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Sarracenia carnivorous plants cannot serve as efficient biological control of the invasive hornet Vespa velutina nigrithorax in Europe

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Abstract

Vespa velutina nigrithorax, an invasive species, a direct result of increased trade and climate change, is spreading rapidly in Europe and endangering entomofauna in general and more alarmingly honeybee (Apis mellifera) populations, and therefore their pollination services. All traps used now, to try to control this species, seem to be not efficient enough and non-selective. However, in the current context of massive disappearance of insects in general, it is urgent to find means of protection for the entomofauna. While no selective trapping is still occurring, we performed a pilot study to test a carnivorous plant as a potential biocontrol tool to trap V. velutina. In our study, we analyzed the hornet-capturing ability of two Sarracenia hybrids (S. juthatip soper and S. evendine) on a 2-years period (2015 and 2016). Our results show that these plants trapped more dipterans than other taxa, and they do not attract many hornets. In such condition, both Sarracenia hybrids cannot therefore be used in a mass trapping system, because they are not selective, and too few hornets are trapped. To maximize captures of V. velutina while minimizing captures of non-target species, other systems need to be thus developed, as traps using hornet pheromone-based baiting.

Keywords

Invasive species, yellow-legged hornet, selective trap, biocontrol, pest management, Sarracenia sp.

Introduction

Biological invasions have occurred frequently around the world, especially in recent decades, mainly due to the increase in human travel and international trade (Levine and D'Antonio 2003; Evans and Oszako 2007; Meyerson and Mooney 2007). Social insects are considered to be particularly invasive because their reproduction and social structure allow them to rapidly exploit new environments (Beggs et al. 2011). They have thus colonized a large range of ecosystems worldwide (Moller 1996; Chapman and Bourke 2001; McGlynn 1999; Beggs et al. 2011). Nine of the 34 *Vespidae* species introduced across the globe are social, and they all have detrimental ecological impacts (Beggs et al. 2011). Introducing a predator into a new ecosystem can have disastrous consequences for local species that have become prey (Williamson and Fitter 1996; Savidge 1987; Wiles et al. 2003; Fritts and Rodda 1998). Not only are invasive social insects a threat to native biodiversity, but they are often also responsible for huge economic losses (Pimentel et al. 2005).

Around 2004, the yellow-legged hornet, *Vespa velutina nigrithorax*, was accidentally introduced into France from China (Rortais et al. 2010; Monceau et al. 2014; Arca et al. 2015). This species has since successfully expanded its range, which now covers more than 80% of France, and is currently colonizing neighboring countries (Spain, Portugal, Italy, Belgium, Germany, Switzerland, Netherlands and Great Britain) (Franklin et al. 2017; Keeling et al. 2017; Robinet et al. 2017, in press). The *V. velutina* invasion in France has caused ecological, economic, and public health problems. The hornet preys on several insect and arthropod taxa, thus potentially affecting biodiversity. It is a predator of the domestic honeybee, *Apis mellifera*, and can result in colony losses, which has significant economic impacts for the apiculture industry (Monceau et al. 2013).

The use of baited traps is generally regarded as the best means to control wasps, although uncontrolled mass trapping induces side effects on non target species (Beggs et al. 2011; Rome et al. 2011). The most used traps, generally baited with beer, kill a huge number of non-target insects, versus only few *V. velutina* (about 1% of the captures on average) (Dauphin and Thomas 2009; Rome et al. 2011). Indeed, eradication campaigns seem inefficient and harmful to biodiversity (Demichelis et al. 2014; Rome et al. 2011), encouraging the development of traps with selective bait. While no selective bait (pheromonal trapping for example) is still occurring, we performed a pilot study to test a carnivorous plant to trap *V. velutina*. Meurget and Perrocheau (2015) observed that carnivorous plants of the genus *Sarracenia* can trap *V. velutina*.

To replace classical traps which demonstrated their non-selectivity (Dauphin and Thomas 2009; Rome et al. 2011), we thus examined the hornet-capturing ability of the *Sarracenia* hybrids previously studied by Meurgey and Perrocheau (2015), over a two-year period, with a view to determining their potential use in *V. velutina* biological control efforts.

Materials and methods

Plant sampling and analysis of trapped insects

Our study focused on two *Sarracenia* hybrids: *S. juthatip soper* and *S. evendine*. They were collected in the botanical garden of Nantes (GPS coordinates 47°13'10"N, 1°32'34'W). These hybrids produce new urns in autumn that will persist and remain active until the emergence of new urns in May. More than 50 plant stools were present in a peat bog of about 60 square meters. Samples were collected twice: 132 and 120 urns were randomly collected on the peat bog surface in October 2015 and 2016 respectively. Since the plants are cultivated in a relatively small numbers and are destined to recreate a bog habitat for the gardens' visitors, it was problematic to sample a larger number of urns for our experiment. Thus, to maximize our sample size while limiting our impact on the bog, we pooled the samples from the two hybrids. Before being opened with a scalpel, urns were frozen at -20 °C for 24 hours to kill any insects inside. The insects inside urns were counted and identified to order.

Hornet nests location

Technicians from the city hall of Nantes and from Bionéo (sarl Prophy Végétal) located *Vespa velutina* nests in and around the botanical garden of Nantes. These data were useful to determinate if *Sarracenia* plants we collected could be present in the foraging area of hornet colonies. According to their flight capacities, workers can forage in an area of 2.000 m around their nest (Sauvard et al. 2018). We performed heatmaps with the software QGIS 2.18.20. We used EPSG: 2154, RGF93/Lambert 93 as reference coordinate system.

Data analysis

We performed data analyses with the software Rstudio (1.0.143 version – 2009–2016 RStudio, Inc.). Analyses were used to compare the relative abundances of the insect orders found in the urns (*Diptera*, *Hymenoptera*, *Lepidoptera*, *Coleoptera* and *Heteroptera*), and in *Hymenoptera* (*Vespa velutina*, *V. crabro*, *Vespula* sp., *Apis* sp. and *Bombus* sp.). To compare the abundances of the insect within each sampling year, we used a chi-squared test and a Wilcoxon signed-rank test for paired samples. We used a Bonferroni correction to reduce type I errors per year (corrected $\alpha = (0.05/10) = 0.005$).

Results

In 2015 and 2016, several *Vespa velutina* colonies were located in Nantes city, and particularly in the botanical garden (Figure 1). In 2015, 1398 insects were present in the

sampled urns of *Sarracenia* plants (mean \pm S.E. insects per urn: 10.59 \pm 0.43): 1082 dipterans, 138 hymenopterans, 106 lepidopterans, 71 coleopterans, and 1 heteropteran. In 2016, 1123 insects were present (mean \pm S.E. insects per urn 9.36 \pm 0.50): 848 dipterans, 17 hymenopterans, 235 lepidopterans, 22 coleopterans, and 1 heteropteran (Figure 2).

The relative abundance of insect orders was analyzed for both experimental years (chi-squared test): in 2015, with *Heteroptera* (one individual), X-squared = 2915.5, df = 4, p-value < 2.2e-16, and without the heteropteran individual, X-squared = 2056.2, df = 3, p-value < 2.2e-16; in 2016, X-squared = 2328, df = 4, p-value < 2.2e-16 and X-squared = 1641.3, df = 3, p-value < 2.2e-16 with or without the heteropteran individual respectively. The comparison of the relative abundance of insect orders for both years combined without heteropteran individuals (one individual per year) showed differences (X-squared = 169.44, df = 3, p-value < 2.2e-16). In 2015 and 2016, dipterans trapped by *Sarracenia* plants were significantly more common than any other taxa (Table 1). However, plants captured significantly more *Vespa velutina* than *V. crabro* (60 *V. velutina* vs 12 *V. crabro* in 2015; 8 *V. velutina* vs 0 *V. crabro* in 2016) (Table 2). The average numbers of *V. velutina* trapped is similar to *Vespula* species in 2015, but is significantly higher in 2016. Number of *V. velutina* trapped is significantly higher than bees in 2015, but not in 2016.

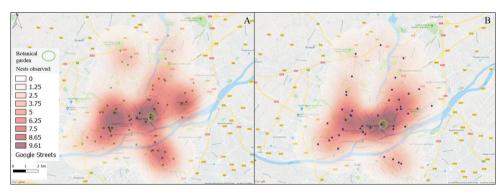


Figure 1. Heatmap of *Vespa velutina*'s nests observed in 2015 (A) and in 2016 (B). The green circle shows the location of the botanical garden where plants were collected.

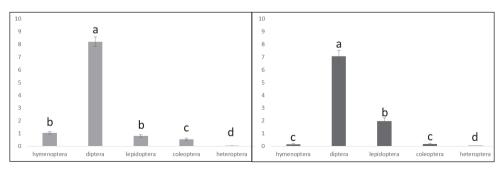


Figure 2. Average number of insects per urns in 2015 (light grey) and 2016 (dark grey). Letters indicate the statistical differences at p=0.005 (Wilcoxon test with Bonferroni correction).

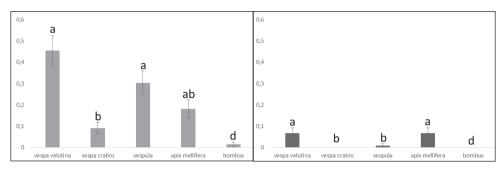


Figure 3. Average number of Hymenoptera per urns in 2015 (light grey) and 2016 (dark grey). Letters indicate the statistical differences at p=0.005 (Wilcoxon test with Bonferroni correction).

Table 1. Comparison (Wilcoxon test) of relative abundances of some insect orders (Diptera, Hymenoptera, Lepidoptera, Coleoptera and Heteroptera) trapped by *Sarracenia* hybrids in 2015 and 2016.

| 2015 / 2016 | Diptera | Hymenoptera | Lepidoptera | Coleoptera |
|-------------|--------------------------------|------------------------|-------------|------------|
| Hymenoptera | Z=672, P<0.001 for both year | | | |
| Lepidoptera | Z=16924, P<0.001 for both year | NS / Z=9678.5, P<0.001 | | |
| Coleoptera | Z=16968, P<0.001 for both year | NS / NS | NS/NS | |
| Heteroptera | Z=17290, P<0.001 for both year | NS / NS | NS/NS | NS/NS |

Table 2. Comparison (Wilcoxon test) of relative abundances of Hymenoptera trapped by *Sarracenia* hybrids in 2015 and 2016.

| 2015 / 2016 | Vespa velutina | Vespa crabro | Vespula sp. | Apis sp. |
|--------------|-----------------------|----------------------|-----------------------|----------------------|
| Vespa crabro | Z=10810, P<0.001 / | | | |
| | Z=7620, p=0.007 | | | |
| Vespula sp. | Z=9571, p=0.07 / | 7 7/00 - 0.002 / NC | | |
| | Z=7560, p=0.03 | Z=7488, p=0.002 / NS | | |
| Apis sp. | Z=10230, P<0.001 / NS | NS / NS | NS / NS | |
| Bombus sp. | Z=11365, P<0.001 / NS | NS / NS | Z=10503, P<0.001 / NS | Z=9903, P<0.001 / NS |

Discussion

Our results show that the carnivorous *Sarracenia* hybrids we analyzed do not attract a lot of *V. velutina* hornets, in spite of the presence of several hornet colonies at proximity. Indeed, *V. velutina* represented just a small percentage of the large numbers of insects captured in the plant urns. In 2015 and 2016, only 4.3% and 0.7% of all the insects captured were *V. velutina*, respectively. Hymenopterans were infrequent; flies were the main prey. Indeed, this result is classical, as ants and flies are the two main prey groups in insectivorous plants (Kato et al. 1993, Adam 1997; Chin et al. 2014). Consequently, these two *Sarracenia* hybrids cannot therefore be used in a mass trapping system because too few hornets are caught in the urns we have dissected. This lack of *V. velutina* trapping is not linked to its absence in the botanical garden, as several nests were located. Sampling efficiency can be defined as a measure of the ability to

count all the insects in a chosen sample (Dent 2000). Trapping efficiency can be thus defined as a measure of the ability to trap all targeted insects (or a significant proportion) in a chosen area; a significant proportion means sufficient number of insects to have a potential impact on the local population. For V. velutina, it means a sufficient number of workers trapped to have a potential impact on the colony fitness. By the end of summer, a colony can reach about 1000 workers (Choi et al. 2012), and a large number leaves the nest to forage. For example, if only 200 workers foraged in the experimental area (botanical garden), and five nests were present in such area 1000 individuals (200×5) could be present near the carnivorous plants. However, in our study, 60 V. velutina in 2015 and 8 in 2016 were trapped. These few individuals trapped (1.2% and 0.16% of the 200 foragers by colony in 2015 and 2016 respectively) should have no impact on colonies fitness and even less at the level of the species. According to such results, the mean number of urns needed to capture a certain percentage of the V. velutina population can be estimated. If more than 50% of foragers from each colony (~500 hornets) need to be trapped to have an impact on the colony fitness, more than 4.000 urns (mean value on the 2-years experiment) are thus necessary for one hornet colony. If five nests were present in the area, more than 20.000 urns would be necessary. If each Sarracenia plant possess about five urns, more than 4.000 plants have to be cultivated. As it is difficult to cultivate such number of Sarracenia, this carnivorous plant could be not a good biological control tool. Moreover, as the Sarracenia hybrids we analyzed are not selective, they would have a huge negative impact on other insect species, i.e. on biodiversity. These plants cannot be used as traps to reduce the predation pressure on apiaries: we observed that a few honeybees had also been trapped in the plants (N=24 in 2015 and N=8 in 2016), which could be problematic if they were to be used in apiaries.

Other species of *Sarracenia* or *Nepenthes* for example should be investigated to determine if these species can trap more hornets than the *Sarracenia* hybrids *S. juthatip soper* and *S. evendine*. The quantification of their attractiveness and the identification of visual and/or olfactory cues they used need to be performed. The aim will be to develop a specific biomimetic trap for *V. velutina*. However, the genus *Nepenthes* uses highly diverse means to attract and capture many different types of insects (Moran et al. 1999 2013; Bonhomme et al. 2011; Gaume et al. 2002 2007; Bazile et al. 2015; Merbach et al. 2001). Attractants range from nectar rewards (Bauer et al. 2009) to volatile compounds (Di Giusto et al. 2010) and visual cues (Moran et al. 1999). Nevertheless, specific mechanisms can target specific insect guilds. For example, the capture of flies is favored by viscoelastic fluids (Bonhomme et al. 2011; Bazile et al. 2015) or translucent tissues acting as light traps (Moran et al. 2012). It could be possible to identify a specific signal, which could selectively attract hornets. Further studies are thus necessary.

In seeking control solutions, it is difficult to strike the right balance between controlling the invasive hornet, its effects on native pollinators, and limiting the deleterious effects of traps on entomofauna. None of the traps currently used seem to show specificity for *V. velutina*. Baited traps are generally regarded as the best means for controlling wasps and are commonly used, although concerns about their use have

been raised because they can have significant effects on non-target species (Rome et al. 2011). To maximize captures of *V. velutina* while minimizing captures of non-target species, traps must demonstrate selectivity. For example, some traps incorporate holes allowing small insects to escape, but they are not selective enough (Turchi and Dérijard 2018). A major advantage by using pheromones as bait, is that they are an effective component of integrated pest management schemes because their use is compatible with that of biological control agents (Howse et al. 1998; Minks and Kirsch 1998). They may provide a way of controlling major hymenopteran pests. Trap development could be informed by recent research, notably by discoveries related to alarm pheromones in *V. velutina auraria* (Cheng et al. 2016), sex pheromone in such species (Wen et al. 2017) and/or chemical signatures (cuticular hydrocarbons) in the French *V. velutina nigrithorax* population (Gévar et al. 2017). These different compounds could be used as bait in selective traps. Their potential should be explored in future studies.

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AUTHOR CONTRIBUTION

MAW analyzed insects traped by plants and wrote the manuscript, RP collected plant samples, ED developed the concept, designed the manuscript and reviewed it. MAW: 45%, RP: 10%, ED: 45%.

| Authors | Contribution | ACI |
|---------|--------------|-------|
| MAW | 0.45 | 1.636 |
| RP | 0.10 | 0.222 |
| ED | 0.45 | 1.636 |

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