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The invasive stink bug Halyomorpha halys affects the reproductive success and the experience-mediated behavioural responses of the egg parasitoid Trissolcus basalis

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Abstract:	Invasive species, because of their lack of co-evolutionary history with recipient communities, can act as “evolutionary traps” causing disconnects between natural enemy behavioural responses and the suitability of the invasive species as a prey resource. Invasion of exotic species in non-native environments may have several ecological effects, including consequences for the experience-mediated behavioural responses of indigenous foragers. Experience is usually thought to help resident species to buffer against negative impacts of new invasive species, including escaping from evolutionary traps. Here we hypothesized that the impact of foraging experience depends on whether an indigenous egg parasitoid can correctly assess the resource suitability of a new invasive species for offspring development. We showed that the invasive stink bug Halyomorpha halys acts as an evolutionary trap for the indigenous egg parasitoid Trissolcus basalis leading to unsuccessful development of ~95% of the eggs laid in this host species. In a mixed scenario in which both the associated resident stink bug Nezara viridula co-occur with the invasive H. halys , we showed that oviposition experience in the low quality invasive host induces in T. basalis similar responses to those of the associated host; thus foraging experience does not lead to avoidance of an evolutionary trap. We discuss parasitoid foraging experience and reproductive success in the light of the evolutionary trap framework with implication for biological control.
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Dear editor,

Please, find submitted our manuscript entitled " **The invasive stink bug *Halyomorpha halys* affects the reproductive success and the experience-mediated behavioural responses of the egg parasitoid *Trissolcus basalis***”. In this paper we show that the invasive stink bug *Halyomorpha halys* acts as an evolutionary trap for *Trissolcus basalis*, an egg parasitoid closely associated with the stink bug *Nezara viridula*. In a series of experiments, we provided behavioural evidence showing that experience on cues associated with *H. halys* affects the subsequent foraging behaviour of *T. basalis.* Yet such responses do not lead to the avoidance of the evolutionary trap, and consequently, do not always maximize the reproductive success of the wasp. We are requesting consideration for publication in BioControl.

Best regards

Antonino Cusumano on the behalf of all co-authors

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The invasive stink bug *Halyomorpha halys* affects the reproductive success and the experience-mediated behavioural responses of the egg parasitoid *Trissolcus basalis*

Abstract

Invasive species, because of their lack of co-evolutionary history with recipient communities, can act as “evolutionary traps” causing disconnects between natural enemy behavioural responses and the suitability of the invasive species as a prey resource. Invasion of exotic species in non-native environments may have several ecological effects, including consequences for the experience-mediated behavioural responses of indigenous foragers. Experience is usually thought to help resident species to buffer against negative impacts of new invasive species, including escaping from evolutionary traps. Here we hypothesized that the impact of foraging experience depends on whether an indigenous egg parasitoid can correctly assess the resource suitability of a new invasive species for offspring development. We showed that the invasive stink bug *Halyomorpha halys* acts as an evolutionary trap for the indigenous egg parasitoid *Trissolcus basalis* leading to unsuccessful development of ~95% of the eggs laid in this host species. In a mixed scenario in which both the associated resident stink bug *Nezara viridula* co-occur with the invasive *H. halys*, we showed that oviposition experience in the low quality invasive host induces in *T. basalis* similar responses to those of the associated host; thus foraging experience does not lead to avoidance of an evolutionary trap. We discuss parasitoid foraging experience and reproductive success in the light of the evolutionary trap framework with implication for biological control.

Introduction

Accidental introduction of exotic species is a common by-product of globalization. Some of these invading organisms are of serious concern for the stability and functioning of ecological processes in their invaded environment (Gandhi and Herms 2010; Vilá et al. 2011). Exotic species may have several direct and indirect effects on indigenous organisms (Kenis et al 2009). For example, they may represent a new resource for indigenous predators, and several studies have focused on the exploitation of exotic prey by natural enemies (Berkvens et al. 2010; Carlsson et al. 2009; Sloggett 2010). Indirect effects between native and exotic hosts or prey can also occur, as for example when species interactions are mediated by a shared natural enemy (apparent competition *sensu* Holt 1977) (Kenis et al. 2009; Redman and Scriber 2000; Settle and Wilson 1990).

Exotic host or prey species can also shape the learning capacity of indigenous foragers. Experience gathered while foraging allows animals to adjust their behavioural responses to variable ecological conditions buffering, under some circumstances, against the introduction of invasive species (Robertson and Blumstein 2019). Experience could be especially important for indigenous foragers encountering “evolutionary traps”, i.e. new, unsuitable prey or host species present in the environment that possess cues similar to those of native, suitable species (Schlaepfer et al. 2002, 2005). How foraging experience may shape the responses of arthropod natural enemies against evolutionary traps is unclear, but it is usually thought to help resident organisms to escape from evolutionary traps (Robertson and Blumstein 2019).

Among natural enemies, insect parasitoids are excellent model organisms to study experience and learning because of the plasticity of their behavioural responses when foraging for resources whose exploitation is tightly linked to the parasitoid’s fitness (Smid and Vet 2016). Parasitoids must find hosts that are scattered throughout complex and heterogenous environments (Aartsma et al. 2019; Meiners and Peri 2013). To successfully locate their hosts, parasitoids can exploit a variety of cues among which chemical cues called infochemicals or semiochemicals, play a key role (Colazza et al. 2014; Fatouros et al. 2008; Vinson et al. 1998). Foraging experience allows parasitoids to dynamically adjust their responses to infochemicals based on how reliable they are associated with the hosts, and fine-tune their foraging strategies accordingly. While there is evidence of invasive host insects acting as evolutionary traps for parasitoids (Abram et al. 2014; Hoogendoorn and Heimpel 2002), experimental evidence on how parasitoid responses change after gathering foraging experience is limited (Bertoldi 2020).

The brown marmorated stink bug *Halyomorpha halys*, (Stål) (Heteroptera: Pentatomidae), is a polyphagous stink bug pest of Asian origin that has invaded both North America and Europe causing major economic losses in diverse crops (Leskey and Nielsen 2018; Rice et al. 2014). From an ecological point of view, *H. halys* has the potential to share the same community structures of resident stink bug pests, especially of other highly polyphagous insects such as the green stink bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae). The egg parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) is the main natural enemy of *N. viridula* and both organisms likely originated from the Palearctic/Ethiopian regions (Jones 1988; Salerno 2000; Talamas et al. 2017; Todd 1989). This wasp has been used worldwide in classical, augmentative and conservation biological control programs (Foti et al 2019, Corrêa-Ferreira and Moscardi 1996; Todd et al. 1989). To locate its pentatomid hosts, scelionid egg parasitoids display an innate arrestment response to cuticular hydrocarbons associated with walking traces (i.e. footprints) of adult stink bugs (Colazza et al. 2014). The hierarchical value of the footprints depends on host gender: *T. basalis* wasps explore intensively those associated with female stink bugs, which are more likely associated with host egg presence (Peri et al. 2013). The innate response to footprints is affected by experience so that the response decreases in intensity when wasps re-encountered patches without hosts (i.e. unrewarded experience), but it can be restored on patches where oviposition occurred (i.e. rewarded experience) (Peri et al. 2006, 2016). The mechanism underlying parasitoids' responsiveness to footprints has been shown to have several characteristics of habituation, a form of non-associative learning (Abram et al. 2017). These characteristics include a decrease of the response intensity following unrewarded experiences in an interval-dependent manner, i.e. wasps display a decline in response which is more rapid and pronounced when footprints are re-encountered without hosts at shorter intervals (Abram et al. 2017).

Because *H. halys* and *N. viridula* already co-occur in several geographical regions of the globe, and the degree of overlap is likely to be extended due to the range expanding status of the invasive species, it can be common for the egg parasitoid *T. basalis* to encounter patches in which egg masses and footprints of both stink bug species are present. Thus *T. basalis* represents an interesting organism to study how experience can shape the responses of resident parasitoids in the presence of invasive species. There is already evidence that *H. halys* may be a potent evolutionary trap for other species of egg parasitoids in invaded areas. For example, in North America ,some indigenous egg parasitoid species, such as *Telenomus podisi* (Ashmed) (Hymenoptera: Scelionidae) and *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae), readily oviposit in *H. halys* eggs, although wasps cannot develop in this invasive stink bug species (Abram et al. 2014; Konopka et al 2018, 2020). On the contrary, other indigenous egg parasitoid species, such as *Ooencyrtus telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae) and *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) which successfully exploit *H. halys* eggs, may selectively benefit from an additional suitable resource present in the environment, thus increasing their reproductive success (Haye et al. 2015; Roversi et al. 2016).

In this study we investigated the consequence of *H. halys* invasion for the fitness and foraging experience in *T. basalis*. In details we explored: 1) the reproductive success of the egg parasitoid on the associated and invasive species in order to assess whether *H. halys* can act as an evolutionary trap for *T. basalis*; 2) the innate response of *T. basalis* to chemical footprints on the invasive stink bug species in order to assess if the egg parasitoid can recognize the chemical cues left by males or females adults; 3) whether a successful oviposition experience by *T. basalis* on *H. halys* eggs affects the subsequent response to chemical footprints of the invasive species at different time intervals. Finally, in a mixed scenario in which both the associated resident stink bug *Nezara viridula* co-occur with the invasive *H. halys*, we evaluated several possible combinations of rewarded (i.e. oviposition) and/or unrewarded (i.e. footprints) experiences.

Materials and methods

Insect colonies

Stink bug colonies of *N. viridula* and *H. halys* were held in insect cages (47.5 × 47.5 × 47.5 cm, BugDorm-44545, MegaView Science Co. Ltd, Taichung, Taiwan), in an environmental room (24 ± 1°C, 70 ± 5% RH, 16 h:8 h L:D), and fed with a diet of sunflower seeds and seasonal fresh organic vegetables. Food was changed every 2–3 days, and separate cages were used for immatures and adults. Paper towels were placed inside each adult cage as ovipositional substrates. Daily collected egg masses were used to maintain the colonies and to carry on the experiments afterwards described. The colonies of *N. viridula* and *H. halys* were established from bugs collected around Palermo, Sicily (southern Italy). The colony of *T. basalis* was established from wasps emerging from sentinel and or naturally laid *N. viridula* egg masses on cultivated and un-cultivated fields in the Palermo area. Parasitoids were kept in 16-ml glass tubes (density = 50-60 wasp/tube), fed with a solution of honey–water (80:20 v/v), inside an incubator (24 ± 1° C, 80 ± 5% RH, 16 h:8 h L:D). *Nezara viridula* egg masses were exposed to parasitoids for 48 h, then the parasitized egg masses were removed and stored for incubation. After the emergence, male and female parasitoids were kept together to allow for mating.

Experiment 1. Reproductive success of egg parasitoids developing in the invasive stink bug species

The aim of the exposure of *H. halys* egg masses to *T. basalis* was to evaluate the potential suitability of exotic host eggs for the egg parasitoid and whether *H. halys* could act as an evolutionary trap (i.e. whether wasps accept *H. halys* eggs at high levels, but their offspring would not emerge). Fresh *H. halys* egg masses (<24h old) were offered to three days old *T. basalis* in 16-ml glass tubes for 24 h. As positive controls we exposed fresh egg masses

(<24h old) of *N. viridula*, the associated host of *T. basalis,* under the same experimental conditions. The host eggs in which parasitoids have successfully oviposited can be identified because *T. basalis* marks the parasitized host by sweeping its ovipositor on the chorion surface. Such marking behaviour is highly correlated with egg deposition (Cusumano et al. 2011; Abram et al. 2014). During the trials high levels of acceptance were observed regardless of the identity of the stink bug eggs (MCF and LM personal observations). The few wasps that rejected the egg masses (i.e. no marking behaviour was observed) within 30 minutes from their release were removed and replaced with new ones.

After 24 h, parasitoids were removed and the exposed egg masses were stored under controlled conditions (24 ± 1° C, 80 ± 5% RH, 16 h:8 h L:D) until parasitoid or nymph emergence. Emergence of parasitoids, mortality of stink bug eggs and emergence of nymphs were recorded. For both treatments (*H. halys* and *N. viridula*), 18 replicates were carried out.

To assess baseline developmental success of both *H. halys* and *N. viridula,* we included additional controls with unexposed egg masses (N=10 for both stink bug species).

To further compare the fitness of egg parasitoids reared on eggs of *H. halys* or *N. viridula*, the size of the emerging wasp females was estimated by measuring the hind tibia length rather than measuring the weight of the wasps due to their minute size. Measurements were taken with the aid of a stereoscope (Zeiss SteREO Discovery.V12) using AxioVision SE64 Rel. 4.9.1 software for image acquisition and analysis. For each group, 30-32 replicates were carried out.

Experiment 2. Egg parasitoid response to chemical footprints of adult stink bugs: general bioassays procedure

In this experiment we evaluated the responses of *T. basalis* females to the chemical footprints left by stink bugs females. The general procedure consisted of bioassays carried out in open arenas made of a sheet of filter paper (20x20·cm; wasp/arena surface ratio: 0.002%). In the middle of each arena, a circular area (6·cm diameter) was defined and exposed for 30·min to a single adult of *H. halys* or *N. viridula*, leaving the surrounding area untreated. This was achieved by constraining the bugs under a steel mesh cover (6·cm diameter, 1·cm high, 0.01·cm mesh) to ensure constant contact of the bug legs with the filter paper and, at the same time, to avoid surface contamination with bug volatiles. Filter papers contaminated by bug's faeces were not used for bioassays. After removing the bug, according with the following experiments, a female wasp was gently released in the middle of the circular area. Experiments started when the wasp displayed the typical arrestment behaviour (i.e. the wasp intensively drummed with the antennae the area contaminated with stink bug footprints) and lasted until it flew away from or walked off the whole arena. Female wasps that did not show the arrestment behaviour, and either flew away or walked off the arena immediately as a consequence of lack of contact of their antennae with the treated area, were excluded from the analysis. Wasp behaviour was monitored with a CCD camera (Sony M370) equipped with a zoom lens and mounted above the centre of the arena and analysed with "Xbug", a video tracking system and motion analysis software (Peri et al 2006). The arrestment responses of the female wasps were quantified over the entire arena (pooling both outside and inside the circular contaminated area) by means of the total arena residence time (s). Experiments were carried out from 09:00·h to 12:00·h in an isolated room at 25 ± 1 °C illuminated by two 18·cm long fluorescent tubes.

Experiment 2a. Egg parasitoid innate responses to chemical footprints of the invasive stink bug species

The aim of this experiment was to investigate whether naive *T. basalis* females can recognize the chemical footprints left by *H. halys*. If a response was observed, we were also interested to see if wasps display host gender discrimination, i.e. if they spend more time on the traces left by female bugs which would normally be of higher hierarchical value, being more likely associated with egg presence (Peri et al. 2006, 2016). Adults of *H. halys* used to contaminate the circular area of the filter paper arena were either males or females taken from the colony and kept isolated about 3·days before the bioassays. Then, naïve wasps were tested on substrates contaminated only with female (F) or male (M) traces following the general bioassay procedure described above. For each treatment 35 replicates were performed

Experiment 2b. Effect of oviposition experience on egg parasitoid responses to chemical footprints of the invasive stink bug species

The aim of the experiment was to evaluate the influence of a successful rewarded experience (i.e. oviposition by *T. basalis* females on eggs of *H. halys*) on the subsequent wasps' behavioural responses to *H. halys* footprints. Naive *T. basalis* females were singly released onto a circular area contaminated with traces of host males or females and with a *H. halys* egg-mass (five to six eggs) in the middle. During this "training" phase, the residence time of the wasp was not recorded since we were interested in the response of experienced wasps. Then, experienced wasps (i.e. those that parasitized at least one egg) were recaptured and kept isolated in a small vial for 1 h or 24 h. They were then tested on another arena treated with *H. halys* according to two treatment combinations: (1) oviposition in the presence of female traces and tested on female traces (F_Ov_F) or (2) oviposition in the presence of male traces and tested on male traces (M_Ov_M). For each treatment 24-31 successful replicates were performed

Experiment 2c. Effects of unrewarded (footprints) and/or rewarded (oviposition) experiences on egg parasitoid response to chemical footprints of the resident or invasive stink bug species

In this experiment, we evaluated several possible combinations of rewarded and unrewarded experience to assess the subsequent wasps' behavioural responses to footprints in a scenario in which both stink bug species are present. Specifically, single *T. basalis* females were subjected to "training phases" by releasing the wasps onto a circular area to gain the following unrewarded and rewarded experiences: a) Contact on *N. viridula* female traces + oviposition experience on *N. viridula* eggs (Tr_Nv+Ov_Nv); b) Contact on *H. halys* female traces + oviposition experience on *N. viridula* eggs (Tr_Hh+Ov_Nv); c) Contact on *N. viridula* female traces + oviposition experience on *H. halys* eggs (Tr_Nv +Ov_Hh); d) Contact on *H. halys* female traces + oviposition experience on *H. halys* eggs (Tr_Hh+Ov_Hh); e) Oviposition experience on *H. halys* eggs (Ov_Hh); f) Oviposition experience on *N. viridula* eggs (Ov_Nv); g) Contact on *H. halys* female traces (Tr_Hh); h) Contact on *N. viridula* female traces (Tr_Nv); as controls naive wasps that had no experience on bug walking traces or oviposition were used. Treatments e) and f) represent unrealistic situations because the presence of stink bug eggs is always associated with walking traces, however we included these two training treatments to experimentally decuple the effects of the bug walking traces and the effects of oviposition. A successful oviposition experience was defined when the wasp marked the parasitized host by sweeping its ovipositor on the chorion surface. The acceptance rate displayed by *T. basalis* females was very high (>90% MCF personal observations), regardless of the host being *N. viridula* or *H. halys*.

During the training phases, the total arena residence time of the wasps was not recorded. Then, the wasp was recaptured and tested after 1 h on filter paper arenas contaminated with female traces of the associated host *N. viridula* (so called "Nv-wasps") or the invasive stink bug *H. halys* (so called "Hh-wasps"). We chose 1 h time interval between training and testing bouts as the effects of experience was "forgotten" at 24 h (see results of experiment 2). The experience of contact on bugs female traces was defined after observing the typical wasp arrestment response on the contaminated area. For each treatment 28-30 successful replicates were performed

Statistical analyses

For experiment 1, we analysed differences in proportional data (eggs abortion, emergence of nymphs and parasitoids) between treatments (*N. viridula* or *H. halys*) with Fisher's Exact Test. Differences in the size of emerged *T. basalis* females between treatments were analysed with a Linear Model (LM) with normal error distribution and identity link function. Residence time data of experiment 2 were not normally distributed (which is typical of time-to-event data) and thus were analysed with General Linear Models (GLMs) fitting gamma error distribution and a reciprocal link function (Crawley 2007). For experiment 2a we used a GLM to test the effect of *H. halys* sex on the residence time of unexperienced wasps. For experiment 2b, we used a GLM with successful oviposition experience on *H. halys* eggs, time interval (1h and 24h) and the time interval × oviposition interaction as explanatory factors using parasitoid residence time as response variable. For experiment 2c, we used a GLM with footprint experience, oviposition experience, stink bug species identity and their interactions as explanatory factors using parasitoid residence time as response variable. Due to a significant 3-way interaction, residence time data were analysed separately depending on whether experienced wasps were tested on *H. halys* footprints (i.e. Hh-wasps) or on *N. viridula* footprints (i.e. Nv-wasps).

Significance of the explanatory factors in GLMs was determined using Likelihood Ratio Tests (LRTs) comparing the full model with and without the factor in question (Crawley 2007). If models detected significant differences amongst factor levels, we proceeded to pairwise comparisons to determine which differed using the *glht* function in the *multcomp* package (Bretz et al. 2010). Significance levels for factors in the LM were derived directly from *F*-tests (Crawley 2007). Model fit was assessed with residual plots. All statistical analyses were performed with R software version 3.1.3 (R Core Team 2013)

Results:

Experiment 1. Reproductive success of egg parasitoids developing in the invasive stink bug species

Baseline levels of stink bug mortality in the absence of parasitism were low for both *H. halys* (7.01%) and *N. viridula* (5.17%) indicating the high viability of stink bug eggs (Fig 1A). *Trissolcus basalis* did develop in the eggs of the non-associated host *H. halys* but a significantly lower percentage of emergence (6.04%) was found when compared with development on the eggs of the associated host *N. viridula* ($\chi^2 = 64.71$, df = 1, $P < 0.001$); A significantly higher percentage (81.34%) of host egg abortion was observed in *H. halys* compared with abortion levels in *N. viridula* ($\chi^2 = 68.21$, df = 1, $P < 0.001$). Such high levels of *H. halys* egg abortion, which differ significantly with baseline levels observed in the absence of parasitism, are an indirect evidence of the high level of acceptance by *T. basalis*. Finally, no statistical differences were observed on the percentage of nymphs emerged between the associated and the non-associated host ($\chi^2 < 0.01$, df = 1, $P = 0.986$) (Fig 1A). The size of *T. basalis* females, as estimated by the hind tibia length, was strongly affected by the treatment ($F = 46.81$, df =1,45 $P < 0.001$). Wasps emerging from eggs of the invasive host *H. halys* were, on average, 23.15 % larger than those emerging from *N. viridula* host eggs (Fig 1B).

Experiment 2a. Egg parasitoid innate responses to chemical footprints of the invasive stink bug species

Naïve *T. basalis* females responded to chemical footprints of the non-associated stink bug species with the typical arrestment response. Wasp residence time was not statistically different when parasitoids were tested on arenas contaminated with *H. halys* female versus *H. halys* male chemical footprints ($\chi^2 = 0.65$, df = 1, $P = 0.398$) (Fig. 2).

Experiment 2b. Effect of successful oviposition experience on egg parasitoid responses to chemical footprints of the invasive stink bug species

The residence time of *T. basalis* wasps was significantly affected by successful oviposition experience (χ^2 = 1.98, df = 1, *P* = 0.075) and the time interval (χ^2 = 2.65, df = 1, *P* = 0.0012) between experience events. Wasps previously rewarded with an oviposition experience on *H. halys* eggs that re-encountered *H. halys* female footprints after 1h showed longer arena residence time compared to rewarded wasps re-encountering *H. halys* male chemical footprints (Fig. 3). Parasitoids showed no discrimination between areas contaminated by chemical footprints left by a host female or host male when the time interval between training and testing bouts was 24h (Fig. 3).

Experiment 2c. Effects of unrewarded (footprints) and/or rewarded (oviposition) experiences on egg parasitoid response to chemical footprints of the resident or invasive stink bug species

Residence time on *H. halys* female traces. The response of *T. basalis* wasps to substrates contaminated with footprints of the non-associated host *H. halys* was affected by oviposition experience (χ^2 =12.01, df = 2, *P* <0.001), by experience on footprints (χ^2 =7.67, df = 2, *P* <0.001) and by the oviposition × footprint interaction (χ^2 =37.98, df = 4, *P* <0.001) (Fig. 4A). Wasps that previously oviposited on the non-associated host in the presence of footprints (i.e. Tr_Nv +Ov_Hh and Tr_Hh+Ov_Hh) achieved high values of residence time similar to naïve wasps, regardless if footprint experience occurred on the native or invasive host (Fig. 4A). On the contrary, when oviposition occurred on the associated host *N. viridula* (Tr_Nv

+Ov_Nv and Tr_Hh+Ov_Nv) lower levels of residence time were found, and again the footprint experience did not affect wasp response (Fig. 4A). The residence time of wasps previously exposed to *N. viridula* oviposition only (Ov_Nv) was not different than residence time of wasps rewarded with oviposition experience on *H. halys* only (Ov_Hh) (Fig. 4A). Finally, wasps spent the lowest residence time when given an unrewarded experience (i.e. no oviposition), especially when trained on the footprints of the non-associated hosts (Tr_Hh) (Fig. 4A).

Residence time on *N. viridula* female traces. The response of *T. basalis* wasps to substrates contaminated with footprints of the associated host *N. viridula* was affected by the previous oviposition experience (χ^2 =7.98, df = 2, P<0.001) and by the oviposition × footprint interaction (χ^2 =4.08, df = 4, P =0.015) whereas no effect of the footprint experience itself was found (χ^2 =0.72, df = 2, P =0.396) (Fig. 4B). Wasps rewarded with oviposition experience on the associated host *N. viridula* (i.e. Tr_Nv+Ov_Nv, Tr_Hh+Ov_Nv, Ov_Nv) showed high levels of responses in terms of residence time, similar to those of naïve wasps (Fig. 4B). An oviposition experience on the invasive species *H. halys* induced overall lower residence times by *T. basalis*, although significant differences were only observed between *H. halys* oviposition only (Ov_Hh) and all *N. viridula* treatments in which oviposition occurred, regardless of the presence or identity of the footprint experience (Fig. 4B). Unrewarded experience decreased the wasp residence times on host footprints, especially when wasps were trained on the footprints of the associated host *N. viridula* (Tr_Nv) (Fig. 4B).

Discussion

In this paper we investigated the ecological consequence of exotic herbivore invasion for the fitness of a non-associated egg parasitoid species. We showed that *H. halys* can act as an evolutionary trap for the egg parasitoid *T. basalis* and that foraging experience by the wasp on cues associated with the invasive stink bug species does not lead to the avoidance of an evolutionary trap.

Our results showed that the egg parasitoid *T. basalis* accepts *H. halys* eggs as ovipositional sites, yet ~95% of the parasitoid eggs failed to develop indicating that *H. halys* represents a clear, poor reproductive investment. In the recent years there has been an increase in the number of studies that documented evolutionary traps in insects (see Robertson et al. 2013 for a review). In egg parasitoids, the taxonomically related *T. podisi* also experienced the invasive *H. halys* as an egg sink although the severity of the trap may be higher for this egg parasitoid species because *T. podisi* parasitoids always failed to successfully develop (Abram et al. 2014). Interestingly, the few *T. basalis* parasitoids that emerged from *H. halys* eggs are almost 25% larger in size, as estimated by the hind tibia length, probably because the wasps benefit from the greater amount of resources contained in *H. halys* eggs compared with the smaller *N. viridula* host eggs (Martorana et al. 2017). Because body size is a good proxy for fitness (Boivin et al. 2010; Cusumano et al. 2016; Roitberg et al. 2001) it is possible that wasps emerging from *H. halys* eggs may have a competitive advantage compared with those developing on the associated host *N. viridula*. This hypothesis suggests potentially “mixed consequences” of attacking an unsuitable host/evolutionary trap as the value of producing larger offspring could partially offset the negative consequences of producing fewer offspring. Such trade-off could be particularly important for species like *T. basalis* that fight over reproductive investments (Field and Calbert 1998).

We also showed that naïve *T. basalis* respond with a typical arrestment behaviour to the walking traces of the invasive stink bug species, suggesting a chemical similarity between contact kairomones of *H. halys* and *N. viridula*, the preferred host of *T. basalis*. However, the similarly between chemical cues of the associated *versus* non-associated host is only partial, because naive *T. basalis* wasps cannot discriminate between the footprints left by male and female adult bugs of *H. halys*. In closely associated egg parasitoid-stink bug systems (i.e. *T. basalis – N. viridula*; *Trissolcus brochymenae – Murgantia histrionica; Trissolcus* sp. – *Graphosoma semipunctatum*), naïve wasps perceive differences in hierarchical value of host chemical residues, with stronger intensity of responses induced by footprints of female bugs, especially if in preovipositional state (Colazza et al. 1999; Peri et al. 2013, 2016; Salerno el al. 2009).

Interestingly, the plasticity of wasp responses depends on experience, as wasps rewarded with an oviposition in *H. halys* eggs learn to discriminate between traces left by females compared with traces left by males. If *H. halys* would be a suitable host for *T. basalis*, this type of experience-induced behavioural response would likely be adaptive as female host traces are a more reliable indicator of the presence of host eggs. However, because the link between cue reliability and resource suitability is broken in evolutionary traps (Schapfer et al. 2005), this response to *H. halys* cues does not maximize the reproductive success of *T. basalis*. The host gender discrimination is time-dependent and it is only displayed when the time interval between wasp training and testing bouts is of 1h, suggesting that the learned information is stored in short term memory (Hoedjes et al. 2011; Margulies et al. 2005).

We also assessed the effect of unrewarded and rewarded experiences on the patch time allocation of the egg parasitoid in “mixed” conditions, i.e. when cues of both *H. halys* and *N. viridula* are present. Species co-occurrence is likely to be more frequent in the future because climate models predict that the invasive stink bug species will continue to spread in North America and Europe (Kriticos et al. 2017; Zhu et al. 2012) thus increasing the degree of overlap with the geographic regions where *N. viridula* and *T. basalis* are already present. Furthermore, *N. viridula* and *H. halys* share many plant hosts so direct overlap is likely to be inevitable. In such mixed scenarios we found that: 1) wasps tested on *H. halys* female traces (i.e. Hh-wasps) achieved high intensity of responses when they experienced a successful oviposition on *H. halys* eggs regardless of the nature of the footprints; 2) wasps tested on *N. viridula* female traces (i.e. Nv-wasps) achieved high intensity of responses when wasps previously oviposited on *N. viridula* regardless of the nature/presence of the footprints. Taken together, these results indicate that *T. basalis* females display the strongest responses when they obtained an oviposition experience on the same stink bug species used for the subsequent tests. A possible explanation for our results is that the oviposition experience overrules the effect of the footprint experience being the former stimulus of higher hierarchical value for the fitness of the wasp (Vinson et al. 1998). Our results challenge the adaptive value of foraging experience as an oviposition reward on the associated, high quality host *N. viridula* does not always trigger the highest residence time in experienced *T. basalis* wasps.

An interesting difference between the behavioural responses of Nv-wasp and Hh-wasp is found when wasp females receive an unrewarded experience (i.e. footprints only) on the same stink bug species used for subsequent testing. In fact, the intensity of the Hh-wasp response decreases much more on *H. halys* footprints (94.21 % reduction compared with naïve wasps) than Nv-wasp response on *N. viridula* (50.75% reduction compared with naïve wasps). This appears to be adaptive because, under unrewarded conditions, *T. basalis* could became quickly habituated and lose motivation to respond to *H. halys* kairomones. Habituation to *H. halys* footprints could, as a consequence, attenuate the risk that *T. basalis* is "trapped" by its behavioural responses to the invasive stink bug species, as long as it is not rewarded by an oviposition experience before habituation takes place.

An invasive exotic species can indirectly affect the interactions between an indigenous parasitoid and its hosts (Heimpel et al. 2003; Desurmont et al. 2014; Kenis et al 2009). Several lines of evidence suggest that the presence of an invasive species such as *H. halys* may interfere with the efficiency of *T. basalis* as biological control agent of *N. viridula* given the fact that: 1) the parasitoid spends time investigating chemical cues associated with the low-suitable resource, time that could be spent foraging for the associated host; 2) the parasitoid wastes ~95% of eggs when oviposition in *H. halys* occurred, eggs that could be invested into associated hosts which yield higher fitness payoffs; 3) the parasitoid appears to value an oviposition reward on *N. viridula* eggs at a similar level (or even lower) than an oviposition in *H. halys* eggs. It would be thus interesting to monitor parasitism rates of *T. basalis* on *N. viridula* in those areas in which *H. halys* has recently established to evaluate if *N. viridula* has become a more destructive pest and whether this is due to insufficient biological control.

It is remarkable to note that our population of *T. basalis*, which originated from individuals collected in Sicily, develops on viable *H. halys* eggs although with a very low success rate, whereas another Italian population (Umbria region) of the wasp cannot do so (Rondoni et al. 2017); such findings suggest that this egg parasitoid species has the potential to "escape" the evolutionary trap. In fact, a recent study conducted in North America showed that another population of *T. basalis* can develop on *H. halys* eggs with relatively high reproductive success (38% of the parasitoids emerged as adults) (Balusu et al. 2019). This means that ovipositing in a *H. halys* egg mass does not constitute a dead end for the parasitoid and, if there will be selective pressure and underlying genetic variation in developmental success for natural selection to act on, it is possible that over evolutionary time *T. basalis* could form a more stable association with the invasive stink bug species. Yet, the impact of *H. halys* for *T. basalis* is difficult to predict and will depend on several ecological aspects including the frequency of encounter with *H. halys* egg masses and the preference of the indigenous parasitoid for eggs of the invasive species over the associated host.

When invasive species are introduced in novel environments, or expand their range towards the poles due to global warming, they interact with native organisms in several direct and indirect ways (Kenis et al. 2009; Vilá et al. 2011). Due to the lack of co-evolutionary history, invasive species can create novel ecological scenarios in which the behavioural responses of indigenous organisms do not maximize fitness (Robertson et al. 2013; Schaper et al. 2005). Here we show that an invasive stink bug species which acts as an evolutionary trap affects the behavioural responses of experienced indigenous egg parasitoids. Our study shows that experience is not necessarily helpful for native species responding to an evolutionary trap because, at least in the short term, it seems to reinforce the maladaptive behaviour (Bertoldi 2020). Future studies should focus on whether, in the long term, this reinforcement could actually make adaptation to the host more likely. This is a topic of particular relevance nowadays in both basic and applied ecology given the increasing frequency of invasion of exotic species in non-native environments.

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Legends

Fig. 1. (A) Development of parasitoids in invasive and resident hosts. Proportion of emerged parasitoids, dead host eggs and emerged stink bug nymphs observed when *Trissolcus basalis* females were exposed to stink bug eggs of the invasive species *Halyomorpha halys* (Hh, white colour) and the resident host *Nezara viridula* (Nv, grey colour) and. Proportion of dead host eggs and emerged stink bug nymphs in unexposed control eggs are shown to display the baseline levels of host mortality and egg viability in both *N. viridula* and *H. halys*. Asterisks indicate significantly different proportions within each developmental outcome (χ^2 tests, $P<0.05$). Solid lines refer to pairwise comparisons between stink bug species whereas dashed lines indicate within species comparisons. **(B) Size of egg parasitoids emerging from invasive and resident hosts**. Hind tibia length of *Trissolcus basalis* developed on stink bug eggs of *Halyomorpha halys* (Hh, white colour) and *Nezara viridula* (Nv, grey colour). Asterisks indicate significant differences (LM, $P<0.05$).

Fig. 2. **Behavioural response of naïve parasitoids to chemical traces of the invasive host**. Mean (+ SE) residence time of *Trissolcus basalis* females encountering for the first time (naïve), *Halyomorpha halys* walking traces left onto a filter paper arena by female (F) or male bugs (M). “ns” above bars indicates no significantly different means (GLM, $P < 0.05$).

Fig. 3. **Behavioural response of parasitoids with rewarded experience to chemical traces of the invasive host**. Mean (+ SE) residence time of *Trissolcus basalis* females allowed to oviposit on *Halyomorpha halys* eggs in the presence of either host female or male traces and then tested after 1h or 24h according to different conditions: F_Ov_F = oviposition in the presence of female traces and tested on female traces; M_Ov_M = oviposition in the presence

of male traces and tested on male traces. Asterisks above bars indicate significantly different means (GLM, $P < 0.05$)

Fig. 4. Behavioural responses of experienced parasitoids to chemical traces left by *Halyomorpha halys* females (4A) or by *Nezara viridula* females (4B). Mean (+ SE) residence time of *Trissolcus basalis* females experienced according to different conditions, rewarded or non rewarded, on the invasive host *Halyomorpha halys* (white bars) or on the resident host *Nezara viridula* (grey bars). Naïve = no experience on host walking traces or oviposition; Tr_Nv+Ov_Nv = Contact on *N. viridula* female traces + successful oviposition experience on *N. viridula* eggs; Tr_Hh+Ov_Nv = Contact on *H. halys* female traces + successful oviposition experience on *N. viridula* eggs; Tr_Nv +Ov_Hh = Contact on *N. viridula* female traces + successful oviposition experience on *H. halys* eggs; Tr_Hh+Ov_Hh = Contact on *H. halys* female traces + successful oviposition experience on *H. halys* eggs; Ov_Hh = successful oviposition experience on *H. halys* eggs; Ov_Nv = successful oviposition experience on *N. viridula* eggs; Tr_Hh = contact on *H. halys* female traces; Tr_Nv = contact on *N. viridula* female traces. Different letters above bars indicate significantly different means (GLM, $P < 0.05$)

Fig. 1

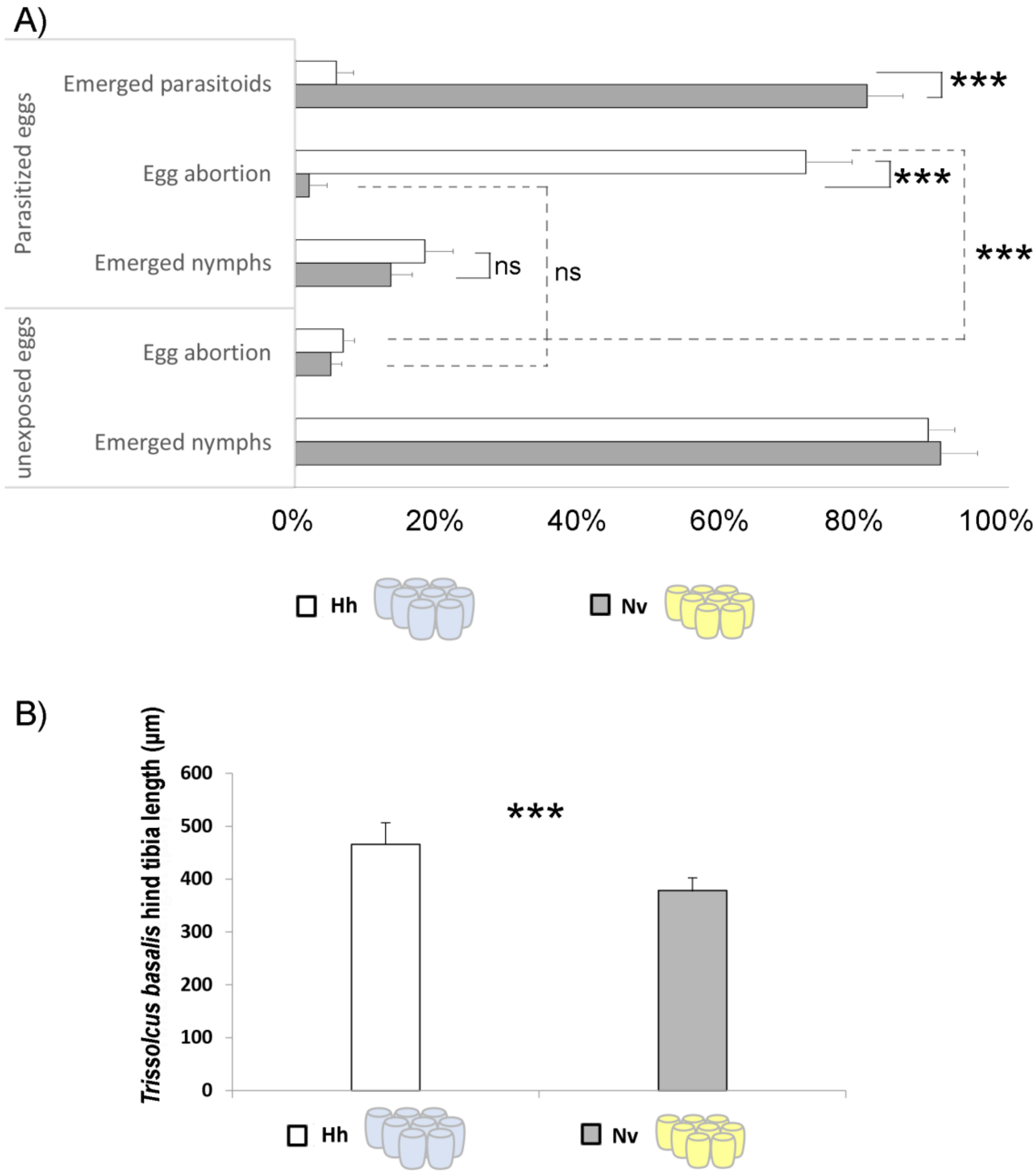

A)
Parasitized eggs
unexposed eggs
Emerged parasitoids
Egg abortion
Emerged nymphs
Egg abortion
Emerged nymphs
***
***
***
ns
ns
0%
20%
40%
60%
80%
100%
Hh
Nv
B)
Trissolcus basalis hind tibia length (µm)
600
500
400
300
200
100
0
***
Hh
Nv

Fig. 2

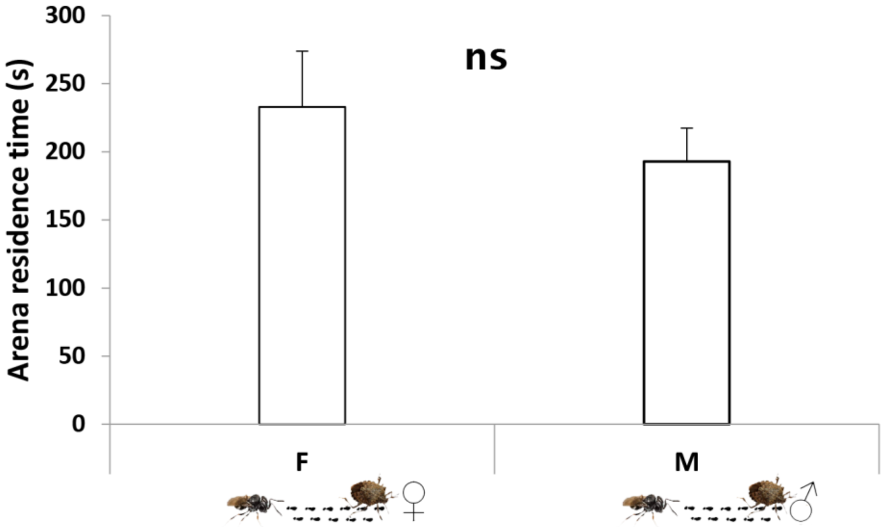
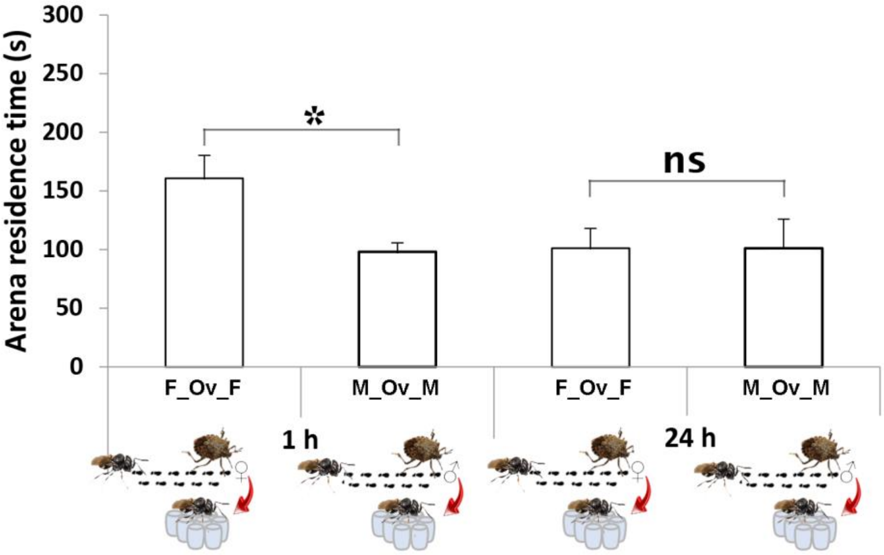


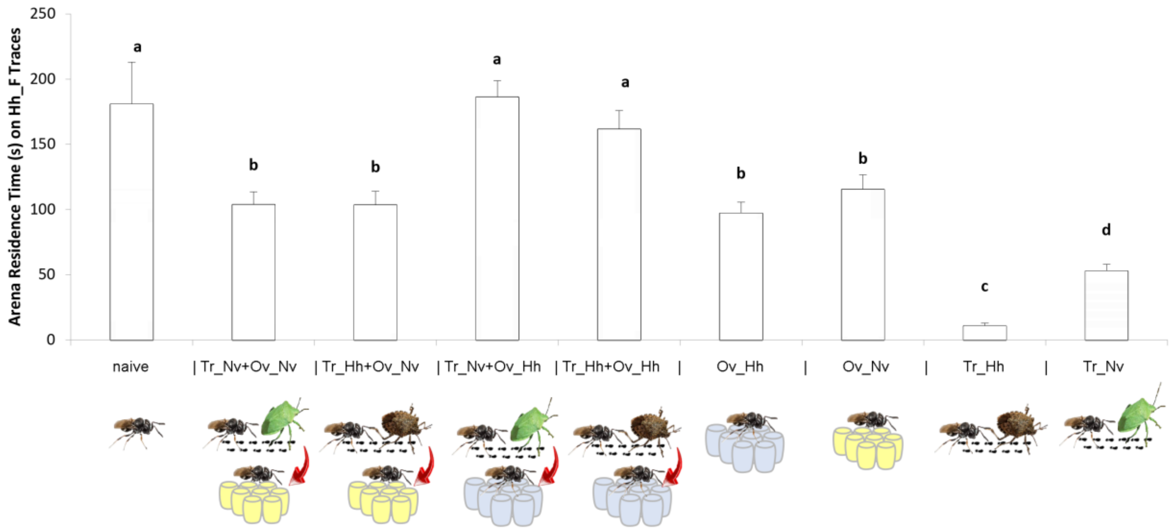
Fig. 3



Arena residence time (s)
300
250
200
150
100
50
0
*
ns
F_Ov_F
M_Ov_M
F_Ov_F
M_Ov_M
1 h
24 h

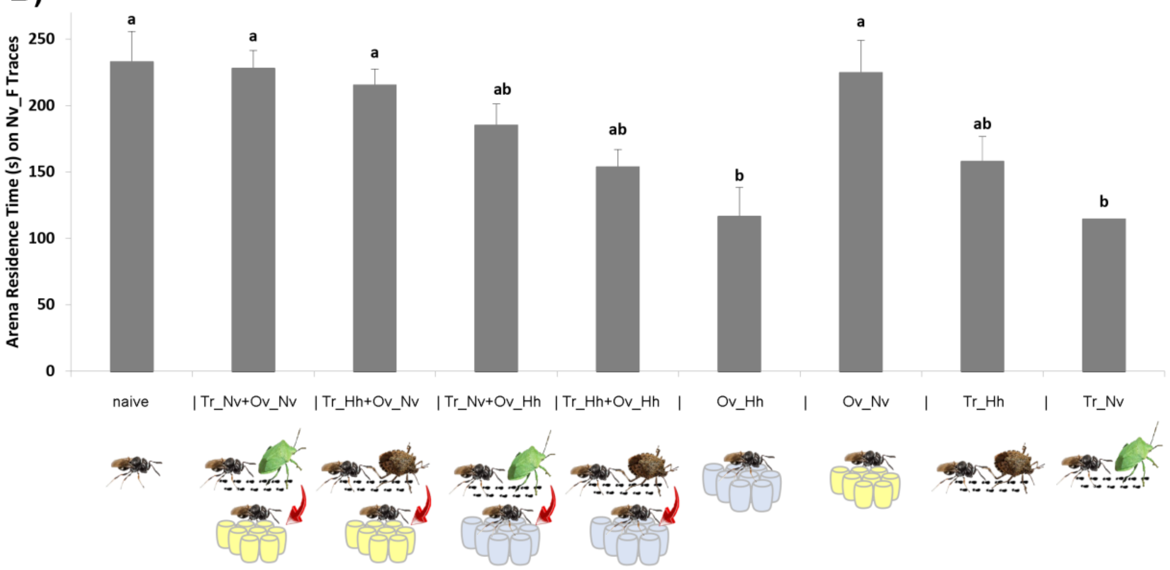
Fig. 4

A)



Arena Residence Time (s) on Hh_F Traces
250
200
150
100
50
0
a
b
b
a
a
b
b
c
d
naive
Tr_Nv+Ov_Nv
Tr_Hh+Ov_Nv
Tr_Nv+Ov_Hh
Tr_Hh+Ov_Hh
Ov_Hh
Ov_Nv
Tr_Hh
Tr_Nv

B)



Arena Residence Time (s) on Nv_F Traces
250
200
150
100
50
0
a
a
a
ab
ab
b
a
ab
b
naive
Tr_Nv+Ov_Nv
Tr_Hh+Ov_Nv
Tr_Nv+Ov_Hh
Tr_Hh+Ov_Hh
Ov_Hh
Ov_Nv
Tr_Hh
Tr_Nv

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Compliance with Ethical Standards

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Compliance with Ethical Standards

There are no ethical concerns regarding the organisms and the topic of this research