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Novel Monitoring and Biological Control of Invasive Insect Pests

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Novel Monitoring and Biological Control of Invasive Insect Pests

by

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Abstract of The Dissertation

Invasive species are alien to the ecosystem under consideration and cause economic or environmental damage or harm to human health. Two alien insects that fit this description are the brown marmorated stink bug, *Halyomorpha halys* and the spotted lanternfly, *Lycorma delicatula.* Both invaders are polyphagous pests that feed on a myriad of plant species and inflict severe crop losses. As sustainable control methods depend on the accurate monitoring of species' invasion and involve the use of natural enemies, we addressed these two facets by exploring novel monitoring techniques and deciphering host-parasitoid interactions for improved integrated pest management. Thus, we adopted 'BugMap', a citizen science initiative that enables students, farmers and everyday citizens to report sightings of *H. halys* from Italy, with emphasis on Trentino-Alto Adige. Aside from fostering citizen participation in scientific endeavors and the enhanced literacy that ensues, BugMap helped uncover the invasion dynamics of *H. halys* and forecast its potential distribution in Trentino, all while coordinating technical monitoring and informing management strategies. The most promising agent currently under study for the classical biological control of *H. halys* is the Asian egg parasitoid *Trissolcus japonicus*. To assess the wasp's potential non-target impacts, we investigated its foraging behavior in response to chemical traces 'footprints' deposited by its main host *H. halys* and by a suboptimal predatory species, the spined soldier bug, *Podisus maculiventris*. Wasps exhibited a 'motivated searching' when in contact with footprints originating from both species. However, *T. japonicus* arrestment was significantly stronger in response to *H. halys* footprints, compared with *P. maculiventris*, implying the presence of underlying chemical cues that shape its natural preferences. A series of GC-MS chemical analyses revealed that *n*-tridecane and (*E*)-2-decenal were more abundant in *H. halys* footprints and are probably the key components utilized by the wasp for short range host location. The function of the aforementioned compounds was studied, *n*-tridecane acted as an arrestant, prolonging *T. japonicus* residence time, whereas (*E*)-2-decenal fulfilled its presumed defensive role and repelled the wasp. These results shed new light on the chemical ecology of *T. japonicus* and help expand the understanding of parasitoid foraging and its implications for classical biological control. Moving to the other invader *L. delicatula*, an egg parasitoid *Anastatus orientalis* was reported attacking it at high rates in its native range in Eastern Asia and may play a key role in reducing its populations there. A series of bioassays revealed that wasps responded to footprints deposited by *L. delicatula* gravid females by initiating a strong searching behavior. Moreover, *A. orientalis* preferred to oviposit in egg masses with intact oothecae, suggesting that the host's egg covering functions as a trigger for *A. orientalis* probing and oviposition. Thus, *A. orientalis* not only overcomes, but also reverses an important line of host structural defense for its own fitness gains. This dissertation discusses the benefits of combining citizen science with traditional monitoring, and the usefulness of decoding host-parasitoid interactions to design more efficacious management strategies of invasive insect pests.

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“Each one of us is a tiny being, permitted to ride on the outermost skin of one of the smaller planets for a few dozen trips around the local star.”

Carl Sagan and Ann Druyan

This dissertation is dedicated to even smaller beings, arthropods, the little things that run our world.

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Chapter 1

General Introduction

1 Invasive species

The Convention on Biological Diversity (CBD) defines invasive alien species as taxa that are introduced, established, naturalized and spread outside of their home range, and whose impacts involve significant harm. Biological invasions have been a lingering threat to human welfare, oftentimes with catastrophic consequences. To name a few, the bubonic plague, the Black Death, the Spanish flu and HIV AIDS swept across continents and claimed millions of lives, but human pathogens are not the sole invasive species involved.

For the past centuries, humans have deliberately or inadvertently traded, transported and introduced plants, animals and other organisms around the globe, and have slowly and perhaps unknowingly rearranged Earth's biotic diversity. Although species have always managed to spread, even without human assistance, but much less often, much more slowly and not nearly as far (Simberloff and Rejmanek, 2011). The industrial revolution yielded technical improvements that have accelerated the ease with which commodities are transported. The world has thus entered a new phase in the magnitude of biological invasions, the 'Era of Globalization' (Hulme, 2009).

1.1 Agriculture and species invasion

Davis and Landis (2011) noted that agriculture and agricultural trade may facilitate biological invasions in various ways. Agriculture acts as a source of invaders when alien species are imported because (1) they offer increased economic returns, (2) satisfy demands for exotic products, (3) feed on or suppress other species, (4) or simply because people like them. Additionally, modern agriculture is considered a sink for invaders due to (1) continuous monoculture that increases agroecosystems' vulnerability to invasions, (2) disturbances caused by agricultural management techniques that opens up biological space for exploitation, (3) resource availability in agricultural settings that helps invasive species thrive and outcompete natives and (4) high connectivity of agricultural landscapes which facilitates the dispersal of invaders.

On the other hand, increased transport of goods and long-distance trade have also revealed another class of invasive species that is probably the most inconspicuous of all. Hitchhikers are those organisms that are dispersed unintentionally and independently of a traded commodity, and whose rate of introduction is set to increase with globalization (Hulme, 2007). Perhaps aquatic species transported via the hulls of boats and ships are one of the most distinguishable of this group (Hulme, 2007). Aside from increased trade, there are other elements that drive biological invasions and help understand why some introduced populations succeed while others fail to establish. For instance, propagule pressure[1] has been implicated in the successful invasion of alien species (Meyerson and Mooney, 2007), and strong evidence for it comes from willful introductions in acclimatization societies that were active in European colonies in the Americas and Australasia. Many New Zealand societies kept detailed records of songbird introductions, the evidence implies that if 10 or fewer birds were introduced, failure is almost guaranteed, while introductions of more than 100 birds were almost always successful (Thompson, 2014). Thus, it is likely that hitchhikers arriving on ships are already present in recipient ports (Levine and D'Antonio, 2003), however, repeated introductions of the same species increase propagule pressure (i.e. genetic diversity of introduced populations), resulting in higher probability that a species will become invasive (Lockwood et al., 2005). Several global (climate change) and local (railways) disturbances are another factor that may be important in facilitating species invasions (Mooney and Hobbs, 2000). Invasive plants are expected to respond with greater growth, higher photosynthetic rates and earlier leaf emergence to rising CO_2 levels compared with native ones (Farnsworth and Meyerson, 2003; Ziska and George, 2004). Anthropogenic climate change affects biological invasions as organisms seek more climatically compatible habitats. For instance, phenological asynchronies, changes in voltinism and range shifts of agricultural insect pests, disease transmitting species (i.e. mosquitos and ticks) and pollinators are occurring

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[1] It includes both the absolute number of individuals introduced to a new system and the number of introduction events that occur.

and expected to increase, with significant impacts on human welfare (Ogden et al., 2014).

Besides these factors, there exists certain characteristics that are common among successful invaders and have been discussed in several studies (Simberloff, 1989; Williamson, 1996). These include a short generation time, high fecundity and growth rates, referred to as an *r*-selected life history. Additionally, a strong dispersal capability, behavioral flexibility, phenotypic plasticity and a wide breadth of hosts enable exotic species to compete with natives for resources and space, facilitating their invasion. In some instances, aesthetic characteristics and human association also play a role in species invasions, as is the case with the eastern grey squirrels in Europe (Bertolino and Genovesi, 2003).

1.2 Effects of invasive species

The extinction of endemic species through the alteration of ecosystem functioning and the reduction of genetic variation are some of the biodiversity threats caused by invasive species (Hulme, 2007; Meyerson and Mooney, 2007). Whereas the full socio-economic costs of such invasions not only include their direct damage or control costs, but also encompass their effects on host ecosystems and on the human populations dependent on them (Perrings et al., 2002). Economic damages are usually more easily deciphered than ecological ones because they are rapidly perceived and reported, also because economic pests are likely to attract more scientific attention (Vilà et al., 2010).

1.2.1 Economic damage

Perrings et al. (2002) suggested that the openness of a country's economy, geography along with its regulatory regimes, and the importance of agriculture, forestry, or tourism all make it more or less vulnerable to invasions by alien species. The first estimate of the cost of damages from 79 invasive species in the USA, for the period spanning from 1906 to 1991, was at US$ 97 billion per year (Office of Technological Assessment, 1993). Pimentel et al. (2000) updated that estimate to US$ 120 billion as annual damage after examining a wider set of invasive species. The total costs of invasive species in Europe is estimated to be well over € 20 billion per year, and even that is an underestimate of the real impact (Pyšek and Richardson, 2010). Annual crop losses due to alien arthropods are estimated at € 2.8 billion in the UK, if we include damages inflicted by pathogens and vertebrates, that would add up to € 3.8 billion per year (Pimentel et al., 2001). Three damaging arthropods in Germany cost the state a minimum of € 12 million per year in losses of stored grains (Reinhardt et al., 2003). Eradication efforts and the removal of host trees of the invasive Asian long-horned beetle, *Anoplophora chinensis* Forster (Coleoptera: Cerambycidae) in Lombardia, Italy, cost the region an estimated € 18 million, apparently without success (Ciampitti and Cavagna, 2014). In New Zealand, 2200 species of invertebrates are estimated to have invaded the island, some are severe agricultural pests such as the Mediterranean fruit fly and the Argentine ant. These latter pests along with the other invasive arthropods inflict about US$ 195 million of crop losses and an additional US$ 242 million control costs (Barlow and Goldson, 2002). According to Sinden et al. (2004), the nationwide impact of weeds alone in Australia amounts to AUS$ 3.9 billion.

1.2.2 Ecological impact

There exists a ubiquitous positive correlation between native species decline and invasive species dominance, but that does not necessarily mean that invasive species are the drivers of the observed phenomenon (Didham et al., 2005). As noted by MacDougall and Turkington (2005), exotic dominance could be a result of invasive species taking advantage of other forms of ecosystem change, such as habitat disturbance.

Genetic effects may result from hybridization between invasive species and native ones, inducing alteration in native genetic resources (Long, 2003; Mallet, 2005). Moreover, alien invertebrate herbivores can have detrimental effects on native plant populations, sometimes driving them to extinction, as is the case with the balsam woolly adelgid, *Adelges piceae* Ratzeburg (Hemiptera: Adelgidae) that is killing vast forests of Fraser fir in North America (Small et al., 2005; Kenis et al., 2009). These invasions often impact trophic cascades, for instance, oak defoliation by the invasive gypsy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae), significantly affected the populations of native birds in North America (Bell and Whitmore, 2000; Gale et al., 2001). Moreover, alien ants show the highest and best-documented records of ecological damage on the native fauna. For example, the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) originating from South America has invaded southern regions of North America, where it threatens several arthropods, reptiles, birds and mammals and also attacks beneficial insects (Morrison, 2002; Holway et al., 2002).

How do we respond to invasive species given the impact they can have? Preventing their entry is surely more effective than attempting to manage them once they arrive (Shine and Williams, 2000). As that is not always the case, early detection and monitoring offers scientists the chance to eradicate or organize strict management techniques that may limit range expansion and potential harm (Hobbs and Humphries, 1995). Large-scale monitoring before and during the invasion, also during and after control attempts, can provide valuable ecological information that would help refine management tactics.

2 Invasive species monitoring and citizen science

Irwin (2002) equated citizen science with a movement to democratize science and bring it closer to the public. Technically, citizen science fosters public participation with professional scientists to gather, submit or analyze data (Bonney, 2016). Biological projects range from national-scale projects, such as the U.K. Open Air Laboratory (OPAL) surveys of trees, insects, or biodiversity (Opalexplorenature.org, 2019), to regional-scale projects such as the Crab Team monitoring of the range expansion of the invasive European green crab in Washington state, USA (Grason et al., 2018).

2.1 Challenges facing traditional monitoring

Invasive species recording, mapping, and monitoring are prerequisites for successful biological invasion risk management (Roura-Pascual et al., 2010). As invasive species continue to spread, a small group of scientists is faced with several challenges to perform long-term surveys that reflect the reality of an invasion and instruct management strategies. Traditional or professional monitoring is costly, as employing scientists, buying and maintaining field equipment and running data analysis facilities, can take up a significant fraction of resources. Its high cost is often a limiting factor in sustaining it over time and is therefore linked to logistic and technical hinders such as covering large geographic areas (Danielsen et al., 2005). Gardiner et al. (2012) estimated the cost (equipment, travel, researcher and student wages, training workshops, and website development) of collecting lady beetles using a sticky card trap in the UK and compared different citizen science approaches with professional monitoring. The authors found that employing traditional monitoring, the costs were US$ 126.62 per trap, whereas only US$ 40.29 and US$ 31.44 were spent on verified science (citizen's observations confirmed by trained experts) and direct science (citizen's data are studied without verification), respectively. Citizen science projects can also be standardized[2] such as the Massachusetts Butterfly Club

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[2] Include volunteer training and preassigned sampling times and locations

surveys or opportunistic[3] such as iNaturalist (iNaturalist, 2019) and eButterfly (eButterfly, 2019) (Soroye et al., 2018).

2.2 Benefits of citizen participation

Given the hurdles faced by traditional monitoring, citizen scientists[4] could help supplement this effort and fill the gaps, provided they have the necessary tools and knowledge to effectively collect and share data. Volunteers could discover invasive species that are not yet detected, as they may have access to lands that are unattainable to professional scientists (Lepczyk, 2005). The increase in data quantity results from the opportunistic recordings that can be collected by a large number of volunteers (Kamenova et al., 2017). By comparing data generated by citizens over 2 weeks with long-term experts-generated data on UK social wasps, namely *Vespa crabro* L. (Hymenoptera: Vespidae), Sumner et al. (2019) found that citizen-generated coverage is comparable to four decades of experts' recordings. As per the quality of such data, some scientists remain skeptical as to whether citizen monitoring activities can reliably detect and adequately characterize ecological change (Penrose and Call, 1995; Brandon et al., 2003). Data variability, over-or under-estimation of species abundance, and misidentification of inconspicuous species are some of the raised concerns (Bray and Shramm, 2001; Barrett et al., 2002). However, data quality and observer error may be reduced through better training and user-friendly

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[3] Does not include mandatory training, volunteers submit observations based on personal decisions on when and where to collect

[4] Volunteers who partner with scientists to solve real world problems

protocols (Lotz and Allen, 2007). Moreover, adopting verified citizen science initiatives, where data are checked by experts or by employing digital technologies such as image recognition, significantly reduces inaccuracies (Gallo and Waitt, 2011).

Widespread Internet access has favored the development of several extensive inventory projects involving networks of volunteers who provide observations following relatively unstructured protocols. One example is FrogID, an expert validated biodiversity database that allows users in Australia to submit acoustic recordings of calling frogs. The large size of Australia and the remoteness within it have long hampered surveying highly threatened taxa such as frogs. Therefore, Rowley et al., (2019) designed this initiative, which enabled them to collect over 30,000 frog recordings in one year, gathering large amounts of data on rare and endangered frog species and detecting and mapping invasive ones. Measham (2007) found that engaging volunteers in such initiatives makes it more likely they collect data relevant to local conservation and management issues. Citizens taking part in such coordinated projects not only enhanced the geographical scope of data collection but also acquired a skillset that benefit societies through increased scientific literacy (Bonney et al., 2009; Silvertown, 2009).

Indeed, participants are destined to increase their understanding of the scientific process and go through improved science and technology literacy (Jenkins, 1999; Trumbull et al., 2000; McKinley, 2017). Brossard et al. (2005) found that participants who placed nest boxes in their house or neighborhoods as part of 'The Birdhouse Network' program, showed statistically significant increases in their knowledge of bird biology. Participants in projects by the National Institute of Invasive Species Science also showed improvement in content knowledge of invasive species, global positioning system (GPS), and vegetation monitoring when using context-specific measures (Crall et al., 2013). Jordan et al. (2011) found that participant's knowledge of invasive plants, their ability to recognize these species and identify their ecological effects, have significantly increased after volunteering for a 2-year project in New York.

2.3 Citizen science and species distribution models

Although citizen science has a centuries-long history, it has seen a recent surge in popularity due to advances in information and communications technology (Miller-Rushing et al., 2012). The ubiquity of smartphones with internet, GPS, and camera capabilities, has made it much easier for citizen volunteers to interact with professional scientists (e.g. through online photos, videos or message boards) and to generate data (e.g. through web interfaces or mobile applications) (Goodchild, 2007; Dickinson et al., 2012).

A geographic information system (GIS) is a framework used to gather, manipulate, process, interrelate and display spatial information (Liebhold et al., 1993; Neteler et al., 2012). In today's fragmented landscapes, the spatio-temporal patterns of invasive species are influenced by a myriad of biotic and abiotic factors. GIS technologies are improving our ability to study invasive species dynamics as influenced by these heterogeneous environments, thus enhancing their invaluable monitoring and management (Hamilton et al., 2007). Habitat suitability maps identify areas where invasive species (1) may actually be present (but are yet undetected), and (2) where they may disperse to in the future, thus providing assistance for planning and prioritizing areas for surveillance (Neteler et al., 2013). Such information can also assist in determining the extent, cost and likelihood of success of a control program. Thus, predictive modelling of a species distribution represents an important tool for invasive species management (Anderson et al., 2003).

Two main approaches are used for modeling the structure and dynamics of the geographic ranges of invasive species:

1. Mechanistic niche modelling: aims to understand, using detailed biophysical approaches, the environmental requirements that make up the fundamental niche of a species (Dormann et al., 2012), wherein hypotheses about niche, dispersal, and (in some cases) biotic interactions are integrated in models
2. Correlative niche modelling: focuses on understanding conditions that allow persistence of species' populations (e.g., climate and vegetation), or on predicting the geographic distribution of the species using statistical techniques (Guisan and Thuiller, 2005; Peterson and Soberón, 2012)

Correlative approaches remain the most frequently used for exploring the determinants of the range of invasive species and their probability of occurrences due to simpler data requirements (Elith et al., 2010). The maximum entropy (MaxEnt) software package (Phillips et al., 2006) is particularly popular in species distribution/environmental niche modeling, with over 1000 applications published since 2006 (Merow et al., 2013). MaxEnt takes a list of species presence locations as input, often called presence-only data, as well as a set of environmental predictors (e.g. precipitation, temperature and land use) across a user-defined landscape that is divided into grid cells (Merow et al., 2013). Recognizing that absence data are rarely available or reliable, MaxEnt uses presence only data, a feature that is appropriate for species distribution modelling and for predicting areas of potential species occurrence (Philips and Elith, 2013). MaxEnt output is continuous, allowing for fine distinctions to be made between the levels of species establishment risk in different areas (Arnold et al., 2014). Some limitations arise when using the maximum entropy model, mainly the possibility of over-fitting[5], however, the 'regularization multiplier' parameter in the software aims to address that (Phillips and Dudík, 2008).

Now that we introduced most of the concepts that will be handled in the coming chapters, we move to our first case study which concerns an invasive insect that has wreaked havoc in invaded territories for the past decades and whose impact was and still is of global magnitude.

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[5] Overfitting occurs when a model fits the calibration data too closely (in environmental space) and therefore, fails to predict independent evaluation data accurately.

3 The invasive brown marmorated stink bug

3.1 Distribution and life history

Halyomorpha halys Stål (Hemiptera: Pentatomidae) is an invasive stink bug that originates from China, Japan and Korea, where it is not considered an important pest (Yanagi and Hagihara, 1980). Outside its native range, it was first detected in Pennsylvania, USA in the mid-1990s (Hoebeke and Carter, 2003) and has now spread to 44 other states (Stopbmsb.org, 2019). It was detected in Ontario, Canada in 2011 and has now spread to four other Canadian provinces (Fogain and Graff, 2011). Faúndez and Rider (2017) reported its presence in private homes in the city of Santiago, Chile. In Europe, *H. halys* was first detected in Zurich, Switzerland in 2007 (Wermelinger et al., 2008), and has ever since expanded its range to include several European and Asian countries, namely Italy, France, Spain, Austria, Germany, Liechtenstein, Albania, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Greece, Hungary, Malta, Poland, Romania, Georgia, Abkhazia, Russia, Serbia, Slovakia, Slovenia, Turkey, Ukraine and Kazakhstan (Gapon, 2016; Zhunisbai et al., 2019; EPPO, 2019). Adults of this species are successful hitchhikers, as they are easily transported in cargo, freight trains and in personal luggage, and have been frequently intercepted in areas not yet invaded such as New Zealand, Australia and the United Kingdom (Walker, 2009; Duthie, 2012; Cianferoni et al., 2018). Indeed, Maistrello et al. (2018) referred to it as a sneaking invasive alien pest, after numerous recordings of *H. halys* in Italy originating from various means of transportation including cars, tractors and trains. Such a capacity to go unnoticed facilitates its fast and passive diffusion, increasing the risk of establishment in diverse territories. In autumn, large aggregations of this bug move to suitable overwintering sites, mainly man-made houses and buildings to shelter from harsh winter conditions (Watanabe et al., 1994). This overwintering behavior makes *H. halys* a pervasive residential nuisance pest. This strong association between overwintering adults and urban settings is thought to reduce winter mortality and play an important role in its successful invasion (Wallner et al., 2014). Adults disperse from overwintering sites in spring in the mid-Atlantic USA and in Italy with numbers peaking in late spring (Bergh et al., 2017; Maistrello et al., 2017). Where adults go after leaving overwintering sites is still poorly understood, but it is thought that they move to nearby vegetation where they start maturation feeding (Haye et al., 2014). Female *H. halys* are synovigenic[6], with oviposition occurring once diapause-terminating cues are met and continue until late summer in Europe (Aldrich et al., 2009; Haye et al., 2014; Leskey and Nielsen, 2018). On average, 28 eggs are laid in masses, predominantly on the underside of leaves with 244 eggs deposited over the life span of a female (Nielsen and Hamilton, 2009). *Halyomorpha halys* has a capacity for bivoltinism throughout the United States and Italy, whereas the completion of one generation per year was suggested by field studies in Switzerland (Haye et al., 2014; Nielsen et al., 2016; Costi et al., 2017). After emergence, 1st instar nymphs remain aggregated around the egg mass to ingest the

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[6] Continue to produce and to mature eggs throughout their life cycle

bacterial endosymbiont *Pantoea carbekii* that is transferred to the egg chorion during oviposition (Taylor et al., 2014). Disruption of *P. carbekii* uptake results in lower survival and fecundity, longer development and aberrant nymphal behavior (Taylor, 2016). *Halyomorpha halys* undergoes five nymphal instars before reaching the adult stage, requiring on average 42 and 33 days at 25 and 30 °C respectively, to develop from egg to adult (Haye et al., 2014).

3.2 Host range and damage

Like most phytophagous pentatomids, *H. halys* is a polyphagous pest with a wide host range encompassing more than 170 plants of different families (Leskey et al., 2012a; Rice et al., 2014). This insect feeds on ornamental shrubs, hardwood trees, row crops and cultivated fruits and vegetable crops of high economic importance such as apple, pear, peach, hazelnut, cherry, grape, sweet corn, pepper and tomato (Smith et al., 2014; Bergmann et al., 2016). All life stages of *H. halys* are present throughout the growing season on various wild and cultivated host plants and readily move among species based on crop phenology as they seek suitable fruiting structures (Martinson et al., 2015; Acebes-Doria et al., 2017). Both nymphs and adults of this pentatomid are ferocious feeders. The severity of *H. halys* feeding damage is dependent on the insect life stage involved, the density of individuals and the phenology of host plants (Cissel et al., 2015; Acebes-Doria et al., 2016). After inserting their stylets in plant parts, *H. halys* inject watery saliva into the plant tissue that breaks down cells and enables consumption of liquified content (Peiffer and Felton, 2014). This behavior results in dry corky tissue below the surface of the feeding site and causes discoloration, depression and distortion of fruiting structures (Nielsen and Hamilton, 2009; Smith et al., 2014). *Halyomorpha halys* feeding on hazelnuts results in shells without a kernel or blank nuts (Hedstrom et al., 2014). The symptoms caused by *H. halys* feeding renders crops unmarketable, which causes severe economic losses for the ornamental and agricultural sector. With apple injury reaching 90% during the 2010 outbreak in North America, US$ 37 million worth of losses were sustained in the mid-Atlantic region on this crop alone (Leskey et al., 2012a). Although economic losses in hazelnuts have yet to be reported in the USA, this industry is expected to suffer US$ 200 million worth of losses since *H. halys* can feed on kernels throughout the entire period of their development and shell thickness is no deterring factor (Hedstrom et al., 2014; CABI, 2019). The National Agricultural Statistics Service (NASS, 2013) estimates that over US$ 40 billion worth of crop losses are expected in the United States. In Europe, some of the most severe agricultural and nuisance pest problems have been recorded in Italy (Bariselli et al., 2016). In the central Italian region of Emilia-Romagna, the estimated damage on pears and peaches amounted to € 120 and € 50 million respectively; while losses in Northern Italy are expected to reach € 350 million on pears, peaches and nectarines (CSO, 2019).

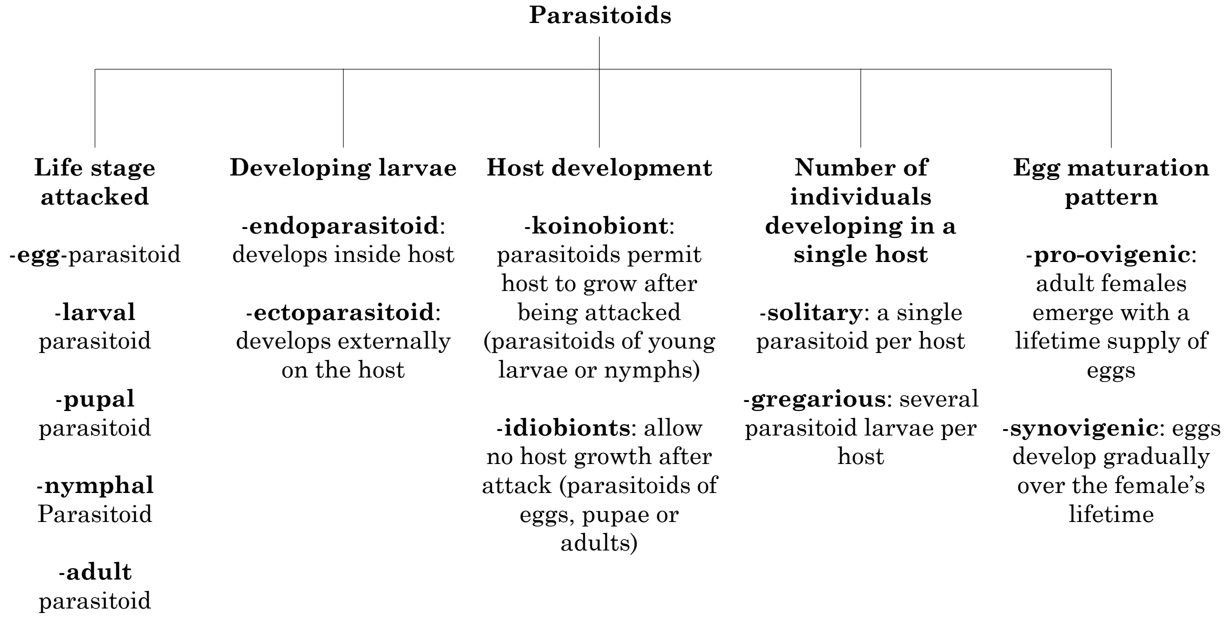
3.3 Management techniques of *Halyomorpha halys*

Controlling high-density populations of this pest have mostly relied on the use of broad-spectrum insecticides, which were found to be only partially effective (Kuhar and Kamminga, 2017). Growers in the mid-Atlantic region increased the number of insecticide applications nearly 4-fold and reduced the interval between treatments during outbreak seasons (Leskey et al., 2012b). The discovery of *H. halys* aggregation pheromone and its integration in different trap designs opened the door for a more effective monitoring and for designing 'attract and kill' techniques (Khrimian et al., 2014; Morrison et al., 2015). The latter involves baiting specific trees with the *H. halys* male secreted aggregation pheromone and treating them regularly with insecticides, a method that successfully reduced low to moderate *H. halys* populations and considerably restricted the pesticide-treated area, thus limiting non-target effects (Morrison et al., 2018). Suckling et al. (2019) designed the novel 'Nazgûl trap' that attracts, traps and kills 3.5-fold more *H. halys* adults and nymphs compared with the equally-baited sticky traps. In an attempt to target *H. halys* adults dispersing to overwintering sites in fall, Bergh and Quinn (2018) showed that installing framed panels of long-lasting insecticide nets was promising in mitigating homeowner nuisance problems. Laboratory and field trials with a series of organically approved insecticides such as neem oil, essential oils and pyrethrins were only slightly effective against *H. halys* (Bergmann and Raupp, 2014) leaving organic growers in desperate need for efficient management tools. One thing for certain is that *H. halys* management cannot depend on one single strategy, it should instead encompass a series of cultural, physical, chemical and biological tactics. In the coming section, I expand further on the topic of biological control, and specifically the biocontrol of *H. halys*.

4 Biological control

Various definitions of biological control may exist and can be withdrawn from a scientific natural phenomenon or from an applied sense. The former denotes one of the major ecological forces of nature, which is the regulation of plant and animal populations by natural enemies (Debach and Rosen, 1991). While the applied definition revolves around 'the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it otherwise would be' (Eilenberg et al., 2001). The major uses of this technique are: (1) biological control of plant pathogens using antagonistic microorganisms and induced plant resistance, (2) biological control of weeds using herbivores and pathogens and (3) biological control of invertebrate pests using predators, parasitoids and pathogens (Eilenberg et al., 2001). As natural enemies are the fundamental pillars of biological control, for insect pests, these organisms can be microbials (nematodes, fungi, bacteria) or macrobials (predators, parasitoids).

Predators are species that have a life stage that kills and eats other living animals for development, sustenance and reproduction; they are typically larger than their prey and require more than one prey to complete development (Van Driesche and Hoddle, 2009). Parasitoids are intermediate life forms between predators and parasites. Gauld et al. (1988) defined them as: "insects whose larvae develop by feeding on or within an arthropod host, and this host is almost always killed by the developing parasitoid larva". Parasitoids have been the most common type of natural enemies introduced against insect pests (Hall and Ehler, 1979), and they can be classified according to several life history traits, that are summarized in Figure 1.1.



Parasitoids

Life stage attacked
- -**egg**-parasitoid
- -**larval** parasitoid
- -**pupal** parasitoid
- -**nymphal** Parasitoid
- -**adult** parasitoid

Developing larvae
- -**endoparasitoid**: develops inside host
- -**ectoparasitoid**: develops externally on the host

Host development
- -**koinobiont**: parasitoids permit host to grow after being attacked (parasitoids of young larvae or nymphs)
- -**idiobionts**: allow no host growth after attack (parasitoids of eggs, pupae or adults)

Number of individuals developing in a single host
- -**solitary**: a single parasitoid per host
- -**gregarious**: several parasitoid larvae per host

Egg maturation pattern
- -**pro-ovigenic**: adult females emerge with a lifetime supply of eggs
- -**synovigenic**: eggs develop gradually over the female's lifetime

Figure 1.1. Parasitoid classification according to their biological traits and to the nature of their interaction with the host.

There are numerous aspects of parasitoid biology that are crucial for their survival and thus are detrimental for the success of any biological control program:

1. Host finding
2. Host recognition and assessment
3. Overcoming and defeating host defenses
4. Regulation of host physiology
5. Patch time allocation

These steps have been intensely studied during the past decades and a rather clear process that leads a parasitoid to its host is now better understood both at the chemical and at the behavioral levels (Vinson, 1984; Vet and Dicke, 1992; Hilker and

Fatouros, 2015). Long distance orientation towards host-infested patches often occurs when parasitoids are in flight and are responding to volatile cues emitted from host-infested plants as a result of herbivory. This indirect defense phenomenon described by Dicke (2009) as a ‘cry for help’, may maximize plant’s Darwinian fitness by attracting the host’s enemies. First evidence of plants recruiting natural enemies appeared almost three decades ago (Dicke and Sabelis, 1988; Dicke et al., 1990), and numerous follow-up studies provided a wealth of information on this phenomenon. Feeding by the beet armyworm larva induced corn plants to release volatiles that are utilized by the larval parasitoid *Cotesia marginiventris* (Cresson) to find their host (Turlings et al., 1990). Colazza et al. (2004) showed that volatiles induced by *Nezara viridula* L. herbivory on faba bean leaves, attracted the species-specific egg parasitoid *Trissolcus basalis* (Wollaston). *Halyomorpha halys* feeding and oviposition on faba bean renders the plants more attractive to generalist parasitoids (Rondoni et al., 2017). Once on a host-infested plant, parasitoids begin orienting visually, by gustation or olfaction over shorter distances, often by walking and using host associated cues referred to as kairomones, to help them track down their target. These kairomones may be emitted from frass, host body parts (cast skin), host secretions (pheromones, defensive compounds, silk) that are found on plant surfaces and alter parasitoid foraging by inducing arrestment, trail following and/or intensified local search (Van Driesche and Hoddle, 2009). Extensive studies on pentatomid trophic interactions found that chemical traces are deposited on leaf surfaces after stink bug walking and are then picked up by their respective foraging parasitoids (Colazza et al., 1999; Colazza et al., 2007; Peri et al., 2016). When encountered, the host's quality is assessed by the foraging parasitoid based on internal and external mechanical and chemical cues. Indicators such as host size, position, shape, chemistry and location in the habitat suggests whether the confronted life stage is an appropriate target. For instance, *Telenomus heliothidis* Ashmead (Hymenoptera: Scelionidae) performs antennal drumming and ovipositor probing to detect external and internal chemicals associated with its host eggs (Strand and Vinson, 1982). The same authors found that glass beads or non-host eggs coated with an extract of the host's eggs stimulated oviposition attempts by the parasitoid. The use of the oothecal glue of the brown banded cockroach by its parasitoid *Comperia merceti* Compere (Hymenoptera: Encyrtidae) is another example of host assessment using mechanical and chemical host associated cues.

4.1 Types of biological control

Before listing the various types of such an approach, it is important to distinguish methods that do not act through populations of natural enemies and do not fall into the category of biological control. Although the use of transgenic pest-resistant plants, the release of sterile males to suppress pest populations, the use of pheromones to disrupt pest mating are all biologically based non-pesticidal methods, they are not biological control. However, these methods do minimize the use of toxic pesticides, which enhances biological control by conserving existing natural enemies (Van Driesche and Hoddle, 2009).

The extent to which natural enemies suppress insect pests is greatly influenced by several farming practices and habitat conditions. **Conservation biological control** is the study and manipulation of such influences (Van Driesche and Hoddle, 2009). This approach aims to protect indigenous biological control agents and provide resources so that they can be more effective (Van Driesche and Bellows, 1996). Györfi (1961) demonstrated this for oak forests in Finland with and without ground vegetation, where outbreaks of the gypsy moth *L. dispar* were far more frequent in forests with a clean forest floor. Györfi studied the corresponding parasitoid complex and found that several alternative caterpillar hosts are available on the ground vegetation and are necessary to sustain the parasitoids in years of gypsy moth scarcity. In commercial agriculture, manipulating crop microclimate, creating overwintering refuges and providing essential food resources are common measures employed to enhance the abundance and activity of resident natural enemies (Wäckers and Steppuhn, 2003; Winkler et al., 2009).

When a biological control agent is released with the expectation that it will multiply and control the pest for an extended period, but not permanently, this is considered **inoculation biological control** (Van Driesche and Hoddle, 2009). It is a common practice in glasshouses, where relatively large numbers of mass reared biocontrol agents are released for the control of whiteflies, leafminers, thrips, aphids and mites and at the end of the season the glasshouse is emptied with no permanent establishment of the introduced organism (van Lenteren, 2000).

Inundation biological control involves the mass production and release of large numbers of the control agent (Bale et al., 2008). The aim of this approach is to create a massive ratio in favor of the natural enemy, which produces a rapid reduction or a local extinction of the pest, somewhat comparable to the use of pesticides (van Lenteren and Bueno, 2003). One example is the mass release of the braconid parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) against the sugarcane borer in several countries of Latin America (van Lenteren and Bueno, 2003). Both **inoculation** and **inundation** can be considered sub-categories of the **augmentation** approach, which usually requires periodical introductions and commercial production of the released agents (van Lenteren, 2000).

Classical biological control is considered by some the only long-term effective method to control non-native pests over large areas. Indeed, it is defined as '*the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control*' (Eilenberg et al., 2001). Because of the early development and use of this strategy since the 1800s, it has been called 'classical' (Greathead, 1994). Originally, classical biological control was focused on managing exotic pests, but has also been successfully applied against native ones (Carruthers and Onsager, 1993), in such a case it is referred to as **novel-association biological control** (Hokkanen and Pimentel, 1989).

4.2 Peaks and valleys of classical biological control

The first major success of a classical biological control program dates back to the 1880s when the ladybird *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae) and the parasitoid *Cryptochaetum iceryae* Williston (Diptera: Cryptochetidae) were imported from Australia for the control of the invasive cottony-cushion scale *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) in the citrus crops of California (DeBach, 1964). Oftentimes, the outcome of a successful biological control program can be dramatic, as invasive pests that once threatened entire regional economies can be reduced to a fraction of their abundance and sustained at low levels without additional costs or input (Messing and Wright, 2006). Another early example is that of the coconut moth *Levuana irridescens* Bethune-Baker (Lepidoptera: Zygaenidae) that attacked coconut plantations in Fiji. A tachinid parasitoid *Ptychomyia remota* Aldrich (Diptera: Tachinidae) was sourced from Malaysia for its control and in 2 years it successfully spread across the whole island and provided complete suppression of this pest (DeBach and Rosen, 1991). One other example is the continental-scale project that involved combatting the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) that first invaded Africa then Southeast Asia, threatening what is considered a staple food by hundreds of millions of people (Norgaard, 1988). The introduction of the carefully selected host-specific and environmentally-adaptable parasitoid *Anagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) that was sourced from South America led to over 50% yield recovery and long-term benefits reaching more than US$ 20.2 billion as well as the likely avoidance of widespread famine, with no measurable negative side effects (Neuenschwander et al., 1989; Zeddies et al., 2001; Wyckhuys et al., 2018). The latter project earned the 1995 World Food Prize for saving millions of lives and billions of dollars across the African continent.

If one was to compare on the basis of specificity, even the most selective insecticide is likely to kill and harm many species of arthropods, herbivores and natural enemies alike, whereas introduced biocontrol agents usually have a narrower host range, killing only one, or a few related species (Bale et al., 2008). Furthermore, the eradication effect of insecticides is strictly limited to the spatio-temporal area within which the chemical is applied, whereas pest regulation by biocontrol agents has an area-wide and long-term effect. As pest control must be cost effective in relation to the crop value, Bale et al. (2008) performed a comparison of costs associated with the development and employment of chemical and biological control. They found that in both respects biological control agents are more cost effective than pesticides, with the development cost alone of an insecticidal molecule amounting to US$ 180 million compared with US$ 2 million for a biocontrol agent.

As problems with insecticides became apparent, namely following the release of Rachel Carson's 1962 *Silent Spring*, biological control was met with enthusiasm and was regarded as an ecologically benign approach for substituting insecticides. However, valid concerns over the ecological risks associated with classical biological control were raised by Howarth (1983; 1991), regarding certain historical malpractices, with evidence of a substantial number of introduced biocontrol agents also attacking non-target species. Hawkins and Marino (1997) documented that across North America, 16% of 313 parasitoid species introduced against holometabolous[7] pests attacked other native species. A tachinid fly *Compsilura concinnata* Meigen (Diptera: Tachinidae) that was introduced in 1906 to North America from Europe to control the gypsy moth, ended up attacking over 100 native lepidopteran species and is implicated in the decline of giant silk moths in the northeastern USA (Boettner et al., 2000; Messing and Wright, 2006). In Hawaii and French Polynesia, the introduction of the predaceous rosy wolf snail to control the giant African snail was later recorded to have strongly impacted endemic tree snails (Messing and Wright, 2006). Many of these poorly planned introductions have tainted the safety record of the more recent and meticulously calculated initiatives. The introduction of a wasp in the genus *Eupelmus* from Guatemala to Hawaii to help control the pepper weevil in the 1930s was carried out with almost certainly no consideration to non-target effects. According to Thompson (2014) it is difficult to predict the reasoning behind such a project since the wasp had no significant effect on the target pest and it turned out to have a huge host range, including other biocontrol agents.

As a result, public funding decreased, and the entire biological control practice went through trying yet necessary reform (Strong and Pemberton, 2000; Messing and Brodeur, 2018; Wyckhuys et al., 2018). It is interesting to note that most of the projects involving introduced biocontrol agents that had severe non-target effects

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[7] Insects that undergo complete metamorphosis

were carried out prior to the establishment of government oversight and strict regulations, afterwards the frequency of non-target impacts has declined dramatically (Messing and Wright, 2006). For instance, on the island of Kauai, there were no records of non-target effects for any parasitoid species released after 1945 (Henneman and Memmot, 2001). Certain species that had a broader host range and were once readily released, would not even be considered under current protocols. Putting the frequency of non-target effects aside, their strength is evidently more difficult to assess. As records of a biocontrol agent attacking an individual is not hard to obtain, but its impact on non-target populations is much harder to measure. Such data have been labelled as "not yet available" or "requires years of painstaking field work", rendering an assessment of impacts on non-target species populations almost impossible (Stiling and Simberloff, 2000).

Therefore, extensive risk assessment protocols need to be fulfilled prior to the deliberate release of any exotic organism into a new environment. Biocontrol practitioners must first ascertain that the harm caused by the target pest warrants biological control and whether this approach is the best means for efficient management (Bale et al., 2008). To that end, surveys in the invasive pest's native range help identify potential natural enemies that are later kept in quarantine facilities to test and ensure their safety for release. Specifically, potential non-target effects encompassed by a biocontrol agent must be fully addressed and anticipated. Therefore, prospective agents undergo studies to assess host-specificity, fundamental and ecological host range, host finding, host selection behavior, dispersal potential and other life cycle parameters. Bale et al. (2008) identified several aspects linked to successful biocontrol agents by evaluating over 100 years of history in agent selection database. The authors concluded that a promising agent must exhibit a strong searching behavior, a preference for the target host, faster development, greater fecundity as well as the completion of more generations per year than the target and must be able to survive at low pest density.

4.3 Biological control of *Halyomorpha halys*

One of the most widely accepted explanations for the success and dominance of invasive species is the Enemy Release Hypothesis (ERH). It predicts that an exotic species will increase its distribution and abundance in an invaded habitat due to the scarcity of natural enemies in the new range (Keane and Crawley, 2002). A variation of the ERH is the Evolution of Increased Competitive Ability hypothesis, where resources that would otherwise be used for defense in the native range, are allocated for reproduction and growth in the invaded environment (Blossey and Nötzold, 1995). *Halyomorpha halys* is assumed to have escaped enemy regulation following invasion of multiple habitats in the US and Europe, as supported by natural enemies' surveys that detected low rates of attack (Jones et al., 2014; Cornelius et al., 2016; Abram et al., 2017).

Gouli et al. (2012) and Parker et al. (2015) evaluated isolates of *Beauveria bassiana* and *Metarhizium anisopliae*, two common entomopathogenic fungi, and found that wettable powder and emulsifiable formulations induced high levels of mortality in *H. halys*. Several generalist predators were recorded sucking or chewing on *H. halys* eggs in field and laboratory trials. The rate of egg feeding varied from 4% up to the consumption of 12.8% of eggs after 48 hours exposure (Ogburn et al., 2016). Opportunistic predators such as Orthopterans are implicated in the feeding on *H. halys* sentinel egg masses (Morrison et al., 2016). Other species of ground beetles, earwigs, jumping spiders and crickets were also found to be reasonably efficient in feeding on *H. halys* egg masses (Rice et al., 2014). Laboratory experiments revealed that the 1st and 2nd *H. halys* nymphal instars were respectively preyed upon by species belonging to the families Reduviidae and Nabidae (Pote and Nielsen, 2017). Vertebrate predators also appear to utilize *H. halys* as food resource, as guano samples from the big brown bat, *Eptesicus fuscus* (Chiroptera: Vespertilionidae) tested positive for *H. halys* DNA (Valentin et al., 2017).

Koppel et al. (2009) conducted field surveys of Pentatomid natural enemies in North America and found that Hymenopteran parasitoids are the main antagonists of endemic stink bug species. Further field studies in its native range, as well as in North America and Europe revealed that the most commonly recovered parasitoids from *H. halys* egg masses belonged to the Scelionidae, Eupelmidae and Encyrtidae families (Yang et al., 2009; Lee et al., 2013; Abram et al., 2017). Endemic species of the genera *Anastatus*, *Telenomus* and *Trissolcus* were the most recovered in the field, providing low and varying rates of parasitization (Haye et al., 2015; Herlihy et al., 2016; Ogburn et al., 2016). Moreover, there exists a physiological unsuitability between the invasive *H. halys* and native egg-parasitoids, such that successful parasitization is hampered, causing *H. halys* eggs to act as an evolutionary trap that reduces the indigenous wasp populations and potentially increases that of the native pentatomids (Abram et al., 2014; Kaser et al., 2018). Hence, classical biological control is warranted, employing the most prominent egg-parasitoid of *H. halys* in its native range, the samurai wasp, *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae). Adventive populations of *T. japonicus* were recorded in several countries (Figure 1.2) where *H. halys* has invaded and is expected to appear in most other regions invaded by *H. halys* (Talamas et al., 2015; Stahl et al., 2018; Sabbatini Peverieri et al., 2018; Abram et al., 2019).

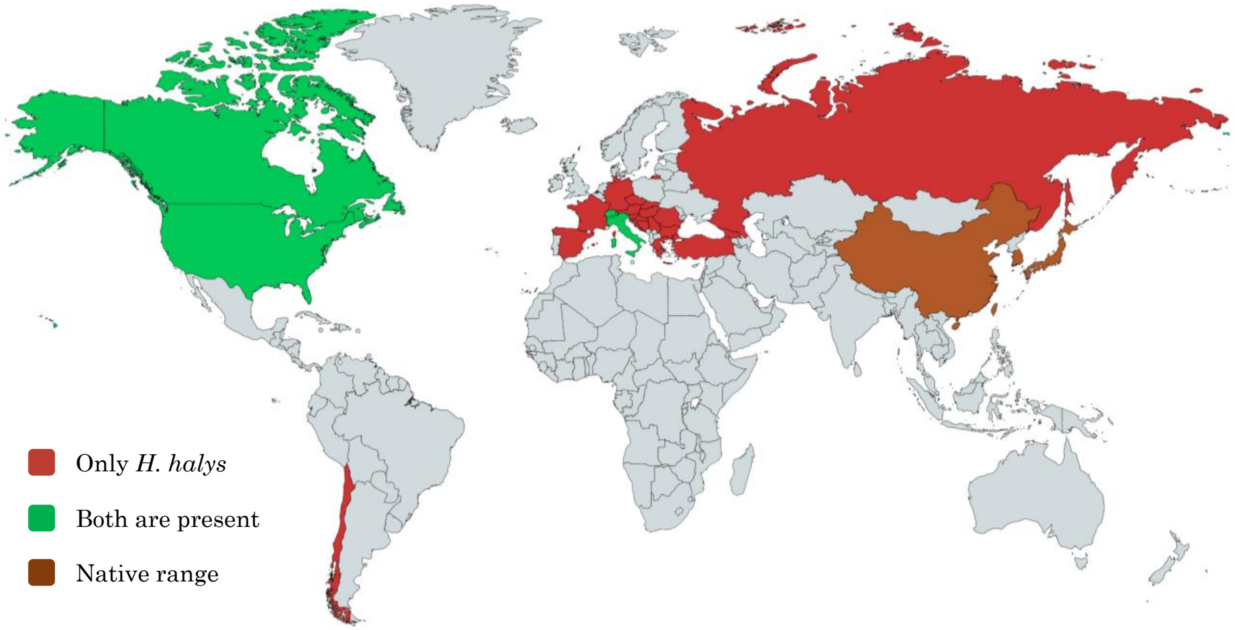

Figure 1.2. A world map showcasing the native range of *Halyomorpha halys* and *Trissolcus japonicus* as well as the regions outside this range where either one or both have established populations.

Thus, farmers, biocontrol practitioners and legislators find themselves in an unorthodox position. The standard approach that would have led to a decision on whether an exotic biocontrol agent is safe for introduction must now assess whether this same agent is safe for redistribution or augmentation. The process must be conducive to a decision that carefully measures the risks and benefits associated with intentionally redistributing *T. japonicus* or allowing it to disperse unassisted. Intending to provide information that would facilitate this decision, we assessed the short-range foraging behavior of *T. japonicus*, a behavior equally important to suitability and host specificity tests (Duan and Messing, 1997). We investigated the chemical ecology of this biocontrol agent with respect to its main host *H. halys* and the predatory spined soldier bug *Podisus maculiventris* Say (Heteroptera: Asopinae). The characteristics of this biocontrol agent and the results of our investigations are elucidated in **chapter 4.**

5 Another invasion nightmare

The following section introduces an insect that very recently invaded northeastern USA, an incident that fell as a stark reminder that under our current globalization trends, the rate of these introductions is only set to increase. The spotted lanternfly, *Lycorma delicatula* White (Hemiptera: Fulgoridae) is a plant hopper native to China, Taiwan and Vietnam (Liu, 1939). This insect gained increased interest in the scientific community shortly after it was detected in the neighboring South Korea in 2004, where it exhibited a rapid increase in geographical distribution and abundance (Kim and Kim, 2005; Jung et al., 2017). According to Park et al. (2012; 2013) who characterized the genetic structure of *L. delicatula* in South Korea, the long-distance movement and expansion of this pest was facilitated by human activity, particularly ground transport. Notwithstanding geographical constraints, *L. delicatula* made its way to the United States, with the first specimen recorded in Berks County, Pennsylvania, in 2014 (Barringer et al., 2015). Despite the eradication efforts by the Pennsylvania Department of Agriculture, this alien insect still managed to expand its range and spread to the neighboring states of Delaware, New York, New Jersey, West Virginia, Maryland, Connecticut, Virginia, Massachusetts and North Carolina (Northeastern IPM, 2020).

5.1 The spotted lanternfly in a nutshell

Lycorma delicatula is a polyphagous phloem feeder that goes through four nymphal instars before reaching the adult stage, all of which are voracious feeders that infest over 70 host plant species, including economically important ones such as grapes, peaches, apples and kiwis (Han et al., 2008; Park et al., 2009; Dara et al., 2015). Damage is inflicted on plants through the depletion of resources and the secretion of large amounts of honeydew, resulting in reduced crop production and sooty mold infestation that renders produce unmarketable (Ding et al., 2006; Leach et al., 2019). Potentially massive risks are posed by this invasion in North America, as the agricultural, ornamental and lumber industries are threatened by future outbreaks (Pennsylvania Department of Agriculture, 2018).

One generation per year is completed by this pest in its native range and in northeastern USA, however, multi-voltinism is a possibility in warmer climatic zones (Lee et al., 2014; Lee et al., 2019). This insect overwinters as eggs that are laid on animate and inanimate objects alike, starting from September until early November and first instars start emerging as early as April in the invaded range (Lee et al., 2014; Dara et al., 2015). In South Korea, around 3 egg masses are found per tree (Lee et al. 2014), in contrast, 197 egg masses were recorded on a single *Ailanthus altissima* tree in the invaded Pennsylvania (Dara et al., 2015). Adult females lay eggs that are clustered together in rows, creating masses of 30-50 eggs that are then covered in a foamy deposit to form an ootheca[8] (Park et al., 2009). Climatic models predicted the global distribution of *L. delicatula*, revealing that it has a high potential of establishment in the USA, Brazil, Mexico, Congo and Japan, whereas lower densities may survive in the UK, Belgium, Switzerland, Spain and Italy (Jung et al., 2017). The authors also found that this pest may establish in southeast Asia, including eastern Australia and New Zealand.

As *L. delicatula* develop from early nymphal stages to adults, their host range narrows, and they exhibit a strong preference towards *A. altissima* in both their native and invaded ranges (Sanyang, 1992; Barringer et al., 2015). Such observations will help improve *L. delicatula* monitoring that currently solely relies on visual inspection, as *A. altissima* volatiles could lead to the development of lures for behaviorally compatible trap designs (Lee and Park, 2013; Lee et al., 2019).

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[8] Egg case that protects eggs from desiccation and from natural enemies

5.2 *Lycorma delicatula* management

Contrary to *H. halys*, a wide range of insecticides seem to inflict very high levels of mortality in *L. delicatula*, with no recorded knockdown and recovery effects. In laboratory trials, several insecticides in the classes of organophosphates, carbamates, pyrethroids and neonicotinoids resulted in 100% mortality in 1st and 2nd instar nymphs, when sprayed directly on the insect at the recommended rate (Shin et al., 2010). In another study, Kim et al. (2010) found that under laboratory conditions, chlorpyrifos, etofenprox, dinotefuran, imidacloprid also resulted in 100% mortality of 3rd instars and adults 48 hours after application. The same group tested the same insecticides in the field and found that they were effective against *L. delicatula* nymphs and adults and that residual activity lasted 14 days after initial application. Most insecticides that were tested against *L. delicatula* eggs had an ovicidal effect that resulted in less than 40% mortality, except for chlorpyrifos that caused 100% mortality at the recommended rate of application (Shin et al., 2010). Environmentally Friendly Agricultural Material (EFAM) tested in South Korea yielded varying mortality rates that were lower than those caused by conventional pesticides in both nymphs and adults (Choi et al., 2012a). Leach et al. (2019) also found that conventional insecticides were effective in managing *L. delicatula* adults and nymphs in semi-field and field bioassays, however, none of the tested organically approved substances provided an effective control.

Although a first response employing chemicals might be effective on the short term, but the risk of acquisition of resistance, non-target effects and a whole other range of environmental hazards render this approach unsustainable. Moreover, anticipated difficulty among organic farmers calls for the need to develop other cultural and biological measures to enhance the long-term management of this pest. Studies are underway to select certain essential oils that provide repellency or attraction for *L. delicatula* to incorporate them in a push-pull management strategy or an attract-and-kill system (Yoon et al., 2011; Lee et al., 2019).

Natural enemies are promising candidates for a long-term management strategy (Yang et al., 2015; Liu and Mottern, 2017). Choi et al. (2012b) tested predatory insects against *L. delicatula* in South Korea and found that several species of assassin bugs (Hemiptera: Reduviidae) were able to kill and consume 8-10 nymphal instars per day. Barringer and Smyers (2016) also observed that wheel bugs and predatory stink bugs are feeding on *L. delicatula* in Pennsylvania. In China, a nymphal parasitoid *Dryinus browni* Ashmead (Hymenoptera: Dryinidae) was identified as an efficient biocontrol agent of *L. delicatula*, providing 12.5-43.5% parasitism (Yan et al., 2017). *Ooencyrtus kuvanae* Howard (Hymenoptera: Encyrtidae) that was introduced to the US in 1908 for gypsy moth biocontrol, was recovered from *L. delicatula* egg masses in Pennsylvania (Liu and Mottern, 2017). Whether this agent will complete development for several generations on this new invasive pest is unknown, especially with the presence of gypsy moth populations in nature. Among egg-parasitoids, *Anastatus orientalis* Yang and Choi (Hymenoptera: Eupelmidae) is currently thought to be the major antagonist of *L. delicatula* in its native range (Lee et al., 2019). It is a solitary endoparasitoid of *L. delicatula* eggs that provides varying degrees of successful parasitization ranging from 30-70% in China and has a female biased sex ratio (Lee et al., 2019). Hou (2013) found that this wasp completes development in 23-33 days at 20-24 °C - much faster than its host - and that *A. orientalis* females produced an average of 232 eggs throughout their lifetime. For these reasons, *A. orientalis* was sourced from the area of origin of the spotted lanternfly, was brought back to quarantine facilities in the USA and is currently being considered for classical biological control in the invaded range.

6 Thesis outline

As per the literature presented in the sections above and the inherent gaps in the monitoring of invasive insect pests and risk assessment of biological control agents, my thesis attempts to address and shed new light on these topics of imminent concern. **Chapter 2** and **chapter 3** introduce a citizen science approach that was designed to monitor and model the distribution of the invasive *H. halys* in Trentino, Italy. BugMap, the adopted mobile application proved instrumental in assisting farmers, scientists and technicians in coordinating and improving management efforts in this North Italian region as well as in raising public awareness and scientific literacy. The outcome of these two chapters underlines the importance of coupling citizen science and traditional monitoring to uncover the current and potential spread of this serious invader. **Chapter 4** expands the knowledge on the chemical ecology of *T. japonicus*, the most promising candidate for the classical biological control of *H. halys*. It describes a comparative study that disentangles the foraging behavior of this agent in response to cues deposited by its main host and by a non-target beneficial stink bug. In this same chapter, the underlying chemical signals that induce this specific foraging behavior are also unearthed and discussed with regard to their significance for host preference and classical biological control. An assessment of the host-parasitoid interactions between the invasive *L. delicatula* and *A. orientalis* are showcased in **chapter 5**. In this latter chapter the boundaries were pushed to explore the evolutionary arms race between the host and its parasitoid, mainly probing the function of *L. delicatula* footprints and ootheca with respect to *A. orientalis* foraging and host acceptance. In **chapter 6**, three other satellite projects that I was involved in are briefly summarized. Finally, A general conclusion of key findings is presented in **chapter 7** along with suggestions for prospective and much needed research that would further advance and materialize the findings of this dissertation.

References

Abram, P.K., Gariepy, T.D., Boivin, G. and Brodeur, J., 2014. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. *Biological Invasions*, *16*(7), pp.1387-1395. DOI:10.1007/s10530-013-0576-y

Abram, P.K., Hoelmer, K.A., Acebes-Doria, A., Andrews, H., Beers, E.H., Bergh, J.C., Bessin, R., Biddinger, D., Botch, P., Buffington, M.L. and Cornelius, M.L., 2017. Indigenous arthropod natural enemies of the invasive brown marmorated stink bug in North America and Europe. *Journal of Pest Science*, *90*(4), pp.1009-1020. DOI: 10.1007/s10340-017-0891-7

Abram, P.K., Talamas, E.J., Acheampong, S., Mason, P.G. and Gariepy, T.D., 2019. First detection of the samurai wasp, Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae), in *Canada. Journal of Hymenoptera Research*, *68*, p.29. DOI: 10.3897/jhr.68.32203

Acebes-Doria, A.L., Leskey, T.C. and Bergh, J.C., 2016. Host plant effects on Halyomorpha halys (Hemiptera: Pentatomidae) nymphal development and survivorship. *Environmental Entomology*, *45*(3), pp.663-670. DOI: 10.1093/ee/nvw018

Acebes-Doria, A.L., Leskey, T.C. and Bergh, J.C., 2017. Temporal and directional patterns of nymphal Halyomorpha halys (Hemiptera: Pentatomidae) movement on the trunk of selected wild and fruit tree hosts in the mid-Atlantic region. *Environmental Entomology*, *46*(2), pp.258-267. DOI: 10.1093/ee/nvw164

Aldrich, J.R., Khrimian, A., Chen, X. and Camp, M.J., 2009. Semiochemically based monitoring of the invasion of the brown marmorated stink bug and unexpected attraction of the native green stink bug (Heteroptera: Pentatomidae) in Maryland. *Florida Entomologist*, *92*(3), pp.483-491. DOI: 10.1653/024.092.0310

Anderson, R.P., Lew, D. and Peterson, A.T., 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. *Ecological Modelling*, *162*(3), pp.211-232. DOI: 10.1016/S0304-3800(02)00349-6

Arnold, J.D., Brewer, S.C. and Dennison, P.E. 2014. Modeling Climate-Fire Connections within the Great Basin and Upper Colorado River Basin, Western United States. *Fire Ecology*, *10*(2), pp.64-75. DOI: 10.4996/fireecology.1002064

Bale, J.S., Van Lenteren, J.C. and Bigler, F., 2008. Biological control and sustainable food production. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1492), pp.761-776. DOI: 10.1098%2Frstb.2007.2182

Bariselli, M., Bugiani, R. and Maistrello, L. 2016. Distribution and damage caused by Halyomorpha halys in Italy. *EPPO Bulletin*, *46*(2), pp.332-334. DOI: 10.1111/epp.12289

Barlow, N.D. and Goldson, S.L., 2002. Alien invertebrates in New Zealand. *Biological Invasions*. *CRC*, *Boca Raton*, pp.195-216.

Barrett, N.S., Edgar, G.J. and Morton, A.J., 2002. Monitoring of Tasmania Inshore Reef Ecosystems. An assessment of the potential for volunteer monitoring programs and summary of change within the Maria Island Marine Reserve from 1992-2002. http://www.tafi.org.au/index.php/site/publications/

Barringer, L.E. and Smyers, E., 2016. Predation of the spotted lanternfly, Lycorma delicatula (White) (Hemiptera: Fulgoridae) by two native Hemiptera. *Entomological News*, *126*(1), pp.71-73. DOI: 10.3157/021.126.0109

Barringer, L.E., Donovall, L.R., Spichiger, S.E., Lynch, D. and Henry, D., 2015. The first new world record of Lycorma delicatula (Insecta: Hemiptera: Fulgoridae). *Entomological News*, *125*(1), pp.20-23. DOI: 10.3157%2F021.125.0105

Bell, J.L. and Whitmore, R.C., 2000. Bird nesting ecology in a forest defoliated by gypsy moths. *The Wilson Journal of Ornithology*, *112*(4), pp.524-531. https://www.jstor.org/stable/4164273

Bergh, J.C. and Quinn, N.F., 2018. Can the dispersal behavior of Halyomorpha halys (Hemiptera: Pentatomidae) inform the use of insecticide-treated netting to mitigate homeowner issues from its fall invasion?. *Environmental Entomology*, *47*(6), pp.1501-1508. DOI: 10.1093/ee/nvy120

Bergh, J.C., Morrison III, W.R., Joseph, S.V. and Leskey, T.C., 2017. Characterizing spring emergence of adult Halyomorpha halys using experimental overwintering shelters and commercial pheromone traps. *Entomologia Experimentalis et Applicata*, *162*(3), pp.336-345. DOI: 10.1111/eea.12539

Bergmann, E.J. and Raupp, M.J., 2014. Efficacies of common ready to use insecticides against Halyomorpha halys (Hemiptera: Pentatomidae). *Florida Entomologist*, *97*(2), pp.791-800. DOI: 10.1653/024.097.0262

Bergmann, E.J., Venugopal, P.D., Martinson, H.M., Raupp, M.J. and Shrewsbury, P.M., 2016. Host plant use by the invasive Halyomorpha halys (Stål) on woody ornamental trees and shrubs. *PloS one*, *11*(2). DOI: 10.1371/journal.pone.0149975

Bertolino, S. and Genovesi, P., 2003. Spread and attempted eradication of the grey squirrel (Sciurus carolinensis) in Italy, and consequences for the red squirrel (Sciurus vulgaris) in Eurasia. *Biological Conservation*, *109*(3), pp.351-358. DOI: 10.1016/S0006-3207(02)00161-1

Blossey, B. and Notzold, R., 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology*, *83*(5), pp.887-889.

Boettner, G.H., Elkinton, J.S. and Boettner, C.J. 2000. Effects of a biological control introduction on three nontarget native species of saturniid moths. *Conservation Biology*, *14*(6), pp.1798-1806. DOI: 10.1111/j.1523-1739.2000.99193.x

Bonney, R., Cooper, C.B., Dickinson, J., Kelling, S., Phillips, T., Rosenberg, K.V. and Shirk, J., 2009. Citizen science: a developing tool for expanding science knowledge and scientific literacy. *BioScience*, *59*(11), pp.977-984. DOI: 10.1525/bio.2009.59.11.9

Bonney, R., Phillips, T.B., Ballard, H.L. and Enck, J.W., 2016. Can citizen science enhance public understanding of science?. *Public Understanding of Science*, *25*(1), pp.2-16. DOI: 10.1177%2F0963662515607406

Brandon, A., Spyreas, G., Molano-Flores, B., Carroll, C. and Ellis, J., 2003. Can volunteers provide reliable data for forest vegetation surveys?. *Natural Areas Journal*, *23*(3), pp.254-262.

Bray, G.S. and Schramm Jr, H.L., 2001. Evaluation of a statewide volunteer angler diary program for use as a fishery assessment tool. *North American Journal of Fisheries Management*, *21*(3), pp.606-615. DOI: 10.1577/1548-8675(2001)021%3C0606:EOASVA%3E2.0.CO;2

Brossard, D., Lewenstein, B. and Bonney, R., 2005. Scientific knowledge and attitude change: The impact of a citizen science project. *International Journal of Science Education*, *27*(9), pp.1099-1121. DOI: 10.1080/09500690500069483

CABI.org. 2019. Home - CABI.org. [online] Available at: https://www.cabi.org/ [Accessed 6 Aug. 2019].

Carruthers, R.I. and Onsager, J.A., 1993. Perspective on the use of exotic natural enemies for biological control of pest grasshoppers (Orthoptera: Acrididae). *Environmental Entomology*, *22*(5), pp.885-903. DOI: 10.1093/ee/22.5.885

Carson, R., 1962. *Silent Spring*. Fawcett Publications, INC., Greenwich, Conn.

Choi, D.S., Kim, D.I., Ko, S.J., Kang, B.R., Park, J.D., Kim, S.G. and Choi, K.J., 2012a. Environmentally-friendly control methods and forecasting the hatching time Lycorma delicatula (Hemiptera: Fulgoridae) in Jeonnam Province. *Korean Journal of Applied Entomology*, *51*(4), pp.371-376. DOI: 10.5656/KSAE.2012.09.0.022

Choi, M.Y., Kim, J.W., Byun, Y.W., Kim, K.H., Lee, S.G. and Park, H.H., 2012b. Study on Control Tactics of a Sporadic Insect Lycorma delicatula in Source Habitats. *National Institute of Agricultural Science, Rural Development of Administration, South Korea.*

Ciampitti, M. and Cavagna, B., 2014. Public awareness: a useful tool for the early detection and a successful eradication of the longhorned beetles *Anoplophora* *chinensis* and *A. glabripennis*. *EPPO Bulletin*, *44*(2), pp.248-250. DOI: 10.1111/epp.12116

Cianferoni, F., Graziani, F., Dioli, P. and Ceccolini, F., 2018. Review of the occurrence of Halyomorpha halys (Hemiptera: Heteroptera: Pentatomidae) in Italy, with an update of its European and World distribution. *Biologia*, *73*(6), pp.599-607. DOI: 10.2478/s11756-018-0067-9

Cissel, W.J., Mason, C.E., Whalen, J., Hough-Goldstein, J. and Hooks, C.R., 2015. Effects of brown marmorated stink bug (Hemiptera: Pentatomidae) feeding injury on sweet corn yield and quality. *Journal of Economic Entomology*, *108*(3), pp.1065-1071. DOI: 10.1093/jee/tov059

Colazza, S., Aquila, G., De Pasquale, C., Peri, E. and Millar, J.G., 2007. The egg parasitoid Trissolcus basalis uses n-nonadecane, a cuticular hydrocarbon from its stink bug host Nezara viridula, to discriminate between female and male hosts. *Journal of Chemical Ecology*, *33*(7), pp.1405-1420. DOI: 10.1007/s10886-007-9300-7

Colazza, S., McElfresh, J.S. and Millar, J.G., 2004. Identification of volatile synomones, induced by Nezara viridula feeding and oviposition on bean spp., that attract the egg parasitoid Trissolcus basalis. *Journal of chemical ecology*, *30*(5), pp.945-964. DOI: 10.1023/B:JOEC.0000028460.70584.d1

Colazza, S., Salerno, G. and Wajnberg, E., 1999. Volatile and contact chemicals released by Nezara viridula (Heteroptera: Pentatomidae) have a kairomonal effect on the egg parasitoid Trissolcus basalis (Hymenoptera: Scelionidae). *Biological Control*, *16*(3), pp.310-317. DOI: 10.1006/bcon.1999.0763

Cornelius, M.L., Dieckhoff, C., Hoelmer, K.A., Olsen, R.T., Weber, D.C., Herlihy, M.V., Talamas, E.J., Vinyard, B.T. and Greenstone, M.H., 2016. Biological control of sentinel egg masses of the exotic invasive stink bug Halyomorpha halys (Stål) in Mid-Atlantic USA ornamental landscapes. *Biological Control*, *103*, pp.11-20. DOI: 10.1016/j.biocontrol.2016.07.011

Costi, E., Haye, T. and Maistrello, L., 2017. Biological parameters of the invasive brown marmorated stink bug, Halyomorpha halys, in southern Europe. *Journal of Pest Science*, *90*(4), pp.1059-1067. DOI: 10.1007/s10340-017-0899-z

Crall, A.W., Jordan, R., Holfelder, K., Newman, G.J., Graham, J. and Waller, D.M., 2013. The impacts of an invasive species citizen science training program on participant attitudes, behavior, and science literacy. *Public Understanding of Science*, *22*(6), pp.745-764. DOI: 10.1177/0963662511434894

CSO Italy Centro Servizi Ortofrutticoli. 2019. CSO Italy Centro Servizi Ortofrutticoli. [online] Available at: https://www.csoservizi.com [Accessed 9 Dec. 2019].

Danielsen, F., Burgess, N.D. and Balmford, A., 2005. Monitoring matters: examining the potential of locally-based approaches. *Biodiversity & Conservation*, *14*(11), pp.2507-2542. DOI: 10.1007/s10531-005-8375-0

Dara, S.K., Barringer, L. and Arthurs, S.P., 2015. Lycorma delicatula (Hemiptera: Fulgoridae): a new invasive pest in the United States. *Journal of Integrated Pest Management*, *6*(1), p.20. DOI: 10.1093/jipm%2Fpmv021

Davis, A.S., Landis, D.A., 2011. Invasive species in agriculture. In: M. Rejmanek and D. Simberloff, eds., *Encyclopedia of Introduced Invasive Species*. Berkeley, CA: University of California Press. pp. 7-11.

DeBach, P. 1964. *Biological Control of Insect Pests and Weeds*. London, UK: Chapman and Hall.

DeBach, P. and Rosen, D. 1991. *Biological Control by Natural Enemies*. CUP Archive.

Dicke, M. and Sabelis, M.W., 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds?. *Functional Ecology*, pp.131-139. DOI: 10.2307/2389687

Dicke, M., 2009. Behavioural and community ecology of plants that cry for help. *Plant, Cell & Environment*, *32*(6), pp.654-665. DOI: 10.1111/j.1365-3040.2008.01913.x

Dicke, M., Van Beek, T.A., Posthumus, M.V., Dom, N.B., Van Bokhoven, H. and De Groot, A.E., 1990. Isolation and identification of volatile kairomone that affects acarine predatorprey interactions Involvement of host plant in its production. *Journal of Chemical Ecology*, *16*(2), pp.381-396. DOI: 10.1007/BF01021772

Dickinson, J.L., Shirk, J., Bonter, D., Bonney, R., Crain, R.L., Martin, J., Phillips, T. and Purcell, K., 2012. The current state of citizen science as a tool for ecological research and public engagement. *Frontiers in Ecology and the Environment*, *10*(6), pp.291-297. DOI: 10.1890/110236

Didham, R.K., Tylianakis, J.M., Hutchison, M.A., Ewers, R.M. and Gemmell, N.J., 2005. Are invasive species the drivers of ecological change?. *Trends in Ecology & Evolution*, *20*(9), pp.470-474. DOI: 10.1016/j.tree.2005.07.006

Ding, J., Wu, Y., Zheng, H., Fu, W., Reardon, R. and Liu, M., 2006. Assessing potential biological control of the invasive plant, tree-of-heaven, Ailanthus altissima. *Biocontrol Science and Technology*, *16*(6), pp.547-566. DOI: 10.1080/09583150500531909

Dormann, C.F., Schymanski, S.J., Cabral, J., Chuine, I., Graham, C., Hartig, F., Kearney, M., Morin, X., Römermann, C., Schröder, B. and Singer, A., 2012. Correlation and process in species distribution models: bridging a dichotomy. *Journal of Biogeography*, *39*(12), pp.2119-2131. DOI: 10.1111/j.1365-2699.2011.02659.x

Duan, J.J. and Messing, R.H., 1997. Biological control of fruit flies in Hawaii: factors affecting non-target risk analysis. *Agriculture and Human Values*, *14*(3), pp.227-236. DOI: 10.1023/A:1007456727655

Duthie, C., Michael, T., Stephenson, B., Yamoah, E. and McDonald, B., 2012. Risk analysis of Halyomorpha halys (brown marmorated stink bug) on all pathways. *Ministry for Primary Industries, Wellington, New Zealand, 57*.

eButterfly. 2019. [online] Available at: http://www.e-butterfly.org/ [Accessed 3 Nov. 2019].

Eilenberg, J., Hajek, A. and Lomer, C., 2001. Suggestions for unifying the terminology in biological control. *BioControl*, *46*(4), pp.387-400. DOI: 10.1023/A:1014193329979

Elith, J., Kearney, M. and Phillips, S., 2010. The art of modelling range-shifting species. *Methods in Ecology and Evolution*, *1*(4), pp.330-342. DOI: 10.1111/j.2041-210X.2010.00036.x

Farnsworth, E.J. and Meyerson, L.A., 2003. Comparative ecophysiology of four wetland plant species along a continuum of invasiveness. *Wetlands*, *23*(4), pp.750-762. DOI: 10.1672/0277-5212(2003)023[0750:CEOFWP]2.0.CO;2

Faúndez, E.I. and Rider, D.A., 2017. The brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) in Chile. *Arquivos Entomolóxicos*, (17), pp.305-307.

Fogain, R. and Graff, S., 2011. First records of the invasive pest, Halyomorpha halys (Hemiptera: Pentatomidae), in Ontario and Quebec. Journal of the *Entomological Society of Ontario*, *142*, pp.45-48. http://www.entsocont.com/pub.htm

Gale, G.A., DeCecco, J.A., Marshall, M.R., McClain, W.R. and Cooper, R.J., 2001. Effects of gypsy moth defoliation on forest birds: an assessment using breeding bird census data. *Journal of Field Ornithology*, *72*(2), pp.291-304. DOI: 10.1648/0273-8570-72.2.291

Gallo, T. and Waitt, D., 2011. Creating a successful citizen science model to detect and report invasive species. *BioScience*, *61*(6), pp.459-465. DOI: 10.1525/bio.2011.61.6.8

Gapon, D.A., 2016. First records of the brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. *Entomological Review*, *96*(8), pp.1086-1088. DOI: 10.1134/S001387381608011X

Gardiner, M.M., Allee, L.L., Brown, P.M., Losey, J.E., Roy, H.E. and Smyth, R.R., 2012. Lessons from lady beetles: accuracy of monitoring data from US and UK citizen-science programs. *Frontiers in Ecology and the Environment*, *10*(9), pp.471-476. DOI: 10.1890/110185

Gauld, I.D., Bolton, B., Huddleston, T., Fitton, M.G., Shaw, M.R., Noyes, J.S., Day, M.C., Else, G.R., Ferguson, N.D.M. and Ward, S.L., 1988. *The Hymenoptera*, pp.1-332.

Gd.eppo.int. 2019. EPPO Global Database. [online] Available at: https://gd.eppo.int/ [Accessed 13 Oct. 2020].

Goodchild, M.F., 2007. Citizens as sensors: the world of volunteered geography. *GeoJournal*, *69*(4), pp.211-221. DOI: 10.1007/s10708-007-9111-y

Gouli, V., Gouli, S., Skinner, M., Hamilton, G., Kim, J.S. and Parker, B.L., 2012. Virulence of select entomopathogenic fungi to the brown marmorated stink bug, Halyomorpha halys (Stål) (Heteroptera: Pentatomidae). *Pest Management Science*, *68*(2), pp.155-157. DOI: 10.1002/ps.2310

Grason, E.W., McDonald, P.S., Adams, J., Litle, K., Apple, J.K. and Pleus, A., 2018. Citizen science program detects range expansion of the globally invasive European green crab in Washington State (USA). *Management of Biological Invasions*, *9*(1), p.39. DOI: 10.3391/mbi.2018.9.1.04

Greathead, D.J. 1994. History of biological control. *Antenna*, *18*(4), 187-199.

Guisan, A. and Thuiller, W., 2005. Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, *8*(9), pp.993-1009. DOI: 10.1111/j.1461-0248.2005.00792.x

Györfi, J., 1961. Beiträge zur Kenntnis der Lebensweise der Chalcididen-Arten in Ungarn. *Zeitschrift für Angewandte Entomologie*, *49*(1-4), pp.207-223.

Hall, R. and Ehler, L.E., 1979. Rate of establishment of natural enemies in classical biological control. *Bulletin of the ESA*, *25*(4), pp.280-283. DOI: 10.1093/besa/25.4.280

Hamilton, R.M., Foster, R.E., Gibb, T.J., Sadof, C.S., Holland, J.D. and Engel, B.A., 2007. Distribution and dynamics of Japanese beetles along the Indianapolis airport perimeter and the influence of land use on trap catch. *Environmental Entomology*, *36*(2), pp.287-296. DOI: 10.1093/ee/36.2.287

Han, J.M., Kim, H., Lim, E.J., Lee, S., Kwon, Y.J. and Cho, S., 2008. Lycorma delicatula (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. *Entomological Research*, *38*(4), pp.281-286. DOI: 10.1111/j.1748-5967.2008.00188.x

Hawkins, B.A. and Marino, P.C., 1997. The colonization of native phytophagous insects in North America by exotic parasitoids. *Oecologia*, *112*(4), pp.566-571. DOI: 10.1007/s004420050346

Haye, T., Abdallah, S., Gariepy, T. and Wyniger, D., 2014. Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, Halyomorpha halys, in Europe. *Journal of Pest Science*, *87*(3), pp.407-418. DOI: 10.1007/s10340-014-0560-z

Haye, T., Fischer, S., Zhang, J. and Gariepy, T., 2015. Can native egg parasitoids adopt the invasive brown marmorated stink bug, Halyomorpha halys (Heteroptera: Pentatomidae), in Europe?. *Journal of Pest Science*, *88*(4), pp.693-705. DOI: 10.1007/s10340-015-0671-1

Hedstrom, C.S., Shearer, P.W., Miller, J.C. and Walton, V.M., 2014. The effects of kernel feeding by Halyomorpha halys (Hemiptera: Pentatomidae) on commercial hazelnuts. *Journal of Economic Entomology*, *107*(5), pp.1858-1865. DOI: 10.1603/EC14263

Henneman, M.L. and Memmott, J., 2001. Infiltration of a Hawaiian community by introduced biological control agents. *Science*, *293*(5533), pp.1314-1316. DOI: 10.1126/science.1060788

Herlihy, M.V., Talamas, E.J. and Weber, D.C., 2016. Attack and success of native and exotic parasitoids on eggs of Halyomorpha halys in three Maryland habitats. *PLoS One*, *11*(3). DOI: 10.1371/journal.pone.0150275

Hilker, M. and Fatouros, N.E., 2015. Plant responses to insect egg deposition. *Annual Review of Entomology*, *60*, pp.493-515. DOI: 10.1146/annurev-ento-010814-020620

Hobbs, R.J. and Humphries, S.E., 1995. An integrated approach to the ecology and management of plant invasions. *Conservation Biology*, *9*(4), pp.761-770. DOI: 10.1046/j.1523-1739.1995.09040761.x

Hoebeke, E.R. and Carter, M.E. 2003. Halyomorpha halys (Stahl) (Heteroptera: Pentatomidae): A polyphagous plant pest from Asia newly detected in North America. *Proceedings of the Entomological Society of Washington*, *105*: 225-237.

Hokkanen, H.M. and Pimentel, D., 1989. New associations in biological control: theory and practice. *The Canadian Entomologist*, *121*(10), pp.829-840. DOI: 10.4039/Ent121829-10

Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D. and Case, T.J., 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics*, *33*(1), pp.181-233. DOI: 10.1146/annurev.ecolsys.33.010802.150444

Hou, Z.R. 2013. *Study On The Lycorma delicatula and Egg Parasitoids*. MS thesis dissertation, Chinese Academy of Forestry, Beijing, China.

Howarth FG. 1983. Classical biological control: panacea or Pandora's Box? *Proceedings of the Hawaiian Entomological Society 24*:239-244.

Howarth, F.G., 1991. Environmental impacts of classical biological control. *Annual Review of Entomology*, *36*(1), pp.485-509. DOI: 10.1146/annurev.en.36.010191.002413

Hulme, P.E., 2007. Biological invasions in Europe: drivers, pressures, states, impacts and responses. *Biodiversity Under Threat*, 25, pp.56-80.

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, *46*(1), pp.10-18. DOI: 10.1111/j.1365-2664.2008.01600.x

iNaturalist. (2019). iNaturalist. [online] Available at: https://www.inaturalist.org/ [Accessed 2 Nov. 2019].

Irwin, A., 2002. *Citizen science: A Study of People, Expertise and Sustainable Development*. Routledge.

Jenkins, E.W., 1999. School science, citizenship and the public understanding of science. *International Journal of Science Education*, *21*(7), pp.703-710. DOI: 10.1080/095006999290363

Jones, A.L., Jennings, D.E., Hooks, C.R. and Shrewsbury, P.M., 2014. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, Halyomorpha halys. *Biological Control*, *78*, pp.61-66. DOI: 10.1016/j.biocontrol.2014.07.011

Jordan, R.C., Gray, S.A., Howe, D.V., Brooks, W.R. and Ehrenfeld, J.G., 2011. Knowledge gain and behavioral change in citizen-science programs. *Conservation Biology*, *25*(6), pp.1148-1154. DOI: 10.1111/j.1523-1739.2011.01745.x

Jung, J.M., Jung, S., Byeon, D.H. and Lee, W.H., 2017. Model-based prediction of potential distribution of the invasive insect pest, spotted lanternfly Lycorma delicatula (Hemiptera: Fulgoridae), by using CLIMEX. *Journal of Asia-Pacific Biodiversity*, *10*(4), pp.532-538. DOI: 10.1016/j.japb.2017.07.001

Kamenova, S., Bartley, T.J., Bohan, D.A., Boutain, J.R., Colautti, R.I., Domaizon, I., Fontaine, C., Lemainque, A., Le Viol, I., Mollot, G. and Perga, M.E., 2017. Invasions toolkit: current methods for tracking the spread and impact of invasive species. In *Advances in Ecological Research* (Vol. *56*, pp. 85-182). Academic Press.

Kaser, J.M., Nielsen, A.L. and Abram, P.K., 2018. Biological control effects of non-reproductive host mortality caused by insect parasitoids. *Ecological Applications*, *28*(4), pp.1081-1092. DOI: 10.1002/eap.1712

Keane, R.M. and Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, *17*(4), pp.164-170. DOI: 10.1016/S0169-5347(02)02499-0

Kenis, M., Auger-Rozenberg, M.A., Roques, A., Timms, L., Péré, C., Cock, M.J., Settele, J., Augustin, S. and Lopez-Vaamonde, C., 2009. Ecological effects of invasive alien insects. *Biological Invasions*, *11*(1), pp.21-45. DOI: 10.1007/s10530-008-9318-y

Khrimian, A., Zhang, A., Weber, D.C., Ho, H.Y., Aldrich, J.R., Vermillion, K.E., Siegler, M.A., Shirali, S., Guzman, F. and Leskey, T.C., 2014. Discovery of the aggregation pheromone of the brown marmorated stink bug (Halyomorpha halys) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. *Journal of Natural Products*, *77*(7), pp.1708-1717. DOI: 10.1021/np5003753

Kim, S. S. and Kim, T. W., 2005. Lycorma delicatula (White) (Hemiptera: Fulgoridae) in Korea. *Lucanus*, *5*, 9-10.

Kim, S.K., Lee, G.Y., Shin, Y.H. and Kim, G.B., 2010. Chemical control effect against spot clothing wax cicada, Lycorma delicatula (Hemiptera: Fulgoridae) nymphs and adults. *The Korean Journal of Pesticide Science*, *14*(4), pp.440-445.

Koppel, A.L., Herbert Jr, D.A., Kuhar, T.P. and Kamminga, K., 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean, and vegetable crops in southeast Virginia. *Environmental Entomology*, *38*(2), pp.375-379. DOI: 10.1603/022.038.0209

Kuhar, T.P. and Kamminga, K., 2017. Review of the chemical control research on Halyomorpha halys in the USA. *Journal of Pest Science*, *90*(4), pp.1021-1031. DOI: 10.1007/s10340-017-0859-7

Leach, H., Biddinger, D.J., Krawczyk, G., Smyers, E. and Urban, J.M., 2019. Evaluation of insecticides for control of the spotted lanternfly, Lycorma delicatula, (Hemiptera: Fulgoridae), a new pest of fruit in the Northeastern US. *Crop Protection*, *124*, p.104833. DOI: 10.1016/j.cropro.2019.05.027

Lee, D.H., Park, Y.L. and Leskey, T.C., 2019. A review of biology and management of Lycorma delicatula (Hemiptera: Fulgoridae), an emerging global invasive species. *Journal of Asia-Pacific Entomology*, *22*(2), pp.589-596. DOI: 10.1016/j.aspen.2019.03.004

Lee, D.H., Short, B.D., Joseph, S.V., Bergh, J.C. and Leskey, T.C., 2013. Review of the biology, ecology, and management of Halyomorpha halys (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environmental Entomology*, *42*(4), pp.627-641. DOI: 10.1603/EN13006

Lee, S.J. and Park, S.C., 2013. Attraction effect against Lycorma delicatula, antioxidant activity and local irritation test of Ailanthus altissima extract. *Korean Journal of Veterinary Research*, *53*(4), pp.231-237. DOI: 10.14405/kjvr.2013.53.4.231

Lee, Y., Jang, M., Kim, J. and Kim, J., 2014. The effect of winter temperature on the survival of lantern fly, Lycorma delicatula (Hemiptera: Fulgoridae) eggs. *Korean Journal of Applied Entomology*, *53*(3), pp.311-315. DOI: 10.5656/KSAE.2014.07.0.034

Lepczyk, C.A., 2005. Integrating published data and citizen science to describe bird diversity across a landscape. *Journal of Applied Ecology*, *42*(4), pp.672-677. DOI: 10.1111/j.1365-2664.2005.01059.x

Leskey, T.C. and Nielsen, A.L., 2018. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. *Annual Review of Entomology*, *63*, pp.599-618. DOI: 10.1146/annurev-ento-020117-043226

Leskey, T.C., Hamilton, G.C., Nielsen, A.L., Polk, D.F., Rodriguez-Saona, C., Bergh, J.C., Herbert, D.A., Kuhar, T.P., Pfeiffer, D., Dively, G.P. and Hooks, C.R., 2012a. Pest status of the brown marmorated stink bug, Halyomorpha halys in the USA. *Outlooks on Pest Management*, *23*(5), pp.218-226. DOI: 10.1564/23oct07

Leskey, T.C., Short, B.D., Butler, B.R. and Wright, S.E., 2012b. Impact of the invasive brown marmorated stink bug, Halyomorpha halys (Stål), in mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. *Psyche: A Journal of Entomology*, 2012. DOI: 10.1155/2012/535062

Levine, J.M. and D'Antonio, C.M., 2003. Forecasting biological invasions with increasing international trade. *Conservation Biology*, *17*(1), pp.322-326. DOI: 10.1046/j.1523-1739.2003.02038.x

Liebhold, A., Luzader, E., Halverson, J. and Elmes, G., 1993. The spatial dynamics of invasions by exotic forest pests. *Proceedings, Spatial Analysis and Forest Pest Management: April* 27-30, 1992, *Mountain Lakes, Virginia*, 175, p.125.

Liu, G., 1939. Some extracts from the history of entomology in China. *Psyche: A Journal of Entomology*, *46*(1), pp.23-28.

Liu, H. and Mottern, J., 2017. An old remedy for a new problem? Identification of Ooencyrtus kuvanae (Hymenoptera: Encyrtidae), an egg parasitoid of Lycorma delicatula (Hemiptera: Fulgoridae) in North America. *Journal of Insect Science*, *17*(1). DOI: 10.1093/jisesa/iew114

Lockwood, J.L., Cassey, P. and Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution*, *20*(5), pp.223-228. DOI: 10.1016/j.tree.2005.02.004

Long, J. L., 2003. Introduced mammals of the world: their history, distribution, and influence. CSIRO publishing.

Lotz, A. and Allen, C.R., 2007. Observer bias in anuran call surveys. *The Journal of Wildlife Management*, *71*(2), pp.675-679. DOI: 10.2193/2005-759

MacDougall, A.S. and Turkington, R., 2005. Are invasive species the drivers or passengers of change in degraded ecosystems?. *Ecology*, *86*(1), pp.42-55. DOI: 10.1890/04-0669

Maistrello, L., Dioli, P., Dutto, M., Volani, S., Pasquali, S. and Gilioli, G., 2018. Tracking the Spread of Sneaking Aliens by Integrating Crowdsourcing and Spatial Modeling: The Italian Invasion of Halyomorpha halys. *BioScience*, *68*(12), pp.979-989. DOI: 10.1093/biosci/biy112

Maistrello, L., Vaccari, G., Caruso, S., Costi, E., Bortolini, S., Macavei, L., Foca, G., Ulrici, A., Bortolotti, P.P., Nannini, R. and Casoli, L., 2017. Monitoring of the invasive Halyomorpha halys, a new key pest of fruit orchards in northern Italy. *Journal of Pest Science*, *90*(4), pp.1231-1244. DOI: 10.1007/s10340-017-0896-2

Mallet, J., 2005. Hybridization as an invasion of the genome. *Trends in Ecology & Evolution*, *20*(5), pp.229-237. DOI: 10.1016/j.tree.2005.02.010

Martinson, H.M., Venugopal, P.D., Bergmann, E.J., Shrewsbury, P.M. and Raupp, M.J., 2015. Fruit availability influences the seasonal abundance of invasive stink bugs in ornamental tree nurseries. *Journal of Pest Science*, *88*(3), pp.461-468. DOI: 10.1007/s10340-015-0677-8

McKinley, D.C., Miller-Rushing, A.J., Ballard, H.L., Bonney, R., Brown, H., Cook-Patton, S.C., Evans, D.M., French, R.A., Parrish, J.K., Phillips, T.B. and Ryan, S.F., 2017. Citizen science can improve conservation science, natural resource management, and environmental protection. *Biological Conservation*, *208*, pp.15-28. DOI: 10.1016/j.biocon.2016.05.015

Measham, T.G., 2007. Building capacity for environmental management: local knowledge and rehabilitation on the Gippsland Red Gum Plains. *Australian Geographer*, *38*(2), pp.145-159. DOI: 10.1080/00049180701392758

Merow, C., Smith, M.J. and Silander Jr, J.A. 2013. A practical guide to MaxEnt for modelling species' distributions: what it does, and what inputs and settings matter. *Ecography*, *36*(10), pp.1058-1069. DOI: 10.1111/j.1600-0587.2013.07872.x

Messing, R. and Brodeur, J., 2018. Current challenges to the implementation of classical biological control. *BioControl*, *63*(1), pp.1-9. DOI: 10.1007/s10526-017-9862-4

Messing, R.H. and Wright, M.G., 2006. Biological control of invasive species: solution or pollution?. *Frontiers in Ecology and the Environment*, *4*(3), pp.132-140. DOI: 10.1890/1540-9295(2006)004[0132:BCOISS]2.0.CO;2

Meyerson, L.A. and Mooney, H.A., 2007. Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, *5*(4), pp.199-208. DOI: 10.1890/1540-9295(2007)5[199:IASIAE]2.0.CO;2

Miller-Rushing, A., Primack, R. and Bonney, R., 2012. The history of public participation in ecological research. *Frontiers in Ecology and the Environment*, *10*(6), pp.285-290. DOI: 10.1890/110278

Mooney, H.A. and Hobbs, R.J., 2000. *Invasive Species in a Changing World*. Island Press, Washington DC.

Morrison III, W.R., Allen, M. and Leskey, T.C., 2018. Behavioural response of the invasive Halyomorpha halys (Hemiptera: Pentatomidae) to host plant stimuli augmented with semiochemicals in the field. *Agricultural and Forest Entomology*, *20*(1), pp.62-72. DOI: 10.1111/afe.12229

Morrison III, W.R., Cullum, J.P. and Leskey, T.C., 2015. Evaluation of trap designs and deployment strategies for capturing Halyomorpha halys (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, *108*(4), pp.1683-1692. DOI: 10.1093/jee%2Ftov159

Morrison III, W.R., Mathews, C.R. and Leskey, T.C., 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. *Biological Control*, *97*, pp.120-130. DOI: 10.1016/j.biocontrol.2016.03.008

Morrison, L.W., 2002. Long-term impacts of an arthropod-community invasion by the imported fire ant, Solenopsis invicta. *Ecology*, *83*(8), pp.2337-2345. DOI: 10.1890/0012-9658(2002)083[2337:LTIOAA]2.0.CO;2

Nass.usda.gov. 2013. USDA - National Agricultural Statistics Service Homepage. [online] Available at: https://www.nass.usda.gov/ [Accessed 15 Dec. 2019].

Neteler, M., Bowman, M.H., Landa, M. and Metz, M., 2012. GRASS GIS: A multi-purpose open source GIS. *Environmental Modelling & Software*, *31*, pp.124-130. DOI: 10.1016/j.envsoft.2011.11.014

Neteler, M., Metz, M., Rocchini, D., Rizzoli, A., Flacio, E., Engeler, L., Guidi, V., Lüthy, P. and Tonolla, M., 2013. Is Switzerland suitable for the invasion of Aedes albopictus?. *PLoS One*, *8*(12). DOI: 10.1371/journal.pone.0082090

Neuenschwander, P.W.N.O., Hammond, W.O., Gutierrez, A.P., Cudjoe, A.R., Adjakloe, R., Baumgärtner, J.U. and Regev, U., 1989. Impact assessment of the biological control of the cassava mealybug, Phenacoccus manihoti Matile-Ferrero (Hemiptera: Pseudococcidae), by the introduced parasitoid Epidinocarsis lopezi (De Santis) (Hymenoptera: Encyrtidae). *Bulletin of Entomological Research*, *79*(4), pp.579-594.

Nielsen, A.L. and Hamilton, G.C., 2009. Life history of the invasive species Halyomorpha halys (Hemiptera: Pentatomidae) in northeastern United States. *Annals of the Entomological Society of America*, *102*(4), pp.608-616. DOI: 10.1603/008.102.0405

Nielsen, A.L., Chen, S. and Fleischer, S.J., 2016. Coupling developmental physiology, photoperiod, and temperature to model phenology and dynamics of an invasive heteropteran, Halyomorpha halys. *Frontiers in Physiology*, *7*, p.165. DOI: 10.3389/fphys.2016.00165

Norgaard, R.B., 1988. The biological control of cassava mealybug in Africa. *American Journal of Agricultural Economics*, *70*(2), pp.366-371. DOI: 10.2307/1242077

NortheastIPM.org. (2020). Northeastern IPM Center. [online] Available at: http://www.northeastipm.org/ [Accessed 4 Jan. 2020].

Ogburn, E.C., Bessin, R., Dieckhoff, C., Dobson, R., Grieshop, M., Hoelmer, K.A., Mathews, C., Moore, J., Nielsen, A.L., Poley, K. and Pote, J.M., 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: a regional assessment. *Biological Control*, *101*, pp.39-51. DOI: 10.1016/j.biocontrol.2016.06.002

Ogden, N.H., Radojevic, M., Wu, X., Duvvuri, V.R., Leighton, P.A. and Wu, J., 2014. Estimated effects of projected climate change on the basic reproductive number of the Lyme disease vector Ixodes scapularis. *Environmental Health Perspectives*, *122*(6), pp.631-638. DOI: 10.1289/ehp.1307799

Opalexplorenature.org., 2019. Home | OPAL. [online] Available at: https://www.opalexplorenature.org/ [Accessed 21 Dec. 2019].

OTA (Office of Technological Assessment) Archive. 1993 | OTA Archive. [online] Available at: https://ota.fas.org/otareports/year/1993a [Accessed 14 Dec. 2019].

Park, J.D., Kim, M.Y., Lee, S.G., Shin, S.C., Kim, J.H. and Park, I.K., 2009. Biological characteristics of Lycorma delicatula and the control effects of some insecticides. *Korean Journal of Applied Entomology*, *48*(1), pp.53-57.

Park, M., Kim, K.S. and Lee, J.H., 2012. Isolation and characterization of eight microsatellite loci from Lycorma delicatula (White) (Hemiptera: Fulgoridae) for population genetic analysis in Korea. *Molecular Biology Reports*, *39*(5), pp.5637-5641. DOI: 10.1007/s11033-011-1368-z

Park, M., Kim, K.S. and Lee, J.H., 2013. Genetic structure of Lycorma delicatula (Hemiptera: Fulgoridae) populations in Korea: implication for invasion processes in heterogeneous landscapes. *Bulletin of Entomological Research*, *103*(4), pp.414-424. DOI: 10.1017/S0007485313000011

Parker, B.L., Skinner, M., Gouli, S., Gouli, V. and Kim, J.S., 2015. Virulence of BotaniGard® to second instar brown marmorated stink bug, Halyomorpha halys (Stål) (Heteroptera: Pentatomidae). *Insects*, *6*(2), pp.319-324. DOI: 10.3390/insects6020319

Peiffer, M. and Felton, G.W., 2014. Insights into the saliva of the brown marmorated stink bug Halyomorpha halys (Hemiptera: Pentatomidae). *PloS ONE*, *9*(2). DOI: 10.1371/journal.pone.0088483

Pennsylvania Department of Agriculture. 2018. PA Dept. of Agriculture. [online] Available at: http://www.agriculture.pa.gov/ [Accessed 7 Nov. 2019].

Penrose, D. and Call, S.M., 1995. Volunteer monitoring of benthic macroinvertebrates: regulatory biologists' perspectives. *Journal of the North American Benthological Society*, *14*(1), pp.203-209. DOI: 10.2307/1467735

Peri, E., Salerno, G., Slimani, T., Frati, F., Conti, E., Colazza, S. and Cusumano, A., 2016. The response of an egg parasitoid to substrate-borne semiochemicals is affected by previous experience. *Scientific Reports*, *6*, p.27098. DOI: 10.1038/srep27098

Perrings, C., Williamson, M., Barbier, E.B., Delfino, D., Dalmazzone, S., Shogren, J., Simmons, P. and Watkinson, A., 2002. Biological invasion risks and the public good: an economic perspective. *Conservation Ecology*, *6*(1). http://www.consecol.org/vol6/iss1/art1/

Peterson, A.T. and Soberón, J., 2012. Species distribution modeling and ecological niche modeling: getting the concepts right. *Natureza & Conservação*, *10*(2), pp.102-107.

Philips, S.J. and Elith, J. 2013. On estimating probability of presence from use–availability or presence–background data. *Ecology*, *94*(6): 1409-1419. DOI: 10.1890/12-1520.1

Phillips, S. and Dudík, M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, *31*(2), pp.161-175. DOI: 10.1111/j.0906-7590.2008.5203.x

Phillips, S.J., Anderson, R.P. and Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3-4), pp.231-259. DOI: 10.1016/j.ecolmodel.2005.03.026

Pimentel, D., Lach, L., Zuniga, R. and Morrison, D., 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience*, *50*(1), pp.53-66. DOI: 10.1641/0006-3568(2000)050[0053:EAECON]2.3.CO;2

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. and Tsomondo, T., 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, *84*(1), pp.1-20. DOI: 10.1016/S0167-8809(00)00178-X

Pote, J.M. and Nielsen, A.L., 2017. Life stage specific predation of Halyomorpha halys (Stål) by generalist predators. *Biological Control*, *114*, pp.1-7. DOI: 10.1016/j.biocontrol.2017.07.007

Pyšek, P. and Richardson, D.M., 2010. Invasive species, environmental change and management, and health. *Annual Review of Environment and Resources*, *35*, pp.25-55. DOI: 10.1146/annurev-environ-033009-095548

Reinhardt, F., Herle, M., Bastiansen, F. and Streit, B., 2003. Economic impact of the spread of alien species in Germany. *Berlin, Germany: Federal Environmental Agency (Umweltbundesamt).*

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T., Hamilton, G., Haye, T. and Herbert, A., 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *Journal of Integrated Pest Management*, *5*(3), pp.A1-A13. DOI: 10.1603/IPM14002

Rondoni, G., Bertoldi, V., Malek, R., Foti, M.C., Peri, E., Maistrello, L., Haye, T. and Conti, E., 2017. Native egg parasitoids recorded from the invasive Halyomorpha halys successfully exploit volatiles emitted by the plant–herbivore complex. *Journal of Pest Science*, *90*(4), pp.1087-1095. DOI: 10.1007/s10340-017-0861-0

Roura-Pascual, N., Krug, R.M., Richardson, D.M. and Hui, C., 2010. Spatially-explicit sensitivity analysis for conservation management: exploring the influence of decisions in invasive alien plant management. *Diversity and Distributions*, *16*(3), pp.426-438. DOI: 10.1111/j.1472-4642.2010.00659.x

Rowley, J.J., Callaghan, C.T., Cutajar, T., Portway, C., Potter, K., Mahony, S., Trembath, D.F., Flemons, P. and Woods, A., 2019. FrogID: Citizen scientists provide validated biodiversity data on frogs of Australia. *Herpetological Conservation and Biology*, *14*(1), pp.155-170.

Sabbatini Peverieri, G.S., Talamas, E., Bon, M.C., Marianelli, L., Bernardinelli, I., Malossini, G., Benvenuto, L., Roversi, P.F. and Hoelmer, K., 2018. Two Asian egg parasitoids of Halyomorpha halys (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: Trissolcus mitsukurii (Ashmead) and Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae). *Journal of Hymenoptera Research*, *67*, p.37. DOI: 10.3897/jhr.67.30883

Sanyang, F., 1992. The eco-geographical distribution of forest insects in China. *Journal of Northeast Forestry University*, *3*(2), pp.13-22.

Shin, Y.H., Moon, S.R., Yoon, C.M., Ahn, K.S. and Kim, G.H., 2010. Insecticidal activity of 26 insecticides against eggs and nymphs of Lycorma delicatula (Hemiptera: Fulgoridae). *The Korean Journal of Pesticide Science*, *14*(2), pp.157-163.

Shine, C. and Williams, N., 2000. *A Guide to Designing Legal and Institutional Frameworks on Alien Invasive Species* (No. 40). IUCN.

Silvertown, J., 2009. A new dawn for citizen science. *Trends in Ecology & Evolution*, *24*(9), pp.467-471. DOI: 10.1016/j.tree.2009.03.017

Simberloff, D. and Rejmánek, M. eds., 2011. *Encyclopedia of Biological Invasions* (No. 3). University of California Press.

Simberloff, D., 1989. Which insect introductions succeed and which fail? In J. A. Drake, H. A. Mooney, F. di Castri, R. H. Groves, F. J. Kruger, M. Rejmanek, and M. Williamson, editors. *Biological Invasions: A Global Perspective*. Wiley, Chichester, UK pp. 61-75.

Sinden, J. A., Jones, R., Hester, S., Odom, D., Kalisch, C., James, R. and Cacho, O., 2004. *The Economic Impact of Weeds in Australia: Report to the CRC for*

Australian Weed Management, CRC for Australian Weed Management, Glen Osmond, S.A

Small, M.J., Small, C.J. and Dreyer, G.D., 2005. Changes in a hemlock-dominated forest following woolly adelgid infestation in southern New England1. *The Journal of the Torrey Botanical Society*, *132*(3), pp.458-470. DOI: 10.3159/1095-5674(2005)132[458:CIAHFF]2.0.CO;2

Smith, J.R., Hesler, S.P. and Loeb, G.M., 2014. Potential impact of Halyomorpha halys (Hemiptera: Pentatomidae) on grape production in the Finger Lakes region of New York. *Journal of Entomological Science*, *49*(3), pp.290-303. DOI: 10.18474/0749-8004-49.3.290

Soroye, P., Ahmed, N. and Kerr, J.T., 2018. Opportunistic citizen science data transform understanding of species distributions, phenology, and diversity gradients for global change research. *Global Change Biology*, *24*(11), pp.5281-5291. DOI: 10.1111/gcb.14358

Stahl, J., Tortorici, F., Pontini, M., Bon, M.C., Hoelmer, K., Marazzi, C., Tavella, L. and Haye, T., 2019. First discovery of adventive populations of Trissolcus japonicus in Europe. *Journal of pest science*, *92*(2), pp.371-379. DOI: 10.1007/s10340-018-1061-2

Stiling, P. and Simberloff, D., 2000. The frequency and strength of nontarget effects of invertebrate biological control agents of plant pests and weeds. In *Nontarget Effects of Biological Control* (pp. 31-43). Springer, Boston, MA.

Stopbmsb.org. 2019. Stop Brown Marmorated Stink Bug - StopBMSB.org. [online] Available at: http://www.stopbmsb.org [Accessed 5 Nov. 2020].

Strand, M.R. and Vinson, S.B., 1982. Source and characterization of an egg recognition kairomone of Telenomus heliothidis, a parasitoid of Heliothis virescens. *Physiological Entomology*, *7*(1), pp.83-90. DOI: 10.1111/j.1365-3032.1982.tb00669.x

Strong, D.R. and Pemberton, R.W., 2000. Biological control of invading species--risk and reform. *Science*, *288*(5473), pp.1969-1970. DOI: 10.1126/science.288.5473.1969

Suckling, D.M., Mazzoni, V., Roselli, G., Levy, M.C., Ioriatti, C., Stringer, L.D., Zeni, V., Deromedi, M. and Anfora, G., 2019. Trapping Brown Marmorated Stink Bugs: "The Nazgûl" Lure and Kill nets. *Insects*, *10*, 433. DOI: 10.3390/insects10120433

Sumner, S., Bevan, P., Hart, A.G. and Isaac, N.J., 2019. Mapping species distributions in 2 weeks using citizen science. *Insect Conservation and Diversity*, *12*(5), pp.382-388. DOI: 10.1111/icad.12345

Talamas, E.J., Herlihy, M.V., Dieckhoff, C., Hoelmer, K.A., Buffington, M., Bon, M.C. and Weber, D.C., 2015. Trissolcus japonicus (Ashmead)(Hymenoptera, Scelionidae) emerges in North America. *Journal of Hymenoptera Research*, *43*, p.119. DOI: 10.3897/JHR.43.4661

Taylor, C.M., 2016. *Understanding the Relationship Between the Brown Marmorated Stink Bug, Halyomorpha halys (Stål), and its Symbiont, Pantoea carbekii, with Implications for Stink Bug Management* (Doctoral dissertation).

Taylor, C.M., Coffey, P.L., DeLay, B.D. and Dively, G.P., 2014. The importance of gut symbionts in the development of the brown marmorated stink bug, Halyomorpha halys (Stål). *PLoS One*, *9*(3). DOI: 10.1371/journal.pone.0090312

Thompson, K. 2014. *Where Do Camels Belong?: The Story and Science of Invasive Species*. Profile Books LTD, London, UK.

Trumbull, D.J., Bonney, R., Bascom, D. and Cabral, A., 2000. Thinking scientifically during participation in a citizen-science project. *Science Education*, *84*(2), pp.265-275. DOI: 10.1002/(SICI)1098-237X(200003)84:2%3C265::AID-SCE7%3E3.0.CO;2-5

Turlings, T.C., Tumlinson, J.H. and Lewis, W.J., 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, *250*(4985), pp.1251-1253. DOI: 10.1126/science.250.4985.1251

Valentin, R.E., Nielsen, A.L., Wiman, N.G., Lee, D.H. and Fonseca, D.M., 2017. Global invasion network of the brown marmorated stink bug, Halyomorpha halys. *Scientific Reports*, *7*(1), pp.1-12. DOI: 10.1038/s41598-017-10315-z

Van Driesche, R. and Hoddle, M., 2009. *Control of Pests and Weeds by Natural Enemies: An Introduction to Biological Control*. John Wiley & Sons.

Van Driesche, R.G. and Bellows, T.S., 1996. Biology of arthropod parasitoids and predators. In *Biological Control* (pp. 309-336). Springer, Boston, MA.

Van Lenteren, J.C. and Bueno, V.H., 2003. Augmentative biological control of arthropods in Latin America. *BioControl*, *48*(2), pp.123-139. DOI: 10.1023/A:1022645210394

van Lenteren, J.C., 2000. A greenhouse without pesticides: fact or fantasy?. *Crop Protection*, *19*(6), pp.375-384. DOI: 10.1016/S0261-2194(00)00038-7

Vet, L.E. and Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, *37*(1), pp.141-172. DOI: 10.1146/annurev.en.37.010192.001041

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., Roy, D. and Hulme, P.E., 2010. How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment*, *8*(3), pp.135-144. DOI: 10.1890/080083

Vinson, S.B., 1984. Parasitoid—host relationship. In *Chemical Ecology of Insects* (pp. 205-233). Springer, Boston, MA.

Wäckers, F.L. and Steppuhn, A., 2003. Characterizing nutritional state and food source use of parasitoids collected in fields with high and low nectar availability. *IOBC WPRS Bulletin*, *26*(4), pp.203-208.

Walker, K., 2009. Brown marmorated stink bug (Halyomorpha halys) Updated on 11/25/2011. http://www.padil.gov.au

Wallner, A.M., Hamilton, G.C., Nielsen, A.L., Hahn, N., Green, E.J., and Rodriguez-Saona, C.R. 2014. Landscape factors facilitating the invasive dynamics and distribution of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae), after arrival in the United States. *PloS ONE*, *9*(5). DOI: 10.1371/journal.pone.0095691

Watanabe, M., Arakawa, R., Shinagawa, Y., and Okazawa, T. (1994). Anti-invading methods against the brown marmorated stink bug, Halyomorpha mista, in houses. *Japanese Journal of Sanitary Zoology*, *45*(4), 311-317. DOI: 10.7601/mez.45.311_1

Wermelinger, B.E.A.T., Wyniger, D. and Forster, B.E.A.T., 2008. First records of an invasive bug in Europe: Halyomorpha halys Stal (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees?. *Mitteilungen-Schweizerische Entomologische Gesellschaft*, *81*(1/2), p.1. DOI: 10.5169/seals-402954

Williamson, M., 1996. Which communities are invaded by which type of species? In *Biological Invasions*. London: Chapman & Hall, pp. 56-64.

Winkler, K., Wäckers, F.L., Kaufman, L.V., Larraz, V. and van Lenteren, J.C., 2009. Nectar exploitation by herbivores and their parasitoids is a function of flower species and relative humidity. *Biological Control*, *50*(3), pp.299-306. DOI: 10.1016/j.biocontrol.2009.04.009

Wyckhuys, K.A., Zhang, W., Prager, S.D., Kramer, D.B., Delaquis, E., Gonzalez, C.E. and Van der Werf, W., 2018. Biological control of an invasive pest eases pressures on global commodity markets. *Environmental Research Letters*, *13*(9), p.094005. DOI: 10.1088/1748-9326/aad8f0

Yan, J.H., Ding, S.M., Qin, X.B. and Wang, F.R., 2017. Observational study on mating and oviposition of parasitoid wasp. *Shandong Science & Technology*. 179, pp.16-18.

Yanagi, T. and Hagihara, Y. 1980. Ecology of the brown marmorated stink bug. *Plant Protection*, *34*, pp.315-326.

Yang, Z.Q., Choi, W.Y., Cao, L.M., Wang, X.Y., and Hou, Z.R., 2015. A new species of Anastatus (Hymenoptera: Eulpelmidae) from China, parasitizing eggs of Lycorma delicatula (Homoptera: Fulgoridae). *Zoological Systematics*, *40*(3), pp.290-302. DOI: 10.11865/zs.20150305

Yang, Z.Q., Yao, Y.X., Qiu, L.F. and Li, Z.X., 2009. A new species of Trissolcus (Hymenoptera: Scelionidae) parasitizing eggs of Halyomorpha halys (Heteroptera: Pentatomidae) in China with comments on its biology. *Annals of the Entomological Society of America*, *102*(1), pp.39-47. DOI: 10.1603/008.102.0104

Yoon, C., Moon, S.R., Jeong, J.W., Shin, Y.H., Cho, S.R., Ahn, K.S., Yang, J.O. and Kim, G.H., 2011. Repellency of lavender oil and linalool against spot clothing wax cicada, Lycorma delicatula (Hemiptera: Fulgoridae) and their electrophysiological responses. *Journal of Asia-Pacific Entomology*, *14*(4), pp.411-416. DOI: 10.1016/j.aspen.2011.06.003

Zeddies, J., Schaab, R.P., Neuenschwander, P. and Herren, H.R., 2001. Economics of biological control of cassava mealybug in Africa. *Agricultural Economics*, *24*(2), pp.209-219. DOI: 10.1016/S0169-5150(00)00064-5

Zhunisbai, R.T., Dinasilov, A.S. and Islamova, R.A., 2019. Brown marmorated stink bug (Halyomorpha halys)-a new invader in the territory of the Republic of Kazakhstan. *Zashchita i Karantin Rasteniĭ*, 10, pp.38-40.

Ziska, L.H. and George, K., 2004. Rising carbon dioxide and invasive, noxious plants: potential threats and consequences. *World Resource Review*, *16*(4), pp.427-447

Coupling traditional monitoring and citizen science to disentangle the invasion of *Halyomorpha halys*

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Abstract

The brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is an invasive pest that expanded its range outside of its original confinements in Eastern Asia, spreading through the United States, Canada and most of the European and Eurasian countries. The invasiveness of this agricultural and public nuisance pest is facilitated by the availability of an array of suitable hosts, an *r*-selected life history and the release from natural enemies in the invaded zones. Traditional monitoring methods are usually impeded by the lack of time and resources to sufficiently cover large geographical ranges. Therefore, the citizen science initiative "BugMap" was conceived to complement and assist researchers in breaking down the behavior of this invasive pest via a user-friendly, freely available mobile application. The collected data were employed to forecast its predicted distribution and to identify the areas at risk in Trentino, Northern Italy. Moreover, it permitted the uncovering of the seasonal invasion dynamics of this insect, besides providing insight on its phenological patterns, life cycle and potential management methods. Hence, the outcomes of this work emphasize the need to further integrate citizens in scientific endeavors, for resolving ecological complications and reducing the gap between the public and science.

Keywords: Pentatomidae; Environmental niche modeling; Citizen Science; Crowdsourcing; MaxEnt; QGIS; Brown marmorated stink bug.

1 Introduction

As defined by the Oxford Dictionary, citizen science (CS) is 'the collection and analysis of data relating to the natural world by members of the general public, typically as part of a collaborative project with professional scientists. Oftentimes in ecological studies, there is a large amount of data to process or an extensive geographic range to cover. This poses a problem for a single researcher or even a small team of researchers (Dickinson et al., 2010). Citizen scientists could help fill this role if provided with the capabilities to effectively assemble and share data.

Having citizens participate in gathering scientific data has several benefits, including improved science and technology literacy among participants and reduced costs (Danielsen et al., 2005). Studies also suggest that engaging citizen volunteers makes it more likely that programs collect data relevant to local conservation and management issues (Danielsen et al., 2005; Measham, 2007). Such data may improve professional predictions on species' future distributions, allowing the timely dissemination of these results to an educated public (Brossard et al., 2005). Volunteering citizens may also have access to lands that may not be accessible to professional scientists, allowing them to discover invasive species not yet detected elsewhere (Lepczyk, 2005).

The field of ornithology has the longest history of CS (Greenwood, 2007), with thousands of amateur and professional ornithologists worldwide. One would assume that arthropods might not be as alluring for the ordinary citizen as much as birds are. Nevertheless, some of the more colorful insects have indeed caught the public's eye. The North American Butterfly Association (NABA) has initiated a program to monitor butterflies, in order to better quantify their range and abundance. Moreover, crowdsourced records on the periodical cicada, *Magicicada* spp., through the website www.magicicada.org, have been used to build mapped distributions of this insect to detect its range changes (Cooley, 2015). Mosquito Alert is another CS project developed in recent years to assist in the monitoring and management of disease carrying mosquitoes, *Aedes albopictus* Skuse (1894) and *Aedes aegypti* Linnaeus (1762). Citizens are invited to report sightings of the insects or of potential breeding sites; this information is communicated to public health managers to monitor and control the spread and damage caused by these "urban *Aedes*" (Palmer et al., 2017).

One of the most documented expressions of global anthropogenic forcing is the human-induced movement of non-native species (Hulme et al., 2008). This phenomenon usually refers to the voluntary or accidental introduction of taxa or genotypes far from their historical distributional areas as a result of trade, tourism, agriculture or biological control programs (Cini et al., 2014; Geslin et al., 2017).

The brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) is an invasive pest that was introduced into the United States from Asia in the mid-1990s (Leskey and Nielsen, 2018). It has spread throughout most of the United States, as well as into Canada (Fogain and Graff, 2011). In Europe, *H. halys* was first detected in 2007 in Zurich, Switzerland (Wermelinger et al., 2008); its range has now expanded to include most of the European and Eurasian countries

(EPPO, 2018). It was first detected in Italy in 2012 in the province of Modena (Bariselli et al., 2016). This pest's feeding on pome fruits results in deformed, symptomatic produce with indents on the surface and corky spots in the flesh, debilitating their marketability (Nielsen and Hamilton, 2009).

Over US$ 21 billion worth of crops in the United States alone have been estimated to be threatened by *H. halys* feeding damage (ODA, 2017). Additional irritation by this pest lies in its overwintering behavior where it tends to aggregate in man-made structures (Inkley, 2012), rendering it a pervasive residential nuisance.

Some of the most severe agricultural and annoyance problems have been recorded in Italy (Bariselli et al., 2016). In the fall of 2017, the Friuli-Venezia Giulia region in North Eastern Italy, witnessed one of the gravest anthropogenic aggregations of the bug in recent years (http://www.udinetoday.it/cronaca/invasione-cimici-marmorata-asiatica-talmassons-medio-basso-friuli.html). In Trentino Alto Adige region in Northern Italy, *H. halys* was first detected in the spring of 2016 (Cesari et al., 2017). Its presence in this region poses an imminent threat to vineyards and especially to the apple industry which accounts for 65% of the Italian apple production (Trentino Agricoltura, 2018).

The recording, mapping and monitoring of invasive species are prerequisites for successful biological invasion risk management (Roura-Pascual et al., 2010). Thus BugMap, a mobile application, was designed with this purpose in mind. It is a CS approach that aims at collecting crowdsourced data on the occurrence of the alien *H. halys* in a newly invaded range. Obtained reports allow species distribution modelling

(SDM), which aims to predict the areas where environmental conditions are suitable for the survival and establishment of the pest (Jackson and Overpeck, 2000). For invasive species management, habitat suitability maps identify areas where invasive species (1) may actually be present, but yet undetected and (2) may disperse to in the future, thus providing assistance for planning and prioritizing areas for surveillance. Such information can also assist in determining the extent, cost and likelihood of success of a control program (Anderson et al., 2003).

However, invasive species distribution models (iSDMs) face special challenges because (1) they typically violate SDMs assumption that the organism is in equilibrium with its environment, and (2) species absence data are often unavailable or believed to be too difficult to interpret (Merow et al., 2013). In general, these modelling methods combine species locality data (geo-referenced coordinates of latitude and longitude from confirmed presence) with environmental variables to create a model of species requirements for the examined variables (Anderson et al., 2003).

Geographic information system (GIS) technologies are enhancing our ability to study and understand the large-scale spatial structure and dynamics of insect populations, as influenced by heterogeneous environments. In the past 20 years, advancements in mapping technology and access to tools that allow us to geo-reference our location allowed for increased acquisition and accuracy of data (Hahn et al., 2016). The ubiquity of the internet, cell phones and wireless technology has led to increasing importance of mobile GIS as a mode of data acquisition, which promoted increased interest in CS and crowdsourcing data (Silvertown, 2009). These technologies offer great potential in entomological research and contribute to the refining of monitoring and management methods of invasive alien pests (Hamilton, 2003; Dminić et al., 2010).

The scope of this study was to evaluate whether the contribution of volunteers would improve the existing monitoring strategies of an alien stink bug, freshly invading their territory and menacing their agricultural production. The effect of user training on the accuracy of citizen reports was evaluated and the amount of crowdsourced data was quantified and compared to reports obtained through traditional monitoring methods. Moreover, we used the collective data registered by both parties through BugMap to disentangle the invasion dynamics and phenology of *H. halys* in Trentino, Northern Italy. In addition, we mapped the potential distribution of this invasive pest based on the integration of both citizen monitoring and traditional methods. We expect this work to provide insight on the importance of such projects and the utility of combining crowdsourced and traditional survey data, for the improvement of ecological monitoring, species distribution mapping and invasive species management programs.

2 Materials and Methods

2.1 Data acquisition

2.1.1 Study area

The study area is located in Trentino, North Eastern Italy in an area covering 6,214 km^2 of land, south of the Alps. It is a mountainous region influenced by a continental climate, with most of the territory lying 1000 m above sea level and around 55% covered by coniferous and deciduous forests. Trentino includes developed touristic, agricultural, industrial and commercial areas that are connected by main roads and railway transport infrastructures, with a population of 537,000 inhabitants concentrated in the plain areas and in the valley floors (ISTAT, 2018). Despite its mountainous nature, agriculture remains one of the most important contributors to this region's economy with over € 800 million of sold agricultural produce in 2013 (Provincia di Trento, 2018). A significant proportion of this agricultural production is at risk from the establishment and spread of *H. halys*.

2.1.2 BugMap, a mobile-based application for crowdsourced *H. halys* reports

BugMap is a free mobile application that was designed by Edmund Mach Foundation bio-informaticians, initiated in autumn 2016 and compatible with both iPhone (Apple Inc. United States, California) and Android (Google LLC. United States, California) operating systems. It is a user-friendly platform that allows citizens to report the presence of this pest whenever encountered. A guidance section was added to familiarize the users with the morphological features of the different life stages of the bug. Notes on its invasive history, potential hosts, overwintering behavior and induced symptoms on host plants caused by *H. halys* feeding are also included. BugMap allows the gathering of information regarding how (trap, beating or visual), when (date) and where (location) the insect was observed.

Reporting users start by (1) either indicating their location on the map in the application or by allowing their geographical coordinates to be automatically registered by BugMap. Next, a simple form must be filled with respect to (2) the date of the sighting. Mandatory segments also include (3) the number of specimens, (4) the phenological stage (adult, nymph, both or unknown), (5) type of sighting (visual, trap or beating methods) and (6) location (inside or outside buildings, garden-hedges, green urban areas, means of transport, bushes, wild areas or agricultural settings). Most importantly, the form needs to be accompanied by (7) a photograph of the suspected insect.

Five experts swiftly assess the reports once submitted, as valid, invalid or unsure (in the case of unclear photographs). To reduce the evaluation bias, each validation is double-checked and amended in case of any doubt by the other experts. Additionally, a feedback section allows experts to send back a message through the application to the users, thanking them for their contribution, explaining the differences between the reported species and *H. halys* in the case of invalidity and in some instances requesting a clearer photograph when diagnosis cannot be made on the basis of the current one.

2.1.3 BugMap campaign

An advertisement campaign was initiated for BugMap shortly after the final design of the platform. Talks were delivered in the University of Trento for Bachelor and Masters' students of applied ecology. In the school of Edmund Mach Foundation, technical days were planned to involve students and technicians in the identification and reporting process of *H. halys*. An exhibition in the Museum of Science of Trento (MUSE) was organized during the "notte dei ricercatori", where the application was introduced to scientists from various fields and to the general public. Presentations and abstracts in conferences (IPM 3.0, First Italian Citizen Science conference) allowed the international community of citizen science and integrated pest management to familiarize with BugMap and understand its significance from an ecological and a social perspective. The appearances of co-authors on Italian television channels helped the dissemination of BugMap to a larger audience outside of the study region.

Various social media platforms such as Instagram (#bugmap) and Facebook (https://www.facebook.com/Bugmap-1926843807640177/) were also employed in order to further the spread of the initiative, with pages created and managed to facilitate the learning of citizens about this ecological monitoring method and on the menace posed by the invasive bug in question. Moreover, flyers (Figure S2.1) depicting the identification and reporting process of the target pest were designed and spread in all of the above-mentioned locations.

2.1.4 Pheromone traps

When the insect was detected in Trentino alto-Adige in the spring of 2016, phytosanitary services of the Trento province, along with Edmund Mach Foundation have placed pheromone traps (RESCUE!®, Sterling International, Inc U.S.A.) in different areas of the province. The traps (n=18) were positioned in various green urban areas, apple and pear orchards and large parking spaces, in an attempt to elucidate the insect's hitchhiking behavior (Figure 2.1). They were used for capturing the stink bug starting from May-June 2016 until September-October 2017, with bi-weekly control. Traps are amended with the *H. halys* aggregation pheromone components, along with a synergist that improves the attractive properties of the mixture, and functions in a 30 meters radius. These traps have a non-toxic mode of action, capturing males, females and all stages of the bug from nymphs to adults.

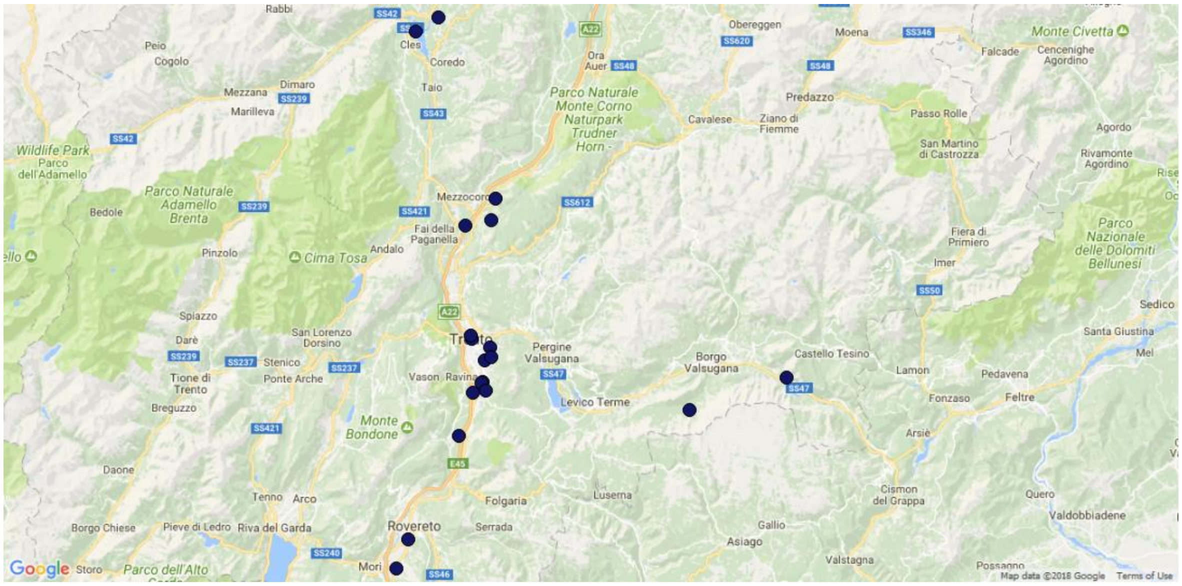

Figure 2.1. An Open Street Map of the area monitored by pheromone traps, dark-blue dots representing the coordinates of the traps placed in orchards, field crops, public parks, outdoor parking spaces, bushes and anthropogenic settings.

2.2 Modeling current and potential distribution of *H. halys* in Trentino

2.2.1 Environmental predictors

A distinct set of environmental parameters with potential effect on the insect's distribution were selected, as described by Capinha and Anastácio (2011). Digital Elevation Model, land-use, hydrography, road network and forest tracks were employed (Table 2.1) and are all freely available at the PAT cartographic portal of the Autonomous Province of Trento (Autonomous Province of Trento, 2017).

Table 2.1. List of the environmental GIS layers included in the analysis.

Index	Spatial resolution	Parameter
Digital elevation model (DEM)[a]	10 m	Slope Aspect Average hours of sun per season
Land use [b]	10 m	Continuous urban fabric Green urban areas Fruit trees and berry plantations
Hydrography	10 m	Distance from rivers Distance from lakes

[a] from the digital elevation model the slope, aspect and hours of sun were calculated and derived using GRASS GIS version 7. These three variables are a good proxy for temperature in a mountainous environment.

[b] land use was classified in GRASS GIS into 30 classes (Table S2.1) to avoid co-linearity and account for the species' ecology.

2.2.2 *Halyomorpha halys* MaxEnt distribution modeling

For modeling the species' distribution, the software MaxEnt was used (MaxEnt version 3.3.3; http://www.cs.princeton.edu/wschapire/maxent/), which is a machine learning algorithm that applies the principle of maximum entropy to predict the potential distribution of species from presence only (PO) data and environmental variables (Merow et al., 2013; Philips et al., 2006). PO data collected from BugMap and pheromone traps were input in MaxEnt, as well as the set of environmental predictors across Trentino landscape. The program attempts to estimate a probability distribution of species occurrence that is closest to uniform while still subject to environmental constraints (Elith et al., 2011). All data have been resampled at 100 meters resolution to increase the speed of calculation in MaxEnt using the jackknife test for determining variables that reduce the model reliability when omitted.

Previous models assessing the potential distribution of *H. halys* worldwide also utilized MaxEnt, using a resolution of 4.5 Km at the equator and strictly employing bioclimatic variables (Zhu et al., 2012).

2.2.3 Accounting for BugMap sampling bias

According to Fourcade et al. (2014), the best methods to increase overall model performance with a travel-time biased data set were (1) Systematic Sampling and (2) providing a bias file to MaxEnt as recommended by the manual (Elith et al., 2011). The bias file was calculated in GRASS (Geographic Resources Analysis Support System) GIS version 7 (2015), using the module for computing a Gaussian kernel from data points with a radius of 500 m and then rescaled to a range of 1 to 20 (Phillips et al., 2009). This raster file represents the sampling effort and it is an input for MaxEnt utilized to weigh the random background data (Elith et al., 2011).

The subsampling grids of 500 m and 1000 m were generated by QGIS (version 2.18.16) vector toolbox and then a single point for each square was randomly sampled and used as input. Four different MaxEnt models were run with default parameters settings, using 30% of data for training as follows: 1) full dataset with no bias file 2) full dataset with bias file 3) systematic sampling over a grid of 500 m 4) systematic sampling over a grid of 1000 m. Receiver operating characteristics analysis (ROC) was performed in R (2015), to compare the Area Under the Curve (AUC) of all the models in order to identify the best bias treatment solution for our case study (Cantor et al., 1999).

2.2.4 Setting the threshold of *H. halys* distribution model

The output from the MaxEnt models is a map of logistic values ranging from 0 to 1; however, the interpretation of this continuous output is not straightforward, so it is a common practice to reclassify the map in a binary format according to a cut-off value. The use of the default 0.5 cut-off value is not recommended (Elith et al., 2011), especially when presence and background data are unbalanced as in our case (Cantor et al., 1999). The ROC plot-based approach was adopted, as suggested by (Cantor et al., 1999; Liu et al., 2005). The analysis of the ROC curve allows to identify the point that maximizes the sensitivity against 1- specificity and that is considered the best classifier for the data (Liu et al., 2005).

3 Results and Discussion

3.1 Citizens' impact

3.1.1 Citizen science VS traditional monitoring

A total of 306 valid *H. halys* registrations were split between those done visually by citizens, and those obtained by traditional monitoring activities such as installment of pheromone traps and tree-beating methods. Volunteers contributed to the monitoring with 250 reports, compared to 56 by technicians (Figure 2.2). The majority of citizen-sightings (214) reported <10 bugs, compared to 31 by technicians. Large aggregations of the insect (>50) were reported by both citizens and technicians.

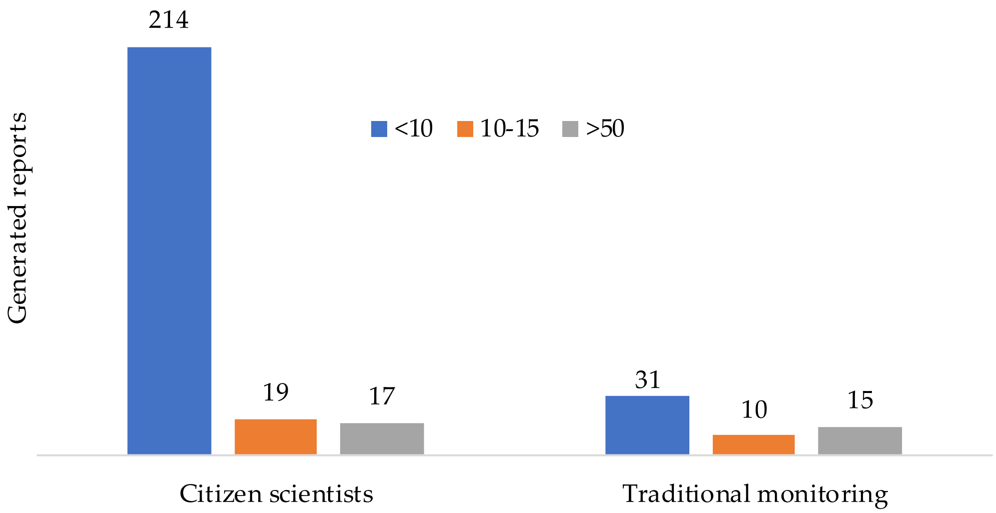

Figure 2.2. A representation of the different BugMap reports registered by citizens and those registered by technicians, adopting traditional monitoring methods (Pheromone traps, tree-beating). Blue bars: <10 specimens; orange bars: 10-15 specimens; grey bars: >50 specimens.

Valid *H. halys* reports were also classified according to the location of the sighting and to the reporting entity, in our case citizen scientists and technicians (traditional monitoring). All the reports from means of transport and almost all reports from buildings (99%) were performed by citizens (Figure 2.3). Similarly, citizen reports far exceeded those registered by technicians in gardens, agricultural and wild areas. On the other hand, traditional monitoring methods reported 63% of the total sightings from green urban areas.

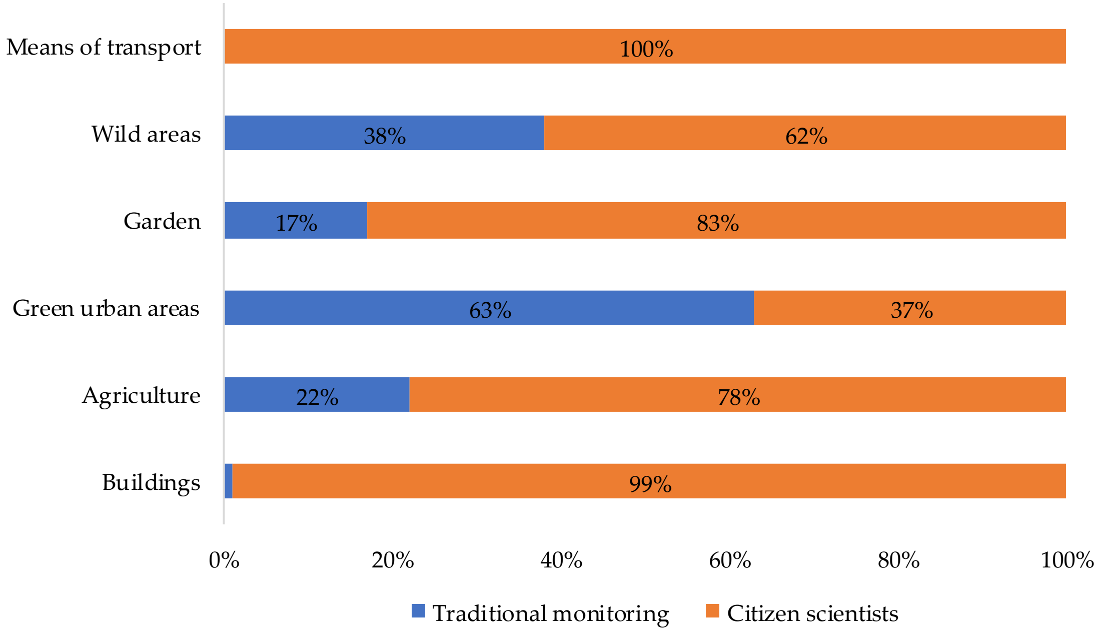

Figure 2.3. A comparison between traditional monitoring and citizen contribution, taking into account the percentage of reports generated by the two parties from various sighting locations.

These results highlight the differences between the two contributing parties, with citizens generating a larger number of reports from more diversified geographical areas. Reports from the general public allowed the identification of a heavily infested nucleus in a public park "Parco Gocciadoro", outside the city of Trento. This knowledge puts forward the possibility to either treat this zone with selective insecticides, or increase trap density to maximize catches, thus reducing the risk of diffusion to nearby agricultural settings. The real-time tracking of the spread and distribution of insects is usually impeded by technical hitches (lack of time, traps and technicians). Acquainted citizens provided insight on the presence of the insect by performing a more intensive sampling of premises that are difficult to reach by a team of scientists. Thus, the complications aforementioned have been alleviated thanks to the involvement of citizens in the monitoring activity (Figure S2.3, S2.4).

These outcomes are in alignment with the proposition of Dickinson et al. (2010), who stated that properly trained volunteers could help fill the role of professional scientists regarding the prediction of species distribution.

The highest number of registrations originated from buildings (157), which might be due to the possibility that citizens are far more likely to encounter the bug in their lodgings during the colder months of the year, and to the association between urban development and the initial phase of *H. halys* establishment and dispersal (Wallner et al., 2014). A total of 64 reports were registered in agricultural areas, followed by green urban areas (52), gardens (23), wild areas (8) and means of transport (2). Out of the 52 registrations from green urban areas, 20 reported large aggregations of insects (>50), along with 11 sightings of 10-15 insects. This can be explained by the availability of an array of desired host plants in public parks that serve as feeding and breeding grounds for *H. halys*, i.e. *Robinia pseudoacacia*, *Fraxinus* sp., *Acer* sp., *Cornus* sp., and *Corylus avellana*, as well as by the proximity of these hosts to overwintering shelters (Maistrello et al., 2016).

A possible means for reducing the populations of this pest is to have citizen volunteers deploy small, pyramid-style pheromone traps to maximize the insect catches in nearby urban settings (Sargent et al., 2014). These preventive control measures should be diapause-aware, meaning that they should be executed in spring and late-fall, to gradually reduce the pest population and minimize the damage on sensitive crops throughout the season (Rossi-Stacconi et al., 2016). *Halyomorpha halys* is an adept hitchhiker, often detected in vehicles and freight shipment (Holtz and Kamminga, 2010; Tindall et al., 2012). Although BugMap reports from means of transportation were low (1%), the first detection of *H. halys* in Trentino can be traced back to a family entering via an infested rental car from the neighboring, pest-ridden Veneto region (Anfora, personal communication). This illustrates how the stowaway behavior of this insect can generate a cascade of social, economic and ecological losses.

3.1.2 The effect of training on user performance

BugMap users can register through Facebook (Fcb), Edmund Mach Foundation (FEM) or remain anonymous. A total of 125 users were registered to the application, 73 of them were active and participated in reporting the insect, while the other 52 were inactive. In order to compare the effect of training on the accuracy of reporting the target insect, users were split between those registered through FEM and those through Fcb. FEM users produced 174 reports, 79% of which were accurate, compared to 71 reports by Fcb users, of which 64% correctly identified *H. halys*. The higher accuracy and performance of FEM users could be due to the hands-on training they received, and to their familiarity in dealing with arthropods, which stems from working in a scientific and agricultural environment. Our results go in accordance with the study of Crall et al. (2010), who noticed that in-person training improved the data collected on invasive species by volunteers. Facebook users performed fairly well, with a reporting accuracy of 64%, meaning that they are generally aware of the alien invasion and that BugMap-based educational tools are helpful, but may need further refining.

Halyomorpha halys adults were often confused with native pentatomids i.e. *Raphigaster nebulosa* Poda, *Dolycoris baccarum* L., both phytophagous species that may be competing with the invasive pest for the occupation of similar ecological niches. On another note, *Troilus luridus* F., *Arma custos* F. and *Pentatoma rufipes* L., also caused confusion, however these species are predators of eggs and juvenile plant pests and may contribute to the biological control of *H. halys*. All the latter organisms look alike to the untrained eye therefore, it comes as no surprise that both FEM and Fcb users generated false reports, corresponding to (21%) and (36%) respectively. This outcome is similar to what was observed by Maistrello et al. (2016), through their citizen science initiative to track *H. halys* via the website of the University of Modena and Reggio Emilia.

3.2 *Halyomorpha halys* invasion

3.2.1 Invasion dynamics in Trentino

The valid BugMap reports were classified seasonally (Figure 2.4), based on the locality of the sighting. A total of 306 valid reports were received during the two-year period since the initiation of BugMap until mid-February 2018. Few reports (n=17) were registered in 2016, the first year of the application release and local invasion, compared to 289 registrations in 2017. This might be due to the rising familiarity of the public with both the insects' invasion and BugMap, or an increasing population of the pest. *Halyomorpha halys* possesses biological characteristics that are common among successful colonists across taxa, including an *r*-selected life history and association with human-modified ecosystems (Kolar and Lodge, 2001). Its population growth can also be due to successful establishment and spread in Trentino by benefiting from host availability and being released from natural enemy pressure, leading to increased population density and fitness (Keane and Crawley, 2002). Similar observations in New Jersey, USA concluded that *H. halys* underwent a population increase of 75% each year, during the period spanning from 2004 to 2011 (Nielsen et al., 2013). An alternative mechanism in invasion ecology which could be linked to this pest's success is the Evolution of Increased Competitive Ability (EICA). This hypothesis states that a reduction in natural enemies could result in the selection for invasive populations that invest less in defense mechanisms and shift resources towards improving growth and fecundity, thereby achieving a competitive advantage over native species (Blossey and Notzold, 1995; Lee and Klasing, 2004).

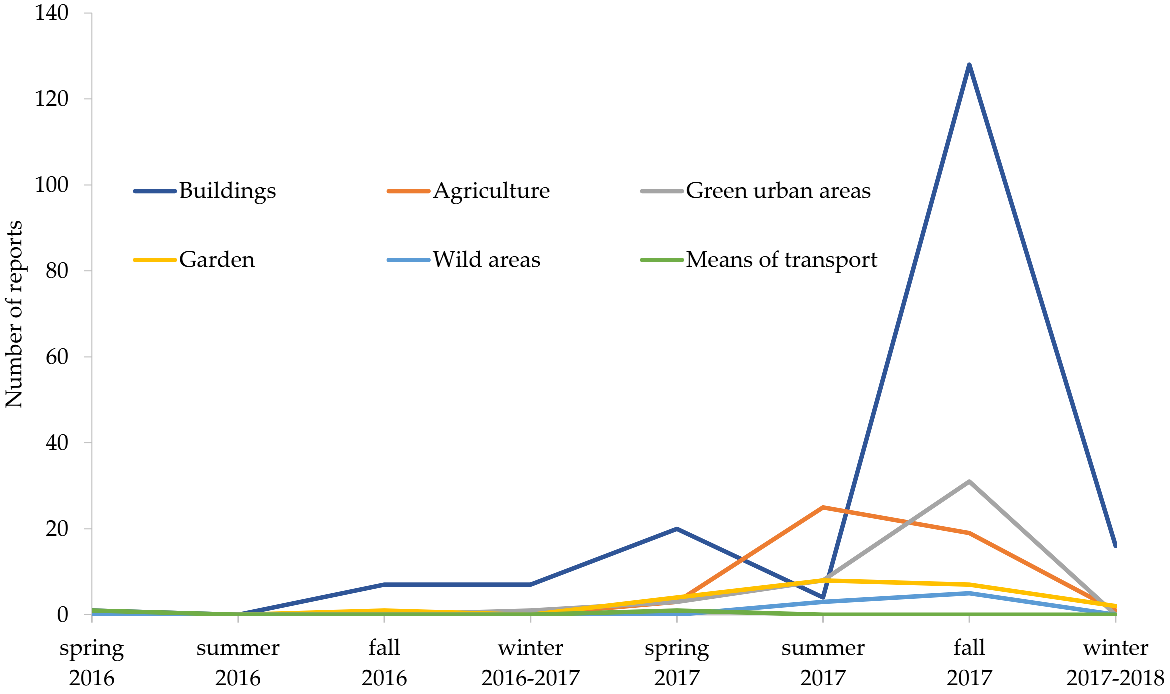

Figure 2.4. A representation of the varying number of valid *H. halys* reports in different localities since the initiation of BugMap in spring 2016 up until February 2018.

The first sightings in 2017 were registered in February-March, with individuals and small aggregations of adults being reported from buildings. Such registrations kept on increasing (n=20) until spring 2017. Afterwards, a decline of reports from anthropogenic structures coincided with an increase of sightings in agricultural areas (n=25) in the summer of 2017, indicating a possible exit from overwintering sites and dispersal onto host plants for feeding and mating. During late-summer and early-fall, there was an upsurge of reports from buildings (n=128) and green urban areas (n=31). This trend can be explained by the possibility that woody ornamental plants spread in public parks and in the vicinity of man-made shelters, provide early-and late-season resources for adults emerging from and returning to overwintering sites (Bergmann et al., 2016).

These findings are analogous with the knowledge on the dynamics of *H. halys*; being a landscape-level pest that moves across habitats throughout the season (Philips et al., 2016). It aggregates, enters in diapause and spends the winter in dead or standing trees and prevalently in anthropogenic structures; a behavior which may result in reduced overwintering mortality (Inkley, 2012). Rising temperatures in spring are believed to be responsible for breaking diapause, whereby insects start moving out of man-made structures into adjacent fields in search for nutritive hosts (Nielsen et al., 2017). During fall, declining temperatures and shorter days trigger the shelter-seeking behavior of the insects (Haye et al., 2014), which could explain the peak of reports from buildings and green urban areas.

3.2.2 Seasonal phenology of *H. halys*

Adults were consistently reported year-long, however, their density dramatically increased during fall 2017, with 102 generated reports (Figure 2.5). The first appearance of nymphs happened in June 2017, with 13 reports during summer, increasing in fall to 38 and disappearing in winter. Reports of both life stages occurred in summer (16) and their incidence increased in fall (34). The number and density of adults from trap catches during fall (13 reports; 5 were >50 insects) were far greater than those generated in spring (3 reports of individual insects) and summer (5 reports; none were > 50 insects). It has been previously noted that all *H. halys* life stages are attracted to the pheromone season-long (Morrison et al., 2016). Our observations of increasing numbers and catch density during fall can probably be explained either by a rising population, or that *H. halys* sensitivity might be higher during this season, given that their ability to aggregate in suitable shelters during that period directly affects their winter survival.

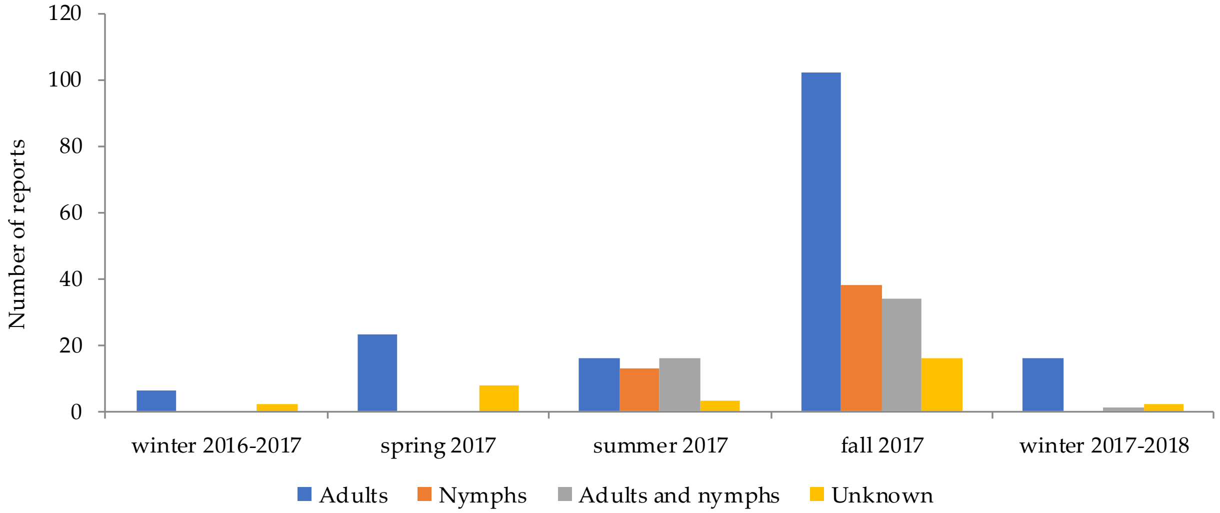

Figure 2.5. The number of sightings of different *H. halys* life stages is showcased with respect to the date of the report. Life stages are classified according to the original reports; "unknowns" are specimens that users were unable to classify.

Our results go in accordance with other observations on the phenology of this pest, in the climatically similar mid-Atlantic region in the United States, where spring-adults also emerge from overwintering sites in late-spring (Bergh et al., 2017). Females are believed to be reproductively immature in early spring, resulting in a delay in reproduction (Nielsen and Hamilton, 2009), hence the first appearance of nymphs in Trentino followed in summer. The decrease of adult observations during summer is probably due to their dispersion onto host plants within the forest edge for early season feeding and perhaps oviposition (Leskey and Nielsen, 2018). Sightings of fall-adults peaked in the period of September to November; this can be related to a seasonal population increase (Wallner et al., 2014), with spring-and summer-adults mating and laying eggs that in turn develop into mature stages. The occurrence and overlapping of adults and nymphal stages during summer and fall, hints towards a bi-voltine life cycle in Trentino, which is consistent with biological studies in other

Italian regions that characterized two *H. halys* generations per year (Maistrello et al., 2016). Based on this data, BugMap can accurately estimate the emergence of the 1st and 2nd generation adults. The rapid decline of nymphal populations in late fall is probably due to fifth instars molting to the adult stage or mortality due to frost (Nielsen and Hamilton, 2009).

3.2.3 Menace to agricultural areas

In 2016, there were no reports of the bug from cultivated zones; it was first sighted in agricultural areas in the spring of 2017, representing the first Trentino case of an open field crop infestation. A total of 64 reports were registered from cultivated areas. Apple orchards constitute the zones with the highest number of *H. halys* sightings (Figure 2.6). Other crops include cherry, peaches and small fruits (17%), vegetable crops (5%) and vineyards (4%). All life stages of the stink bug (adults and nymphs) were found in orchards, vegetable crops and vineyards.

Most reports (79%) from agricultural areas were registered during late-summer and early-fall (September and October). This can be related to the fact that this period, with temperatures ranging between 17-19 °C, represents the most preferable feeding time of the year for *H. halys* (Wiman et al., 2014). Studies on the severity and damage inflicted on key crops found that late-season apple is more susceptible to economic injury caused by *H. halys* feeding (Acebes-Doria et al., 2016; Joseph et al., 2015). The overlap between these two notions is particularly alarming, given the ubiquity of late-season apple orchards throughout the Trentino territory; raising the need to control this pest before harvest time to minimize losses. A possible means of control would be the exploitation of the behavioral ecology of this insect, through the adoption of a border-based attract-and-kill technique (Morrison et al., 2016). The latter enhances the strong 'edge-effect' exhibited by the insect (the tendency to inhabit trees at the orchard perimeter), by baiting select border row trees with pheromone traps, and subsequently treating them with effective insecticides. This method was found efficient in arresting *H. halys* in a 2.5 m radius around baited trees, and damage to fruit was significantly reduced in the remainder of the apple orchard.

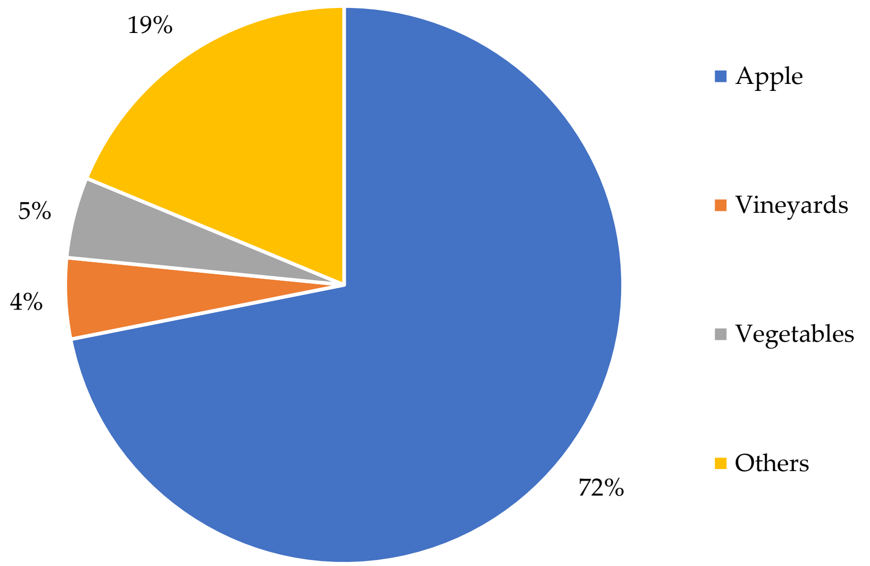

Figure 2.6. A display of the proportion of reports from different agricultural cultivations, with n=64 reports from agricultural cultivations.

3.3 *Halyomorpha halys* projected distribution in Trentino

3.3.1 MaxEnt-generated suitability map

The occurrence data collected on BugMap platform exhibit a well-known geographical bias pattern termed travel-time bias (Table S2.5) (Fourcade et al., 2014;

McCarthy et al., 2012). Reports are especially concentrated in the urban area of Trento and in the neighboring villages, while records from open field are rare. After trials with different bias treatment methods, the best performing MaxEnt model corresponded to systematic sampling over a grid of 500 m, utilizing 144 valid reports that were used to train (70%) and to test (30%) the model. The aforementioned model had the highest AUC value for training data with 0.982, AUC test data: 0.970 (full dataset AUC training: 0,980, AUC test: 0,974; full dataset with bias file AUC training: 0,976, AUC test: 0,970; systematic sampling 1000 m AUC training: 0,982, AUC test: 0,963). The Jackknife test for assessing variable contribution revealed that the parameters most affecting the dispersal of the bug are the digital terrain model, urban land use and distance from houses and from streets, respectively (Figure S2.6). Therefore, the high suitability of the Adige valley across Trentino can probably be explained by its appropriate elevation, as well as by the prevalent agricultural-urban interfaces in Trento (Figure 2.7). For a polyphagous species that browses across landscapes tracking crop phenology, these diverse rural boundaries may facilitate *H. halys* population growth by offering diverse host plants that meet its nutritional requirements, in addition to natural and human-made overwintering structures (Nielsen and Hamilton, 2009; Wallner et al., 2014; Acebes-Doria et al., 2016; Venugopal et al., 2014). Studies on *H. halys* haplotype diversity in North America and Europe revealed that Italy housed the second most diverse population of the bug, with 2-8 haplotypes represented in Emilia-Romagna, Piemonte and Veneto regions. This suggests that there is an ongoing invasion in Italy, with frequent reintroductions of the bug from several localities (Morrison et al., 2017). Through the Adige valley passes one of the main traffic corridors (Brennero) linking Italy to the rest of Europe, thus *H. halys* populations in this valley are thought to be periodically augmented by stratified diffusion via human transportation (i.e. movement of plants, goods and contaminated cargo) from the heavily infested Central Italian regions.

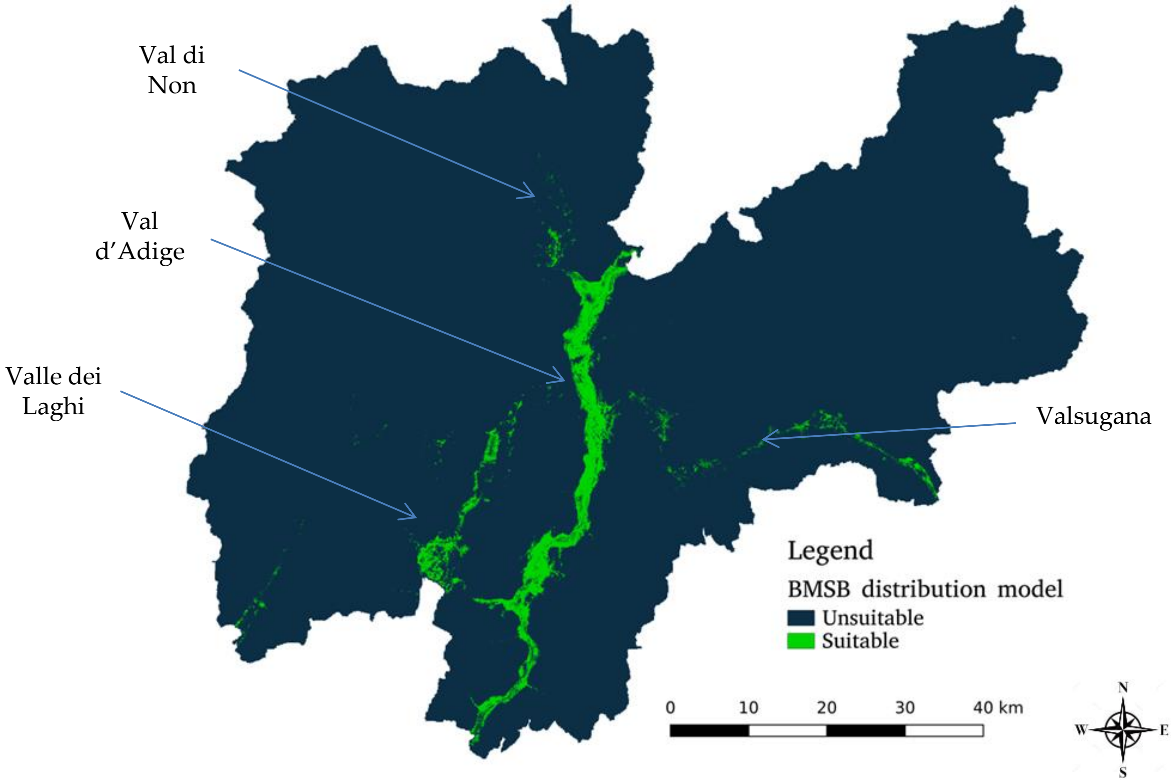

Figure 2.7. A suitability map of Trentino generated by MaxEnt, illustrating the areas suitable for the establishment of *H. halys* (BMSB). ROC analysis was performed on the results of the 500 m sub-sampled model allowing the identification of the best cut-off value at 0.26 with a classification accuracy of 0.92. The logistic output of the map from MaxEnt was reclassified according to the best cut-off in two classes: unsuitable habitat below 0.26 of the logistic output and suitable habitat above that value.

To the West of the Adige, lies another suitable area which is Valle dei Laghi, nearby Garda Lake. This is a predominantly touristic zone characterized by a unique

Mediterranean climate; aspects that constitute major driving forces for the passive flow and establishment of the bug.

North of the map, a scattered suitability is projected for Val di Non, one of the most important apples growing regions of Trentino. Given that *H. halys* is a chill-intolerant species and mortality due to cold stress commences at temperatures as high as 4 °C (Cira et al., 2016), therefore the ascending altitudinal gradient in Val di Non might be hampering the capacity of *H. halys* to spend the winter and overcome cumulative cold temperatures in this region. This might explain the unsuitability for *H. halys* establishment in this critical zone for the time being. On another note, the registration of several valid BugMap reports from this area may be due to a possible source to sink population dynamic, with Val di Non populations being replaced each spring by migrants from the southern, highly suitable Adige valley. Such a behavior has been previously observed in Alberta for the diamondback moth *Plutella xylostella* L. (Dosdall, 1994). Therefore, we propose intensification of the monitoring activities in this region, and appeal for citizens and farmers to stay on guard for early-season inoculations, as spring-adults are easier to manage than late-season populations (Leskey et al., 2014). The impacts of climate change on the distribution of *H. halys* have been assessed and a Northward expansion of its suitable range is projected for Europe, as well as an increase of the number of annual generations (Kistner, 2017). These predictions indicate that in the absence of adequate control measures and lack of co-evolved natural enemies, *H. halys* will increasingly jeopardize agricultural areas around the world.

The suitable Eastern strip in Trentino corresponds to Valsugana. This area might be appropriate for *H. halys* establishment due to its richness in small fruits production and various horticultural crops, besides, its confinement to the East by the pest infested Friuli and Veneto regions, poses a ceaseless re-introduction risk of new individuals.

The global model produced by Zhu et al. (2012), is helpful in understanding the suitable areas for this insect's establishment. They indicated that the whole Italian peninsula is suitable for setting up breeding populations, whereas our regional fine-scale model accounts for the extreme altitudinal variation and land morphology in Trentino, while offering monitoring and management support for affected areas.

3.3.2 Nationwide involvement

A total of 431 reports were obtained from the whole of Italy, of which 306 were validated as accurate sightings of *H. halys*. Traditional monitoring methods were only employed in Trentino, and the contribution of citizens was also mostly focused in this same freshly invaded North Italian region. Therefore, 244 reports originated from Trentino, while the remaining 62 sightings were registered via BugMap in other Italian regions (Figure 2.8) namely: Veneto, Lombardia, Friuli, Piemonte, Liguria, Emilia Romagna, Toscana, Lazio and Basilicata.

The serendipitous reports from different parts of Italy indicate that although the application was not advertised there, however, protagonists from the general public exhibited awareness, excitement and motivation for addressing national ecological issues. This behavior suggests a rising environmental democracy, the notion of making science more accessible to the public and scientists more aware of local knowledge and public enthusiasm (Conrad and Hilchey, 2011). Moreover, the registration of *H. halys* in regions where it has not been previously detected represents an early warning that can be communicated to phytosanitary services and collaborators. The latter entities are then advised to establish monitoring strategies and start the employment of preventive techniques to limit the spread and potential damage that can be inflicted by this pest.

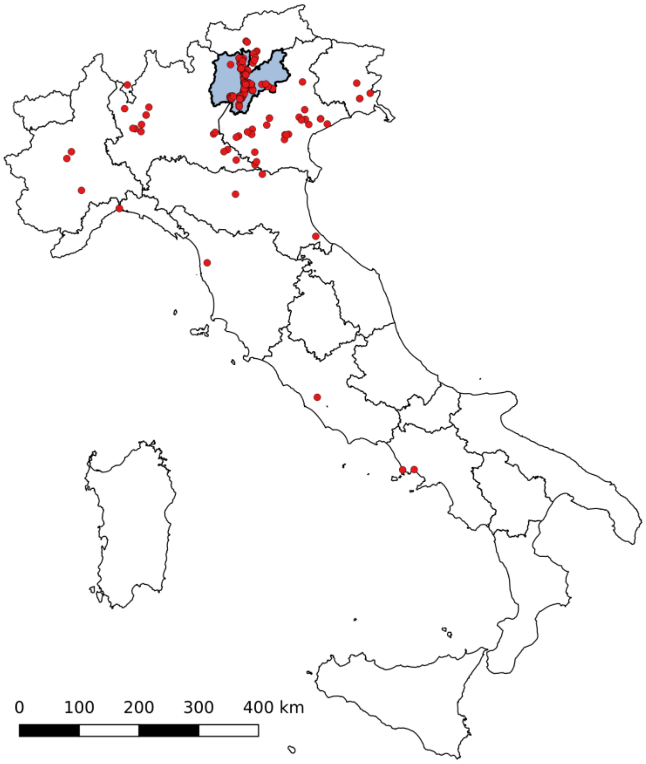

Figure 2.8. A map of Italy showcasing *H. halys* reports from the targeted Trentino (light blue) along with scattered registrations (red dots) from various Italian regions, offering an early warning for unsuspecting communities.

4 Conclusions

This study demonstrated that the coupling of volunteer-collected data with traditional ecological surveys is indeed instrumental for the improvement of existing and future monitoring programs and is worthy of the term ‘monitoring 2.0’ (Figure 2.9). Harnessing the capabilities of citizens helped uncover the invasion pattern and potential dissemination of the brown marmorated stink bug in Trentino. Although young and in its early stages, BugMap has proven efficient in stimulating scientific literacy and aided in raising public awareness regarding local ecological and economic efforts, a cornerstone towards a more active scientific citizenship. Future activities will aim at (1) elucidating the nutritional requirements of *H. halys* by projecting BugMap reports onto GIS layers of plant species in the urban area of Trento; (2) refining the MaxEnt models by including abiotic parameters such as temperature and humidity; (3) expanding BugMap domain to farther geographical boundaries and other invasive species; (4) utilize BugMap data to assess the accuracy of the different invasive species modeling approaches currently employed and (5) develop an image recognition algorithm for identifying and validating the increasing flux of BugMap reports.

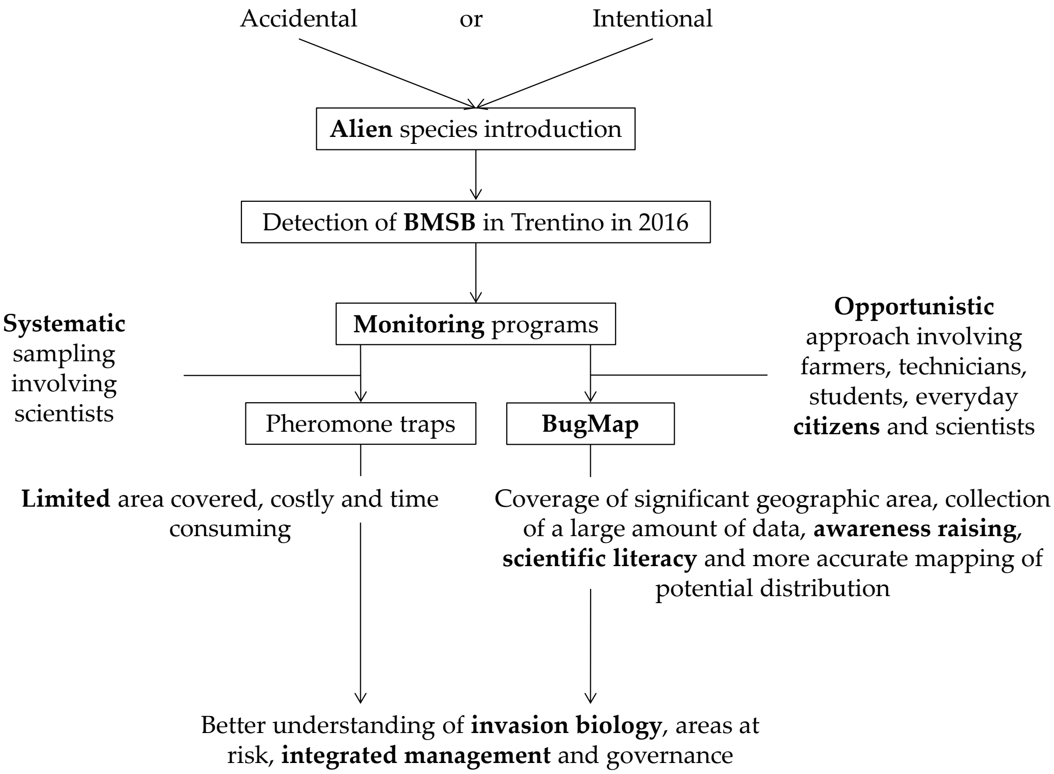

Figure 2.9. A summary of the workflow adopted in Trentino for tracking the spread of the invasive *H. halys* (BMSB), termed "Monitoring 2.0", that potentially constitutes a model for future programs.

Select supplementary material

PROVINCIA AUTONOMA DI TRENTO

FONDAZIONE EDMUND MACH

BugMap

Un'applicazione smartphone per monitorare la presenza della cimice asiatica che sta invadendo il Trentino

#bugmap

1

Scarica BugMap gratuitamente su Google Play Store
www.http://meteo.fmach.it/meteo/bugMap.php

2

Hai identificato una **cimice asiatica?**

3

Ogni cittadino può **segnalare** la presenza dell'insetto con BugMap!

BANDO "I COMUNICATORI STAR DELLA SCIENZA" - Legge provinciale 2 agosto 2005, n. 14, art. 22

Figure S2.2. BugMap flyer.

Table S2.1. Land use classes embedded in the environmental parameters of the MaxEnt model.

Nomenclature	**Class**
Continuous Urban fabric	1
Discontinuous urban fabric	2
Quarry	3
Dump sites	4
Green urban areas	5
Arable land	6
Vineyards	7
Fruit trees and berry plantations	8
Olive groves	9
Meadows	10
Heterogenous agricultural areas	11
Uncultivated vegetation	12
Broad-leaved forests	13
Coniferous forests	14
Natural pastures and high-altitude grasslands	15
Moors and heathland	16
Shrubs and dwarf pine	17
Schlerophyllous vegetation	18
Tree-lined grasslands	19
Tree-lined pasture	20
Bare rocks	21
Wooded cliffs	22
Perennial glaciers	23
Inland wetlands	24
Inland marshes	25
Peat bogs	26
Riversides and outcropping soils	27
Artificial waterbodies	28
Natural watercourse	29
Lakes	30

The remaining supplementary material can be found online at https://www.mdpi.com/2220-9964/7/5/171. Figure S2.3: BugMap real time tracking, Figure S2.4: Citizen monitoring and traditional monitoring; Table S2.5: Travel-time bias. Figure S2.6: Jackknife test for variable contribution

References

Acebes-Doria, A.L., Leskey, T.C. and Bergh, J.C., 2016. Host plant effects on Halyomorpha halys (Hemiptera: Pentatomidae) nymphal development and survivorship. *Environmental Entomology*, *45*(3), pp.663-670. DOI: 10.1093/ee/nvw018

Anderson, R.P., Lew, D. and Peterson, A.T., 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. *Ecological Modelling*, *162*(3), pp.211-232. DOI: 10.1016/S0304-3800(02)00349-6

Anfora, G. (Trento University, Trento, Trentino, Italy). Personal Communication, 2018.

Autonomous Province of Trento. 2017. http://www.territorio.provincia.tn.it/portal/server.pt/community/cartografia_di_base/260/cartografia_di_base/19024 (Accessed on August 6 2017).

Bariselli, M., Bugiani, R. and Maistrello, L., 2016. Distribution and damage caused by Halyomorpha halys in Italy. *Eppo Bulletin*, *46*(2), pp.332-334. DOI: 10.1111/epp.12289

Bergh, J.C., Morrison III, W.R., Joseph, S.V. and Leskey, T.C., 2017. Characterizing spring emergence of adult Halyomorpha halys using experimental overwintering shelters and commercial pheromone traps. *Entomologia Experimentalis et Applicata*, *162*(3), pp.336-345. DOI: 10.1111/eea.12539

Bergmann, E.J., Venugopal, P.D., Martinson, H.M., Raupp, M.J. and Shrewsbury, P.M., 2016. Host plant use by the invasive Halyomorpha halys (Stål) on woody ornamental trees and shrubs. *PLOS One*, *11*(2), p.e0149975. DOI: 10.1371/journal.pone.0149975

Blossey, B. and Notzold, R., 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology*, *83*(5), pp.887-889. DOI: 10.2307/2261425

Brossard, D., Lewenstein, B. and Bonney, R., 2005. Scientific knowledge and attitude change: The impact of a citizen science project. *International Journal of Science Education*, *27*(9), pp.1099-1121. DOI: 10.1080/09500690500069483

Cantor, S.B., Sun, C.C., Tortolero-Luna, G., Richards-Kortum, R. and Follen, M., 1999. A comparison of C/B ratios from studies using receiver operating characteristic curve analysis. *Journal of Clinical Epidemiology*, *52*(9), pp.885-892. DOI: 10.1016/s0895-4356(99)00075-x

Capinha, C. and Anastácio, P., 2011. Assessing the environmental requirements of invaders using ensembles of distribution models. *Diversity and Distributions*, *17*(1), pp.13-24. DOI: 10.1111/j.1472-4642.2010.00727.x

Cesari, M., Maistrello, L., Piemontese, L., Bonini, R., Dioli, P., Lee, W., Park, C.G., Partsinevelos, G.K., Rebecchi, L. and Guidetti, R., 2018. Genetic diversity of the brown marmorated stink bug Halyomorpha halys in the invaded territories of Europe and its patterns of diffusion in Italy. *Biological Invasions*, *20*(4), pp.1073-1092. DOI: 10.1007/s10530-017-1611-1

Cini, A., Anfora, G., Escudero-Colomar, L.A., Grassi, A., Santosuosso, U., Seljak, G. and Papini, A., 2014. Tracking the invasion of the alien fruit pest Drosophila suzukii in Europe. *Journal of Pest Science*, *87*(4), pp.559-566. DOI: 10.1007/s10340-014-0617-z

Cira, T.M., Venette, R.C., Aigner, J., Kuhar, T., Mullins, D.E., Gabbert, S.E. and Hutchison, W.D., 2016. Cold tolerance of Halyomorpha halys (Hemiptera: Pentatomidae) across geographic and temporal scales. *Environmental Entomology*, *45*(2), pp.484-491. DOI: 10.1093/ee/nvv220

Conrad, C.C. and Hilchey, K.G., 2011. A review of citizen science and community-based environmental monitoring: issues and opportunities. *Environmental Monitoring and Assessment*, *176*(1-4), pp.273-291. DOI: 10.1007/s10661-010-1582-5

Cooley, J.R., 2015. The Distribution of Periodical Cicada (Magicicada) Brood I in 2012 with Previously Unreported Disjunct Populations (Hemiptera: Cicadidae, Magicicada). *American Entomologist*, *61*(1), pp.51-56. DOI: 10.1093/ae/tmv004

Crall, A.W., Newman, G.J., Jarnevich, C.S., Stohlgren, T.J., Waller, D.M. and Graham, J., 2010. Improving and integrating data on invasive species collected by citizen scientists. *Biological Invasions*, *12*(10), pp.3419-3428. DOI: 10.1007/s10530-010-9740-9

Danielsen, F., Burgess, N.D. and Balmford, A., 2005. Monitoring matters: examining the potential of locally-based approaches. *Biodiversity & Conservation*, *14*(11), pp.2507-2542. DOI: 10.1007/s10531-005-8375-0

Dickinson, J.L., Zuckerberg, B. and Bonter, D.N., 2010. Citizen science as an ecological research tool: challenges and benefits. *Annual Review of Ecology, Evolution, and Systematics*, *41*, pp.149-172. DOI: 10.1146/annurev-ecolsys-102209-144636

Dminić, I., Kozina, A., Bažok, R. and Barčić, J.I., 2010. Geographic information systems (GIS) and entomological research: a review. *Journal of Food, Agriculture & Environment*, *8*(2), pp.1193-1198.

Dosdall, L.M., 1994. Evidence for successful overwintering of diamondback moth, Plutella xylostella (L.) (Lepidoptera: Plutellidae), in Alberta. *The Canadian Entomologist*, *126*(1), pp.183-185. DOI: 10.4039/Ent126183-1

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E. and Yates, C.J., 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, *17*(1), pp.43-57. DOI: 10.1111/j.1472-4642.2010.00725.x

EPPO Global Database. Available online: https://gd.eppo.int (Accessed on 13 January 2018)

Fogain, R. and Graff, S., 2011. First records of the invasive pest, Halyomorpha halys (Hemiptera: Pentatomidae), in Ontario and Quebec. *Journal of the Entomological Society of Ontario*, *142*, pp.45-48.

Fourcade, Y., Engler, J.O., Rödder, D. and Secondi, J., 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. *PLOS One*, *9*(5), p.e97122. DOI: 10.1371/journal.pone.0097122

Geslin, B., Gauzens, B., Baude, M., Dajoz, I., Fontaine, C., Henry, M., Ropars, L., Rollin, O., Thébault, E. and Vereecken, N.J., 2017. Massively introduced managed species and their consequences for plant–pollinator interactions. In *Advances in Ecological Research* (Vol. 57, pp. 147-199). Academic Press. DOI: 10.1016/bs.aecr.2016.10.007

GRASS Development Team. 2015. Geographic Resources Analysis Support System (GRASS) Software, Version 7.0. Open Source Geospatial Foundation, http://grass.osgeo.org

Greenwood, J.J., 2007. Citizens, science and bird conservation. *Journal of Ornithology*, *148*(1), pp.77-124. DOI: 10.1007/s10336-007-0239-9

Hahn, N.G., Kaufman, A.J., Rodriguez-Saona, C., Nielsen, A.L., LaForest, J. and Hamilton, G.C., 2016. Exploring the spread of brown marmorated stink bug in New Jersey through the use of crowdsourced reports. *American Entomologist*, *62*(1), pp.36-45. DOI: 10.1093/ae/tmw007

Hamilton, R.M. **2003.** Remote sensing and GIS studies on the spatial distribution and management of Japanese beetle adults and grubs. PhD dissertation, Purdue University, West Lafayette, IN.

Haye, T., Abdallah, S., Gariepy, T. and Wyniger, D., 2014. Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, Halyomorpha halys, in Europe. *Journal of Pest Science*, *87*(3), pp.407-418. DOI: 10.1007/s10340-014-0560-z

Holtz, T. and Kamminga, K., 2010. Qualitative analysis of the pest risk potential of the brown marmorated stink bug (BMSB), Halyomorpha halys (Stål), in the United States. *United States Department of Agriculture: APHIS*.

https://www.michigan.gov/documents/mda/BMSB_Pest_Risk_Potential_-_USDA_APHIS_Nov_2011_344862_7.pdf

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J. and Pyšek, P., 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology*, *45*(2), pp.403-414. DOI: 10.1111/j.1365-2664.2007.01442.x

Inkley, D.B., 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). *Journal of Entomological Science*, *47*(2), pp.125-130. DOI: 10.18474/0749-8004-47.2.125

Istat-Istituto Nazionale di Statistica. http://www.istat.it/en/ (Accessed on 16 February 2018).

Jackson, S.T. and Overpeck, J.T., 2000. Responses of plant populations and communities to environmental changes of the late Quaternary. *Paleobiology*, *26*(S4), pp.194-220. DOI: 10.1666/0094-8373(2000)26[194:ROPPAC]2.0.CO;2

Joseph, S.V., Nita, M., Leskey, T.C. and Christopher Bergh, J., 2015. Temporal effects on the incidence and severity of brown marmorated stink bug (Hemiptera: Pentatomidae) feeding injury to peaches and apples during the fruiting period in Virginia. *Journal of Economic Entomology*, *108*(2), pp.592-599. DOI: 10.1093/jee/tou059

Keane, R.M. and Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, *17*(4), pp.164-170. DOI: 10.1016/S0169-5347(02)02499-0

Kistner, E.J., 2017. Climate change impacts on the potential distribution and abundance of the brown marmorated stink bug (Hemiptera: Pentatomidae) with special reference to North America and Europe. *Environmental Entomology*, *46*(6), pp.1212-1224. DOI: 10.1093/ee/nvx157

Kolar, C.S. and Lodge, D.M., 2001. Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, *16*(4), pp.199-204. DOI: 10.1016/S0169-5347(01)02101-2

Lee, K.A. and Klasing, K.C., 2004. A role for immunology in invasion biology. *Trends in Ecology & Evolution*, *19*(10), pp.523-529. DOI: 10.1016/j.tree.2004.07.012

Lepczyk, C.A., 2005. Integrating published data and citizen science to describe bird diversity across a landscape. *Journal of Applied Ecology*, *42*(4), pp.672-677. DOI: 10.1111/j.1365-2664.2005.01059.x

Leskey, T.C. and Nielsen, A.L., 2018. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. *Annual Review of Entomology*, *63*, pp.599-618. DOI: 10.1146/annurev-ento-020117-043226

Leskey, T.C., Short, B.D. and Lee, D.H., 2014. Efficacy of insecticide residues on adult Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. *Pest Management Science*, *70*(7), pp.1097-1104. DOI: 10.1002/ps.3653

Liu, C., Berry, P.M., Dawson, T.P. and Pearson, R.G., 2005. Selecting thresholds of occurrence in the prediction of species distributions. *Ecography*, *28*(3), pp.385-393. DOI: 10.1111/j.0906-7590.2005.03957.x

Maistrello, L., Dioli, P., Bariselli, M., Mazzoli, G.L. and Giacalone-Forini, I., 2016. Citizen science and early detection of invasive species: phenology of first occurrences of Halyomorpha halys in Southern Europe. *Biological Invasions*, *18*(11), pp.3109-3116. DOI: 10.1007/s10530-016-1217-z

McCarthy, K.P., Fletcher Jr, R.J., Rota, C.T. and Hutto, R.L., 2012. Predicting species distributions from samples collected along roadsides. *Conservation Biology*, *26*(1), pp.68-77. DOI: 10.1111/j.1523-1739.2011.01754.x

Measham, T.G., 2007. Building capacity for environmental management: local knowledge and rehabilitation on the Gippsland Red Gum Plains. *Australian Geographer*, *38*(2), pp.145-159. DOI: 10.1080/00049180701392758

Merow, C., Smith, M.J. and Silander Jr, J.A., 2013. A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, *36*(10), pp.1058-1069. DOI: https://doi.org/10.1111/j.1600-0587.2013.07872.x

Morrison, W.R., Lee, D.H., Short, B.D., Khrimian, A. and Leskey, T.C., 2016. Establishing the behavioral basis for an attract-and-kill strategy to manage the invasive Halyomorpha halys in apple orchards. *Journal of Pest Science*, *89*(1), pp.81-96. DOI: 10.1007/s10340-015-0679-6

Morrison, W.R., Milonas, P., Kapantaidaki, D.E., Cesari, M., Di Bella, E., Guidetti, R., Haye, T., Maistrello, L., Moraglio, S.T., Piemontese, L. and Pozzebon, A., 2017. Attraction of Halyomorpha halys (Hemiptera: Pentatomidae) haplotypes in North America and Europe to baited traps. *Scientific Reports*, *7*(1), p.16941. DOI: 10.1038/s41598-017-17233-0

Nielsen, A.L. and Hamilton, G.C., 2009. Life history of the invasive species Halyomorpha halys (Hemiptera: Pentatomidae) in northeastern United States. *Annals of the Entomological Society of America*, *102*(4), pp.608-616. DOI: 10.1603/008.102.0405

Nielsen, A.L., Fleischer, S., Hamilton, G.C., Hancock, T., Krawczyk, G., Lee, J.C., Ogburn, E., Pote, J.M., Raudenbush, A., Rucker, A. and Saunders, M., 2017. Phenology of brown marmorated stink bug described using female reproductive development. *Ecology and Evolution*, *7*(17), pp.6680-6690. DOI: 10.1002/ece3.3125

Nielsen, A.L., Holmstrom, K., Hamilton, G.C., Cambridge, J. and Ingerson–Mahar, J., 2013. Use of black light traps to monitor the abundance, spread, and flight behavior of Halyomorpha halys (Hemiptera: Pentatomidae). *Journal of Economic Entomology*, *106*(3), pp.1495-1502. DOI: 10.1603/EC12472

Oregon Department of Agriculture: Pest Alert: Brown Marmorated Stink Bug Halyomorpha halys. 2017. http://www.oregon.gov/ODA/Pages/default.aspx (Accessed on 4 December 2017).

Oxford English Dictionary. "art, n.1." *OED Online*, Oxford University Press, January 2018, www.oed.com/viewdictionaryentry/Entry/11125. Accessed 21 January 2018.

Palmer, J.R., Oltra, A., Collantes, F., Delgado, J.A., Lucientes, J., Delacour, S., Bengoa, M., Eritja, R. and Bartumeus, F., 2017. Citizen science provides a reliable and scalable tool to track disease-carrying mosquitoes. *Nature Communications*, *8*(1), p.916. DOI: 10.1038/s41467-017-00914-9

Philips, C.R., Kuhar, T.P., Dively, G.P., Hamilton, G., Whalen, J. and Kamminga, K., 2016. Seasonal abundance and phenology of Halyomorpha halys (Hemiptera: Pentatomidae) on different pepper cultivars in the Mid-Atlantic (United States). *Journal of Economic Entomology*, *110*(1), pp.192-200. DOI: 10.1093/jee/tow256

Phillips, S.J., Anderson, R.P. and Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3-4), pp.231-259. DOI: 10.1016/j.ecolmodel.2005.03.026

Phillips, S.J., Dudík, M., Elith, J., Graham, C.H., Lehmann, A., Leathwick, J. and Ferrier, S., 2009. Sample selection bias and presence-only distribution models: implications for background and pseudo-absence data. *Ecological Applications*, *19*(1), pp.181-197. DOI: 10.1890/07-2153.1

Provincia di Trento. http://www.statweb.provincia.tn.it/ (Accessed on 20 February 2018).

R Core Team. 2015 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rossi-Stacconi, M.V., Kaur, R., Mazzoni, V., Ometto, L., Grassi, A., Gottardello, A., Rota-Stabelli, O. and Anfora, G., 2016. Multiple lines of evidence for reproductive winter diapause in the invasive pest Drosophila suzukii: useful clues for control strategies. *Journal of Pest Science*, *89*(3), pp.689-700. DOI: 10.1007/s10340-016-0753-8

Roura-Pascual, N., Krug, R.M., Richardson, D.M. and Hui, C., 2010. Spatially-explicit sensitivity analysis for conservation management: exploring the influence of decisions in invasive alien plant management. *Diversity and Distributions*, *16*(3), pp.426-438. DOI: 10.1111/j.1472-4642.2010.00659.x

Sargent, C., Martinson, H.M. and Raupp, M.J., 2014. Traps and trap placement may affect location of brown marmorated stink bug (Hemiptera: Pentatomidae) and increase injury to tomato fruits in home gardens. *Environmental Entomology*, *43*(2), pp.432-438. DOI: 10.1603/EN13237

Silvertown, J., 2009. A new dawn for citizen science. *Trends in Ecology & Evolution*, *24*(9), pp.467-471. DOI: 10.1016/j.tree.2009.03.017

Tindall, K.V., Fothergill, K. and McCormack, B., 2012. Halyomorpha halys (Hemiptera: Pentatomidae): a first Kansas record. *Journal of the Kansas Entomological Society*, *85*(2), pp.169-170. DOI: https://doi.org/10.2317/JKES120129.1

Trentino Agricoltura. 2018 Available online: http://www.trentinoagricoltura.it/Trentino-Agricoltura/Settori2/Ortofrutticoltura/Mela (Accessed on 9 January 2018).

Venugopal, P.D., Coffey, P.L., Dively, G.P. and Lamp, W.O., 2014. Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges. *PLOS One*, *9*(10), p.e109917. DOI: 10.1371/journal.pone.0109917

Wallner, A.M., Hamilton, G.C., Nielsen, A.L., Hahn, N., Green, E.J. and Rodriguez-Saona, C.R., 2014. Landscape factors facilitating the invasive dynamics and distribution of the brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae), after arrival in the United States. *PLOS One*, *9*(5), p.e95691. DOI: 10.1371/journal.pone.0095691

Wermelinger, B.E.A.T., Wyniger, D. and Forster, B.E.A.T., 2008. First records of an invasive bug in Europe: Halyomorpha halys Stål (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees?. *Mitteilungen-Schweizerische Entomologische Gesellschaft*, *81*(1/2), p.1. DOI: 10.5169/seals-402954

Wiman, N.G., Walton, V.M., Shearer, P.W. and Rondon, S.I., 2014. Electronically monitored labial dabbing and stylet 'probing' behaviors of brown marmorated stink bug, Halyomorpha halys, in simulated environments. *PLOS One*, *9*(12), p.e113514. DOI: 10.1371/journal.pone.0113514

Zhu, G., Bu, W., Gao, Y. and Liu, G., 2012. Potential geographic distribution of brown marmorated stink bug invasion (Halyomorpha halys). *PLoS One*, *7*(2), p.e31246. DOI: 10.1371/journal.pone.0031246

Monitoring 2.0: update on the *Halyomorpha halys* invasion of Trentino

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Abstract

"BugMap" is a citizen science mobile application that provides a platform for amateur and expert scientists to report sightings of two invasive insect pests, the tiger mosquito *Aedes albopictus* Skuse (Diptera: Culicidae) and the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae). The latter is a notorious pest of fruit trees, vegetables, ornamentals and row crops, inflicting severe agricultural and ecological disturbances in invaded areas. Our approach consists of coupling traditional monitoring with citizen science to uncover *H. halys* invasion in Trentino. The project was initiated in 2016 and the first results were reported in 2018. Here we revisit our initiative four years after its adoption and unravel new information related to the invader dispersal and overwintering capacity. We found that our previous model predicted the current distribution of *H. halys* in Trentino, with an accuracy of 72.5%. A new MaxEnt model was generated by pooling all reports received so far, providing a clearer perspective on areas at risk of the stink bug establishment in this North Italian region. The information herein presented is of immediate importance for enhancing monitoring strategies of this pest and for refining its integrated management tactics.

Keywords: citizen science; Northern Italy; invasive insect pests; crowdsourcing; long-term monitoring; brown marmorated stink bug

1 Introduction

Disentangling a species' invasion requires extensive sampling and surveys across large spatial and temporal scales, which can be challenging and costly for a small group of scientists trying to track invasive species. Citizen science is a potentially powerful means to overcome some of these challenges, whereby it enlists the public in collecting a large amount of data across an array of habitats and locations (Dickinson et al., 2010). Another major strength of citizen science is that it can aid in raising public awareness regarding local ecological issues and promote a more active scientific citizenship (Danielsen et al., 2005). Catalyzed by access to technology and the internet, citizen science is on the rise, with thousands of projects worldwide (https://scistarter.org/), yet, few involve agricultural pests (Ryan et al., 2018). Numerous projects were initiated in the USA and UK to monitor the species richness of "Lady Beetles" (UKLS; www.ladybird-survey.org; LLP; http://lostladybug.org and BLBB; http://ladybeetles.osu.edu). Another example is the Monarch Larva Monitoring Project (MLMP), a trinational long-term project concerning the monarch butterfly, *Danaus plexippus* and involving volunteers from across the United States, Canada and Mexico in monarch research (https://monarchlab.org/mlmp).

The brown marmorated stink bug, *Halyomorpha halys* is an invasive pest that feeds on over 300 plants, including economically important fruit trees, vegetables, row crops, as well as ornamentals (Lee et al., 2013; Rice et al., 2014). This insect is native to Eastern Asia, but its occupied range expanded outside of its areas of origin, probably due to global anthropogenic forcing (Hulme et al., 2008), thus spreading through most of the United States, Canada and numerous European and Eurasian countries (EPPO, 2019). In the Southern Hemisphere it has been recorded in Chile (Faúndez and Rider, 2017) and intercepted many times in Australia (Horwood et al., 2019) and New Zealand (Vandervoet et al., 2019). Feeding by *H. halys* results in deformed, symptomatic produce with indents on the surface and corky spots in the flesh, hampering marketability (Nielsen and Hamilton, 2009). Severe crop losses were registered in the USA and Europe, reaching millions of dollars per year (Leskey and Nielsen, 2018), as well as serious nuisance problems due to large *H. halys* aggregations in man-made structures (Inkley, 2012). Some of the most acute agricultural and nuisance problems were recorded in Italy (Bariselli et al., 2016; Maistrello et al., 2016). Besides its polyphagy, *H. halys* is capable of long-distance flight (Lee and Leskey, 2015) and hitchhiking on both animate and inanimate objects alike, making it a harder to control and to monitor landscape level pest.

About four years ago, we attempted to couple traditional monitoring strategies with a citizen science approach, whereby students, farmers, technicians and everyday citizens can report sightings of *H. halys* via ‘BugMap’, a freely available mobile application. The collected data were then employed to forecast its predicted distribution and to identify areas at risk in Trentino, instructing technical monitoring and management efforts. This tactic permitted the uncovering of *H. halys* seasonal invasion dynamics, phenological patterns and potential management methods in this

North Italian region. Monitoring 2.0: coupling traditional monitoring and citizens science, has thus proved effective in unraveling *H. halys* invasion in Trentino (Malek et al., 2018). The objective of this update is to utilize the newly recorded data points, to verify the accuracy of our previously calculated model on the projected distribution of the invasive bug in Trentino. We also reveal new information regarding the seasonal activity of the bug, namely its overwintering capacity in areas of high economic interest. We also aim to verify and highlight the far-reaching capacity of citizen science in such an initiative, spanning all Italian territories.

2 Materials and methods

2.1 Study area and BugMap campaign

Our study focuses on Trentino, North Eastern Italy, which is characterized by a continental climate and covering 6214 km^2 south of the Alps. The BugMap team has been generally advertising for the mobile application in the whole of Italy, with special attention to Trentino. Several social media platforms were used to actively disseminate information on the pest and the application, engaging followers year-round. This initiative was further circulated in several scientific and socio-technical events that took place in museums, in national and international conferences (Table 3.1).

Table 3.1. A summary of main events revolving around the BugMap campaign

Type of event	**Audience**	**Interactions**
Social media platforms	Local and International users	- BugMap Facebook: https://www.facebook.com/Bugmap-1926843807640177/ - BugMap Instagram: #bugmap #citizenscience #halyomorpha #downloadbugmap #cimice #stinkbug
Scientific gatherings	Researchers, PhD students and conference participants	- "Arbovirosis: emerging diseases": scientific workshop, Trento, Italy 2019 - "CitSci2019": international citizen science conference, Raleigh, North Carolina, (USA) 2019 - "The First Italian Citizen Science Conference", Rome, Italy 2017 - "IPM 3.0" Riva del Garda, Italy, 2017
Scholastic events	Students and pupils of various schools in Trento province	- "*Citizen science for kids! Alla scoperta della cimice asiatica"* - "hands-on activities on invasive insect species" - "*Let's make goals! The week of sustainable goals*" - "*Trentino for Biodiversity*"
Technical days and outreach activities	Citizens, researchers and journalists	- "*La Giornata Tecnica "Frutticoltura delle Valli del Noce"* - "*Green Week*": Natural History museum of Trento - "*The European Research Night of Trento"*: Natural History museum of Trento - "*Porte Aperte*": Fondazione Edmund Mach - "*Trento Economy Festival 2019"*: Aliens without borders - "Magazine article": Cassa rurale della Valsugana
Television and radio appearances	National and international citizens	- RaiTre Geo, October 2017

2.2 Citizen reports and seasonal classification

Since the initiation of this mobile application, the BugMap team, which includes technicians, computer scientists, science communicators, modelers and entomologists, has been receiving citizen feedback and constantly working towards enhancing citizen involvement. Even though the steps to follow are the same as the ones described in Malek et al. (2018), registering an *H. halys* sighting was made easier by refining map visualization, image upload and ameliorating overall user-experience.

Recorded sightings between February 21st, 2018 and October 1st, 2019 were seasonally classified according to the North Italian climate. In Trentino, 799 valid BugMap reports were obtained during that period, and were projected in QGIS (version 2.18.16) on the *H. halys* distribution model that we previously generated. These new 799 records of the species were used to validate the first model, developed using 144 record from the previous survey.

For the generation of BugMap reports' map of Italy and for computing the new MaxEnt model, we utilized all valid datapoints recorded thus far, spanning from May 2016 to October 2019.

2.3 *Halyomorpha halys* MaxEnt species distribution

Using the same environmental predictors applied in Malek et al. (2018), such as Digital Elevation Model, land-use, hydrography, road networks and forest tracks, along with all valid BugMap reports obtained so far, we modeled *H. halys* distribution in MaxEnt (v. 3.3.3) and ran Jackknife test to determine the variables that reduce the model reliability when omitted. The latter software attempts to estimate a probability distribution of species occurrence that is closest to uniform while still subject to environmental constraints (Elith et al., 2011).

In order to increase the model performance, we followed the recommendations of Fourcade et al. (2014), who suggested undertaking a systematic sampling of the records when the data are biased according to travel time. Performing a systematic sampling over a 500 m grid was found to be the best method to account for BugMap sampling bias (Malek et al., 2018), and we again selected randomly a single point for each mesh of the grid. In the end we ran the model using a total of 699 presence locations: 490 points were used for training the model and 209 (30%) for testing.

In order to determine the optimal cut-off value, we followed the ROC plot-based approach, allowing us to identify the point that maximizes the sensitivity of our model against 1-specificity (Elith et al., 2011; Cantor et al., 1999; Liu et al., 2005).

3 Results

3.1 Model accuracy

The logistic output of the previously generated map from MaxEnt was back then classified according to the cut-off value into two classes: unsuitable habitat below 0.26 of the logistic output and suitable habitat above that value. Projecting the new BugMap reports on the old model, we find that it predicts the distribution of the bug in Trentino with 72.5% accuracy, with 580 out of 799 new reports falling in the projected distribution range (Figure 3.1).

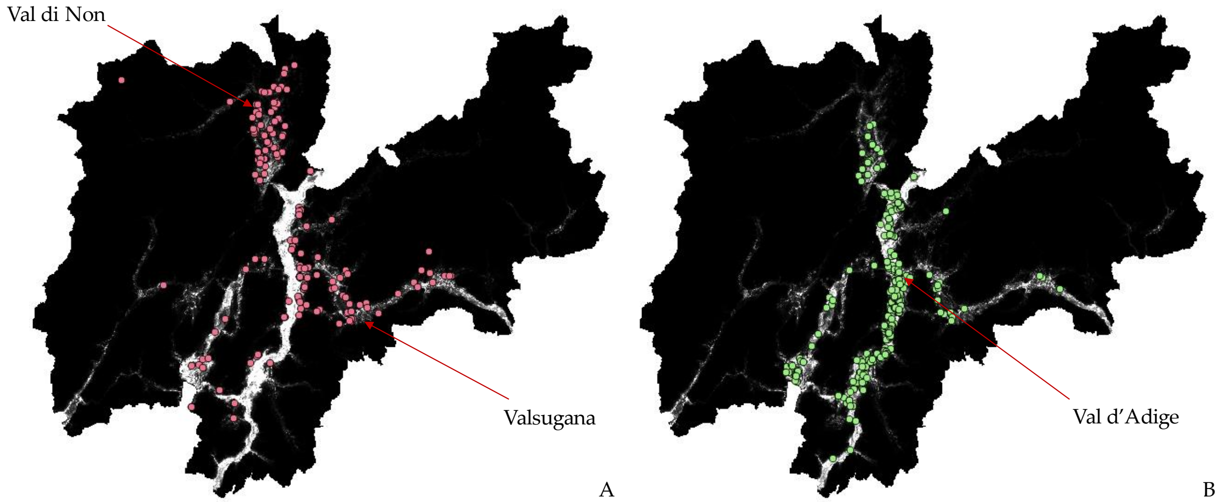

Figure 3.1. Maps showing the new BugMap reports falling outside (A) and inside (B) the previously predicted distribution range of *H. halys* in Trentino.

3.2 Seasonal distribution

Halyomorpha halys was reported all year round from most urban and agricultural areas of Trentino. In spring 2018 and 2019, 146 reports were recorded from Trentino (Figure 3.2), reflecting a seven-fold increase compared with reports recorded in 2016 and 2017. During the summer of 2016 and 2017, 49 reports were registered, compared with 260 reports in 2018 and 2019. Summer and autumn seem to be the seasons when both citizens and technicians record most *H. halys* sightings, with 32.5 % and 40.9 % of reports, respectively. During spring and summer, most reports were registered from the highly suitable Val d'Adige, while in autumn, an upsurge of BugMap reports was noted from Val di Non, an economically important agricultural area. Scattered sightings of the bug were registered during winter from all urban and agricultural valleys of the North Italian region.

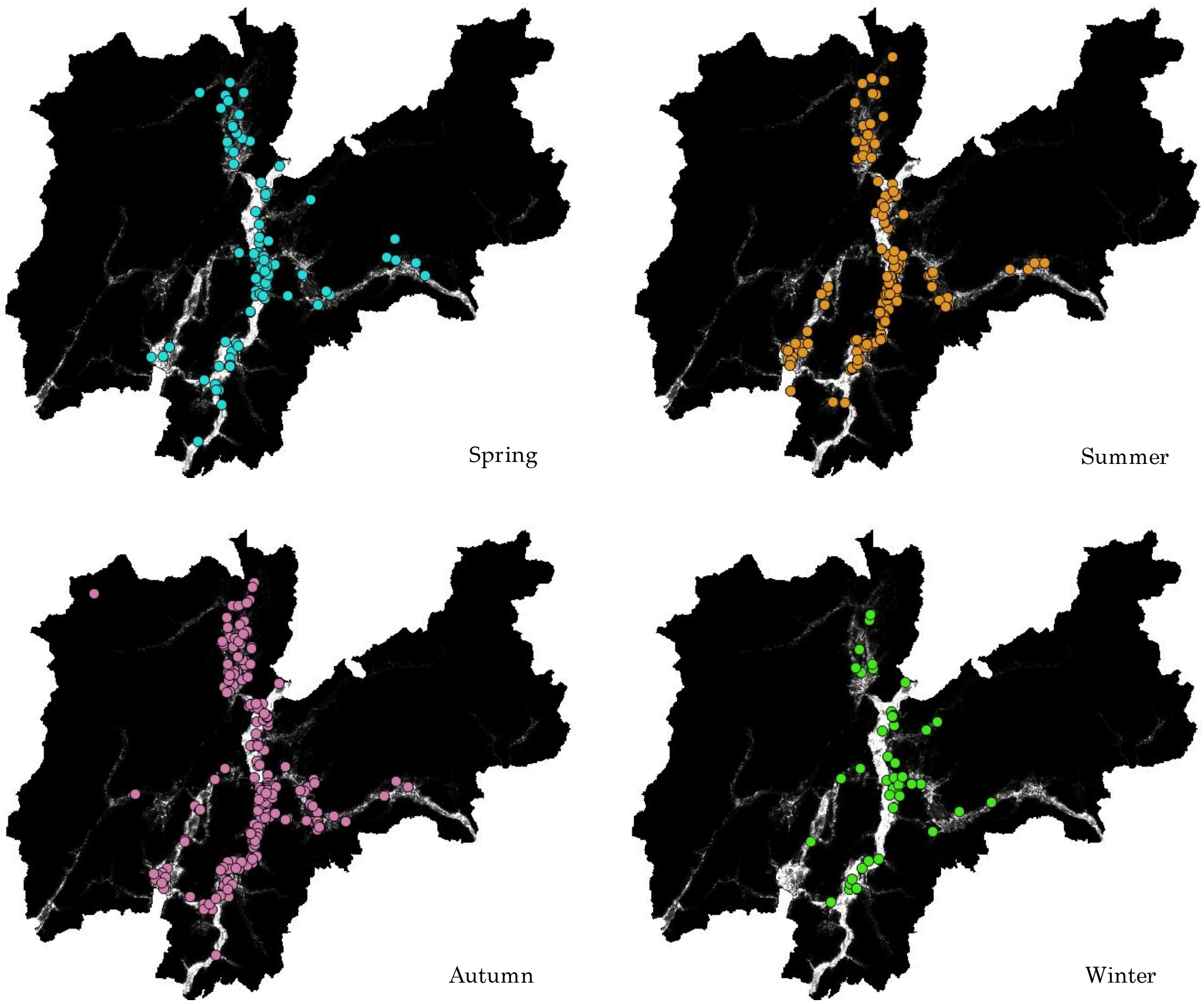

Figure 3.2. Seasonal distribution of *H. halys* in Trentino, according to traditional monitoring and new BugMap reports. Spring and summer maps show 2018 and 2019 records, while autumn and winter maps show records of 2018.

3.3 Italian distribution

Halyomorpha halys reports have been increasing throughout the Italian territories, with 259 new recordings obtained from Italian regions other than Trentino in 2019, compared with 62 recorded reports in 2017 and 2018 (Figure 3.3). Adjoining North Italian regions such as Veneto and Lombardy have seen an upsurge in recordings of the invasive stink bug compared with the previous years. Valid

BugMap reports were also registered for the first time in Umbria, Marche, Abruzzo, Molise, Puglia, Calabria, Sicily and Sardinia. Sporadic reports have also occurred from nearby countries such as Switzerland, Slovenia, Croatia and Hungary.

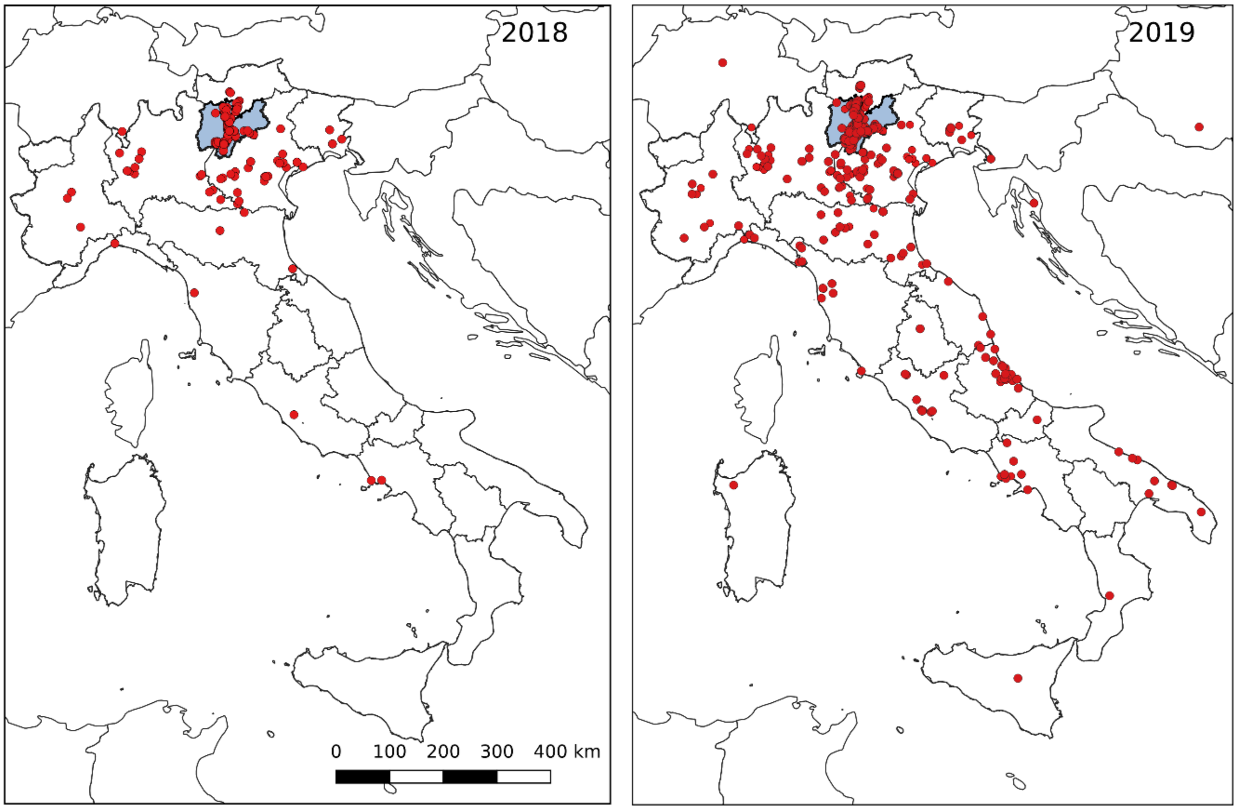

Figure 3.3. Map of Italy and neighboring countries, showing reports since the initiation of BugMap until February 2018 (left) and until October 2019 (right).

3.4 New MaxEnt-generated suitability map

The occurrence data collected on our platform were treated for travel-time bias by systematic sampling of random points over a 500 m grid. For training the model, 490 points were used while 209 records were used for testing it, the model had an AUC for training data: 0.956 and for test data: 0.943. The 2018 and 2019 suitability maps are shown below (Figure 3.4). The Jackknife test for assessing variable contribution revealed that the parameters that most affected the dispersal of the bug are the digital terrain model, land use and distance from houses and from streets.

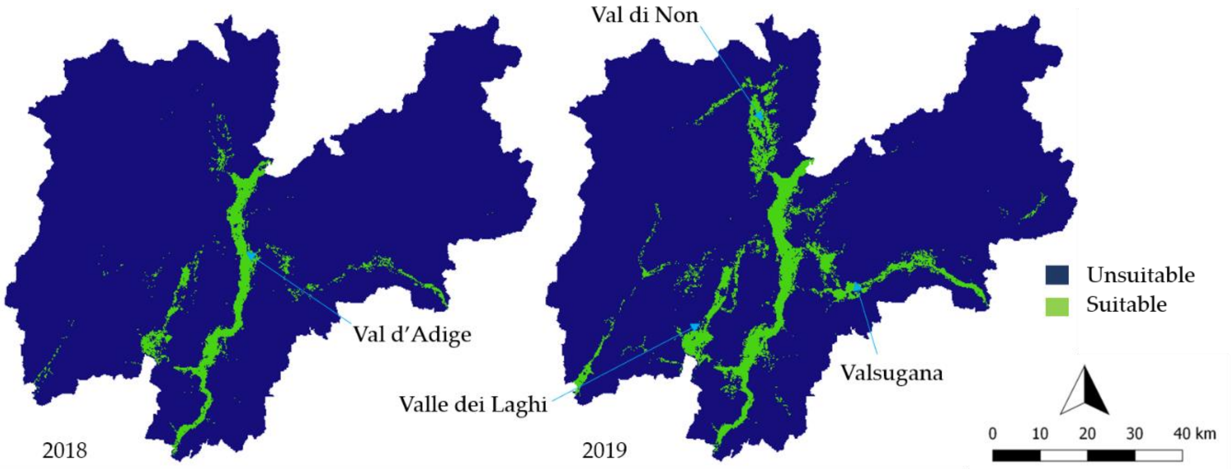

Figure 3.4. Comparative MaxEnt models of the 2018 and 2019 *H. halys* projected distribution in Trentino, highlighting suitable and unsuitable areas at risk of pest establishment.

4 Discussion and Conclusion

BugMap is the only citizen science initiative specifically designed to monitor *H. halys* in Italy while employing a highly accessible, user-friendly and freely available mobile application that facilitates volunteer participation and involvement. The only comparable system that was adopted by researchers in the central Italian region of Emilia Romagna, also integrated systematic sampling and crowdsourcing. Maistrello et al. (2016) worked in close collaboration with amateur entomologists, University students, phytosanitary services and volunteers of the general public, encouraging them to collect *H. halys* specimens and/or take high quality photographs and send them to project leaders along with a detailed annotation. In a more recent study, they referred to *H. halys* as a sneaking hitchhiking alien pest (Maistrello et al., 2018), a denomination well-earned as 13 sightings of both adults and nymphs were recorded via BugMap in different means of transportation (cars, tractors and train stations). The capacity of this pest to go unnoticed in such means facilitates its fast spread and passive diffusion along urban and agricultural landscapes, increasing the risk of establishment in diverse territories.

Our previously generated MaxEnt model (Malek et al., 2018) successfully predicted with 72.5 % accuracy, the current distribution of *H. halys* in Trentino. Mainly, our 2018 model projected a scattered suitability for Val di Non (Figure 3.1) as an area at risk of *H. halys* establishment. This is probably due to the high altitudinal gradient in this agricultural valley, with elevations ranging from 268 to 2999 m. We previously speculated that *H. halys* would probably be incapable of overwintering there, but it would rather have a source to sink population dynamic, with seasonal re-infestations from the highly suitable Val d'Adige. The continuing BugMap reports shed new light on this issue, showing that all year long numerous *H. halys* sightings were recorded from this northernmost region of Trentino, with 81.8 % of winter reports originating from buildings, thus rectifying our previously held assumptions. As has been suggested by several researchers (Kiritani, 2006; Wallner et al., 2014), the latter finding highlights the crucial *H. halys* association and aggregation in man-made structures, in maximizing survivorship and perhaps reducing Allee effect. Kistner (2017) projected that the impacts of global warming may cause a northward expansion of *H. halys* invasion and an increase in the number of annual generations. Therefore, the rise of BugMap reports from Val di Non might also be due to more favorable environmental conditions in 2018-2019 and better climatic suitability compared with the first years of our monitoring.

Val di Non is an area of high economic importance due to the prevalence of about 9.000 hectares of apple orchards. After the generation of our previous model (Malek et al., 2018), we organized technical days and trainings for farmers from that area, stressing the necessity of intensifying monitoring activities. Therefore, the increase of BugMap reports from Val di Non might be due to an *H. halys* population increase in the past two years, to a more active citizen involvement or to both. This observation shows the importance of translating citizen participation and effort into practical agricultural advice to properly orient monitoring and management. All the other main regions of Trentino predicted to be suitable for *H. halys* establishment, namely Val d'Adige and Valle dei Laghi, have seen a great increase of the bug population in spring, summer and autumn (Figure 3.2). Climatic suitability, agricultural-urban interfaces, host plant availability and diffusion via human transportation, are all factors that may contribute to the establishment and expansion of *H. halys* in the aforementioned-regions.

Our new MaxEnt model, generated using all BugMap reports registered so far, revealed an expansion of the area under risk of stink bug establishment in Trentino (Figure 3.4). This territorial spread and landscape correlates go in accordance with a study by Wallner et al. (2014), who found that *H. halys* range expansion is associated with urban land use, agricultural/urban interfaces and human habitat. Specifically, the main agricultural regions of Trentino, Val di Non and Valsugana are now seen as highly suitable for *H. halys*, suggesting that in coming years the damage inflicted on crops are expected to rise there. A means to avoid increasing crop losses could be to adopt pre-emptive measures based on our model, instructing increased monitoring in critical periods of the year. Specifically, early detection during spring and implementation of appropriate control measures as adults emerging from overwintering are more susceptible to insecticides (Leskey et al., 2014). Also, the first appearance of nymphs is considered a key point in the management strategy, to prevent *H. halys* from establishing in the orchards early in the growing season (Krawczyk G., personal communication, 2019). Global *H. halys* distribution models are indeed useful for predicting areas climatically suitable and offering host plants that facilitate the establishment of this invader (Zhu et al., 2012; Kriticos et al., 2017). However, our fine scale-model presents information of direct concern to farmers and citizens in affected areas, while accounting for urban and agricultural land use and extreme altitudinal variation in this mountainous North Italian region.

In the course of time spanning from February 2018 until October 2019, valid BugMap reports were registered for the first time from numerous Italian regions (Figure 3.3). Northern, Central and Southern Italian regions are now threatened by *H. halys* establishment and accompanying damage to agricultural and ornamental industries. Although the hands-on training provided by the BugMap campaign was focused primarily in Trentino, however, the general public from other Italian regions positively reacted to social media posts and exhibited increased awareness and interest for taking part in this issue of local and national concern. Zhu et al., (2012) indicated that the entire Italian peninsula is suitable for *H. halys* establishment and for setting up breeding populations. Therefore, the surge of reports from these regions could then be linked to an increased public engagement or to the stink bug's expansion throughout Italian territories or both.

Crowdsourcing geospatial information on *H. halys* has proven efficient for predicting its distribution and for instructing decision making in geographical areas ravaged by this invader. So much so, that in 2018 we applied the stink bug approach to the invasive tiger mosquito, aiming to better its monitoring and management in this region. Notwithstanding geographical limitations, BugMap was adopted by volunteers all around Italy in a show of elevated environmental awareness and a high scientific citizenship. Elements that contributed to its success are a user-friendly platform for facilitating stink bug reporting, an active informative campaign, close collaboration among amateur and expert scientists and transformation of long-term monitoring into a time- and cost-effective reality. The BugMap approach can now be regarded as a model that may be applied in other communities struggling with their own biological invasions, ranging from invasive plants, lizards, mammals, insects and other environmental hazards.

References

Bariselli, M., Bugiani, R. and Maistrello, L., 2016. Distribution and damage caused by Halyomorpha halys in Italy. *EPPO Bulletin*, *46*(2), pp.332-334. DOI: 10.1111/epp.12289

Cantor, S.B., Sun, C.C., Tortolero-Luna, G., Richards-Kortum, R. and Follen, M., 1999. A comparison of C/B ratios from studies using receiver operating characteristic curve analysis. *Journal of Clinical Epidemiology*, *52*(9), pp.885-892. DOI: 10.1016/S0895-4356(99)00075-X

Danielsen, F., Burgess, N.D. and Balmford, A., 2005. Monitoring matters: examining the potential of locally-based approaches. *Biodiversity & Conservation*, *14*(11), pp.2507-2542. DOI: 10.1007/s10531-005-8375-0

Dickinson, J.L., Zuckerberg, B. and Bonter, D.N., 2010. Citizen science as an ecological research tool: challenges and benefits. *Annual Review of Ecology, Evolution, and Systematics*, *41*, pp.149-172. DOI: 10.1146/annurev-ecolsys-102209-144636

Elith, J., Phillips, S.J., Hastie, T., Dudík, M., Chee, Y.E. and Yates, C.J., 2011. A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, *17*(1), pp.43-57. DOI: 10.1111/j.1472-4642.2010.00725.x

EPPO 2019. European and Mediterranean Plant Protection Organization, EPPO Global Database. (https://www.eppo.int) (accessed 12 October 2019).

Faúndez, E.I. and Rider, D.A., 2017. The brown marmorated stink bug Halyomorpha halys (Stål, 1855) (Heteroptera: Pentatomidae) in Chile. *Arquivos Entomolóxicos*, (*17*), pp.305-307.

Fourcade, Y., Engler, J.O., Rödder, D. and Secondi, J., 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: a performance assessment of methods for correcting sampling bias. *PLOS One*, *9*(5), p.e97122. DOI: 10.1371/journal.pone.0097122

Horwood, M., Milnes, J.M. and Cooper, W.R., 2019. Brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae), detections in Western Sydney, New South Wales, Australia. *Austral Entomology*, *58*(4), pp.857-865. DOI: 10.1111/aen.12421

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J. and Pyšek, P., 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology*, 45(2), pp.403-414. DOI: 10.1111/j.1365-2664.2007.01442.x

Inkley, D.B., 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). *Journal of Entomological Science*, *47*(2), pp.125-130. DOI: 10.18474/0749-8004-47.2.125

Kiritani, K., 2006. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. *Population Ecology*, *48*(1), pp.5-12. DOI: 10.1007/s10144-005-0225-0

Kistner, E.J., 2017. Climate change impacts on the potential distribution and abundance of the brown marmorated stink bug (Hemiptera: Pentatomidae) with special reference to North America and Europe. *Environmental Entomology*, *46*(6), pp.1212-1224. DOI: 10.1093/ee/nvx157

Krawczyk, G. 2019. (Pennsylvania State University, Pennsylvania, USA). Personal communication.

Kriticos, D.J., Kean, J.M., Phillips, C.B., Senay, S.D., Acosta, H. and Haye, T., 2017. The potential global distribution of the brown marmorated stink bug, Halyomorpha halys, a critical threat to plant biosecurity. *Journal of Pest Science*, *90*(4), pp.1033-1043. DOI: 10.1007/s10340-017-0869-5

Lee, D.H. and Leskey, T.C., 2015. Flight behavior of foraging and overwintering brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae). *Bulletin of Entomological Research*, *105*(5), pp.566-573. DOI: 10.1017%2FS0007485315000462

Lee, D.H., Short, B.D., Joseph, S.V., Bergh, J.C. and Leskey, T.C., 2013. Review of the biology, ecology, and management of Halyomorpha halys (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environmental Entomology*, *42*(4), pp.627-641. DOI: 10.1603/EN13006

Leskey, T.C. and Nielsen, A.L., 2018. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. *Annual Review of Entomology*, *63*, pp.599-618. DOI: 10.1146/annurev-ento-020117-043226

Leskey, T.C., Short, B.D. and Lee, D.H., 2014. Efficacy of insecticide residues on adult Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) mortality and injury in apple and peach orchards. *Pest Management Science*, *70*(7), pp.1097-1104. DOI: 10.1002/ps.3653

Liu, C., Berry, P.M., Dawson, T.P. and Pearson, R.G., 2005. Selecting thresholds of occurrence in the prediction of species distributions. *Ecography*, *28*(3), pp.385-393. DOI: 10.1111/j.0906-7590.2005.03957.x

Maistrello, L., Dioli, P., Bariselli, M., Mazzoli, G.L. and Giacalone-Forini, I., 2016. Citizen science and early detection of invasive species: phenology of first occurrences of Halyomorpha halys in Southern Europe. *Biological Invasions*, *18*(11), pp.3109-3116. DOI: 10.1007/s10530-016-1217-z

Maistrello, L., Dioli, P., Dutto, M., Volani, S., Pasquali, S. and Gilioli, G., 2018. Tracking the Spread of Sneaking Aliens by Integrating Crowdsourcing and Spatial Modeling: The Italian Invasion of Halyomorpha halys. *BioScience*, *68*(12), pp.979-989. DOI: 10.1093/biosci/biy112

Malek, R., Tattoni, C., Ciolli, M., Corradini, S., Andreis, D., Ibrahim, A., Mazzoni, V., Eriksson, A. and Anfora, G., 2018. Coupling traditional monitoring and citizen science to disentangle the invasion of Halyomorpha halys. *ISPRS International Journal of Geo-Information*, *7*(5), p.171. DOI: 10.3390/ijgi7050171

Nielsen, A.L. and Hamilton, G.C., 2009. Life history of the invasive species Halyomorpha halys (Hemiptera: Pentatomidae) in northeastern United States. *Annals of the Entomological Society of America*, *102*(4), pp.608-616. DOI: 10.1603/008.102.0405

Rice, K.B., Bergh, C.J., Bergmann, E.J., Biddinger, D.J., Dieckhoff, C., Dively, G., Fraser, H., Gariepy, T., Hamilton, G., Haye, T., Herbert, A., Hoelmer, K., Hooks, C. R., Jones, A., Krawczyk, G., Kuhar, T., Martinson, H., Mitchell, W., Nielsen, A. L., Pfeiffer, D. G., Raupp, M. J., Rodriguez-Saona, C., Shearer, P., Shrewsbury, P., Venugopal, P. D., Whalen, J., Wiman, N. G., Leskey, T. C. and Tooker J. F., 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). *Journal of Integrated Pest Management*, *5*(3), pp. A1-A13. DOI: 10.1603/IPM14002

Ryan, S.F., Adamson, N.L., Aktipis, A., Andersen, L.K., Austin, R., Barnes, L., Beasley, M.R., Bedell, K.D., Briggs, S., Chapman, B. and Cooper, C.B., Corn, J.O., Creamer, N.G., Delborne, J.A., Domenico, P., Driscoll, E., Goodwin, J., Hjarding, A., Hulbert, J.M., Isard, S., Just, M.G., Kar Gupta, K., López-Uribe, M.M., O'Sullivan, J., Landis, E.A., Madden, A.A., McKenney, E.A., Nichols, L.M., Reading, B.J., Russell, S., Sengupta, N., Shapiro, L.R., Shell, L.K., Sheard, J.K., Shoemaker, D.D., Sorger, D.M., Starling, C., Thakur, S., Vatsavai, R.R., Weinstein, M., Winfrey, P. and Dunn, R.R., 2018. The role of citizen science in addressing grand challenges in food and agriculture research. *Proceedings of the Royal Society*, *285*(1891), p.20181977. DOI: 10.1098/rspb.2018.1977

Vandervoet, T.F., Bellamy, D.E., Anderson, D. and MacLellan, R., 2019. Trapping for early detection of the brown marmorated stink bug, Halyomorpha halys, in New Zealand. *New Zealand Plant Protection*, *72*, pp.36-43. DOI: 10.30843/nzpp.2019.72.316

Wallner, A.M., Hamilton, G.C., Nielsen, A.L., Hahn, N., Green, E.J. and Rodriguez-Saona, C.R., 2014. Landscape factors facilitating the invasive dynamics and distribution of the brown marmorated stink bug, Halyomorpha halys (Hemiptera:

Pentatomidae), after arrival in the United States. *PLOS One*, *9*(5), p.e95691. DOI: 10.1371/journal.pone.0095691

Zhu, G., Bu, W., Gao, Y. and Liu, G., 2012. Potential geographic distribution of brown marmorated stink bug invasion (Halyomorpha halys). *PLOS One*, *7*(2), p.e31246. DOI: 10.1371/journal.pone.0031246

Trissolcus japonicus foraging behavior: implications for host preference and classical biological control

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In review

Abstract

The brown marmorated stink bug, *Halyomorpha halys,* is an alien pest native to East Asia, which in the past two decades has invaded USA, Europe and other countries around the globe, causing severe economic losses and public nuisance. The Asian egg parasitoid *Trissolcus japonicus* is the most promising agent currently under study for the classical biological control of *H. halys*. The foraging behavior of this wasp was investigated in response to chemical traces - 'footprints' - deposited by its host *H. halys* and by a suboptimal predatory host species, the spined soldier bug, *Podisus maculiventris*. Motion tracking software (Ethovision) was employed to record and analyze the behavior of *T. japonicus* on stink bug-contaminated and on control substrates. Wasps exhibited a 'motivated searching' behavior (i.e. longer residence time, slower walking velocity, higher angular velocity and coverage of greater distances) in response to footprints originating from females and from males of both species compared to blank controls. However, this searching behavior was significantly more intense on *H. halys* footprints, compared to those of *P. maculiventris*. Moreover, *T. japonicus* significantly intensified its searching on footprints of all mobile nymphal instars of *H. halys*, but not on those of *P. maculiventris* nymphs. Additionally, the longevity of *H. halys* female trails was assessed, and footprints remained bioactive, eliciting a consistent searching behavior in the wasp for 72 hours after initial deposition. A series of GC-MS chemical analyses revealed components of these trails, with *n*-tridecane and (*E*)-2-decenal found to be the most abundant, and probably the key components of the kairomone utilized by the wasp for short range host location. Solutions of the synthetic compounds were tested both together and singly. The blend of *n*-tridecane and (*E*)-2-decenal (4: 1) significantly prolonged the residence time of *T. japonicus*, as did *n*-tridecane alone, whereas (*E*)-2-decenal significantly shortened the wasp's residence time on filter paper. These results are discussed in the context of host preference and classical biological control.

Keywords: Biological control; Invasive pest; Kairomone; Risk assessment; Chemical ecology; Trophic interaction.

1 Introduction

The ecology of information is the study of how organisms provide, acquire, and use information in decision-making to manage their lives and secure current and future reproductive success (Schmidt et al., 2010). Faced with environmental complexity, organisms can acquire information about their physical and biotic environment (or future environment) that reduces uncertainty and improves their likelihood of increasing fitness (Stephens, 1989). Upon emergence in a new complex environment, female parasitoids are faced with several challenges related to mate and host finding to ensure offspring survival. Thus, parasitoid fitness is strongly dependent on their ability to locate hosts in a timely manner, before the target life stage becomes unsuitable due to rapid development (Godfray, 1994). Parasitoids of the third trophic level often use herbivore-induced plant volatiles (HIPVs) and/or oviposition-induced plant volatiles (OIPVs) as long-range cues to locate their inconspicuous hosts (Mumm and Dicke, 2010; Hilker and Fatouros, 2015). After landing on a host-infested plant, parasitoids exploit short-range and/or contact kairomones such as gustatory, visual and olfactory cues (Vet and Dicke, 1992; Aartsma et al., 2018). During this phase of parasitoid foraging, chemical traces originating from the host (termed indirect host-related cues) elicit a ‘motivated searching’ and play an important role in finding and locating life stages suitable for oviposition (Colazza et al., 1999; Colazza et al., 2007; Peri et al., 2016).

When exotic organisms are introduced into new environments, novel interactions encompassing several trophic levels may arise and lead to unpredictable ecological consequences. Laboratory host range studies are useful in predicting the likelihood of non-target parasitism by introduced biological control agents, but they often overestimate their ecological host range (i.e. the number of host species that are parasitized in the field) (Babendreier et al., 2003; Haye et al., 2005). Such overestimates may hamper the development of successful classical biological control programs, especially because of strict regulatory limitations on the importation and release of natural enemies (Mason et al., 2013). Success of a biological control program utilizing egg parasitoids is largely dependent upon the behavioral decisions made by the biological control agent in searching for and parasitizing host eggs (Mills and Wajnberg, 2008). According to Duan and Messing (1997), behavioral tests are as important as suitability tests when assessing host specificity.

A relevant system for such an investigation is that of the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), its main egg-parasitoid, the samurai wasp, *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae) and an indigenous North American predatory stink bug, the spined soldier bug, *Podisus maculiventris* Say (Hemiptera: Pentatomidae). The latter is a generalist predator known to feed on 50 insect species (McPherson et al., 1980), including important agricultural pests, notably *H. halys* eggs (Pote and Nielsen, 2017). *Halyomorpha halys* is an invasive pest that feeds on over 170 plants, including economically important fruit trees, vegetables, row crops, as well as ornamentals (Lee et al., 2013). This insect is native to Eastern Asia, but its occupied range expanded outside of its areas of origin, probably due to global anthropogenic forcing (Hulme et al., 2008), thus spreading through most of the United States, and into Canada, Chile, and numerous European and Eurasian countries (EPPO, 2019). Feeding by *H. halys* results in deformed, symptomatic produce with indents on the surface and corky spots in the flesh, hampering marketability (Nielsen and Hamilton, 2009). It is estimated that over US$ 21 billion worth of crops in the United States alone are threatened by *H. halys* feeding damage (ODA, 2016). Besides being highly polyphagous, *H. halys* is capable of long-distance flight and dispersal (Lee and Leskey, 2015) making it a hard-to-control landscape level pest. Phenological models and laboratory studies predicted the capacity for bivoltinism throughout the United States and Italy (Costi et al., 2017; Nielsen et al., 2016), matching field observations (Acebes-Doria et al., 2019), whereas the completion of only one generation per year was suggested by field studies in Switzerland (Haye et al., 2014). Additionally, *H. halys* tends to aggregate in man-made structures in late autumn to protect itself from harsh winter conditions (Inkley, 2012), rendering it a pervasive residential nuisance.

Managing outbreaks and high-density populations of this pest relies mostly on the extensive use of broad-spectrum insecticides that were found to be only partially effective and which disrupted already established IPM programs (Kuhar and Kamminga, 2017). An integrated approach has since been in development, with trap crops, attract and kill, insecticidal nets and other techniques being evaluated and adapted for its control. Indigenous natural enemies (predators, parasitoids and entomopathogens) across North America and Europe were found to be incapable of significantly reducing this pest's populations in most cropping systems (Abram et al., 2017). Moreover, an evolutionary mismatch expressed by physiological incompatibilities between native egg-parasitoids and *H. halys* is potentially causing the latter's egg masses to act as an evolutionary trap, reducing the indigenous wasp populations, and potentially increasing that of the native pentatomids (Abram et al., 2014; Kaser et al., 2018). For these reasons, classical biological control may be the best management option for this pest at a landscape scale, based upon *T. japonicus*, the most effective egg parasitoid of *H. halys* in its native range in Eastern Asia (Yang et al., 2009).

Trissolcus japonicus is a solitary endoparasitoid of *H. halys* eggs throughout its native range. It can complete multiple generations per year and has a highly female-biased sex ratio (Qiu et al., 2007; Yang et al., 2009) with parasitism rates in China reaching up to 90% (Zhang et al., 2017). Based on CLIMEX models, Avila and Charles (2018) projected that *T. japonicus* will naturally spread in all places where *H. halys* is known to occur and in most, if not all, areas where *H. halys* has been predicted to expand. Adventive *T. japonicus* populations, presumed to be accidently introduced, were first detected in the USA in Maryland in 2014 (Talamas et al., 2015) and subsequently recorded in Virginia, West Virginia, Delaware, New Jersey, New York, Oregon, Washington, and the District of Columbia (Leskey and Nielsen, 2018), and most recently in Pennsylvania, Ohio, Michigan, California and Utah (NE IPM, 2019). It has also been reported in the Canadian provinces of British Columbia (Abram et al., 2019) and Ontario (Gariepy and Talamas, 2019). In Europe, the first report of *T. japonicus* populations emerged in 2018 after field surveys in Switzerland detected the wasp for two consecutive years in three different locations (Stahl et al., 2019). Shortly after, reports of the wasp's presence followed from the neighboring Lombardia region in Northern Italy (Sabbatini Peverieri et al., 2018). Field surveys along with choice and no-choice laboratory studies revealed that *T. japonicus* is capable of parasitizing multiple pentatomid species, with a fundamental host range that includes predatory stink bugs such as *Arma custos* F., *A. chinensis* Fallou and *P. maculiventris* (Zhang et al., 2017; Haye et al., 2019). Botch and Delfosse (2018) found that *T. japonicus* exhibited strong preference for its naturally associated host *H. halys* when reared on its eggs. However, when reared on non-target hosts including *P. maculiventris*, the wasp showed reduced host specificity that came with measurable trade-offs with respect to brood-size and fertility, suggesting specialization to *H. halys*. A recent study by Boyle et al. (2019) showed that *T. japonicus* responds more actively to adult *H. halys* footprints deposited on various leaf substrates compared with those of *P. maculiventris*, but that study did not examine the chemical components influencing the wasp's response.

Therefore, in this study we investigated the behavioral preferences of *T. japonicus* in response to indirect host-related and-suboptimal-host-related cues, namely to the "footprints" (chemical residues deposited on substrates after passage of adults and nymphs) of *H. halys* and *P. maculiventris* respectively. We also assessed the longevity of female *H. halys* footprint: the duration for which it remains bioactive.

Finally, we identified the underlying chemicals shaping *T. japonicus* foraging behavior and decoded the role of the two main detected compounds.

2 Materials and Methods

2.1 Insects

Field-collected and laboratory-reared *H. halys* and *P. maculiventris* adults and nymphs were kept in walk-in rearing chambers at the USDA ARS Beneficial Insects Introduction Research Unit in Newark, DE, under controlled environmental conditions (24 ± 2°C, 60% R.H., 16:8 L:D). *Halyomorpha halys* were reared in screened cages (35 x 42 x 47 cm) containing fresh (2-3 weeks) green bean plants, *Phaseolus vulgaris* L., as substrates for egg deposition. They were also provided with hulled sunflower seeds, fresh green beans, grapes, slices of apples and carrots for food and water-soaked pieces of cotton. *Podisus maculiventris* were provided with green beans, greater wax worms, *Galleria mellonella* L., and water-soaked pieces of cotton. Pieces of foam were hung from the lid served as substrates for oviposition. All plant and food material were changed bi-weekly or as needed. For footprint extracts, *H. halys* used were obtained from the USDA ARS Invasive Insect Biocontrol and Behavior Laboratory colony in Beltsville, MD, reared under similar conditions and provided with fresh green beans hulled raw sunflower seeds and buckwheat seed as well as water ad libitum. *Podisus maculiventris* used for extracts also came from the Beltsville IIBBL laboratory colony and were reared similarly as above. Starter colonies from a population of *Trissolcus japonicus* originally collected in Beijing in

2007 were reared and maintained at the USDA ARS Newark quarantine facility under controlled conditions (24 ± 2°C, 40% R.H., 16:8 L:D). Wasps were reared on *H. halys* egg masses attached to card stock using sand-sprinkled double-sided sticky tape in 10-dram snap cap vials and provided with a drop of honey on the lid.

2.2 Footprint foraging bioassay

All experiments were carried out in the quarantine facility of USDA-ARS laboratories in Newark DE, at 23±1 ºC and 35±10 % RH. Step (1): to collect its chemical footprints, a single stink bug was placed in an arena consisting of a petri dish, confined in a plastic container and motivated to walk on a filter paper (4.25 cm ⌀) for 30 minutes. Whenever the insect rested and stopped walking, the container was gently rotated to stimulate continued movement. Step (2): the contaminated filter paper was then transferred to a sterile glass petri dish (8 cm ⌀) which was placed on a LED light pad (MEDALight LP-400N, Technical Lamps Ltd, Middlesex, United Kingdom) providing illumination from below to optimize visibility and contrast. Directly above the center of the light pad, a camera (ICD-49, Ikegami Tsushinki Co., Ltd., Tokyo, Japan) was mounted on a portable tripod and connected to a video monitor and desktop PC. Step (3): a single 2-4 day-old, mated, naïve (i.e. had no previous experience of stink bug chemicals except for the *H. halys* egg mass from which it emerged) *T. japonicus* female was placed in the arena and confined under a plastic lid (0.5 cm height). Using the motion tracking software EthoVision XT 8.0 (Noldus Information Technology, Wageningen, The Netherlands) the behavior of the wasp was recorded, tracked and processed in real-time. Step (1) was repeated using single gravid females, males, 5th instars, 4th instars, 3rd instars and 2nd instars of either *H. halys* and *P. maculiventris*. The variables measured included the wasp's residence time (time in seconds spent on the filter paper), mean linear velocity (mm/s), angular velocity (°/s) and distance moved (mm). These variables are characteristic of a wasp's motivated searching, which starts with a motionless period with the antennae kept in contact with the surface, followed by drumming of the substrate and alteration in the orthokinetic and klinotaxic locomotion, and increased turning frequency (Gardner and van Lenteren, 1986; Peri et al., 2006; Colazza et al., 2007). Recording commenced 2 seconds after the wasp was detected on the filter paper by the software and stopped after a maximum of 10 minutes or after the wasp left the substrate for a cumulative 15 seconds. Each wasp was used for only one replicate, and 30 replicates were conducted for each treatment. Control treatments were conducted using non-contaminated filter paper. For all treatments, each filter paper was used for 5 replicates.

2.3 Footprint longevity

The duration for which *H. halys* female footprints elicit a motivated searching behavior in *T. japonicus* was assessed up to 4 days after initial deposition. In total, 44 filter papers were contaminated following the same above-described method in step (1). A subset of 11 treated filter papers were exposed to 11 unique 2-4-day-old naïve, mated *T. japonicus* females at day 1 (within 1 hour after footprint-contamination) and treated filter papers were alternated with untreated controls. The remaining footprint-contaminated filter papers were individually stored in closed plastic petri dishes inside a climatic chamber (24°C, 16h L: 8h D and 70% RH) until needed. At each consecutive treatment (days after exposure), 11 treated filter papers were utilized at day 2, 3 and 4 (24, 48 and 72 hours after initial footprint-deposition, respectively).

2.4 Chemical extraction and identification

For extraction of the chemical footprints of the different life stages of *P. maculiventris* and *H. halys*, we slightly modified the exposure method described in step (1). We used sterile Pyrex petri dishes (150 x 15 mm) that were washed, cleaned with acetone and placed into an oven at 140 °C to remove any remnants of volatile compounds. Afterwards, instead of exposing the 4.25 cm ⌀ filter paper to one bug, we contaminated a 150 mm ⌀ filter paper by confining 20 bugs and allowing them to walk for 30 minutes. Filter papers were then removed with sterile forceps, inspected for fecal deposits and if any were observed, the contaminated section was cut out with sterile scissors rinsed with acetone. Five filter papers, on which no stink bug walked, were similarly enclosed in a petri dish and considered as controls. Filter papers were then cut into four equal parts, rolled, inserted into the anterior part of a sterile glass tube and washed by applying 0.5 ml dichloromethane, of which 100 µl were kept for GC-MS analyses and the rest stored at -80 °C and later utilized for behavioral bioassays. The chemical analyses were conducted using HP-5 MS UI (30 m x 320 µm x 0.25 µm) column on Agilent technologies 7890B GC System coupled to a 5977B GC/MSD in EI mode. Samples were handled by Agilent technologies 7693 Autosampler and injected in a pulsed-splitless mode. Inlet temperature was 200°C and the transfer line was set at 270 °C. Oven temperature was set at 40 °C, held for 5 min, then programed to 270 °C at 10 °C /min; the carrier gas was He at 2 ml/min.

2.5 Behavioral responses of *T. japonicus* to *n*-tridecane and (*E*)-2-decenal

For testing the effects of synthetic *n*-tridecane and (*E*)-2-decenal (Sigma-Aldrich, Atlanta, GA), aliquots containing 0.2 nl of each compound in 100 µl of dichloromethane were individually applied on sterile filter papers (4.25 cm ⌀) and allowed to dry for 2 min. Afterwards, 2-4 days old naïve, mated *T. japonicus* females were placed on the filter paper and their behavior was recorded as described above. The two compounds were tested alone and in two blends of opposite ratios (4: 1 and 1: 4), with *n*-tridecane: (*E*)-2-decenal (1.6: 0.4 and 0.4: 1.6 nl/ml), respectively, using the same above-mentioned concentration. Controls consisted of 100 µl aliquots of the solvent dichloromethane.

2.6 Statistical analysis

All statistical analyses were conducted in R (v. 3.4.3). For *T. japonicus* foraging behavior, the distributions of all response variable values for each treatment were evaluated for normality using a Shapiro Wilk test (α = 0.05), and if required, appropriate transformations were made. The behavior of *T. japonicus* in response to footprints of *H. halys* females and *P. maculiventris* females were compared with controls, interspersing the treatments that correspond to each tested life stage (i.e. 5 trials with *H. halys* followed by 5 trials with *P. maculiventris* followed by 5 controls).

The same was done for the remaining treatments, such as male stink bugs, as well as 5th, 4th, 3rd and 2nd instar nymphs. A non-parametric Wilcoxon rank sum test (Mann-Whitney *U* test) was used to compare different treatments, when data were not normally distributed under any transformation utilized. When data were normally distributed or became so after logarithmic or square root transformations, Student's t-test was utilized.

3 Results

3.1 *Trissolcus japonicus* foraging behavior on adult footprints

In general, wasps resided for a significantly longer time, had a slower walking velocity, higher angular velocity and covered greater distances on filter papers contaminated by *H. halys* footprints compared with *P. maculiventris* and with controls.

3.1.1 Residence time

In response to adult footprints, wasps were retained for significantly longer durations on *H. halys* females (574.03 ± 12.24 s Mean ± SEM) vs *P. maculiventris* females (219.75 ± 38.37 s; W= 34, P<0.001) and vs controls (68.08 ± 15.67; W= 462, P<0.001). The wasps also spent significantly more time on footprints of *P. maculiventris* females vs controls (W=80, P<0.001). The residence time of *T. japonicus* was significantly longer on footprints from *H. halys* males (539.57 ± 22.07 s) vs controls (64.26 ± 12.77; W= 648, P<0.001), *H. halys* males vs *P. maculiventris* males

(144.16 ± 26.26 s; W= 13, P<0.001) also on *P. maculiventris* males vs controls (W= 154.5, P= 0.006). Results are summarized in Figure 4.1.

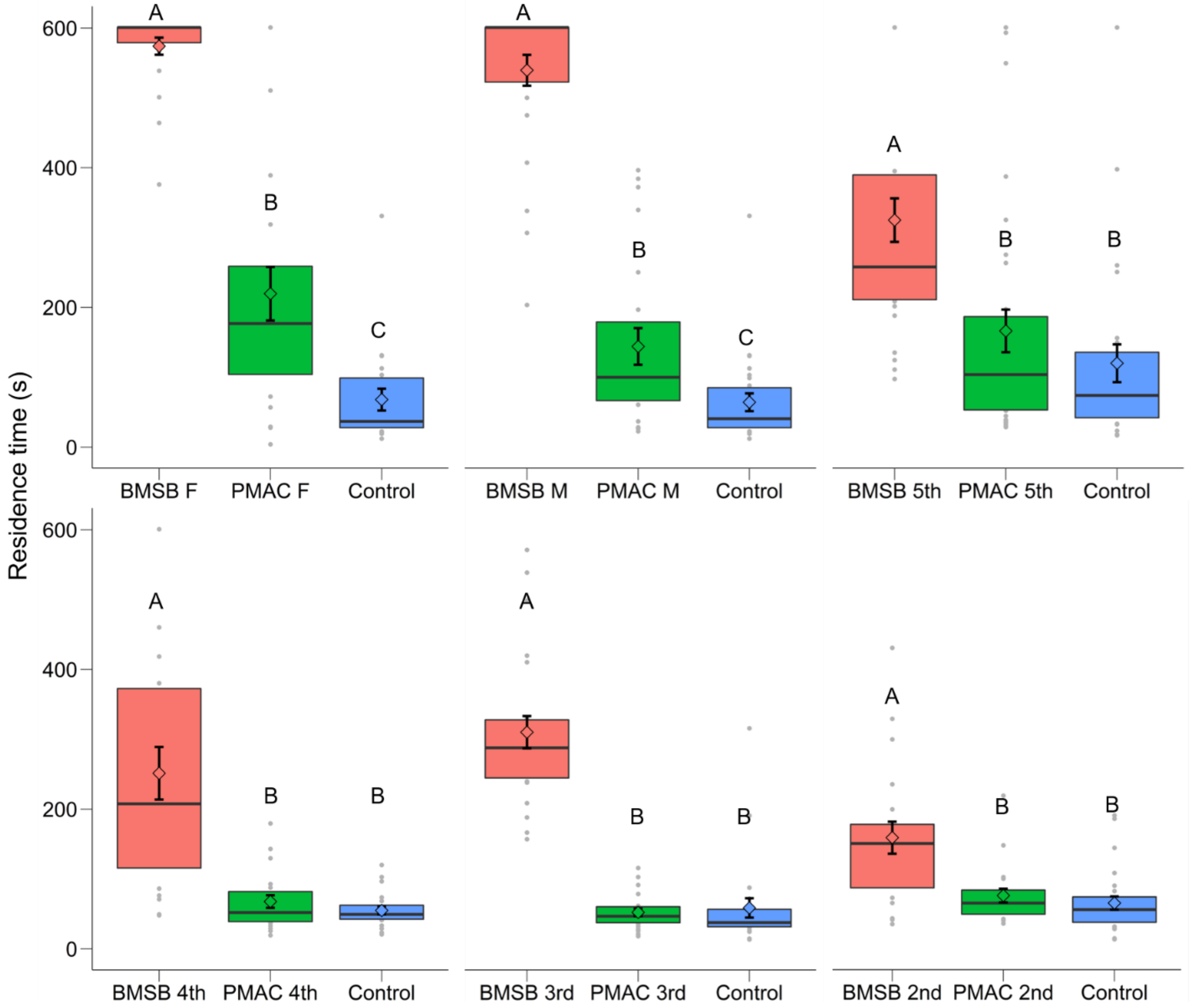

Figure 4.1. Residence time of female *T. japonicus* on footprint-contaminated substrate from different life stages of both *H. halys* (BMSB) and *P. maculiventris* (PMAC) females, males, 5th, 4th, 3rd and 2nd instars. Different letters express statistical difference between groups. Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual wasps per treatment.

3.1.2 Walking velocity

The wasps walked significantly more slowly on *H. halys* female footprints (4.30 ± 0.79 mm/s) compared to controls (11.25 ± 1.36; $t_{38.64}$=-4.36, P<0.001), *H. halys* females vs *P. maculiventris* females (5.21 ± 0.94 mm/s; $t_{39.89}$=-0.63, P<0.001), also when comparing *P. maculiventris* females with controls ($t_{39.72}$=-3.53, P=0.001) (Figure 4.2). They also walked significantly more slowly on footprints of *H. halys* males (2.56 ± 0.28 mm/s) vs control (12.33 ± 1.35; W= 37.5, P<0.001), *H. halys* males vs *P. maculiventris* males (8.33 ± 1.29 mm/s; W= 69.5, P<0.001) and *P. maculiventris* males vs control (W= 175, P=0.022).

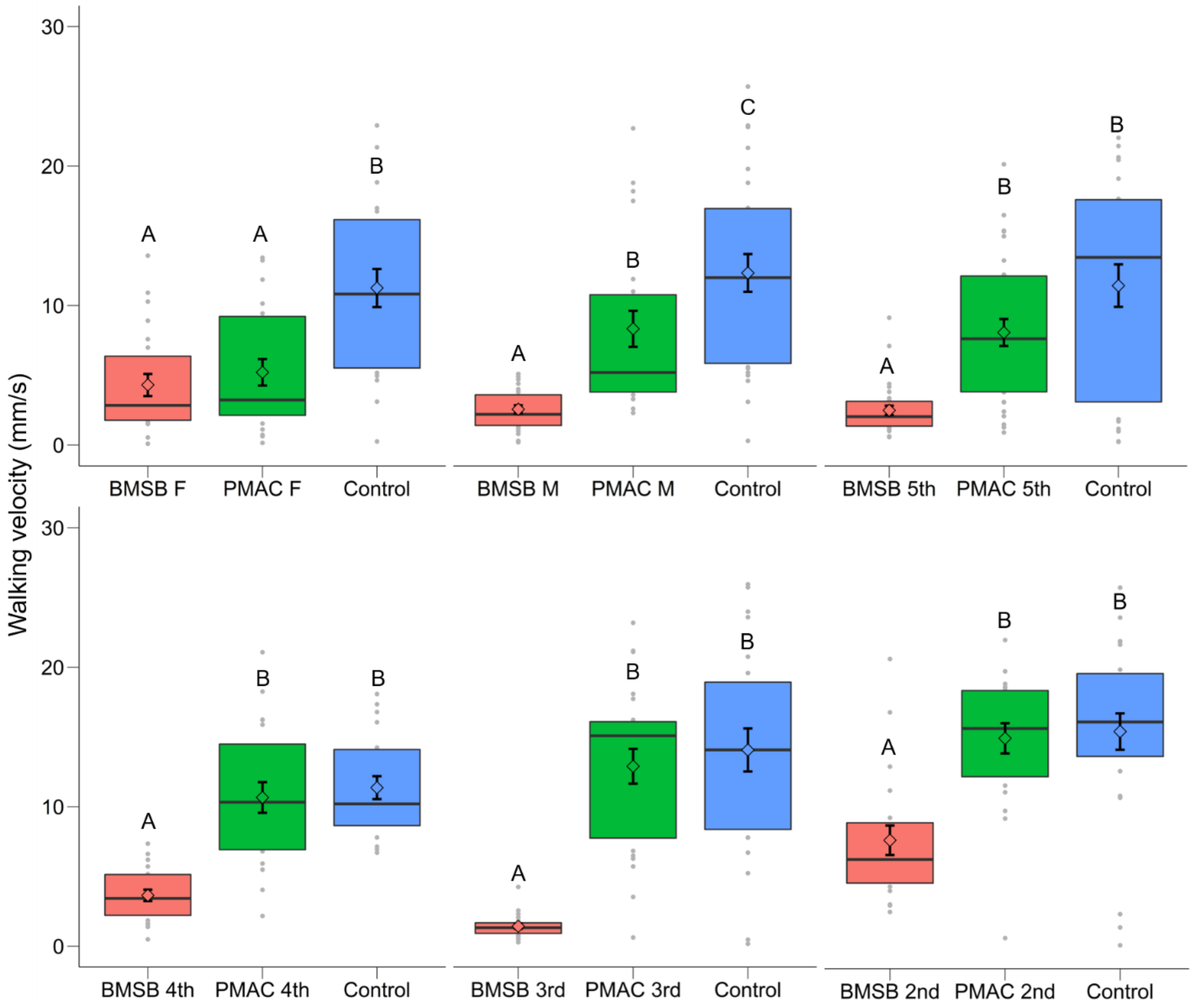

Figure 4.2. Walking velocity (mm/s) of female *T. japonicus* on substrate contaminated by footprints of *H. halys* and *P. maculiventris* life stages. Different letters express statistical difference between groups. Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual wasps per treatment.

3.1.3 Angular velocity

Angular velocity of *T. japonicus* was higher on footprints from *H. halys* females (117.14 ± 3.58 deg/s) vs controls (65.70 ± 11.06; $t_{7.29}$=4.42, P=0.002) and on *P. maculiventris* vs controls (123.91 ± 6.65 deg/s, $t_{10.6}$=4.5, P<0.001); no statistical difference was detected when comparing *H. halys* to *P. maculiventris* footprints (P>0.05) (Figure 4.3). The wasps had a higher angular velocity on footprints from *H. halys* males (130.74 ± 5.98 deg/s) vs controls (79.24 ± 12.01; W= 215, P<0.001), *H. halys* males vs *P. maculiventris* males (94.93 ± 5.41 deg/s, W= 483, P<0.001), but not on *P. maculiventris* males vs controls (P>0.05).

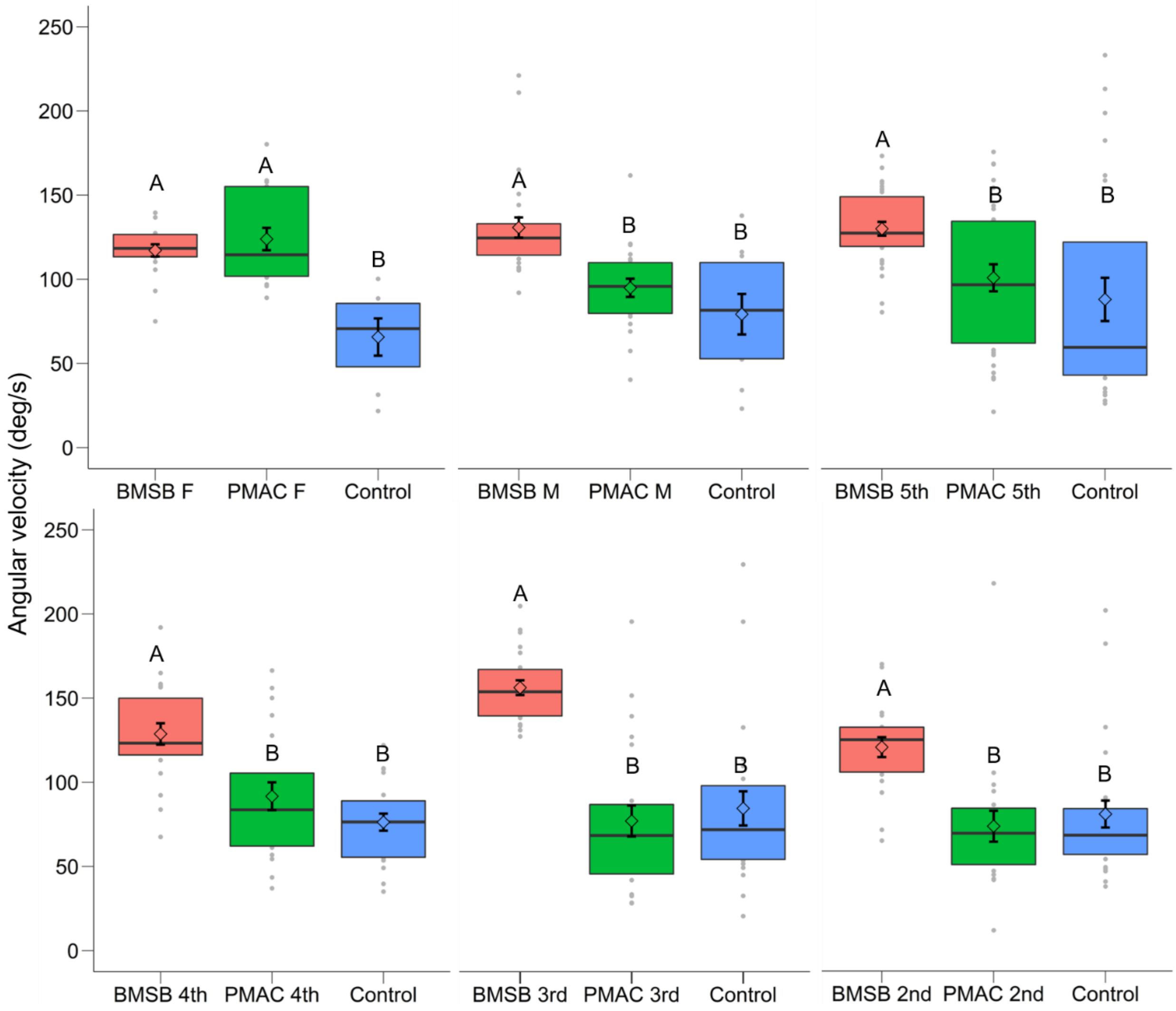

Figure 4.3. Angular velocity (deg/s) of female *T. japonicus* on substrate contaminated by footprints of *H. halys* and *P. maculiventris*. Different letters express statistical difference between groups. Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual wasps per treatment.

3.1.4 Distance moved

Trissolcus japonicus covered significantly greater distances when exposed to footprints originating from *H. halys* females (1532.65 ± 234.08 mm) vs controls (207.68 ± 37.37; $t_{40.46}$=7.31, P<0.001), *H. halys* females vs *P. maculiventris* females (485.82 ± 117.95 mm; $t_{32.83}$=4.11, P<0.001), but not to footprints from *P. maculiventris* females vs controls ($t_{30.04}=0.87$, $P=0.38$) (Figure 4.4). Greater distances were covered on footprints from *H. halys* males (1026 ± 120.85 mm) vs controls (199.73 ± 30.55; $t_{47.9}=7.4$, $P<0.001$), *H. halys* males vs *P. maculiventris* males (410.12 ± 58.98 mm; $t_{44.8}=4.02$, $P<0.001$) and *P. maculiventris* males vs controls ($t_{45.6}=3.7$, $P<0.001$).

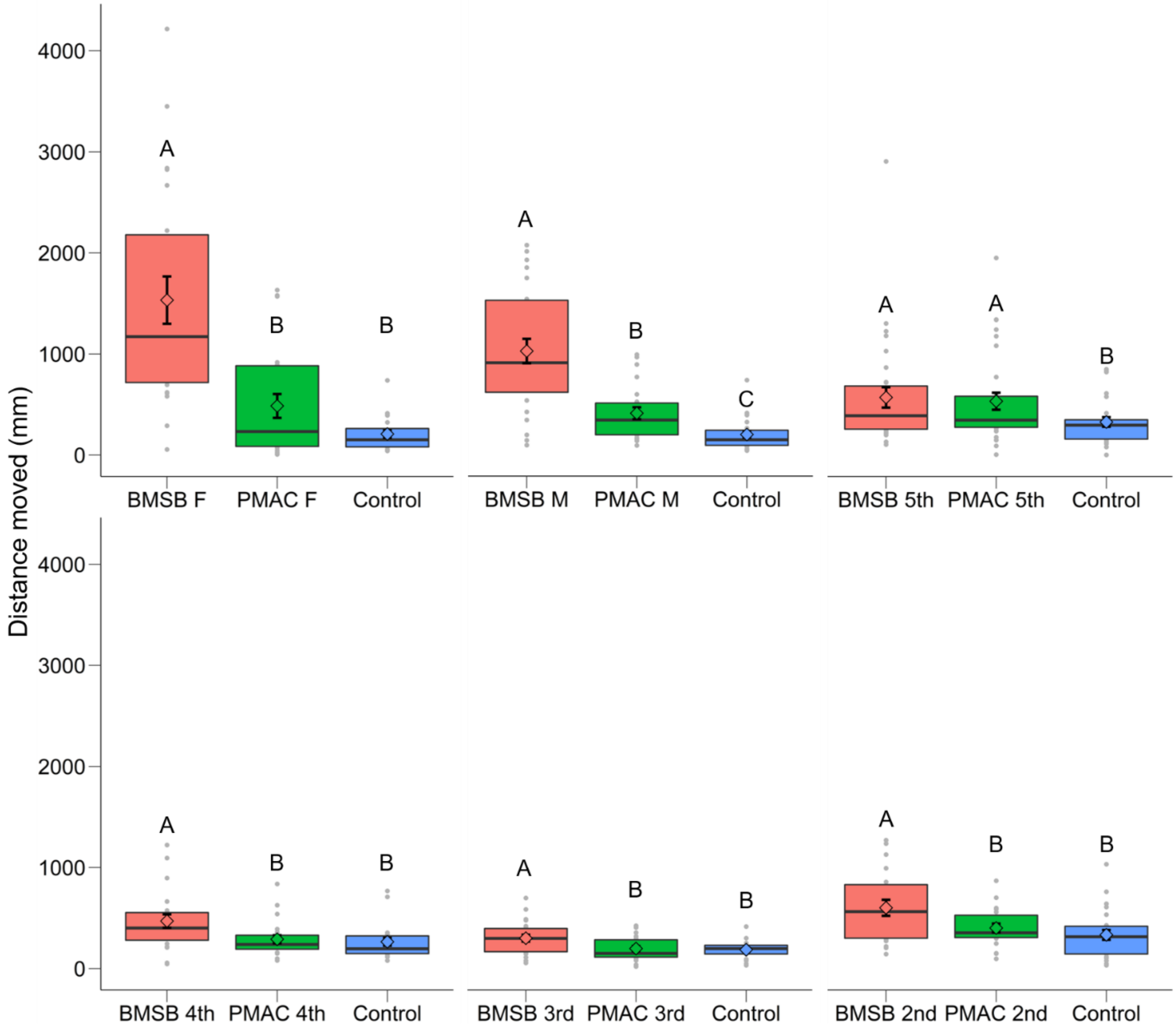

Figure 4.4. Distance moved (mm) by female *T. japonicus* on substrate contaminated by footprints of *H. halys* and *P. maculiventris*. Different letters express statistical difference between groups. Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N=25 individual wasps per treatment.

3.2 *Trissolcus japonicus* foraging behavior on nymphal footprints

In response to footprints from 5th, 4th, 3rd and 2nd nymphal instars of both species, the wasps exhibited a significantly stronger arrestment response on those footprints originating from *H. halys* compared with *P. maculiventris* and with controls. However, no statistical difference was observed when comparing all the studied variables of *T. japonicus* behavior on *P. maculiventris* footprints vs controls. Results presented in Figures 4.1, 4.2, 4.3 and 4.4.

3.2.1 5th nymphal instars

The wasps' residence time was significantly longer on *H. halys* (325.02 ± 31.18 s) vs controls (120.15 ± 27.01 s; W= 651, P<0.001), it walked significantly more slowly on *H. halys* (2.48 ± 0.33 mm/s) vs controls (11.42 ± 1.52 mm/s; W= 154, P<0.001), it had a higher angular velocity on *H. halys* (130.02 ± 4.08 deg/s) vs controls (88.02 ± 12.86 deg/s; $t_{28.84}$= 3.11, P=0.004) and covered greater distance on *H. halys* (569.27 ± 100.6 mm) vs controls (327.13 ± 47.31 mm; W= 508, P= 0.031). Similarly, the wasp spent significantly more time on *H. halys* vs *P. maculiventris* (166.37 ± 30.53 s; W= 728,5, P<0.001), it walked significantly more slowly on *H. halys* vs *P. maculiventris* (8.06 ± 0.96 mm/s; W= 140, P<0.001), it had a higher angular velocity on *H. halys* vs *P. maculiventris* (100.88 ± 8.03 deg/s; $t_{43.06}$= 3.23, P=0.002), with no difference in the distance covered (P>0.05).

3.2.2 4th nymphal instars

The residence time of *T. japonicus* was significantly longer on substrate contaminated with *H. halys* (251.39 ± 37.66 s) vs controls (55.01 ± 5.56; W= 408, P<0.001), the wasp had significantly slower velocity on *H. halys* (3.64 ± 0.41 mm/s) vs controls (11.36 ± 0.82; $t_{39.85}$= -8.93, P<0.001), it had a higher angular velocity on *H. halys* (128.79 ± 6.33 deg/s) vs controls (76.39 ± 5.07 deg/s; $t_{38.18}$= 6.45, P<0.001), and covered greater distance on *H. halys* (470.29 ± 66.17 mm) vs controls (264.97 ± 38.65 mm; $t_{35.46}$= 2.26, P= 0.029). The wasp also spent significantly more time on *H. halys* vs *P. maculiventris* (67.77 ± 9.06 s; W= 384, P<0.001), it walked significantly more slowly on *H. halys* vs *P. maculiventris* (10.66 ± 1.1 mm/s; $t_{34.4}$= -6.34, P<0.001), had a higher angular velocity on *H. halys* vs *P. maculiventris* (91.74 ± 8.28 deg/s; $t_{37.42}$= 3.55, P=0.001), with no significant difference in the distance covered on *H. halys* vs *P. maculiventris* footprints.

3.2.3 3rd nymphal instars

Trissolcus japonicus resided for significantly more time on *H. halys* (310.25 ± 23.04 s) vs controls (58.65 ± 13.86 s; W= 510, P<0.001), it walked significantly more slowly on *H. halys* (1.42 ± 0.17 mm/s) vs controls (14.06 ± 1.54 mm/s; W= 45, P<0.001), it had a higher angular velocity on *H. halys* (156.31 ± 4.37 deg/s) vs controls (84.53 ± 10.13 deg/s; W= 482, P<0.001) and covered greater distance on *H. halys* (300.69 ± 33.94 mm) vs controls (186.41 ± 17.27 mm; $t_{38.6}$= 2.81, P= 0.007). With regard to the wasp's behavior on *H. halys* vs *P. maculiventris* footprints, it spent significantly more time on *H. halys* vs *P. maculiventris* (52.31 ± 5.2 s; W= 529, P<0.001), it walked significantly more slowly on *H. halys* vs *P. maculiventris* (12.89 ± 1.24 mm/s; W=21, P<0.001), it had a higher angular velocity on *H. halys* vs *P. maculiventris* (77.07 ± 9.15 deg/s; W= 490, P<0.001), also covered greater distances on *H. halys* vs *P. maculiventris* footprints (197.66 ± 25.52 mm; $t_{43.75}$= 2.42, P= 0.019).

3.2.4 2nd nymphal instars

The residence time of *T. japonicus* was significantly longer on substrate contaminated by *H. halys* (158.99 ± 22.91 s) vs controls (65.36 ± 9.45 s; $t_{40.28}$=4.38, P<0.001), its walking velocity was significantly slower on *H. halys* (7.67 ± 1.05 mm/s) vs controls (15.44 ± 1.30 mm/s; W= 92, P<0.001), its angular velocity was higher on *H. halys* (120.74 ± 5.91 deg/s) vs controls (80.93 ± 8.05 deg/s; $t_{40.89}$= 4.39, P<0.001) and covered greater distances on *H. halys* (601.31 ± 78.86 mm) vs controls (333.73 ± 48.02 mm; $t_{38.75}$= 3.06, P=0.003). When comparing the wasp's behavior on *H. halys* and *P. maculiventris* footprints, it spent significantly more time on *H. halys* vs *P. maculiventris* (76.08 ± 9.75 s; $t_{33.43}$=3.45, P=0.0015), had significantly slower walking velocity on *H. halys* vs *P. maculiventris* (14.95 ± 1.08 mm/s; W= 58, P<0.001), had a significantly higher angular velocity on *H. halys* vs *P. maculiventris* (73.67 ± 9.2 deg/s; $t_{30.11}$= 4.61,P<0.001) and covered greater distances on *H. halys* vs *P. maculiventris* (401.41 ± 43.25 mm; $t_{33.45}$= 2.05, P= 0.047).

3.3 Footprint longevity

Using *T. japonicus* horizontal mobility and arrestment as a proxy for the longevity of *H. halys* female footprints, we recorded an almost unchanging behavior when comparing the wasp's arrestment response on treated filter papers up to 4 days after initial exposure (Figure 4.5). There was no significant difference ($P>0.05$) in the wasp's residence time, walking velocity, angular velocity and distance moved between the four different treatments (Day 1, 2, 3 and 4).

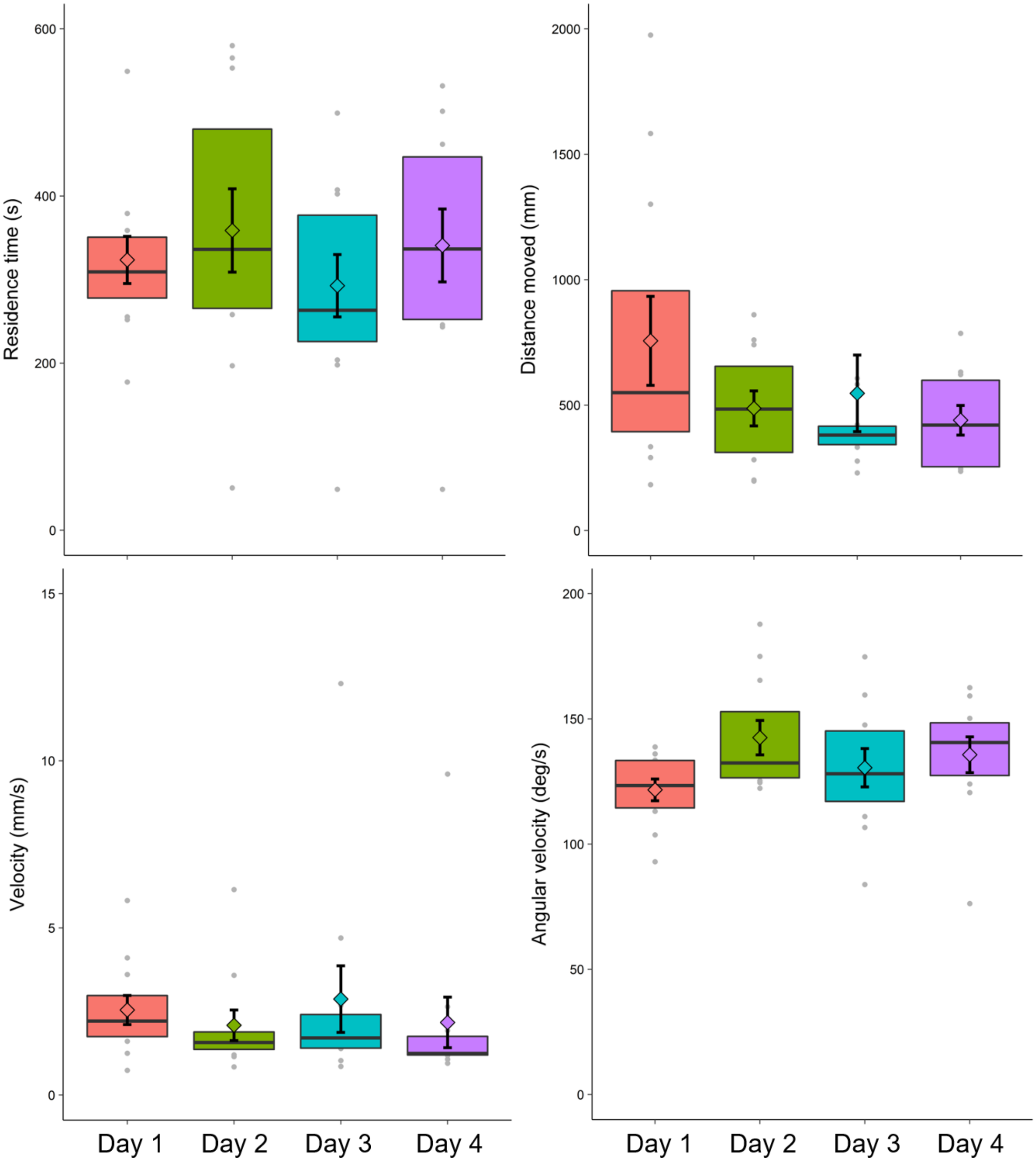

Figure 4.5. Box plots showing the four studied variables of female *T. japonicus* arrestment response up to 4 days after initial exposure. Boxplots represent the interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N= 11 individual wasp per treatment.

3.4 Chemical footprint identity

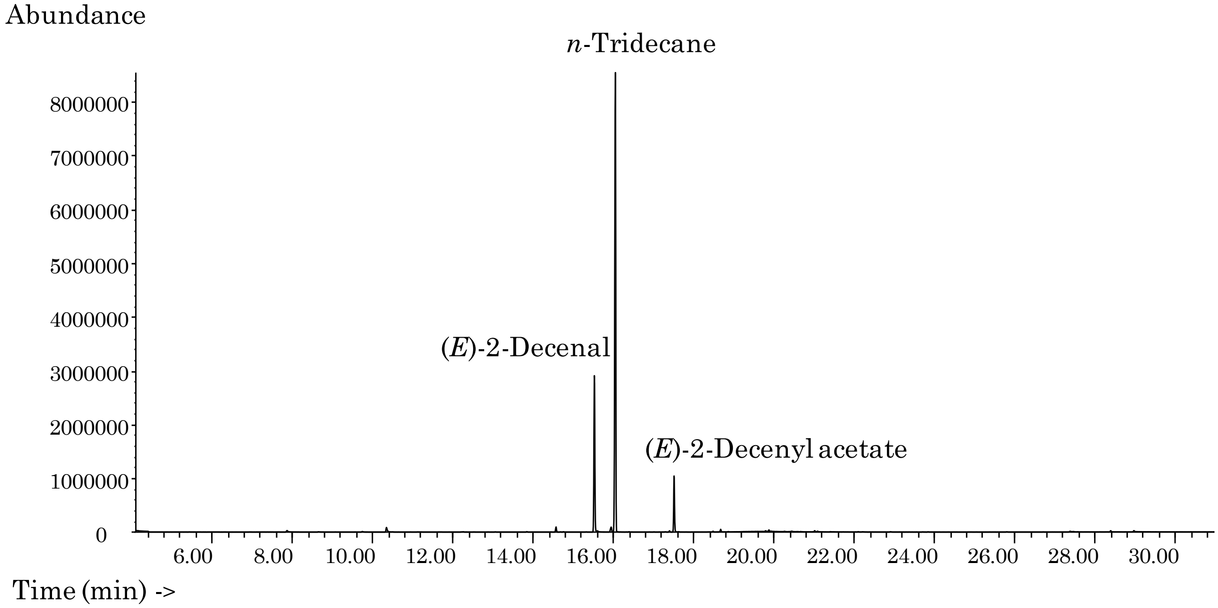

Figure 4.6. GC-MS total ion chromatogram of *H. halys* female footprints. *n*-tridecane and (*E*)-2-decenal were identified by matching retention times and mass-spectra those of authentic standards. (*E*)-2-Decenyl acetate was identified using NIST mass-spectral library

Chemical analyses revealed the presence of *n*-tridecane and (*E*)-2-decenal in all tested life stages of *H. halys*, while much smaller amounts of these compounds were detected in *P. maculiventris* footprints (Figure 4.6). Both *n*-tridecane and (*E*)-2-decenal were most abundant in male *H. halys* extracts compared with those of other life stages from both species (table 4.1).

Table 4.1. Mean ± SEM of absolute *n*-tridecane and (*E*)-2-decenal in *H. halys* and *P. maculiventris* extracts.

Life stage	**N**	***n*-tridecane (μg)**	**(*E*)-2-decenal (μg)**
H. halys female	10	9.50 ± 3.49	2.70 ± 0.97
H. halys male	10	44.01 ± 13.17	14.14 ± 4.54
H. halys 5th instar	10	5.79 ± 1.16	3.18 ± 0.87
P. maculiventris female	10	0.26 ± 0.24	0.025 ± 0.025
P. maculiventris male	10	0.13 ± 0.13	0.019 ± 0.019
P. maculiventris 5th instar	10	2.40 ± 1.83	0.004 ± 0.002

3.5 Response to synthetic compounds

According to Zhong et al. (2017) (*E*)-2-decenyl acetate did not elicit an electrophysiological response in female *T. japonicus*, therefore it was excluded from our behavioral tests. Only residence time was measured for experiments involving *T. japonicus* response to synthetic *n*-tridecane and (*E*)-2-decenal (Figure 4.7). The average time spent by the wasp on *n*-tridecane (61.24 ± 7.60 s) treated filter papers was significantly longer compared with the control (40.62 ± 4.22 s; $t_{17.20}$=2.36, P=0.029). The residence time of *T. japonicus* was shortened on (*E*)-2-decenal (26.06 ± 1.45 s) compared with controls (40.62 ± 4.22 s; $t_{16.44}$=-3.23, P=0.005). The blend of *n*-tridecane: (*E*)-2-decenal (4:1) significantly prolonged the residence time of the wasp (91.95 ± 15.29 s) compared with the controls (34.73 ± 1.95; $t_{14.51}$=6.12, P<0.001). No statistical difference was found when comparing the residence time of the wasp on *n*-tridecane: (*E*)-2-decenal blend (1:4) with controls (P>0.05).

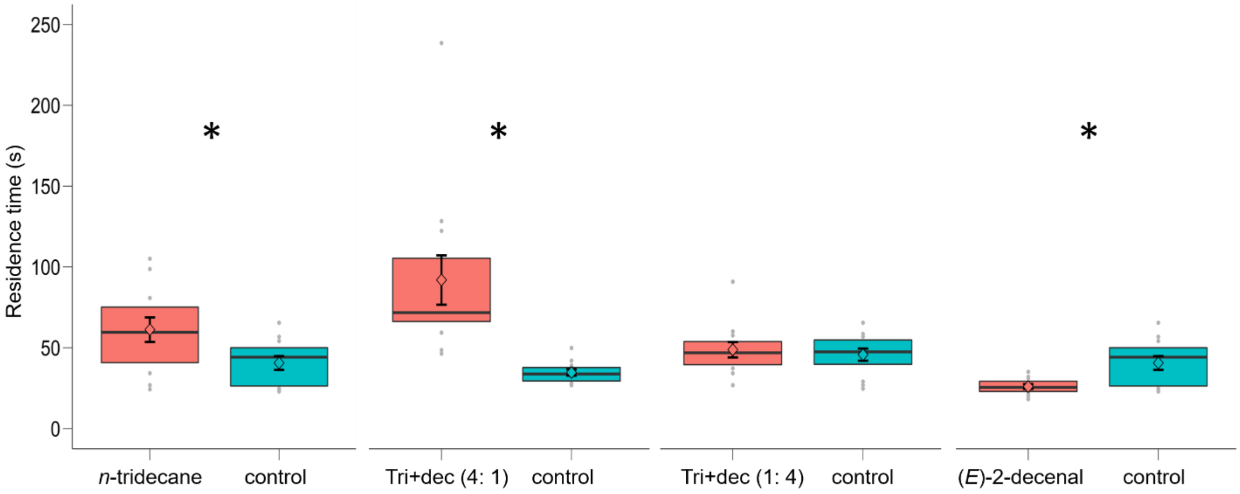

Figure 4.7. Box plots representing the effect of n-tridecane: (E)-2-decenal blends and single compounds on the residence time of female Trissolcus japonicus. Asterisks indicate significant difference according to Student's t-test. Box plots show interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Gray dots represent data points that fell outside the interquartile range. N= 12 individual wasps per treatment

4 Discussion

Female parasitoids are motivated to optimize their host-selection behavior because it is directly linked to their reproductive success, since the host is the only food source for their offspring during their immature development (van Alphen and Vet, 1986). Therefore, studies on parasitic wasps have played a major role in shaping and testing foraging theory (Godfray, 1994).

Samurai wasp behavior in our studies differed greatly on treated compared to untreated substrates. Parasitoids observed on control substrate walked fast in relatively straight lines interspersed with erratic turns and exited the arena fairly quickly. When wasps experienced target or nontarget stimuli, they altered their orthokinetic (i.e. speed of movement dependent upon the intensity of the stimulus) and locomotory behavior, and clearly initiated an arrestment response. The latter is characterized by a decrease in linear walking velocity, an increase in residence time, angular velocity and distance covered on footprint-contaminated substrate (Peri et al., 2006; Rostás and Wölfling, 2009; Konopka et al., 2018). Peri et al. (2013) showed that *Trissolcus* egg parasitoids only responded positively to footprints of hosts with which they are normally associated. *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) exhibited an arrestment response to footprints of its host *Nezara viridula* L. (Heteroptera: Pentatomidae) (Colazza et al., 1999), while the generalist *Ooencyrtus telenomicida* Vassiliev (Hymenoptera: Encyrtidae) did not respond to footprint contact kairomones of *N. viridula* (*Peri* et al*.,* 2011). Rostás et al. (2008) also demonstrated that *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) parasitoids respond to chemical footprints of its host *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), with a characteristic host recognition behavior, displayed as antennal drumming of the host-contaminated substrate. In a recent study, *Anastatus orientalis* Yang and Gibson (Hymenoptera: Eupelmidae) exhibited a motivated searching in response to footprints from females of the invasive spotted lanternfly, *Lycorma delicatula* White (Hemiptera: Fulgoridae) (Malek et al., 2019). In our bioassays, *T. japonicus* exhibited a significantly stronger arrestment in response to footprints originating from its optimal host *H. halys*, compared with the suboptimal *P. maculiventris*. For example, when on *P. maculiventris* contaminated substrate *T. japonicus* resided for 1/3 the time it spent on *H. halys* footprints. Moreover, in response to nymphal footprints, *T. japonicus* displayed a motivated searching only to *H. halys* but not to *P. maculiventris*. Such a behavior would seem to indicate detection of a negligible cue, which translates into an efficient strategy for moving between patches of hosts (Bell and Tobin, 1982). Thus, the weaker arrestment to *P. maculiventris* footprints suggests low current profitability, which may become potentially higher by searching elsewhere in the habitat (Thiel and Hoffmeister, 2004).

Studies aimed at determining *T. japonicus* physiological and ecological host range revealed its capacity to recognize, accept and successfully develop in several species of stink bugs. Efforts in China, North America and Europe found that closely related species in the pentatomid subfamily Asopinae such as *Arma chinensis*, *Podisus maculiventris* and *Arma custos* respectively, were highly suitable developmental hosts for *T. japonicus* (Zhang et al., 2017; Hedstrom et al., 2017; Haye et al., 2019; Gariepy and Talamas, 2019). The nontarget effects on these predatory stink bugs, classified according to Kuhlmann et al. (2006) as “safeguard species”, raises more concern than effects on other herbivores. Bertoldi et al. (2019) tested the attraction of *T. japonicus* to tomato plants subjected to feeding and oviposition by either *H. halys* or *P. maculiventris*. They showed that female wasps were significantly more attracted to plant odors induced by *H. halys* feeding and egg laying, whereas no significant attraction was detected when comparing clean plants with those subjected to *P. maculiventris* herbivory. Their results suggest that naïve *T. japonicus* reared on *H. halys* exploit cues associated with its coevolved host but not those related to the novel *P. maculiventris* association. Haye et al. (2019) further investigated the fundamental host range of *T. japonicus* in Europe, revealing that of the twelve nontarget European Pentatomidae species tested, all were accepted by *T. japonicus* and eleven were suitable for parasitoid development in no-choice tests. When paired choice-tests in semi-natural large arenas were performed, egg masses of *Arma custos* F. (Heteroptera: Pentatomidae), *Graphosoma lineatum* and *Acrosternum heegeri* Fieber (Heteroptera: Pentatomidae) were significantly less parasitized when exposed together with *H. halys* egg masses. Their results confirm those of Bertoldi et al. (2019), that additional complexity (host plants with naturally laid egg masses, feeding damage and stink bug footprints) can substantially reduce the degree of nontarget host parasitism in controlled laboratory conditions. When reared on *P. maculiventris*, the residence time of *T. japonicus* on footprints of *H. halys* was not different than on those of *P. maculiventris*, however, egg masses of *P. maculiventris* were significantly less parasitized than those of *H. halys* (Boyle, 2017). Botch and Delfosse (2018) found that when reared on *H. halys*, *T. japonicus* showed a stronger preference for it compared with the nontarget stink bugs *P. maculiventris* and *Thyanta custator acerra* McAtee (Heteroptera: Pentatomidae). However, when reared on these same nontarget hosts, *T. japonicus* females showed reduced host specificity, and not only were their progeny significantly smaller in size, but also of a lower number, indicating significant fitness costs. It remains unclear whether habitat preference, competition with native egg-parasitoids or phenology of nontarget species may limit nontarget parasitism; therefore, risk assessment studies dealing with these aspects are warranted. Although it is predicted that *T. japonicus* may detect and parasitize *P. maculiventris* or other native pentatomid species, the aforementioned findings suggest that female wasps will adopt a searching strategy in the field that brings about optimal fitness gains, and consequently reduced probability of nontarget parasitization.

Walking by *H. halys* females for 30 minutes left sufficient amounts of infochemicals on the filter papers to induce a strong arrestment response even four days after initial deposition. This persistence of female footprints is comparable to the period during which *H. halys* eggs are still viable for parasitization, but further studies in natural conditions are required to confirm this result. Hemptinne et al. (2001) reported that females *Adalia bipunctata* L. (Coleoptera: Coccinellidae) avoid laying eggs on filter papers contaminated by conspecific larval tracks for at least ten days. In contrast, the response of the parasitic wasp *Cephalonomia tarsalis* Ashmead (Hymenoptera: Bethylidae) to trail traces of its host, the saw-toothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) vanished 30 min after larvae had been removed from the filter paper (Collatz and Steidl, 2008). Therefore, as suggested by Rostás and Wölfling (2009), the bioactivity of kairomonal footprints is expected to vary with the perceiving insect, substrate, storage conditions, and nature and concentration of deposited chemicals.

Pentatomids are commonly known as “stink bugs” because they respond to disturbances or aggression by producing irritating defensive secretions with a disagreeable odor from dorsal abdominal glands or metathoracic glands (Aldrich, 1988). Our present study showed that *n*-tridecane and (*E*)-2-decenal were the two main compounds extracted from footprints of females, males and 5th instar nymphs of *H. halys*, while only trace amounts were detected in *P. maculiventris* extracts. These two compounds, along with (*E*)-2-decenyl acetate, dodecane and (*E*)-2-octenal have been previously identified from *H. halys* volatiles and from other stink bug metathoracic secretions (Kitamura et al., 1984; Fávaro et al., 2011; Solomon et al., 2013; Harris et al., 2015; Fraga et al., 2017; Zhong et al., 2017). They are thought to have defensive roles, mediating behaviors among individual pentatomids, predators, and natural enemies (Borges and Aldrich, 1992; Weber et al., 2017). The variable amounts secreted by the different sexes and life stages of *H. halys* may be linked to their differing functions at the given life stages and physiological states of the bugs. Nixon et al. (2018) sampled diapausing and diapause-disrupted aggregations of *H. halys* and found that the predominant compounds included *n*-tridecane, 4-oxo-(*E*)-2-hexenal, and (*E*)-2-decenal, which suggests that these aggregations secrete and possibly passively exude these compounds even in the absence of any disturbance (Harris et al., 2015).

In our behavioral studies, synthetic *n*-tridecane alone and in a blend with (*E*)-2-decenal (4: 1 respectively) significantly prolonged the residence time of *T. japonicus*. In contrast, (*E*)-2-decenal alone shortened it and a blend of *n*-tridecane and (*E*)-2-decenal (1: 4) had no effect on the wasp's behavior compared with the solvent control. Our findings suggest that *n*-tridecane acts as an arrestant, while (*E*)-2-decenal is functioning as a deterrent. Our results are in accordance with those of Zhong et al. (2017), who showed that *T. japonicus* females and *H. halys* males were attracted by *n*-tridecane but strongly repelled by (*E*)-2-decenal. In another study, (*E*)-2-decenal was also repellent to *N. viridula* 1st instar nymphs, suggesting an alarm function (Fucarino et al., 2004). (*E*)-2-decenal not only exhibited fungistatic effect and inhibited spore germination of entomopathogenic fungi in the genera *Metarhizium*, *Beauveria*, and *Isaria* (Pike, 2014), but was also found to display antibacterial activity as both *Staphylococcus aureus* and *Escherichia coli* were significantly inhibited by it (Sagun et al., 2016).

A study by Fraga et al. (2017) found that *n*-tridecane is a major compound released by *H. halys* adults and nymphs, and by *H. halys*-damaged bean pods. They also confirmed that it attracted *Orius insidiosus* Say (Hemiptera: Anthocoridae) in olfactometer assays and field trials but contributed only to arresting the predator rather than increasing prey consumption. Harris et al. (2015) stated that *n*-tridecane inhibited aggregation pheromone production by individual *H. halys* males, while Lockwood and Story (1985; 1987) suggested that it functions as nymphal aggregation pheromone at low doses but as an alarm pheromone at high doses in *N. viridula*. Other authors have stated that *n*-tridecane and similar hydrocarbons function as solvents or carriers rather than inherently bioactive compounds (Calam and Youdeowei, 1968; Kment and Vilimova, 2010). Interestingly, coupling (*E*)-2-decenal with *n*-tridecane strongly deterred the feeding activity of *H. halys* in laboratory trials (Zhang et al., 2016).

5 Conclusions

Our bioassays indicate that the samurai wasp *T. japonicus* employs an infochemical detour (sensu Vet and Dicke, 1992), responding to host cues that might lead to the target life stage. Wasps reacted to contact kairomones by activating a significantly stronger arrestment to *H. halys* footprints compared with *P. maculiventris* footprints. While this preference for *H. halys* cues might indicate lower chances of *P. maculiventris* parasitization in the field, negative nontarget effects cannot be excluded. We found that *H. halys* female footprints persist on contaminated filter papers for at least four days, meaning that *T. japonicus* can use these cues for the period when eggs are still suitable for parasitoid development, which declines significantly after host eggs are more than about three days old (Yang et al., 2018). Further research is required to understand whether this timeline varies under natural conditions and on various plant substrates. Our chemical analyses revealed that *n*-tridecane and (*E*)-2-decenal are the two major compounds of *H. halys* and *P. maculiventris* footprints. The former prolonged *T. japonicus* residence time, whereas the latter shortened it, suggesting that wasps are eavesdropping on *n*-tridecane for host location, while (*E*)-2-decenal performs its presumed defensive role and repels the parasitoid. These findings shed new light on the potential applications of *n*-tridecane in agroecosystems, where it could be used to attract or arrest natural enemies, to improve parasitoid surveys or to provide refugia that may assist in their conservation or augmentation. Further studies are required in this regard to pinpoint the optimal ratios and concentrations that can be utilized for field testing. As *T. japonicus* appears to be following *H. halys* through its invasive journey, first to North America (Talamas et al., 2015) then to Europe (Stahl et al., 2018), efforts to relocate and redistribute it in the USA are already underway (Jentsch, 2017). Risks and rewards resulting from introducing this biocontrol agent or assisting its establishment in *H. halys* invaded territories need to be weighed with care, also accounting for the risks of not releasing it and instead waiting for it to disperse on its own. If this agent can help reduce *H. halys* populations, this probably means that the use of broad-spectrum insecticides–that surged under *H. halys* outbreaks, are environmentally harmful, disruptive to IPM programs, cause extensive nontarget effects and linked to global insect decline–would also be reduced.

References

Aartsma, Y., Cusumano, A., de Bobadilla, M.F., Rusman, Q., Vosteen, I. and Poelman, E.H., 2019. Understanding insect foraging in complex habitats by comparing trophic levels: insights from specialist host-parasitoid-hyperparasitoid systems. *Current Opinion in Insect Science*, *32*, pp.54-60. DOI: 10.1016/j.cois.2018.11.001

Abram, P.K., Gariepy, T.D., Boivin, G. and Brodeur, J., 2014. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. *Biological Invasions*, *16*(7), pp.1387-1395. DOI: 10.1007/s10530-013-0576-y

Abram, P.K., Hoelmer, K.A., Acebes-Doria, A., Andrews, H., Beers, E.H., Bergh, J.C., Bessin, R., Biddinger, D., Botch, P., Buffington, M.L. and Cornelius, M.L., 2017. Indigenous arthropod natural enemies of the invasive brown marmorated stink bug in North America and Europe. *Journal of Pest Science*, *90*(4), pp.1009-1020. DOI: 10.1007/s10340-017-0891-7

Abram, P.K., Talamas, E.J., Acheampong, S., Mason, P.G. and Gariepy, T.D., 2019. First detection of the samurai wasp, Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae), in *Canada. Journal of Hymenoptera Research*, *68*, p.29. DOI: 10.3897/jhr.68.32203

Acebes-Doria, A.L., Agnello, A.M., Alston, D.G., Andrews, H., Beers, E.H., Bergh, J.C., Bessin, R., Blaauw, B.R., Buntin, G.D., Burkness, E.C., Chen, S., Cottrell, T.E., Daane, K.M., Fann, L.E., Fleischer, S.J., Guédot, C., Gut, L.J., Hamilton, G.C., Hilton, R., Hoelmer, K.A., Hutchison, W.D., Jentsch, P., Krawczyk, G., Kuhar, T.P., Lee, J.C., Milnes, J.M., Nielsen, A.L., Patel, D.K., Short, B.D., Sial, A.A., Spears, L.R., Tatman, K., Toews, M.D., Walgenbach, J.D., Welty, C., Wiman, N.G., Van Zoeren, J. and Leskey, TC., 2019. Season-long monitoring of the brown marmorated stink bug (Hemiptera: Pentatomidae) throughout the United States using commercially available traps and lures. *Journal of Economic Entomology*, toz240. DOI: 10.1093/jee/toz240

Aldrich, J.R., 1988. Chemical ecology of the Heteroptera. *Annual Review of Entomology*, *33*(1), pp.211-238. DOI: 10.1146/annurev.en.33.010188.001235

Avila, G.A. and Charles, J.G., 2018. Modelling the potential geographic distribution of Trissolcus japonicus: a biological control agent of the brown marmorated stink bug, Halyomorpha halys. *BioControl*, *63*(4), pp.505-518. DOI: 10.1007/s10526-018-9866-8

Babendreier, D., Kuske, S. and Bigler, F., 2003. Non-target host acceptance and parasitism by Trichogramma brassicae Bezdenko (Hymenoptera: Trichogrammatidae) in the laboratory. *Biological Control*, *26*(2), pp.128-138. DOI: 10.1016/S1049-9644(02)00121-4

Bell, W.J. and Tobin, T.R., 1982. Chemo-orientation. *Biological Reviews*, *57*(2), pp.219-260. DOI: 10.1111/j.1469-185X.1982.tb00369.x

Bertoldi, V., Rondoni, G., Brodeur, J. and Conti, E., 2019. An egg parasitoid efficiently exploits cues from a coevolved host but not those from a novel host. *Frontiers in Physiology*, *10*, p.746. DOI: 10.3389/fphys.2019.00746

Borges, M. and Aldrich, J.R., 1992. Instar-specific defensive secretions of stink bugs (Heteroptera: Pentatomidae). *Experientia*, *48*(9), pp.893-896. DOI: 10.1007/BF02118429

Botch, P.S. and Delfosse, E.S., 2018. Host-acceptance behavior of Trissolcus japonicus (Hymenoptera: Scelionidae) reared on the invasive Halyomorpha halys (Heteroptera: Pentatomidae) and nontarget species. *Environmental Entomology*, *47*(2), pp.403-411. DOI: 10.1093/ee/nvy014

Boyle, S.M., 2017. Novel techniques for evaluating the potential host range of candidate biological control agent Trissolcus japonicus (Hymenoptera: Platygastridae). *M.Sc. thesis*, University of Delaware.

Boyle, S.M., Weber, D.C., Hough-Goldstein, J. and Hoelmer, K.A., 2019. Host kairomones influence searching behavior of Trissolcus japonicus (Hymenoptera:

Scelionidae), a parasitoid of Halyomorpha halys (Heteroptera: Pentatomidae). *Environmental Entomology*, nvz155, DOI: 10.1093/ee/nvz155

Calam, D.H. and Youdeowei, A., 1968. Identification and functions of secretion from the posterior scent gland of fifth instar larva of the bug Dysdercus intermedius. *Journal of Insect Physiology*, *14*(8), pp.1147-1158. DOI: 10.1016/0022-1910(68)90054-1

Colazza, S., Aquila, G., De Pasquale, C., Peri, E. and Millar, J.G., 2007. The egg parasitoid Trissolcus basalis uses n-nonadecane, a cuticular hydrocarbon from its stink bug host Nezara viridula, to discriminate between female and male hosts. *Journal of Chemical Ecology*, *33*(7), pp.1405-1420. DOI: 10.1007/s10886-007-9300-7

Colazza, S., Salerno, G. and Wajnberg, E., 1999. Volatile and contact chemicals released by Nezara viridula (Heteroptera: Pentatomidae) have a kairomonal effect on the egg parasitoid Trissolcus basalis (Hymenoptera: Scelionidae). *Biological Control*, *16*(3), pp.310-317. DOI: 10.1006/bcon.1999.0763

Collatz, J. and Steidle, J.L., 2008. Hunting for moving hosts: Cephalonomia tarsalis, a parasitoid of free-living grain beetles. *Basic and Applied Ecology*, *9*(4), pp.452-457. DOI: 10.1016/j.baae.2007.04.002

Costi, E., Haye, T. and Maistrello, L., 2017. Biological parameters of the invasive brown marmorated stink bug, Halyomorpha halys, in southern Europe. *Journal of Pest Science*, *90*(4), pp.1059-1067. DOI: 10.1007/s10340-017-0899-z

Duan, J.J. and Messing, R.H., 1997. Biological control of fruit flies in Hawaii: factors affecting non-target risk analysis. *Agriculture and Human Values*, *14*(3), pp.227-236. DOI: 10.1023/A:1007456727655

EPPO 2019. European and Mediterranean Plant Protection Organization, EPPO Global Database. (https://www.eppo.int) (accessed 22 September 2019).

Fávaro, C.F., Rodrigues, M.A.D.M., Aldrich, J.R. and Zarbin, P.H., 2011. Identification of semiochemicals in adults and nymphs of the stink bug Pallantia macunaima Grazia (Hemiptera: Pentatomidae). *Journal of the Brazilian Chemical Society*, *22*(1), pp.58-64. DOI: 10.1590/S0103-50532011000100007

Fraga, D.F., Parker, J., Busoli, A.C., Hamilton, G.C., Nielsen, A.L. and Rodriguez-Saona, C., 2017. Behavioral responses of predaceous minute pirate bugs to tridecane, a volatile emitted by the brown marmorated stink bug. *Journal of Pest Science*, *90*(4), pp.1107-1118. DOI: 10.1007/s10340-016-0825-9

Fucarino, A., Millar, J.G., McElfresh, J.S. and Colazza, S., 2004. Chemical and physical signals mediating conspecific and heterospecific aggregation behavior of first instar stink bugs. *Journal of Chemical Ecology*, *30*(6), pp.1257-1269. DOI: 10.1023/B:JOEC.0000030276.32665.cb

Gardner, S.M. and Van Lenteren, J.C., 1986. Characterisation of the arrestment responses of Trichogramma evanesces. *Oecologia*, *68*(2), pp.265-270. DOI: 10.1007/BF00384798

Gariepy, T.D. and Talamas, E.J., 2019. Discovery of Trissolcus japonicus (Hymenoptera: Scelionidae) in Ontario, Canada. *The Canadian Entomologist*, *151*(6), pp.824-826. DOI: 10.4039/tce.2019.58

Godfray, H.C.J., 1994. *Parasitoids: behavioral and evolutionary ecology* (Vol. 67). Princeton University Press.

Harris, C., Abubeker, S., Yu, M., Leskey, T. and Zhang, A., 2015. Semiochemical production and laboratory behavior response of the brown marmorated stink bug, Halyomorpha halys. *PLOS One*, *10*(11), p.e0140876. DOI: 10.1371/journal.pone.0140876

Haye, T., Abdallah, S., Gariepy, T. and Wyniger, D., 2014. Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, Halyomorpha halys, in Europe. *Journal of Pest Science*, *87*(3), pp.407-418. DOI: 10.1007/s10340-014-0560-z

Haye, T., Goulet, H., Mason, P.G. and Kuhlmann, U., 2005. Does fundamental host range match ecological host range? A retrospective case study of a Lygus plant bug parasitoid. *Biological Control*, *35*(1), pp.55-67. DOI: 10.1016/j.biocontrol.2005.06.008

Haye, T., Moraglio, S.T., Stahl, J., Visentin, S., Gregorio, T. and Tavella, L., 2019. Fundamental host range of Trissolcus japonicus in Europe. *Journal of Pest Science*, pp.1-12. DOI: 10.1007/s10340-019-01127-3

Hedstrom, C., Lowenstein, D., Andrews, H., Bai, B. and Wiman, N., 2017. Pentatomid host suitability and the discovery of introduced populations of Trissolcus japonicus in Oregon. *Journal of Pest Science*, *90*(4), pp.1169-1179. DOI: 10.1007/s10340-017-0892-6

Hemptinne, J.L., Lognay, G., Doumbia, M. and Dixon, A.F.G., 2001. Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, Adalia bipunctata (Coleoptera: Coccinellidae). *Chemoecology*, *11*(1), pp.43-47. DOI: 10.1007/PL00001831

Hilker, M. and Fatouros, N.E., 2015. Plant responses to insect egg deposition. *Annual Review of Entomology*, *60*, pp.493-515. DOI: 10.1146/annurev-ento-010814-020620

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J., Pyšek, P., Roques, A., Sol, D., Solarz, W. and Vilà, M., 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology*, *45*(2), pp.403-414. DOI: 10.1111/j.1365-2664.2007.01442.x

Inkley, D.B., 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). *Journal of Entomological Science*, *47*(2), pp.125-130. DOI: 10.18474/0749-8004-47.2.125

Jentsch, P., 2017. Expanding the Range of the Samurai Wasp, *Trissolcus japonicus*, in New York Orchards. http://nyshs.org/wp-content/uploads/2018/04/Jentsch-Pages-31-36-from-NYFQ-Winter-Book-2017.pdf (accessed 14 August 2019)

Kaser, J.M., Nielsen, A.L. and Abram, P.K., 2018. Biological control effects of non-reproductive host mortality caused by insect parasitoids. *Ecological Applications*, *28*(4), pp.1081-1092. DOI: 10.1002/eap.1712

Kitamura, C., Wakamura, S. and Takahashi, S., 1984. Identification and functions of ventral glands secretion of some Heteroptera. *Applied Entomology and Zoology*, *19*(1), pp.33-41. DOI: 10.1303/aez.19.33

Kment, P. and Vilimova, J., 2010. Thoracic scent efferent system of Pentatomoidea (Hemiptera: Heteroptera): a review of terminology. *Zootaxa*, *2706*(1), pp.1-77. DOI: 10.11646/zootaxa.2706.1.1

Konopka, J.K., Poinapen, D., Gariepy, T. and McNeil, J.N., 2018. Understanding the mismatch between behaviour and development in a novel host-parasitoid association. *Scientific Reports*, *8*(1), p.15677. DOI: 10.1038%2Fs41598-018-33756-6

Kuhar, T.P. and Kamminga, K., 2017. Review of the chemical control research on Halyomorpha halys in the USA. *Journal of Pest Science*, *90*(4), pp.1021-1031. DOI: 10.1007/s10340-017-0859-7

Kuhlmann, U., Schaffner, U. and Mason, P.G., 2006. Selection of non-target species for host specificity testing. *Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment*. CABI Publishing, Wallingford, UK, pp.15-37.

Lee, D.H. and Leskey, T.C., 2015. Flight behavior of foraging and overwintering brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae). *Bulletin of Entomological Research*, *105*(5), pp.566-573. DOI: 10.1017%2FS0007485315000462

Lee, D.H., Short, B.D., Joseph, S.V., Bergh, J.C. and Leskey, T.C., 2013. Review of the biology, ecology, and management of Halyomorpha halys (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environmental Entomology*, *42*(4), pp.627-641. DOI: 10.1603/EN13006

Leskey, T.C. and Nielsen, A.L., 2018. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. *Annual Review of Entomology*, *63*, pp.599-618. DOI: 10.1146/annurev-ento-020117-043226

Lockwood, J.A. and Story, R.N., 1985. Bifunctional pheromone in the first instar of the southern green stink bug, Nezara viridula (L.) (Hemiptera: Pentatornidae): its characterization and interaction with other stimuli. *Annals of the Entomological Society of America*, *78*(4), pp.474-479. DOI: 10.1093/aesa/78.4.474

Lockwood, J.A. and Story, R.N., 1987. Defensive secretion of the southern green stink bug (Hemiptera: Pentatomidae) as an alarm pheromone. Annals of the *Entomological Society of America*, *80*(5), pp.686-691. DOI: 10.1093/aesa/80.5.686

Malek, R., Kaser, J.M., Broadley, H.J., Gould, J., Ciolli, M., Anfora, G. and Hoelmer, K.A., 2019. Footprints and Ootheca of Lycorma delicatula Influence Host-Searching and-Acceptance of the Egg-Parasitoid Anastatus orientalis. *Environmental Entomology*, *48*(6), pp.1270-1276. DOI: 10.1093/ee/nvz110

Mason, P.G, Kabaluk, J.T., Spencer, B., et al., 2013. Regulation of biological control in Canada. In: Mason PG, Gillespie DR, (Eds). *Biological control programmes in Canada 2001–2012*. Wallingford: CABI Publishing; 2013. pp. 1-5.

McPherson, J.E., 1980. A list of the prey species of Podisus maculiventris (Hemiptera: Pentatomidae). *Great Lakes Entomologist*, *13*(1), pp.17-24.

Mills, N.J. and Wajnberg, E., 2008. Optimal foraging behavior and efficient biological control methods. *Behavioral ecology of insect parasitoids: From theoretical approaches to field applications*, pp.3-30. DOI: 10.1002/9780470696200.ch1

Mumm, R. and Dicke, M., 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Canadian Journal of Zoology*, *88*(7), pp.628-667. DOI: 10.1139/Z10-032

Nielsen, A.L. and Hamilton, G.C., 2009a. Seasonal occurrence and impact of Halyomorpha halys (Hemiptera: Pentatomidae) in tree fruit. *Journal of Economic Entomology*, *102*(3), pp.1133-1140. DOI: 10.1603/029.102.0335

Nielsen, A.L., Chen, S. and Fleischer, S.J., 2016. Coupling developmental physiology, photoperiod, and temperature to model phenology and dynamics of an invasive Heteropteran, Halyomorpha halys. *Frontiers in Physiology*, *7*, p.165. DOI: 10.3389/fphys.2016.00165

Nixon, L.J., Morrison, W.R., Rice, K.B., Brockerhoff, E.G., Leskey, T.C., Guzman, F., Khrimian, A., Goldson, S. and Rostas, M., 2018. Identification of volatiles released by diapausing brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae). *PLOS One*, *13*(1), p.e0191223. DOI: 10.1371/journal.pone.0191223

Northeast IPM Center. 2019. U.S. Map of Field Recoveries of Trissolcus japonicus. http://www.stopbmsb.org/biological-control/samurai-wasp-trissolcus-japonicus/. (accessed on 13 November 2019).

Oregon Department of Agriculture: Pest Alert: Brown Marmorated Stink Bug Halyomorpha halys. http://www.oregon.gov/ODA/Pages/default.aspx (accessed on 4 October 2019).

Peri, E., Cusumano, A., Agrò, A. and Colazza, S., 2011. Behavioral response of the egg parasitoid Ooencyrtus telenomicida to host-related chemical cues in a tritrophic perspective. *BioControl*, *56*(2), pp.163-171. DOI: 10.1007/s10526-010-9323-9

Peri, E., Frati, F., Salerno, G., Conti, E. and Colazza, S., 2013. Host chemical footprints induce host sex discrimination ability in egg parasitoids. *PLOS One*, *8*(11), p.e79054. DOI: 10.1371/journal.pone.0079054

Peri, E., Salerno, G., Slimani, T., Frati, F., Conti, E., Colazza, S. and Cusumano, A., 2016. The response of an egg parasitoid to substrate-borne semiochemicals is affected by previous experience. *Scientific Reports*, *6*, p.27098. DOI: 10.1038/srep27098

Peri, E., Sole, M.A., Wajnberg, E. and Colazza, S., 2006. Effect of host kairomones and oviposition experience on the arrestment behavior of an egg parasitoid. *Journal of Experimental Biology*, *209*(18), pp.3629-3635. DOI: 10.1242/jeb.02416

Pike, T.J., 2014. *Interactions between the invasive brown marmorated stink bug, Halyomorpha halys (Hemiptera: Pentatomidae), and entomopathogenic fungi* (Doctoral dissertation). University of Maryland College Park

Pote, J.M. and Nielsen, A.L., 2017. Life stage specific predation of Halyomorpha halys (Stål) by generalist predators. *Biological Control*, *114*, pp.1-7. DOI: 10.1016/j.biocontrol.2017.07.007

Qiu, L.F., Yang, Z. and Tao, W., 2007. Biology and population dynamics of Trissolcus halyomorphae. *Scientia Silvae Sinicae*, *43*(11), pp.62-65. DOI:

Rostás, M. and Wölfling, M., 2009. Caterpillar footprints as host location kairomones for Cotesia marginiventris: persistence and chemical nature. *Journal of Chemical Ecology*, *35*(1), pp.20-27. DOI: 10.1007/s10886-009-9590-z

Rostás, M., Ruf, D., Zabka, V. and Hildebrandt, U., 2008. Plant surface wax affects parasitoid's response to host footprints. *Naturwissenschaften*, *95*(10), p.997. DOI: 10.1007/s00114-008-0411-y

Sabbatini Peverieri, G.S., Talamas, E., Bon, M.C., Marianelli, L., Bernardinelli, I., Malossini, G., Benvenuto, L., Roversi, P.F. and Hoelmer, K., 2018. Two Asian egg parasitoids of Halyomorpha halys (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: Trissolcus mitsukurii (Ashmead) and Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae). *Journal of Hymenoptera Research*, *67*, p.37. DOI: 10.3897/jhr.67.30883

Sagun, S., Collins, E., Martin, C., Nolan, E.J. and Horzempa, J., 2016. Alarm odor compounds of the brown marmorated stink bug exhibit antibacterial activity.

Journal of Pharmacognosy and Natural Products, *2*(3). DOI: 10.4172%2F2472-0992.1000119

Schmidt, K.A., Dall, S.R. and Van Gils, J.A., 2010. The ecology of information: an overview on the ecological significance of making informed decisions. *Oikos*, *119*(2), pp.304-316. DOI: 10.1111/j.1600-0706.2009.17573.x

Solomon, D., Dutcher, D. and Raymond, T., 2013. Characterization of Halyomorpha halys (brown marmorated stink bug) biogenic volatile organic compound emissions and their role in secondary organic aerosol formation. *Journal of the Air & Waste Management Association*, *63*(11), pp.1264-1269. DOI: 10.1080/10962247.2013.819047

Stahl, J., Tortorici, F., Pontini, M., Bon, M.C., Hoelmer, K., Marazzi, C., Tavella, L. and Haye, T., 2019. First discovery of adventive populations of Trissolcus japonicus in Europe. *Journal of Pest Science*, *92*(2), pp.371-379. DOI: https://doi.org/10.1007/s10340-018-1061-2

Stephens, D.W., 1989. Variance and the value of information. *The American Naturalist*, *134*(1), pp.128-140. DOI: 10.1086/284969

Talamas, E.J., Herlihy, M.V., Dieckhoff, C., Hoelmer, K.A., Buffington, M., Bon, M.C. and Weber, D.C., 2015. Trissolcus japonicus (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. *Journal of Hymenoptera Research*, *43*, p.119. DOI: 10.3897/JHR.43.4661

Thiel, A. and Hoffmeister, T.S., 2004. Knowing your habitat: linking patch-encounter rate and patch exploitation in parasitoids. *Behavioral Ecology*, *15*(3), pp.419-425. DOI: 10.1093/beheco/arh030

van Alphen, J.J.M. and Vet, L.E.M., 1986. An evolutionary approach to host finding and selection. In Insect parasitoids: 13th symposium of the Royal Entomological Society of London, 18-19 September 1985 at the Department of Physics Lecture Theatre, Imperial College, London / edited by Jeff Waage, David Greathead (pp. 23-61). London: Academic Press.

Vet, L.E. and Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, *37*(1), pp.141-172. DOI: 10.1146/annurev.en.37.010192.001041

Weber, D.C., Morrison, W.R., Khrimian, A., Rice, K.B., Leskey, T.C., Rodriguez-Saona, C., Nielsen, A.L. and Blaauw, B.R., 2017. Chemical ecology of Halyomorpha halys: discoveries and applications. *Journal of Pest Science*, *90*(4), pp.989-1008. DOI: 10.1007/s10340-017-0876-6

Yang, S.Y., Zhan, H.X., Zhang, F., Babendreier, D., Zhong, Y.Z., Lou, Q.Z., Zhong, Y. and Zhang, J.P., 2018. Development and fecundity of Trissolcus japonicus on fertilized and unfertilized eggs of the brown marmorated stink bug, Halyomorpha halys. *Journal of Pest Science*, *91*(4), pp.1335-1343. DOI: 10.1007/s10340-018-0998-5

Yang, Z.Q., Yao, Y.X., Qiu, L.F. and Li, Z.X., 2009. A new species of Trissolcus (Hymenoptera: Scelionidae) parasitizing eggs of Halyomorpha halys (Heteroptera: Pentatomidae) in China with comments on its biology. *Annals of the Entomological Society of America*, *102*(1), pp.39-47. DOI: 10.1603/008.102.0104

Zhang, A., Harris, C. and Leskey, T.C., US Department of Agriculture, 2016. *Methods and compositions for deterring feeding/repelling the brown marmorated stink bug (BMSB), Halyomorpha halys*. U.S. Patent 9,320,280.

Zhang, J., Zhang, F., Gariepy, T., Mason, P., Gillespie, D., Talamas, E. and Haye, T., 2017. Seasonal parasitism and host specificity of Trissolcus japonicus in northern China. *Journal of Pest Science*, *90*(4), pp.1127-1141. DOI: 10.1007/s10340-017-0863-y

Zhong, Y.Z., Zhang, J.P., Ren, L.L., Tang, R., Zhan, H.X., Chen, G.H. and Zhang, F., 2017. Behavioral responses of the egg parasitoid Trissolcus japonicus to volatiles from adults of its stink bug host, Halyomorpha halys. *Journal of Pest Science*, *90*(4), pp.1097-1105. DOI: 10.1007/s10340-017-0884-6

Footprints and ootheca of *Lycorma delicatula* influence host-searching and -acceptance of the egg-parasitoid *Anastatus orientalis*

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Abstract

The spotted lanternfly *Lycorma delicatula* White (1845) (Hemiptera: Fulgoridae) is an invasive insect that was first reported in North America in Berks county Pennsylvania, USA in 2014. It is a polyphagous phloem feeder that attacks over 70 plant species, threatening the agricultural, lumber and ornamental industries of North America. Infestations of the pest have been reported in several U.S. counties, and a lack of endemic predators and parasitoids feeding on *L. delicatula* suggests a release from natural enemies in the invaded range. An egg parasitoid *Anastatus orientalis* (Hymenoptera: Eupelmidae) was reported attacking *L. delicatula* at high rates in its native range and may play a key role in reducing its populations there. To better understand the foraging behavior of *A. orientalis*, a series of behavioral experiments were conducted to determine successful parasitism and behavioral responses to traces left by adult *L. delicatula* and to the oothecae which cover their eggs. Our results suggest that wasps detected chemical traces left by *L. delicatula* adults while walking on surfaces and exhibited a strong arrestment response. Moreover, wasps preferred to oviposit in egg masses with intact oothecae. The implications of these findings are herein discussed with regard to the exploitation of host kairomones by foraging wasps, as well as to its ability to overcome host structural defenses.

Keywords: Invasive species, Host-parasitoid interactions, Fulgoridae, Foraging behavior, Spotted lanternfly

1 Introduction

The spotted lanternfly, *Lycorma delicatula*, is a new invasive insect in North America that is native to China, Taiwan and Vietnam, and is also invasive in Japan, and Korea (Liu et al., 2017). Detections of this species in South Korea in 2004 followed by subsequent observations of large populations sparked increased interest in its biology, ecology, taxonomy, pest status and control (Kim et al., 2013). It was first detected in North America in Berks county, Pennsylvania, in 2014, and as of October 2018, it has also been reported in Delaware, New York, Virginia, New Jersey, Maryland, Connecticut and Massachusetts (Leach et al., 2019). The European and Mediterranean Plant Protection Organization categorized it in 2016 as an A1 pest (quarantine pests absent from the EPPO region), in anticipation of its potential arrival in Europe (EPPO, 2018).

Lycorma delicatula is a polyphagous phloem feeder whose recorded host range includes more than 70 plant species and vines in 25 different families, such as grape, apple, birch, hops, cherry, maple, poplar, and stone fruits (Kim et al., 2011; Dara et al., 2015). It has a univoltine life cycle in China and in invaded regions, it overwinters as eggs laid in masses consisting of several rows that are covered in a foamy deposit to form an ootheca. Egg masses are deposited on the smooth surfaces of living and dead trees, rocks and man-made structures (Figure 5.1). Nymphs emerge in spring and early summer and pass through four developmental instars before reaching the adult winged stage in July and August (Han, 2008). All life stages feed via a piercingsucking mouthpart on a wide range of host plants, which narrows as the insect matures, with adults exhibiting a strong attraction to the tree of heaven *Ailanthus altissima* just before egg-laying in October (Kim et al., 2011). This attraction to *A. altissima* can be used to increase the chances of detecting *L. delicatula* in the early stages of the invasion, and key volatiles of this host plant can lead to the development of lures and behaviorally compatible traps (Lee et al., 2019).

Figure 5.1. Dozens of *L. delicatula* egg masses laid on the underside of rocks on a stone wall (a) and on a dead standing tree (b). (Image by Anna Dougherty/USDA-ARS).

Lycorma delicatula does not inflict direct damage on fruits, but feeds instead on branches and stems, causing them to wilt and lose vigor, which may result in plant death. Puncture sites result in wounds that weep sap along the bark and constitute entry points for pathogens. The insect secretes large amounts of honeydew, which together with the oozing wounds promote the growth of sooty mold that can coat leaf surfaces and fruits, thus interfering with photosynthesis and reducing plant growth, crop yield and fruit quality (Han et al., 2008). Significant economic impacts have been recorded in grapevine orchards in South Korea, and mold-contaminated table grapes are deemed unmarketable (Lee et al., 2009). Abundant *L. delicatula* populations in

South Korea also cause public annoyance as the insects intrude into people's homes and neighboring orchards in autumn (Han et al., 2008); this has been seen in the Pennsylvania infestation as well (Spears and Mull, 2019). Potentially massive risks are posed by this invasive insect in North America; for example, Pennsylvania alone has US$ 18 billion worth of agricultural commodities, such as apples, grapes and hardwoods, that are threatened by future outbreaks (Pennsylvania Department of Agriculture, 2018).

Non-chemical control of *L. delicatula* includes tree banding with sticky traps, to catch nymphs and adults walking up the trees to reach the new, softer tissue. However, this method catches indiscriminately, with preliminary trials reporting impacts on non-target organisms such as birds, reptiles and other arthropods (Tracy Leskey, personal communication). Another management method is to remove the insect's preferred host plant *A. altissima*, through the application of herbicides (Pennsylvania Department of Agriculture, 2018). Several contact insecticides such as deltamethrin and fenitrothion, have been shown to have quick and high lethality against *L. delicatula* (Park et al., 2009). However, nearby populations can re-infest treated areas, thus the use of systemic insecticides, such as dinotefuran and imidacloprid, was proposed (Kim et al., 2011). Leach et al. (2019) found that chlorpyrifos provided 100% mortality of *L. delicatula* overwintering egg masses, while very low efficacy was obtained from OMRI-listed compounds, indicating anticipated difficulties among organic producers in controlling this pest.

In North America, the impact of natural enemies on *L. delicatula* populations remains ambiguous. Predation on this species is scarce, possibly due to the adult camouflaged appearance of the forewings (Figure 5.2), or to natural chemical defenses acquired by feeding on *A. altissima* and sequestering cytotoxins (Kang et al., 2011). Moreover, sequential behavioral defenses were recorded in *L. delicatula* in response to predator attack, such as rapid escape jumping, deimatic display that reveals its contrasting hindwings and black/yellow banded abdomen as well as death-feigning (Kang et al., 2016). Two fungal entomopathogens *Beauveria bassiana* and *Batkoa major* have caused a coepizootic which lead to localized collapse of a *L. delicatula* population in eastern United States (Clifton et al., 2019) and are now being evaluated for use as biocontrol agents. Also, field surveys confirmed that *Ooencyrtus kuvanae* (Hymenoptera: Encrytidae) parasitized eggs of *L. delicatula* in Pennsylvania (Liu and Mottern, 2017). In China and South Korea, native nymphal- and egg-parasitic wasps, such as *Dryinus* sp. nr. *browni* Ashmead (Hymenoptera: Dryinidae) (Yang, 1994) and *Anastatus orientalis* respectively, appear to be important in biological control. The latter is currently thought to be the major parasitoid species of *L. delicatula* in its native range, it provides varying degrees of successful parasitization ranging from 30 to 80 % depending on the region sampled and has a female biased sex ratio (Choi et al., 2014; Yang et al., 2015; Lee et al., 2019). *Anastatus orientalis* overwinters as mature larvae inside host eggs, with two eclosion periods reported in China, one in April-May and the second in August-September (Hou, 2013).

Female parasitoid wasps often rely on chemical cues to locate their hosts in multitrophic contexts and may exploit plant synomones and host volatiles for long-range location of infested patches (Hilker and Fatouros, 2015). The detection of their target life stage also depends on their ability to detect host pheromones, allomones and kairomones (Vet and Dicke, 1992; Fatouros et al., 2008). The first report of a parasitoid wasp using chemical traces to find its host was that of *Trichogramma evanescens* that exhibited an arrestment behavior when exposed to chemical trails left by the grain moth *Sitotroga cerealella* (Laing, 1937). Rostás and Wölfling (2009) found that female *Cotesia marginiventris* perform characteristic antennation when in contact with cuticular hydrocarbons from their larval host *Spodoptera frugiperda* footprints. Extensive studies on scelionid parasitic wasps and their heteropteran hosts have also increased our knowledge on the finely tuned exploitation of direct- and indirect-host cues, including reliability and detectability (Colazza et al., 1999; Conti et al., 2004; Peri et al., 2006; Salerno et al., 2009).

To uncover the foraging strategy of *A. orientalis* and its ability to locate infested patches with potential host eggs, bioassays were conducted to record and analyze parasitoid behavior when exposed to *L. delicatula* “footprints” (chemical compounds deposited on substrates after passage of adult). Moreover, to better grasp the role of the ootheca and how the wasp responds to it, the sequential assessment, acceptance behavior and parasitization of eggs by *A. orientalis* was evaluated when the parasitoids were exposed to covered versus uncovered egg masses.

Figure 5.2. A cryptic *L. delicatula* adult in a resting state with forewings providing camouflage that serve in predator avoidance. (Image by Anna Dougherty/USDA-ARS).

2 Materials and Methods

2.1 Insects

Spotted lanternfly field mated adults were collected in November 2018, in Marsh Creek Park, Downingtown PA (40°03'52.9"N 75°43'48.5"W), after citizens reported sighting the insect in this area. Specimens were transported to the USDA-ARS quarantine facility in Newark, DE, and maintained on field collected *A. altissima* stems in growth chambers (24°C, 16h L: 8h D and 70%RH). Populations of the egg-parasitoid *A. orientalis* originally collected from *L. delicatula* egg masses in China by J. Gould, were maintained on the same host in quarantine culture at the APHIS laboratory in Massachusetts and were shipped to the ARS laboratory in Delaware for these studies. *Lycorma delicatula* egg masses subjected to the egg-assessment and choice-test experiments were collected in February 2019 from the same above-mentioned location, thus were approximately 3 months old and possessed a hardened ootheca.

2.2 Foraging behavior bioassays

All experiments were carried out in the quarantine facility of USDA-ARS laboratories in Newark DE, at 23±1 ºC and 35±10 % RH. Step (1): a single gravid adult female *L. delicatula* was placed in an arena consisting of a petri dish, confined in a plastic container and forced to walk on a filter paper (4.25 cm ⌀) for 30 minutes, to collect its chemical footprints. Whenever the insect rested and stopped walking, the container was gently rotated to motivate continued movement. Step (2): the contaminated filter paper was then transferred to a sterile glass petri dish (8 cm ⌀) which was placed on a LED light pad (MEDALight LP-400N, Technical Lamps Ltd, Middlesex, United Kingdom) providing illumination from below to optimize visibility and contrast. Directly above the center of the light pad, a camera (ICD-49, Ikegami Tsushinki Co., Ltd., Tokyo, Japan) was mounted on a portable tripod and connected to a video monitor and desktop PC (Figure 5.3). Step (3): a single 2-4 day-old, mated, naïve –had no previous experience of an egg mass– *A. orientalis* female was placed in the arena and confined under a plastic lid (0.5 cm height). Using the motion tracking software EthoVision XT 8.0 (Noldus Information Technology, Wageningen, The Netherlands) the behavior of the wasp was recorded, tracked and processed in real-time. The variables studied included the wasp's residence time (time spent on the filter paper), mean linear velocity (mm/s), angular velocity (°/s) and distance moved (mm). Recording commenced 2 seconds after the wasp was detected by the software on the filter paper and stopped 15 seconds after it left the paper. Each wasp was used for one replicate only, and 25 replicates were conducted for each treatment. A control treatment consisted of a non-contaminated filter paper. For both treatments, each filter paper was used for 5 replicates, and 25 replicates were conducted for each treatment. We tested for an effect of the order in which wasps were placed on the filter paper to see if the footprints might have a diminishing effect with time, or if the previous presence of wasps walking on the filter paper might impact behavior of subsequent wasps. For all response variables except residence time, we used multiple regression models to test for a main effect of wasp sequence and for an interaction between sequence and treatment, after controlling for block. Because residence time did not meet the multiple regression assumption of normality under any transformation used, we performed a Kruskal-Wallis test to evaluate the effect of wasp sequence.

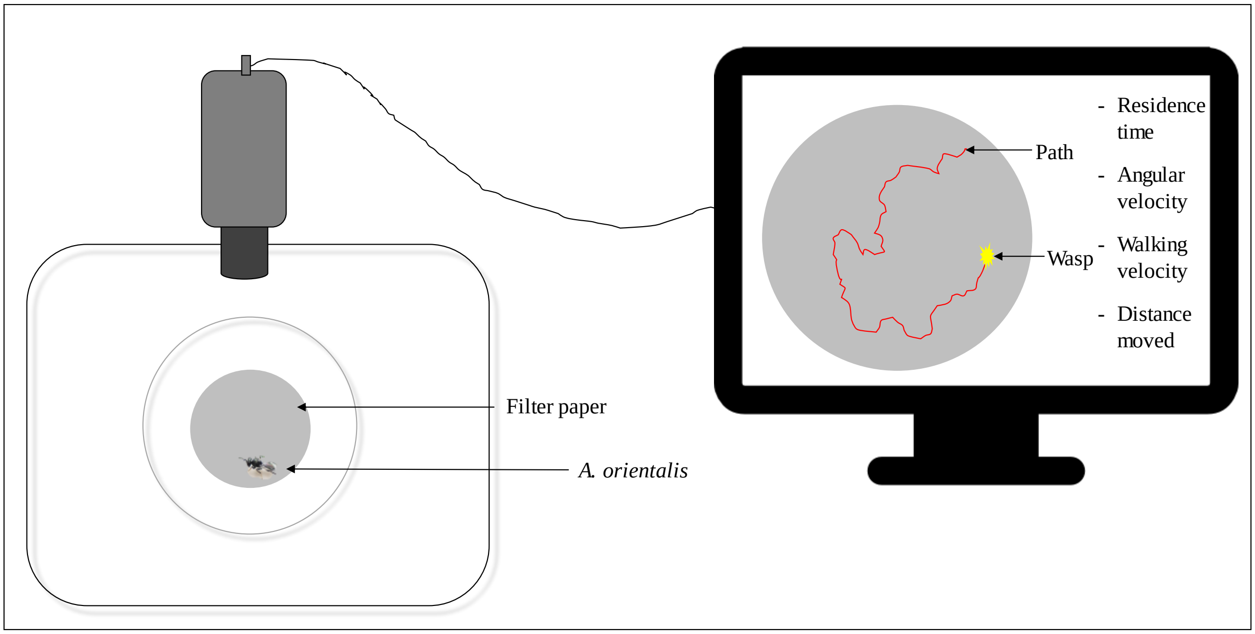

Figure 5.3. Diagram of the experimental setup concerning the foraging behavior of the wasp, with the monitor displaying the recorded path and variables.

2.3 Egg-assessment and-acceptance

In order to decode the wasp's ovipositional sequence and assess the role of the foam egg-covering (ootheca) in providing ovipositional signals or protection, the behavior of the wasp was observed and recorded on egg masses covered by *L. delicatula* secretions and on artificially uncovered ones (Figure 5.4). The uncovering process consisted of brushing the egg mass with a fine brush for 1 minute, the time needed to remove the foam covering and fully expose all underlying eggs. First, a subset of egg masses was presented to wasps, in order to identify the different steps leading to oviposition. The steps observed were as follows: (1) antennal contact- contact with the egg mass by at least one wasp antennae; (2) antennation and arrestment- wasp slows down its movement and intensifies antennation of the egg surface; (3) probing- insertion of the ovipositor in an egg for a brief period (0-2 minutes); (4) oviposition/abdominal oscillation- insertion of the ovipositor in an egg for a long period (>9 minutes), with abdominal vibrations and oscillations likely corresponding to the deposition of an egg, as has been shown for Trichogrammatidae (Kaiser et al., 1987), Encrytidae (Strand, 1989) and Mymaridae (van Baaren et al., 1995). All egg masses in this experiment were on pieces of dry wood on which the eggs were naturally deposited in the field. The arena for observations consisted of an egg mass in a glass petri dish under a binocular microscope, where a single 4-5-day-old, mated, naïve *A. orientalis* female was gently placed and confined by a plastic lid (0.5 cm height). Observations lasted for 25 minutes, starting when the wasp contacted the egg mass. Both the wasp and the egg mass were replaced for each replicate, with a total of 12 replicates for each treatment.

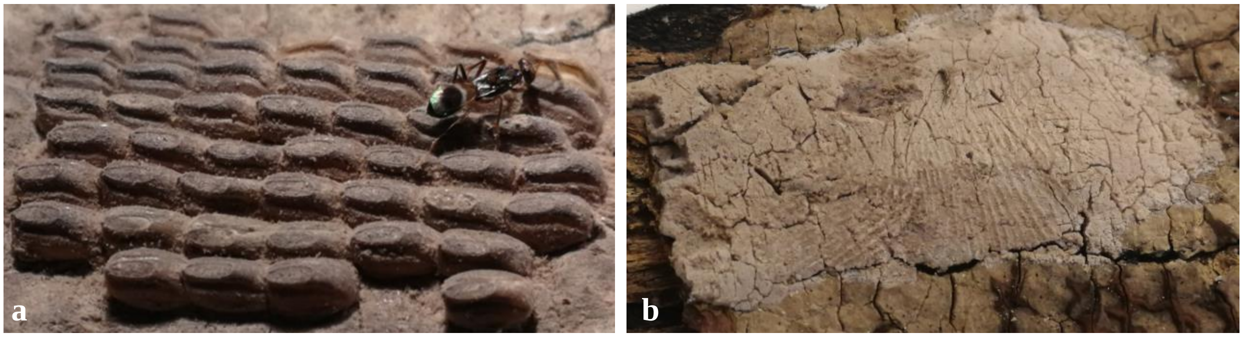

Figure 5.4. A female *A. orientalis* examining an uncovered egg mass (a). An intact egg mass within ootheca laid on a piece of bark.

2.4 Parasitism choice test of covered vs uncovered egg masses

To follow up on parasitoid ovipositional preferences, *A. orientalis* wasps were set up in a choice test with *L. delicatula* egg masses with an ootheca covering and without. Thirty replicates of one newly emerged (<24 hours old) female and male *A. orientalis* were each placed in a rearing container (16-oz plastic deli cup with mesh lid). The wasps were given one-week pre-oviposition under 'Beijing-conditions' that cycled from 25˚C to 14˚C daily with 13.5 hours of light and 65% RH. They had honey streaked on the mesh lid. If any male died during this week, it was replaced. Following the one-week pre-oviposition, two *L. delicatula* egg masses were introduced to each cup, one with an intact ootheca while the other had the ootheca brushed off, as previously described. Wasps were allowed to oviposit for one week, then they were removed, and the egg masses were separated into two distinct cups to monitor subsequent emergence of *L. delicatula* nymphs and of *A. orientalis* progeny (emerged and un-emerged wasps) from each type of egg mass independently. The eggs were maintained under 'Beijing-conditions' for one month, then were moved to 25˚C constant temperature and 16 hours of light to encourage wasp emergence. Hatching of *L. delicatula* nymphs and *A. orientalis* wasps was counted and un-hatched eggs were dissected to determine their fate.

2.5 Statistical analysis

For *A. orientalis* foraging behavior, the distributions of all response variable values for each treatment were evaluated for normality using a Shapiro Wilk test ($a = 0.05$), and if required, appropriate transformations were made. The angular velocity (P= 0.52) and linear walking velocity (P= 0.13) data were normally distributed. The distance data was not normally distributed (P<0.001), therefore logarithmic transformation ($\log_{10}$) was applied. Residence time was not normally distributed under any transformation utilized, so a non-parametric test was used. Two-tailed Student's t-tests were then used to compare the response of *A. orientalis* on footprint-contaminated filter papers and on controls, except for residence time, for which we utilized a Wilcoxon rank-sum test. For egg assessment and acceptance, the frequencies of each parasitoid behavior on covered and uncovered egg masses were compared using generalized linear models with binomial distributions. Regarding the parasitism choice-test, differences in the number of wasp offspring in each type of egg mass (removed ootheca vs intact ootheca) were compared using a one-sided t-test. All statistical analyses were performed in R (v. 3.4.3).

3 Results

3.1 Foraging behavior

The behavior of the wasps varied greatly on the treated substrate compared to the controls. Significant differences were observed between the two treatments regarding the residence time, distance moved, and linear walking velocity (Figure 5.5). On average, the wasp's residence time on footprint-contaminated filter papers was over fourfold (mean 318.6 ± 30.2 s SEM) than that spent on the control (65.6 ± 6.5 s) with (W= 13; P<0.0001). The distance moved by the wasp was significantly greater on the footprint-contaminated filter paper (259.4 ± 29.7 cm) when compared to the control (73.7 ± 10.7 cm) (t_{48}=7.34; P<0.0001). The linear walking velocity of *A. orientalis* was significantly slower on footprint-contaminated filter paper (11.6 ± 0.8 mm/s) compared to the control (18.65 ± 1.01 mm/s) (t_{48}=5.28; P<0.0001). There was no significant difference between the angular velocity of the wasp on footprint-contaminated substrate (115.2 ± 4.7 °/s) compared to controls (108.3 ± 6.7 °/s)

(t_{48}=0.84; P>0.402). There was no main or interaction effect of the order in which wasps were placed on the filter papers (p>0.05 for all response variables).

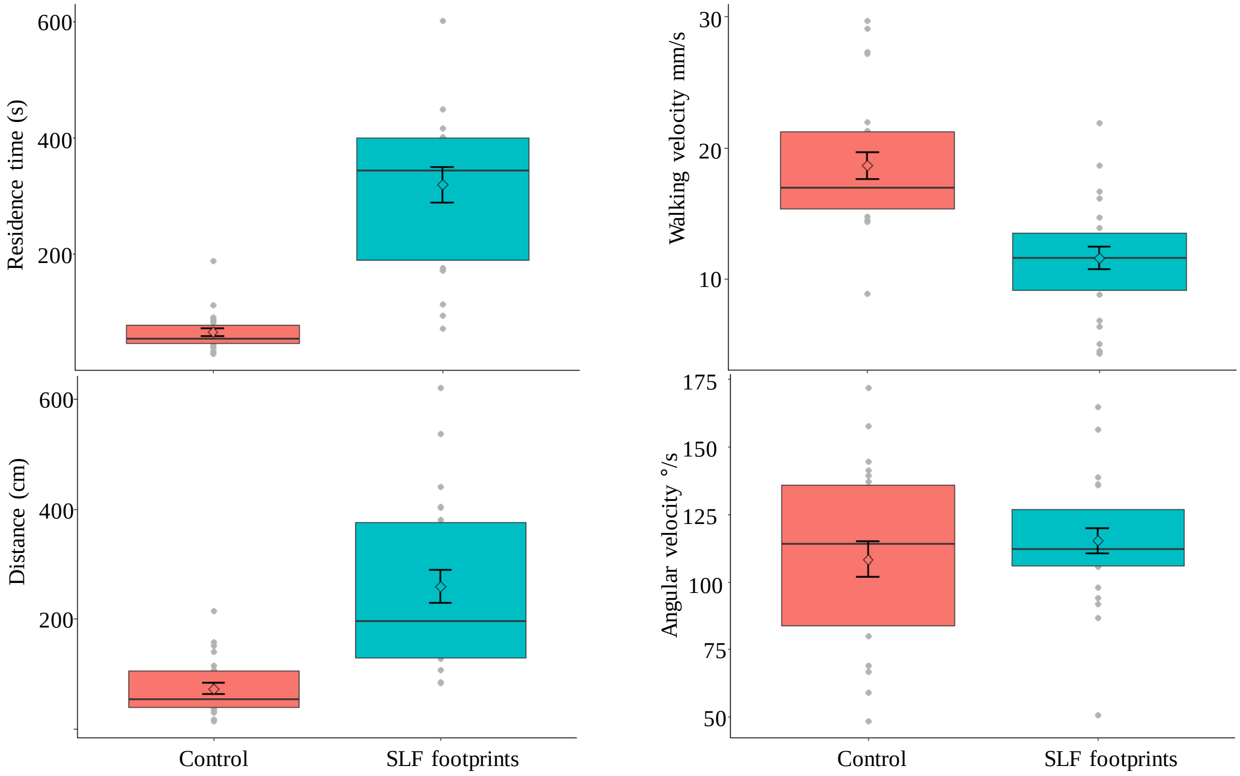

Figure 5.5. Boxplots showing residence time, linear walking velocity, distance covered and angular velocity of *A. orientalis* on footprint-contaminated substrate and on control. The first three of these recorded variables were statistically different from controls, whereas only angular velocity was not. Boxes represent interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean ± SEM. Grey dots represent data points that fall outside the interquartile range. N=25 replicate/treatment.

3.2 Egg-assessment and -acceptance

A total of 12 egg masses were presented to 12 unique *A. orientalis* females for each treatment. Our results summarized below (Table 5.1) reveal that for both treatments, all wasps encountered the egg mass and exhibited an arrestment behavior, characterized by a slower motion and intense antennation of the substrate. Covered egg masses were probed with significantly higher frequency (66.6%)

compared to uncovered ones (16.6%) (P= 0.0197). Inferred oviposition instances were also significantly more frequent on covered egg masses (58.3%) rather than on uncovered ones (16.6%) (P= 0.0451).

Table 5.1. Ovipositional sequence, egg assessment and acceptance by *A. orientalis* of *L. delicatula* covered and uncovered egg masses.

SLF egg mass	Contact	Antennation/ arrestment	Probing	Oviposition/ oscillation
Covered	12	12	8*	7*
Uncovered	12	12	2	2

N=12, * $P<0.05$

3.3 Parasitism choice test of covered vs uncovered egg masses

Significant differences were observed between the two treatments, with fewer wasp progeny produced in egg masses that had the ootheca covering removed (17.57 ± 2.05), compared to egg masses with the ootheca left intact (23.63 ± 2.18) ($t_{57.7}$=-2.0253, P=0.0237).

4 Discussion

Insect parasitoids locate their hosts through sequences of behavioral steps that are mainly mediated by chemical cues that can be directly or indirectly related to the host (Vinson, 1998). On control substrates (non-contaminated filter papers), female *A. orientalis* residence times were short, they walked rapidly, and they consequently explored only small sections of the substrate. The parasitoid reacted to chemical footprints deposited by *L. delicatula* gravid females by altering orthokinetic (i.e.

speed of movement dependent upon the intensity of the stimulus) and locomotory behavior, and clearly initiated an arrestment response. The latter is characterized by a decrease in linear walking velocity, and an increase in residence time and distance covered on footprint-contaminated substrate (Peri et al., 2006; Rostás and Wölfling, 2009; Konopka et al., 2018). For scelionid egg-parasitoids, an increase in angular velocity (directional change) is also associated with an arrestment response (Colazza, 1999); however, this was not observed for *A. orientalis*, perhaps due to its larger size compared to scelionids. Nonetheless, the wasps exhibited an intensified searching behavior, prolonging their residence time and slowly covering greater distances on patches containing cues that might lead them to their host eggs.

Our results suggest that cuticular hydrocarbons or other chemicals from *L. delicatula* might be deposited on the substrate where the insect walked, potentially through its tarsal adhesive pads, or via abdominal contact. Consequently, *A. orientalis* switches to a state of "motivated searching" (Vinson, 1998), restricting the search to an area where eggs are more likely to be found. The *L. delicatula* females tested in the present study were gravid, so the wasps may have been detecting cues more closely proximate to oviposition. Salerno et al. (2009) found that *Trissolcus brochymenae* Ashmead (Hymenoptera: Scelionidae) residence time on substrate contaminated by mated *Murgantia histrionica* Hahn (Hemiptera: Pentatomidae) female footprints was significantly longer than that of mated males and unmated males and females. Further investigations of mated and unmated *L. delicatula* females and males are needed to better understand this aspect of wasp foraging.

Peri et al. (2013) showed that *Trissolcus* egg parasitoids only responded positively to footprints of hosts they are normally associated with. For instance, *T. brochymenae* only responded to footprints of *M. histrionica*, not to those of *Graphosoma semipunctatum* Fabricius (Hemiptera: Pentatomidae) nor to those of *Nezara viridula* L. (Hemiptera: Pentatomidae). In contrast, *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) only responded to footprints of its host *N. viridula* and not to the other heteropteran species tested. Insect host-parasitoid interactions are shaped by natural selection (Godfray, 1994), thus, the strength of *A. orientalis* arrestment on *L. delicatula* footprints may be considered a result of a pairwise co-evolution and warrants further testing.

Concentrated egg depositions in the form of oothecae (sometimes referred to as egg cases or egg pods) are common among dictyopterans (cockroaches, termites, and praying mantises), orthopterans, and even some coleopterans (Grimaldi and Engel, 2005). Oothecae likely evolved to protect the eggs from desiccation, predators, and parasitoids (Salas-Araiza et al., 2013). Our study revealed that *A. orientalis* probed and oviposited more frequently through the ootheca, rather than in artificially uncovered egg masses. This suggests that both mechanical, and chemical cues present in the ootheca may serve as triggers for probing and oviposition. For example, Vinson (1986) found that *Tetrastichus hagenowii* Ratzeburg (Hymenoptera: Eulophidae) relies on mucopolysaccharides and calcium oxalates as kairomones for the recognition of its host *Periplaneta americana* L. (Blattodea: Blattidae), but only when these cues are present on curved surfaces. Thus, the absence of an ootheca in our uncovered egg masses may have been suboptimal for host recognition and acceptance by *A. orientalis*. Although not systematically investigated here, it is worth mentioning that throughout our observations of parasitoids presented with uncovered eggs, several instances were recorded where the wasps probed the wood on which the eggs were laid, even after having antennated the exposed eggs (Figure 5.6).

Figure 5.6. *Anastatus orientalis* female probing the wooden plant material onto which *L. delicatula* egg masses were deposited, with ovipositor clearly inserted in non-host tissue.

It might be that while *A. orientalis* adults are chewing their way out of *L. delicatula* ootheca, they ingest or acquire chemical cues that later influence their ovipositional preferences. Hopkins' host selection principle (HHSP) states that phytophagous and parasitic insects will prefer to continue to breed on hosts onto which they had been laid and developed (Hopkins, 1916;1917). This notion was refined and expanded – and also heavily criticized in later studies, as reviewed by

Barron (2001). Corbet (1985) proposed that adults may respond to certain chemical cues acquired in the larval environment during early imaginal stages, termed ‘chemical legacy’. This host-associated learning occurs as the parasitoid emerges from the host, or shortly after emergence (Giunti et al., 2015). The adult females *A. orientalis* used in the present study had emerged from egg masses with intact oothecae, and this may have influenced host preference.

Our parasitism choice test revealed that *L. delicatula* eggs contained within an ootheca are significantly more suitable than uncovered egg masses for *A. orientalis* successful parasitism and development. *Anastatus orientalis* enters diapause and overwinters as mature larvae in *L. delicatula* egg masses (Hou, 2013). The preference for eggs within ootheca indicates that not only is the wasp using its host for reproduction but could also be benefiting from the egg case for the protection of its own progeny from harsh winter conditions. An arms race metaphor might be appropriate in this context, with *A. orientalis* managing to circumvent the structural defense of *L. delicatula* ootheca to its own likely fitness gains, shedding light on the potential co-evolution between these two species. The consequences of such a behavior on the likelihood of effective control by *A. orientalis* requires further investigation and field validation.

Our results provide insight into the foraging behavior of *A. orientalis*, a potential candidate for classical (importation) biological control of invasive *L. delicatula*. The former perceives chemical footprints of *L. delicatula* and invests vital time and energy in searching for host eggs when these traces are present. Further studies on the identity of the compounds eliciting such responses may be helpful in designing future parasitoid surveys. Future attention to the wasp's response to long-range cues and behavior in complex natural environments is also well-deserved. The preferences of the wasp are no less adaptive than its host's defensive strategies, with eggs in oothecae more frequently probed and stung than exposed ones. This warrants further chemical analyses on the composition of the oothecal covering to better understand its role and function. The higher parasitism frequency of egg masses with oothecae may inform and improve rearing methods of this biological control agent. Future studies on the host range and life cycle of this wasp will improve our understanding of its host-specificity and ecological risk-assessment for its potential introduction against this newly emerging invader. This study was made possible through access to biosecurity containment facilities and foreign exploration to gather natural enemies of *L. delicatula* in its native range. Early initiation of investigations aimed toward importation biological control provide valuable ecological insights, which can inform integrated pest management tactics, irrespective of whether a safe agent is successfully screened and released.

References

Barron, A.B., 2001. The life and death of Hopkins' host-selection principle. *Journal of Insect Behavior*, *14*(6), pp.725-737. DOI: 10.1023/A:1013033332535

Choi, M.Y., Yang, Z.Q., Wang, X.Y., Tang, Y.L., Hou, Z.R., Kim, J.H. and Byeon, Y.W., 2014. Parasitism rate of egg parasitoid Anastatus orientalis (Hymenoptera: Eupelmidae) on Lycorma delicatula (Hemiptera: Fulgoridae) in China. *Korean Journal of Applied Entomology*, *53*(2), pp.135-139. DOI: 10.5656/KSAE.2014.01.1.075

Clifton, E.H., Castrillo, L.A., Gryganskyi, A. and Hajek, A.E., 2019. A pair of native fungal pathogens drives decline of a new invasive herbivore. *Proceedings of the National Academy of Sciences*, *116*(19), pp.9178-9180. DOI: 10.1073/pnas.1903579116

Colazza, S., Salerno, G. and Wajnberg, E., 1999. Volatile and contact chemicals released by Nezara viridula (Heteroptera: Pentatomidae) have a kairomonal effect on the egg parasitoid Trissolcus basalis (Hymenoptera: Scelionidae). *Biological Control*, *16*(3), pp.310-317. DOI: 10.1006/bcon.1999.0763

Conti, E., Salerno, G., Bin, F. and Vinson, S.B., 2004. The role of host semiochemicals in parasitoid specificity: a case study with Trissolcus brochymenae and Trissolcus simoni on pentatomid bugs. *Biological Control*, *29*(3), pp.435-444. DOI: 10.1016/j.biocontrol.2003.08.009

Corbet, S.A., 1985. Insect chemosensory responses: a chemical legacy hypothesis. *Ecological Entomology*, *10*(2), pp.143-153. DOI: 10.1111/j.1365-2311.1985.tb00543.x

Dara, S.K., Barringer, L. and Arthurs, S.P., 2015. Lycorma delicatula (Hemiptera: Fulgoridae): a new invasive pest in the United States. *Journal of Integrated Pest Management*, *6*(1), p.20. DOI: 10.1093/jipm/pmv021

EPPO, 2019. European and Mediterranean Plant Protection Organization, EPPO Global Database. (https://www.eppo.int) (Accessed: 22 March 2019).

Fatouros, N.E., Dicke, M., Mumm, R., Meiners, T. and Hilker, M., 2008. Foraging behavior of egg parasitoids exploiting chemical information. *Behavioral Ecology*, *19*(3), pp.677-689. DOI: 10.1093/beheco/arn011

Giunti, G., Canale, A., Messing, R.H., Donati, E., Stefanini, C., Michaud, J.P. and Benelli, G., 2015. Parasitoid learning: current knowledge and implications for biological control. *Biological Control*, *90*, pp.208-219. DOI: 10.1016/j.biocontrol.2015.06.007

Godfray, H.C.J. and Godfray, H.C.J., 1994. *Parasitoids: Behavioral and Evolutionary Ecology* (Vol. 67). Princeton University Press.

Grimaldi, D. and M.S. Engel. 2005. *Evolution of the Insects*. Cambridge University Press, New York.

Han, J.M., Kim, H., Lim, E.J., Lee, S., Kwon, Y.J. and Cho, S., 2008. Lycorma delicatula (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. *Entomological Research*, *38*(4), pp.281-286. DOI: 10.1111/j.1748-5967.2008.00188.x

Hilker, M. and Fatouros, N.E., 2015. Plant responses to insect egg deposition. *Annual Review of Entomology*, *60*, pp.493-515. DOI: 10.1146/annurev-ento-010814-020620

Hopkins, A.D. 1917. A discussion of C. G. Hewitt's paper on "insect behaviour." *Journal of Economic Entomology*, *10*, pp.92-93.

Hopkins, A.D., 1916. Economic investigations of the scolytid bark and timber beetles of North America. *U.S. Department of Agriculture Program of Work for 1917*, p. 353.

Hou, Z.R. 2013. Study on the Lycorma delicatula and egg parasitoids (Doctoral dissertation, MS thesis dissertation, Chinese Academy of Forestry, Beijing, China).

Kaiser, L., M. Pham-Delegue, N. Hawlitzki and C. Masson. 1987. Comportement de recherche et d'oviposition de *Trichogramma maidis* Pint. et Voeg. (Hym: Trichogrammatidae): Analyse quantitative en conditions contrflres. *Comptes Rendus de l'Académie des Sciences*. *305*(111): 95-99.

Kang, C., Moon, H., Sherratt, T.N., Lee, S.I. and Jablonski, P.G., 2016. Multiple lines of anti-predator defence in the spotted lanternfly, Lycorma delicatula (Hemiptera: Fulgoridae). *Biological Journal of the Linnean Society*, *120*(1), pp.115-124. DOI: 10.1111/bij.12847

Kang, C.K., Le, S.I. and Jablonski, P.G., 2011. Effect of sex and bright coloration on survival and predator-induced wing damage in an aposematic lantern fly with startle display. *Ecological Entomology*, *36*(6), pp.709-716. DOI: 10.1111/j.1365-2311.2011.01319.x

Kim, H., Kim, M., Kwon, D.H., Park, S., Lee, Y., Huang, J., Kai, S., Lee, H.S., Hong, K.J., Jang, Y. and Lee, S., 2013. Molecular comparison of Lycorma delicatula (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan. *Journal of Asia-Pacific Entomology*, *16*(4), pp.503-506. DOI: 10.1016/j.aspen.2013.07.003

Kim, J.G., Lee, E.H., Seo, Y.M. and Kim, N.Y., 2011. Cyclic behavior of Lycorma delicatula (Insecta: Hemiptera: Fulgoridae) on host plants. *Journal of Insect Behavior*, *24*(6), p.423. DOI: 10.1007/s10905-011-9266-8

Konopka, J.K., Poinapen, D., Gariepy, T. and McNeil, J.N., 2018. Understanding the mismatch between behaviour and development in a novel host-parasitoid association. *Scientific Reports*, *8*(1), p.15677. DOI: 10.1038%2Fs41598-018-33756-6

Laing, J., 1937. Host-finding by insect parasites. 1. Observations on the finding of hosts by Alysia manducator, Mormoniella vitripennis and Trichogramma evanescens. *The Journal of Animal Ecology*, *6*(2), pp.298-317. DOI: 10.2307/1186

Leach, H., Biddinger, D.J., Krawczyk, G., Smyers, E. and Urban, J.M., 2019. Evaluation of insecticides for control of the spotted lanternfly, Lycorma delicatula, (Hemiptera: Fulgoridae), a new pest of fruit in the Northeastern US. *Crop Protection*, *124*, pp.104833. DOI: 10.1016/j.cropro.2019.05.027

Lee, D.H., Park, Y.L. and Leskey, T.C., 2019. A review of biology and management of Lycorma delicatula (Hemiptera: Fulgoridae), an emerging global invasive species. *Journal of Asia-Pacific Entomology*, *22*(2), pp. 589-596. DOI: 10.1016/j.aspen.2019.03.004

Lee, J.E., Moon, S.R., Ahn, H.G., Cho, S.R., Yang, J.O., Yoon, C.M. and Kim, G.H., 2009. Feeding behavior of Lycorma delicatula (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. *Korean Journal of Applied Entomology*, *48*(4), pp.467-477.

Liu, H., Hoelmer, K.A. and Gould, J.S., 2017. Natural enemies of the spotted lanternfly in Asia and North America. USDA Interagency Forum on Invasive Species. https://www.ars.usda.gov/research/publications/publication/?seqNo115=338695. (Accessed: 25 February 2019).

Park, J.D., Kim, M.Y., Lee, S.G., Shin, S.C., Kim, J.H. and Park, I.K., 2009. Biological characteristics of Lycorma delicatula and the control effects of some insecticides. *Korean Journal of Applied Entomology*, *48*(1), pp.53-57.

Pennsylvania Department of Agriculture. 2018. Spotted Lanternfly. https://extension.psu.edu/spotted-lanternfly (Accessed: 13 March 2019).

Peri, E., Frati, F., Salerno, G., Conti, E. and Colazza, S., 2013. Host chemical footprints induce host sex discrimination ability in egg parasitoids. *PLOS ONE*. *8*(11): e79054. DOI: 10.1371/journal.pone.0079054

Peri, E., Sole, M.A., Wajnberg, E. and Colazza, S., 2006. Effect of host kairomones and oviposition experience on the arrestment behavior of an egg parasitoid. *Journal of Experimental Biology*, *209*(18), pp.3629-3635. DOI: 10.1242/jeb.02416

Rostás, M. and Wölfling, M., 2009. Caterpillar footprints as host location kairomones for Cotesia marginiventris: persistence and chemical nature. *Journal of Chemical Ecology*, *35*(1), pp.20-27. DOI: 10.1007/s10886-009-9590-z

Salas-Araiza, M.D., Mackay, W.P., Valdez-Carrasco, J., Salazar-Solís, E. and Martínez-Jaime, O.A., 2013. Characterization and Comparison of the Eggs of Seven Species of Mexican Grasshoppers 1. *Southwestern Entomologist*, *38*(2), pp.267-275. DOI: 10.3958/059.038.0210

Salerno, G., Frati, F., Conti, E., De Pasquale, C., Peri, E. and Colazza, S., 2009. A finely tuned strategy adopted by an egg parasitoid to exploit chemical traces from host adults. *Journal of Experimental Biology*, *212*(12), pp.1825-1831. DOI: 10.1242/jeb.028308

Spears, L.R. and A.M.M. Mull. 2019. *Spotted Lanternfly [Lycorma delicatula (White)].* Utah State University Extension and Utah Plant Pest Diagnostic Laboratory.

Strand, M.R., 1989. Oviposition behavior and progeny allocation of the polyembryonic wasp Copidosoma floridanum (Hymenoptera: Encyrtidae). *Journal of Insect Behavior*, *2*(3), pp.355-369. DOI: 10.1007/BF01068061

Van Baaren, J., Nénon, J.P. and Boivin, G., 1995. Comparison of oviposition behavior of a solitary and a gregarious parasitoid (Hymenoptera: Mymaridae). *Journal of Insect Behavior*, *8*(5), pp.671-686. DOI: 10.1007/BF01997237

Vet, L.E. and Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, *37*(1), pp.141-172. DOI: 10.1146/annurev.en.37.010192.001041

Vinson, S.B. and Piper, G.L., 1986. Source and characterization of host recognition kairomones of Tetrastichus hagenowii, a parasitoid of cockroach eggs.

Physiological Entomology, *11*(4), pp.459-468. DOI: 10.1111/j.1365-3032.1986.tb00437.x

Vinson, S.B., 1998. The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control*, *11*(2), pp.79-96. DOI: 10.1006/bcon.1997.0601

Xiao, G.R., 1992. *Forest Insects of China*. Forestry Publishing House, Beijing.

Yang, C., 1994. Descriptions of Dryinus lycormae sp. n. and its biological notes (Hymenoptera Dryinidae). *Fauna and Taxonomy of Insects in Henan*, *1*, pp.37-44.

Yang, Z.Q., Choi, W.Y., Cao, L.M., Wang, X.Y. and Hou, Z.R., 2015. A new species of Anastatus (Hymenoptera: Eulpelmidae) from China, parasitizing eggs of Lycorma delicatula (Homoptera: Fulgoridae). *Zoological Systematics*, *40*(3), pp.290-302. DOI: 10.11865/zs.20150305

Satellite projects

Native egg parasitoids recorded from the invasive *Halyomorpha halys* successfully exploit volatiles emitted by the plant–herbivore complex

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Summary

When an accidentally introduced pest establishes in the invaded area, native natural enemies may adapt to the new host. A decade after the accidental introduction to Europe of the brown marmorated stink bug, *Halyomorpha halys*, two generalist native egg parasitoids, have been recorded from this invasive pest in the field. Both *Anastatus bifasciatus* (Hymenoptera: Eupelmidae) and *Ooencyrtus telenomicida* (Hymenoptera: Encyrtidae) can complete development to the adult stage within the new host. *Trissolcus basalis* (Hymenoptera: Scelionidae), which is not associated with *H. halys* in the field and cannot successfully complete development on this invader's eggs, has a narrower host range with preference for the green stink bug, *Nezara viridula*. Given the new potential associations set to happen in the invaded ranges, we investigated the long-range foraging of these wasps

when stimulated by volatiles from *H. halys* adults alone or from *Vicia faba* plants subjected to herbivory (feeding + oviposition) by *H. halys*. Both wasps *A. bifasciatus* and *O. telenomicida* positively responded to adult *H. halys* male volatiles and to *H. halys*-induced plant volatiles, indicating ability to exploit cues associated with the new host for egg location. *Trissolcus basalis* responds to plants induced by *N. viridula* but not to those induced by *H. halys*, suggesting that the plant volatiles are species specific and that the wasp is more specialized on the former trophic system. For *A. bifasciatus* and *O. telenomicida*, two generalist egg parasitoids, their capacity to exploit oviposition-induced plant volatiles suggests they adapted to use a wider range of cues for host-location. In this study, *V. faba* plants responded to herbivory by *H. halys* through activation of indirect defenses involving the release of volatiles that are attractive to indigenous natural enemies. The capacity of these native wasps to utilize invasive host-associated cues, offers hope for their potential as augmentative biological control agents against *H. halys* in Europe.

Vicia faba plants respond to oviposition by invasive *Halyomorpha halys* activating direct defenses against offspring

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Summary

The invasive stink bug *Halyomorpha halys* is established in many European and American agro-ecosystems, where it causes severe crop losses. Potential control measures might include enhancement of plant defenses. When attacked by herbivorous insects that oviposit on it, the plant may respond by priming direct defenses, which might affect the development of future brood. *Halyomorpha halys* attacks numerous plant species in the invaded areas. Here, we investigated whether *Vicia faba* plants challenged by *H. halys* females can impair the development of the pest's offspring through the activation of induced direct defenses. We measured the weight and dimension of *H. halys* nymphs that developed on oviposition-experienced plants after 7 and 17 days from hatching. Nymphs that developed on oviposition-experienced plants weighed less compared to those that developed either on control plants or on plants solely subjected to *H. halys* feeding, and third instars showed shorter dimensions (tibia length). This finding suggests that *V. faba* plants are elicited by the egg mass of the pest, activating in response physiological processes

that directly result in hampering juveniles' development. Molecular investigations revealed that in oviposition-experienced plants, higher and more rapid expression of two jasmonic acid-dependent genes (cysteine proteinase inhibitor gene and NAI1) was detected, possibly due to a mechanism of priming. This indicates that the rapid activation of these genes is associated with the release of proteins that when ingested by feeding nymphs, hamper digestion and proper development. Increased expression of the salicylic acid-responsive PR1 gene was also detected in egg-experienced plants, although the response was delayed compared to JA-dependent genes, suggesting an interplay between the JA and SA-dependent pathways. Our results imply that an early warning mechanism is activated in *V. faba* plants after detection of *H. halys* egg mass, allowing for a direct and swift response against hatching brood. The identification of the exact elicitors of this plant defensive mechanism may offer practical applications for reducing pest damages and improving management of this invasive pest.

A closer look at the antennae of the invasive *Halyomorpha halys*: fine structure of the sensilla

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Summary

The antennae of the brown marmorated stink bug, *Halyomorpha halys* play an important role not only in detecting food and mates but also in short-range location of conspecifics when aggregating for diapause. The morphology and distribution of antennal sensilla of *H. halys* were investigated at an ultrastructural level using scanning and transmission electron microscopy approaches. Adults have 5-segmented antennae, made up of a scape, a 2-segmented pedicel and two flagellomeres, while 5th instar nymphs have shorter, 4-segmented antennae, with only one pedicel segment. Five types of sensilla are distinguished, based on their shape, length and basal width and the presence of basal socket and pores: sensilla basiconica (types A, B, C, D and E), sensilla coeloconica, sensilla trichoidea and sensilla chaetica (types A and B). Sexual dimorphism was not observed in this species, with respect to the morphological features and abundance of sensilla. The most abundant sensory structures are sensilla trichoidea showing characteristics typical of olfactory sensilla, whereas the least abundant are sensilla coeloconica which may be involved in thermo- and hygro-reception. Basiconic sensilla type A are solely identified on the antennae of 5th instar nymphs, where they presumably have a dual

mechanosensory-gustatory role. Morphological description and mapping data of these sensilla lay the foundation for future trials using the single sensillum recording technique to advance our understanding of the underlying mechanisms of *H. halys* sensory physiology and chemical ecology. Moreover, this would push forward the development of potent attractant (such as host-derived volatiles) and repellent formulations that increase the trapping efficacy of *H. halys* in agricultural and urban settings.

Chapter 7

Conclusions and future perspectives

The fast spread of *H. halys* outside its native range and the severe damage that accompanied its invasion, called for the establishment of integrated management techniques to mitigate its economic and ecological impacts. As pest monitoring is imperative for a successful management, and traditional surveys of invasive species are laborious, costly and geographically confined, "BugMap" was designed to complement this task and alleviate these hurdles. This citizen science initiative allowed for the gathering of a large amount of data on the whereabouts of *H. halys* mainly in Trentino, but also in other Italian regions. The collected information permitted the uncovering of *H. halys* invasion dynamics in this North Italian region, decoding its movement from and to overwintering shelters, all while shedding new light on its dispersal, phenology and other life cycle parameters. The georeferenced recordings of this bug were employed to forecast its distribution at a fine scale and to identify potential invasion pathways as well as key areas at risk from its establishment. Revisiting the data after almost a year and a half revealed over 1000 additional recordings of this invasive stink bug. These new reports indicated that *H. halys* is in the expansion phase of its invasion, exploiting man-made structures, wild and cultivated plants, to further disperse and increase its population. BugMap also engaged citizens in numerous regions to report sightings of *H. halys*, thereby contributing to its early detection that is crucial for increasing chances of containment. The data communicated to farmers, technicians, and citizens, were instrumental in coordinating efforts including trap placement, increased monitoring, timing of sprays and other physical, cultural and biological management tactics. Widening the scope of BugMap to include other invasive species in Trentino such as the tiger mosquito is already in place, aiding phytosanitary services to improve the management of the disease-carrying invader. This freely available and user-friendly platform can be replicated with a high chance of success in other areas suffering from their comparable invasions. Lastly, coupling traditional monitoring and citizen science proved effective in enhancing invasive species monitoring, improving IPM strategies as well as involving citizens in local and regional scientific inquiries, thus increasing science and technology literacy in affected societies.

With *Trissolcus japonicus* following *H. halys* throughout its invasion route, a better understanding of host-parasitoid chemical ecology is necessary to evaluate risks associated with the natural or human-assisted spread of this exotic biological control agent. Thus, a comparative study of the wasp's response to cues originating from *H. halys* and from the new sub-optimal host *P. maculiventris* was carried out to better understand this novel trophic association. The wasp responded to footprints of adults of both species; however, it exhibited a much stronger arrestment in response to *H. halys* kairomones compared with *P. maculiventris*. Moreover, an intense arrestment was only recorded in response to *H. halys* nymphs, but not to *P. maculiventris* nymphs. The latter finding suggests that not only *P. maculiventris* cues are deemed suboptimal by the wasp, but also that *T. japonicus* may seek areas infested by all life stages of its main host, limiting its search to zones where target eggs are more likely to be found. *Halyomorpha halys* footprints remained bioactive and elicited an intense searching behavior in *T. japonicus* for at least four days after initial deposition. Further studies on the relevance of this result are needed, to determine whether this persistence interval is linked to the period during which *H. halys* eggs are still viable for parasitization. A series of chemical analyses and bioassays revealed the identity of the kairomone components utilized by *T. japonicus* for host finding. The stink bug defensive compound (*E*)-2-decenal shortened the residence time of *T. japonicus*, thereby fulfilling its role and repelling the wasp. In contrast, *T. japonicus* residence time was prolonged by *n*-tridecane, suggesting that this compound functions as an arrestant that is utilized by the wasp for improved host location. Further studies to determine the concentrations suitable for field applications are needed, as these may help improve parasitoid surveys, create natural enemy refugia and contribute to *T. japonicus* conservation and augmentation in pest-infested areas. The methodology applied in this line of research provided crucial information on the parasitoid's foraging behavior, its chemical preferences and potential repercussions on classical biological control. Although *T. japonicus* foraging was found to be less intense in response to *P. maculiventris* cues, non-target impacts cannot be excluded. These risks should be contextualized, as there are also major perils associated with inaction and the failure to implement biological control in a timely manner, making the consideration of an overall risk/benefit equation more necessary. With the continued and increased pesticide applications to control and keep *H. halys* populations in check, risks on water bodies, soil, air, biodiversity and ecosystem functions are inevitable. A strong case can be made that biological control agents contribute to a reduction of pesticide use, bringing forth inherent economic and environmental benefits. The difficult decision to release or redistribute *T. japonicus* - perform an assisted biological invasion - in *H. halys* infested territories or allow it to spread naturally, must then be made with imperfect knowledge. After the detection of *T. japonicus* in Northern Italy, financial, political and legislative pressure grew, which led agricultural extension services and Fondazione Edmund Mach researchers to plan its inoculative redistribution in the spring of 2020. Irrespective of the management potential and/or non-target risks, one thing is certain that interactions of this biological control agent with native fauna and flora will be occupying ecologists, conservationists, biologists and biological control practitioners for years to come.

The increasing frequency and rate of biological invasions recently raised a new insect to the scientific and public attention, the spotted lanternfly *Lycorma delicatula*. Foreign explorations in its native range resulted in the identification of a potential candidate for classical biological control, the Asian egg parasitoid *Anastatus orientalis*. As interactions between these two organisms are poorly understood, we designed an investigation that would disentangle this relationship and provide a better understanding of its implications for successful biological control. Bioassays of *A. orientalis* foraging behavior suggest that after walking on substrate, *L. delicatula* deposit chemical traces which are consequently used by the wasp as a host searching cue. Future studies on the nature of long-and-short range kairomones used during parasitoid foraging may prove beneficial in refining surveys. *Lycorma delicatula* females evolved egg-deposition in oothecal masses, likely to protect their progeny from harsh winter conditions and from natural enemies. The function of the pest's ootheca was inspected with regard to its effect on the foraging wasp; systematic visual observations disclosed that *A. orientalis* probes and oviposits more frequently in eggs contained within an ootheca. These findings imply that *A. orientalis* detects and uses mechanical and/or chemical cues present on the ootheca as a trigger for egg laying, thereby overcoming host structural defenses. Parasitism choice tests between egg masses with and without an ootheca showed that higher wasp progeny was recovered from *L. delicatula* egg masses that had the ootheca intact. Thus, *A. orientalis* may have evolved to use the host egg case to confer protection for its own progeny. The wasp's preference for eggs within ootheca ensues direct practical implications for improving parasitoid rearing techniques. Although studies on this biological control agent are still in their infancy, this research provided novel information on the evolutionary arms race taking place in this pairwise interaction and proved highly informative for integrated pest management tactics.

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The brown marmorated stink bug, *Halyomorpha halys* and the spotted lanternfly, *Lycorma delicatula* are invasive polyphagous pests from Eastern Asia. As sustainable control methods depend on the accurate monitoring of species' invasion and involve the use of natural enemies, we addressed these two facets by exploring novel monitoring techniques and deciphering host-parasitoid interactions. We adopted 'BugMap', a citizen science initiative that enables students, farmers and everyday citizens to report sightings of *H. halys* from Italy, with emphasis on Trentino-Alto Adige. Aside from fostering citizen participation in scientific endeavors and the enhanced literacy that ensues, BugMap helped uncover the invasion dynamics of *H. halys* and forecast its potential distribution, all while informing management strategies. The most promising agent currently under study for the classical biological control of *H. halys* is the Asian egg parasitoid *Trissolcus japonicus*. To assess this wasp's non-target impacts, we investigated its behavior in response to chemical traces 'footprints' deposited by its main host *H. halys* and by a suboptimal predatory species, the spined soldier bug, *Podisus maculiventris*, and found that it exhibited a strong natural preference for *H. halys*. A series of chemical analyses and behavioral bioassays revealed the identity and function of two putative components utilized by the wasp for short range host location, elucidating the chemical ecology of this biocontrol agent. Moving to the other invader *L. delicatula*, an egg parasitoid *Anastatus orientalis* was reported attacking it at high rates in its native range. Behavioral bioassays showed that wasps responded to footprints of *L. delicatula* by initiating a strong searching behavior. Moreover, *A. orientalis* preferred to oviposit in egg masses with intact oothecae, suggesting that the egg covering is a trigger for host-acceptance. Thus, *A. orientalis* not only overcomes, but also reverses an important line of host structural defense. These findings are discussed throughout the chapters of this dissertation.

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