided parts (left or right, neck or tip) were counted, and the number of eggs hatched after 5 days was also recorded. Each treatment was replicated 20 times.

Observation of egg-laying behavior and analysis of oviposition efficiency

During the control experiments, the egg-laying movements of 15 females on the tail and middle sections were observed, and the entire egg-laying process was recorded using a Micro video recording system (HDR-SR11E, Sony, Japan). When the start of the egg-laying movement was observed, an electronic timer (Deli, China) was used to determine how long females took to lay one egg on the tail or middle section.

Statistical analysis

Microsoft Excel (version 16.39) was used to record data. The analysis was conducted using Prism 8 (version 8.4.0) and SPSS (version 26.0). One-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparison test was used to analyze the differences in egg number between different parts or subsections of the KBPs. A t-test was used to compare the total number of eggs and the egg-laying efficiency between treatments.

Results

Oviposition site selection preferences

A total of 97.9% of the eggs were laid on the seam of the KBPs, and only 2.1% of the eggs were laid on the face ((Figure 1A; $t = 59.0$, $df = 19$, $P < 0.0001$). Moreover, significant differences in egg numbers were observed between different KBP parts. More eggs were laid on the seam of tail and middle than on the seam of head, and the highest percentage of eggs was laid on tail (Figure 1A; $F_{2,119} = 44.8$, $P < 0.0001$), i.e., more than half of the total eggs (50.9%). Overall *O. strigicollis* females laid more eggs on the seam of the KBPs, and specifically on the tails.

Influence of restricting KBP access on the oviposition site selection in *Orius strigicollis*

A non-choice experiment was conducted with tail covered. In this treatment, the total number of eggs on each pod was dramatically lower than that in the control (Figure 1B; 130.2 ± 7.1 vs. 55.1 ± 3.0 individuals, $t = 11.2$, $df = 18$, $P < 0.0001$). A choice assay was also performed with tail covered for one pod and another pod presented uncovered. In this case, the mean number of eggs per replicate was 87.6 ± 6.73 individuals, which was also significant lower than found in the control (Figure 1C; $t = 5.7$, $df = 13$, $P < 0.0001$). The data indicated that *O. strigicollis* females did not lay more eggs on other parts of the KBP when the tail parts were unavailable.

Differences in egg number and egg hatching rate at different oviposition sites except for the head part of the KBPs

The section of the right tail (Tail-R) was found to contain the majority of eggs, next to the section of the left middle (Middle-L) (Figure 1D, $F_{3,159} = 69.1$, $P < 0.0001$). It was further showed that the section of the right tip (Tip-R) where *O. strigicollis* most preferred to lay its eggs (Figure 1E; $t = 11.2$, $df = 19$, $P < 0.0001$). The egg hatching rates on different sections were not significantly different, and all were higher than 80% (Figure 2A, $F_{4,104} = 0.23$, $P = 0.921$). Therefore, we indicate that the factors that influence the selection of oviposition sites may not be those that restrict the hatching or survival of eggs.

Observation of egg-laying behavior and efficiency analysis

The average time that each female consume laying one egg on the tip was significantly shorter than the time to lay an egg on the middle section (Figure 2B, $t = 6.0$, $df = 14$, $P < 0.0001$). We observed that laying eggs on the right tip seam was more efficient than laying eggs in another section (Figure 2C/D), and indicate that the larger or less neat the surface contour of the part is, the more conducive to *O. strigicollis* females to obtain the point of action when oviposition.

Discussion

Postmating behaviour such as oviposition sites seletion, is observed in many insect species and is important for the reproduction of these species (*Thompson, 1988*). For example, *Gryllus texensis* and some myrmecophilous butterfly species choose a suitable oviposition site for the survival of their offspring (*Stahlschmidt & Adamo, 2013*). In this study, we found that *O. strigicollis* females selected the seam of the KBPs rather than the face for egg laying. Such a preference difference for a different site on the same type of tissue or unit is common in oviposition site seletion. For instance, the lepidopteran multivoltine leafminers, *Phyllocnistis* sp., prefer to lay eggs on only the lower-surface epidermal layer of the primary shoots, switching to lammas shoots when they appear later in the season (*Ayabe et al., 2017*). The longhorn beetle, *Glenea cantor*, preferentially select the upper section of kapok trees first for oviposition according to the bark moisture content from the top to the bottom of the trees (*Lu et al., 2011*). For *O. strigicollis*, we found that the number of eggs laid gradually decreased from the tail to the head of the KBPs, and that the egg numbers at the tip accounted for more than half of the total number of eggs laid. Subdividing the different positions at the tail part of the KBPs, we found that the right-side tip seam was the primary site for oviposition. Further experiments were conducted to elucidate the hierarchy of preference for egg-laying females and identify the factors that influence it.

The results of the choice experiments suggest that first, when one of the preferred parts was unavailable, the total number of eggs laid decreased; second, when none of the preferred parts were available, the number of total eggs laid dropped rapidly. Although the left middle seam remained available for oviposition, this site did not replace the preferred site; instead, unexpectedly, the number of eggs laid significantly decreased when the preferred site was unavailable. A previous study on mosquitoes suggested that the decreased oviposition rate observed on highly enriched leaves may be due to a pungent odor that is caused by the extreme anoxic environment and repels gravid female mosquitoes (*Hoekman et al., 2007*). Similar behavior was also observed in peach twig borers, *Anarsia lineatella*, and female adults can determine whether peach fruits were fresh and viable for oviposition so that their larvae can have enough time to develop into adults before the peach fruits decompose (*Sidney et al., 2008*). These examples suggest that *O. strigicollis* females may have the ability to assess whether the oviposition substrate is favorable for oviposition.

Previous studies found that insects tend to lay eggs on well-nourished hosts or tissue to ensure the healthy development and survival of their offspring (*Jeong et al., 2016*; *Malheiro et al., 2018*; *Mitchell et al.,* *2019*). Here, we found that *O. strigicollis* laid the most eggs at the tip of the KBPs, which indicated this location to be their preferred oviposition site. The egg hatching rate is an important biological index used to measure host fitness or the suitability of oviposition substrates (*Murai et al., 2001*; *Bonte & Clercq, 2010*; *Krug & Sosa, 2019*), and it is also the most intuitive criterion to judge (*Castane & Zalom, 1994*). Therefore, we further analyzed the hatching rates of eggs laid on different parts of the KBPs (tail vs. middle). Data showed that the hatching rates on these four sections (Middle L and R, Tail L and R) were not significantly different. We suggest that the factors that influence the selection of oviposition sites may not be those that restrict the hatching or survival of eggs. Additionally, we found that the eggs were embedded in the KBP tissue, and the lid of the egg was opened when it hatched. Embedding the eggs may simply protect the eggs from predation or parasitism and from abiotic factors as well as stabilizing the eggs or keeping them in a moist environment (*Shapiro & Ferkovich, 2006*).

Based on our observation of the entire egg-laying process of the females and our measurements of the time required for the females to lay eggs, we suggest that females select the tip of the KBPs as their first oviposition site to achieve higher egg-laying efficiency, reducing their time spent ovipositing also reduces their risk of predation and allows more time for foraging and perching (*Martens, 2001*; *Philippe et al., 2015*). Furthermore, we suggest that increased egg-laying efficiency is due to the ‘ergonomics’ of this egg-laying position. The females must use force to insert their eggs into the KBP. To achieve this, they need anchor points for both their propodeum and metapodium to push against to gain the required power. Comparing the seam at the tip and in the middle section of the KBPs, the females were able to clasp the tip of the KBPs using their propodeum. This allowed oviposition in KBPs with much greater ease (see Supplement 2 for more details). In contrast, because the side of the KBP is nearly flat, the females are required to use more strength and expend more energy to insert their eggs there. There is a similar explanation for the low egg distribution on the seam on the other side; compared with the preferred seam, the other seam is relatively shallow, and more energy might be required for the females to lay their eggs inside it. Similar observations and speculations were also mentioned briefly by Shapiro and Ferkovich (2006), who speculated that the female adults of *O. insidiosus* may need to take advantage of the internal angles or surface irregularities to gain leverage into the ovipositor.

Conclusions

The physical features of each site are ultimately reflected in the corresponding egg-laying efficiency. In other words, the more ‘comfortable’ the females were, the higher their egg-laying efficiency. The behavioral mechanism of the preference of *O. insidiosus* females for oviposition site on the KBPs was found and identified, which is conducive to our later development of artificial media or mold to attract *O. insidiosus* to lay eggs, so as to provide key technical support for the massive propagation and industrialization of *O. insidiosus* in the future.

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References

Ali S, Zhu Q, Jaleel W, Rehman SU, Rasheed MA, Khan MM, Islam Y, Hafeez M, Zhou X. 2020. Determination of fitness traits of *Orius strigicollis* Poppius (Hemiptera: Anthocoridae) on *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) using two-sex life table analysis. *PeerJ* 8: e9594 DOI: 10.7717/peerj.9594

Ayabe Y, Minoura T, Hijii N. 2017. Oviposition site selection by a lepidopteran leafminer in response to heterogeneity of leaf surface conditions: structural traits and microclimates: Leafminer oviposition site selection. *Ecol Entomol* 42: 294–305 DOI 10.1111/een.12387

Barbosa-Andrade CL, Andrade DJ, Matos STS, Nogueira L, Cividanes FJ. 2019. Selection of oviposition sites by ground beetles *Abaris basistriata* and *Selenophorus seriatoporus* for mass rearing purposes. *BioControl* 64: 315–321 DOI 10.1007/s10526-019-09930-8

Benedict JH, Leigh TF, Hyer AH. 1983. *Lygus Hesperus* (Heteroptera: Miridae) oviposition behavior, growth, and survival in relation to cotton trichome density. *Environ Entomol* 12: 331–335 DOI 10.1093/ee/12.2.331

Bond JG, Arredondo-Jimenez JI, Rodriguez MH, Quiroz-Martinez H, Williams T. 2005. Oviposition habitat selection for a predator refuge and food source in a mosquito. *Ecol Entomol* 30: 255–263 DOI 10.1111/j.0307-6946.2005.00704.x

Bonte M, De Clercq P. 2010. Impact of artificial rearing systems on the developmental and reproductive fitness of the predatory bug, *Orius laevigatus*. *J Insect Sci* 10: 104 DOI 10.1673/031.010.10401

Bonte M, De Clercq P. 2011. Influence of predator density, diet and living substrate on developmental fitness of *Orius laevigatus*. *J Appl Entomol* 135: 343–350 DOI 10.1111/j.1439-0418.2010.01554.x

Castane C, Zalom FG. 1994. Artificial oviposition substrate for rearing *Orius insidiosus* (Hemiptera, Anthocoridae). *Biol Control* 4: 88-91 DOI 10.1006/bcon.1994.1015

Choh Y, Sabelis MW, Janssen A. 2015. Distribution and oviposition site selection by predatory mites in the presence of intraguild predators. Exp Appl Acarol 67: 477–491 DOI 10.1007/s10493-015-9970-8

Choh Y, Takabayashi J. 2007. Predator avoidance in phytophagous mites: response to present danger depends on alternative host quality. *Oecologia* 151: 262–267 DOI 10.1007/s00442-006-0590-1

Cocuzza GE, Clercq PD, De Veire MV, Cock AD, Degheele D, Vacante V. 1997. Reproduction of *Orius laevigatus* and *Orius albidipennis* on pollen and *Ephestia kuehniella* eggs. *Entomol Exp Appl* 1: 101–104 DOI 10.1046/j.1570-7458.1997.00118.x

Coll M. 1996. Feeding and ovipositing on plants by an omnivorous insect predator. *Oecologia* 105: 214–220 DOI 10.1007/BF00328549

Griffen ML, Yeargan KV. 2002. Factors potentially affecting oviposition site selection by the lady beetle *Coleomegilla maculate* (Coleoptera: Coccinellidae). *Environ Entomol* 31: 112–119 DOI 10.1603/0046-225X-31.1.112

Grostal P, Dicke M. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behav Ecol* 10: 422–427 DOI 10.1603/0046-225X-31.1.112

Hoekman D, Terhorst C, Bauer A, Braun S, Gignac P, Hopkins R, Joshi S, Laskis K, Sanscrainte N, Travis J, Miller TE. 2007. Oviposition decreased in response to enriched water: a field study of the pitcher-plant mosquito, *Wyeomyia smithii*. *Ecol Entomol* 32: 92–96 DOI 10.1111/j.1365-2311.2006.00840.x

Isenhour DJ, Yeargan KV. 1982. Oviposition sites of *Orius insidiosus* (Say) and *Nabis* spp. in soybean (Anthocoridae and Nabidae). *J Kansas Entomol Soc* 55: 65-72 DOI 10.1016/0022-474X(82)90029-7

Jeong YT, Oh SM, Shim J, Seo JT, Kwon JY, Moon SJ. 2016. Mechanosensory neurons control sweet sensing in Drosophila. *Nat Commun* 7: 12872 DOI 10.1038/ncomms12872

Krug P, Sosa AJ. 2019. Mother knows best: plant polyploidy affects feeding and oviposition preference of the alligator weed biological control agent, *Agasicles hygrophila*. *BioControl* 64: 623–632 DOI 10.1007/s10526-019-09959-9

Li X, Geng S, Zhang Z, Zhang J, Li W, Huang J, Lin W, Bei Y, Lu Y. 2018. Species-specific aggregation pheromones contribute to coexistence in two closely related thrips species. *B Entomol Res* 109: 119–126 DOI 10.1017/S0007485318000366

Lu W, Wang Q, Tian MY, Xu J, Qin AZ, He L, Jia B, Cai JJ. 2011. Host selection and colonization strategies with evidence for a female produced oviposition attractant in a longhorn beetle. *Environ Entomol* 40: 1487–1493 DOI 10.1603/EN10280

Lundgren JG, Fergen JK. 2006. The oviposition behavior of the predator *Orius insidiosus*: acceptability and preference for different plants. *BioControl* 51: 217–227 DOI 10.1007/s10526-005-0609-2

Lundgen JG, Fergen JK, Riedell WE. 2007. The influence of plant anatomy on oviposition and reproductive success of the omnivorous bug *Orius insidiosus*. *Anim Behav* 75: 1495–1502 DOI 10.1016/j.anbehav.2007.09.029

Markheiser A, Rid M, Biancu S, Gross J, Hoffmann C. 2017. Physical factors influencing the oviposition behaviour of *European grapevine* moths *Lobesia botrana* and *Eupoecilia ambiguella*. *J Appl Entomol* 142: 201-210 DOI 10.1111/jen.12423

Martens A. 2001. Initial preference of oviposition sites: discrimination between living and dead plant material in *Sympecma fusca* and *Coenagrion caerulescens* (Odonata: Lestidae, Coenagrionidae). *Eur J Entomol* 98: 121–123 DOI 10.14411/eje.2001.021

Malheiro R, Susana C, Pinheiro L, Baptista P, Pereira J. 2018. Olive cultivar and maturation process on the oviposition preference of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). *B Entomol Res* 109: 1–11 DOI 10.1017/S0007485318000135

Minks AK, Harrewijg P, Helle W. 1989. Aphids: Their biology, natural enemies and control. *Q Rev Biol* 64(2): 208–209 DOI 10.1016/0378-4290(89)90065-8

Mitchell TS, Shephard AM, Kalinowski CR, Kobiela ME, Snell-Rood EC. 2019. Butterflies do not alter oviposition or larval foraging in response to anthropogenic increases in sodium. *Anim Behav* 154: 121–129 DOI 10.1016/j.anbehav.2019.06.015

Murai T, Naraim Y, Sugiura N. 2001. Utilization of germinated broad bean seeds as an oviposition substrate in mass rearing of the predatory bug, *Orius sauteri* (Poppius) (Heteroptera: Anthocoridae). *Appl Entomol Zool* 36: 489–494 DOI 10.1303/aez.2001.489

Notter-Hausmann C, Dorn S. 2010. Relationship between behavior and physiology in an invasive pest species: Oviposition site selection and temperature-dependent development of the oriental fruit moth (Lepidoptera: Tortricidae). *Environ Entomol* 39: 561–569 DOI 10.1603/EN09231

Philippe L, Besnard A, Natalia M. 2015. Initial preference for plant species and state during oviposition site selection by an odonate. *Entomol Sci* 18: 377–382 DOI 10.1111/ens.12130

Puysseleyr VD, Hofte M. 2011. Ovipositing *Orius laevigatus* increase tomato resistance against *Frankliniella occidentalis* feeding by inducing the wound response. *Arthropod- Plant Inte* 5: 71-80 DOI 10.1007/s11829-010-9117-0

Reich P, Downes B. 2003. Experimental evidence for physical cues involved in oviposition site selection of lotic hydrobiosid caddis flies. *Oecologia* 136: 465–475 DOI 10.1007/s00442-003-1284-6

Rouault G, Battisti A, Roques A. 2007. Oviposition sites of the cypress seed bug *Orsillus maculatus* and response of the egg parasitoid *Telenomus* gr. *Floridanus. BioControl* 52: 9 DOI 10.1007/s10526-006-9002-z

Saitoh F, Choh Y. 2018. Do intraguild prey protect their eggs from intraguild predators that share their oviposition site? Anim Behav 140: 49–55 DOI 10.1016/j.anbehav.2018.04.005

Yang LH. 2006. Periodical cicadas use light for oviposition site selection. *P Roy Soc B-Biol Sci* 273: 2993–3000 DOI 10.1098/rspb.2006.3676

Sengonca C, Ahmadi K, Blaeser P. 2008. Biological characteristics of *Orius similis* Zheng (Heteroptera, Anthocoridae) by feeding on different aphid species as prey. *J Plant Dis Protect* 115: 32–38 DOI 10.1098/rspb.2006.3676

Shapiro JP, Ferkovich SM. 2006. Oviposition and isolation of viable eggs from Orius insidiosus in a Parafilm and water substrate: Comparison with green beans and use in enzyme-linked immunosorbent assay. *Ann Entomol Soc Am* 99: 586–591 DOI 10.1038/sj.ijo.0802417

Sidney M, Brown K, Judd GJR, Gries G. 2008. Stimuli affecting selection of oviposition sites by female peach twig borer, *Anarsia lineatella Zeller* (Lepidoptera: Gelechiidae. *J Appl Entomol* 132: 538–544 DOI 10.1111/j.1439-0418.2008.01292.x

Simmons AT, Gurr GM. 2004. Trichome-based host plant resistance of *Lycopersicon* species and the biocontrol agent *Mallada signata*: are they compatible? *Entomol Exp Appl* 113: 95–101 DOI 10.1111/j.0013-8703.2004.00210.x

Stahlschmidt ZR, Adamo SA. 2013. Warm and cozy: temperature and predation risk interactively affect oviposition site selection. *Anim Biol* 86: 553–558 DOI 10.1016/j.anbehav.2013.06.009

Thompson JN. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomol Exp Appl* 47: 3–14 DOI 10.1111/j.1570-7458.1988.tb02275.x

Zhang S, Zhu F, Zheng X, Lei C, Zhou X. 2012. Survival and developmental characteristics of the predatory bug *Orius similis* (Hemiptera: Anthocoridae) fed on *Tetranychus cinnabarinus* (Acari: Tetranychidae) at three constant temperatures. *Eur J Entomol* 109: 503–508 DOI 10.14411/eje.2012.063

Zhou XM, Zhu F, Li H, Lei CL. 2006. Effect of temperature on development of *Orius similis* Zheng (Hemiptera: Anthocoridae) and on its predation activity against *Aphis gossypii* Glover (Hemiptera: Aphididae). *Pan Pacific Entomologist* 82: 97–102.

Figure 1

The preference of oviposition sites on kidney bean pods (KBPs) in *Orius strigicollis.* (How to choose “delivery room”?)

A) The percentage of eggs laid on the seam and face of the KBPs and the percentage of eggs laid on the seam in the three different parts. Each bar represents the mean + SEM ($N = 20$). ** indicates a significant difference ($P < 0.0001$, Student’s *t*-test); different letters indicate significant differences ($P < 0.0001$, one-way ANOVA test followed by a Tukey-Kramer test).

B) Comparing of the mean number of eggs (+ SEM) laid on treatment (tail covered or restricting access) and the control (uncovered) by nonchoices assay ($N_{control} = 20$, $N_{treatment} = 19$). ** indicates significant differences ($P < 0.0001$, Student’s *t*-test).

C) Comparing of the mean number of eggs (+ SEM) laid on treatment (tail covered or restricting access) and the control (uncovered) by choices assay ($N_{control} = 20$, $N_{treatment} = 14$). ** indicates significant differences ($P < 0.0001$, Student’s *t*-test).

D) Comparison of the percentage of eggs laid on the right and left sides of KBP tail and middle sections. Each bar represents the mean + SEM ($N = 20$); different letters indicate significant differences ($P < 0.0001$, one-way ANOVA test followed by a Tukey-Kramer test).

E) Comparison of the percentage of eggs laid on the tip and neck sections. Each bar represents the mean + SEM ($N = 20$); ** indicates significant differences ($P < 0.0001$, Student’s *t*-test). [p]

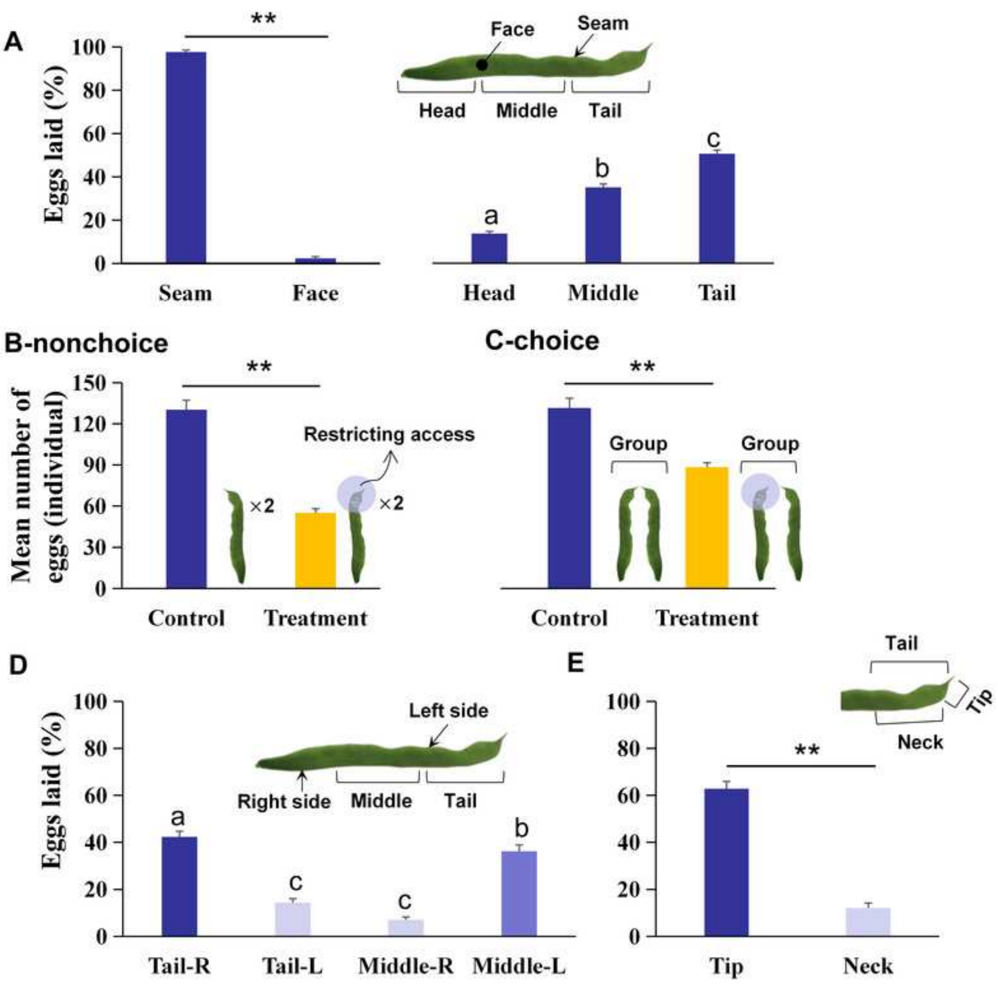

A
**
Eggs laid (%)
100
80
60
40
20
0
Seam
Face
Face
Seam
Head
Middle
Tail
a
b
c
Head
Middle
Tail
B-nonchoice
Mean number of eggs (individual)
150
120
90
60
30
0
**
×2
Restricting access
×2
Control
Treatment
C-choice
**
Group
Group
Control
Treatment
D
Eggs laid (%)
100
80
60
40
20
0
Left side
Right side
Middle
Tail
a
c
c
b
Tail-R
Tail-L
Middle-R
Middle-L
E
Tail
Tip
Neck
100
80
60
40
20
0
**
Tip
Neck

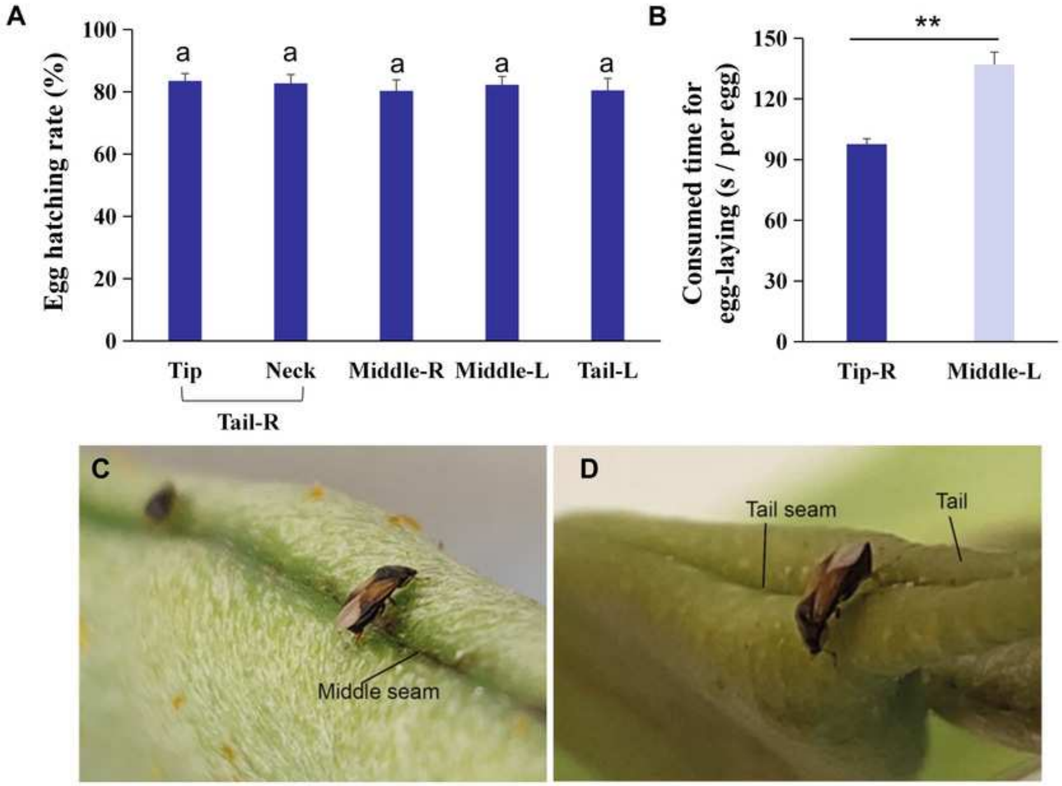
Figure 2

Behavioral mechanisms of oviposition site selection on kidney bean pods (KBPs) in *Orius strigicollis.* (Why do *O. strigicollis* choose this way?)

A) Hatching rates of eggs laid on five parts, i.e., tip, neck, right middle, left middle, and left tail. Same letters indicate no significant differences ($P > 0.05$, one-way ANOVA test followed by a Tukey-Kramer test). Each bar represents the mean + SEM ($N = 20$).

B) The time consumed (seconds) for females to lay one egg on the right tip and left middle sections. Each bar represents the mean + SEM ($N = 15$). ** indicates significant differences ($P < 0.0001$, Student’s *t*-test).

C/D) Photograph of female adult ready to lay eggs on the seam of the middle and tip sections. [p]


A
Egg hatching rate (%)
100
80
60
40
20
0
a
a
a
a
a
Tip
Neck
Middle-R
Middle-L
Tail-L
Tail-R
B
Consumed time for egg-laying (s / per egg)
150
120
90
60
30
0
**
Tip-R
Middle-L
C
Middle seam
D
Tail seam
Tail