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Étude des leurres visuels et olfactifs des plantes carnivores du genre *Sarracenia* et de leurs rôles dans la capture du frelon asiatique, *Vespa velutina*, en vue de l'élaboration d'un piège biomimétique.

Corentin Dupont

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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

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École doctorale GAIA – Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau

Unité de recherche

AMAP - botAnique et Modélisation de l'Architecture des Plantes et des végétations

Étude des leurres visuels et olfactifs des plantes
carnivores du genre *Sarracenia* et de leurs rôles dans la
capture du frelon asiatique, *Vespa velutina*, en vue de
l'élaboration d'un piège biomimétique.

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Le 28 Juin 2022

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Résumé

Le frelon asiatique, *Vespa velutina*, une espèce envahissante, menace les abeilles domestiques, déjà en déclin, et leurs services de pollinisation, et présente un coût pour l'environnement et l'économie. Les campagnes d'éradication, peu efficaces et dommageables pour la biodiversité, incitent à développer des pièges plus sélectifs. En Amérique du Nord, la plante à urnes, *Sarracenia leucophylla*, piège de nombreuses proies volantes, et en France ses hybrides horticoles piègent le frelon asiatique. Alors qu'aucun piégeage phéromonal ne voit le jour, cette thèse explore la possibilité de s'inspirer du piège naturel de ces plantes carnivores pour lutter contre le frelon.

Les objectifs étaient (1) de caractériser les signaux visuels et olfactifs de 4 espèces de sarracénies présentant un gradient de parenté avec *S. leucophylla*, (2) d'identifier et tester les signaux et autres traits du piège les plus performants dans l'attraction et la capture du frelon. Les couleurs des urnes ont été mesurées en spectrophotométrie et leurs contrastes modélisés pour le système visuel du frelon. Les odeurs des urnes (n=39) et des plantes (n=31) ont été récoltées par headspace et analysées en GC-MS. Elles ont été testées en olfactométrie, avec les odeurs du miel et de l'abeille, sur le frelon (406 tests). L'efficacité de capture de ces plantes (12 plantes / 96 urnes) a été mesurée et filmée lors d'une expérience en laboratoire et évaluée durant 3 ans sur le terrain (n=223).

Il ressort que les 4 espèces de sarracénies ont un signal odorant généraliste incluant terpènes, benzenoïdes et dérivés d'acides gras. Elles présentent cependant des spécificités dans leurs leurres visuels et olfactifs qui expliquent une part importante des différences dans leur composition en proies. On observe de véritables syndromes de piégeage avec les espèces aux urnes courtes, peu contrastées et dont les odeurs sont riches en dérivés d'acides gras qui piègent surtout des fourmis, comme *S. purpurea*, et celles aux urnes longues et étroites, avec des aréoles blanches contrastées, des émissions d'odeurs importantes riches en monoterpènes, qui piègent surtout des hyménoptères volants, comme *S. X leucophylla*.

Ces sarracénies diffèrent également dans leur attractivité et dans leur efficacité de capture du frelon asiatique. *S. purpurea* ne l'attire ni visuellement ni olfactivement et n'en capture jamais tandis que *S. X leucophylla* et *S. X Juthatip soper* en capturent fréquemment en automne. *S. mitchelliana* qui, avec *S. X Juthatip soper*, émet les odeurs les plus attractives pour le frelon, a des taux de captures intermédiaires, qui s'expliquent par de faibles taux de rétention. Ces deux espèces ont également les profils d'odeurs de plante les plus similaires. Quant aux odeurs très concentrées de *S. X leucophylla*, elles pourraient éliciter une attraction à distance et une répulsion à proximité, d'après les comportements « tout puis rien » observés en olfactométrie. Ses forts taux de visites et de capture en laboratoire et sur le terrain suggèrent que son odeur n'est jamais aussi concentrée et donc potentiellement répulsive que dans l'olfactomètre. L'expérience en laboratoire et la mesure des traits des urnes a mis en évidence d'autres traits efficaces dans l'attraction du frelon comme les aréoles blanches de l'opercule et la sécrétion de nectar au niveau de l'entrée du piège, et ceux liés à sa capture comme la forme longue et étroite du piège et la présence de microstructures responsables d'une texture glissante qui favorisent la chute des insectes dans le piège.

La comparaison des profils d'odeurs des espèces attractives et non-attractives nous ont permis d'identifier les composés organiques volatiles à tester dans l'avenir pour l'élaboration d'un appât attractif. Les contrastes de clarté, les microstructures responsables du caractère glissant des surfaces sont autant de sources d'inspiration pour l'élaboration d'un piège biomimétique, écologique, et efficace.

Mots clés : frelon asiatique, *Vespa velutina*, espèce exotique envahissante, prédation, déclin des abeilles, plante carnivores, *Sarracenia*, *S. purpurea*, *S. leucophylla*, attraction, leurres, signaux sensoriels, odeurs, COVs, couleurs, contrastes, piège, surface glissante, biomimétisme, bioinspiration, contrôle biologique.

Abstract

The invasive Asian hornet, *Vespa velutina*, threatens the already declining honey bees and their pollination services, and presents a cost to the environment and the economy. Eradication campaigns are ineffective and damaging to biodiversity, calling for the development of more selective traps. In North America, the pitcher plant, *Sarracenia leucophylla*, traps many flying prey, and in France its horticultural hybrids trap the Asian hornet. While no pheromone trapping is emerging, this thesis explores the possibility of drawing inspiration from the natural trap of these carnivorous plants to fight against the hornet.

The objectives were (1) to characterize the visual and olfactory signals of 4 species of pitcher plants with a gradient of relatedness to *S. leucophylla*, (2) to identify and test the signals and other trap features most effective in attracting and capturing the hornet. The colours of the pitchers were measured using spectrophotometry and their contrasts modelled for the hornet's visual system. The odours of the pitchers (n=39) and plants (n=31) were collected by headspace and analysed in GC-MS. They were tested in olfactometry, with the odours of honey and bee, on the hornet (406 tests). The capture efficiency of these plants (12 plants / 96 pitchers) was measured and filmed during a laboratory experiment and evaluated during 3 years in the field (n=223).

The 4 species of pitcher plants revealed to have a generalist odorant signal including terpenes, benzenoids and fatty acid derivatives. However, they present specificities in their visual and olfactory lures, which explain an important part of the differences in their prey composition. We observe real trapping syndromes with species with short pitchers, with few contrasts, the odours of which are rich in fatty acid derivatives that trap mainly ants, like *S. purpurea*, and those with long and narrow pitchers, with contrasting white areoles, important odour emissions rich in monoterpenes, that trap mainly flying hymenoptera, like *S. X leucophylla*.

These pitcher plants also differ in their attractiveness and capture efficiency for the Asian hornet. *S. purpurea* attracts neither visually nor olfactory and never captures them, whereas *S. X leucophylla* and *S. X Juthatip* soper frequently capture them in autumn. *S. mitchelliana*, which together with *S. X Juthatip* soper emits the most attractive odours to hornets, has intermediate capture rates, which is explained by low retention rates. These two species also have the most similar plant odour profiles. As for the highly concentrated odours of *S. X leucophylla*, they could elicit attraction at large distance and repulsion at close range, based on the all-then-nothing behaviours observed in olfactometry. Its high visitation and capture rates in laboratory and field suggest that its odour is never as concentrated and therefore potentially repulsive as in the olfactometer. The laboratory experiment and the measurement of the pitchers' features highlighted other traits effective in attracting the hornet such as the white areoles of the operculum and the nectar secretion at the trap entrance, and those related to its capture, such as the long and narrow shape of the trap and the presence of microstructures responsible for a slippery texture that favours the fall of insects in the trap. The comparison of odour profiles of attractive and non-attractive species allowed us to identify volatile organic compounds to be tested in the future for the development of an attractive bait. The brightness contrasts, the microstructures responsible for the slipperiness of the surfaces are sources of inspiration for the development of a biomimetic, biodiversity-friendly and effective trap.

Key words: Asian hornet, *Vespa velutina*, invasive alien species, predation, bee decline, carnivorous plant, *Sarracenia*, *S. purpurea*, *S. leucophylla*, attraction, lures, sensory cues, odours, VOCs, colours, contrast, trap, slippery surface, biomimetics, bioinspiration, biological control.

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INTRODUCTION

Avec le changement global, à l'ère de l'anthropocène, les invasions biologiques sont de plus en plus nombreuses avec un coût écologique et économique important. Le frelon asiatique, un exemple d'espèce exotique envahissante, se répand en Europe et s'en prend aux abeilles domestiques déjà en déclin et aux services de pollinisation pourtant inestimables. En introduction, nous allons ainsi présenter ce prédateur, les dégâts qu'il occasionne, les moyens de lutte actuels, ainsi que la plante carnivore qui pourrait nous servir de source d'inspiration pour le piéger, puis nous détaillerons plus précisément les objectifs de la thèse.

1. Biologie du frelon asiatique, *Vespa velutina*

a. Description et origine du frelon asiatique

Dans le monde, on recense au total 22 espèces différentes de frelons, la plupart vivent en Asie (Carpenter & Kojima, 1997) et seulement deux de ces espèces sont naturellement présentes en Europe. *Vespa velutina* ou « frelon asiatique » (**Figure 1**), dont le premier spécimen a été décrit par Lepeletier en 1836 sur l'île de Java en Indonésie, fait partie de ces nombreux frelons originaires d'Asie (Villemant *et al.*, 2006). Son aire de répartition naturelle s'étend sur les régions tropicales et subtropicales du Sud-Est de l'Asie, du nord de l'Inde à la Chine et de la péninsule indochinoise à l'archipel indonésien (**Figure 2**, Perrard *et al.* (2014)). Au sein de l'espèce, on répertorie une douzaine de sous-espèces à la coloration très variable réparties sur cette aire (**Figure 2**, Van der Vecht (1957); Carpenter & Kojima (1997); Perrard *et al.* (2014)). *Vespa velutina nigrithorax* est la sous-espèce arrivée en Europe en 2004, elle vit habituellement au nord de l'Inde, en Chine et dans les montagnes d'Indonésie (**Figure 2**, Carpenter & Kojima (1997); Rome *et al.* (2011b)). *Vespa velutina nigrithorax*, comme son nom l'indique, se caractérise par son corps entièrement brun-noir à l'exception de fins liserés jaunes sur les segments abdominaux et un quatrième segment abdominal entièrement jaune-

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orangé (**Figure 1**). On le surnomme également « frelon à pattes jaunes » en raison de la coloration jaune des extrémités de ces pattes. Sa face est elle aussi orangée tandis que la tête et les antennes sont entièrement noires (**Figure 1**, Villemant *et al.* (2006) Rome & Villemant (2017)). Chez ce frelon comme chez les autres guêpes sociales, il existe deux castes, les femelles reproductrices (reines ou fondatrices) et les ouvrières (Rome *et al.*, 2015). Chez *Vespa velutina*, ces deux castes sont très peu différenciables morphologiquement du fait d'un dimorphisme de taille très peu marqué au contraire d'autres espèces comme par exemple *Vespa crabro* ou *Vespa tropica* (Perrard *et al.*, 2012). On peut néanmoins différencier les jeunes fondatrices des ouvrières au moment de leur émergence par leur poids du fait de la présence plus importante de graisse dans leur corps pour passer l'hiver (Cappa *et al.*, 2019a). Aucun polythéisme de caste (i.e. division des tâches selon la caste) n'est observé chez le frelon asiatique mais un polythéisme d'âge, comme chez les abeilles domestiques, est fortement suspecté (Monceau *et al.*, 2013b).

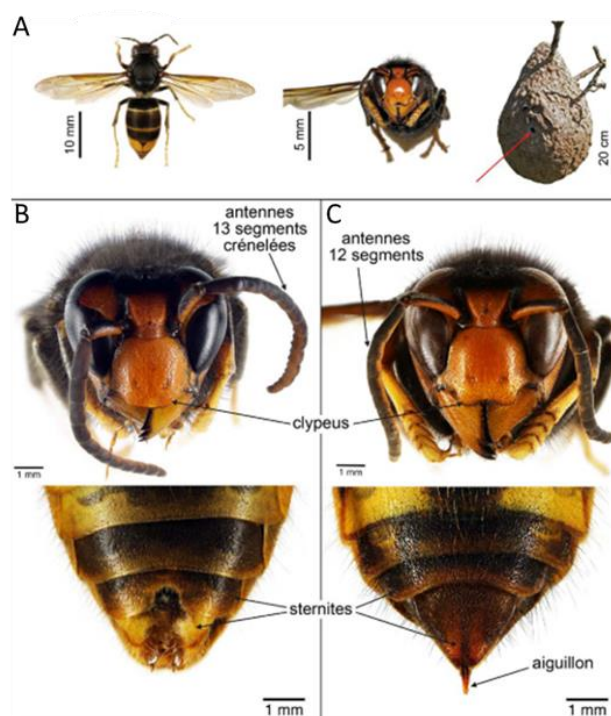


Figure 1. (A) *Vespa velutina nigrithorax* en vue dorsale et frontale et son nid dont l'entrée est marquée par la flèche, (B) Mâle de *Vespa velutina nigrithorax*, en haut tête en vue frontale, en bas vue ventrale des derniers segments abdominaux (sternites). (C) Femelle de *Vespa velutina nigrithorax*, en haut tête en vue frontale, en bas vue ventrale des derniers sternites. Image de Quentin Rome provenant de Rome & Villemant (2017).

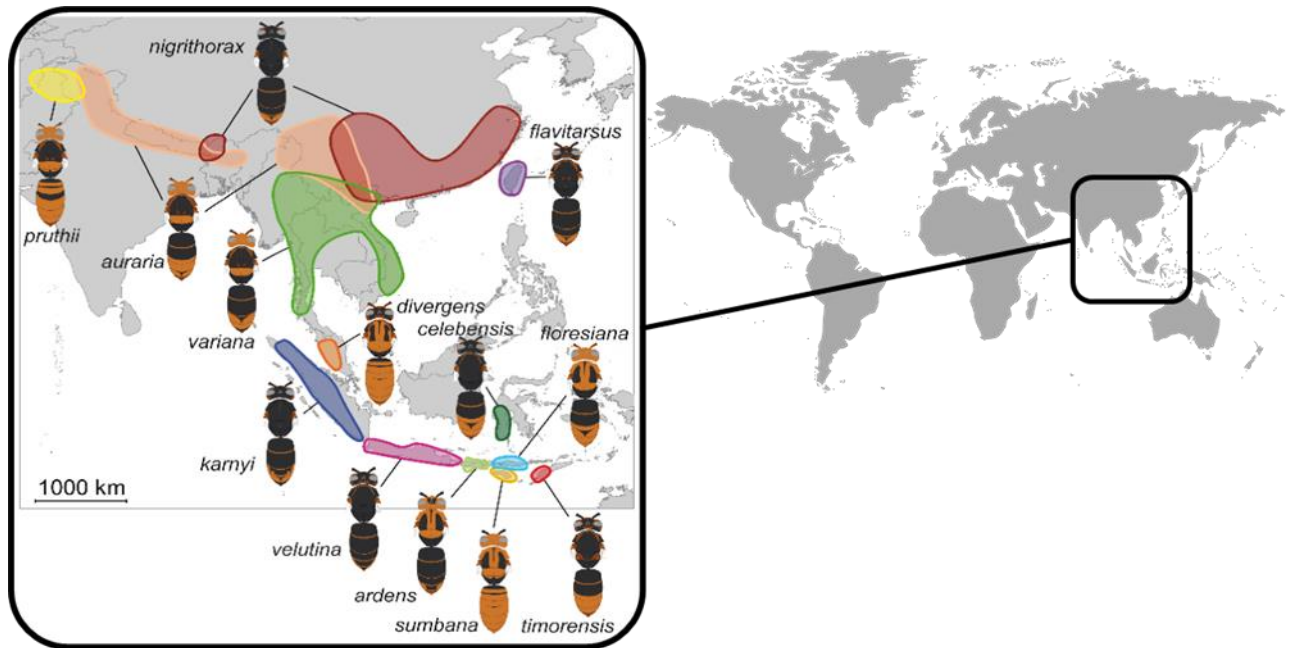


Figure 2. Distribution connue des différents morphes de couleur de *Vespa velutina* en Asie du Sud-Est Image de Perrard *et al.* (2014).

b. Développement saisonnier du frelon en région tempérée

En Asie continentale, le frelon se développe sous des climats comparables à ceux du sud de l'Europe (Villemant *et al.*, 2006). Habituellement dans des conditions tropicales, leurs colonies se développent tout au long de l'année de manière asynchrone (Edwards, 1980; Matsuura & Yamane, 1990). En milieu tempéré, les colonies se développent selon un cycle saisonnier dont la fin est marquée par l'arrivée du froid hivernal auquel seules les jeunes fondatrices survivent. Le cycle commence au printemps avec ces fondatrices, ces dernières construisent seules un nid embryonnaire de quelques centimètres dans lequel elles pondent leurs premiers œufs pour donner naissance aux premières ouvrières. Le nid et le premier couvain sont alors entretenus par la jeune reine mais ce sont les ouvrières qui prennent le relais petit à petit dans la gestion, l'alimentation, la construction et la défense du nid. La reine ne se consacre par la suite plus qu'à la ponte. Le nid lui, enveloppé de larges coquilles remplies d'air et formées par l'ajout successif de couches de papier cartonné constituées de salive et de fibres végétales va s'agrandir au fil des saisons jusqu'à atteindre sa taille maximale en fin d'automne (Perrard *et al.*, 2009). C'est au début de l'automne que l'activité du nid atteindra son maximum (Monceau *et al.*,

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2017b). A l'approche de l'hiver, la reine va commencer à produire des individus sexués fertiles, mâles et femelles. Les mâles se différencient des femelles par leurs plus longues antennes (Perrard et al., 2009). Au total environ 300 femelles et 900 mâles pourront être ainsi produits au sein d'un même nid (Rome et al., 2015). Après accouplement, les jeunes fondatrices vont partir à la recherche d'un endroit sûr pour passer l'hiver avant de renouveler le cycle l'année suivante (**Figure 3**, Monceau *et al.* (2013c)). Au final, un nid de frelon asiatique peut atteindre 40 à 50 cm de diamètre et produire en moyenne 6000 individus au total mais les plus gros nids pourraient atteindre 80 cm et produire plus de 15 000 individus (Villemant *et al.*, 2011b). Les nids sont en général ronds ou en forme de poire avec une ouverture latérale (à la différence de *V. crabro* dont le nid est à ouverture basale) et généralement perché à 15 - 20 m du sol au sommet des arbres (Rodríguez-Flores *et al.*, 2019). Dans les zones anthropisées on peut également trouver des nids dans les haies ou dans les constructions humaines comme les caves ou les greniers (Rome et al., 2011b).

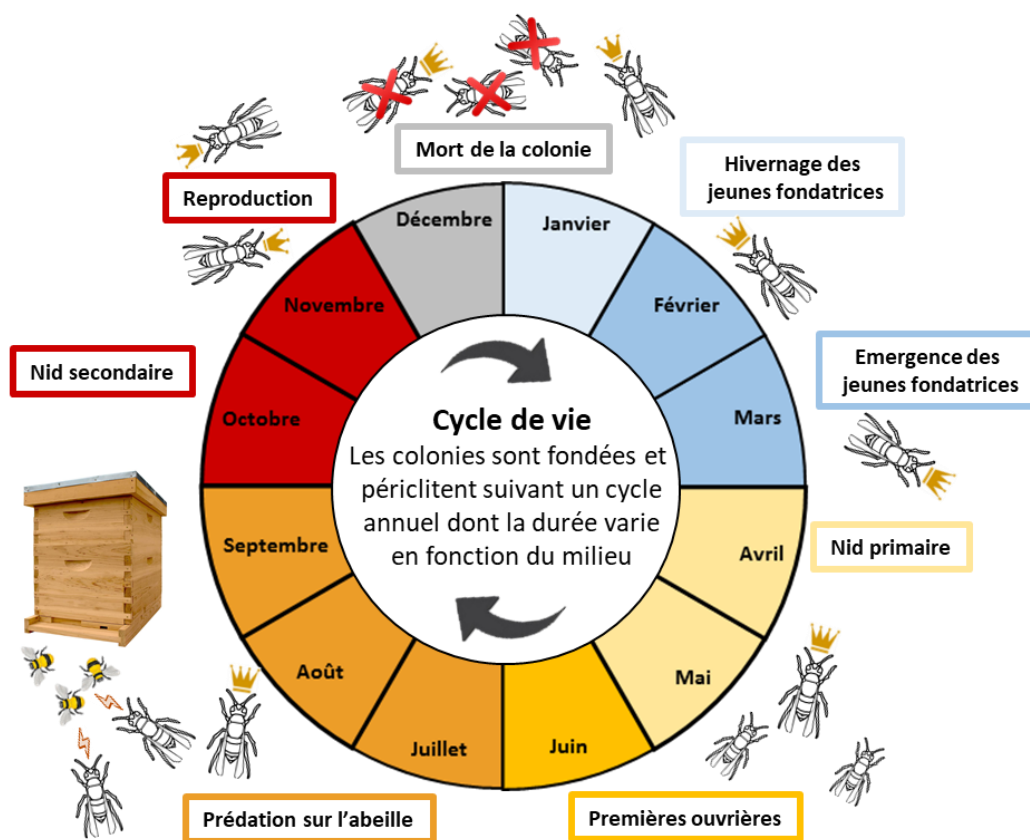


Figure 3. Cycle de vie de *Vespa velutina nigrithorax* dans les régions envahies française. D'après Monceau *et al.* (2014)

c. Activité et vie de la colonie

Les activités extérieures du nid commencent dès le matin à partir du moment où la température est supérieure à 10°C et se termine au crépuscule (Perrard et al., 2009). Le travail des ouvrières pendant la journée est varié ; à l'intérieur du nid il consiste à garder le nid, le nettoyer, nettoyer les larves ou les autres ouvrières. A haute température, les frelons ventilent aussi le nid grâce à l'« *evaporative cooling* » en régurgitant de l'eau sur le nid et en l'évaporant avec le battement de leurs ailes (Perrard et al., 2009). A l'extérieur, le travail des ouvrières consiste à explorer et rechercher des ressources comme de l'eau, de la pulpe végétale pour la construction (Edwards, 1980) et de la nourriture. Les frelons adultes ont besoin de carbohydrates (ou glucides) pour se nourrir (Edwards, 1980), ils les trouvent généralement en consommant des fruits, du nectar de fleur et la sève des arbres (Matsuura & Yamane, 1990). Les larves par contre réclament une grande quantité de protéines, les frelons les trouvent parfois sur des cadavres comme la viande et le poisson des marchés (Richter, 2000) mais la plupart du temps en se nourrissant d'autres insectes qu'ils chassent (Edwards, 1980). En échange de ces protéines, les larves produisent pour les ouvrières un liquide riche en sucres, en protéines et en acides aminés (Verdasca *et al.*, 2021). Ce comportement d'échange de nourriture par contact buccal ou trophallaxie incite les ouvrières à nourrir régulièrement leurs larves (Jeong *et al.*, 2020). Ainsi une estimation de la consommation d'une seule colonie en une saison représente environ 11 kg en biomasse d'insectes chassés par les ouvrières (Villemant et al., 2011b).

2. Invasion et propagation

La base de données mondiales sur les espèces envahissantes (GISD) reconnaît actuellement comme envahissante dans le monde entier, 468 espèces de plantes et 377 espèces animales. Parmi ces espèces animales, 81 sont des insectes, ce qui fait de ce groupe le groupe le plus représenté sans compter que les invasions par les insectes sont sûrement très sous-estimées par rapport à celles des plantes (Hill *et al.*, 2016). En Europe en 2009, un inventaire des espèces exotiques envahissantes (Delivering Alien Invasive Species Inventory for Europe) a relevé plus de 1300 espèces d'insectes (Roques *et al.*, 2009).

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Parmi ces insectes, les espèces qui présentent une organisation sociale ont un avantage certain pour l'invasion de nouvelles niches écologiques de par la flexibilité et l'efficacité de leur mode de reproduction et d'exploitation des ressources (Chapman & Bourke, 2001; Bertelsmeier, 2021). La fourmi d'Argentine est l'un des exemples les plus connus car elle est présente aujourd'hui sur tous les continents et forme dans les régions envahies des super colonies qui lui confèrent d'impressionnantes capacités de dispersion (Suarez *et al.*, 1999; Suarez *et al.*, 2001).

a. Introduction du frelon en France et sa propagation en Europe

Le frelon asiatique, *Vespa velutina*, est le premier frelon envahisseur à s'être implanté avec succès en Europe (Rome *et al.*, 2011b). D'autres évènements d'introduction accidentelle ont déjà eu lieu par le passé, par exemple avec le frelon oriental *Vespa orientalis* en Belgique et au Royaume-Uni, mais aucune n'a conduit à l'implantation de l'espèce (Edwards, 1982). Néanmoins la découverte récente d'ouvrières de *V. orientalis* et d'un nid dans le quartier de la Cabucelle à Marseille en France fin 2021 pourrait témoigner d'un nouveau début d'invasion (Gereys *et al.*, 2021). On a observé le frelon asiatique et son nid en France pour la première fois en 2004 dans le Lot-et-Garonne (**Figure 4**, Haxaire *et al.* (2006); Villemant *et al.* (2006)). L'origine de l'invasion reste incertaine mais d'après les témoignages et l'analyse des séquences ADN des frelons envahisseurs, on pense qu'il aurait été introduit accidentellement via l'arrivée d'une seule reine fécondée importée dans des poteries par bateau depuis l'est de la Chine (Villemant *et al.*, 2006; Arca *et al.*, 2015). Les années suivantes, de nombreux témoignages semblent indiquer que *V. velutina* était dès 2006 très largement répandu dans quatre départements d'Aquitaine et dans la périphérie. Il s'est ensuite rapidement propagé dans le reste de la France (Villemant *et al.*, 2011a; Villemant *et al.*, 2011b), ainsi après les 3 nids signalés dans le Lot-et-Garonne en 2005, c'est au final 1637 nids qui ont été signalés sur 32 départements 4 ans plus tard en 2009 (Villemant *et al.*, 2011a). On sait que la propagation rapide du frelon dans toute l'Europe est le résultat de ses importantes capacités de dispersion mais a aussi été favorisée par des infrastructures humaines comme les autoroutes (Robinet *et al.*, 2017; Verdasca *et al.*, 2021). Le frelon

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a ensuite rapidement poursuivie son expansion dans d'autres pays d'Europe avec une première observation au nord de l'Espagne en 2010 (Castro & Pagola-Carte, 2010), au Portugal (Carvalho *et al.*, 2020) et en Belgique (Bruneau, 2011) en 2011, en Italie en 2013 (Demichelis *et al.*, 2014), en Allemagne en 2014 (Witt, 2015), au Royaume-Uni en 2016 (Budge *et al.*, 2017), en Suisse en 2017 (Rome, 2017) et au Luxembourg en 2020 (Ries *et al.*, 2021). Aujourd'hui il est présent sur l'ensemble du territoire Français, et dans toute l'Europe de l'Ouest (**Figure 4**).

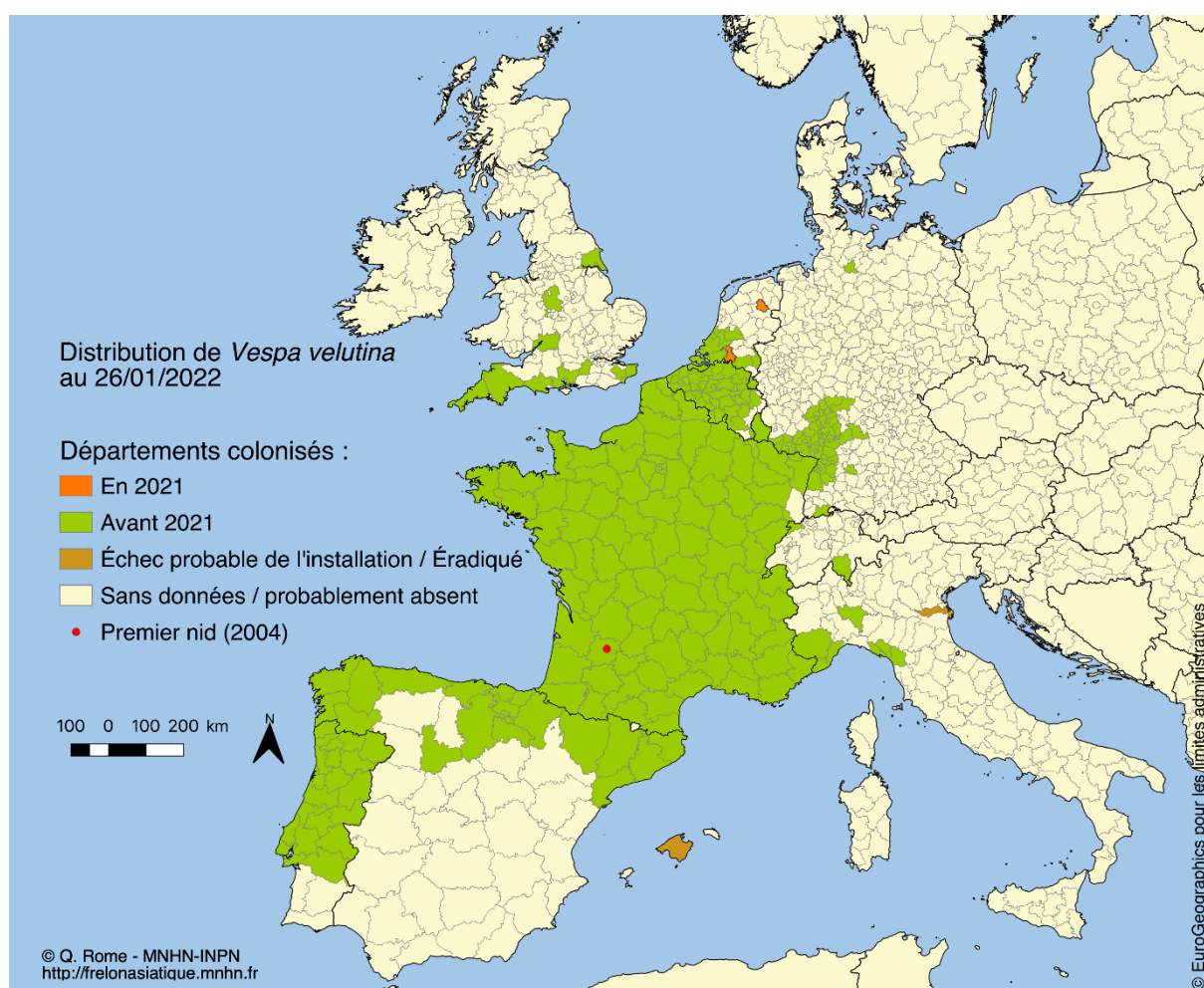


Figure 4. Aire de répartition connue de *Vespa velutina nigrithorax* au 26/01/2022 en Europe de l'Ouest. Image de Quentin Rome pour le MNHN <https://frelonasiatique.mnhn.fr/> produites à partir des sources suivantes : INPN (France), Dirección General de Calidad y Evaluación Ambiental y Medio Natural, N. Vicens (DDGI), S. Rojas Nossa (Univ. Vigo) (Espagne), ICNF, NATIVA et SOS Vespa (Portugal), CARI et CiEi / DGARNE (Belgique), CREA et Università de Torino (Italie), R. Witt, M. Husemann (Univ. Hamburg) (Allemagne), NNSS et NBU (Grande Bretagne), Waarneming.nl (Pays-Bas), Service sanitaire apicole suisse (Suisse), Administration de la Nature et des Forêt et MNHNL (Luxembourg), EASIN (UE).

b. Les futures expansions en Europe et dans le monde

De nombreuses données semblent indiquer que l'aire d'invasion du frelon est loin d'avoir atteint ses limites. En effet, des modèles basés sur les données climatiques et sur les zones actuellement colonisées par le frelon asiatique montrent qu'à l'avenir, l'invasion pourrait encore s'étendre dans tout l'Ouest de l'Europe et notamment le long des côtes, milieu à priori très propice à son développement (Villemant et al., 2011a). Il pourrait alors progresser vers les zones côtières des Balkans, de la Turquie et du Proche-Orient. Des modèles semblables avec la prise en compte du changement climatique montrent aussi une augmentation de l'aire propice à l'invasion dans l'Europe centrale et l'Europe de l'Est (Barbet-Massin *et al.*, 2013). Au niveau mondial, la répartition potentielle du frelon correspond fortement à la répartition actuelle d'une autre guêpe sociale envahissante, la guêpe germanique *Vespula germanica* (Villemant et al., 2011a). Les modèles prédisent ainsi que l'invasion pourrait se produire dans de nombreuses autres parties du monde en cas de nouvelle introduction accidentelle. Ce frelon est d'ailleurs aussi un envahisseur dans certaines régions d'Asie, notamment en Corée du sud depuis 2003 (Kim *et al.*, 2006; Choi *et al.*, 2012) et au Japon depuis 2012 (Ueno, 2014). A raison d'une progression de 10 à 20 km par an depuis 2003, l'invasion s'étend néanmoins beaucoup moins rapidement en Corée (Jung, 2012) qu'en France par exemple (Villemant et al., 2011b). Cette différence de progression pourrait s'expliquer par la présence de 6 autres espèces de frelons en Corée dont *Vespa mandarinia* potentiel grand compétiteur et grand prédateur de guêpes et d'abeilles (Matsuura & Yamane, 1990; Yoshimoto & Nishida, 2009).

3. Conséquences de l'invasion

a. Un facteur de pression supplémentaire sur l'abeille domestique

Vespa velutina est un prédateur bien connu des abeilles mellifères dans sa région d'origine (Van der Vecht, 1957). En Asie, *V. velutina* peut ainsi détruire 20 à 30% d'une colonie d'abeilles domestiques orientales, *Apis cerana* (Qun, 2001). Il pratique une stratégie de chasse très efficace qu'on appelle le

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« *bee-hawking* » (Tan *et al.*, 2007), elle consiste à rester en vol stationnaire devant l'entrée d'une ruche pour capturer les ouvrières qui entrent et qui sortent (Monceau *et al.*, 2013a). Après avoir capturé sa proie, le frelon la découpe avec ses mandibules pour ne récupérer que le thorax dont il forme ensuite une pelote riche en protéines qu'il ramène au nid afin de nourrir ses larves (Perrard *et al.*, 2009). Sa proie naturelle, *A. cerana*, a cependant mis en place une stratégie coordonnée de défense, elle aussi très efficace, contre ce prédateur, le « *heat-balling* » (Ken *et al.*, 2005). En cas d'attaque, cette stratégie consiste à submerger rapidement le frelon avec une masse compacte d'ouvrières qui, en vibrant des ailes, augmentent la température au sein de la boule ainsi formée pour atteindre 45°C en moins de 5 min (Ken *et al.*, 2005), le tout en produisant du dioxyde de carbone en quantité (Sugahara & Sakamoto, 2009). Cette réaction entraîne rapidement la mort de l'agresseur mais pas celle des abeilles capables de supporter des températures supérieures.

En France, sûrement à cause de son récent contact avec ce nouveau prédateur, la mise en place d'un tel comportement a été observée de manière épisodique chez *Apis mellifera* mais reste bien moins efficace (Arca *et al.*, 2014). A titre de comparaison, la boule thermique de *A. mellifera* rassemble une fois et demi moins d'ouvrières que celle de *A. cerana* et monte moins rapidement et moins haut en température (Arca *et al.*, 2014). Il a été montré également que *A. cerana* contrairement à *A. mellifera* prépare cette défense dès l'apparition d'un prédateur à l'entrée du nid en augmentant la température corporelle de plusieurs ouvrières avant même de s'agglutiner sur ce dernier (Tan *et al.*, 2010). Les faibles capacités de défense de l'abeille domestique en Europe font qu'elle subit une pression de prédation bien plus forte de la part de *V. velutina* que ces congénères asiatiques. Le frelon bénéficie de cette faiblesse et va d'ailleurs préférentiellement chasser *A. mellifera* quand *A. cerana* est présente dans un même rucher (Tan *et al.*, 2007). Cette prédation sur l'abeille est plus intense à la fin de l'été, lorsque la population au sein du nid de frelons est à son maximum et lorsque que le couvain des futurs individus fertiles a besoin d'être nourri (Monceau *et al.*, 2013a; Villemant *et al.*, 2014). Au cours de cette période, on peut alors observer jusqu'à 20 frelons chasser devant une seule ruche en simultané (Requier *et al.*, 2019). La pression du frelon sur les colonies d'abeilles ne se traduit pas

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uniquement en termes de prédation directe des individus (Monceau *et al.*, 2013d) mais sa présence à l'entrée de la ruche entraîne en parallèle une augmentation du stress de la colonie (Leza *et al.*, 2019) ainsi qu'une paralysie dans la recherche de nourriture du fait de la diminution des activités de vol à l'extérieur (Requier *et al.*, 2019). Les conséquences de cette paralysie pour les abeilles sont lourdes et conduisent parfois à long terme à la mort de la colonie faute d'approvisionnement (Requier *et al.*, 2019).

b. Impact général sur l'entomofaune et les pollinisateurs

Chez les frelons, il est commun d'observer un comportement opportuniste malgré une spécialisation sur certaines proies (Matsuura & Yamane, 1990). Par exemple le frelon européen, *V. crabro*, se nourrit préférentiellement de cigales au Japon (Matsuura, 1984), ou encore *Vespa ducalis* s'attaque principalement à de petites guêpes sociales (Sakagami & Fukushima, 1957), tandis que le frelon géant, *V. mandarinia*, se spécialise également sur les abeilles domestiques. Cette tendance à une spécialisation partielle chez les frelons pour les proies abondantes localement participerait à une exploitation optimum des ressources. C'est un avantage évolutif fort pour les guêpes sociales qui ont besoin de beaucoup de protéines pour nourrir leurs larves. Malgré une tendance à se spécialiser sur l'abeille domestique, d'autres pollinisateurs comme les abeilles sauvages ou les mouches de la famille des syrphidés représente une part importante du spectre de proies du frelon asiatique (Villemant *et al.*, 2011b). L'identification précise des proies à partir de 2151 pelotes provenant de différents nids par la combinaison d'approches morphologiques et de « *barcoding* » (technique d'identification moléculaire permettant la caractérisation génétique d'un échantillon d'individus à partir de courtes séquences d'ADN), a démontré le régime généraliste provenant d'un comportement opportuniste de *V. velutina* (Rome *et al.*, 2021). Il chasse en effet une grande diversité d'organismes (159 espèces identifiées au moins et 411 espèces estimées) dont en majorité des abeilles domestiques (38.1%), des mouches (29.9%) et d'autres guêpes sociales (19.7%) (Rome *et al.*, 2021). Il a aussi été montré que le spectre de proies du frelon dépendait énormément de l'environnement du nid, la capture d'abeilles

domestiques étant beaucoup plus importante en milieu urbain qu'en milieu forestier (**Figure 5**, Villemant *et al.* (2011b)). Ainsi en tant qu'opportuniste, il semblerait qu'il cible principalement les proies localement abondantes (Rome *et al.*, 2021) suggérant, malgré son fort impact sur l'abeille domestique, un impact plus faible sur les espèces sauvages. Par exemple (Carisio *et al.*, 2018) n'ont pas démontré d'effet négatif du frelon sur l'abondance et la diversité d'abeilles sauvages dans les zones envahies même si des études à plus long terme restent à mettre en place. Il ne faut néanmoins pas négliger l'effet indirect de ce prédateur sur les populations de pollinisateurs puisque des études ont montré qu'il interférait indirectement sur les processus de pollinisation des insectes (Leza *et al.*, 2019; Rojas-Nossa & Calvino-Cancela, 2020).

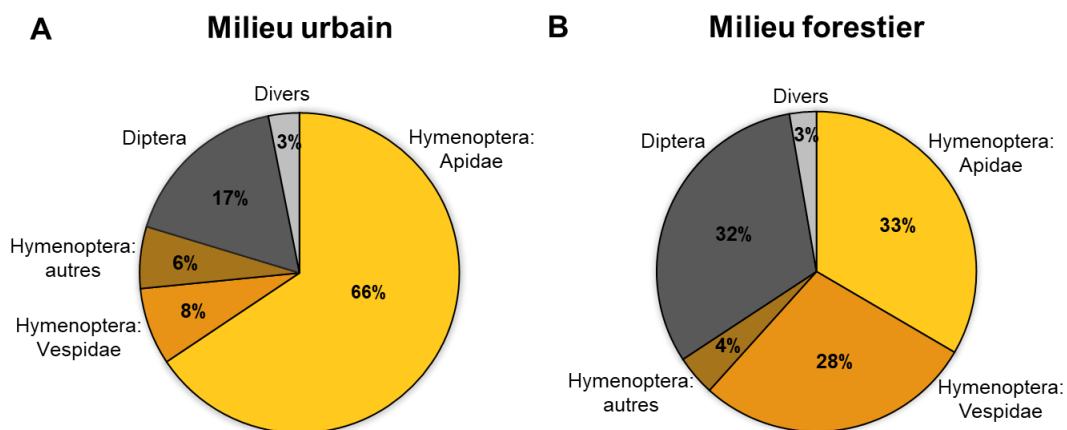


Figure 5. Proportion des principaux groupes de proies de *Vespa velutina nigrithorax* selon le type d'habitat : **(A)** urbain et **(B)** forestier. D'après (Villemant *et al.*, 2011b).

c. Compétition avec les autres prédateurs endémiques

Les arthropodes prédateurs généralistes représentent souvent une menace forte pour les écosystèmes qu'ils envahissent. Via la compétition pour les ressources, la prédation intragilde ou encore l'apport de parasites et de maladies, ils affectent fortement les autres prédateurs présents dans le milieu envahi. De plus, les prédateurs envahissants prolifèrent souvent beaucoup plus rapidement que leurs homologues endémiques, perturbant ainsi l'équilibre prédateurs-proies déjà en place (Snyder & Evans, 2006). Le frelon asiatique ne fait pas exception et représente un compétiteur sérieux pour les autres

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frelons et prédateurs d'insectes. Dans sa zone d'invasion en Corée, 7 ans après son introduction, *V. velutina* était l'espèce de frelon la plus abondante (37%) dans la région de Busan malgré la présence de 6 autres espèces natives. En outre, certaines espèces comme *V. simillima* ou *V. mandarinia* y ont vu, depuis, leurs populations diminuer respectivement de 20% et 10% (Choi et al., 2012). De même au Japon sur l'île de Tsushima, *V. velutina* semble affecter négativement les autres espèces de frelons (Ikegami et al., 2020). Sur cette même île, il a même été montré qu'une partie des reines de *V. simillima* (43% de reines capturées) s'accouplaient avec des mâles de l'espèce envahissante, *V. velutina*, tandis qu'en parallèle, aucune reine de *V. velutina* ne semblaient s'être s'accouplée avec des mâles d'une autre espèce. L'effet de cette interférence reproductive sur les populations indigènes de *V. simillima* reste à quantifier mais elle pourrait aussi bien participer à leur diminution (Yamasaki et al., 2019).

En France, c'est l'unique frelon endémique, le frelon européen, *V. crabro*, qui est susceptible d'être affecté par l'invasion de *V. velutina*. Leurs niches écologiques sont semblables par certains aspects (Monceau et al., 2015), et notamment leurs préférences alimentaires (Cini et al., 2018). Tous deux sont parfois charognards, amateurs de fruits mûrs, prédateurs généralistes d'arthropodes et surtout d'abeilles. Néanmoins *V. crabro* est largement moins spécialisé sur l'abeille domestique et ses attaques devant les ruches reste anecdotiques par rapport à *V. velutina* (Baracchi et al., 2010). La pression de prédation moindre de *V. crabro* sur les ruches pourrait notamment s'expliquer par la plus petite taille de ses colonies. En effet, *V. crabro*, bien que supérieur en taille à *V. velutina* (Rortais et al., 2010), produit des colonies avec environ trois fois moins d'individus (Archer, 2008; Villemant et al., 2011b). En termes de reproduction, les reines de *V. velutina* sont aussi potentiellement plus précoces et fécondes que les reines de *V. crabro*. (Poidatz et al., 2018a). Cela explique la forte propagation de *V. velutina* mais cela pourrait aussi lui donner l'avantage en cas de compétition. Les deux espèces présentent par contre des habitudes de nidification différentes, si *V. velutina* construit ses nids de préférence dans la canopée des arbres (Villemant et al., 2011b), *V. crabro*, lui, niche presque exclusivement dans des espaces protégés tels que les cavités du sol et les creux des arbres (Spradbery, 1973). Il semble également que les ouvrières de *V. crabro* présentent une meilleure défense

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immunitaire que les ouvrières de *V. velutina* (Cini et al., 2018), ceci sûrement à cause du fort degré de consanguinité de ce dernier en lien avec l'unique épisode d'invasion (Darrouzet et al., 2015b). Paradoxalement, il a par contre été montré que les capacités de défenses immunitaires des fondatrices de frelon asiatique était supérieur à celui de ses ouvrières ainsi qu'aux fondatrices du frelon européen (Cappa et al., 2021). Malgré le fort potentiel compétitif de *V. velutina*, son impact sur le prédateur natif reste finalement encore peu connu en Europe. Une étude sur 4 ans dans une région envahie de l'Italie se veut rassurante car n'a pas mis en évidence une diminution des populations de *V. crabro* en présence de *V. velutina*, supposant que l'espèce native a probablement évité ou minimisé les interactions de compétition (Carisio et al., 2020).

d. Impact sur l'homme et sur l'économie

Le caractère opportuniste du frelon fait qu'il nidifie volontiers à proximité de l'homme. Les caves, greniers et abris de jardin représentent souvent des abris de choix pour les jeunes fondatrices (Villemant et al., 2006; Monceau & Thiéry, 2017). Cette proximité, sa taille imposante et sa réputation d'agresseur amplifiée par les médias font que le frelon asiatique est souvent considéré comme dangereux pour l'humain. Cependant, comme *V. crabro*, il ne montre pas particulièrement de comportements agressifs envers l'homme au-delà d'un périmètre de défense de 5 m autour de son nid (de Haro et al., 2010). D'après les données des Centres Antipoison et de Toxicovigilance, une seule observation avérée d'envenimation a pu être relevée en France jusqu'en 2010 depuis son introduction (de Haro et al., 2010). De 2009 à 2013, 4 décès liés à une piqûre de frelon asiatique ont été recensés dans le système d'information commun des centres antipoison dont seulement 1 pour lequel l'espèce avait été formellement identifiée. Aussi ces 4 décès ont tous été des conséquences d'un choc anaphylactique et 3 des patients présentaient déjà des antécédents d'allergie aux venins d'hyménoptères (Viriot et al., 2015). Les Centres Antipoison et de Toxicovigilance insistent ainsi sur le fait que *Vespa velutina* est très probablement dangereux pour l'homme dans trois circonstances particulières qui sont les mêmes qu'avec les espèces d'hyménoptère autochtones : piqûres multiples,

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piqûre unique avec localisation sur les muqueuses ou encore patient allergique au venin d'hyménoptère. Par contre, à la différence de nos hyménoptères locaux, le frelon asiatique, en cas d'attaque, projette des sécrétions de venin. Il arrive que ces projections atteignent les yeux, ce fut le cas pour 29 personnes de 2009 à 2021 dont 80% concernaient des pompiers et des exterminateurs : des professionnels souvent confrontés aux nids de frelons (Laborde-Castérot *et al.*, 2021). Malgré les accidents, concernant souvent des personnes allergiques ou cardiaques, il ne représenterait donc pas un réel danger sanitaire pour l'homme. L'abeille domestique, les guêpes et le frelon d'Europe sont responsables de beaucoup plus d'accidents en France (Rome, 2019) et de 2004 à 2010, le nombre de cas d'envenimations par piqûres d'hyménoptères dans les départements affectés par l'invasion n'avait d'ailleurs pas augmenté depuis son arrivée (de Haro *et al.*, 2010). Il est à noter cependant que de récentes études se veulent plus prudentes quant à la minimisation des risques et montrent par exemple une forte implication de la présence de l'espèce exotique, *V. velutina*, dans les cas recensés de réaction allergique en Espagne (Feas, 2021; Vidal *et al.*, 2021).

D'un point de vue économique, alors que l'apiculture en Europe souffre déjà d'un déclin notable sous la pression de multiples facteurs impactant les abeilles domestiques, comme les pesticides, les parasites, les maladies, ou les mauvaises pratiques apicoles (Ellis *et al.*, 2010; Neumann & Carreck, 2010; VanEngelsdorp D & Meixner, 2010), la pression de *V. velutina nigrithorax* sur les colonies déjà affaiblies apparaît, en France pour le moins, comme un nouveau facteur de déclin avec un coût économique élevé pour une industrie déjà fragilisée (Monceau *et al.*, 2013c). Aucune véritable donnée sur les pertes directes liées au frelon n'existe pour le moment mais à titre d'exemple, Kennedy *et al.* (2018) estiment que l'établissement de *V. velutina* au Royaume-Uni entraînerait au moins 20 à 30 % de pertes dans la production de miel et coûterait à l'économie britannique entre 30 et 45 millions de livres sterling. De plus, de nombreux apiculteurs en France témoignent de la pénibilité toujours plus importante de leur situation depuis l'arrivée du frelon (**Figure 6**). En 2010, l'Union Nationale pour l'Apiculture Française a estimé que 30% des ruches avait été détruites ou affaiblies en Gironde à cause de *V. velutina nigrithorax* (Monceau & Thiery, 2016). La pression du frelon sur les ruchers en France et

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en Europe est généralement d'autant plus impactante que la plupart des ruchers sont d'assez petite taille, avec moins de 30 ruches et souvent dans des zones rurales ou urbaines (Rodríguez-Flores et al., 2019), milieux fort appréciés du frelon.

Il a été montré aussi que lorsqu'une espèce envahissante se propage sur un territoire, les coûts de son contrôle et de sa gestion augmentent de façon exponentielle (Williams *et al.*, 2010). La lutte mise en place contre ce frelon représente ainsi un coût déjà énorme : entre 2006 et 2015, 23 millions d'euros ont été investis dans la destruction des nids en France et le coût annuel augmente à mesure que la propagation de l'espèce s'intensifie (Barbet-Massin *et al.*, 2020). Une estimation montre qu'il pourrait atteindre 11,9 millions d'euros en France, 9 millions d'euros en Italie et 8,6 millions d'euros au Royaume-Uni si l'espèce poursuivait sa route dans les zones qu'on considère aujourd'hui adaptées à son implantation (Barbet-Massin et al., 2020).



Figure 6. Récolte du miel sur nos ruches du site d'expérimentation à Lavalette, Montpellier, France avec l'aide de Jean-Luc Delon, président du GDSA 34 (Groupement de Défense Sanitaire Apicole de l'Hérault).

4. La lutte contre le frelon asiatique

V. velutina nigrithorax représente une menace sérieuse pour la biodiversité et les écosystèmes des régions envahies, d'autant plus qu'il y présente de grandes capacités de reproduction et de dispersion ainsi qu'un large choix de régimes alimentaires et d'habitats qui favorise sûrement son expansion rapide. Les menaces que représente le frelon pour l'environnement ainsi que pour l'économie font qu'il a été classé en France dans la liste des espèces nuisibles de deuxième catégorie en 2012 et dans la liste des espèces exotiques envahissantes préoccupantes pour l'Union européenne en 2016 (Reg. EU 1141/2016). Les espèces exotiques envahissantes sont définies comme "des espèces exotiques dont l'introduction ou la propagation menace ou impacte négativement la biodiversité et les services écosystémiques associés". Au Japon, le ministère de l'Environnement l'a ajouté à la liste des espèces exotiques envahissantes en 2015 (Kishi & Goka, 2017). Même si son éradication semble possible à petite échelle, c'est par exemple le cas des îles de l'archipel occidental Méditerranéen où l'introduction était récente et pour lesquelles un plan global de contrôle a été très rapidement mis en place (Leza *et al.*, 2021). Elle reste cependant illusoire dans la plupart des régions envahies car impraticable lorsque les populations ont atteint un certain niveau d'invasion. Dans le monde, la majorité des tentatives d'éradication des Vespides invasifs se sont d'ailleurs soldées par des échecs (Beggs *et al.*, 2011). Pour réduire l'impact local et éventuellement la progression du frelon asiatique, plusieurs méthodes et stratégies de lutte ont été envisagées et mises en place depuis son arrivée en France (Monceau *et al.*, 2014).

a. Localisation et destruction des nids

Une des principales méthodes de lutte consiste en un contrôle mécanique. Celle-ci consiste à localiser et à détruire directement les nids de frelons pour limiter leur prolifération et leur impact local sur l'entomofaune. Les nids restent cependant difficiles à détecter tôt car ils sont souvent cachés dans les arbres durant le printemps et l'été. On estime ainsi qu'on arrive à repérer moins de 5% des nids de frelons au total (Robinet *et al.*, 2017). La plupart des nids sont donc seulement découverts et détruits

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trop tard, en automne après la chute des feuilles, une fois que les ouvrières ont déjà fait leurs ravages sur les abeilles, que les colonies sont en fin de vie et que les individus sexués ont déjà été produits. Diverses méthodes pour repérer les nids plus tôt sont envisagées et mises en pratiques. La triangulation par exemple est une technique qui a été couramment utilisée pour repérer les nids de guêpes. Elle consiste à capturer au moins trois spécimens, puis à les relâcher à différents endroits et à observer la direction de leur vol. Les hyménoptères capturés et stressés ont tendance à retourner directement à leur nid, ainsi si les trois individus proviennent du même nid, il y a de fortes chances que les trois directions qu'ils ont prises se croisent en un point qui donnera la position du nid (Turchi & Derijard, 2018). De récentes études visent à améliorer cette façon de procéder. Kennedy et al. (2018) par exemple ont mis en avant une technique de radio-télémetrie permettant de suivre avec précision des ouvrières de frelons retournant à leur nid. L'utilisation de la science participative pour le repérage semble lui aussi se montrer efficace via l'intermédiaire d'application comme « Vespapp » (Leza et al., 2021). L'efficacité d'une telle approche a d'ailleurs été démontrée sur d'autres insectes comme la punaise marbrée, ravageuse de culture, par rapport à des méthodes traditionnelles comme les pièges à phéromones (Malek *et al.*, 2018). C'est aussi la mise en place de la participation citoyenne qui a permis de suivre précisément la progression du frelon asiatique en Europe (Rome et al., 2011b; Rome & Villemant, 2017).

Le nid, une fois découvert, pose encore le problème de sa destruction car il est bien souvent difficile à atteindre. Le tir au fusil souvent pratiqué, en plus d'être dangereux, est inefficace car ne garantit pas la mise à mort de la reine qui, même en son absence, n'empêche pas la colonie de survivre un temps (Turchi & Derijard, 2018). Les traitements au poison comme la perméthrine ou le dioxyde de soufre sont plus efficaces mais dangereux pour d'autres animaux comme les oiseaux, susceptibles de se nourrir des larves contaminées. Des alternatives moins dangereuses sont en développement comme l'utilisation d'insecticides naturels tels que la terre de diatomée (Turchi & Derijard, 2018) ou encore l'utilisation de vapeur d'eau bouillante (Ruiz-Cristi *et al.*, 2020). Néanmoins ces deux dernières années, on relève la destruction de plus 50 000 nids sur le territoire français par an et l'invasion de

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semble pas régresser pour autant. Pour espérer un résultat et à cause du fort potentiel invasif du frelon, cette forme de lutte nécessiterait une coordination et une organisation à grande échelle, entre régions, dans toutes la France et l'Europe, ce qui s'avère extrêmement difficile à mettre en place.

b. Exploiter les ennemis naturels du frelon

La lutte biologique consiste à introduire un pathogène, un prédateur ou encore un parasite dans l'objectif de réduire l'abondance des populations invasives. Les prédateurs naturels de *V. velutina* sont assez rares cependant. On a pu observer un seul cas de prédation de nid de *V. velutina* en France par un oiseau insectivore, la bondrée apivore *Pernis apivorus*, un oiseau assez peu commun (Monceau *et al.*, 2013c). En Corée du sud, la Martre à gorge jaune, un mammifère se nourrissant souvent de guêpes est suspecté chasser les frelons mais là encore cela reste épisodique (Kim & Choi, 2021). Comme beaucoup d'autres guêpes sociales, les frelons sont au final des proies souvent évitées du fait qu'ils se défendent en piquant et sont souvent répugnants au goût à cause de leur venin (Kauppinen & Mappes, 2003). Il pourrait être envisageable d'exploiter les parasites naturels du frelon plutôt que ses prédateurs. On a par exemple constaté la mort de fondatrices par infestation du parasitoïde Européen, *Conops vesicularis*, mais seuls deux nids étaient concernés par cette observation (Darrouzet *et al.*, 2015a). De même on a pu observer une infestation par le nématode, *Pheromermis vesparum*, mais là aussi dans deux cas seulement (Villemant *et al.*, 2015). Peut-être plus prometteuses, des observations récentes de *Varroa* ont été faites sur le frelon asiatique (Sanchez & Arias, 2021). Le *Varroa* est un acarien parasite qui se nourrit en général de l'hémolymphe des abeilles du genre *Apis* (Anderson & Trueman, 2000) causant ainsi d'importants dommages aux d'abeilles domestiques, en plus d'être un vecteur de virus pouvant causer la mort de colonies entières (Anderson & Trueman, 2000; Mordecai *et al.*, 2016). Le *Varroa* pourrait ainsi affecter les colonies de frelons mais malheureusement aussi se propager par ce potentiel nouveau vecteur (Sanchez & Arias, 2021). Enfin, on a récemment détecté de nouveaux virus chez *V. velutina* comme le virus de l'aile déformée assez problématique chez les abeilles (Dalmon *et al.*, 2019). Mais là encore, dans quelle mesure ces virus peuvent servir de contrôles

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biologiques sans pour autant menacer les abeilles via un potentiel nouveau vecteur que représente le frelon ? Au final, beaucoup d'études restent à mener pour envisager le contrôle du frelon asiatique grâce à ses ennemis naturels car la question de l'efficacité de la démarche et la transmission potentielle de parasites à d'autres espèces comme le frelon européen ou l'abeille implique de faire preuve de beaucoup de prudence.

c. Compter sur la défense des abeilles

En France, l'apiculture a longtemps sélectionné les races d'abeilles domestiques pour leur docilité, (Monceau *et al.*, 2013c) avec un comportement plus calme et doux à l'encontre de l'homme (Arca *et al.*, 2014). Cette sélection de souches « douces » chez les abeilles, couplée au fait que le contact du prédateur avec les abeilles est récent, est une des explications à l'inexistence de stratégie de défense efficace chez *A. mellifera* (Arca *et al.*, 2014). On suppose que c'est cette forte concentration de proies sans défense qu'elles représentent qui font des abeilles domestiques la cible préférée du frelon asiatique (Tan *et al.*, 2012; Arca *et al.*, 2014). Il a été montré que les populations qui survivent le plus longtemps en présence de frelon sont les populations aux comportements les plus défensifs d'abeilles domestiques (Monceau *et al.*, 2017a). Les comportements collectifs de défenses des abeilles sont très variables d'une population à l'autre (Wray *et al.*, 2011). Il est donc assez envisageable pour les apiculteurs de sélectionner des souches plus agressives pour réduire l'impact de *V. velutina*. Monceau *et al.* (2017a) ont récemment observé de rares occurrences de boules thermiques sur les frelons, chose qui n'avait jusqu'alors été observée sur *Apis mellifera* que sur de simples leurres de prédateurs immobiles (Arca *et al.*, 2014). Il reste à déterminer si le peu d'observations de ce comportement s'explique par sa rareté ou sa nouveauté dans les populations d'abeilles. On sait également que certaines sous-espèces d'*A. mellifera* sont capables de se défendre efficacement contre le frelon, par exemple la sous-espèce, *Apis mellifera cyprina*, habituée aux contacts avec le frelon oriental, *V. orientalis*, est capable d'asphyxier ses assaillants (Papachristoforou *et al.*, 2007) ou encore *Apis mellifera ligustica* complète même sa stratégie de boule thermique par la projection de venin pour se

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défendre de *V. crabro* (Baracchi et al., 2010). D'autres comportements plus défensifs existent aussi : certaines colonies de *Apis mellifera cypria* plutôt que de favoriser l'attaque et le conflit, protègent l'entrée de leur ruche avec des agrégats d'ouvrières et des murs de propolis (Papachristoforou et al., 2011). Cette stratégie est d'ailleurs déjà plus ou moins mise en place artificiellement par les apiculteurs qui utilisent des muselières (Bonfond et al., 2021), un dispositif placé à l'entrée des ruches constituant en général un grillage avec une maille assez fine pour laisser passer l'abeille mais pas le frelon. Ce dispositif empêchant les frelons d'entrer dans la ruche et reculant son périmètre d'attaque semble sans impact négatif sur la production de miel et la reproduction (Blanco et al., 2021) et réduit drastiquement la paralysie des abeilles causée par la présence du frelon (Requier et al., 2020). Les muselières sont d'autant plus intéressantes qu'elles sont peu chères et respectueuses de l'environnement.

5. Le piégeage

Le piégeage a longtemps soulevé de nombreuses questions, étant donné le cycle de vie du frelon (**Figure 3**), il faut savoir qui piéger parmi les ouvrières, les fondatrices ou encore les mâles, à quels moments les piéger et comment les piéger.

a. Qui piéger ?

Le piégeage des reines chez les hyménoptères sociaux semble assez peu efficace. En Nouvelle-Zélande le piégeage des reines de *Vespula germanica* et *Vespula vulgaris* n'a eu aucun impact sur le nombre de nids installés la saison suivante (Monceau et al., 2012). De même chez *V. velutina* la destruction massive des reines fondatrices au printemps semble n'avoir pratiquement aucun effet sur la densité des nids pour les mois d'été suivants (Villemant et al., 2011b). En effet, une colonie de frelon asiatique peut produire plus de 400 reines fondatrices mais le faible taux de survie naturel fait que seules quelques-unes survivront de toute façon (Villemant et al., 2011b). La survie de quelques colonies seulement est néanmoins suffisante au maintien et à la multiplication des populations (Haxaire &

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Villemant, 2010). Il est aussi très probable que les fondatrices entrent en compétition pour les sites de nidification, il n'est pas rare d'observer plusieurs fondatrices mortes sur le sol au pied des nids primaires. Piéger les fondatrices pourrait donc être inutile voir contre-productif en diminuant la compétition intraspécifique naturellement existante entre les futures reines. (Monceau et al., 2012). Cependant une étude plus récente mettant en avant la forte densité et la répartition aléatoire des nids suggère que la compétition intraspécifique est peu présente à l'échelle de la population en France (Monceau & Thiéry, 2017). De la même manière, un piégeage massif des ouvrières ne semble pas être la meilleure méthode pour contrôler les populations de Vespides. En effet, retirer 50 à 75 % des adultes dans un nid de *Polistes* n'induit qu'une réduction de 29 % à 34 % de la taille de la colonie (Toft & Harris, 2004). Le piégeage à l'heure actuelle ne doit donc être utilisé que pour limiter localement l'impact de la prédation de *V. velutina nigrithorax* sur les ruchers (Rome et al., 2011a).

b. Comment piéger ?

Différentes formes et types de piège ont été développés depuis l'arrivée du frelon. Les pièges classiques les plus souvent utilisés sont constitués d'une partie supérieure de bouteille en plastique coupée et retournée sur la partie inférieure appâtée avec de la bière ou du vin et du sirop de fruits rouges (**Figure 7A, B**). Ce type de piégeage pour protéger les ruches s'est jusqu'ici montré très peu efficace (Decante, 2014). D'autres pièges plus sophistiqués ont, depuis, vu le jour. Par exemple le piège à nasse ressemblant à un casier de pêche et formé d'une boîte dotée de deux entrées constituées d'un cône percé de trous (**Figure 7C**) ou encore la harpe électrique (**Figure 7D**) qui consiste en un système de grille électrique basse tension qui immobilise les frelons au passage et les fait tomber dans un réservoir d'eau. Ce type de piège permet grâce à un maillage spécifique de laisser passer sans problème les plus petits insectes et un fabricant de harpe indique ainsi une grande spécificité du dispositif qui piège entre 60% et 80% de frelons asiatiques (Lubat, 2017). Des chiffres à considérer avec prudence néanmoins car l'efficacité de ces pièges n'a pas encore été testée de manière indépendante. Un autre moyen de lutte basé sur les appâts consiste à utiliser des appâts empoisonnés, les ouvrières ainsi attirées iront contaminer le nid. Cette méthode a été utilisée par le passé dans la lutte contre *V.*

germanica avec un succès certain (Sackmann *et al.*, 2001) mais aucune étude ne concerne *V velutina*.

De plus de par le danger que cette méthode représente pour le reste de la faune locale, elle a été interdite en France (Turchi & Derijard, 2018). Quel que soit le type de piège utilisé au final, pour qu'il se montre efficace, il doit être associé à un appât pour le frelon.



Figure 7. Différents dispositifs de pièges utilisés dans la lutte contre *Vespa velutina nigrithorax* : **(A)** le piège classique confectionné à partir de bouteilles en plastique, **(B)** le piège VespaCatch® produit et commercialisé par Vêto-pharma, **(C)** le piège à nasse et **(D)** la harpe électrique.

c. Quels appâts ?

Les pièges actuels appâtés avec de la bière sucrée en général sont inefficaces. En effet diverses études démontrent leur piètre sélectivité vis-à-vis du frelon asiatique (Dauphin & Thomas, 2009; Rome *et al.*, 2011a; Rodríguez-Flores *et al.*, 2019; Liroy *et al.*, 2020; Sanchez & Arias, 2021). Sanchez & Arias (2021) montrent par exemple qu'au total 74 différents taxa d'insectes ont été retrouvés dans les pièges à

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frelons classiques, la plupart étant des diptères (93.26% pour 53 taxa) et des hyménoptères (3.91%) mais aussi beaucoup de frelons européens (1.45%) contre seulement 2.23% de frelon asiatique. Même si l'espèce la plus abondante dans ces pièges est une mouche invasive, *Drosophila suzukii* (36.07%), de nombreux autres taxa jouent un rôle important en tant que pollinisateurs, décomposeurs, prédateurs ou parasitoïdes. De même dans une autre étude, Rojas-Nossa et ses collègues montraient que dans les pièges classiques *V. velutina* représentait moins de 1% des captures contre beaucoup de diptères, d'hyménoptères et de lépidoptères natifs (Rojas-Nossa *et al.*, 2018). Parmi ces espèces beaucoup d'insectes aussi ne sont même pas chassés par le frelon lui-même, cela implique que le simple fait de piéger est dangereux pour une entomofaune qui n'aurait de toute façon pas souffert de la présence du frelon (Haxaire & Villemant, 2010). Finalement, pour maximiser les captures de *V. velutina* et minimiser le nombre d'insectes non ciblés, le piégeage doit être effectué avec des pièges combinant une sélection mécanique et des appâts sélectifs. De tels pièges appâtés par exemple avec du jus de nid d'abeille fermenté, placés de juillet à novembre à proximité de ruches dans des ruchers attaqués par le frelon, ont capturé environ 40 % de *V. velutina* (Rome *et al.*, 2011a).

d. S'inspirer du frelon pour trouver un appât sélectif

Si l'attraction du frelon pour l'odeur d'abeille, ses phéromones et les produits de la ruche a été démontré (Couto *et al.*, 2014), de nombreuses équipes de recherche s'intéressent aussi aux phéromones produites par le frelon lui-même. Chez les insectes les réponses phéromonales sont en général très spécifiques (Roelofs & Comeau, 1969), ce qui fait de ces molécules de bonnes candidates pour un appât sélectif. Cappa et ses collègues ont étudié la phéromone présente dans le venin du frelon comme potentielle phéromone sexuelle mais n'ont montré aucune différence dans sa composition et sa quantité entre les fondatrices et les ouvrières ainsi qu'aucune attraction particulière des mâles vis-à-vis de cette phéromone (Cappa *et al.*, 2019b). Dans une étude précédente il avait été néanmoins montré que la phéromone d'alarme contenue dans le venin, en plus de déclencher une attaque, était très attractive pour les ouvrières (Cheng *et al.*, 2017). Aussi chez la sous-espèce de frelon

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asiatique, *V. velutina auraria*, avait été identifiés au niveau des glandes sternales des composés très attractifs pour les mâles qui s'apparenteraient à une phéromone sexuelle (Wen *et al.*, 2017). Sur la base de ce travail, Cappa et ses collègues ont ainsi montré chez *V. velutina nigrithorax* des différences de sécrétion de la glande sternale entre fondatrices et ouvrières avec la présence de certains composés propres aux fondatrices (Cappa *et al.*, 2019b). Contrairement à la phéromone sexuelle tirée du venin, ces sécrétions se sont révélées attractives pour les mâles qu'elles proviennent de l'une ou l'autre des castes.

L'utilisation d'une phéromone sexuelle pour attirer les frelons et notamment les individus sexués se heurte néanmoins à quelques barrières. Elle implique déjà de bien connaître le comportement d'accouplement de l'espèce étudiée car c'est de lui dont va dépendre la potentielle efficacité de la phéromone. En effet, chez diverses espèces de frelon, l'accouplement peut avoir lieu loin du nid, ce qui suggère la nécessité d'une signalisation à longue distance (Matsuura & Yamane, 1990) et donc d'une phéromone efficace en terme d'attraction. Par exemple les mâles du frelon européen, *V. crabro*, ont des stratégies différentes dans leur recherche de partenaire. Certains patrouillent les sites de nidification, tandis que d'autres surveillent les endroits ensoleillés dans la végétation, à une bien plus grande distance des nids (Spiewok *et al.*, 2006). De ce fait, bien qu'une phéromone sexuelle de contact à courte distance ait été identifiée chez *V. crabro* (Batra, 1980), ces phéromones ont une utilité limitée en tant qu'attractif sexuel pour les pièges. En revanche chez d'autres espèces, comme *Vespa mandarinia*, l'accouplement ne se fait qu'à l'entrée des nids (Matsuura & Yamane, 1990). Le comportement d'accouplement chez *V. velutina nigrithorax* est encore peu connu mais quelques observations suggèrent qu'il se ferait à l'intérieur du nid (Cappa *et al.*, 2019b). En effet la grande majorité des futures reines sont déjà accouplées lorsqu'elles quittent le nid (Poidatz *et al.*, 2018b) ; ensuite des agrégations de mâles en recherche de partenaires n'ont jamais été observées sur le terrain (Monceau *et al.*, 2013c) contrairement à la sous-espèce *auraria* (Wen *et al.*, 2017) ; et enfin la population européenne présente un niveau élevé de mâles diploïdes et de consanguinité (Darrouzet *et al.*, 2015b). Un autre problème pour l'efficacité de l'utilisation des phéromones émises par le frelon

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en tant qu'appât attractif est l'hétérogénéité probable de ces phéromones au sein des populations Thiéry *et al.* (2018). On sait que cette hétérogénéité existe déjà au niveau des hydrocarbures cuticulaires par exemple, malgré la faible diversité génétique de la population européenne de frelon asiatique, Gévar *et al.* (2017) ont montré, que la signature chimique des individus était très variable selon leur caste, leur sexe et leur colonie. De même Haouzi *et al.* (2021) ont examiné les profils chimiques des nids et ont montré que les profils d'odeurs de chaque nid leur était propre. Enfin, on sait que les abeilles *A. cerana* sont capables de détecter l'odeur de leur prédateur, *V. velutina*, (Wang *et al.*, 2016), il faut donc se poser la question de à quel point l'utilisation d'appât phéromonal à proximité des ruches pourrait perturber les abeilles domestiques.

e. S'inspirer des plantes pour trouver un appât sélectif

Le frelon asiatique est connu pour visiter de nombreuses fleurs, Ueno l'a observé au Japon se nourrir du nectar floral de 27 espèces végétales différentes appartenant à 15 familles (Ueno, 2015). Mais il existe aussi des relations spécifiques entre les vespides et les plantes, parfois presque aussi spécifiques que celles intervenant dans la reconnaissance sexuelle au sein d'une même espèce. Il a été montré par exemple que des orchidées pollinisées exclusivement par des guêpes ou des frelons émettaient des composés spécifiques pour les attirer (Brodmann *et al.*, 2008; Brodmann *et al.*, 2009). Ces orchidées exploitent les biais sensoriels de ces prédateurs en imitant les phéromones d'abeilles (leurs proies) ou encore les composés émis par les tissus végétaux endommagés par des herbivores auxquels ils ont l'habitude de répondre en tant qu'ennemis naturels de nombreux herbivores et donc auxiliaires des plantes (Brodmann *et al.*, 2008; Brodmann *et al.*, 2009). En ce qui concerne *V. velutina*, il a été mis en avant l'existence d'un mécanisme de dispersion des graines de *Stemosa tuberosa* en Chine par les ouvrières de frelon, le tout grâce à l'émission d'odeurs attractives par les plantes (Chen *et al.*, 2018). Cette étude suggère que l'existence de relations très spécifiques entre plantes et frelons comme la "vespichorie", terme défini seulement depuis 1996, sont sûrement largement sous-estimées dans les interactions plantes-insectes. S'inspirer des plantes pour la recherche d'un appât attractif n'est donc en soi pas une mauvaise idée. De plus, récemment des hybrides horticoles de sarracénies, une

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plante carnivore originaire d'Amérique (Juniper *et al.*, 1989), ont été observés dans un jardin botanique français piéger beaucoup de frelons asiatiques parmi d'autres Vespidés et Diptères (**Figure 8**, Meurgey & Perrocheau (2015)) Certaines de ces sarracénies sont dans leur milieu naturel aussi connues pour capturer de nombreux hyménoptères, principalement des fourmis (Heard, 1998; Newell & Nastase, 1998) mais aussi des guêpes, c'est le cas notamment de *S. leucophylla* (Gibson, 1983). Certains taxons introduits dans des tourbières en Europe ont également été observés piéger des frelons européens (Adlassnig *et al.*, 2009). Par conséquent, nous soulevons ici l'hypothèse que ces sarracénies seraient un modèle intéressant en vue de la recherche de signaux impliqués dans l'attraction du frelon asiatique.

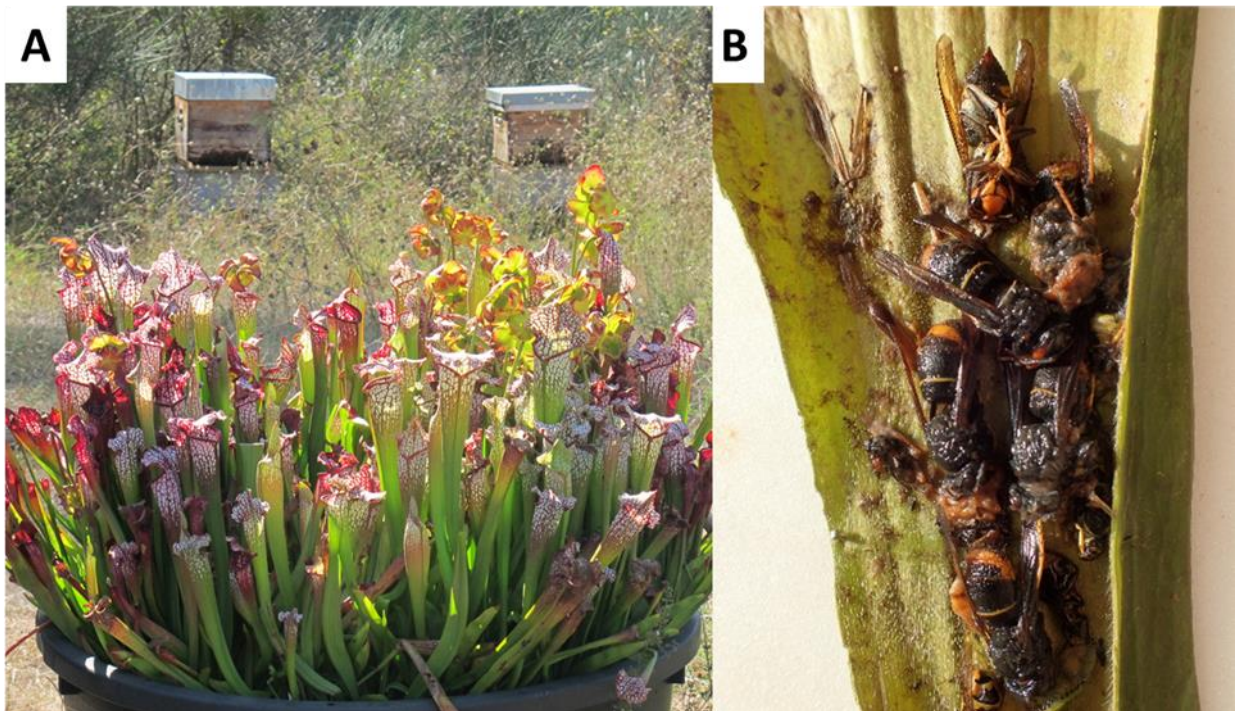


Figure 8. (A) Pièges foliaires et fleurs de *Sarracenia X. leucophylla* non loin des ruches sur le terrain d'expérimentation et de capture à Lavalette, Montpellier, France. **(B)** Contenu de l'intérieur d'une urne de sarracénie majoritairement composé de frelons asiatiques.

6. Les sarracénies

a. La carnivorie chez les plantes

La carnivorie chez les plantes est une adaptation remarquable qui est apparue indépendamment au moins neuf fois au sein des Angiospermes (Givnish, 2015). Cette adaptation se définit par la production chez la plante de feuilles modifiées en pièges qui sont spécialisées dans l'émission de signaux attractifs, la capture, la digestion de proies et l'absorption des nutriments dérivés de ces proies (Givnish, 1989; Juniper et al., 1989). De cette manière, la carnivorie permet aux plantes de survivre et de se reproduire dans les milieux humides, ensoleillés et pauvres en nutriments où elle se développent (Darwin, 1875; Juniper *et al.*, 1989; Ellison & Adamec, 2018) en leur permettant de se nourrir de divers arthropodes et en majorité d'insectes. On peut noter quelques exceptions chez certaines espèces très spécialisées comme *Nepenthes rajah* qui se nourrit des fèces de petits mammifères (Greenwood *et al.*, 2011). Chez les plantes carnivores, les pièges foliaires sont de formes diverses et variées. Ainsi on connaît le piège à mâchoires de la Dionée attrape-mouche (Forterre et al., 2005), les pièges collants à mucilage (Adlassnig et al., 2010), les pièges en forme d'urnes (McPherson & Schnell, 2011) ou même les pièges à aspiration (Knight & Frost, 1991). Certains de ces pièges sont très similaires et sont apparus plusieurs fois indépendamment au cours de l'évolution, témoignant d'exemples remarquables de convergence évolutive (Albert *et al.*, 1992; Thorogood *et al.*, 2018).

b. Les espèces de sarracénies et leurs aires de répartition

La famille Sarraceniaceae est entièrement carnivore et comprend trois genres, *Heliophora* avec 15 espèces confinées dans les hauts plateaux (tepuy) de Guyane et du Venezuela, *Darlingtonia* avec l'unique espèce, *D. californica*, que l'on trouve en Oregon et en Californie et le genre *Sarracenia* le plus récemment divergé avec 11 espèces que l'on trouve surtout dans la plaine côtière du Sud-Est des Etats-Unis dont *S. purpurea* qui présente l'aire de répartition la plus grande s'étendant jusqu'au Nord des Etats-Unis et au Sud du Canada (Stephens et al. (2015b), **Figure 9**). Ces sarracénies se trouvent en

général dans les habitats typiques de tourbières ou encore des savanes humides de Pin pauvres en nutriments (McPherson & Schnell, 2011).

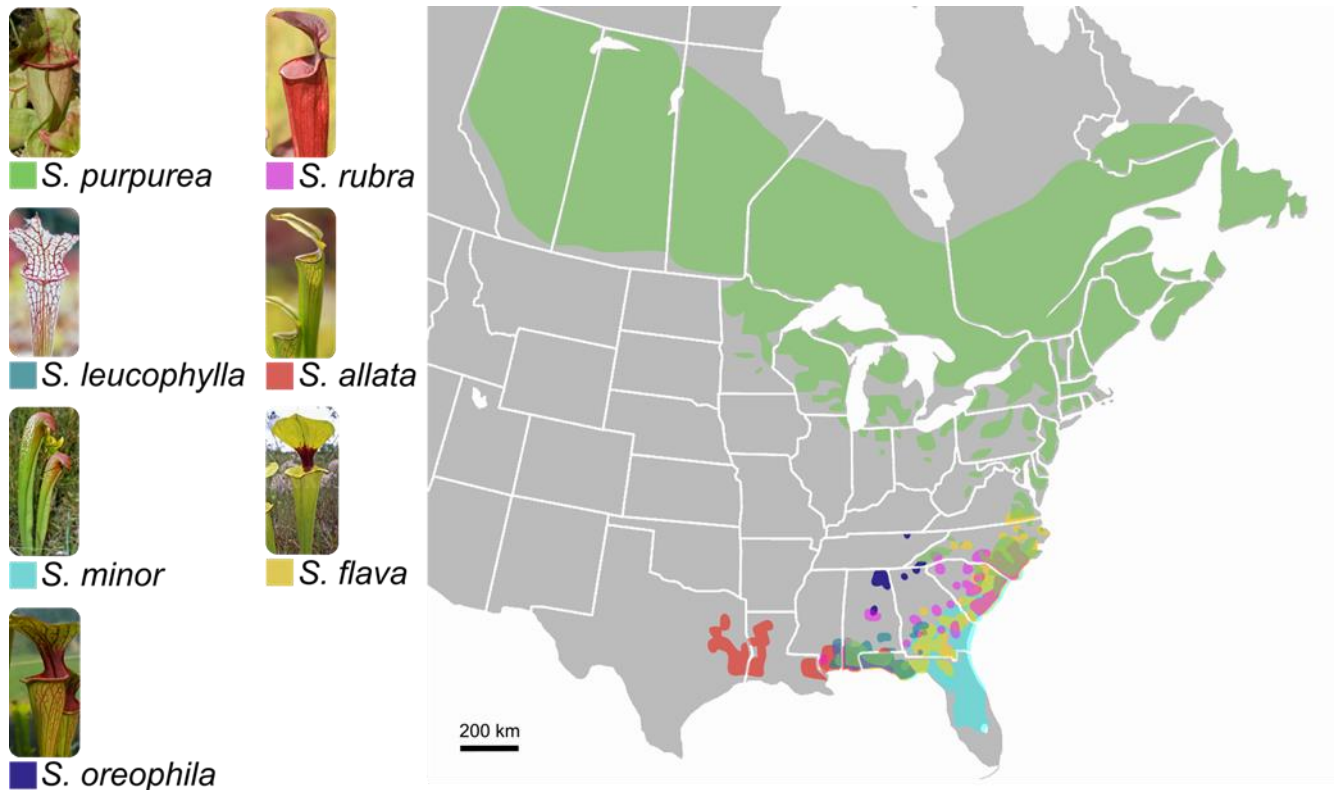


Figure 9. Aire de répartition des principales espèces de sarracénie en Amérique du Nord.

c. Le piège et les leurres des sarracénies

Les sarracénies font partie des plantes à urnes (Juniper *et al.*, 1989; McPherson & Schnell, 2011; Ellison & Adamec, 2018). On relève avec *Sarracenia*, au total 5 genres différents de plantes à urnes dont *Nepenthes*, *Darlingtonia*, *Heliophora* et *Cephalotus* tous présentant leurs propres particularités morphologiques (Arber, 1941). Leurs points communs sont leurs feuilles modifiées en urnes creuses dans lesquelles les proies tombent et sont digérées (Schnell, 2002). L'urne de sarracénie se décompose en différentes grandes zones macroscopiques (**Figure 10**) : le péristome (le bord de l'ouverture), l'opercule surmontant l'ouverture, et le corps qui constitue le reste de l'urne (Juniper *et al.*, 1989). Chacune de ces parties joue un rôle bien défini dans la carnivorie. Ainsi la stratégie de carnivorie des

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urnes de sarracénie semble fonctionner en trois étapes : les signaux attractifs se composant de divers bouquets d'odeurs (Miles *et al.*, 1975; Jaffé *et al.*, 1995; Jürgens *et al.*, 2009), aux touches souvent florales et fruitées (Jürgens *et al.*, 2009) ou différents patrons de colorations (Newell & Nastase, 1998; Green & Horner, 2007; Schaefer & Ruxton, 2008) incluant parfois même des ultra-violets (Joel *et al.*, 1985) incitent les visiteurs à venir sur la partie supérieure de l'urne où se trouve le nectar extra-floral (Juniper *et al.*, 1989) généralement sur le péristome (collerette entourant l'ouverture de l'urne) et la base de l'opercule (Adams & Smith, 1977; Deppe *et al.*, 1999; Bennett & Ellison, 2009). La chute des visiteurs venus se nourrir de nectar est ensuite provoquée par la morphologie de l'urne et la présence de microstructures glissantes (Adams & Smith, 1977). Enfin les proies piégées sont digérées par les enzymes directement secrétées par l'urne ou par un microbiote et un 'macrobiote' vivant à l'intérieur de l'urne (Anderson & Midgley, 2003; Król *et al.*, 2012).

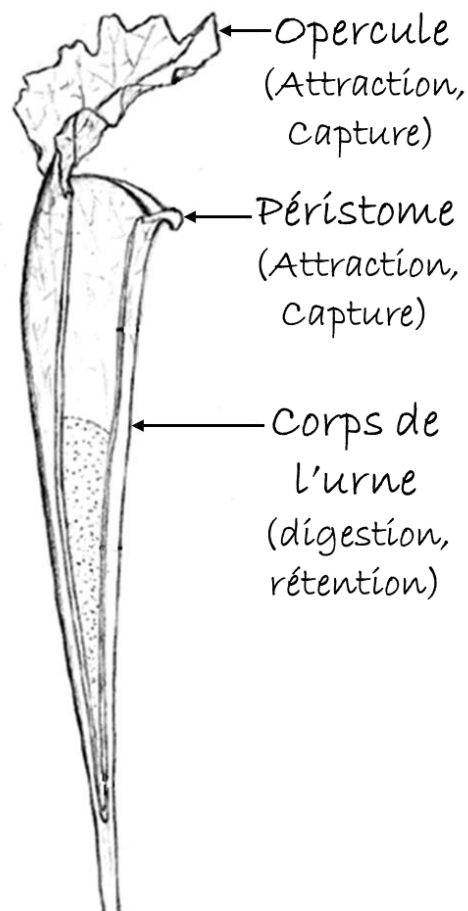


Figure 10. Schéma d'une urne de sarracénie

d. Le rôle des leurres dans l'attraction

Chez les sarracénies, la contribution précise des différents signaux à l'attraction reste assez peu comprise et même souvent sujette à controverse. Par exemple l'effet de la coloration des urnes sur l'attraction et la capture des insectes a été mis en avant par certains (Newell & Nastase, 1998; Schaefer & Ruxton, 2008) mais réfuté par d'autres (Green & Horner, 2007; Sheridan *et al.*, 2021). Ou encore dans une expérience avec *Sarracenia purpurea*, le nectar extra-floral, souvent associé à la coloration rouge de ces plantes, s'est avéré être le facteur d'attraction des proies, plutôt que la coloration rouge elle-même (Bennett & Ellison, 2009). Décomposer l'implication de chaque trait de l'urne s'avère difficile au final car ces traits sont souvent corrélés et forment ensemble ce qu'on appelle le syndrome carnivore (Juniper *et al.*, 1989). Différents syndromes de piégeage quant à eux sont associés chez les *Nepenthes* à des différences de régime alimentaire, les différents traits des urnes des différentes espèces leur permettant de cibler plus spécifiquement certains types de proies (Pavlovič *et al.*, 2007; Gaume *et al.*, 2016). Cette spécialisation, somme toute partielle, vers des proies spécifiques s'expliquerait en partie par une adaptation à la faune présente dans l'environnement des plantes ou par une ségrégation de niche des espèces en réponse à une compétition interspécifique pour les ressources trophiques (Gaume *et al.*, 2016).

Chez les *Sarracenia*, la plupart des espèces semblent piéger principalement des fourmis, si bien que les urnes de sarracénies, qu'elles appartiennent à une espèce ou à une autre, sont considérées par certains auteurs comme des pièges 'passifs' non dans le sens communément employé de 'pièges sans mouvement mécanique', mais dans le sens de 'pièges capturant au hasard ce qui est directement disponible et abondant', (Ellison & Gotelli, 2009). Les fourmis étant les insectes sociaux les plus ubiquistes des strates terrestres, elles seraient ainsi la cible privilégiée et passive de ces pièges. Cette vision est cependant contredite par certaines études qui reportent des différences interspécifiques notables dans les spectres de proies tant au niveau taxonomique (e.g. Stephens *et al.* (2015a)) que dans la taille des proies (e.g. Gibson (1983)). Cette vision simpliste viendrait du fait que la plupart des études se sont focalisées sur *S. purpurea* qui est l'espèce la plus étendue (**Figure 9**) et connue pour

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piéger essentiellement des fourmis et des diptères (Cresswell, 1991; Heard, 1998), alors que d'autres plus rarement étudiées, comme *S. leucophylla*, sont réputées piéger aussi beaucoup de gros hyménoptères volants (Gibson, 1983). Peu d'études ont cherché à expliquer ces différences. Chez les sarracénies, il existe des interactions de compétition entre certaines espèces (Gibson, 1983; Folkerts, 1992) vivant en sympatrie (cf aires de répartition communes dans la **Figure 9**) qui pourraient expliquer des spécialisations à des types de proies particuliers. Par ailleurs, si on s'intéresse aux facteurs proximaux, c'est plus souvent la morphologie que les signaux attractifs qui a été supposée responsable de la variation des spectres de proies chez ces plantes (Gibson, 1983; Stephens *et al.*, 2015a; Sheridan *et al.*, 2021). Les études qui en revanche ont étudié le rôle des signaux, ne s'intéresse jamais à l'effets de ces traits sur la composition des proies mais seulement à la variation intraspécifique de la quantité totale de proies capturées (Green & Horner, 2007; Wallen, 2008; Bhattarai & Horner, 2009).

C'est pourquoi dans cette thèse, toujours avec l'objectif de trouver des signaux attractifs pour le frelon asiatique nous avons aussi cherché à mieux comprendre le rôle des signaux dans le syndrome carnivore chez les sarracénies mais aussi leur implication dans la spécialisation des captures à l'échelle interspécifique.

7. Objectifs de la thèse

L'objectif principal de la thèse est de comprendre le fonctionnement des leurres visuels et olfactifs des sarracénies ainsi que des traits liés à la capture de grosses proies volantes chez ces plantes carnivores dans le but d'identifier prioritairement les signaux les plus à mêmes de leurrer, et éventuellement les traits les plus à même de piéger, le frelon asiatique, *Vespa velutina*, espèce envahissante en Europe et grande prédatrice d'abeilles domestiques, *Apis mellifera*.

Nous avons pour cela exploité la diversité morphologique qu'offrent ces plantes dans la nature et en horticulture et mené un travail de recherche sur 4 taxa de sarracénies qui diffèrent dans leurs traits visuels et morphologiques. Il s'agit de deux espèces naturelles, *S. purpurea* subsp. *venosa* et

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l'hybride *S. X mitchelliana* = *S. purpurea* X *S. leucophylla*, ainsi que deux hybrides horticoles, *S. X Juthatip soper* = (*S. leucophylla* X *S. purpurea*) X *S. leucophylla* et *S. X leucophylla* = *S. leucophylla* X *S. X Juthatip soper*. Le choix de ces quatre taxa a été réalisé avec l'aide de Jean-Jacques Labat, pépiniériste spécialiste des plantes carnivores et fournisseur et conseiller du jardin botanique de Nantes où ont été observées les premières captures de frelons asiatiques. Plusieurs critères ont été retenus pour le choix de ces sarracénies, comme le fait de conserver des pièges en automne, au moment du pic d'activité du frelon, et le fait de différer dans leurs aspects visuels et leurs captures. Ces 4 taxa forment un gradient de parenté entre deux espèces naturelles, *S. purpurea* et *S. leucophylla* (**Figure 11**) elles-mêmes à priori très différentes dans leurs signaux et leurs captures, l'une piégeant principalement des fourmis, l'autre reportée piéger beaucoup d'hyménoptères volants (Gibson, 1983; Ellison & Gotelli, 2009). Nous supposons que la variabilité de leurs signaux, de leurs captures et de la capture du frelon en particulier nous permettra de décrypter au mieux le rôle de ces signaux dans l'attraction du prédateur et d'amorcer des pistes de recherche pour un appât spécifiquement attractif pour le frelon.

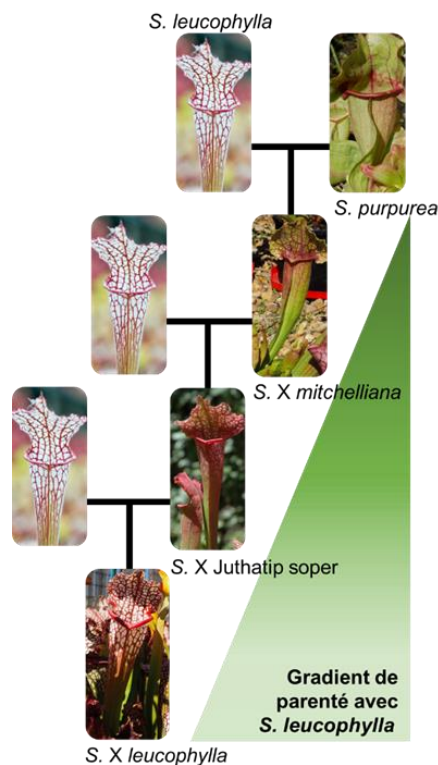


Figure 11. Les différents hybrides de sarracénies étudiées au cours de cette thèse, leur origine génétique et leur gradient de parenté avec les espèces naturelles, *S. leucophylla* et *S. purpurea*.

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Nous avons ainsi dans un premier temps cherché à caractériser les signaux olfactifs et visuels de ces 4 sarracénies, et dans un deuxième temps testé leur efficacité dans l'attraction et la capture du frelon asiatique en laboratoire et sur le terrain, dans le but d'identifier les traits les plus pertinents pour le développement d'un piège biomimétique artificiel à son encontre. Les deux premiers chapitres sont ainsi consacrés à la caractérisation des signaux mis en place par les sarracénies pour leurrer leurs proies, les deux suivants à tester l'efficacité de ces signaux et traits dans l'attraction et la capture du frelon asiatique.

(1) Caractérisation des signaux sensoriels émis par les sarracénies responsables de l'attraction des insectes

Dans le **chapitre 1**, nous avons cherché à étudier en détail le bouquet d'odeurs des urnes des sarracénies afin de connaître plus précisément la nature et la composition de ce signal ainsi que sa variation potentielle d'une espèce à l'autre. Nous avons également cherché à mieux connaître l'influence de ce signal sur la composition en proies des urnes et surtout à dissocier son rôle de celui de la morphologie souvent invoquée pour expliquer les variations de capture chez ces plantes. Nous avons donc procédé à des mesures morphologiques des urnes des 4 taxa de sarracénies, à l'échantillonnage de leurs odeurs suivi de leurs analyses en GC-MS ainsi qu'à l'identification des proies capturées sur le terrain. L'étude de ces questions a abouti à l'écriture d'un article : **Volatile organic compounds influence prey composition in *Sarracenia* carnivorous plants.**

Dans le **chapitre 2**, nous avons cherché à étudier les signaux visuels des sarracénies, comme perçus par le système de vision des hyménoptères volants comme le frelon. Nous avons plus précisément cherché à savoir si la présence d'alvéoles claires sur les urnes (motifs délimités par les veines de l'urne dans sa partie distale) consistait un signal particulièrement fort pour ces insectes en termes de contraste par rapport au reste de l'urne ou au fond environnant. Nous avons également cherché à mieux connaître l'influence de ces signaux visuels sur la composition en proies des urnes en regardant notamment si le contraste des alvéoles claires était relié à la capture d'hyménoptères volants

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indépendamment de la morphologie du piège. Nous avons pour cela caractérisé les contrastes de couleur et de clarté des différents motifs colorés des urnes des 4 taxa de sarracénies grâce à des mesures de spectrophotométrie et à un modèle de vision du frelon et avons procédé à l'identification des proies capturées par leurs urnes sur le terrain. L'étude de ces questions a abouti à l'écriture d'un article: **Variation in colour signals among *Sarracenia* pitcher plants and the potential role of areoles in the attraction of flying Hymenoptera.**

(2) Test et identification des signaux efficaces dans l'attraction et la capture du frelon asiatique

Dans le **chapitre 3**, nous avons cherché à évaluer les capacités d'attraction et de piégeage du frelon asiatique par les urnes des sarracénies en conditions de confinement. Nous avons plus particulièrement cherché à connaître les traits impliqués dans l'attraction du frelon parmi la production de nectar et la coloration des urnes dans une cage où les odeurs des plantes sont supposées se mélanger. De même nous avons cherché à connaître les traits impliqués dans la capture et la rétention de grandes proies volantes comme le frelon parmi différents traits morphologiques et les microstructures des urnes. Nous avons donc observé 50 frelons dans une cage contenant les 4 différents taxa de sarracénies et des témoins constitués de fausses urnes dont les caractéristiques morphologiques ont été mesurées et nous avons décompté un certain nombre d'événements comme les visites, les chutes dans les urnes, les fuites des urnes ainsi que le taux de capture de chaque plante. L'étude de ces questions a abouti à l'écriture d'un article : **Capture efficiency of *Sarracenia* pitcher plants towards the Asian hornet, *Vespa velutina*, under laboratory conditions : contribution of lid visual signals, nectar and pitcher shape.**

Enfin, nous avons cherché dans le **chapitre 4** à évaluer les capacités d'attraction pour le frelon asiatique des odeurs émises par les sarracénies notamment par rapport aux odeurs du miel et de l'abeille déjà connues pour être attractives. Nous avons pour cela analysé les profils d'odeurs des plantes entières des 4 taxa de sarracénies. Nous avons ensuite testé le comportement du frelon asiatique vis-à-vis des

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de ces plantes, du miel et de l'abeille dans un dispositif d'olfactométrie constitué d'un tube en Y. Nous avons défini différents critères et modèles pour évaluer cette attractivité afin de mieux appréhender le comportement d'attraction complexe dans un dispositif simplifié. Afin de comparer leurs capacités d'attraction du frelon avec leurs taux de capture, nous avons également sur 3 années consécutives et pour trois saisons différentes prélevé des urnes des 4 taxa de sarracénies sur le terrain pour évaluer leurs succès de capture de frelons asiatiques. L'étude de ces questions a abouti à l'écriture d'un article : **Olfactory attraction of the Asian hornet, *Vespa velutina*, by *Sarracenia* pitcher plants.**

Dans une synthèse des résultats, nous avons ensuite discuté de l'apport de notre travail dans la compréhension des traits et mécanismes qui ont promu l'évolution et la diversification morphologique des sarracénies, des avancées qu'ont permis notre étude dans le domaine de la communication visuelle et olfactive du frelon avec les plantes et nous avons discuté des traits à retenir pour l'élaboration d'un appât et d'un piège à l'encontre du frelon asiatique tout esquisant les perspectives de recherche pour le développement d'un tel piège.

Chapitre 1

**Volatile organic compounds influence prey
composition in *Sarracenia* carnivorous plants**

Résumé

Les sarracénies présentent des différences interspécifiques dans l'aspect visuel de leurs pièges et de façon moins marquée dans les proies qu'elles capturent. C'est le plus souvent la morphologie des urnes qui a été invoquée pour expliquer la ségrégation des proies et l'atténuation de la compétition pour les ressources trophiques. Nous avons émis l'hypothèse que les odeurs des sarracénies jouaient également un rôle important. Nous avons d'abord comparé la composition des odeurs et des proies de quatre espèces cultivées ensemble : *Sarracenia purpurea*, *S. X mitchelliana*, *S. X leucophylla* et *S. X Juthatip soper*. Nous avons ensuite effectué des analyses confrontant les variables quantitatives liées aux plantes et aux proies afin de démêler les contributions de la morphologie et de l'odeur dans la variation des proies. Les odeurs des sarracénies étaient aussi diverses que celles des fleurs généralistes, émettant à la fois des terpènes, des dérivés d'acides gras et des benzénoïdes, mais présentées des différences notables entre les espèces. Les analyses de similarité des COVs ont révélé des spécificités taxonomiques chez *Sarracenia*, tout comme les analyses de similarité des proies, soulignant la nature non aléatoire des captures. *S. X leucophylla* s'est distingué en étant l'espèce la plus spécialisée pour les insectes volants comme les abeilles et les papillons de nuit et en libérant plus de monoterpènes connus pour attirer les visiteurs des fleurs. *S. X Juthatip soper* a piégé autant d'abeilles mais moins de papillons de nuit, les sesquiterpènes contribuant moins à son odeur. Les fourmis et les diptères étaient les principales proies des deux autres, avec des odeurs dominées par les dérivés d'acides gras. Une analyse de corrélation canonique a montré que les quantités des différents groupes de proies peuvent être déduites à 98 % des quantités des classes d'odeurs et des dimensions de l'urne. Elle a aussi révélé deux syndromes : les fourmis associées aux dérivés d'acides gras et aux urnes courtes, les insectes volants aux monoterpènes, aux benzénoïdes et aux urnes hautes. Selon les régressions multiples de Poisson chez *S. X leucophylla*, l'émission de dérivés d'acides gras et la longueur des urnes expliquaient la plupart des variations dans les captures de fourmis ; les monoterpènes et la longueur des urnes expliquaient la plupart des variations dans les captures d'abeilles et de papillons de nuit ; les monoterpènes seuls expliquaient la plupart des variations dans les captures de diptères et de guêpes. Par conséquent, nos résultats indiquent que les sarracénies ne sont pas des pièges passifs et suggèrent que les odeurs sont un facteur clé dans la composition de leur régime alimentaire.

Volatile organic compounds influence prey composition in *Sarracenia* carnivorous plants.

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Abstract

Sarracenia pitcher plants display interspecific differences in prey, so far only explained by pitcher morphology. We hypothesized that pitcher odours play a role in prey composition. We first compared odour and prey compositions among *Sarracenia* taxa grown together, forming a kinship gradient from *S. purpurea* known to capture primarily ants towards *S. leucophylla* known to capture many flying insects: *S. purpurea*, *S. X mitchelliana*, and *S. X Juthatip soper* & *S. X leucophylla* horticultural hybrids. We then measured several pitcher traits to disentangle the contributions of morphology and odour to prey variation. The pitcher odours were as diverse as those of generalist-pollinated flowers but with notable differences among taxa, reflecting their relatedness. VOC similarity analyses revealed taxon specificities, that mirrored those revealed by prey similarity analyses. *S. X leucophylla* stood out by being more specialised in flying insects like bees and moths and by releasing more monoterpenes known to attract flower visitors. *S. X Juthatip soper* trapped as many bees but fewer moths, sesquiterpenes contributing less to its scent. Ants and Diptera were the main prey of the other two with fatty-acid-derivative-dominated scents. Quantities of the different prey groups can be inferred 98% from quantities of the odour classes and pitcher dimensions. Two syndromes were revealed: ants associated with fatty-acid-derivatives and short pitchers; flying insects associated with monoterpenes, benzenoids and tall pitchers. In *S. X leucophylla*, emission rate of fatty-acid-derivatives and pitcher length explained most variation in ant captures; monoterpenes and pitcher length explained most variation in bee and moth captures; monoterpenes alone explained most variation in Diptera and wasp captures. Our results suggest that odours are key factors of the diet composition of pitcher plants. They support the hypothesis of perceptual exploitation of insect biases in carnivorous plants and provide new insights into the olfactory preferences of insect groups.

Introduction

Plants are sedentary organisms and have developed over the course of evolution a particularly diverse and effective language for communicating at a distance with each other or with organisms from other kingdoms: odours with an alphabet made of volatile organic compounds [1, 2]. Plants communicate with each other and use particular combinations of VOCs to warn their neighbours of herbivore attacks, or, conversely, to inhibit the germination of their competitors [3]. Plants also communicate with other organisms, by emitting repellent odours to deter pathogens [4, 5] and herbivores [6, 7] or by emitting COVs attractive to insects, which often perform essential functions in mutualistic relationships with them, such as defense against herbivores [8], pollination [9], seed dispersal [10], and even nutrition in carnivorous plants [11, 12]. Carnivorous plants, to overcome the lack of nutrients in the soils where they grow, do indeed supplement their diet with essential nutrients obtained from insects and other arthropods that they attract, capture and digest with their highly-modified leaves [13-15]. Attraction is thus the first, but not least, component of the carnivorous syndrome in these specialised plants [13].

A group of carnivorous plants, the so-called pitcher plants, includes the well-known Sarraceniaceae from the Americas and the Nepenthaceae from Southeast Asia. The results of some studies comparing insect prey or inquiline numbers of pitcher traps *versus* control traps suggest that pitchers are not simple pitfall traps, highlighting the importance of attraction in these carnivorous plants [16, 17]. Attraction in these pitcher plants is actually satisfied not only by olfactory signals [18-20] but also by other lures, such as nectar guides [21, 22] and colour patterns [23-25]. Olfactory cues have received comparatively little attention. A few old studies used tissue extraction to investigate the odour of pitcher plants, but this method also collected non-volatile compounds [18, 26] and thus did not provide information on the composition of the emitted bouquet. A more recent study used the same method to compare metabolites among Sarraceniaceae species and also investigated their scent profile but the investigation was then focused on the search for the volatile alkaloid coniine [27]. Only a few studies have actually examined the volatile compounds in the odour bouquets of *Sarracenia* [19, 28] and *Nepenthes* [20] pitchers and have shown some similarity with flower scents. The role of pitcher scent in attracting insects has been put forward in *Sarracenia flava* and *Sarracenia leucophylla* where

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significant correlation between fly visits and VOC emission rates have been found [19] and has been demonstrated experimentally via olfactometry on ants and flies in *Nepenthes rafflesiana* [20] confirming earlier hypotheses [29, 30].

Odours play an important role in the pollination systems of flower plants and the VOC composition of flowers sometimes characterises species-specific pollination systems or whole syndromes attracting a particular type of animals (e.g. bees in melittophile plants with an often terpinoid-rich cue; flies in myophile plants with odors often rich in aliphatics and nitrogen-compounds, butterflies in psychophile plants with a benzenoid-rich cue, bats in chiropterophile plants with sulfur-containing fetid odors) [31-33]. Thus, one may wonder whether in carnivorous pitcher plants, which show some inter-specific partitioning of prey [34], especially in those growing in sympatry where interspecific competition is high [17, 35, 36], odour plays a role in the differences observed in their prey spectra.

In *Nepenthes* pitcher plants, in addition to pitcher morphology and visual characteristics [23] as well as trapping mechanisms [37, 38], odour is strongly suspected to participate in prey segregation (i.e. substantial variation in prey spectra) [17]. A large part of the differences in the amounts of flying and terrestrial insects trapped in pitchers can be explained by differences in VOC emission [20]. In *Sarracenia*, morphology has hitherto been mainly invoked to explain these differences in prey capture [35, 39, 40]. But Ellison and Gotelli reported only few differences in prey spectra among *Sarracenia* species. They even concluded that these carnivorous pitcher plants would act as passive traps [41], since the relative abundance of the different groups of prey arthropods trapped by the pitcher plants did not differ from the relative abundance of arthropods found in their environment. Their meta-analysis concluded that catches were random, which leaved little room for olfactory signals.

On the contrary, we hypothesise that olfactory signals play an important role in the prey composition of *Sarracenia* pitcher plants and that catches are not random. To test this hypothesis, we explored differences in odour emission and prey capture in four *Sarracenia* taxa grown together under the same biotic and abiotic conditions and differing by pitcher morphologies. The taxa, including two natural species and two horticultural hybrids formed a kinship gradient from *S. purpurea* (known to capture mainly ants [42, 43] towards *S. leucophylla* (known to capture many flying insects [36])). We

then investigated the extent to which the amounts of various classes of compounds emitted by pitchers, classified according to their biosynthetic pathways, explained the variations in captures of different insect groups.

Material and methods

Plant taxa and growing conditions

We considered four *Sarracenia* taxa differing in visual aspect and leaf shape (Fig 1). All the studied plants were horticultural specimens obtained from the carnivorous plant nursery “Nature and Paysages” (Peyrusse-Massas, Gers, France) at the same juvenile stage and replanted in the beginning of April 2016 in the experimental station of AMAP (Montpellier, France). Two of the taxa were natural species: *S. purpurea* subsp. *venosa* and *S. X mitchelliana* = *S. purpurea* X *S. leucophylla*. The two others were horticultural hybrids: *S. X Juthatip soper* = (*S. leucophylla* x *S. purpurea*) X *S. leucophylla* and *S. X leucophylla* = *S. leucophylla* X *S. X Juthatip soper*. The plants of the four taxa were grown outdoors in polyculture in the same 1m³ rectangular container (one row of plants per taxon). The container was filled with a peat mixture consisting of 2/3 blond peat and 1/3 sand on a background of clay balls in a sunny place. It was regularly watered with demineralized water to match their natural moist and mineral-poor environment. The study took place in the south of France during the summer of 2016, characterised by rather hot temperatures typical of the Mediterranean climate.



Fig 1. Pitchers of the four studied *Sarracenia* taxa. (A) *S. purpurea*, (B) *S. X mitchelliana*, (C) *S. X Juthatip soper*, (D) *S. X leucophylla*

Collection of volatile organic compounds

Plant odours were collected under natural light and ambient temperature by the dynamic headspace method and the adsorption-desorption technique. We collected the odours of a total of 39 pitchers on 16 different plants (7-13 pitchers per taxon see S1 Table) in 13 extraction sessions haphazardly carried out in the morning or afternoon of 7 days between 27/07/2016 and 25/08/2016. Each studied pitcher was enclosed in a polyethylene terephthalate bag (Nalophane®, Kalle Nalo GmbH, Würsthüllen, Germany) tightly closed with cotton string. Airflow was maintained through the bags by two standard 12-V air pumps connected with silicone tubes to flowmeters. Pure air, filtered by charcoal filters, was flown into the bags at 60 mL/min and extracted at 40 mL/min through silicone tubing. The difference in flow made it possible to cope with the inevitable leaks and prevent outside air from entering the system. Pure air was flown into the bags for 10 min before starting dynamic extraction. The adsorbent traps attached to silicone tubing and put above pitcher aperture in the collection bags were ChromatoProbe® quartz microvials of Varian Inc. (length: 15 mm; inner diameter: 2 mm, previously cut closed-end) filled with 3 mg of a 1:1 mix of Tenax-TA and Carbotrap® (60–80 and 20–40 mesh, respectively; Sigma Aldrich,

Munich, Germany). Empty bags with similar traps were used as ambient controls to check for possible contaminations during each set of VOC collections. ChromatoProbe® samples were stored at -20°C in a freezer until analysis, which was made within 1 month of collection. One microliter of a solution of two internal standards (n-Nonane and n-Dodecane, 108 and 114 ng/ μL , respectively) was added to each ChromatoProbe® trap before scent extraction to ensure that samples did not suffer loss during collection and sample processing. Dose-response curves using standard compounds representing each class of VOCs found in the pitchers have also been processed to correct for the quantitative losses during sample processing. These two approaches allowed us to carry out reliable quantitative analyses and thereby comparisons of emission rates between pitchers.

Chemical analysis

Samples were analysed with a gas chromatograph (GC, Trace™ 1310, Thermo Scientific™ Milan, Italy) coupled to a mass spectrometer (ISQ™ QD Single Quadrupole, Thermo Scientific™ Milan, Italy) and an Optima 5-MS capillary column (30 m, 0.25-mm internal diameter, 0.25- μm film thickness, Machery-Nagel, Düren, Germany). Absorbent traps were handled with a Multi-purpose sampler (Gerstell, Mülheim, Germany) and desorbed with a double stage desorption system, composed of a Thermal Desorption Unit (TDU) and a Cold Injection System (CIS) (Gerstell, Mülheim, Germany). First, filters were desorbed in splitless mode with a temperature of 250°C on the CIS trap cooled at -80°C by liquid nitrogen. Then, the CIS trap was heated to 250°C with a 1:4 split ratio to inject the compounds into the column. The carrier gas used was helium at 1 ml/min. Oven temperature was held at 40°C for 3 min, increased from 40 to 200°C at a rate of $5^{\circ}\text{C}/\text{min}$ and from 200 to 250°C at $10^{\circ}\text{C}/\text{min}$, and finally held for 2 min. The temperature of the transfer line and the ion source of the mass spectrometer were 250 and 200°C , respectively. The acquisition was from 38 to 350 m/z , at a 70-eV ionization energy.

We used the standard retention times of C8 to C20 *n*-alkanes (Alkanes standard solution, 04070, Sigma Aldrich®) to convert retention times into retention indexes. We identified volatile organic compounds by comparing mass spectra with those of database (NIST 2007 MS library, Wiley 9th edition) and retention indexes reported in the literature [44]. Identifications were done using netCDF converted files on the GC-MS solution software (v4.11 SU1, Shimadzu Corporation, Kyoto, Japan).

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Peaks of identified VOCs were confirmed and integrated in each sample using Xcalibur® software (Thermo Scientific™ Milan, Italy). Compounds present in the controls were considered to be contaminants and their quantity was subtracted from each associated sample.

Pitcher morphological measurements and prey capture analysis

22 pitchers out of the 39 analysed for their scents were also analysed for their prey contents and pitcher morphology. Thus, at the end of 4 days of odour sampling (corresponding to 7 sampling sessions) during the second half of August, a total of 22 pitchers of 11 plants (*S. X leucophylla* = 9, *S. X Juthatip soper* = 6, *S. X mitchelliana* = 3 and *S. purpurea* = 4) were cut and measured for pitcher length and aperture width (see Fig 1). Prey content was preserved in 70% ethanol and identified by Claire Villemant, at least to the family level for all taxonomic orders except Lepidoptera and Diptera, whose soft bodies were more digested preventing sometimes more precise identification. To obtain a more representative sample of pitcher mensurations and prey contents for each taxon, the 25th of August 2016, we further carried out the measurements and prey identification of 19 other pitchers belonging to 18 plants (*S. X leucophylla* = 2, *S. X Juthatip soper* = 4, *S. X mitchelliana* = 7 and *S. purpurea* = 6). Unfortunately, it was not possible to sample the odours of these further pitchers.

Statistical analysis

First, we compared pitcher scent and pitcher prey composition among the four different taxa. We therefore performed non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarities of relative proportions (of VOCs or prey groups), using the “metaMDS” function from the R package “Vegan” [45]; The significance of differences in scent and prey patterns among taxa was assessed by permutational multivariate analyses of variance (PERMANOVA) with 999 random permutations using the “adonis2” function from the R package “Vegan” and pairwise comparisons using “pairwise.adonis2” function from the R package “pairwiseAdonis” [46]. P-values were adjusted for multiple comparisons using the Holm’s method. We also considered the variable ‘Plant identity’ nested within the variable ‘Plant taxon’ in our analyses.

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Second, we used general linear mixed models to investigate whether the total emission rate and those of the different compound classes (benzenoids, fatty acid derivatives, monoterpenoids and sesquiterpenoids) varied according to the taxon considered. We analysed several dependent variables, i.e. the total emission rate (absolute quantity of VOCs), the emission rates of the four classes of compounds and the relative quantities of the four classes of compounds released by pitchers, with taxon as explanatory variable. As some of the pitchers belonged to the same plant and several samples were taken in the same sampling session, we considered the variables plant identity and sampling session as random variables. Absolute and relative quantities were square-root transformed to achieve normality of residuals.

Third, we used the same approach to investigate whether the total number of prey items and the number of prey items for different taxonomic groups varied according to the taxon considered. We thus analysed with general mixed models the total number of prey individuals trapped by pitchers, the number of flying prey individuals, the number of bees, moths, Diptera (flies, midges and mosquitoes), wasps (solitary, social and parasitoid wasps) and ants trapped by pitchers as dependent variables, testing taxon as an explanatory fixed variable and sampling day and plant identity as random variables. As the four taxa differed significantly in pitcher length (S1 Fig), which was likely to influence the number of prey [35, 39, 40], we corrected prey number by pitcher length in the analyses and considered this variable as a density (number of individuals / pitcher length). Density was square-root transformed to achieve normality of residuals.

Finally, we explored whether prey spectra can be explained by scent spectra in addition to pitcher morphology. We first investigated this for the four taxa using the pitchers for which we got both scent and measurement data, using a canonical correlation analysis (CCA) and the R package “CCA” [47]. This analysis aimed to explain the relationship between prey variables, i.e. the insect number of the main prey groups, and plant variables, which were the absolute VOC quantities of each compound class produced by pitchers as well as pitcher length and aperture width. Investigating further the correlations between plant taxa and prey variables observed in the CCA using general linear models implied testing too many taxon-variable interactions. Hence, we decided to focus on *S. X leucophylla* for which we had

the highest sample size. Using multiple regression models with a Poisson distribution and a log-link function, we explored which variables –the absolute quantities of each compound classes and/or the morphological measures - best explained the variation in individual captures for each prey group (ants, Diptera, wasps, bees, moths and beetles) observed in this taxon. Since pitcher length and aperture width were highly correlated within the same taxon (e.g. *S. X leucophylla*, S4 Table) and, unlike length, width did not differ between taxa, we considered only pitcher length in these analyses for reasons of parcimony.

For all linear models, backward selection and Type III tests were carried out and we selected the best model with the lowest AIC (Akaike criterium). Mixed models were estimated with the REML (Restricted Maximum Likelihood) method using the function “lmer” from the R package “lme4” [48]. Models were then adjusted with the ML (Maximum Likelihood) method and update function to get a better estimate of the coefficients for the fixed factors. For the last five Poisson multiple regression models only, a stepwise (forward-backward) selection of variables was preferred because it is a classical and appropriate procedure when several quantitative variables and a small sample size are involved. Indeed, by keeping fewer variables than the backward selection, it favours omission of variables that contributes little to the model, which is a favoured procedure because although it slightly biases the least square estimates, it also decreases the variance and mean square error of all least square estimates [49]. For each significant factor, post-hoc tests were carried out between any two factor levels, when necessary, using the function “contrast” from the R package “emmeans” [50]. P-values were adjusted for multiple comparisons using the Holm’s method. Statistics were performed using the software R version 4.0.3 [51]. Model assumptions were checked by plotting residuals against fitted values.

Results

In summer 2016 in Montpellier, the pitchers of *Sarracenia* cultivated plants were found to release rich and complex odours, including a wide diversity of compounds ranging from 41 to 65 identified VOCs depending on taxon, with an average emission rate of 46 to 155 ng/h (S1 Table, n=39 pitchers). The VOCS released belong to four classes of compounds, monoterpenes, sesquiterpenes, benzenoids and

fatty acid derivatives (S1 Table). On the other hand, pitchers trapped up to 10 orders of animals, mostly insects (6 orders) with an average of 25 ± 27 insect individuals per pitcher ($n=41$), among which, hymenopterans were dominant (46%) followed by dipterans (26%), lepidopterans (11%) and coleopterans (9%). As the non-insect groups represented only 4 individuals they were not considered in the analyses.

Interspecific variation in scent emission

The pitchers of the same taxa grouped together based on the resemblance of their scent profile in terms of VOC composition (results of NMDS in Fig 2A) and the PERMANOVA (Table 1) showed significant differences among taxa in the VOC profiles (Fig 2A). *Sarracenia* taxa thus differed globally in the odour bouquets of their pitchers (Fig 2A). However, there was a high intra-specific variability, shown by the sometimes-high distances in the scent profile between pitchers of the same taxon (Fig 2A), and also significant effects of “Plant identity” and “Sampling session” random variables in the analyses of compound-class emission (Table 2). Therefore, only the odours of *S. X leucophylla* pitchers came out to be significantly different from those of the three other taxa (S2 Table). The odours of *S. X Juthatip soper* differed significantly from those of *S. X mitchelliana*. The odours of *S. X mitchelliana* revealed to be different from those of the two previous ones but not from those of *S. purpurea*. This highest singularity of *S. X leucophylla* regarding COV composition can be explained by the particularly high emission rate of monoterpenoids in this latter taxon (Fig 3A), which was significantly higher ($P < 0.01$) than in the other three taxa, independently of any effects of sampling session and plant identity, which were considered in the model (Table 2). *S. X leucophylla* also stood out in terms of VOC diversity since up to 65 VOCs were found for this taxon, whereas there were no more than 56 VOCs identified in the other three taxa (S1 Table). The benzenoid class was particularly rich in this taxon, where twice as many compounds were found as in the other three taxa (S1 Table). In terms of emission rate, *Sarracenia* taxa did not vary significantly neither for benzenoids, nor for fatty acid derivatives, except weakly ($P=0.04$) for sesquiterpenes, for which the emission rate was the lowest in *S. X Juthatip soper* (Fig 3A). Yet there were more marked interspecific differences in the relative amounts of the four classes of compounds. The share of benzenoids in the bouquet of *S. X Juthatip soper*, which had the lowest emission rate, was

the highest but, statistically, it only came out to be higher than that of *S. X mitchelliana* with an almost significant trend ($P=0.05$, Fig 3B). *S. X leucophylla* released a significantly lower proportion of fatty acid derivatives than the other three taxa and a significantly higher proportion of monoterpenes. *S. X Juthatip soper* displayed the second highest proportion of monoterpenes. Finally, the share of sesquiterpenes in the blend was the highest for *S. X mitchelliana* and the lowest for *S. X Juthatip soper* (Fig 3B). A total of 71 VOCs produced by the four *Sarracenia* taxa were identified (S1 Table). We observed some notable differences in the predominant compounds in the four plant taxa (Fig 3B). The bouquet of *S. X leucophylla* was clearly dominated by the monoterpenes, myrcene, (E)- β -ocimene and limonene; that of *S. X Juthatip soper* by (E)- β -ocimene and by the benzenoid esters, ethyl benzoate and methyl benzoate, that of *S. X mitchelliana* was dominated by the monoterpene, *p*-cymene, the sesquiterpene, β -caryophyllene and the two aldehydes, nonanal and decanal; while that of *S. purpurea* was dominated by *p*-cymene, β -caryophyllene and the fatty acid derivatives, decanal, nonanal and ethyl-octanoate.

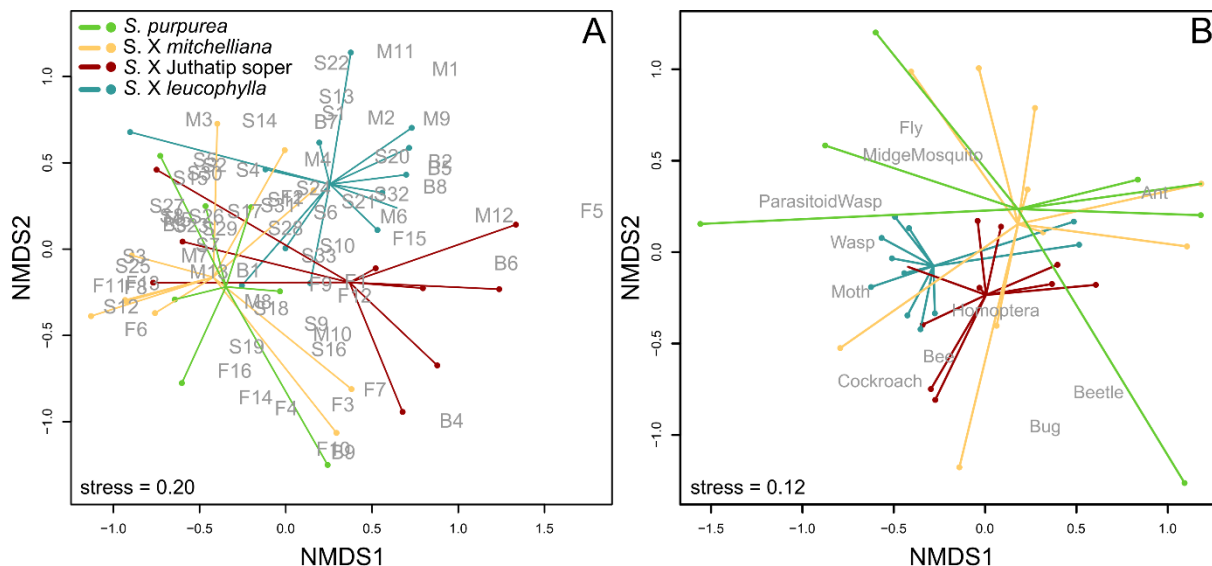


Fig 2. Similarity analyses of odour and prey composition of the *Sarracenia* taxa. Distribution of *Sarracenia* pitchers according to the similarity of their odour (A) and prey (B) spectra in terms of relative amounts of VOCs emitted and prey groups captured, respectively. The non-metric multi-dimensional scaling (NMDS) based on Bray-Curtis dissimilarity index showed a globally significant difference between *Sarracenia* taxa for both odour and prey spectra according to permutational multivariate analyses of variance considering also the effect of plant identity ($P<0.001$ in each case, see S2 Table for pairwise comparisons). The stress value was given to evaluate the quality of the ordination fit to the observed data. The VOCs are referred as Fi, Bi, Mi, Si, where i is the COV number and F, B, M and S refer to Fatty acid derivatives, Benzenoids, Monoterpenes and Sesquiterpenes respectively.

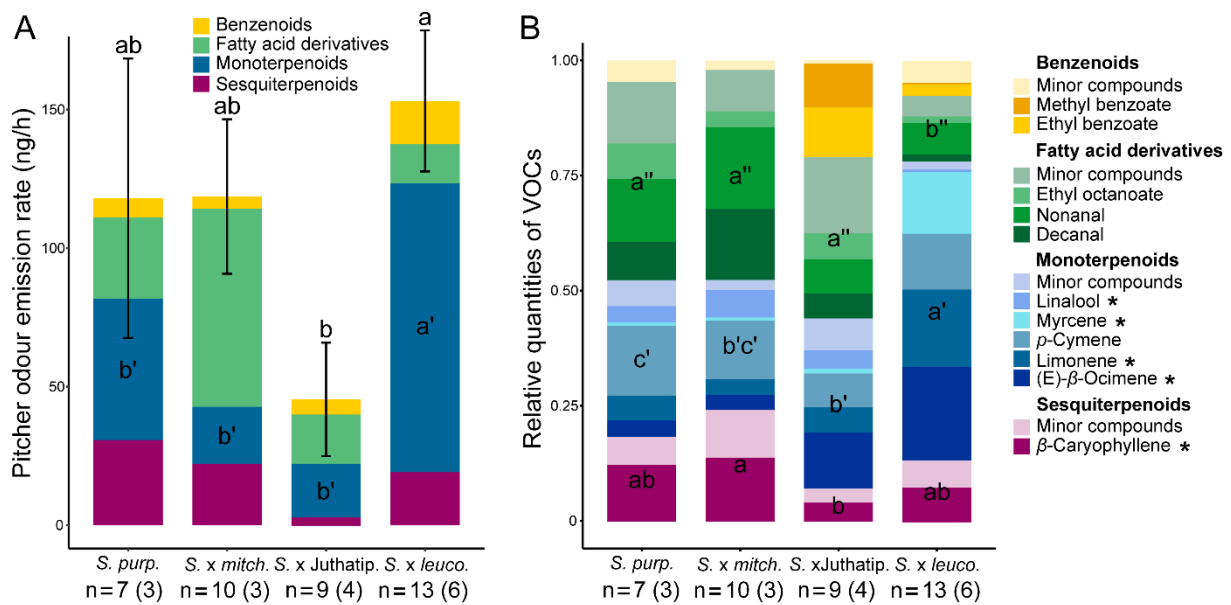


Fig 3. Odour profiles of the *Sarracenia* taxa. Odour profiles of pitchers of the four taxa in terms of emission rates (A) and VOC relative quantities (B). Mean values are presented with their associated standard errors (A). Only major VOCs representing more than 5% of the quantity of the odour bouquet were detailed (B). “*” indicates extremely common compounds in flower scents in Angiosperms (i.e. present in more than half of the seed plant families after Knudsen 2006). Different letters show statistically significant differences ($P < 0.05$) in means between plant taxa for absolute (A) and relative (B) quantities of each class of VOCs according to the mixed linear models considering also the effects of the random variables “Sampling session” and ‘Plant identity” and using pairwise tests with Holm’s correction for multiple comparisons. The number of sampled pitchers is shown with the number of plants in brackets.

Table 1. Result of similarity analyses of odour and prey composition of the *Sarracenia* taxa. Factors of variation for the relative amounts of VOCs emitted (A) and prey trapped (B) by pitchers, from the results of the two PERMANOVAs. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$. Contrasts are detailed in S2 Table.

Dependent variable	(A) VOCs emitted			(B) Prey trapped		
	F	df	P-value	F	df	P-value
Explanatory variables						
Plant taxon	3.94	3	< 0.001 ***	4.90	3	< 0.001 ***
Plant identity	1.84	12	0.002 **	1.60	22	0.035 *

Table 2. Interspecific differences in odour analyses. Factors of variation for (A) the emission rate (sqrt-transformed) of the odour bouquet and those of the different classes of VOCs composing the bouquet and (B) the relative quantities (sqrt-transformed) of each VOC class emitted. Random variables retained in models are presented in italics. “Chisq” values refer to type-III Walds tests for mixed models, “F” values refer to type-III F tests for non-mixed models performed when random variables were non-significant. *: P<0.05, **: P<0.01, and ***: P<0.001.

Dependent variable	(A) Emission rate of VOC classes					(B) Relative quantities of VOC classes					
	Explanatory variables	Chisq	Df	Df resid.	P-value	Explanatory variables	Chisq	F	Df	Df resid.	P-value
Total VOCs	Plant taxon <i>Plant identity</i>	13.53	3	33	0.0036 **						
Benzenoids	Plant taxon <i>Plant identity</i>	5.04	3	33	0.1688	Plant taxon -	2.72	3	35	0.0593	
Fatty acid derivatives	Plant taxon <i>Sampling session</i>	1.29	3	33	0.7307	Plant taxon <i>Sampling session</i>	29.92	3	33	0.0093 **	
Monoterpenes	Plant taxon <i>Sampling session</i>	40.84	3	33	<0.001 ***	Plant taxon <i>Sampling session</i>	41.18	3	33	<0.001 ***	
Sesquiterpenes	Plant taxon <i>Plant identity</i>	8.55	3	33	0.0359 *	Plant taxon <i>Plant identity</i>	11.49	3	33	<0.001 ***	

Interspecific variation in prey capture

Pitchers from the same taxa were grouped together based on the resemblance of their prey spectra (Fig 2B) and the PERMANOVA showed significant differences among taxa in prey composition (Table 1), with a structure of prey resemblance broadly similar to the structure of odour resemblance (Figs 2A and 2B, S2 Table). The prey compositions of *S. X leucophylla* and *S. X Juthatip soper* were significantly different from those of *S. X mitchelliana* and *S. purpurea* (S2 Table) and almost significantly different from each other (P=0.06). *S. X purpurea* and *S. X mitchelliana*, which were grouped together, also both displayed higher intra-specific variance in their prey profile, compared to the other two taxa (Fig 2B, Table 1). The total number of prey individuals significantly differed according to plant taxon even after correction by pitcher length (Table 3A). *S. X leucophylla* and *S. X Juthatip soper* trapped a higher density of prey, especially of flying insects such as dipterans, moths and also bees than *S. X mitchelliana* and *S. purpurea* (Fig 4A, S3 Table). Bees trapped were mostly solitary bees with a clear dominance (92± 5%) of sweat bees (Halictidae). *S. X mitchelliana* trapped higher densities of bees than *S. purpurea* (S3

Table). Moths (especially Noctuidae *sensu lato*) were trapped in even greater densities in *S. X leucophylla* than in *S. X Juthatip soper* (S3 Table). The flies, which composed 84% of the dipterans and of which only 23% could be identified were, for the identified part, mostly composed of Phoridae detritivores (29%), Calliphoridae scavengers (18%), and Syrphidae pollinators (28%). The hoverflies (Syrphidae) were found almost exclusively in the pitchers of *S. X leucophylla* and *S. X Juthatip soper*. By contrast, the densities of ants and beetles did not differ significantly among the four taxa (Table 3). In terms of proportions, the dominant prey groups also changed according to the taxon considered (Table 3B, Fig 4B). While ants and Diptera constituted the two most abundant groups in *S. purpurea* and *S. X mitchelliana*, they reached only 40% in *S. X leucophylla* and *S. X Juthatip soper* (with among these two groups, a dominance of Diptera in *S. X leucophylla* and a dominance of ants in *S. X Juthatip soper*), the remainder being composed mainly of bees (Fig 4B). The share of bees was significantly higher for *S. X leucophylla* and *S. X Juthatip soper* than the other two, *S. purpurea* did not trap any bees (Table 3B, Fig 4B). The share of moths was also the highest for *S. X leucophylla*, the lowest for *S. X mitchelliana* and intermediate for *S. X Juthatip soper* and *S. purpurea* (Table 3B, Fig 4B).

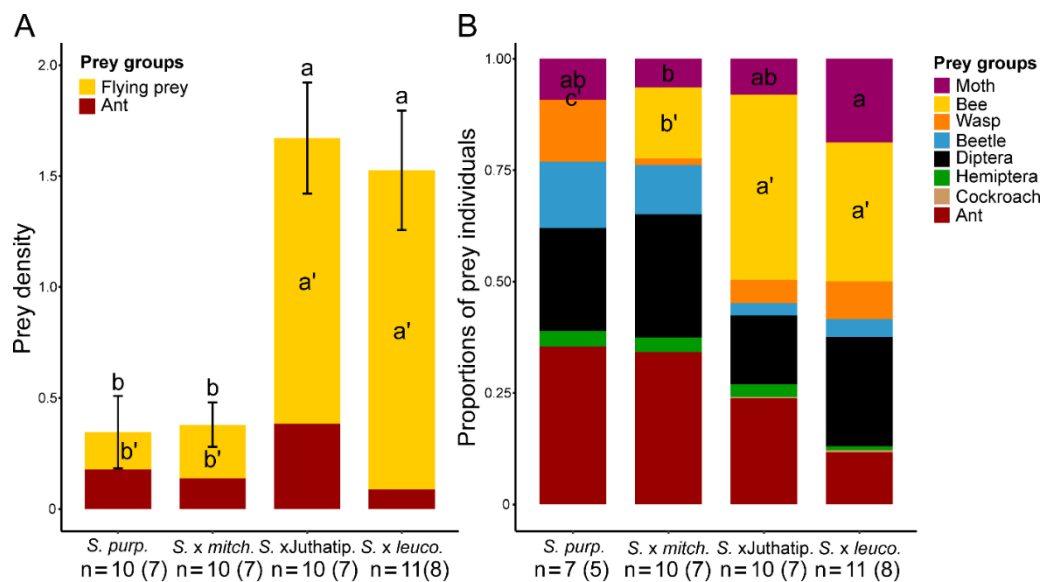


Fig 4. Prey profiles of the four *Sarracenia* taxa. Prey profiles of pitchers of the four taxa in terms of prey densities (Mean prey number/pitcher length \pm SE, A) and relative numbers of individuals captured for each prey groups (B). Different letters show statistically significant differences in means between plant taxa for prey densities and proportions ($P < 0.05$) according to the mixed linear models considering also the effects of the random variables “Sampling date” and ‘Plant identity” and using pairwise tests with Holm’s correction for multiple comparisons. Contrast for prey densities are detailed in S3 Table. Three pitchers of *S. purpurea* did not trap any prey and were therefore excluded from proportion analyses.

Table 3. Interspecific difference in prey captures. Factors of variation in (A) the density, i.e. number of individuals / pitcher length (sqrt-transformed) of the different insect groups trapped by pitchers and (B) the relative quantities (sqrt-transformed) of these groups of prey in terms of number of individuals. Random variables retained in models are presented in italics. “Chisq” values refer to type-III Walds tests for mixed models, “F” values refer to type-III F tests for non-mixed models performed when random variables were non-significant *: P<0.05, **: P<0.01, and ***: P<0.001. Contrasts for prey densities are detailed in S3 Table.

Dependent variable	(A) Densities of prey groups					(B) Proportions of prey groups					
	Explanatory variables	Chisq	Df	Df resid.	P-value	Explanatory variables	Chisq	F	Df	Df resid.	P-value
Total prey	Plant taxon <i>Sampling day</i>	61.55	3	35	<0.001 ***						
Flying prey	Plant taxon <i>Sampling day</i>	101.58	3	35	<0.001 ***						
Ants	Plant taxon <i>Sampling day</i>	6.31	3	35	0.0976	Plant taxon <i>Sampling day</i>	3.22		3	32	0.3590
Bees	Plant taxon <i>Sampling day</i>	191.77	3	35	<0.001 ***	Plant taxon -		21.18	3	34	<0.001 ***
Moths	Plant taxon <i>Sampling day</i>	51.86	3	35	<0.001 ***	Plant taxon <i>Plant identity</i>	12.08		3	32	0.0071 **
Diptera	Plant taxon <i>Sampling day</i>	33.12	3	35	<0.001 ***	Plant taxon <i>Sampling day</i>	0.83		3	32	0.8429
Wasps	Plant taxon <i>Sampling day</i>	21.58	3	35	<0.001 ***	Plant taxon <i>Plant identity</i>	9.19		3	32	0.0269 *
Beetles	Plant taxon <i>Plant identity</i>	2.22	3	35	0.5276	Plant taxon -		0.50	3	34	0.6875

Correlations between odour, morphology and prey capture

According to the CCA (Fig 5A), the first two canonical correlations $r_1 = 0.98$ and $r_2 = 0.76$ were close to one meaning that plant and prey variables were highly correlated. There was a strong positive correlation of axis 1 with pitcher length, monoterpenoid and benzenoid emission rates on the one hand and with the number of flying prey (bees, moths, Diptera and wasps) on the other hand. Furthermore, axis 1 was negatively correlated with the number of ants trapped and the emission rate of fatty acid derivatives. Axis 1 opposed *S. X leucophylla* to the other three taxa (Fig 5B), this comparatively longer-leaf taxon being associated on the one hand with the release of higher amounts of monoterpenoids and benzenoids and fewer amounts of fatty acid derivatives, and on the other hand with more captures of

flying insects and fewer captures of ants. Beetles, among flying insects, were an exception and contributed more to axis 2, which was also positively correlated, but to a lesser extent, with pitcher length and abundance of other prey since all the quantitative insect-variables were localised in the positive section delimited by this axis. Axis 2 separated *S. X mitchelliana* and *S. purpurea* shorter-leaved and less capture-efficient taxa from the two long-leaved *S. X leucophylla* and *S. X Juthatip soper*.

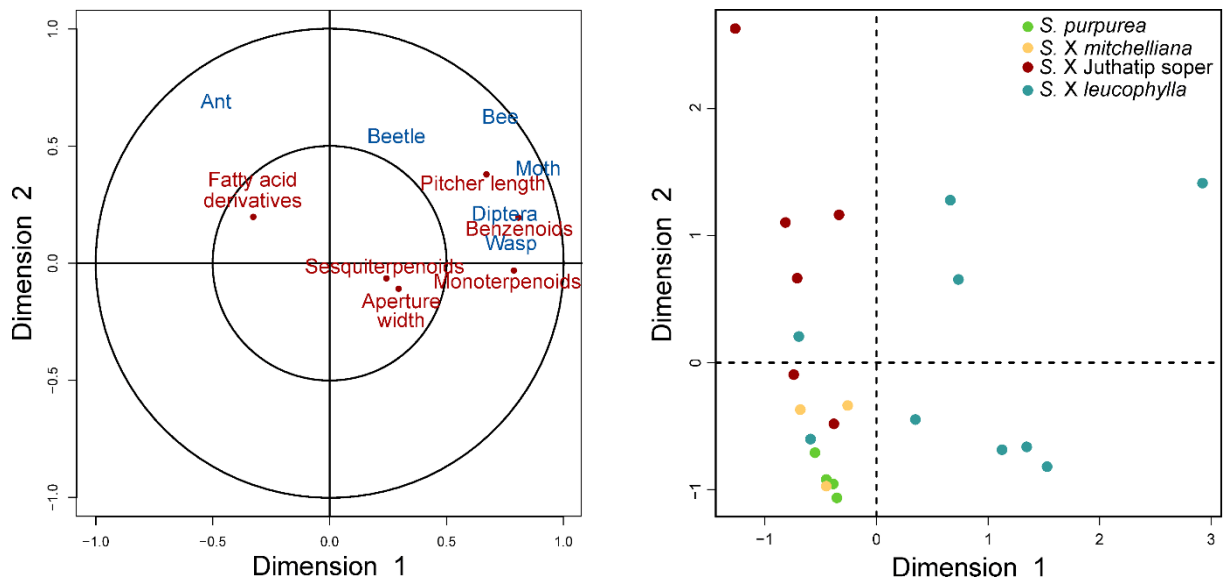


Fig 5. Canonical correlation analysis of plant and prey quantitative variables. Canonical correlation analysis between plant quantitative variables pertaining to odour & morphology and prey quantitative variables pertaining to the different insect groups trapped. (A) Representation of the plant (red) and prey (blue) variables on the correlation circle of the first two canonical axes. (B) Representation of the pitchers on the first two canonical axes.

When considering *S. X leucophylla* only, we also found that both odour and pitcher morphology explained a high part of prey composition, and odour even more than pitcher morphology (Table 4, Fig 6). Regarding pitcher morphology, the number of ants trapped in pitchers decreased when pitcher length increased while the number of bees and moths increased when pitcher length increased (Table 4, Fig 6). As for odours, higher emission rates of fatty acid derivatives were associated with higher captures of ants while higher emission rates of monoterpenoids were associated with higher captures of bees, moths, Diptera and wasps (Table 4, Fig 6). The captures of Diptera and wasps were correlated only to olfactory traits and not to pitcher morphology. Moreover, while sesquiterpene and benzenoid emissions were highly correlated with bee and moth captures (S4 Table), they were not retained by the model because they explained less variation in the captures of bees and moths than did monoterpene emission and

pitcher length variables to which they were also correlated (S4 Table). Conversely, beetle capture was not significantly dependent on either pitcher morphology or pitcher odour traits (Table 4, Fig 6).

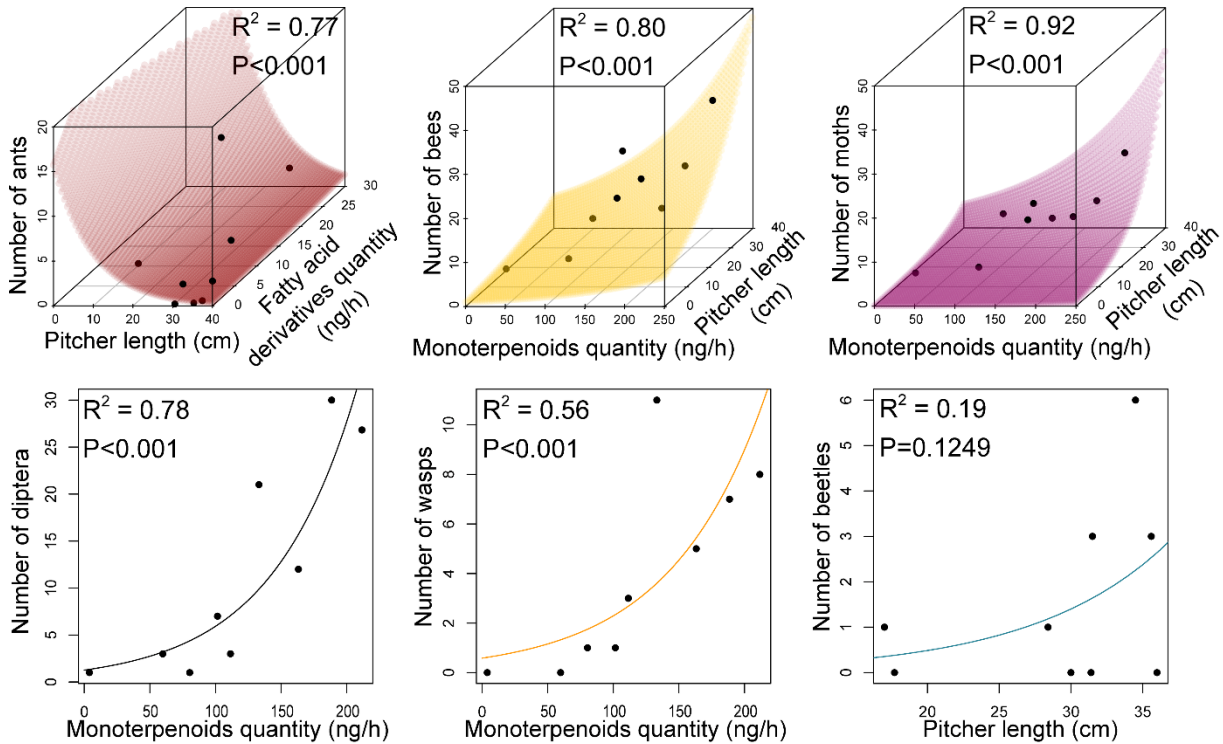


Fig 6. Relationships between prey capture and morphology & odour of *S. X leucophylla* pitchers. Relationships between prey numbers for the main groups of *S. X leucophylla* insect prey and the plant morphological and olfactory quantitative traits that were retained in the multiple Poisson regression models (Table 4). McFadden's pseudo- R^2 ($1 - (\text{residual deviance} / \text{null deviance})$) and overall significance of the models are indicated.

Table 4. Effect of pitcher traits on prey-group captures in *S. X leucophylla*. Effects of the variables retained in the best Poisson multiple regression models to explain variation in the number of ants, bees, moths, Diptera, wasps and beetles trapped in pitchers of *S. X leucophylla*.

Dependent variable	Explanatory variables	Estimate (±S.E.)	P-value	AIC	Null model AIC
Number of ants	Intercept	2.76 (±0.95)	0.0036 **	29.4	45.6
	Pitcher length	-0.10 (±0.03)	0.0034 **		
	Fatty acid derivative quantity	0.05 (±0.02)	0.0232 *		
Number of bees	Intercept	-0.83 (±0.77)	0.2804	55.0	101.1
	Pitcher length	0.08 (±0.03)	0.0029 **		
	Monoterpene quantity	0.01 (±0.02)	<0.001 ***		
Number of moths	Intercept	-2.27 (±1.16)	0.0502	38.8	69.7
	Pitcher length	0.10 (±0.04)	0.0072 **		
	Monoterpene quantity	0.01 (±0.002)	0.0012 **		
Number of diptera	Intercept	0.24 (±0.35)	0.5000	58.3	127.8
	Monoterpene quantity	0.02 (±0.002)	<0.001 ***		
Number of wasps	Intercept	-0.54 (±0.56)	0.3370	42.3	60.6
	Monoterpene quantity	0.01 (±0.003)	<0.001 ***		
Number of beetles	Intercept	-2.84 (±2.00)	0.1569	35.8	38.0
	Pitcher length	0.11 (±0.06)	0.0844		

Discussion

Our results based on a detailed study of the volatile organic compounds and prey individuals of some 40 pitchers of *S. purpurea*, *S. X mitchelliana* and further *S. leucophylla*-backcrossed hybrids show that these pitcher plants are quite generalist in their attraction systems and prey diet. However, despite high intraspecific variability, pitchers display taxon specificities in their scent and prey compositions, with similar inter-taxa differences in both odour and prey composition. We further show that the amounts in the blend of the different classes of volatile organic compounds, and to a lesser extent the length of the pitcher, explain the main differences observed in prey composition. These results show that catches are not random and suggest that odours contribute to control catches.

A generalist scent signal with notable interspecific differences

The studied *Sarracenia* pitcher-shaped leaves release an odour rich in VOCs with a mean emission rate of 46 to 155 ng/h depending on taxon, which is in the range of flower emission rates [1, 52]. Odours released by leaves usually have much narrower spectra and lower emission rates [53, 54]. The bouquets released by the four *Sarracenia* taxa were composed of fatty acid derivatives, benzenoids and terpenoids. Such a composition is more typical of blends of generalist-pollination systems [1, 33, 53], and characterizes also the traps of other carnivorous plants [11, 20]. Having a generalist olfactory signal is certainly advantageous for pitcher plants, which strongly rely on arthropods to obtain the nutrients indispensable to their growth [55]. A generalist scent is expected to increase prey diversification, otherwise the carnivorous plants could experience periods of starvation due to seasonality effects in the availability of specific insects.

In spite of this overall generalist character, some more specific syndromes seem to characterise the scents of the different taxa. *S. X leucophylla*, certainly displays the most flower-like scent of all taxa, because of the intensity of its perfume, and the release in high quantities of very common floral compounds. The monoterpenes, myrcene, (E)- β -ocimene and limonene, which dominated their bouquet, are present in the flower scents of two-thirds of the Angiosperm families [1]. They are often found in flower scents pollinated by bees and/or lepidopterans [31, 33], which actually constituted half of the catches of *S. X leucophylla*. High emission rates of myrcene, a powerful attractant of Noctuidae *s.l.* [56], the predominant group of lepidopteran prey in these *Sarracenia* pitchers, are even a specificity of this taxon. In their study of *S. leucophylla* native species, Jürgens also described a floral scent with abundant monoterpenes but with higher quantity of benzenoids than found in this study [19]. In the field, *S. leucophylla*, being genetically close to *S. X leucophylla* backcrossed three times with *S. leucophylla*, was reported to trap mostly flying insects of large sizes including bees [35], sometimes with a dominance of flies [36], results which are consistent with our own observations on *S. X leucophylla*. It is thus probably a striking example, like *Nepenthes rafflesiana* in Southeastern Asia, of carnivorous plants where pitcher-shaped leaves biochemically “mimic” flowers [20], or at least exploit the sensory biases

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of generalist insect-pollinators [57]. By contrast, the scents of both *S. purpurea* and *S. X mitchelliana*, quite similar to each other, were dominated by fatty acid derivatives, and presented in their odour bouquet some characteristics of the myophile syndrome [33]. Their fatty acid derivatives are mainly the two aldehydes, nonanal and decanal, as well as alkanes and alkenes. While nonanal can effectively be associated with the attraction of carrion flies [58], nonanal and decanal (the latter with a more citrus-smell), are most often herbivore-induced plant volatiles known to attract insect predators [59] or host-seeking parasitoid wasps [60]. Predators and parasitoid compose a significant part of the prey in *S. purpurea* and *S. X mitchelliana* among ants, wasps and beetles. Alkanes and alkenes, which might also come from insects themselves and not exclusively from the plants, are also largely used in chemical communication in ants [61, 62], some alkanes even known to trigger recruitment behaviour [61] in ants being the major prey group of these two plant taxa. Most previous studies on prey capture in *S. purpurea* also report a dominance of ants [21, 42, 43, 63] except some, which report a dominance of flies [64, 65]. The scents of *S. X mitchelliana* and *S. purpurea* also tend to have a greater proportion of sesquiterpenes than the other two plant taxa in their odour bouquets with a predominance of *p*-cymene and β -caryophyllene. These two terpenes are known to attract, in addition to some bees or moths [1], parasitoid wasps in response to herbivore attacks [66, 67]. β -caryophyllene is also a common sesquiterpene with a spicy note that, when associated with fatty acid derivatives, attracts saprophagous and/or carrion flies [33], two also abundant groups of flies found in the pitchers of these two taxa. Furthermore, *p*-cymene and β -caryophyllene were two compounds particularly abundant in starving plants of the Venus flytrap [11], which as its name suggests captures mainly flies. As for *S. X Juthatip soper*, this taxon presents an olfactory syndrome halfway between the generalist-flower syndrome of *S. X leucophylla* and the fly & ant syndrome of *S. purpurea* and *S. X mitchelliana*. Indeed, its odour is less strong than that of *S. X leucophylla* and thereby less typical of flowers, but it keeps important shares of benzenoids, monoterpenes and fatty acid derivatives, which could still contribute to the attraction of flower visitors such as bees and moths, in addition to ants and Diptera. However, two dominant benzenoids in this taxa were methyl benzoate and ethyl benzoate, two compounds more commonly found in fermented fruits and known to attract fruit flies such as Drosophilidae [68] or Tephritidae [69], making its odour thus more fruity than floral.

Prey variability globally mirrors scent variability at both intra and interspecific levels

The NMDS analysis shows that pitcher scents from the same taxon are overall more similar than pitcher scents from different taxa. However, it also illustrates a rather high variability in scent composition within the same taxon. In their meta-analysis, Delle-Vedove *et al.* highlighted the importance of intraspecific variation in floral scent in Angiosperms [70], where individual genetics, flower ontogeny, climatic parameters, interactions with insects are factors that can affect floral scent. Similar factors may influence pitcher scent. Part of the observed “within” variation might indeed reflect differences among individuals or among days or timings of odour sampling, as suggested by our analyses, which often retained the random variables "Plant identity" and "Sampling session" in the models on the relative amounts of the different classes of compounds. For example, the influence of temperature and humidity on monoterpene emission [71] might explain the effect of the session of scent sampling on this compound class. The observed intra-specific variation may also reflect a variation linked to pitcher age or physiology. Indeed, the traps are likely to release their odour only when they need to feed and therefore reduce and/or change it after substantial captures, during the digestive process. Supporting this, is the report of a reduction of terpene emission in fed-traps compared to unfed-traps in an experiment carried out on the famous Venus fly-trap, *Dionaea* [11].

Furthermore, the NMDS graphs also show that the interspecific variation in prey composition roughly reflects that in scents. Indeed, in both ordinations (i.e. based on scent and prey profiles), the centroids of *S. X purpurea* and *S. X mitchelliana* are almost superposed, and those of *S. X leucophylla* and *S. X Juthatip* are closer to each other than to the other two. While *S. X purpurea* and *S. X mitchelliana* are closer to fatty acid derivatives and sesquiterpenes on the one hand, and closer to ants and flies on the other hand, *S. X leucophylla* and *S. X Juthatip* are closer to benzenoid and monoterpene compounds on the one hand and closer to flower visitors on the other. Interestingly, the distribution of taxa according to their odour and prey profiles also reflects their degrees of relatedness, including similar structures of odour and prey dissimilarities among taxa (Fig 2). This supports both the hypothesis of a genetic control of their odour patterns or at least their biosynthetic pathways and the

hypothesis that odour ‘selects’ for prey. In other words, the horticultural hybrids would share the olfactory characteristics of their parents and the pitcher plants would exploit the sensory biases of insect groups in the same manner as their native counterparts and/or closest relatives, the sensory characteristics of which would have originally evolved to adapt to the ecological constraints and prey availability of their native environments.

At the intra-specific level, the highest variability in NMDS prey profile observed in *S. purpurea* and *S. X mitchelliana* is somehow also observed in the NMDS scent profile, suggesting that prey differences also mirror odour differences at the intraspecific level, but the variability is even higher for prey in these two taxa. High unevenness in prey capture was also reported in the field for *S. purpurea* [64]. An explanation, in addition to the scent-variability hypothesis, would be that capture is maybe less efficient in the shorter-leaved *S. purpurea* and *S. X mitchelliana* than in *S. X leucophylla* and *S. X Juthatip soper*, that would conversely capture more efficiently the insects which they attract. This latter hypothesis is supported by two reports. Newell and Nastase reported high escape rates in the short-leaved *S. purpurea* [42], while on the contrary, in the native and long-leaved *S. leucophylla*, the escape rates were very low [72]. The strong correlation between the quantities of compound classes and prey groups that we find for the longest-leaved *S. X leucophylla* further argue for a stronger link between prey and odour for pitchers of longer sizes. It also highlights the existence in carnivorous plants of different traits linked to attraction and trapping that play in concert to form trapping syndromes, as proposed by Juniper et al. [13] and further evidenced in *Nepenthes* genus [17].

An overlooked but potentially important role of odours in the diet composition of pitcher plants

Of the signals involved in communication, odour is probably the most cryptic to humans, who primarily use their visual sense [73] and it may thus have been largely overlooked. In addition, odour is often correlated with other plant characteristics, making it difficult to disentangle its effect from others. Furthermore, it cannot be precisely described without a technological tool, i.e. the GC-MS. Its effect may also be not easily pointed out because different compounds that form the blend can have opposite

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effects on different guilds of insects. *Sarracenia* plants are not spared from these issues. The positive correlation revealed in this study between certain odour components such as the amounts of benzenoids and terpenes with pitcher length (S4 Table) could explain why the effect of odour could have remained unnoticed. Indeed, pitcher morphology, a set of traits easier to measure, was often shown to be “the” driver of prey abundance. Thus, some authors have already shown a correlation between pitcher length and prey amount in different *Sarracenia* species (Cresswell [65], Heard [43] in *S. purpurea*, Bhattarai and Horner [16] Green and Horner [74] in *Sarracenia alata* and Sheridan [40] in *S. leucophylla*). However, only Bhattarai and Horner obtained data, which let them suppose that this positive correlation was also due to “a greater quantity of attractants produced by larger pitchers”. Yet these authors did not test for any difference linked to insect guilds and/or taxonomy. Only Gibson [35] reported in *S. leucophylla* a positive correlation between pitcher size and prey number only for flying insects, which corroborates our own results.

An experimental approach involving olfactory tests is needed to get definitive proofs that odours are initial drivers of prey composition. Moreover, we cannot rule out the hypothesis according to which the observed positive associations between odour and prey patterns might be an artefact resulting from the process of human hybridization of two of these taxa. However, a number of arguments does not support the hypothesis that these patterns were observed by chance. First, the observed odour profiles seem to roughly reflect the genetics of these plants, chosen because they formed a gradient of relatedness from *S. purpurea* towards *S. leucophylla*. Indeed, their odours concomitantly show an increasing gradient in the relative amount of monoterpenes commonly found in flowers and a decreasing gradient in the relative amount of fatty acid derivatives. Second, it was found that the natural species *S. purpurea* and *S. leucophylla* emit mainly fatty acid derivatives for the former and floral compounds for the latter [19], exactly as we find for *S. purpurea* and *S. X leucophylla*, the taxon the most related to *S. leucophylla*. Next, we similarly find that ants are the main prey of *S. purpurea* as observed in its native environment and that flying insects are the main prey of *S. X leucophylla*, exactly like its most closely related natural species, *S. leucophylla* [36, 72]. Moreover, the high strength of the observed correlations does not reflect the intervention of chance either. The canonical correlation analysis carried out on the four taxa shows

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that quantities of the different prey groups can be inferred 98% from quantities of the odour classes and dimensions of their pitchers. Taking pitcher size into account, the CCA illustrates how the number of ants is then positively correlated with the quantity of fatty acid derivatives and negatively correlated with the quantity of monoterpenes and benzenoids. Conversely, the number of flying insects is positively correlated with the quantity of monoterpenes and benzenoids. Moreover, this analysis shows a spatial separation of the taxa according to the first two canonical axes. The two axes separate from each other *S. X leucophylla*, *S. X Juthatip soper*, and the remaining couple, *S. purpurea* and *S. X mitchelliana*. These results are confirmed at the intra-specific level in *S. X leucophylla*, for which these odour components explain systematically a higher part of the variation in prey captures than does pitcher size, for each prey group separately. They also confirm the correlation between ant number and fatty acid derivative quantity, and show that the quantity of monoterpenes, among those of benzenoids and sesquiterpenes, which are mutually correlated, is the key odour component explaining variation of the different groups of flying prey tested except beetles, for which no significant correlation is found. While the negative correlation with pitcher size of ants, ground-dwelling insects, is easier to understand, the positive correlation of flying insects may be explained either by a greater attraction because taller pitchers are probably more conspicuous to flying insects, or by a greater retention of flying insects in taller and usually slender pitchers (personal observations CD, DG, LG, TH).

The positive correlation of ants with fatty acid derivatives is not surprising since ants use some of them in their chemical communication for nestmate recognition [62] or as pheromones [61] and since they are often released by plants, which attract ants as bodyguards [75]. Interestingly, the lower pitchers of the vine *Nepenthes rafflesiana*, which trap mostly ants release greater percentages of fatty acid derivatives than the upper pitchers, which target flying insects, mostly flower visitors, with a blend rich in benzenoids and monoterpenes, thus presenting a compelling similarity with *S. X leucophylla* [20]. Bees and moths, important pollinators, are indeed known to be attracted by monoterpenes and benzenoids, which are typical flower components [31, 33, 76]. Ants constitute only a small proportion ($12\% \pm 7$) of the prey in *S. X leucophylla* and their abundance turns to be negatively correlated with the quantity of monoterpenes in the CCA analysis including all taxa. Whether this is because the share of fatty acid derivatives is significantly lower in the scent of this taxon or because ants are repelled by a

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high abundance of typical flower compounds such as limonene (the second most abundant compound in this taxon) as suggested by an earlier study [77] remains to be clarified. Gibson reports that the native *S. leucophylla* captures some small insects, but collectively these contribute little to the total catch biomass, to which many larger insects contribute much more [35, 72]. This suggests that ants constitute also a small portion of its prey. Resource partitioning between *Sarracenia* species is likely to occur in the bogs of southeastern USA where competition has been shown to be high between *S. leucophylla* and other *Sarracenia* species [35]. However, Ellison and Gotelli did not find strong evidence of resource partitioning among five *Sarracenia* species living in sympatry because they turned to trap mostly ants [41]. But in the *Sarracenia* community considered, several long-leaved species were absent, such as *S. alata*, *S. alabamensis*, *S. oreophila* and *S. minor* tall form, which are though described by Gibson to produce attractive odours” and to live in sympatry with *S. leucophylla*. Ants are ubiquitous and may thus constitute a rather reliable source of food for pitcher plants, but the carnivorous plants are more likely to compete for bigger prey, which are more nutritive but also rarer, as suggested by Darwin [78] and pointed out by Gibson [35]. Analysis of the prey composition and odour pattern of these species would certainly help to better understand the evolutionary ecology of these carnivorous plants.

The question as to why *S. X Juthatip soper*, which emits fewer VOCs than *S. X leucophylla*, still attract as many flying insects, especially bees as *S. X leucophylla*, warrants further exploration. The stronger share of fatty acid derivatives in its blend associated to an equivalent share in benzenoids than *S. X leucophylla* could be an explanation. Indeed, Kantsa *et al.* in their meta-analysis reported the importance of the percentage of aliphatic for bee attraction [79]. But something else must also be at play. Other traits like colour signal is also an important aspect of attraction in the trapping syndrome of carnivorous plants [13]. In particular, pitcher red coloration has been proposed to play a role in prey attraction in *Sarracenia* pitcher plants [24, 42]. However, this hypothesis has been refuted by some authors [21, 40] arguing that it is not easy to disentangle the contribution of the different features since they are often combined to form whole trapping syndromes, with many traits targeting specific guilds of animals or characterising specific diets [17, 80].

In conclusion, our study shows that the odours of *Sarracenia* pitchers are rich in VOCs, combining compounds from three biosynthetic pathways, with the pitchers of *S. X mitchelliana* and *S. purpurea* having an odour dominated by fatty acid derivatives, and the pitchers of the two taxa genetically closest to *S. leucophylla* having the most diverse and floral odours. This confirms the trends observed in the natural *S. purpurea* on the one hand and *S. leucophylla* on the other hand. Our study further brings together a set of presumptions that, in addition to pitcher morphology, olfactory cues play an important role in the diet composition of *Sarracenia* pitcher plants with monoterpenes and benzenoids associated with flying insects, and fatty acid derivatives more specifically associated with ants. Such trends observed in a non-native environment on *S. purpurea* and *S. leucophylla* backcrossed hybrids, which confirm the insect trends observed on *S. purpurea* and *S. leucophylla* native species in their own environments, provide support to the perceptual exploitation hypothesis [81]. Carnivorous plants would thus exploit insect sensory biases, i.e. general sensory preferences related to insect guild and/or taxonomy, a hypothesis, which contrary to what mimicry *sensu stricto* implies, does not rely on consistent spatio-temporal association between species [82]. Experimental studies on native species in their own environment and integrating different traits together, including visual ones, would certainly clarify and complement the different trapping syndromes of these carnivorous plants and shed light on the eco-evolutionary mechanisms underlying them.

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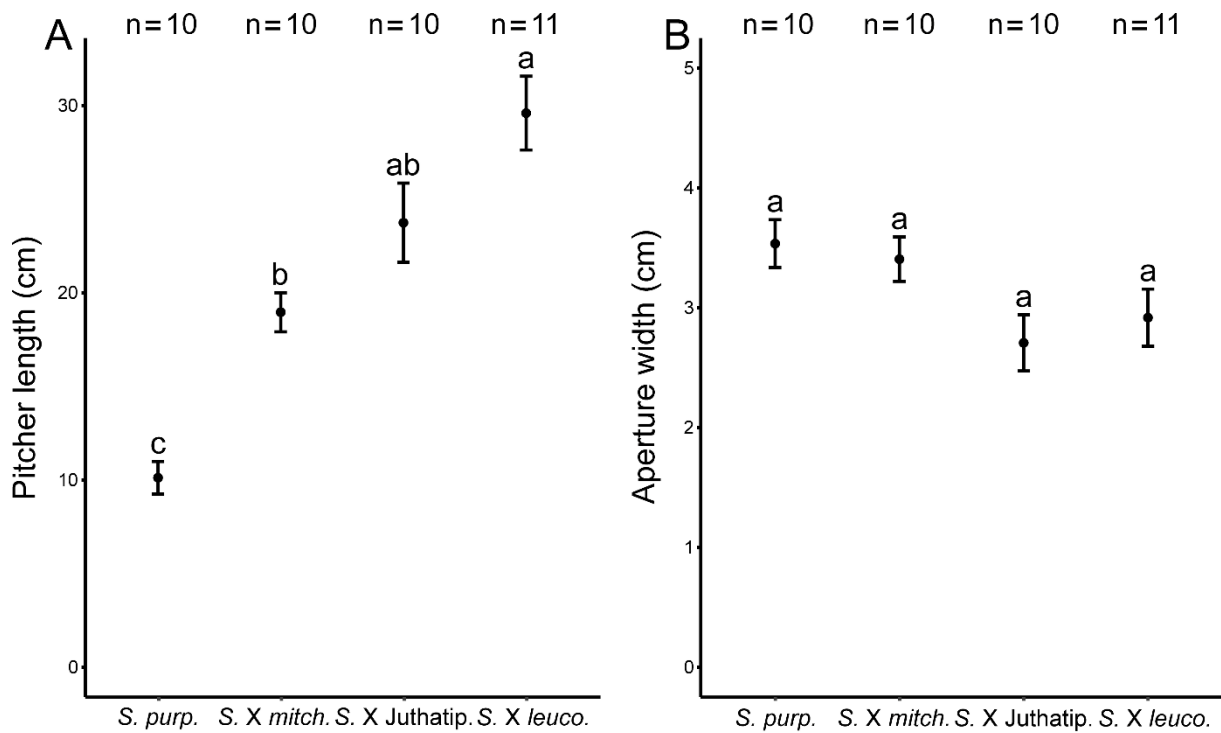
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Supporting information



S1 Fig. Variation of morphology among taxa. Pitcher length (A) and aperture width (B) in the four *Sarracenia* taxa. Mean values are presented with their associated standard errors. Different letters show statistically significant differences in means between plant taxa ($P < 0.05$).

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S1 Table. VOC composition of *Sarracenia taxa*. Mean relative amounts (% \pm SE) of VOCs in the odour bouquet of the four *Sarracenia taxa*. VOCs are listed according to their class and calculated Linear Retention Indices (LRI). Mass fragments for unknown compounds were listed with the molecular ion first, followed by the base peak and other fragments in order of decreasing abundance. IA : Identification Attempt, * : identified with comparison with synthetic standards. Occurrence (Occ.) refers to the proportion of pitchers in which the VOC was found.

			<i>S. purpurea</i>	<i>S. X mitchelliana</i>	<i>S. X Juthatip soper</i>	<i>S. X leucophylla</i>	
Number of individuals sampled			7	10	9	13	
Total number of compounds			47	56	41	65	
Mean quantity of scent emitted (ng/h)			118.01 (± 51.14)	118.63 (± 28.42)	45.94 (± 20.90)	154.64 (± 26.88)	Occ. (%)
Code	FATTY ACID DERIVATIVES	LRI					
F1	Oct-1-ene	794	0.18 (± 0.16)	0.05 (± 0.03)	0.32 (± 0.20)	0.22 (± 0.17)	56
F2	Heptanal	896	0.05 (± 0.05)	0.07 (± 0.07)		0.20 (± 0.13)	23
F3	Oct-1-en-3-ol*	981	4.06 (± 1.88)	1.48 (± 1.22)	2.64 (± 1.76)	0.61 (± 0.33)	51
F4	Octan-2-one	988	0.09 (± 0.09)	0.63 (± 0.47)			13
F5	Dec-1-ene	991			4.82 (± 4.82)		3
F6	Octanal	1006	4.82 (± 3.03)	1.48 (± 0.53)	1.92 (± 1.06)	0.29 (± 0.16)	51
F7	Undec-1-ene	1092	1.95 (± 1.76)	0.48 (± 0.48)	1.91 (± 0.83)	0.56 (± 0.36)	26
F8	Nonanal*	1108	13.66 (± 3.69)	17.77 (± 5.90)	7.41 (± 3.84)	6.89 (± 3.19)	51
F9	Dodec-1-ene	1192		2.44 (± 0.72)	1.29 (± 0.68)	0.28 (± 0.14)	49
F10	Ethyl-octanoate	1196	7.67 (± 5.13)	3.45 (± 1.89)	5.63 (± 3.80)	1.37 (± 0.78)	41
F11	Decanal*	1208	8.31 (± 4.32)	15.38 (± 5.09)	5.41 (± 3.16)	1.47 (± 0.77)	46
F12	Tridec-1-ene	1289	0.89 (± 0.42)	1.03 (± 0.41)	3.03 (± 0.90)	0.53 (± 0.20)	77
F13	Undecanal	1310	0.12 (± 0.05)	0.67 (± 0.28)	0.14 (± 0.10)	0.27 (± 0.18)	33
F14	Tetradec-1-ene	1388	0.54 (± 0.44)	0.11 (± 0.05)	0.09 (± 0.06)	0.10 (± 0.09)	36
F15	(Z)-Jasmone	1402		0.19 (± 0.14)	0.32 (± 0.32)	1.09 (± 0.66)	23
F16	Dodecanal	1406	0.63 (± 0.53)	0.31 (± 0.15)	0.09 (± 0.07)	0.34 (± 0.28)	33
BENZENOIDS							
B1	Benzaldehyde*	971		0.11 (± 0.11)		0.28 (± 0.28)	5
B2	Benzyl alcohol	1042				0.46 (± 0.30)	8
B3	Acetophenone	1072	3.51 (± 2.06)	1.72 (± 1.18)	0.49 (± 0.49)	0.45 (± 0.45)	18
B4	Methyl benzoate	1102			9.39 (± 5.55)	0.37 (± 0.37)	15
B5	Phenylethanol*	1121				1.03 (± 0.73)	5
B6	Ethyl benzoate	1178			10.86 (± 7.29)	2.49 (± 1.11)	18
B7	Methyl salicylate*	1202	0.56 (± 0.36)			2.34 (± 1.23)	23
B8	Isopropyl benzoate	1212				0.03 (± 0.03)	3
B9	Isobutyl Benzoate	1337	0.59 (± 0.59)	0.21 (± 0.14)	0.23 (± 0.15)	0.05 (± 0.05)	15
MONOTERPENOIDS							
M1	Sabinene	978				0.23 (± 0.17)	15
M2	Myrcene*	991	0.75 (± 0.29)	0.66 (± 0.44)	1.11 (± 0.66)	13.45 (± 2.95)	56

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M3	<i>p</i> -Cymene*	1030	15.13 (±6.87)	12.75 (±6.18)	7.33 (±6.31)	12.07 (±6.49)	41
M4	Limonene*	1035	5.41 (±1.59)	3.41 (±1.60)	5.50 (±2.62)	16.81 (±3.13)	74
M5	(<i>Z</i>)- β -Ocimene*	1036	0.01 (±0.01)		2.18 (±2.17)	0.20 (±0.08)	28
M6	(<i>E</i>)- β -Ocimene*	1048	3.58 (±1.43)	3.27 (±1.21)	12.14 (±3.31)	20.25 (±3.57)	72
M7	(<i>Z</i>)-Linalool oxide (furanoid form)	1076	0.53 (±0.38)	1.48 (±0.61)	0.62 (±0.47)	0.03 (±0.02)	33
M8	(<i>E</i>)-Linalool oxide (furanoid form)	1091	4.87 (±2.92)		1.83 (±1.33)		13
M9	Terpinolene	1091				0.24 (±0.17)	5
M10	Linalool*	1102	3.52 (±1.71)	5.96 (±2.03)	3.92 (±2.29)	0.48 (±0.22)	67
M11	allo-Ocimene	1131				0.32 (±0.13)	13
M12	(<i>E,E</i>)-Cosmene	1135	0.13 (±0.13)	0.51 (±0.30)	2.18 (±1.76)	0.66 (±0.19)	36
M13	(<i>E</i>)-Linalool oxide (pyranoid form)	1179	0.09 (±0.08)	0.16 (±0.05)	0.08 (±0.06)	0.12 (±0.08)	36
SESQUITERPENOIDS							
S1	α -Ylangene	1374		0.34 (±0.20)	0.48 (±0.30)	0.67 (±0.53)	28
S2	α -Santalene	1381	0.18 (±0.09)		0.04 (±0.04)	0.17 (±0.08)	23
S3	Cyclosativene	1385		0.27 (±0.19)		0.05 (±0.05)	10
S4	α -Copaene*	1389	0.26 (±0.24)	0.26 (±0.14)	0.02 (±0.02)	0.12 (±0.06)	33
S5	β -Bourbonene	1398		0.04 (±0.04)		0.03 (±0.03)	8
S6	isoCaryophyllene ^{IA}	1420	0.05 (±0.05)	0.06 (±0.06)		0.01 (±0.01)	8
S7	(<i>Z</i>)- α -Bergamotene	1423	0.00 (±0.00)	0.02 (±0.02)	0.04 (±0.04)	0.02 (±0.02)	10
S8	β -Ylangene	1434		0.35 (±0.23)		0.12 (±0.10)	10
S9	β -Caryophyllene*	1437	12.28 (±5.47)	13.93 (±5.19)	3.57 (±2.44)	7.51 (±1.85)	79
S10	(<i>E</i>)- β -Bergamotene	1439	0.02 (±0.02)	1.49 (±0.59)	2.13 (±0.81)	0.01 (±0.01)	36
S11	β -Copaene	1442	0.02 (±0.02)	0.20 (±0.12)		0.24 (±0.15)	21
S12	Geranylacetone*	1451	1.85 (±1.62)	0.75 (±0.30)	0.33 (±0.28)	0.03 (±0.03)	41
S13	(<i>E</i>)- β -Farnesene	1456		0.28 (±0.28)		0.09 (±0.09)	5
S14	unknown Sesquiterpene 1 (91, 105, 161, 119, 81, 204)	1464		0.18 (±0.18)		0.21 (±0.17)	10
S15	ϵ -Muuroolene	1467		0.06 (±0.06)		0.03 (±0.03)	5
S16	α -Humulene*	1473	0.18 (±0.18)	0.14 (±0.14)		0.10 (±0.06)	13
S17	γ -Muuroolene unknown Sesquiterpene 2	1488		0.42 (±0.30)			5
S18	(93, 119, 105, 69, 161, 204)	1493	1.76 (±1.23)	0.07 (±0.07)		1.23 (±0.52)	33
S19	Germacrene D*	1498		1.42 (±0.71)		0.10 (±0.08)	26
S20	γ -Amorphene	1504		0.11 (±0.11)		0.12 (±0.09)	8
S21	(<i>E,E</i>)- α -Farnesene	1507	0.38 (±0.26)	0.05 (±0.05)	0.11 (±0.11)	0.45 (±0.17)	26
S22	β -Curcumene	1509	0.01 (±0.01)	0.10 (±0.07)		0.05 (±0.05)	13
S23	α -Muuroolene	1511	0.04 (±0.04)	0.08 (±0.05)	0.01 (±0.01)	0.01 (±0.00)	18
S24	β -Bisabolene	1516	0.02 (±0.02)	0.06 (±0.06)			5

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S25	γ -Cadinene	1528	0.15 (\pm 0.15)	0.36 (\pm 0.20)		0.04 (\pm 0.03)	15
S26	δ -Cadinene	1530	0.15 (\pm 0.15)	0.79 (\pm 0.38)		0.03 (\pm 0.03)	23
S27	(Z)-Calamenene	1534	0.14 (\pm 0.14)	0.24 (\pm 0.13)	0.01 (\pm 0.01)	0.02 (\pm 0.02)	15
S28	(E)-Cadina-1,4-diene	1548	0.14 (\pm 0.08)	0.21 (\pm 0.13)	0.05 (\pm 0.05)	0.29 (\pm 0.14)	41
S29	α -Cadinene	1552		0.11 (\pm 0.07)			5
S30	α -Calacorene	1558		1.75 (\pm 0.67)	0.27 (\pm 0.21)	0.30 (\pm 0.22)	28
S31	(E)-Nerolidol*	1566	0.10 (\pm 0.07)	0.09 (\pm 0.07)	0.05 (\pm 0.04)	0.08 (\pm 0.04)	38
S32	Caryophyllene oxide	1575	0.01 (\pm 0.01)			0.01 (\pm 0.01)	5
S33	Hydroxysesquicineole ^{IA}	1598	0.63 (\pm 0.43)			1.28 (\pm 0.74)	18

S2 Table. Post-hoc tests of similarity analyses in odour and prey composition of the different *Sarracenia* taxa. Results of the pairwise comparisons between taxa associated to the permutational multivariate analyses of variance on the relative amounts of VOCs emitted (A) and prey group trapped (B) by pitchers (Fig 1). P-values were adjusted for multiple comparisons with the Holm's correction, *: P<0.05, **: P<0.01, and ***: P<0.001. The plant effect ('Plant identity' nested within 'Plant taxon') was also accounted for.

Dependent variable	Explanatory variables	A) VOC emitted			(B) Prey trapped		
		F	Df	adjusted P-value	F	Df	adjusted P-value
<i>S. purpurea</i> vs <i>S. X leucophylla</i>	Plant taxon	5.68	1	0.002 **	6.41	1	0.004 **
	Plant identity	1.56	7	0.050 *	1.29	9	0.242
<i>S. purpurea</i> vs <i>S. X Juthatip soper</i>	Plant taxon	1.82	1	0.100	6.16	1	< 0.001 ***
	Plant identity	1.26	5	0.168	1.41	10	0.206
<i>S. purpurea</i> vs <i>S. X mitchelliana</i>	Plant taxon	1.46	1	0.199	1.28	1	0.299
	Plant identity	3.37	4	0.002 **	1.40	11	0.146
<i>S. X mitchelliana</i> vs <i>S. X leucophylla</i>	Plant taxon	9.26	1	< 0.001 ***	7.13	1	0.002 **
	Plant identity	2.54	7	< 0.001 ***	1.79	12	0.090
<i>S. X mitchelliana</i> vs <i>S. X Juthatip soper</i>	Plant taxon	2.69	1	0.032 *	6.70	1	0.004 **
	Plant identity	2.24	5	0.005 **	2.58	13	0.016 *
<i>S. X Juthatip soper</i> vs <i>S. X leucophylla</i>	Plant taxon	3.30	1	0.002 **	2.84	1	0.064
	Plant identity	1.19	8	0.202	1.20	11	0.276

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S3 Table. Post-hoc tests for prey density analyses according to taxa. Results of the pairwise comparisons between taxa associated to the models carried out to explain the variation in the sqrt-transformed density (number of individuals / pitcher length) of the different insect groups trapped by pitchers. Contrasts were tested between plant taxa using pairwise tests. P-values were adjusted for multiple comparisons with the Holm's correction.

Dependent variable	Plant taxon	Estimate (\pm SE)	Df	Contrasts
Density of Total prey	<i>S. purpurea</i>	0.30 (\pm 0.16)	18.1	b
	<i>S. X mitchelliana</i>	0.48 (\pm 0.16)	17.9	b
	<i>S. X Juthatip soper</i>	1.16 (\pm 0.16)	17.9	a
	<i>S. X leucophylla</i>	1.16 (\pm 0.16)	15.5	a
Flying prey	<i>S. purpurea</i>	0.09 (\pm 0.16)	10.6	b
	<i>S. X mitchelliana</i>	0.32 (\pm 0.17)	11	b
	<i>S. X Juthatip soper</i>	0.96 (\pm 0.17)	11	a
	<i>S. X leucophylla</i>	1.10 (\pm 0.17)	10.2	a
Ants	<i>S. purpurea</i>	0.22 (\pm 0.12)	23.1	a
	<i>S. X mitchelliana</i>	0.29 (\pm 0.12)	27.2	a
	<i>S. X Juthatip soper</i>	0.50 (\pm 0.12)	27.2	a
	<i>S. X leucophylla</i>	0.21 (\pm 0.11)	21.2	a
Bees	<i>S. purpurea</i>	-0.12 (\pm 0.10)	11.4	c
	<i>S. X mitchelliana</i>	0.15 (\pm 0.11)	11.8	b
	<i>S. X Juthatip soper</i>	0.77 (\pm 0.11)	11.8	a
	<i>S. X leucophylla</i>	0.70 (\pm 0.11)	10.8	a
Moths	<i>S. purpurea</i>	-0.01 (\pm 0.08)	17.5	c
	<i>S. X mitchelliana</i>	0.05 (\pm 0.08)	17.1	c
	<i>S. X Juthatip soper</i>	0.29 (\pm 0.08)	17.1	b
	<i>S. X leucophylla</i>	0.48 (\pm 0.08)	14.8	a
Diptera	<i>S. purpurea</i>	0.03 (\pm 0.12)	11.9	b
	<i>S. X mitchelliana</i>	0.14 (\pm 0.12)	12	b
	<i>S. X Juthatip soper</i>	0.37 (\pm 0.12)	12	a
	<i>S. X leucophylla</i>	0.52 (\pm 0.11)	10.8	a
Wasps	<i>S. purpurea</i>	0.08 (\pm 0.08)	20	b
	<i>S. X mitchelliana</i>	-0.02 (\pm 0.08)	19.5	b
	<i>S. X Juthatip soper</i>	0.17 (\pm 0.08)	19.5	ab
	<i>S. X leucophylla</i>	0.32 (\pm 0.08)	16.6	a
Beetles	<i>S. purpurea</i>	0.07 (\pm 0.05)	20.7	a
	<i>S. X mitchelliana</i>	0.14 (\pm 0.06)	19.7	a
	<i>S. X Juthatip soper</i>	0.13 (\pm 0.06)	19.7	a
	<i>S. X leucophylla</i>	0.15 (\pm 0.05)	15.1	a

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S4 Table. Correlation between odour, morphology and prey capture parameters in *S. X leucophylla*. Correlation table relative to odour variables (emission rate of fatty acid derivatives, benzenoids, monoterpenoids and sesquiterpenoids), morphology variables (pitcher length and aperture width) and number of ants, bees, moths, Diptera, wasps and beetles trapped in pitchers of *S. X leucophylla*. The correlation coefficients between each pair of variables estimated using the Pearson method, are presented with their associated P-values, *: P<0.05, **: P<0.01, and ***: P<0.001.

Variables	Fatty. emission rate	Benz. emission rate	Mono. emission rate	Sesqui. emission rate	Pitcher length	Aperture width
Benzenoids emission rate	0.02					
Monoterpenoids emission rate	-0.04	0.78*				
Sesquiterpenoids emission rate	-0.20	0.86**	0.57			
Pitcher length	-0.43	0.45	0.58	0.52		
Aperture width	-0.29	0.69*	0.67*	0.66	0.92***	
Number of ants	0.69*	-0.22	-0.28	-0.32	-0.78*	-0.71*
Number of bees	-0.17	0.84**	0.80**	0.86**	0.66	0.75*
Number of Diptera	-0.26	0.75*	0.86**	0.53	0.51	0.67*
Number of moths	-0.17	0.91***	0.85**	0.89**	0.72*	0.85**
Number of wasps	-0.18	0.67*	0.77*	0.35	0.40	0.52
Number of beetles	-0.37	0.18	0.11	0.49	0.39	0.27

S5 Table. Effect of pitcher traits on prey-group captures in *S. X leucophylla*: second-best model.

Effects of the variables retained in the second-best Poisson multiple regression model to explain variation in the number of ants, bees, moths, Diptera, wasps and beetles trapped in pitchers of *S. X leucophylla*.

Dependent variable	Explanatory variables	Estimate (±S.E.)	P-value	AIC	Null model AIC
Number of ants	Intercept	3.77 (±1.36)	0.0058 **	30.2	45.6
	Pitcher length	-0.15 (±0.07)	0.0188 *		
	Fatty acid derivative quantity	0.05 (±0.02)	0.0372 *		
	Benzenoid quantity	0.02 (±0.02)	0.2790		
Number of bees	Intercept	-1.45 (±0.99)	0.1453	55.8	101.1
	Pitcher length	0.09 (±0.03)	0.0026 **		
	Monoterpene quantity	0.01 (±0.002)	<0.001 ***		
	Fatty acid derivative quantity	0.01 (±0.01)	0.2762		
Number of moths	Intercept	-3.37 (±1.55)	0.0296 *	39.2	69.7
	Pitcher length	0.13 (±0.05)	0.0054 **		
	Monoterpene quantity	0.01 (±0.002)	0.0024 **		
	Fatty acid derivative quantity	0.02 (±0.02)	0.2075		
Number of diptera	Intercept	0.47 (±0.37)	0.1960	57.4	127.8
	Monoterpene quantity	0.01 (±0.002)	<0.001 ***		
	Fatty acid derivative quantity	-0.02 (±0.01)	0.1050		
Number of wasps	Intercept	-0.87 (±0.65)	0.1850	42.4	60.6
	Monoterpene quantity	0.02 (±0.004)	<0.001 ***		
	Sesquiterpene quantity	-0.01 (±0.01)	0.1810		
Number of beetles	Intercept	-1.46 (±2.12)	0.4891	36.4	38.0
	Pitcher length	0.07 (±0.06)	0.2580		
	Fatty acid derivative quantity	-0.05 (±0.05)	0.2950		

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**Variation in colour signals among *Sarracenia*
pitcher plants and the potential role of areoles in
the attraction of flying Hymenoptera**

Résumé

Les plantes carnivores du genre *Sarracenia* présentent au niveau de leurs urnes une grande diversité de caractéristiques visuelles, mais la perception par les insectes et le rôle de ces caractéristiques dans l'attraction ont été peu étudiés. Ces plantes varient également dans la composition de leurs proies, certaines espèces piégeant davantage d'hyménoptères volants, comme les abeilles. Pour tester l'hypothèse d'un lien entre la variabilité du signal visuel et la capacité de ségrégation des proies et pour identifier les signaux susceptibles d'attirer les hyménoptères volants, nous avons caractérisé les motifs de couleur de 32 urnes appartenant à quatre espèces, modélisé leur perception par les hyménoptères volants et examiné les proies qu'elles piègent. Les urnes des quatre espèces différaient par leurs couleurs, notamment deux espèces aux grandes urnes qui présentaient des alvéoles claires, dont la couleur et la clarté contrastaient fortement avec le fond végétal et avec les autres zones de l'urne aux yeux des hyménoptères volants. Ces espèces ont piégé une forte proportion d'hyménoptères volants. Cela suggère que les alvéoles contrastées pourraient constituer un leurre visuel pour les hyménoptères volants, rendant les plantes particulièrement visibles pour ces insectes. Le nombre de proies capturées différait aussi en fonction des caractéristiques visuelles de l'urne, de son stade, de sa morphologie et de la saison. Des études sur les visiteurs des urnes seront nécessaires pour mieux comprendre le lien entre la capture des proies et les caractéristiques visuelles d'attraction des urnes.

Variation in colour signals among *Sarracenia* pitcher plants and the potential role of areoles in the attraction of flying Hymenoptera

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Abstract

Sarracenia insectivorous plants show a diversity of visual features in their pitchers but their perception by insects and their role in attraction, have received little attention. They also vary in prey composition, with some species trapping more flying Hymenoptera, such as bees. To test the hypothesis of a link between visual signal variability and prey segregation ability, and to identify which signal could attract flying Hymenoptera, we characterised, the colour patterns of 32 pitchers belonging to four taxa, modelled their perception by flying Hymenoptera, and examined the prey they trapped. The pitchers of the four taxa differed in colour patterns, with notably two long-leaved taxa displaying clear areoles, which contrasted strongly in colour and brightness with the vegetative background and with other pitcher areas in the eyes of flying Hymenoptera. These taxa trapped high proportion of flying hymenoptera. This suggests that contrasting areoles may act as a visual lure for flying Hymenoptera, making plants particularly visible to these insects. Prey capture also differed according to pitcher stage, morphology, season and visual characteristics. Further studies on prey visitation are needed to better understand the link between prey capture and attraction feature.

Introduction

Angiosperms, the most diverse group of plants, underwent explosive radiation during the Cretaceous in a coevolutionary process with insect pollinators (Grimaldi, 1999; Van der Niet & Johnson, 2012). These flowering plants have thus evolved different combinations of nectar rewards, and sensory signals, such as visual and olfactory cues, which attract a diversity of pollinators (Kevan & Baker, 1983; Dobson, 1994; Lunau & Maier, 1995; Chittka & Raine, 2006). Visual and olfactory signals often operate synergistically in attracting pollinators (Raguso, 2008). Some plants, such as orchid species, have even evolved ‘dishonest’ signals that deceive pollinators, by exploiting their sensory biases (Schaefer & Ruxton, 2009) and/or by using deceit mimicry of nectar or sexual mate (Dafni, 1984; Schiestl, 2010), without any reward. Carnivorous plants are particularly efficient at exploiting the sensory biases of generalist insect-pollinators because their leaves often resemble flowers in their visual and olfactory traits such as colour patterns (Moran *et al.*, 1999; Schaefer & Ruxton, 2008; Moran *et al.*, 2012b), even in the UV range (Joel *et al.*, 1985; Moran, 1996; Kurup *et al.*, 2013; Golos, 2020), nectar guides (Dress *et al.*, 1997; Bennett & Ellison, 2009), and perfume (Jaffé *et al.*, 1995; Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010). The most adorned plants regarding these floral features attract a wide range of flower-visiting insects (Joel, 1988; Di Giusto *et al.*, 2010). Part of the attracted insects are then trapped with an arsenal of leaf traits, as diverse as snap-traps (Forterre *et al.*, 2005), viscoelastic mucilage (Gaume & Forterre, 2007; Adlassnig *et al.*, 2010) and slippery surfaces (Gaume *et al.*, 2004; Bauer *et al.*, 2013). They are digested by endogenous and/or exogenous proteolytic enzymes, and ultimately supplement carnivorous plants with essential nutrients, which often lack in the soil where they grow (Ellison & Adamec, 2018).

One group of carnivorous plants, the so-called pitcher plants, includes the well-known Sarraceniaceae from the Americas and the Nepenthaceae from Southeast Asia. These plants exhibit several morphological and physiological adaptations, such as pitcher-like leaves of different sizes and shapes (Cresswell, 1993; Bhattarai & Horner, 2009; Gaume *et al.*, 2016), different attractive visual or olfactory signals (Moran *et al.*, 1999; Di Giusto *et al.*, 2010) and capture devices (Bonhomme *et al.*, 2011). However, it is difficult to disentangle the contribution of the different features since they are

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often combined to form whole trapping syndromes, which target specific guilds of animals or characterise specific diets (Pavlovič *et al.*, 2007; Gaume *et al.*, 2016).

Compared to olfactory cues (Jaffé *et al.*, 1995; Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010), the visual cues of pitcher plants as perceived by insects have received no attention to our knowledge and their role in attraction is the subject of controversy. For instance, pitcher red colouration has been proposed to play an important role in prey attraction by some authors (Newell & Nastase, 1998; Schaefer & Ruxton, 2008) but not by others (Green & Horner, 2007). In an experiment with *Sarracenia purpurea* (Linnaeus), extrafloral nectar, often associated to red colouration in carnivorous plants (Bennett & Ellison, 2009; Gaume *et al.*, 2016), was shown to account for prey attraction, rather than the red colouration itself (Bennett & Ellison, 2009). Yet, colour signals should not be overlooked since they operate at larger distances than nectar and are thus needed to attract flying insects. Moreover, many pitcher plants sport a large colour diversity with contrasting patterns and not just bicolor patterns with nuances of green and red (Moran *et al.*, 1999), which suggests that visual signals are important components of the attractive system in these plants. For instance, clear white areoles present in some carnivorous plants (Schnell, 2002; McPherson, 2006) have been shown to function as light lures helping insect capture in *Sarracenia minor* (Walter) (McGregor *et al.*, 2016), and in the Sumatran *Nepenthes aristolochioides* (Jebb & Cheek) (Moran *et al.*, 2012a). Yet, according to Schaefer & Ruxton (2014), these areoles only play a role in attracting insects to pitcher plants, but have no role in prey capture. The role, if any, of clear areoles in attracting prey – a prerequisite for capture in carnivorous plants – remains to be clarified. As visual contrasts are important features of attraction in pollinating systems (Spaethe *et al.*, 2001), we hypothesise that clear areoles are better perceived by flying prey since they are more conspicuous against the green vegetation background and the remaining parts of the plant. We test this hypothesis by exploring the variation in colouration in four *Sarracenia* pitcher plants of different pitcher morphology and visual characteristics, especially regarding the areoles, and by modelling how these colours are perceived by flying Hymenoptera. We secondly investigated whether clear areoles play a role in attracting this kind of insects to the pitcher by examining the relationship between the composition in prey trapped by pitchers and the visual signals they displayed, as seen by flying Hymenoptera.

Material and methods

Plant taxa and growing conditions

We considered four *Sarracenia* taxa differing in the visual aspect and leaf shape (Fig. 1). Two were natural species: *S. purpurea* subsp. *venosa* (Rafinesque) and *S. x mitchelliana* = *S. purpurea* x *S. leucophylla* hybrid. *S. purpurea*, which does not bear clear areoles, mostly traps ants and comparatively few flying hymenopterans (Cresswell, 1991; Heard, 1998; Newell & Nastase, 1998; Bhattarai & Horner, 2009). The two others were horticultural hybrids with clear areoles: *S. x Juthatip soper* = (*S. leucophylla* x *S. purpurea*) x *S. leucophylla* and *S. x leucophylla* = *S. leucophylla* x *S. x Juthatip soper*. The long-leaved *Sarracenia leucophylla* (Rafinesque) characterized by clear areoles has been observed to trap a large amount of bees in the field (Gibson, 1983a) and its horticultural hybrids have been observed to trap wasps and hornets in large amounts (Meurgey & Perrocheau, 2015). Plants were grown in polyculture in a 6 m² container filled with a peat mixture consisting of 2/3 blond peat and 1/3 sand on a background of clay balls. Containers were placed outdoors in the experimental station of AMAP (Montpellier, France) in a sunny place. They were regularly watered with demineralized water to match their natural moist and mineral-poor environment.

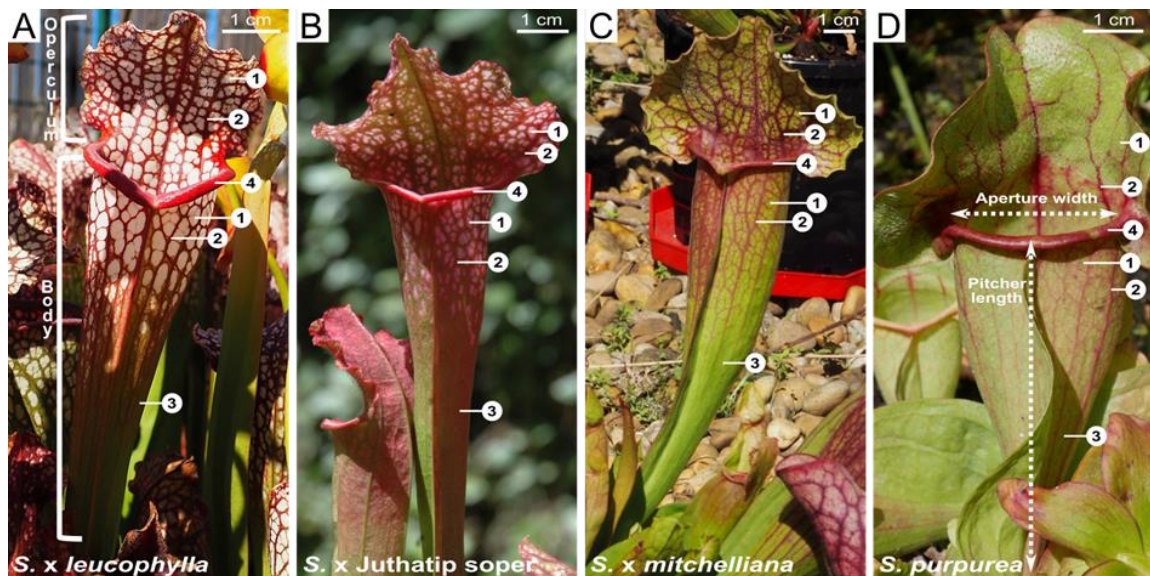


Figure 1. Pitchers of the four *Sarracenia* taxa: (A) *S. x leucophylla*, (B) *S. x Juthatip soper*, (C) *S. x mitchelliana*, (D) *S. purpurea* and their different areas: areoles (1) and veins (2) on the operculum, areoles (1), veins (2) and tube (3) on the pitcher body, and the peristome (4). For all species, areoles are defined as the areas between the veins, in the pitcher body or in the operculum. Areoles appear white (A), pink (B), or green (C, D) to a human eye. Aperture width and pitcher length, shown in (D) were measured in all pitchers.

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Pitcher morphological measurements and prey capture

Pitchers were marked the day they opened and four pitcher stages were thus scored (1: young, 2: middle, 3: mature, 4: old) corresponding to 5 ± 1 , 15 ± 3 , 40 ± 5 , and 60 ± 10 days after pitcher opening, respectively. We measured a total of 32 pitchers on 21 plants (8 pitchers per taxon). We took a first set of measurements for 24 pitchers of different stages belonging to 5-6 plants of each of the four taxa in mid-August 2016 (summer), and a second set of 8 pitchers of stage 2 belonging to two plants of each of the four taxa in October 2016 (autumn). The plants measured in autumn were different from those measured in summer. We measured pitcher length and aperture width (see Fig. 1D) before taking colour measurements (see below). These measurements were compared among taxa using one-way ANOVAs. We then collected pitcher content and preserved it in 70% ethanol. Claire Villemant identified prey individuals to the species level when possible, and at least always to the family or super-family level, depending on the stage of the digestive process. It was always possible to make the distinction between flying Hymenoptera (e. g. social or solitary bees and wasps, hornets, parasitoid wasps and sawflies) and crawling Hymenoptera (ants).

Pitcher colour measurement and analysis

To study the visual signals of the carnivorous plants, we measured the reflectance spectra of the 32 pitchers using spectrophotometry. The light produced by a Deuterium-Halogen light source (Avalight) was conducted to the sample through an optic probe (FCR-7UV200-2-45-ME, terminated by a quartz transparent window cut at 45° guaranteeing a constant distance between the sample and the light beam). The light reflected by the sample was conducted through the probe to a spectrometer (Ocean Optics USB 2000). Measurements were taken between 300 and 700 nm, which included the range of flying Hymenoptera sensitivity, considered to be between 300 nm and 650 nm, as in honeybees (De Ibarra *et al.*, 2014). In each of the 32 pitchers, we took 4 to 5 repeated spectral measurements of the following six areas: areoles of the body, veins of the body, peristome, areoles of the operculum, veins of the operculum and tube (Fig. 1).

Sarracenia pitcher plants trap mainly bees, wasps and hornets as flying Hymenoptera (Gibson, 1983a; Farnsworth & Ellison, 2008; Meurgey & Perrocheau, 2015), and all have similar trichromatic

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vision (Peitsch *et al.*, 1992) with similar spectral sensitivity (Peitsch *et al.*, 1992; Briscoe & Chittka, 2001; De Ibarra *et al.*, 2014). Hence, we chose the hornet *Vespa crabro* (Linnaeus) to model how *Sarracenia* colours are perceived by flying Hymenoptera, using Vorobyev & Osorio's discriminability model (Vorobyev & Osorio, 1998) to compute the visual contrasts produced by a pitcher colour seen against a green grass background or against another pitcher colour. We took the D65 standard illuminant as the irradiance spectrum of an open habitat, the reflectance spectrum of a colour area of a *Sarracenia* pitcher plant, the mean reflectance spectrum of green grass as a visual background (Gomez, personal data) for whenever the contrast against the background was computed. We built flying Hymenoptera photoreceptor absorptance curves by using photoreceptor peaks measured for hornet by Peitsch *et al.* (1992), that is 336, 436 and 536 nm for the S, M, and L photoreceptors respectively. We assumed a neural noise and ω_i -values of 0.13, 0.06 and 0.12 for S, M, and L receptors respectively, as in Vorobyev & Osorio (1998). We assumed that brightness was detected by L receptors (Lehrer, 1993).

We first computed the colour and brightness contrasts displayed by the various pitcher areas against the mean green grass background. These contrasts are used to quantify how visible the pitcher plant stands out against its natural background, and has been used to better understand pollinator attraction to plants, as in orchids for instance (Streinzer *et al.*, 2009; Streinzer *et al.*, 2010). These authors computed colour and brightness contrasts to understand attraction at short and long distances respectively, as Hymenoptera use colour signal at close range and brightness signal to detect small objects or objects at larger distances, respectively (Giurfa *et al.*, 1996; Dyer *et al.*, 2008). Second, we computed the colour and brightness contrasts between any two pitcher areas. Aguiar *et al.* (2020) considered an equivalent of colour contrast against the background of sepals, petals and labellum of tropical orchids pollinated by bees, but also the colour contrast between each floral piece and showed the importance of intrafloral colour patterns in pollinator perception, which could serve as a guide to the flower centre. Colour patterns between pitcher areas could similarly help attracting prey to the trap (Moran, 1996). Regarding pitcher areas, areoles of the pitcher body and areoles of the operculum had similar colouration and yielded similar contrasts, they were thus pooled into a common 'areoles' category. Likewise, the veins of the pitcher body and the veins of the operculum had similar colouration and yielded similar contrasts, and were pooled into a common 'veins' category. Hence the variable

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pitcher area had four levels: areoles, veins, peristome, and tube. All computations were performed with the R package *pavo* version 2 (Maia *et al.*, 2019).

Statistical analyses

We analysed variation in plant colouration and variation in prey capture using a mixed model approach as it is well suited to repeated observations. All statistics were performed using the software R version 4.0.3 (R Core Team, 2020). Model assumptions were checked by plotting residuals versus fitted values.

First, we explored to which extent the colour contrast and the brightness contrast displayed by a pitcher area against the green background varied with plant taxon, pitcher stage and pitcher area. We took the colour contrast or the brightness contrast of all spectral measurements as the dependent variable, a normal error distribution, and Plant identity and Pitcher identity nested within Plant identity as random effects. We tested plant taxon, pitcher stage, season, pitcher area, and all relevant two-way and three-way interactions when possible (for season, only the simple effect could be included). Both dependent variables were square-root transformed to match residual normality.

Second, considering the contrasts between any two pitcher areas, we explored to which extent the colour contrast and the brightness contrast displayed by a pitcher area against another varied with plant taxon, pitcher stage and the pitcher area considered. We took the colour contrasts or the brightness contrasts between pitcher areas as the dependent variable, a normal error distribution, and Plant identity and Pitcher identity nested within Plant identity as random effects. We tested plant taxon, pitcher stage, season, pair of areas (each combination of two areas contrasted with each other), and all relevant two-way and three-way interactions when possible (for season, only the simple effect could be included). Both dependent variables were square-root transformed to match residual normality.

Finally, we tested whether the number of prey trapped was linked to plant taxon, pitcher stage, pitcher morphology and season. The total number of prey individuals and the total number of crawling Hymenoptera trapped were analysed using Generalized Linear Models (GLM) assuming a Poisson distribution with a log-link function. We tested, as explanatory variables, Plant taxon, Pitcher Stage, Pitcher length, Aperture width, the ratio Pitcher length / Aperture width, and Capture area (as defined in Bhattarai & Horner (2009) i.e. the area of a circle of diameter Aperture width) and Season. For

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morphological variables, which were partly correlated (for instance the ratio Pitcher length / Aperture width was negatively correlated with Pitcher length : correlation-coefficient = -0.83, $t = -8.27$, p -value < 0.001), we chose to include in the same model only the least correlated variables, that is Pitcher length and Aperture width (which were not correlated : correlation-coefficient = -0.20, $t = -1.15$, p -value = 0.261). For this analysis, an outlier was identified and excluded (a *S. x leucophylla* pitcher with 153 prey individuals including 54 ants).

Furthermore, we tested whether the number of flying Hymenoptera prey trapped was also linked to visual signals. The number of flying Hymenoptera was analysed using similar Poisson regression models. As Plant taxon and Pitcher stage absorbed the variation in visual signals, they were not included in the analysis. Season was retained as it was likely to affect the abundance of prey available. As the visual signals measured were numerous compared to the sample size, we focused on the variables of interest, i.e. the contrasts related to areoles and also included in the analysis only the least correlated contrasts (<50%, see Supporting Information Fig. S1). Hence, we tested the effects of Season, Pitcher length, Aperture width, Areoles brightness contrast with background, Areoles-Peristome colour contrast, Areoles-Peristome brightness contrast, Areoles-Tube colour contrast.

For all models, we selected the best model with the lowest AIC, and we did that for the fixed and random effects separately. In all mixed models, only Pitcher identity was retained as a random effect. Backward selection of models and type III tests were carried out. For each significant factor, we estimated the regression coefficients and post-hoc tests were carried out between any two factor levels, when necessary.

Results

Pitcher morphology and colouration

Plant taxa differed both in morphology and colouration (Fig. 1, Supporting Information Fig. S2, Fig. S3). *S. x leucophylla* and *S. x Juthatip* soper tended to have long pitchers with a narrow aperture (Fig. S3) and well-defined clear areoles, which respectively appeared white and pink to a human eye (Fig. 1, Fig. S3). The other two taxa, *S. x mitchelliana* and *S. purpurea*, had short pitchers with a large aperture

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(Fig. S3), and areas defined as areoles had a green colouration to a human eye, which was similar to that of the pitcher tube (Fig. 1, Fig. S2). In all taxa, the tube generally reflected light in the middle wavelengths while the peristome and veins reflected more in the long wavelengths, appearing respectively green and red to a human eye (Fig. 1, Fig. S2).

Contrasts with a green background

The visual contrasts – colour contrast and brightness contrast – produced by pitcher areas against a green background as seen by flying Hymenoptera varied with taxon and stage (Table 1). More specifically, to flying Hymenoptera, peristome and tube showed no significant difference in colour contrast between taxa (Fig. 2A, (Supporting Information Table S1), and veins and tube showed no significant difference in brightness contrast between taxa (Fig. 2B, Supporting Information Table S2). Conversely, areoles and veins differed in colour contrast and areoles and peristome differed in brightness contrast between taxa (Fig. 2, Table S1, S2). The veins contrasted more in colour with a green background in *S. x mitchelliana* than in *S. x leucophylla*. The peristome contrasted more in brightness with a green background in *S. x leucophylla* than in *S. x Juthatip soper*. The areoles contrasted more in colour with a green background in *S. x leucophylla* and *S. x Juthatip soper* than in *S. x mitchelliana* and *S. purpurea* while *S. x leucophylla* had the most contrasting areoles of all four taxa. Hence, areoles accounted for most of the visual differences observed between taxa as they differed both in colour and brightness among taxa (Fig. 2). Yet, the area that contrasted most strongly against a green background was not always the same from one taxon to another (Table S1, S2): for the two long-leaved taxa, *S. x leucophylla* and *S. x Juthatip soper*, areoles were the most contrasting area either for colour or for brightness (Table S1, S2). Conversely, the most contrasting area for colour was the veins for *S. x mitchelliana* while it was the peristome for *S. purpurea* (Table S1, S2).

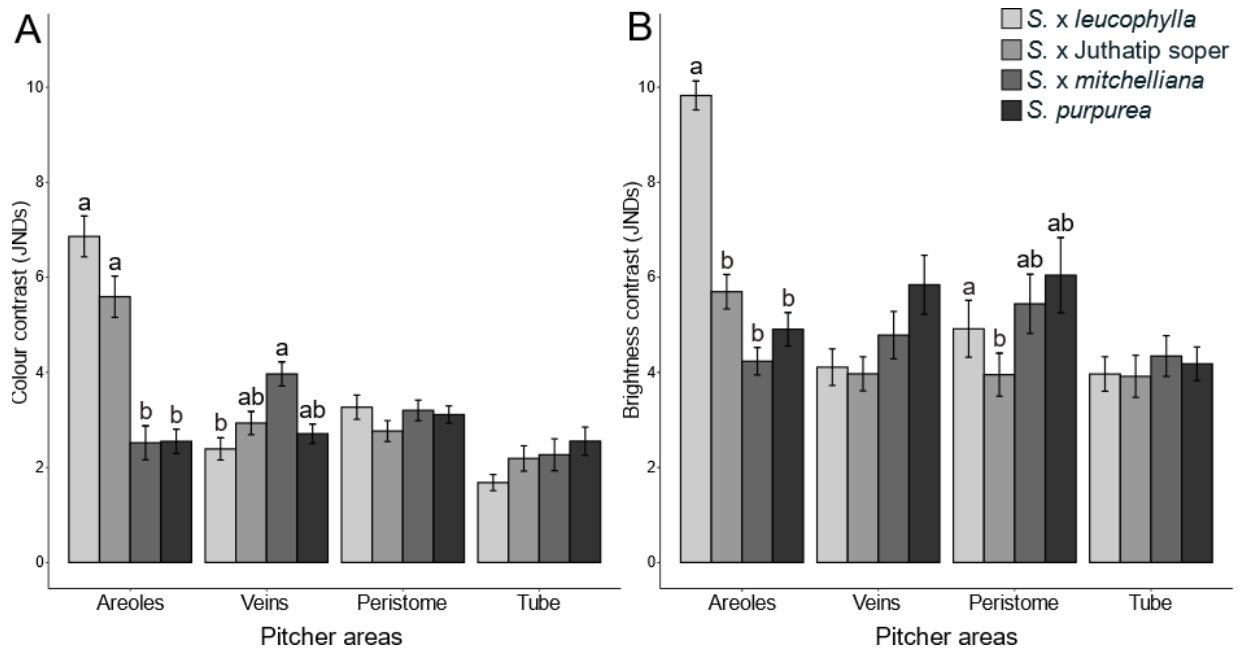


Figure 2. Variation in colour contrast (A) and brightness contrast (B) displayed by the different pitcher areas against the green background, as perceived by flying Hymenoptera. Mean values are presented with their associated standard errors. Contrasts are expressed in Just Noticeable Differences (JNDs). Different letters above bars show statistically significant differences in means between plant taxa for the contrasts of each specific area, no letter meaning that differences are not statistically different ($p > 0.05$).

The change in colour and brightness contrasts with pitcher stage was not similar in all taxa (Table 1). However, areoles were the area most contrasting in colour at stage 1 (~5 days after pitcher opening) and at stage 2 (~15 days after pitcher opening) while the peristome was the area most contrasting in colour at stage 3 (~40 days after pitcher opening) and at stage 4 (~60 days after pitcher opening) (Supporting Information Fig. S4A, Table S1). Regarding brightness, areoles were the most contrasting pitcher areas against background at stage 1, 3 and 4 but only the second most contrasting areas at stage 2, veins being the most contrasting areas for this stage (Fig. S4B, Table S2). Season also directly affected the colour but not brightness contrasts with background, the colour contrasts being more pronounced in autumn than in summer (Table 1, Table S1).

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Table 1. Factors of variation in colour and brightness contrasts with a green background. Only the best models are presented. Both dependent variables were square-root transformed to match residuals normality. “Chisq” values refer to type III Wald chi-square tests. Symbols describe various levels of p-values *: p<0.05, **: p<0.01, and ***: p<0.001. Contrasts are detailed in ESM, Tables S1 (colour contrast) and S2 (brightness contrast). “ - “ means that the variable was not retained in the best model. “ : ” refers to the interaction between variables.

Explanatory variables	Colour contrast				Brightness contrast			
	Chisq	Df	p-value		Chisq	Df	p-value	
Intercept	80.11	1	<0.001	***	74.33	1	<0.001	***
Plant taxon	9.69	3	0.0214	*	1.38	3	0.7107	
Area	32.28	3	<0.001	***	15.68	3	0.0013	**
Pitcher stage	3.06	3	0.3823		5.27	3	0.1532	
Season	31.79	1	<0.001	***	-	-	-	
Plant taxon: Area	72.44	9	<0.001	***	26.96	9	0.0014	**
Plant taxon: Pitcher stage	11.20	9	0.2622		17.55	9	0.0407	*
Area: Pitcher stage	45.83	9	<0.001	***	40.48	9	<0.001	***
Plant taxon: Area : Pitcher stage	103.08	27	<0.001	***	136.96	27	<0.001	***

Contrasts among pitcher areas

The visual contrasts – colour contrast and brightness contrast –between pitcher areas seen by flying Hymenoptera varied with taxon and stage (Table 2). More specifically, as seen by flying Hymenoptera, areoles produced a higher colour contrast with any other area in *S. x leucophylla* than in the 3 other taxa (Fig. 3A). These contrasts were also generally higher for *S. x Juthatip soper* than for *S. x mitchelliana* and *S. purpurea* but this was not the case for the areoles-veins contrast, which was not significantly different between *S. x Juthatip soper* and *S. x mitchelliana* (Fig. 3A). Areoles displayed a generally higher brightness contrast against any other area in *S. x leucophylla* than in the 3 other taxa but this was not the case for the areoles-veins contrast, which was not significantly different between *S. x leucophylla* and *S. x mitchelliana* and for the areoles-tube contrast, which was not significantly different between *S. x leucophylla* and *S. x Juthatip soper* (Fig. 3B). Overall, colour contrasts values between any two pitcher areas were always higher when they involved the areoles than when they involved any other areas for these two taxa (Supporting Information Table S3, Table S4). Yet, the pair of areas that displayed the strongest contrasts was not always the same from one taxon to another (Table S3, Table S4). For the two long-leaved taxa, *S. x leucophylla* and *S. x Juthatip soper*, the strongest contrast for colour or

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brightness was produced by areoles against peristome (Table S3, Table S4). For *S. x mitchelliana* the strongest colour contrast was produced by veins against tube and peristome against tube and the strongest brightness contrast was produced between any red areas (peristome or veins) against any green area (areoles or tube). For *S. purpurea*, the strongest colour or brightness contrast was produced by areoles against peristome and peristome against tube, the brightness contrast being higher between areoles and peristome than between tube and peristome.

The differences in contrast produced by each pair of areas depended on the pitcher stage (Table 2, Supporting Information Fig. S5) but areoles and peristome always produced the highest contrast at stages 2 and 3 regarding colour and at stages 1, 2 and 3 regarding brightness (Table S3, S4). At stage 4, the most contrasting pairs of areas regarding colour and brightness contrasts involved the peristome against both areoles and tube (Table S3, S4). Overall, pitcher stages did not show many differences (Table 2), but differences occurred according to the pair of areas considered (Table 2, Fig. S5). Pitchers of stage 1 showed a weaker brightness contrast than pitchers of other stages, and the contrast displayed by areoles or tube against peristome or veins was always the lowest for that stage (Table S4). Regarding contrasts produced by areoles against peristome, stage 2 was the highest for colour but stage 3 was the highest for brightness. Moreover, stage 2 displayed the highest brightness contrast between areoles and veins, while the most brightness contrasting pairs of areas of stages 3 and 4 were peristome and tube. Finally, colour and brightness contrasts between areas varied with season and were generally more pronounced in autumn than in summer (Table 2, S3, and S4).

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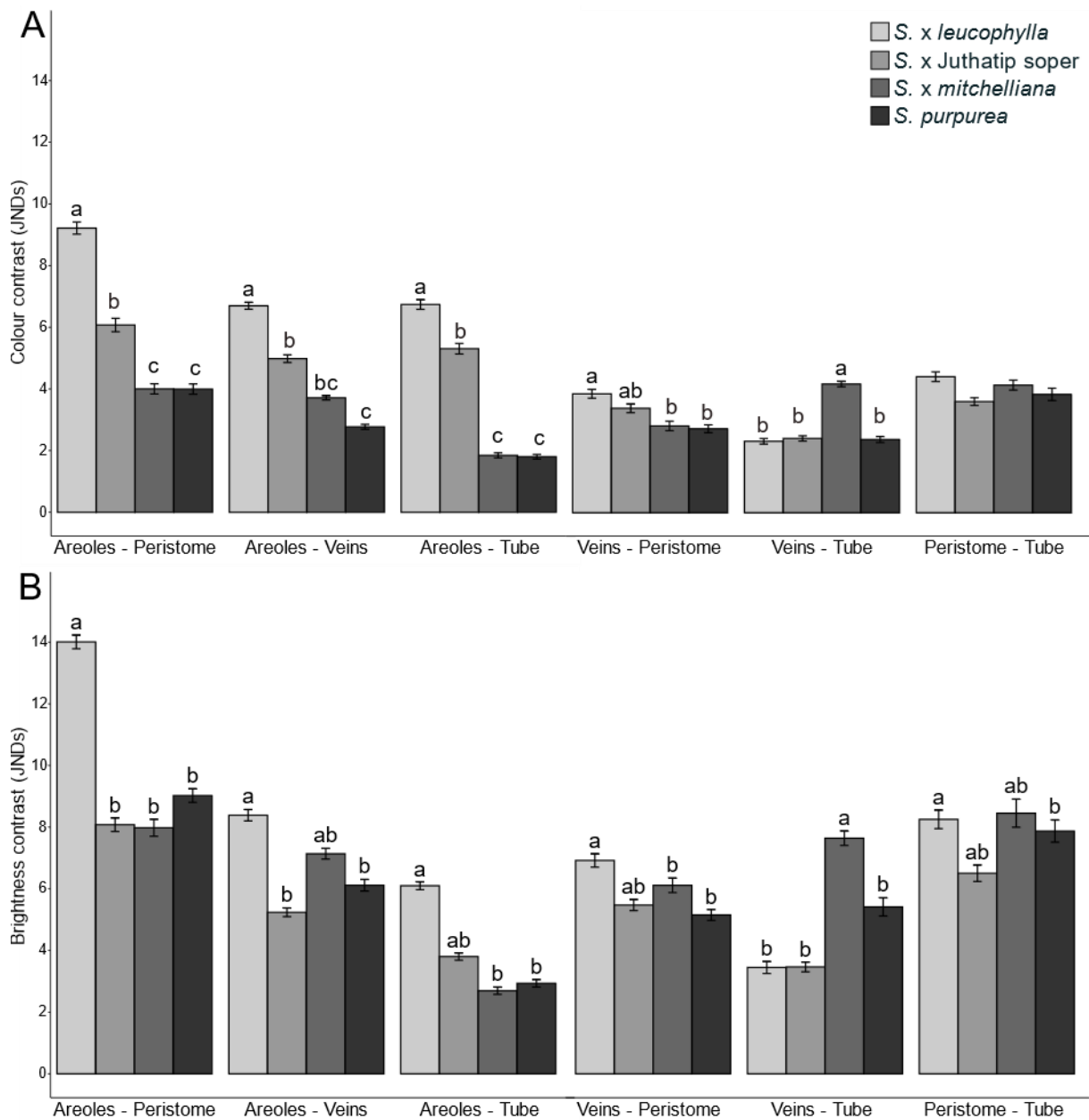


Figure 3. Variation among plant taxa in colour contrast (A) and brightness contrast (B) between any two pitcher areas (areoles, peristome, veins, or tube), as perceived by flying Hymenoptera. Mean values are presented with their associated standard errors. Contrasts are expressed in Just Noticeable Differences (JNDs). Different letters above bars show statistically significant differences in means between plant taxa for the contrasts of each specific area, no letter meaning that differences are not statistically different ($p > 0.05$).

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Table 2. Factors of variation in colour and brightness contrasts between two any pitcher areas. The best model is presented. Both dependent variables were square-root transformed to match residual normality. “Chisq” values refer to type III Wald chi-square tests. *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$. Contrasts are detailed in ESM, Table S3 for colour contrast and S4 for brightness contrast. “ : ” refers to the interaction between variables.

Explanatory variables	Colour contrast			Brightness contrast		
	Chisq	Df	p-value	Chisq	Df	p-value
Intercept	132.85	1	<0.001 ***	55.23	1	<0.001 ***
Plant taxon	13.14	3	0.0043 **	2.55	3	0.4662
Pair of areas	347.46	5	<0.001 ***	259.38	5	<0.001 ***
Pitcher stage	2.12	3	0.5481	4.09	3	0.2520
Season	56.42	1	<0.001 ***	7.03	1	0.0080 **
Plant taxon: Pair of areas	553.75	15	<0.001 ***	174.22	15	<0.001 ***
Plant taxon: Pitcher stage	30.21	9	<0.001 ***	6.45	9	0.6941
Pair of areas: Pitcher stage	241.65	15	<0.001 ***	199.66	15	<0.001 ***
Plant taxon: Pair of areas : Pitcher stage	1114.93	45	<0.001 ***	674.58	45	<0.001 ***

Prey capture and pitcher feature

Prey spectra varied with plant taxon (Supporting Information Fig. S6). The prey trapped by pitchers were mostly composed of flying Hymenoptera and Diptera in *S. x leucophylla* ($35 \pm 8\%$, $23 \pm 10\%$); flying Hymenoptera, ants (crawling Hymenoptera) in *S. x Juthatip soper* ($39 \pm 7\%$, $39 \pm 8\%$); ants and Diptera in *S. x mitchelliana* ($32 \pm 13\%$ and $21 \pm 8\%$) and ants in *S. purpurea* ($36 \pm 16\%$). Flying Hymenoptera trapped were mostly solitary bees ($93 \pm 6\%$) with a clear dominance of sweet bees (Halictidae).

The total number of prey individuals and crawling Hymenoptera caught in pitchers depended on plant taxon and pitcher stage. Likewise, pitcher morphology affected the total number of prey individuals (Supporting Information Table S5). Pitchers of *S. x leucophylla* and *S. x Juthatip soper* trapped the highest total number of prey individuals while pitchers of *S. x mitchelliana* and *S. purpurea* trapped the lowest number of prey individuals (Fig. 4, Table 3). As regards crawling Hymenoptera, *S. x Juthatip soper* pitchers trapped more individuals than other taxa (Fig. 4, Table 3). Pitchers of stage 1 trapped the lowest number of prey individuals and crawling Hymenoptera individuals while pitchers of stage 3 trapped the highest total number of prey individuals followed by pitchers of stage 2 (Table 3). Stage 4 trapped more crawling Hymenoptera individuals than other stages (Table 3). Regarding morphology, the total number of prey individuals increased with aperture width and pitcher length (Table 3).

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The number of flying Hymenoptera individuals caught in pitchers depended on season, visual signals and pitcher morphology (Table S5). Regarding morphology, the number of flying Hymenoptera individuals increased with pitcher length (Table 3). Pitchers also trapped less flying Hymenoptera individuals in autumn than in summer (Table 3). In terms of visual signals, the colour contrast between areoles and tube had a positive effect on the number of flying hymenopterans caught in pitchers while the colour contrast between areoles and peristome and the areoles brightness contrast with background had a negative one (Table 3).

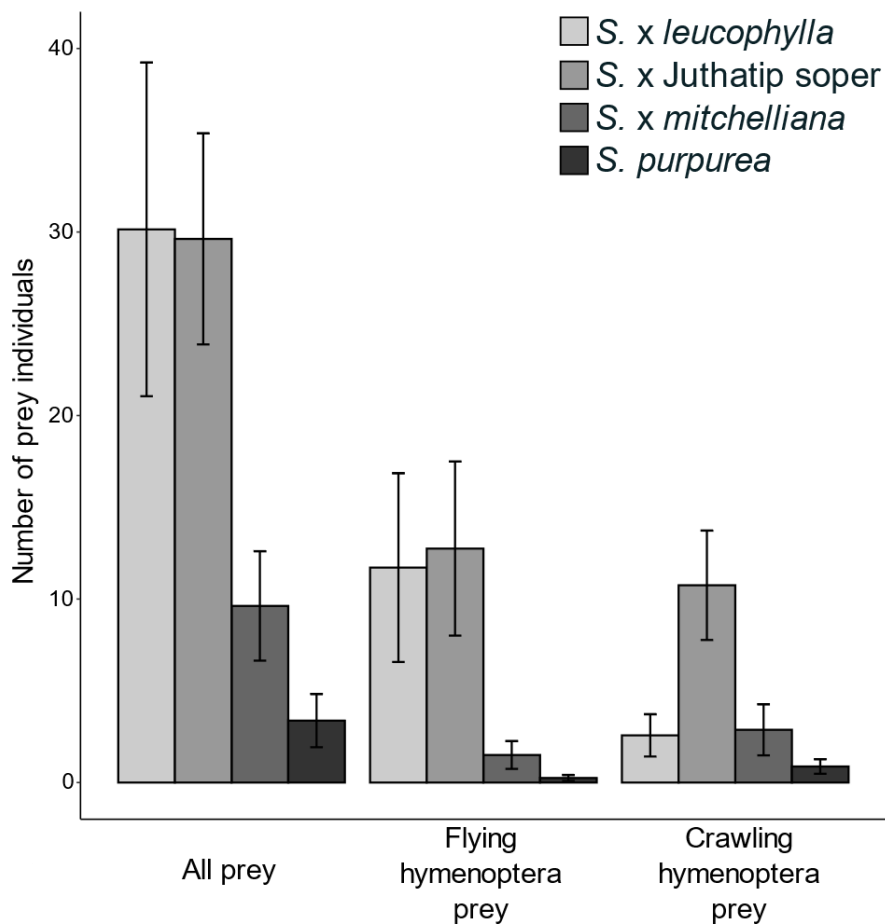


Figure 4. Variation among plant taxa in the number of prey individuals captured. Mean values are presented with their associated standard errors. We consider all prey, flying Hymenoptera prey and crawling Hymenoptera prey (ants).

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Table 3. Effects of covariates included in the best linear models presented in Table S5 to explain the variations in the number of prey individuals, the number of flying Hymenoptera individuals and the number of crawling Hymenoptera individuals caught in the pitchers. We used a GLM with a Poisson distribution. P-values were adjusted for multiple comparisons with the Bonferroni correction, *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$. For qualitative variables, we tested the contrast between two levels of factor or between 2 gathered levels of factor. For instance, we tested whether the number of prey individuals in *S. x leucophylla* was higher than the number of prey individuals in *S. x Juthatip soper* and it was significantly different (p -value < 0.001). For quantitative variables, which were centred and scaled before analysis to assess relative effect sizes, we showed the estimated regression coefficients of the model.

Dependent variable	Explanatory variables	Contrast on factor	Coefficient (\pm standard error)	z-ratio	adjusted p-value
Number of prey individuals	Plant taxon	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.07 (± 0.11)	-0.60	1.0000
		<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.85 (± 0.20)	4.34	<0.001 ***
		<i>S. x leucophylla</i> > <i>S. purpurea</i>	1.21 (± 0.30)	4.02	<0.001 ***
		<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.92 (± 0.16)	5.76	<0.001 ***
		<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	1.27 (± 0.27)	4.79	<0.001 ***
		<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.36 (± 0.24)	1.47	0.8454
	Stage	Stage 1 > (2, 3, 4)	-1.64 (± 0.22)	-7.42	<0.001 ***
		Stage 2 > (1, 3, 4)	0.39 (± 0.13)	2.99	0.0112 *
		Stage 3 > (1, 2, 4)	0.92 (± 0.11)	8.65	<0.001 ***
		Stage 4 > (1, 2, 3)	0.33 (± 0.18)	1.84	0.2635
	Aperture width		0.20 (± 0.07)	3.09	0.0020 **
	Pitcher length		0.57 (± 0.08)	6.76	<0.001 ***
Number of flying Hymenoptera individuals	Season	autumn > summer	-2.75 (± 0.53)	-5.17	<0.001 ***
	Areoles-Tube colour contrast		0.92 (± 0.24)	3.86	<0.001 ***
	Areoles brightness contrast		-0.34 (± 0.12)	-2.81	0.0050 **
	Areoles-Peristome colour contrast		-0.47 (± 0.08)	-5.77	<0.001 ***
	Pitcher length		0.78 (± 0.13)	6.19	<0.001 ***
Number of crawling Hymenoptera individuals	Plant taxon	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-1.44 (± 0.26)	-5.55	<0.001 ***
		<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.12 (± 0.32)	-0.39	1.0000
		<i>S. x leucophylla</i> > <i>S. purpurea</i>	1.07 (± 0.45)	2.40	0.0997
		<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	1.32 (± 0.23)	5.62	<0.001 ***
		<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	2.51 (± 0.39)	6.38	<0.001 ***
		<i>S. x mitchelliana</i> > <i>S. purpurea</i>	1.19 (± 0.43)	2.76	0.0351 *
	Stage	Stage 1 > (2, 3, 4)	-1.91 (± 0.58)	-3.27	0.0043 **
		Stage 2 > (1, 3, 4)	0.30 (± 0.26)	1.18	0.9547
		Stage 3 > (1, 2, 4)	0.59 (± 0.25)	2.41	0.0629
		Stage 4 > (1, 2, 3)	1.02 (± 0.28)	3.63	0.0011 **

Discussion

Pitcher colouration and attraction of flying hymenoptera

Beyond the broad range of colouration patterns within pitchers and between plant taxa, our findings support the hypothesis that the clear areoles – a characteristic of the two long-leaved taxa related to *S. leucophylla*, *S. x leucophylla* and *S. x Juthatip soper* – may potentially help *Sarracenia* pitcher plants attracting flying Hymenoptera. (i) Clear areoles contrasted strongly both in colour and brightness with the other parts of the pitcher and were the areas that contrasted the most against the green background, making them the most conspicuous area in the eyes of flying Hymenoptera. Flying Hymenoptera, such as honeybees and bumblebees, use both colour and brightness signals for flower detection (Giurfa *et al.*, 1996; Giurfa & Vorobyev, 1998; Dyer *et al.*, 2008). Brightness contrast is used for small or distant objects (subtending a small visual angle in the eye) while colour contrast is used for large or close objects (subtending a large visual angle in the eye) (Giurfa *et al.*, 1996; Dyer *et al.*, 2008). Hence, bright areoles may play a role as a long-distance attractant for flying Hymenoptera, because they make the upper part with the aperture of the trap more visible compared to the rest of the pitcher. (ii) Furthermore, the two taxa that sported clear areoles also trapped more prey in total and trapped a majority of flying hymenoptera compared to the two smallest-leaved taxa without clear areoles, *S. x mitchelliana* and *S. purpurea*, that trapped a majority of ants. (iii) In addition, the colour contrast between areoles and tube explained an important part of the variation in the number of flying Hymenoptera trapped, the number of captures increasing with this contrast. On the other hand, we observed a negative effect of areoles brightness contrast and areoles-peristome colour contrast. Such a seemingly contradictory effect can be explained by the fact that colour and brightness contrasts are not particularly correlated. The pitchers that contrasted strongly in colour between areoles and tube are not the ones that contrasted the most in brightness for their areoles or in colour between areoles and peristome. In any case, the effect of the areoles-tube contrast outweighed the other two negative effects combined and is thus the most reliable effect. Considering the spectrum of attracted insects instead of the spectrum of trapped insects would have helped to better assess the relative contribution of the interacting effects, since only a few insects attracted are bound to be trapped (Joel, 1988; Newell & Nastase, 1998). However, attraction is a

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prerequisite to capture and our results support the hypothesis that areoles help attraction by contrasting highly in colour against the tube, a result coherent with that found by Schaefer & Ruxton (2014). *Sarracenia* form dense groups of plants (Gibson, 1983b) and areoles are often seen against a background of other pitcher tubes. Areoles would thus lure flying insects to the deadly traps, as also shown by McGregor *et al.* (2016) and Moran *et al.* (2012a). Although we cannot exclude a role of areoles as light traps, in this study we suggest that areole high visual contrasts may help long-range attraction, a potential role, which has rarely been considered previously. Most of the insects caught in the traps of carnivorous plants are hymenopterans and dipterans (Juniper *et al.*, 1989; Ellison & Gotelli, 2009). While Hymenoptera cannot detect long wavelengths (red) and use brightness to detect red colours since the latter poorly reflect at shorter wavelengths, appearing them dark (Martínez-Harms *et al.*, 2010), many flies have a tetrachromatic or even pentachromatic vision (Kooi *et al.*, 2021) and they are able to see red wavelengths both in colour and brightness. While most studies regarding carnivorous pitcher plants have overlooked how pitcher colours are perceived by potential prey (Newell & Nastase, 1998; Green & Horner, 2007; Schaefer & Ruxton, 2008; Bennett & Ellison, 2009), our results underline the importance of red colouration, already considered in these previous studies, in enhancing the visual contrast even seen by flying Hymenoptera. Indeed, for all plant taxa, whether for colour or brightness, the peristome produced one of the most important contrast either against the tube or against the areoles. The red peristome, which borders the mouth of the carnivorous pitcher, is a strategic area (Moran, 1996; Bohn & Federle, 2004; Kurup *et al.*, 2013) and any contrasting patterns that concerns this area is of high advantage in terms of plant fitness, as it is likely to favour insect guidance to the deadly trap. In the two short-leaf plants *S. x mitchelliana* and *S. purpurea*, the red peristome provides similar brightness and colour contrast with green areoles or with green tube. In the two long-leaved plants, *S. x leucophylla* and *S. x Juthatip soper*, the red peristome contrasts more in brightness and in colour with white-pinkish areoles than with green tube, which increases pitcher visibility. Areoles when distinct in colouration from the pitcher tube, add to pitcher visibility and are associated to higher prey capture in the two long-leaved plants compared to the two short-leaf plants.

In our study, all measured pitcher areas show very poor UV-reflection in all taxa (Fig. S2), thus showing virtually no contrast in the UV range. Whether a UV contrast may exist locally between highly-

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absorbing (like the UV-induced fluorescence documented in Sarraceniaceae by Golos (2020)) and highly-reflecting areas remains to be studied. Such highly-localised UV-contrasts may help orientation, as suggested in Kurup *et al.* (2013), but their role, if any, in attraction and capture is controversial and remains to be elucidated (Jansen, 2017).

Influence of other pitcher traits on prey capture and composition

Besides colouration, our results show that pitcher morphology likely plays a role in prey capture. Pitchers with a longer size and a larger aperture trapped significantly more prey in total. This is consistent with the results of Cresswell (1993) and Heard (1998) on *S. purpurea* and of Green & Horner (2007) and Bhattarai & Horner (2009) on *S. alata*, who showed that larger pitchers trapped more prey. Bhattarai & Horner (2009) supposed that the positive relationship often observed between pitcher size and prey capture was due to a greater quantity of attractants produced by larger pitchers and not merely a larger area of capture. Indeed, the capture area as defined by Bhattarai & Horner (2009) did not better explain variation in our models than aperture width. Larger pitcher apertures also mean larger surface areas of lid, which was the most nectar-rewarding area of the pitchers in the studied plants (TH, JP and LG personal observations). Nevertheless, previous studies were limited to a single taxon. By considering different taxa, we showed that the effect of pitcher length was still more important than the effect of aperture width. The hypothesis of Bhattarai & Horner (2009) is therefore plausible on an intraspecific scale, but on an interspecific scale, the shape of the trap must also play an important role in prey capture.

Pitcher length was also positively linked to the number of flying Hymenoptera individuals, which suggests that taller pitchers may be more efficient at retaining flying Hymenoptera and/or may be more attractive, for instance by their colour signals as shown above. The conspicuousness of taller pitchers can arise from their size and/or their colouration: here clear areoles were only present in long-leaved taxa. Both aspects – size and colouration – can be under selection. Such multi-component signals are frequent in the contexts where plant appearance is under selection. For instance, in a prey-pollinator conflict regarding sundew carnivorous plants, El-Sayed *et al.* (2016) showed that in addition to different colouration between flower and trap, spatial separation is necessary to attract pollinator to the flowers

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rather than to the traps. Likewise, in *Nepenthes liana*, which explore both terrestrial and aerial strata, two pitcher types “lower” and “upper” are produced, each one targeting a different group of prey, the “upper” type trapping more flying insects than the “lower” type (Moran, 1996; Di Giusto *et al.*, 2008; Gaume *et al.*, 2016). Similarly, in long-leaved *Sarracenia* plants, pitchers are likely more accessible to flying insects. In *Nepenthes rafflesiana* (Jack.), this is also explained by the emission by “upper” pitchers of volatile compounds, which attract a diversity of flying insects (Di Giusto *et al.*, 2010). Whether *Sarracenia* taxa differ in their emission of odour attractive to prey remains to be studied.

Variation in visual signals and prey capture over pitcher life span

Our study also show that both colour contrasting patterns and prey capture changed during pitcher life span. We observed an increase in capture at stage 2 and a peak at stage 3 for the number of prey individuals. Although our sampling is weak for stages 1 and 4, mixed models control for unbalanced observations and show that capture increases over the first part of pitcher life (stages 1 to 3) and later (stage 4) decreases, which implies that when aging, pitchers allocated their resources more to the digestive process and less to attraction and capture. Heard (1998) observed a similar peak in prey capture in 12-33-day-old pitchers (corresponding to stages 2 and 3 in our study) in *S. purpurea*. Several studies on pitcher plants have reported that the accumulation of ammonia released by dead prey when prey capture is too important leads to digestion disruption and pitcher putrefaction (Clarke & Kitching, 1995). Increase in ammonia with prey capture has been recorded for *Sarracenia* (Wakefield *et al.*, 2005), suggesting prey capture should not exceed a threshold. Enzyme activity increases with pitcher age in *Sarracenia* (Luciano & Newell (2017);_hydrolase secretion increases after pitcher opening for *S. purpurea* but stops in absence of prey according to Gallie & Chang (1997). Hence, younger stages are likely specialized in attracting and capturing prey, a hypothesis that is supported by the variation in colouration that we observed. (i) Areoles are the most contrasting area for colour in younger stages (1 and 2) while peristome is the most contrasting area for colour in older stages, (ii) areoles-peristome contrast is stronger at stage 2 for colour and stronger at stage 3 for brightness and areoles-veins brightness contrast is stronger at stage 2 (iii) areoles against peristome produced the highest contrast on

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pitchers for colour at stages 2, 3 and for brightness at stages 1, 2, 3, while at stage 4 areoles do not contrast more than tube against peristome for colour and brightness.

Variation in colouration with pitcher age has been mentioned by Bhattarai & Horner (2009) and has been already shown in *S. alata* by Horner *et al.* (2012). Leaf colour plasticity is already known in pitcher plants: nitrogen supply or food intake has been shown to affect foliar reflectance pattern (Moran & Moran, 1998; Yoon *et al.*, 2019) or chlorophyll activity (Farnsworth & Ellison, 2008). Therefore, it remains an open question whether the change in colouration of pitchers as they age is a consequence of prey capture. We also observed a seasonal variation in pitcher colouration with more pronounced contrasts in autumn, although our design allows us to this only for stage 2 pitchers. In parallel, pitchers seem to capture more flying hymenoptera individuals in summer than in autumn. Bees representing the majority of flying hymenoptera trapped by our pitchers. Like many other Hymenoptera, bees are thermophile species and are more active in summer than in autumn. Summer good weather conditions maximize time spent foraging (Vicens & Bosch, 2000). As season did seem to have neither an effect on the total number of prey individuals nor on the total number of ants, an increase in the capture of other non-Hymenoptera prey is likely to have compensated for the observed decrease in the capture of flying Hymenoptera (see Supporting Information Fig. S7). An increase in the capture of non-Hymenoptera flower visitors for instance may result from a reduced availability of flowers redirecting them toward nectar-producing pitchers - or from pitcher attraction towards different groups of insects with various visual ability - or capture efficiency, a question that deserves proper study. Yet, as we focused on the relation between areoles and flying Hymenoptera and used a specific vision model for that purpose, it is difficult to consider the effect of visual signals on the whole prey spectrum. For instance, ant workers, which represent a large part of the prey spectra of *Sarracenia* are often dichromate (Aksoy & Camlitepe, 2018). It would therefore be interesting to model their vision to better understand pitcher colours as perceived by a completely different type of prey, especially since their capture variation among stage and taxon differed a lot from that of flying Hymenoptera. Moreover, carnivorous plants have been suggested to lure prey by other attractants such as nectar (Bennett & Ellison, 2009) and odours (Jürgens *et al.*, 2009). For example, Bhattarai & Horner (2009) showed that volatiles from *Sarracenia* pitchers attract a majority of flying insects but few crawling ones, suggesting an important role of odours in the

attraction of flying prey. Studies of these other attractants could help us to better understand the variation in prey capture and complete our knowledge on the attraction strategy of *Sarracenia* pitcher plants.

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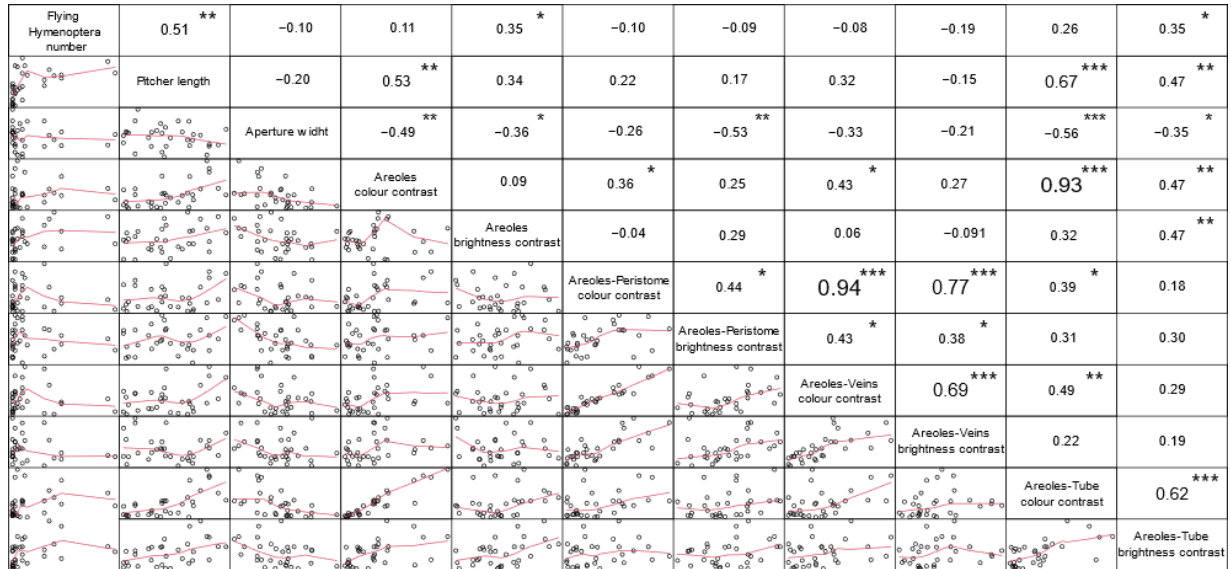
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Supporting information

Figure S1. Correlation table relative to visual areole-contrast variables, size variables (Pitcher length and Aperture Width) and number of flying Hymenoptera. The correlation coefficients between each pair of variables, estimated using the Pearson method, are presented with their associated p-values, *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.



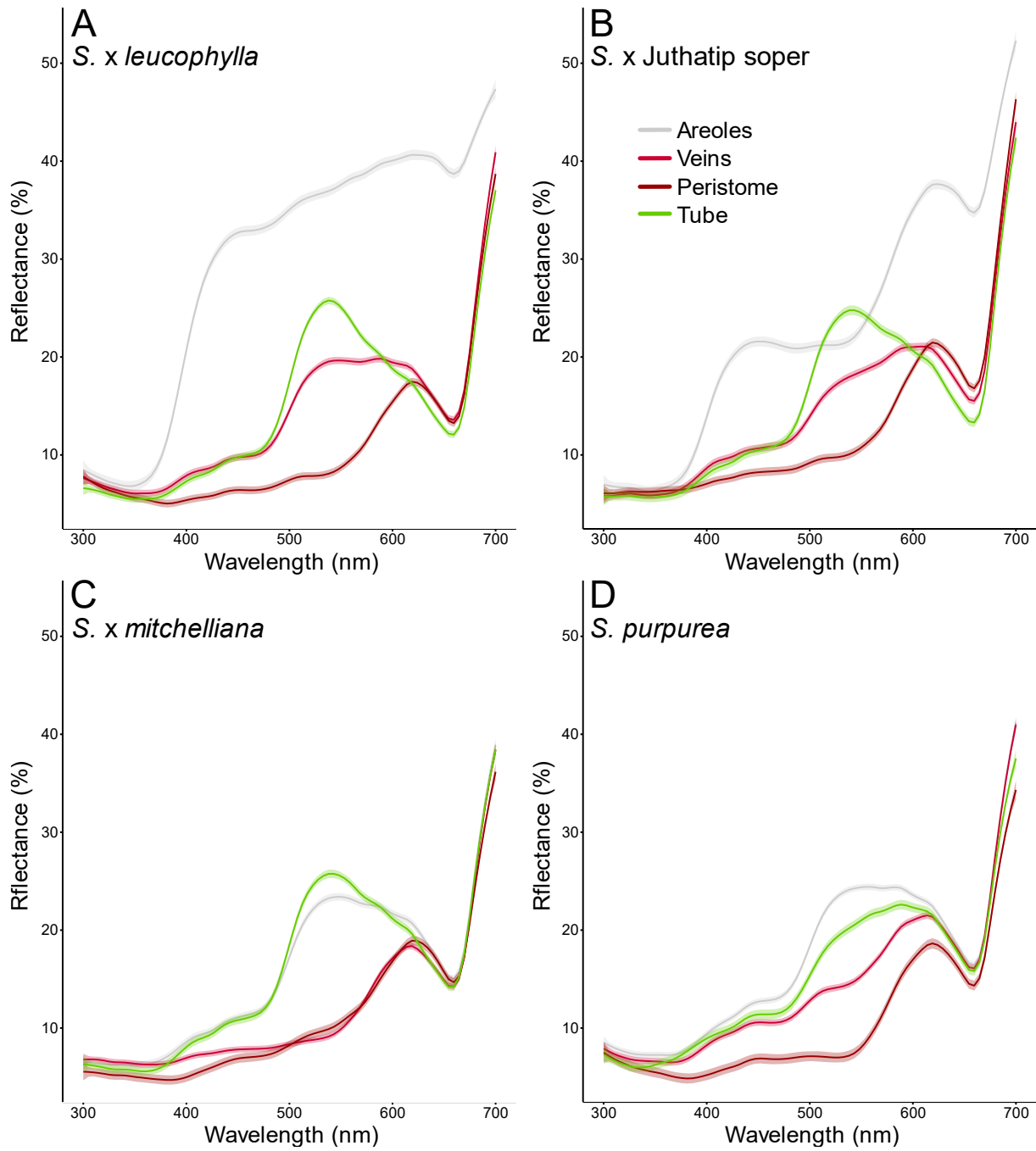


Figure S2. Mean reflectance spectra (\pm SE) of the pitcher areas in the four *Sarracenia* taxa: (A) *S. x leucophylla*, (B) *S. x Juthatip soper*, (C) *S. x mitchelliana*, (D) *S. purpurea*: areoles, veins, peristome and tube. For each taxon and pitcher area, we presented the estimated means and confident intervals of all spectral measurements obtained for all stages and seasons.

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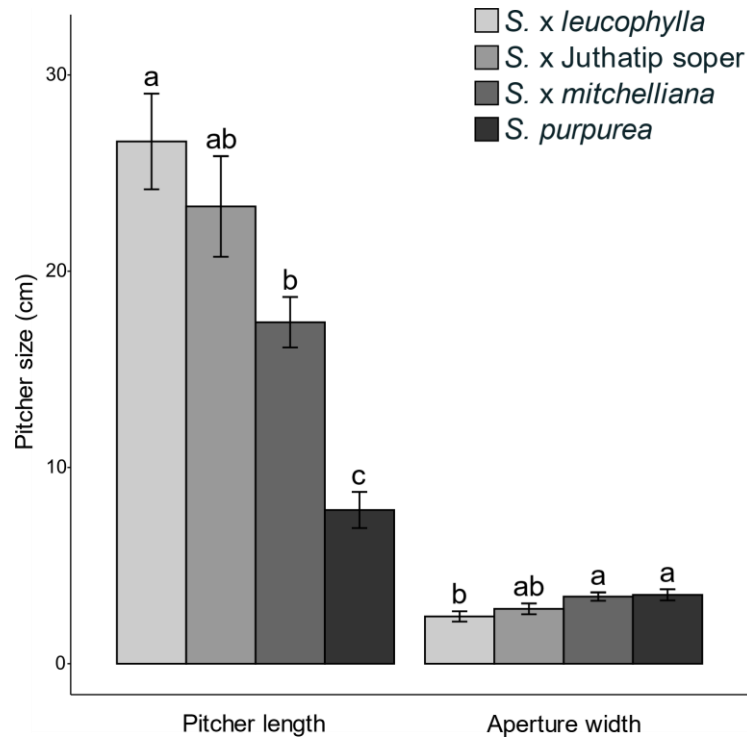


Figure S3. Variation among plant taxa of pitcher length and pitcher width. Mean values are presented with their associated standard errors. For each measurement, different letters above bars show statistically significant differences in means between plant taxa ($p < 0.05$). For instance, pitcher length is significantly greater in *S. x leucophylla* than in *S. x mitchelliana* ($a \neq b$) and greater than in *S. purpurea* ($a \neq c$) while the length of the pitchers of *S. x Juthatip soper* is not different from that of *S. x leucophylla* or of *S. x mitchelliana* (ab is not different from a or from b).

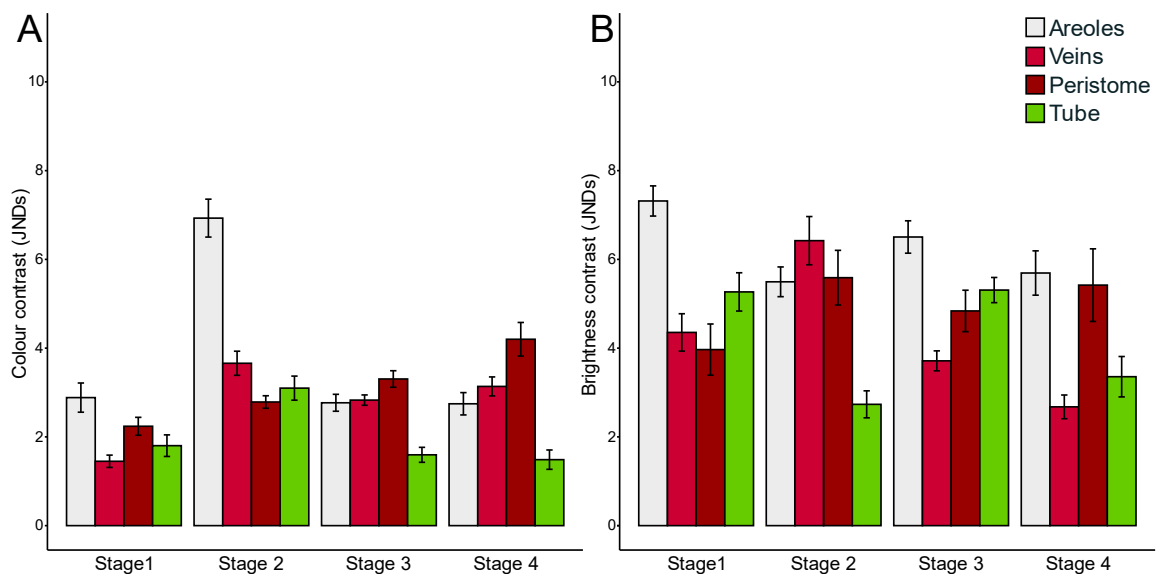


Figure S4. Variation among pitcher stages of colour contrast (A) and brightness contrast (B) displayed by the different pitcher areas against the green background, as perceived by flying Hymenoptera. Mean values are presented with their associated standard errors. Contrasts are expressed in Just Noticeable Differences (JNDs). Colours of the graphs do not correspond to the actual colours of the pitcher areas.

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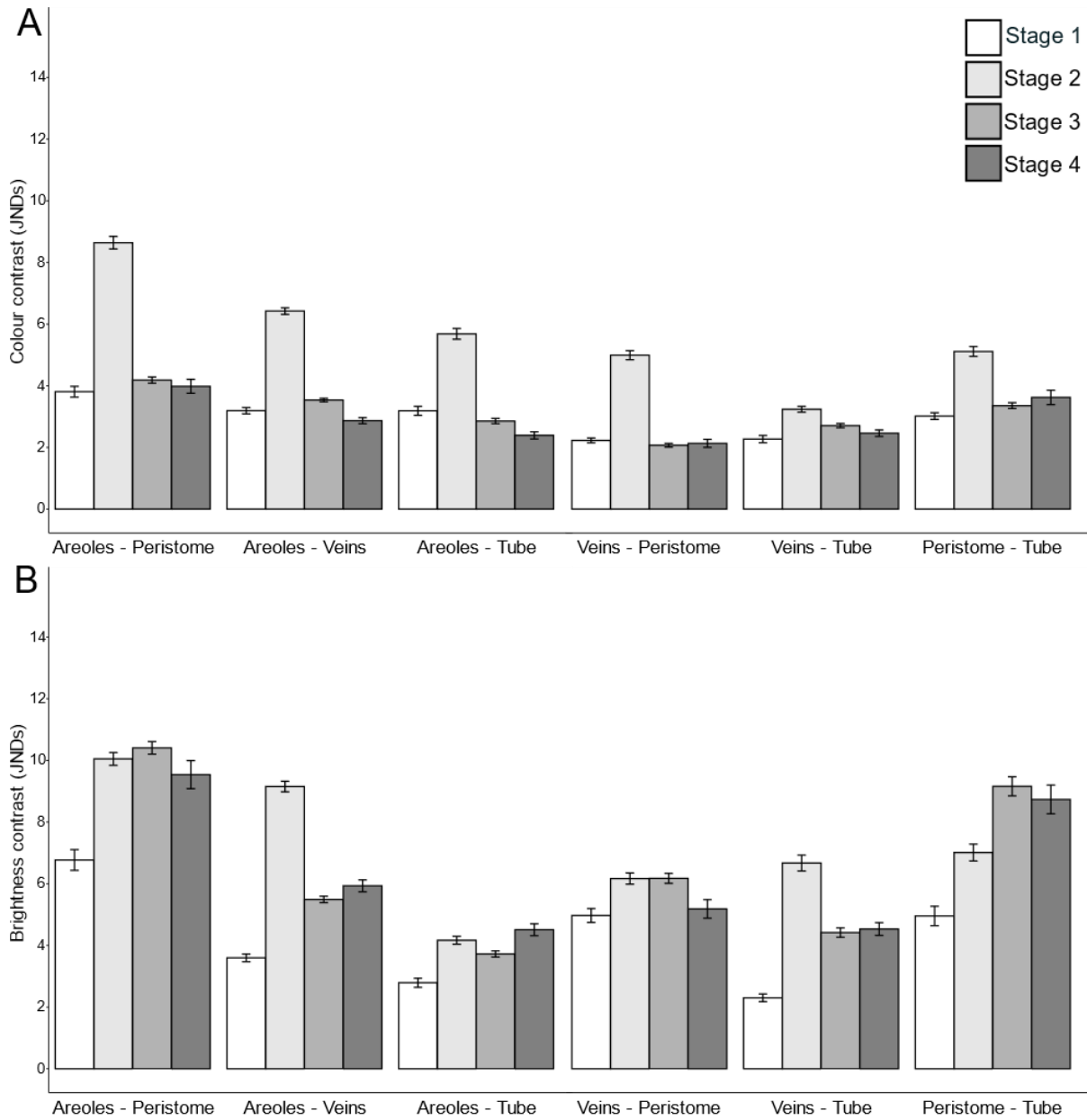
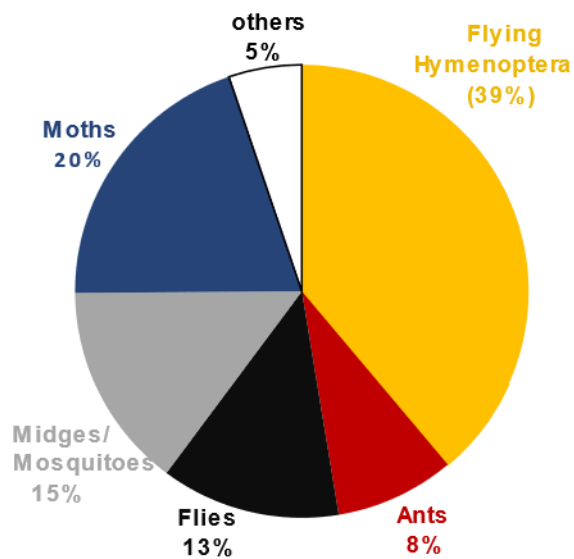
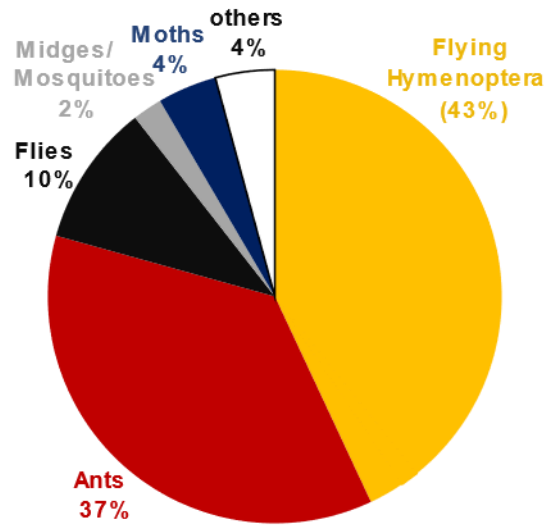


Figure S5. Variation among pitcher stages of colour contrast (A) and brightness contrast (B) between any two pitcher areas (areoles, peristome, veins, or tube), as perceived by flying Hymenoptera. Mean values are presented with their associated standard errors. Contrasts are expressed in Just Noticeable Differences (JNDs).

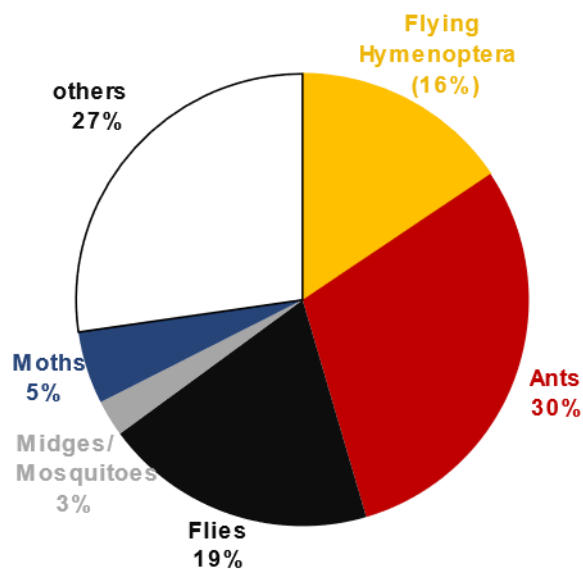
A *S. x leucophylla*



B *S. x Juthatip soper*



C *S. x mitchelliana*



D *S. purpurea*

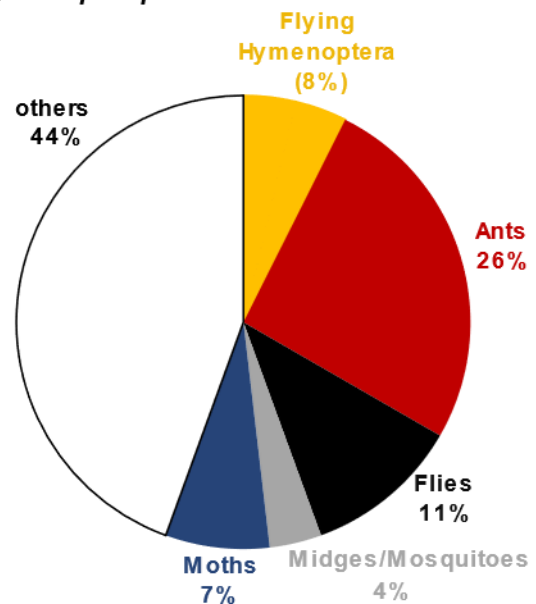


Figure S6. Total proportion of prey trapped in all pitchers for the four *Sarracenia* taxa: (A) *S. x leucophylla*, (B) *S. x Juthatip soper*, (C) *S. x mitchelliana*, (D) *S. purpurea*. Prey could be Diptera (Flies in black or Midges/Mosquitoes in grey), flying Hymenoptera (Bees, Bumblebees, Solitary Bees, Wasps and Parasitoid wasps in yellow), or crawling Hymenoptera (ants in red), Lepidoptera (Moths in blue) or other prey (in white, including Coleoptera, Hemiptera, Blattodea, Springtails, Snails and Spiders).

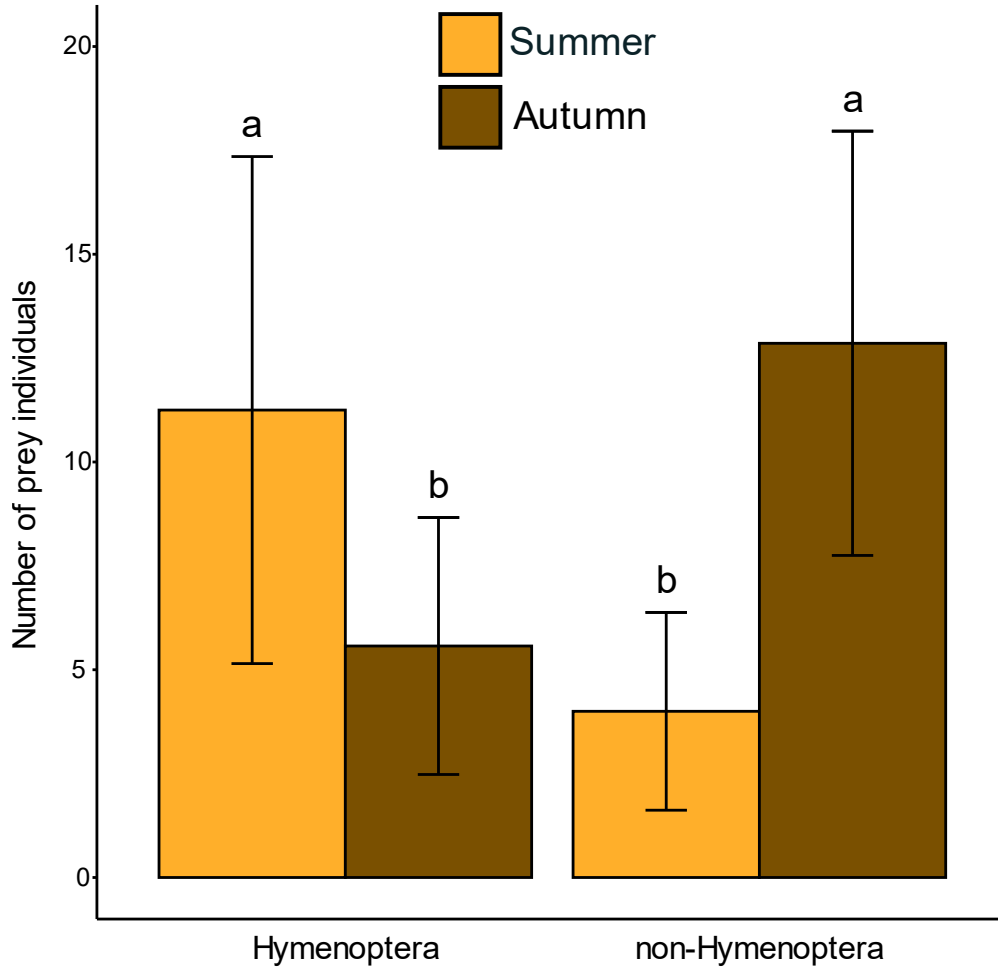


Figure S7. Seasonal variation in the number of Hymenoptera and non-Hymenoptera individuals captured. Mean values are presented with their associated standard errors. We consider only prey captured in pitchers at stage 2 because it was the only stage sampled for the two seasons (n = 4 in summer, n = 7 in autumn). For each prey category, different letters above bars show statistically significant differences in means between seasons ($p < 0.05$), as output by one-way ANOVAs.

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Table S1. Results of the post-hoc tests associated to the best linear mixed model presented in Table 1 to explain the variation in colour contrast with a green background. The variable Colour contrast was square-root transformed to match residual normality. P-values were adjusted for multiple comparisons with the Bonferroni correction, *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$. Within a level of factor 1, we tested the contrast between two levels of factor 2 or between 2 gathered levels of factor 2. For instance, for areoles, we tested whether the colour contrast of *S. x leucophylla* was higher than the colour contrast of *S. x Juthatip soper* and it was not significantly different (p-value=0.585). Symbol “-“ means that the contrasts were tested overall and not within a level of another factor. For instance, we tested whether the colour contrast of *S. x leucophylla* was higher overall than the colour contrast of *S. x Juthatip soper*.

Level on factor 1	Contrast on factor 2	Coefficient (± standard error)	t- ratio	adjusted p-value	
-	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.08 (±0.15)	0.51	1.0000	
-	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.08 (±0.15)	0.55	1.0000	
-	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.11 (±0.15)	0.74	1.0000	
-	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.01 (±0.15)	0.04	1.0000	
-	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.03 (±0.15)	0.22	1.0000	
-	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.03 (±0.15)	0.18	1.0000	
-	Areoles > Veins	0.23 (±0.04)	6.22	<0.001	***
-	Areoles > Peristome	0.08 (±0.04)	1.68	0.5605	
-	Areoles > Tube	0.47 (±0.04)	10.60	<0.001	***
-	Veins > Peristome	-0.15 (±0.04)	-3.37	0.0048	**
-	Veins > Tube	0.24 (±0.04)	5.52	<0.001	***
-	Peristome > Tube	0.4 (±0.05)	7.67	<0.001	***
-	Stage 1 > Stage 2	0.08 (±0.18)	0.43	1.0000	
-	Stage 1 > Stage 3	-0.15 (±0.15)	-1.02	1.0000	
-	Stage 1 > Stage 4	-0.24 (±0.18)	-1.34	1.0000	
-	Stage 2 > Stage 3	-0.23 (±0.15)	-1.55	0.8456	
-	Stage 2 > Stage 4	-0.32 (±0.18)	-1.77	0.5784	
-	Stage 3 > Stage 4	-0.09 (±0.15)	-0.62	1.0000	
-	autumn > summer	0.87 (±0.15)	5.64	<0.001	***
Areoles	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.29 (±0.16)	1.81	0.5085	
Areoles	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	1 (±0.16)	6.32	<0.001	***
Areoles	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.98 (±0.16)	6.20	<0.001	***
Areoles	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.71 (±0.16)	4.53	0.0012	**
Areoles	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.69 (±0.16)	4.40	0.0016	**
Areoles	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.02 (±0.16)	-0.13	1.0000	
Veins	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.15 (±0.16)	-0.94	1.0000	
Veins	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.51 (±0.16)	-3.24	0.0241	*
Veins	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.25 (±0.16)	-1.58	0.7796	
Veins	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.36 (±0.16)	-2.31	0.1898	
Veins	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.1 (±0.16)	-0.64	1.0000	
Veins	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.26 (±0.16)	1.67	0.6621	
Peristome	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.24 (±0.17)	1.41	1.0000	

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Peristome	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.08 (±0.17)	0.47	1.0000	
Peristome	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.03 (±0.17)	-0.15	1.0000	
Peristome	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.16 (±0.17)	-0.92	1.0000	
Peristome	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.27 (±0.17)	-1.56	0.7747	
Peristome	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.11 (±0.17)	-0.62	1.0000	
Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.08 (±0.17)	-0.47	1.0000	
Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.25 (±0.17)	-1.44	0.9696	
Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.27 (±0.17)	-1.58	0.7498	
Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.17 (±0.17)	-0.96	1.0000	
Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.19 (±0.17)	-1.10	1.0000	
Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.02 (±0.17)	-0.14	1.0000	
<i>S. x leucophylla</i>	Areoles > (Veins, Peristome, Tube)	0.92 (±0.07)	14.02	<0.001	***
<i>S. x leucophylla</i>	Veins > (Areoles, Peristome, Tube)	-0.43 (±0.07)	-6.67	<0.001	***
<i>S. x leucophylla</i>	Peristome > (Areoles, Veins, Tube)	0.17 (±0.08)	2.11	0.1422	
<i>S. x leucophylla</i>	Tube > (Areoles, Veins, Peristome)	-0.66 (±0.08)	-8.37	<0.001	***
<i>S. x Juthatip soper</i>	Areoles > (Veins, Peristome, Tube)	0.64 (±0.06)	10.02	<0.001	***
<i>S. x Juthatip soper</i>	Veins > (Areoles, Peristome, Tube)	-0.14 (±0.06)	-2.15	0.1260	
<i>S. x Juthatip soper</i>	Peristome > (Areoles, Veins, Tube)	-0.06 (±0.08)	-0.69	1.0000	
<i>S. x Juthatip soper</i>	Tube > (Areoles, Veins, Peristome)	-0.45 (±0.08)	-5.59	<0.001	***
<i>S. x mitchelliana</i>	Areoles > (Veins, Peristome, Tube)	-0.3 (±0.06)	-4.62	<0.001	***
<i>S. x mitchelliana</i>	Veins > (Areoles, Peristome, Tube)	0.35 (±0.06)	5.46	<0.001	***
<i>S. x mitchelliana</i>	Peristome > (Areoles, Veins, Tube)	0.17 (±0.08)	2.00	0.1854	
<i>S. x mitchelliana</i>	Tube > (Areoles, Veins, Peristome)	-0.22 (±0.08)	-2.74	0.0253	*
<i>S. purpurea</i>	Areoles > (Veins, Peristome, Tube)	-0.24 (±0.06)	-3.71	<0.001	***
<i>S. purpurea</i>	Veins > (Areoles, Peristome, Tube)	0.04 (±0.06)	0.63	1.0000	
<i>S. purpurea</i>	Peristome > (Areoles, Veins, Tube)	0.35 (±0.08)	4.33	<0.001	***
<i>S. purpurea</i>	Tube > (Areoles, Veins, Peristome)	-0.15 (±0.08)	-1.90	0.2331	
Areoles	Stage 1 > (2, 3, 4)	-0.07 (±0.15)	-0.49	1.0000	
Areoles	Stage 2 > (1, 3, 4)	0.29 (±0.15)	2.01	0.2415	
Areoles	Stage 3 > (1, 2, 4)	-0.16 (±0.11)	-1.43	0.6787	
Areoles	Stage 4 > (1, 2, 3)	-0.06 (±0.15)	-0.41	1.0000	
Veins	Stage 1 > (2, 3, 4)	-0.37 (±0.15)	-2.41	0.1022	
Veins	Stage 2 > (1, 3, 4)	-0.29 (±0.15)	-2.00	0.2470	
Veins	Stage 3 > (1, 2, 4)	0.27 (±0.11)	2.48	0.0897	
Veins	Stage 4 > (1, 2, 3)	0.39 (±0.15)	2.54	0.0778	
Peristome	Stage 1 > (2, 3, 4)	-0.14 (±0.17)	-0.84	1.0000	
Peristome	Stage 2 > (1, 3, 4)	-0.7 (±0.15)	-4.50	<0.001	***
Peristome	Stage 3 > (1, 2, 4)	0.26 (±0.12)	2.18	0.1501	
Peristome	Stage 4 > (1, 2, 3)	0.57 (±0.17)	3.42	0.0074	**
Tube	Stage 1 > (2, 3, 4)	0.17 (±0.17)	1.01	1.0000	
Tube	Stage 2 > (1, 3, 4)	-0.13 (±0.15)	-0.86	1.0000	

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Tube	Stage 3 > (1, 2, 4)	0 (±0.12)	0.01	1.0000	
Tube	Stage 4 > (1, 2, 3)	-0.04 (±0.17)	-0.22	1.0000	
Stage 1	Areoles > (Veins, Peristome, Tube)	0.29 (±0.08)	3.63	0.0012	**
Stage 1	Veins > (Areoles, Peristome, Tube)	-0.31 (±0.08)	-3.94	<0.001	***
Stage 1	Peristome > (Areoles, Veins, Tube)	0.12 (±0.1)	1.22	0.8938	
Stage 1	Tube > (Areoles, Veins, Peristome)	-0.1 (±0.1)	-1.03	1.0000	
Stage 2	Areoles > (Veins, Peristome, Tube)	0.76 (±0.05)	16.72	<0.001	***
Stage 2	Veins > (Areoles, Peristome, Tube)	-0.13 (±0.05)	-2.87	0.0166	*
Stage 2	Peristome > (Areoles, Veins, Tube)	-0.33 (±0.06)	-5.85	<0.001	***
Stage 2	Tube > (Areoles, Veins, Peristome)	-0.3 (±0.06)	-5.20	<0.001	***
Stage 3	Areoles > (Veins, Peristome, Tube)	0.01 (±0.05)	0.12	1.0000	
Stage 3	Veins > (Areoles, Peristome, Tube)	0.13 (±0.05)	2.93	0.0138	*
Stage 3	Peristome > (Areoles, Veins, Tube)	0.32 (±0.06)	5.74	<0.001	***
Stage 3	Tube > (Areoles, Veins, Peristome)	-0.46 (±0.06)	-8.15	<0.001	***
Stage 4	Areoles > (Veins, Peristome, Tube)	-0.02 (±0.08)	-0.26	1.0000	
Stage 4	Veins > (Areoles, Peristome, Tube)	0.13 (±0.08)	1.60	0.4445	
Stage 4	Peristome > (Areoles, Veins, Tube)	0.52 (±0.1)	5.11	<0.001	***
Stage 4	Tube > (Areoles, Veins, Peristome)	-0.62 (±0.1)	-6.29	<0.001	***
<i>S. x leucophylla</i>	Stage 1 > (2, 3, 4)	-0.24 (±0.28)	-0.88	1.0000	
<i>S. x leucophylla</i>	Stage 2 > (1, 3, 4)	-0.09 (±0.22)	-0.41	1.0000	
<i>S. x leucophylla</i>	Stage 3 > (1, 2, 4)	0.07 (±0.2)	0.37	1.0000	
<i>S. x leucophylla</i>	Stage 4 > (1, 2, 3)	0.26 (±0.28)	0.94	1.0000	
<i>S. x Juthatip</i>					
soper	Stage 1 > (2, 3, 4)	0 (±0.28)	-0.01	1.0000	
<i>S. x Juthatip</i>					
soper	Stage 2 > (1, 3, 4)	-0.16 (±0.22)	-0.71	1.0000	
<i>S. x Juthatip</i>					
soper	Stage 3 > (1, 2, 4)	0.4 (±0.2)	2.00	0.2542	
<i>S. x Juthatip</i>					
soper	Stage 4 > (1, 2, 3)	-0.24 (±0.28)	-0.85	1.0000	
<i>S. x mitchelliana</i>	Stage 1 > (2, 3, 4)	0.04 (±0.28)	0.14	1.0000	
<i>S. x mitchelliana</i>	Stage 2 > (1, 3, 4)	-0.28 (±0.22)	-1.28	0.8834	
<i>S. x mitchelliana</i>	Stage 3 > (1, 2, 4)	-0.01 (±0.2)	-0.05	1.0000	
<i>S. x mitchelliana</i>	Stage 4 > (1, 2, 3)	0.25 (±0.28)	0.91	1.0000	
<i>S. purpurea</i>	Stage 1 > (2, 3, 4)	-0.21 (±0.28)	-0.75	1.0000	
<i>S. purpurea</i>	Stage 2 > (1, 3, 4)	-0.3 (±0.22)	-1.37	0.7655	
<i>S. purpurea</i>	Stage 3 > (1, 2, 4)	-0.08 (±0.2)	-0.39	1.0000	
<i>S. purpurea</i>	Stage 4 > (1, 2, 3)	0.58 (±0.28)	2.11	0.2081	
Stage 1	<i>S. x leucophylla</i> > <i>S. x Juthatip</i> soper	-0.1 (±0.36)	-0.29	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.13 (±0.36)	-0.36	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.08 (±0.36)	0.23	1.0000	
Stage 1	<i>S. x Juthatip</i> soper > <i>S. x mitchelliana</i>	-0.03 (±0.36)	-0.07	1.0000	
Stage 1	<i>S. x Juthatip</i> soper > <i>S. purpurea</i>	0.18 (±0.36)	0.52	1.0000	
Stage 1	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.21 (±0.36)	0.59	1.0000	
Stage 2	<i>S. x leucophylla</i> > <i>S. x Juthatip</i> soper	0.12 (±0.21)	0.60	1.0000	

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Stage 2	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.22 (±0.21)	1.09	1.0000
Stage 2	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.27 (±0.21)	1.29	1.0000
Stage 2	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.1 (±0.21)	0.48	1.0000
Stage 2	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.14 (±0.21)	0.69	1.0000
Stage 2	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.04 (±0.21)	0.20	1.0000
Stage 3	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.17 (±0.21)	-0.81	1.0000
Stage 3	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.14 (±0.21)	0.69	1.0000
Stage 3	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.22 (±0.21)	1.06	1.0000
Stage 3	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.31 (±0.21)	1.50	0.9213
Stage 3	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.39 (±0.21)	1.87	0.4831
Stage 3	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.08 (±0.21)	0.37	1.0000
Stage 4	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.45 (±0.36)	1.25	1.0000
Stage 4	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.08 (±0.36)	0.24	1.0000
Stage 4	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.14 (±0.36)	-0.38	1.0000
Stage 4	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.36 (±0.36)	-1.01	1.0000
Stage 4	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.58 (±0.36)	-1.63	0.7396
Stage 4	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.22 (±0.36)	-0.62	1.0000

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Table S2. Results of the post-hoc tests associated to the best linear mixed model presented in Table 1 to explain the variation in brightness contrast with a green background. The variable Brightness contrast was square-root transformed to match residual normality. P-values were adjusted for multiple comparisons with the Bonferroni correction, *: p<0.05, **: p<0.01, and ***: p<0.001. Within a level of factor 1, we tested the contrast between two levels of factor 2 or between 2 gathered levels of factor 2. For instance, for areoles, we tested whether the brightness contrast of *S. x leucophylla* was higher than the brightness contrast of *S. x Juthatip soper* and we found that it was significantly different (associated with a corrected p-value=0.0034). Symbol “-” means that the contrasts were tested overall and not within a level of another factor. For instance, we tested whether the brightness contrast of *S. x leucophylla* was higher than the brightness contrast of *S. x Juthatip soper* overall.

Level on factor 1	Contrast on factor 2	Coefficient (± standard error)	t- ratio	adjusted p-value	
-	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.32 (±0.14)	2.21	0.2481	
-	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.23 (±0.14)	1.57	0.8025	
-	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.14 (±0.14)	0.95	1.0000	
-	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.09 (±0.14)	-0.63	1.0000	
-	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.18 (±0.14)	-1.26	1.0000	
-	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.09 (±0.14)	-0.63	1.0000	
-	Areoles > Veins	0.5 (±0.07)	7.63	<0.001	***
-	Areoles > Peristome	0.35 (±0.08)	4.28	<0.001	***
-	Areoles > Tube	0.45 (±0.08)	5.65	<0.001	***
-	Veins > Peristome	-0.15 (±0.08)	-1.91	0.3381	
-	Veins > Tube	-0.05 (±0.08)	-0.61	1.0000	
-	Peristome > Tube	0.11 (±0.09)	1.14	1.0000	
-	Stage 1 > Stage 2	0.17 (±0.14)	1.21	1.0000	
-	Stage 1 > Stage 3	0.06 (±0.14)	0.44	1.0000	
-	Stage 1 > Stage 4	0.25 (±0.18)	1.44	1.0000	
-	Stage 2 > Stage 3	-0.11 (±0.1)	-1.08	1.0000	
-	Stage 2 > Stage 4	0.08 (±0.14)	0.56	1.0000	
-	Stage 3 > Stage 4	0.19 (±0.14)	1.32	1.0000	
Areoles	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.67 (±0.18)	3.74	0.0034	**
Areoles	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.95 (±0.18)	5.27	<0.001	***
Areoles	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.81 (±0.18)	4.52	<0.001	***
Areoles	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.28 (±0.18)	1.55	0.7744	
Areoles	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.14 (±0.18)	0.77	1.0000	
Areoles	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.14 (±0.18)	-0.78	1.0000	
Veins	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.01 (±0.18)	-0.04	1.0000	
Veins	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.02 (±0.18)	-0.11	1.0000	
Veins	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.3 (±0.18)	-1.68	0.6086	
Veins	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.01 (±0.18)	-0.07	1.0000	
Veins	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.29 (±0.18)	-1.66	0.6352	
Veins	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.28 (±0.18)	-1.58	0.7395	
Peristome	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.54 (±0.22)	2.44	0.1009	

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Peristome	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.09 (±0.22)	0.39	1.0000	
Peristome	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.14 (±0.22)	0.65	1.0000	
Peristome	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.45 (±0.22)	-2.00	0.2879	
Peristome	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.39 (±0.22)	-1.78	0.4682	
Peristome	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.06 (±0.22)	0.25	1.0000	
Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.07 (±0.22)	0.32	1.0000	
Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.11 (±0.22)	-0.49	1.0000	
Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.11 (±0.22)	-0.49	1.0000	
Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.18 (±0.22)	-0.79	1.0000	
Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.18 (±0.22)	-0.80	1.0000	
Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0 (±0.22)	0.00	1.0000	
<i>S. x leucophylla</i>	Areoles > (Veins, Peristome, Tube)	1.02 (±0.12)	8.57	<0.001	***
<i>S. x leucophylla</i>	Veins > (Areoles, Peristome, Tube)	-0.57 (±0.12)	-4.87	<0.001	***
<i>S. x leucophylla</i>	Peristome > (Areoles, Veins, Tube)	0 (±0.15)	0.00	1.0000	
<i>S. x leucophylla</i>	Tube > (Areoles, Veins, Peristome)	-0.45 (±0.14)	-3.14	0.0070	**
<i>S. x Juthatip soper</i>	Areoles > (Veins, Peristome, Tube)	0.54 (±0.12)	4.71	<0.001	***
<i>S. x Juthatip soper</i>	Veins > (Areoles, Peristome, Tube)	-0.14 (±0.11)	-1.20	0.9151	
<i>S. x Juthatip soper</i>	Peristome > (Areoles, Veins, Tube)	-0.29 (±0.14)	-2.02	0.1733	
<i>S. x Juthatip soper</i>	Tube > (Areoles, Veins, Peristome)	-0.11 (±0.15)	-0.78	1.0000	
<i>S. x mitchelliana</i>	Areoles > (Veins, Peristome, Tube)	0.06 (±0.12)	0.48	1.0000	
<i>S. x mitchelliana</i>	Veins > (Areoles, Peristome, Tube)	-0.24 (±0.12)	-2.07	0.1547	
<i>S. x mitchelliana</i>	Peristome > (Areoles, Veins, Tube)	0.19 (±0.15)	1.23	0.8698	
<i>S. x mitchelliana</i>	Tube > (Areoles, Veins, Peristome)	0 (±0.15)	0.00	1.0000	
<i>S. purpurea</i>	Areoles > (Veins, Peristome, Tube)	0.12 (±0.11)	1.05	1.0000	
<i>S. purpurea</i>	Veins > (Areoles, Peristome, Tube)	0.01 (±0.11)	0.09	1.0000	
<i>S. purpurea</i>	Peristome > (Areoles, Veins, Tube)	-0.01 (±0.14)	-0.07	1.0000	
<i>S. purpurea</i>	Tube > (Areoles, Veins, Peristome)	-0.12 (±0.14)	-0.83	1.0000	
Areoles	Stage 1 > (2, 3, 4)	0.39 (±0.17)	2.29	0.1089	
Areoles	Stage 2 > (1, 3, 4)	-0.28 (±0.12)	-2.37	0.0916	
Areoles	Stage 3 > (1, 2, 4)	0.03 (±0.12)	0.22	1.0000	
Areoles	Stage 4 > (1, 2, 3)	-0.13 (±0.17)	-0.79	1.0000	
Veins	Stage 1 > (2, 3, 4)	0.08 (±0.17)	0.47	1.0000	
Veins	Stage 2 > (1, 3, 4)	0.49 (±0.12)	4.18	<0.001	***
Veins	Stage 3 > (1, 2, 4)	-0.11 (±0.12)	-0.89	1.0000	
Veins	Stage 4 > (1, 2, 3)	-0.47 (±0.17)	-2.79	0.0326	*
Peristome	Stage 1 > (2, 3, 4)	-0.26 (±0.21)	-1.26	0.8413	
Peristome	Stage 2 > (1, 3, 4)	0.11 (±0.15)	0.75	1.0000	
Peristome	Stage 3 > (1, 2, 4)	-0.02 (±0.15)	-0.13	1.0000	
Peristome	Stage 4 > (1, 2, 3)	0.17 (±0.21)	0.81	1.0000	
Tube	Stage 1 > (2, 3, 4)	0.46 (±0.21)	2.21	0.1186	
Tube	Stage 2 > (1, 3, 4)	-0.6 (±0.15)	-4.05	<0.001	***

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Tube	Stage 3 > (1, 2, 4)	0.41 (±0.15)	2.82	0.0235	*
Tube	Stage 4 > (1, 2, 3)	-0.27 (±0.21)	-1.32	0.7619	
Stage 1	Areoles > (Veins, Peristome, Tube)	0.66 (±0.14)	4.61	<0.001	***
Stage 1	Veins > (Areoles, Peristome, Tube)	-0.32 (±0.14)	-2.29	0.0899	
Stage 1	Peristome > (Areoles, Veins, Tube)	-0.46 (±0.18)	-2.57	0.0411	*
Stage 1	Tube > (Areoles, Veins, Peristome)	0.12 (±0.17)	0.69	1.0000	
Stage 2	Areoles > (Veins, Peristome, Tube)	0.22 (±0.08)	2.70	0.0286	*
Stage 2	Veins > (Areoles, Peristome, Tube)	0.33 (±0.08)	4.00	<0.001	***
Stage 2	Peristome > (Areoles, Veins, Tube)	0.15 (±0.1)	1.46	0.5846	
Stage 2	Tube > (Areoles, Veins, Peristome)	-0.7 (±0.1)	-6.80	<0.001	***
Stage 3	Areoles > (Veins, Peristome, Tube)	0.38 (±0.08)	4.66	<0.001	***
Stage 3	Veins > (Areoles, Peristome, Tube)	-0.42 (±0.08)	-5.12	<0.001	***
Stage 3	Peristome > (Areoles, Veins, Tube)	-0.13 (±0.1)	-1.25	0.8488	
Stage 3	Tube > (Areoles, Veins, Peristome)	0.17 (±0.1)	1.61	0.4280	
Stage 4	Areoles > (Veins, Peristome, Tube)	0.48 (±0.14)	3.35	0.0034	**
Stage 4	Veins > (Areoles, Peristome, Tube)	-0.53 (±0.14)	-3.69	<0.001	***
Stage 4	Peristome > (Areoles, Veins, Tube)	0.32 (±0.18)	1.75	0.3245	
Stage 4	Tube > (Areoles, Veins, Peristome)	-0.27 (±0.18)	-1.51	0.5278	
<i>S. x leucophylla</i>	Stage 1 > (2, 3, 4)	-0.27 (±0.27)	-1.01	1.0000	
<i>S. x leucophylla</i>	Stage 2 > (1, 3, 4)	-0.24 (±0.19)	-1.27	0.8835	
<i>S. x leucophylla</i>	Stage 3 > (1, 2, 4)	0.19 (±0.19)	1.00	1.0000	
<i>S. x leucophylla</i>	Stage 4 > (1, 2, 3)	0.33 (±0.27)	1.20	0.9928	
<i>S. x Juthatip</i>					
soper	Stage 1 > (2, 3, 4)	0.13 (±0.27)	0.49	1.0000	
<i>S. x Juthatip</i>					
soper	Stage 2 > (1, 3, 4)	-0.03 (±0.19)	-0.17	1.0000	
<i>S. x Juthatip</i>					
soper	Stage 3 > (1, 2, 4)	0.15 (±0.19)	0.77	1.0000	
<i>S. x Juthatip</i>					
soper	Stage 4 > (1, 2, 3)	-0.25 (±0.27)	-0.91	1.0000	
<i>S. x mitchelliana</i>	Stage 1 > (2, 3, 4)	0.21 (±0.27)	0.79	1.0000	
<i>S. x mitchelliana</i>	Stage 2 > (1, 3, 4)	-0.27 (±0.19)	-1.39	0.7262	
<i>S. x mitchelliana</i>	Stage 3 > (1, 2, 4)	0.25 (±0.19)	1.31	0.8256	
<i>S. x mitchelliana</i>	Stage 4 > (1, 2, 3)	-0.2 (±0.28)	-0.72	1.0000	
<i>S. purpurea</i>	Stage 1 > (2, 3, 4)	0.58 (±0.27)	2.15	0.1859	
<i>S. purpurea</i>	Stage 2 > (1, 3, 4)	0.27 (±0.19)	1.43	0.6840	
<i>S. purpurea</i>	Stage 3 > (1, 2, 4)	-0.28 (±0.19)	-1.44	0.6723	
<i>S. purpurea</i>	Stage 4 > (1, 2, 3)	-0.58 (±0.27)	-2.14	0.1886	
Stage 1	<i>S. x leucophylla</i> > <i>S. x Juthatip</i> soper	0.01 (±0.35)	0.04	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.14 (±0.35)	-0.39	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.51 (±0.35)	-1.44	1.0000	
Stage 1	<i>S. x Juthatip</i> soper > <i>S. x mitchelliana</i>	-0.15 (±0.35)	-0.43	1.0000	
Stage 1	<i>S. x Juthatip</i> soper > <i>S. purpurea</i>	-0.52 (±0.35)	-1.47	0.9583	
Stage 1	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.37 (±0.35)	-1.04	1.0000	
Stage 2	<i>S. x leucophylla</i> > <i>S. x Juthatip</i> soper	0.16 (±0.2)	0.78	1.0000	

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Stage 2	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.25 (±0.2)	1.20	1.0000
Stage 2	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.25 (±0.2)	-1.24	1.0000
Stage 2	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.09 (±0.2)	0.42	1.0000
Stage 2	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.41 (±0.2)	-2.02	0.3586
Stage 2	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.5 (±0.2)	-2.44	0.1550
Stage 3	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.35 (±0.2)	1.72	0.6201
Stage 3	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.18 (±0.2)	0.89	1.0000
Stage 3	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.49 (±0.2)	2.39	0.1708
Stage 3	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.17 (±0.2)	-0.83	1.0000
Stage 3	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.14 (±0.2)	0.68	1.0000
Stage 3	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.31 (±0.2)	1.50	0.9048
Stage 4	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.75 (±0.35)	2.12	0.2950
Stage 4	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.62 (±0.36)	1.74	0.5913
Stage 4	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.82 (±0.35)	2.31	0.2012
Stage 4	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.13 (±0.36)	-0.36	1.0000
Stage 4	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.07 (±0.35)	0.19	1.0000
Stage 4	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.2 (±0.36)	0.55	1.0000

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Table S3. Results of the post-hoc tests associated to the best linear mixed model, presented in Table 2, to explain the variation in colour contrast between any two pitcher areas. The variable colour contrast was square-root transformed to match residual normality. P-values were adjusted for multiple comparisons with the Bonferroni correction. Symbols describe various levels of p-values *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$. Within a level of factor 1, we tested the oriented contrast between two levels of factor 2 or between 2 grouped levels of factor 2. For instance, for the contrast produced by areoles against veins, we tested whether the colour contrast of *S. x leucophylla* was higher than the colour contrast of *S. x Juthatip soper* and it was significantly different (p-value=0.0039). Symbols “-“ mean that the oriented contrasts were tested overall and not within a level of another factor. For instance, we tested whether the colour contrast of *S. x leucophylla* was higher than the colour contrast of *S. x Juthatip soper* overall.

Level on factor 1	Contrast on factor 2	Coefficient (\pm standard error)	t-ratio	adjusted p-value	
-	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.31 (± 0.1)	3.11	0.0427	*
-	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.47 (± 0.1)	4.69	0.0017	**
-	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.64 (± 0.1)	6.35	<0.001	***
-	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.16 (± 0.1)	1.58	0.8049	
-	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.33 (± 0.1)	3.24	0.0327	*
-	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.17 (± 0.1)	1.66	0.7099	
-	Stage 1 > Stage 2	-0.05 (± 0.12)	-0.41	1.0000	
-	Stage 1 > Stage 3	-0.03 (± 0.1)	-0.32	1.0000	
-	Stage 1 > Stage 4	0.03 (± 0.12)	0.26	1.0000	
-	Stage 2 > Stage 3	0.02 (± 0.1)	0.18	1.0000	
-	Stage 2 > Stage 4	0.08 (± 0.12)	0.67	1.0000	
-	Stage 3 > Stage 4	0.06 (± 0.1)	0.64	1.0000	
-	Areoles-Veins > Areoles-Peristome	-0.22 (± 0.02)	-13.49	<0.001	***
-	Areoles-Veins > Areoles-Tube	0.16 (± 0.02)	10.16	<0.001	***
-	Areoles-Veins > Veins-Peristome	0.34 (± 0.02)	21.06	<0.001	***
-	Areoles-Veins > Veins-Tube	0.35 (± 0.02)	22.01	<0.001	***
-	Areoles-Veins > Peristome-Tube	0.03 (± 0.02)	1.34	1.0000	
-	Areoles-Peristome > Areoles-Tube	0.38 (± 0.02)	20.47	<0.001	***
-	Areoles-Peristome > Veins-Peristome	0.56 (± 0.02)	29.82	<0.001	***
-	Areoles-Peristome > Veins-Tube	0.57 (± 0.02)	30.68	<0.001	***
-	Areoles-Peristome > Peristome-Tube	0.25 (± 0.02)	10.80	<0.001	***
-	Areoles-Tube > Veins-Peristome	0.18 (± 0.02)	9.54	<0.001	***
-	Areoles-Tube > Veins-Tube	0.19 (± 0.02)	10.23	<0.001	***
-	Areoles-Tube > Peristome-Tube	-0.14 (± 0.02)	-5.95	<0.001	***
-	Veins-Peristome > Veins-Tube	0.01 (± 0.02)	0.60	1.0000	
-	Veins-Peristome > Peristome-Tube	-0.31 (± 0.02)	-13.70	<0.001	***
-	Veins-Tube > Peristome-Tube	-0.33 (± 0.02)	-14.27	<0.001	***
-	summer > autumn	-0.8 (± 0.11)	-7.51	<0.001	***
Areoles-Veins	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.43 (± 0.1)	4.19	0.0039	**
Areoles-Veins	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.64 (± 0.1)	6.21	<0.001	***

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Areoles-Veins	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.91 (±0.1)	8.83	<0.001	***
Areoles-Veins	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.21 (±0.1)	2.03	0.3569	
Areoles-Veins	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.48 (±0.1)	4.65	0.0015	**
Areoles-Veins	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.27 (±0.1)	2.62	0.1099	
Areoles-Peristome	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.72 (±0.11)	6.69	<0.001	***
Areoles-Peristome	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	1.18 (±0.11)	10.98	<0.001	***
Areoles-Peristome	<i>S. x leucophylla</i> > <i>S. purpurea</i>	1.09 (±0.11)	10.16	<0.001	***
Areoles-Peristome	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.46 (±0.11)	4.32	0.0022	**
Areoles-Peristome	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.37 (±0.11)	3.48	0.0156	*
Areoles-Peristome	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.09 (±0.11)	-0.86	1.0000	
Areoles-Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.37 (±0.11)	3.46	0.0159	*
Areoles-Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	1.08 (±0.11)	10.12	<0.001	***
Areoles-Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	1.19 (±0.11)	11.16	<0.001	***
Areoles-Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.71 (±0.11)	6.66	<0.001	***
Areoles-Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.82 (±0.11)	7.70	<0.001	***
Areoles-Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.11 (±0.11)	1.03	1.0000	
Veins-Peristome	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.16 (±0.11)	1.48	0.9419	
Veins-Peristome	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.42 (±0.11)	3.94	0.0053	**
Veins-Peristome	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.35 (±0.11)	3.26	0.0250	*
Veins-Peristome	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.26 (±0.11)	2.47	0.1388	
Veins-Peristome	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.19 (±0.11)	1.79	0.5366	
Veins-Peristome	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.07 (±0.11)	-0.69	1.0000	
Veins-Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.06 (±0.11)	-0.53	1.0000	
Veins-Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.58 (±0.11)	-5.45	<0.001	***
Veins-Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.08 (±0.11)	0.73	1.0000	
Veins-Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.52 (±0.11)	-4.92	<0.001	***
Veins-Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.13 (±0.11)	1.26	1.0000	
Veins-Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.66 (±0.11)	6.18	<0.001	***
Peristome-Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.27 (±0.11)	2.38	0.1558	
Peristome-Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.11 (±0.11)	0.95	1.0000	
Peristome-Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.24 (±0.11)	2.13	0.2640	
Peristome-Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.16 (±0.11)	-1.41	1.0000	
Peristome-Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.03 (±0.11)	-0.25	1.0000	
Peristome-Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.13 (±0.11)	1.17	1.0000	
<i>S. x leucophylla</i>	Areoles-Veins > Areoles-Peristome	-0.47 (±0.03)	-13.78	<0.001	***
<i>S. x leucophylla</i>	Areoles-Veins > Areoles-Tube	0 (±0.03)	0.06	1.0000	
<i>S. x leucophylla</i>	Areoles-Veins > Veins-Peristome	0.61 (±0.03)	18.15	<0.001	***
<i>S. x leucophylla</i>	Areoles-Veins > Veins-Tube	0.99 (±0.03)	30.16	<0.001	***
<i>S. x leucophylla</i>	Areoles-Veins > Peristome-Tube	0.37 (±0.04)	9.02	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Areoles-Tube	0.47 (±0.04)	12.27	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Veins-Peristome	1.08 (±0.04)	27.93	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Veins-Tube	1.46 (±0.04)	38.43	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Peristome-Tube	0.84 (±0.05)	18.50	<0.001	***
<i>S. x leucophylla</i>	Areoles-Tube > Veins-Peristome	0.61 (±0.04)	16.01	<0.001	***
<i>S. x leucophylla</i>	Areoles-Tube > Veins-Tube	0.99 (±0.04)	26.51	<0.001	***

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<i>S. x leucophylla</i>	Areoles-Tube > Peristome-Tube	0.37 (±0.04)	8.25	<0.001	***
<i>S. x leucophylla</i>	Veins-Peristome > Veins-Tube	0.38 (±0.04)	10.18	<0.001	***
<i>S. x leucophylla</i>	Veins-Peristome > Peristome-Tube	-0.24 (±0.05)	-5.25	<0.001	***
<i>S. x leucophylla</i>	Veins-Tube > Peristome-Tube	-0.62 (±0.04)	-13.88	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Areoles-Peristome	-0.19 (±0.03)	-5.81	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Areoles-Tube	-0.06 (±0.03)	-1.95	0.7761	
<i>S. x Juthatip soper</i>	Areoles-Veins > Veins-Peristome	0.33 (±0.03)	10.48	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Veins-Tube	0.5 (±0.03)	15.77	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Peristome-Tube	0.21 (±0.04)	4.97	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Areoles-Tube	0.12 (±0.04)	3.32	0.0135	*
<i>S. x Juthatip soper</i>	Areoles-Peristome > Veins-Peristome	0.52 (±0.04)	14.06	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Veins-Tube	0.69 (±0.04)	18.61	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Peristome-Tube	0.39 (±0.05)	8.61	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Tube > Veins-Peristome	0.39 (±0.04)	10.68	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Tube > Veins-Tube	0.56 (±0.04)	15.23	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Tube > Peristome-Tube	0.27 (±0.05)	5.89	<0.001	***
<i>S. x Juthatip soper</i>	Veins-Peristome > Veins-Tube	0.17 (±0.04)	4.62	<0.001	***
<i>S. x Juthatip soper</i>	Veins-Peristome > Peristome-Tube	-0.13 (±0.05)	-2.78	0.0806	
<i>S. x Juthatip soper</i>	Veins-Tube > Peristome-Tube	-0.29 (±0.05)	-6.51	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Areoles-Peristome	0.07 (±0.03)	2.02	0.6497	
<i>S. x mitchelliana</i>	Areoles-Veins > Areoles-Tube	0.44 (±0.03)	13.61	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Veins-Peristome	0.39 (±0.03)	11.62	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Veins-Tube	-0.23 (±0.03)	-7.22	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Peristome-Tube	-0.16 (±0.04)	-3.79	0.0022	**
<i>S. x mitchelliana</i>	Areoles-Peristome > Areoles-Tube	0.37 (±0.04)	9.75	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Peristome > Veins-Peristome	0.32 (±0.04)	8.20	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Peristome > Veins-Tube	-0.3 (±0.04)	-7.87	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Peristome > Peristome-Tube	-0.23 (±0.05)	-4.84	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Tube > Veins-Peristome	-0.05 (±0.04)	-1.36	1.0000	
<i>S. x mitchelliana</i>	Areoles-Tube > Veins-Tube	-0.67 (±0.04)	-18.02	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Tube > Peristome-Tube	-0.6 (±0.05)	-12.80	<0.001	***
<i>S. x mitchelliana</i>	Veins-Peristome > Veins-Tube	-0.62 (±0.04)	-16.24	<0.001	***
<i>S. x mitchelliana</i>	Veins-Peristome > Peristome-Tube	-0.55 (±0.05)	-11.53	<0.001	***
<i>S. x mitchelliana</i>	Veins-Tube > Peristome-Tube	0.07 (±0.05)	1.47	1.0000	
<i>S. purpurea</i>	Areoles-Veins > Areoles-Peristome	-0.3 (±0.03)	-9.36	<0.001	***
<i>S. purpurea</i>	Areoles-Veins > Areoles-Tube	0.28 (±0.03)	8.81	<0.001	***
<i>S. purpurea</i>	Areoles-Veins > Veins-Peristome	0.04 (±0.03)	1.35	1.0000	
<i>S. purpurea</i>	Areoles-Veins > Veins-Tube	0.15 (±0.03)	4.91	<0.001	***
<i>S. purpurea</i>	Areoles-Veins > Peristome-Tube	-0.3 (±0.04)	-7.29	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Areoles-Tube	0.57 (±0.04)	15.64	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Veins-Peristome	0.34 (±0.04)	9.23	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Veins-Tube	0.45 (±0.04)	12.29	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Peristome-Tube	-0.01 (±0.05)	-0.14	1.0000	
<i>S. purpurea</i>	Areoles-Tube > Veins-Peristome	-0.23 (±0.04)	-6.42	<0.001	***
<i>S. purpurea</i>	Areoles-Tube > Veins-Tube	-0.12 (±0.04)	-3.35	0.0121	*

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<i>S. purpurea</i>	Areoles-Tube > Peristome-Tube	-0.58 (±0.05)	-12.77	<0.001	***
<i>S. purpurea</i>	Veins-Peristome > Veins-Tube	0.11 (±0.04)	3.06	0.0329	*
<i>S. purpurea</i>	Veins-Peristome > Peristome-Tube	-0.34 (±0.05)	-7.59	<0.001	***
<i>S. purpurea</i>	Veins-Tube > Peristome-Tube	-0.46 (±0.05)	-10.06	<0.001	***
Stage 1	Areoles-Veins > Areoles-Peristome	-0.15 (±0.04)	-3.70	0.0032	**
Stage 1	Areoles-Veins > Areoles-Tube	0.03 (±0.04)	0.86	1.0000	
Stage 1	Areoles-Veins > Veins-Peristome	0.27 (±0.04)	6.97	<0.001	***
Stage 1	Areoles-Veins > Veins-Tube	0.28 (±0.04)	7.32	<0.001	***
Stage 1	Areoles-Veins > Peristome-Tube	-0.01 (±0.05)	-0.14	1.0000	
Stage 1	Areoles-Peristome > Areoles-Tube	0.18 (±0.05)	3.97	0.0011	**
Stage 1	Areoles-Peristome > Veins-Peristome	0.42 (±0.05)	9.22	<0.001	***
Stage 1	Areoles-Peristome > Veins-Tube	0.43 (±0.05)	9.53	<0.001	***
Stage 1	Areoles-Peristome > Peristome-Tube	0.14 (±0.06)	2.54	0.1665	
Stage 1	Areoles-Tube > Veins-Peristome	0.24 (±0.05)	5.31	<0.001	***
Stage 1	Areoles-Tube > Veins-Tube	0.25 (±0.04)	5.58	<0.001	***
Stage 1	Areoles-Tube > Peristome-Tube	-0.04 (±0.05)	-0.75	1.0000	
Stage 1	Veins-Peristome > Veins-Tube	0.01 (±0.04)	0.22	1.0000	
Stage 1	Veins-Peristome > Peristome-Tube	-0.28 (±0.05)	-5.11	<0.001	***
Stage 1	Veins-Tube > Peristome-Tube	-0.29 (±0.05)	-5.33	<0.001	***
Stage 2	Areoles-Veins > Areoles-Peristome	-0.37 (±0.02)	-16.07	<0.001	***
Stage 2	Areoles-Veins > Areoles-Tube	0.23 (±0.02)	10.03	<0.001	***
Stage 2	Areoles-Veins > Veins-Peristome	0.33 (±0.02)	14.36	<0.001	***
Stage 2	Areoles-Veins > Veins-Tube	0.73 (±0.02)	32.15	<0.001	***
Stage 2	Areoles-Veins > Peristome-Tube	0.25 (±0.03)	8.51	<0.001	***
Stage 2	Areoles-Peristome > Areoles-Tube	0.6 (±0.03)	22.61	<0.001	***
Stage 2	Areoles-Peristome > Veins-Peristome	0.69 (±0.03)	26.35	<0.001	***
Stage 2	Areoles-Peristome > Veins-Tube	1.1 (±0.03)	41.76	<0.001	***
Stage 2	Areoles-Peristome > Peristome-Tube	0.62 (±0.03)	19.14	<0.001	***
Stage 2	Areoles-Tube > Veins-Peristome	0.1 (±0.03)	3.74	0.0027	**
Stage 2	Areoles-Tube > Veins-Tube	0.5 (±0.03)	19.15	<0.001	***
Stage 2	Areoles-Tube > Peristome-Tube	0.02 (±0.03)	0.68	1.0000	
Stage 2	Veins-Peristome > Veins-Tube	0.41 (±0.03)	15.41	<0.001	***
Stage 2	Veins-Peristome > Peristome-Tube	-0.08 (±0.03)	-2.38	0.2608	
Stage 2	Veins-Tube > Peristome-Tube	-0.48 (±0.03)	-14.96	<0.001	***
Stage 3	Areoles-Veins > Areoles-Peristome	-0.14 (±0.02)	-6.25	<0.001	***
Stage 3	Areoles-Veins > Areoles-Tube	0.24 (±0.02)	10.41	<0.001	***
Stage 3	Areoles-Veins > Veins-Peristome	0.46 (±0.02)	20.13	<0.001	***
Stage 3	Areoles-Veins > Veins-Tube	0.26 (±0.02)	11.36	<0.001	***
Stage 3	Areoles-Veins > Peristome-Tube	0.04 (±0.03)	1.33	1.0000	
Stage 3	Areoles-Peristome > Areoles-Tube	0.38 (±0.03)	14.48	<0.001	***
Stage 3	Areoles-Peristome > Veins-Peristome	0.6 (±0.03)	22.94	<0.001	***
Stage 3	Areoles-Peristome > Veins-Tube	0.4 (±0.03)	15.29	<0.001	***
Stage 3	Areoles-Peristome > Peristome-Tube	0.18 (±0.03)	5.65	<0.001	***
Stage 3	Areoles-Tube > Veins-Peristome	0.22 (±0.03)	8.40	<0.001	***
Stage 3	Areoles-Tube > Veins-Tube	0.02 (±0.03)	0.84	1.0000	

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Stage 3	Areoles-Tube > Peristome-Tube	-0.2 (±0.03)	-6.20	<0.001	***
Stage 3	Veins-Peristome > Veins-Tube	-0.2 (±0.03)	-7.54	<0.001	***
Stage 3	Veins-Peristome > Peristome-Tube	-0.42 (±0.03)	-13.10	<0.001	***
Stage 3	Veins-Tube > Peristome-Tube	-0.22 (±0.03)	-6.88	<0.001	***
Stage 4	Areoles-Veins > Areoles-Peristome	-0.22 (±0.04)	-5.55	<0.001	***
Stage 4	Areoles-Veins > Areoles-Tube	0.15 (±0.04)	3.89	0.0015	**
Stage 4	Areoles-Veins > Veins-Peristome	0.31 (±0.04)	7.61	<0.001	***
Stage 4	Areoles-Veins > Veins-Tube	0.14 (±0.04)	3.41	0.0098	**
Stage 4	Areoles-Veins > Peristome-Tube	-0.17 (±0.05)	-3.27	0.0164	*
Stage 4	Areoles-Peristome > Areoles-Tube	0.38 (±0.05)	8.16	<0.001	***
Stage 4	Areoles-Peristome > Veins-Peristome	0.53 (±0.05)	11.30	<0.001	***
Stage 4	Areoles-Peristome > Veins-Tube	0.36 (±0.05)	7.75	<0.001	***
Stage 4	Areoles-Peristome > Peristome-Tube	0.05 (±0.06)	0.93	1.0000	
Stage 4	Areoles-Tube > Veins-Peristome	0.15 (±0.05)	3.31	0.0143	*
Stage 4	Areoles-Tube > Veins-Tube	-0.02 (±0.05)	-0.42	1.0000	
Stage 4	Areoles-Tube > Peristome-Tube	-0.33 (±0.06)	-5.70	<0.001	***
Stage 4	Veins-Peristome > Veins-Tube	-0.17 (±0.05)	-3.72	0.0030	**
Stage 4	Veins-Peristome > Peristome-Tube	-0.48 (±0.06)	-8.31	<0.001	***
Stage 4	Veins-Tube > Peristome-Tube	-0.31 (±0.06)	-5.36	<0.001	***
Stage 1	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.14 (±0.25)	-0.57	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.19 (±0.25)	0.76	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.34 (±0.25)	1.36	1.0000	
Stage 1	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.33 (±0.25)	1.33	1.0000	
Stage 1	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.48 (±0.25)	1.93	0.4394	
Stage 1	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.15 (±0.25)	0.60	1.0000	
Stage 2	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.21 (±0.14)	1.49	0.9426	
Stage 2	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.67 (±0.14)	4.71	0.0017	**
Stage 2	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.51 (±0.14)	3.53	0.0179	*
Stage 2	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.46 (±0.14)	3.22	0.0342	*
Stage 2	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.29 (±0.14)	2.05	0.3519	
Stage 2	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.17 (±0.14)	-1.17	1.0000	
Stage 3	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.28 (±0.14)	1.96	0.4101	
Stage 3	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.31 (±0.14)	2.18	0.2753	
Stage 3	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.79 (±0.14)	5.56	<0.001	***
Stage 3	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.03 (±0.14)	0.21	1.0000	
Stage 3	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.51 (±0.14)	3.60	0.0158	*
Stage 3	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.48 (±0.14)	3.38	0.0244	*
Stage 4	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.91 (±0.25)	3.65	0.0139	*
Stage 4	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.73 (±0.25)	2.93	0.0619	
Stage 4	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.93 (±0.25)	3.76	0.0111	*
Stage 4	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.18 (±0.25)	-0.73	1.0000	
Stage 4	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.03 (±0.25)	0.11	1.0000	
Stage 4	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.21 (±0.25)	0.83	1.0000	
Areoles-Veins	Stage 1 > (2, 3, 4)	-0.07 (±0.1)	-0.68	1.0000	
Areoles-Veins	Stage 2 > (1, 3, 4)	0.16 (±0.1)	1.64	0.4805	

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Areoles-Veins	Stage 3 > (1, 2, 4)	0.07 (± 0.07)	0.94	1.0000
Areoles-Veins	Stage 4 > (1, 2, 3)	-0.16 (± 0.1)	-1.63	0.4902
Areoles-Peristome	Stage 1 > (2, 3, 4)	-0.16 (± 0.1)	-1.59	0.5123
Areoles-Peristome	Stage 2 > (1, 3, 4)	0.36 (± 0.1)	3.56	0.0099 **
Areoles-Peristome	Stage 3 > (1, 2, 4)	-0.04 (± 0.07)	-0.48	1.0000
Areoles-Peristome	Stage 4 > (1, 2, 3)	-0.16 (± 0.1)	-1.52	0.5816
Areoles-Tube	Stage 1 > (2, 3, 4)	0.11 (± 0.1)	1.02	1.0000
Areoles-Tube	Stage 2 > (1, 3, 4)	0.08 (± 0.1)	0.76	1.0000
Areoles-Tube	Stage 3 > (1, 2, 4)	-0.03 (± 0.07)	-0.41	1.0000
Areoles-Tube	Stage 4 > (1, 2, 3)	-0.15 (± 0.1)	-1.46	0.6447
Veins-Peristome	Stage 1 > (2, 3, 4)	0.02 (± 0.1)	0.23	1.0000
Veins-Peristome	Stage 2 > (1, 3, 4)	0.18 (± 0.1)	1.81	0.3532
Veins-Peristome	Stage 3 > (1, 2, 4)	-0.09 (± 0.07)	-1.17	1.0000
Veins-Peristome	Stage 4 > (1, 2, 3)	-0.12 (± 0.1)	-1.14	1.0000
Veins-Tube	Stage 1 > (2, 3, 4)	0.03 (± 0.1)	0.24	1.0000
Veins-Tube	Stage 2 > (1, 3, 4)	-0.34 (± 0.1)	-3.42	0.0134 *
Veins-Tube	Stage 3 > (1, 2, 4)	0.19 (± 0.07)	2.56	0.0776
Veins-Tube	Stage 4 > (1, 2, 3)	0.13 (± 0.1)	1.23	0.9329
Peristome-Tube	Stage 1 > (2, 3, 4)	-0.02 (± 0.11)	-0.20	1.0000
Peristome-Tube	Stage 2 > (1, 3, 4)	-0.13 (± 0.1)	-1.29	0.8463
Peristome-Tube	Stage 3 > (1, 2, 4)	0.05 (± 0.08)	0.68	1.0000
Peristome-Tube	Stage 4 > (1, 2, 3)	0.1 (± 0.11)	0.93	1.0000
<i>S. x leucophylla</i>	Stage 1 > (2, 3, 4)	-0.37 (± 0.19)	-1.91	0.3034
<i>S. x leucophylla</i>	Stage 2 > (1, 3, 4)	0.04 (± 0.15)	0.24	1.0000
<i>S. x leucophylla</i>	Stage 3 > (1, 2, 4)	0.01 (± 0.14)	0.09	1.0000
<i>S. x leucophylla</i>	Stage 4 > (1, 2, 3)	0.32 (± 0.19)	1.65	0.4772
<i>S. x Juthatip soper</i>	Stage 1 > (2, 3, 4)	0.24 (± 0.19)	1.26	0.9133
<i>S. x Juthatip soper</i>	Stage 2 > (1, 3, 4)	0.17 (± 0.15)	1.13	1.0000
<i>S. x Juthatip soper</i>	Stage 3 > (1, 2, 4)	0.06 (± 0.14)	0.42	1.0000
<i>S. x Juthatip soper</i>	Stage 4 > (1, 2, 3)	-0.47 (± 0.19)	-2.45	0.1080
<i>S. x mitchelliana</i>	Stage 1 > (2, 3, 4)	0.02 (± 0.19)	0.08	1.0000
<i>S. x mitchelliana</i>	Stage 2 > (1, 3, 4)	-0.23 (± 0.15)	-1.49	0.6249
<i>S. x mitchelliana</i>	Stage 3 > (1, 2, 4)	0.23 (± 0.14)	1.68	0.4565
<i>S. x mitchelliana</i>	Stage 4 > (1, 2, 3)	-0.02 (± 0.19)	-0.09	1.0000
<i>S. purpurea</i>	Stage 1 > (2, 3, 4)	0.04 (± 0.19)	0.22	1.0000
<i>S. purpurea</i>	Stage 2 > (1, 3, 4)	0.22 (± 0.15)	1.44	0.6844
<i>S. purpurea</i>	Stage 3 > (1, 2, 4)	-0.19 (± 0.14)	-1.40	0.7251
<i>S. purpurea</i>	Stage 4 > (1, 2, 3)	-0.07 (± 0.19)	-0.36	1.0000

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Table S4. Results of the post-hoc tests associated to the best linear mixed model, presented in Table 2, to explain the variation in brightness contrast between any two pitcher areas. The variable brightness contrast was square-root transformed to match residual normality. P-values were adjusted for multiple comparisons with the Bonferroni correction. Symbols describe various levels of p-values *: p<0.05, **: p<0.01, and ***: p<0.001. Within a level of factor 1, we tested the oriented contrast between two levels of factor 2 or between 2 grouped levels of factor 2. For instance, for the contrast produced by areoles against veins, we tested whether the brightness contrast of *S. x leucophylla* was higher than the brightness contrast of *S. x Juthatip soper* and it was significantly different (p-value=0.0136). Symbols “-“ mean that the oriented contrasts were tested overall and not within a level of another factor. For instance, we tested whether the brightness contrast of *S. x leucophylla* was higher than the brightness contrast of *S. x Juthatip soper* overall.

Level on factor 1	Contrast on factor 2	Coefficient (± standard error)	t-ratio	adjusted p-value	
-	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.52 (±0.16)	3.20	0.0357	*
-	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.45 (±0.16)	2.81	0.0790	
-	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.55 (±0.16)	3.40	0.0238	*
-	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.06 (±0.16)	-0.39	1.0000	
-	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.03 (±0.16)	0.20	1.0000	
-	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.09 (±0.16)	0.59	1.0000	
-	Stage 1 > Stage 2	-0.29 (±0.2)	-1.47	0.9718	
-	Stage 1 > Stage 3	-0.49 (±0.16)	-3.04	0.0489	*
-	Stage 1 > Stage 4	-0.47 (±0.2)	-2.39	0.1803	
-	Stage 2 > Stage 3	-0.2 (±0.16)	-1.25	1.0000	
-	Stage 2 > Stage 4	-0.18 (±0.2)	-0.93	1.0000	
-	Stage 3 > Stage 4	0.02 (±0.16)	0.11	1.0000	
-	Areoles-Veins > Areoles-Peristome	-0.58 (±0.03)	-21.31	<0.001	***
-	Areoles-Veins > Areoles-Tube	0.48 (±0.03)	17.87	<0.001	***
-	Areoles-Veins > Veins-Peristome	0.08 (±0.03)	3.02	0.0386	*
-	Areoles-Veins > Veins-Tube	0.39 (±0.03)	14.56	<0.001	***
-	Areoles-Veins > Peristome-Tube	-0.27 (±0.04)	-7.73	<0.001	***
-	Areoles-Peristome > Areoles-Tube	1.07 (±0.03)	33.91	<0.001	***
-	Areoles-Peristome > Veins-Peristome	0.67 (±0.03)	21.06	<0.001	***
-	Areoles-Peristome > Veins-Tube	0.98 (±0.03)	31.13	<0.001	***
-	Areoles-Peristome > Peristome-Tube	0.31 (±0.04)	8.12	<0.001	***
-	Areoles-Tube > Veins-Peristome	-0.4 (±0.03)	-12.81	<0.001	***
-	Areoles-Tube > Veins-Tube	-0.09 (±0.03)	-2.95	0.0477	*
-	Areoles-Tube > Peristome-Tube	-0.75 (±0.04)	-19.68	<0.001	***
-	Veins-Peristome > Veins-Tube	0.31 (±0.03)	9.92	<0.001	***
-	Veins-Peristome > Peristome-Tube	-0.35 (±0.04)	-9.18	<0.001	***
-	Veins-Tube > Peristome-Tube	-0.66 (±0.04)	-17.34	<0.001	***
-	summer > autumn	-0.45 (±0.17)	-2.65	0.0181	*
Areoles-Veins	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.59 (±0.17)	3.60	0.0136	*
Areoles-Veins	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.34 (±0.17)	2.03	0.3510	

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Areoles-Veins	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.54 (±0.17)	3.25	0.0290	*
Areoles-Veins	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.26 (±0.16)	-1.57	0.8092	
Areoles-Veins	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.06 (±0.16)	-0.36	1.0000	
Areoles-Veins	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.2 (±0.16)	1.22	1.0000	
Areoles-Peristome	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	1.07 (±0.17)	6.24	<0.001	***
Areoles-Peristome	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	1.36 (±0.17)	7.91	<0.001	***
Areoles-Peristome	<i>S. x leucophylla</i> > <i>S. purpurea</i>	1.05 (±0.17)	6.16	<0.001	***
Areoles-Peristome	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.29 (±0.17)	1.69	0.6405	
Areoles-Peristome	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.01 (±0.17)	-0.09	1.0000	
Areoles-Peristome	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.3 (±0.17)	-1.78	0.5443	
Areoles-Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.49 (±0.17)	2.85	0.0614	
Areoles-Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.78 (±0.17)	4.57	0.0012	**
Areoles-Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.74 (±0.17)	4.32	0.0022	**
Areoles-Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.29 (±0.17)	1.72	0.6104	
Areoles-Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.25 (±0.17)	1.47	0.9513	
Areoles-Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.04 (±0.17)	-0.25	1.0000	
Veins-Peristome	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.48 (±0.17)	2.84	0.0633	
Veins-Peristome	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.57 (±0.17)	3.30	0.0219	*
Veins-Peristome	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.62 (±0.17)	3.61	0.0111	*
Veins-Peristome	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.08 (±0.17)	0.48	1.0000	
Veins-Peristome	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.13 (±0.17)	0.78	1.0000	
Veins-Peristome	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.05 (±0.17)	0.29	1.0000	
Veins-Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	-0.05 (±0.17)	-0.27	1.0000	
Veins-Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.77 (±0.17)	-4.53	0.0014	**
Veins-Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	-0.19 (±0.17)	-1.10	1.0000	
Veins-Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.73 (±0.17)	-4.27	0.0025	**
Veins-Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.14 (±0.17)	-0.83	1.0000	
Veins-Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.59 (±0.17)	3.43	0.0167	*
Peristome-Tube	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.5 (±0.18)	2.76	0.0645	
Peristome-Tube	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.45 (±0.18)	2.45	0.1303	
Peristome-Tube	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.52 (±0.18)	2.90	0.0475	*
Peristome-Tube	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.05 (±0.18)	-0.30	1.0000	
Peristome-Tube	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.02 (±0.18)	0.13	1.0000	
Peristome-Tube	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.08 (±0.18)	0.43	1.0000	
<i>S. x leucophylla</i>	Areoles-Veins > Areoles-Peristome	-1.09 (±0.06)	-19.12	<0.001	***
<i>S. x leucophylla</i>	Areoles-Veins > Areoles-Tube	0.35 (±0.06)	6.27	<0.001	***
<i>S. x leucophylla</i>	Areoles-Veins > Veins-Peristome	0.03 (±0.06)	0.57	1.0000	
<i>S. x leucophylla</i>	Areoles-Veins > Veins-Tube	1.01 (±0.06)	18.33	<0.001	***
<i>S. x leucophylla</i>	Areoles-Veins > Peristome-Tube	-0.27 (±0.07)	-3.94	0.0012	**
<i>S. x leucophylla</i>	Areoles-Peristome > Areoles-Tube	1.44 (±0.06)	22.40	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Veins-Peristome	1.12 (±0.06)	17.34	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Veins-Tube	2.1 (±0.06)	32.97	<0.001	***
<i>S. x leucophylla</i>	Areoles-Peristome > Peristome-Tube	0.82 (±0.08)	10.72	<0.001	***
<i>S. x leucophylla</i>	Areoles-Tube > Veins-Peristome	-0.32 (±0.06)	-5.00	<0.001	***
<i>S. x leucophylla</i>	Areoles-Tube > Veins-Tube	0.66 (±0.06)	10.56	<0.001	***

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<i>S. x leucophylla</i>	Areoles-Tube > Peristome-Tube	-0.62 (±0.08)	-8.26	<0.001	***
<i>S. x leucophylla</i>	Veins-Peristome > Veins-Tube	0.98 (±0.06)	15.53	<0.001	***
<i>S. x leucophylla</i>	Veins-Peristome > Peristome-Tube	-0.3 (±0.08)	-4.02	<0.001	***
<i>S. x leucophylla</i>	Veins-Tube > Peristome-Tube	-1.28 (±0.07)	-17.15	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Areoles-Peristome	-0.61 (±0.05)	-11.43	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Areoles-Tube	0.24 (±0.05)	4.48	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Veins-Peristome	-0.08 (±0.05)	-1.48	1.0000	
<i>S. x Juthatip soper</i>	Areoles-Veins > Veins-Tube	0.37 (±0.05)	6.95	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Veins > Peristome-Tube	-0.37 (±0.07)	-5.27	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Areoles-Tube	0.86 (±0.06)	13.70	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Veins-Peristome	0.54 (±0.06)	8.67	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Veins-Tube	0.98 (±0.06)	15.90	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Peristome > Peristome-Tube	0.25 (±0.08)	3.25	0.0177	*
<i>S. x Juthatip soper</i>	Areoles-Tube > Veins-Peristome	-0.32 (±0.06)	-5.17	<0.001	***
<i>S. x Juthatip soper</i>	Areoles-Tube > Veins-Tube	0.13 (±0.06)	2.08	0.5705	
<i>S. x Juthatip soper</i>	Areoles-Tube > Peristome-Tube	-0.61 (±0.08)	-7.95	<0.001	***
<i>S. x Juthatip soper</i>	Veins-Peristome > Veins-Tube	0.45 (±0.06)	7.31	<0.001	***
<i>S. x Juthatip soper</i>	Veins-Peristome > Peristome-Tube	-0.29 (±0.08)	-3.79	0.0023	**
<i>S. x Juthatip soper</i>	Veins-Tube > Peristome-Tube	-0.74 (±0.08)	-9.69	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Areoles-Peristome	-0.07 (±0.06)	-1.17	1.0000	
<i>S. x mitchelliana</i>	Areoles-Veins > Areoles-Tube	0.79 (±0.05)	14.69	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Veins-Peristome	0.26 (±0.06)	4.71	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Veins > Veins-Tube	-0.1 (±0.05)	-1.82	1.0000	
<i>S. x mitchelliana</i>	Areoles-Veins > Peristome-Tube	-0.16 (±0.07)	-2.23	0.3884	
<i>S. x mitchelliana</i>	Areoles-Peristome > Areoles-Tube	0.86 (±0.06)	13.45	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Peristome > Veins-Peristome	0.33 (±0.07)	5.02	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Peristome > Veins-Tube	-0.03 (±0.06)	-0.52	1.0000	
<i>S. x mitchelliana</i>	Areoles-Peristome > Peristome-Tube	-0.1 (±0.08)	-1.20	1.0000	
<i>S. x mitchelliana</i>	Areoles-Tube > Veins-Peristome	-0.53 (±0.06)	-8.31	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Tube > Veins-Tube	-0.89 (±0.06)	-14.28	<0.001	***
<i>S. x mitchelliana</i>	Areoles-Tube > Peristome-Tube	-0.96 (±0.08)	-12.11	<0.001	***
<i>S. x mitchelliana</i>	Veins-Peristome > Veins-Tube	-0.36 (±0.06)	-5.65	<0.001	***
<i>S. x mitchelliana</i>	Veins-Peristome > Peristome-Tube	-0.42 (±0.08)	-5.30	<0.001	***
<i>S. x mitchelliana</i>	Veins-Tube > Peristome-Tube	-0.06 (±0.08)	-0.80	1.0000	
<i>S. purpurea</i>	Areoles-Veins > Areoles-Peristome	-0.57 (±0.05)	-10.78	<0.001	***
<i>S. purpurea</i>	Areoles-Veins > Areoles-Tube	0.55 (±0.05)	10.40	<0.001	***
<i>S. purpurea</i>	Areoles-Veins > Veins-Peristome	0.11 (±0.05)	2.13	0.4970	
<i>S. purpurea</i>	Areoles-Veins > Veins-Tube	0.29 (±0.05)	5.42	<0.001	***
<i>S. purpurea</i>	Areoles-Veins > Peristome-Tube	-0.28 (±0.07)	-4.09	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Areoles-Tube	1.12 (±0.06)	18.23	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Veins-Peristome	0.68 (±0.06)	11.11	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Veins-Tube	0.86 (±0.06)	13.94	<0.001	***
<i>S. purpurea</i>	Areoles-Peristome > Peristome-Tube	0.29 (±0.08)	3.76	0.0025	**
<i>S. purpurea</i>	Areoles-Tube > Veins-Peristome	-0.44 (±0.06)	-7.12	<0.001	***
<i>S. purpurea</i>	Areoles-Tube > Veins-Tube	-0.26 (±0.06)	-4.29	<0.001	***

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<i>S. purpurea</i>	Areoles-Tube > Peristome-Tube	-0.83 (±0.08)	-10.95	<0.001	***
<i>S. purpurea</i>	Veins-Peristome > Veins-Tube	0.17 (±0.06)	2.83	0.0704	
<i>S. purpurea</i>	Veins-Peristome > Peristome-Tube	-0.4 (±0.08)	-5.21	<0.001	***
<i>S. purpurea</i>	Veins-Tube > Peristome-Tube	-0.57 (±0.08)	-7.49	<0.001	***
Stage 1	Areoles-Veins > Areoles-Peristome	-0.64 (±0.07)	-9.59	<0.001	***
Stage 1	Areoles-Veins > Areoles-Tube	0.26 (±0.07)	3.93	0.0013	**
Stage 1	Areoles-Veins > Veins-Peristome	-0.32 (±0.07)	-4.84	<0.001	***
Stage 1	Areoles-Veins > Veins-Tube	0.39 (±0.06)	6.03	<0.001	***
Stage 1	Areoles-Veins > Peristome-Tube	-0.25 (±0.08)	-3.00	0.0400	*
Stage 1	Areoles-Peristome > Areoles-Tube	0.9 (±0.08)	11.74	<0.001	***
Stage 1	Areoles-Peristome > Veins-Peristome	0.32 (±0.08)	4.22	<0.001	***
Stage 1	Areoles-Peristome > Veins-Tube	1.03 (±0.08)	13.63	<0.001	***
Stage 1	Areoles-Peristome > Peristome-Tube	0.39 (±0.09)	4.19	<0.001	***
Stage 1	Areoles-Tube > Veins-Peristome	-0.58 (±0.08)	-7.62	<0.001	***
Stage 1	Areoles-Tube > Veins-Tube	0.13 (±0.08)	1.77	1.0000	
Stage 1	Areoles-Tube > Peristome-Tube	-0.51 (±0.09)	-5.55	<0.001	***
Stage 1	Veins-Peristome > Veins-Tube	0.71 (±0.07)	9.49	<0.001	***
Stage 1	Veins-Peristome > Peristome-Tube	0.07 (±0.09)	0.71	1.0000	
Stage 1	Veins-Tube > Peristome-Tube	-0.65 (±0.09)	-7.05	<0.001	***
Stage 2	Areoles-Veins > Areoles-Peristome	-0.18 (±0.04)	-4.75	<0.001	***
Stage 2	Areoles-Veins > Areoles-Tube	1 (±0.04)	26.01	<0.001	***
Stage 2	Areoles-Veins > Veins-Peristome	0.56 (±0.04)	14.63	<0.001	***
Stage 2	Areoles-Veins > Veins-Tube	0.58 (±0.04)	15.10	<0.001	***
Stage 2	Areoles-Veins > Peristome-Tube	0.39 (±0.05)	7.92	<0.001	***
Stage 2	Areoles-Peristome > Areoles-Tube	1.18 (±0.04)	26.64	<0.001	***
Stage 2	Areoles-Peristome > Veins-Peristome	0.74 (±0.04)	16.79	<0.001	***
Stage 2	Areoles-Peristome > Veins-Tube	0.76 (±0.04)	17.19	<0.001	***
Stage 2	Areoles-Peristome > Peristome-Tube	0.57 (±0.05)	10.59	<0.001	***
Stage 2	Areoles-Tube > Veins-Peristome	-0.44 (±0.04)	-9.85	<0.001	***
Stage 2	Areoles-Tube > Veins-Tube	-0.42 (±0.04)	-9.45	<0.001	***
Stage 2	Areoles-Tube > Peristome-Tube	-0.61 (±0.05)	-11.17	<0.001	***
Stage 2	Veins-Peristome > Veins-Tube	0.02 (±0.04)	0.40	1.0000	
Stage 2	Veins-Peristome > Peristome-Tube	-0.17 (±0.05)	-3.12	0.0270	*
Stage 2	Veins-Tube > Peristome-Tube	-0.19 (±0.05)	-3.45	0.0084	**
Stage 3	Areoles-Veins > Areoles-Peristome	-0.92 (±0.04)	-24.21	<0.001	***
Stage 3	Areoles-Veins > Areoles-Tube	0.38 (±0.04)	10.03	<0.001	***
Stage 3	Areoles-Veins > Veins-Peristome	-0.13 (±0.04)	-3.48	0.0076	**
Stage 3	Areoles-Veins > Veins-Tube	0.3 (±0.04)	7.71	<0.001	***
Stage 3	Areoles-Veins > Peristome-Tube	-0.68 (±0.05)	-13.87	<0.001	***
Stage 3	Areoles-Peristome > Areoles-Tube	1.31 (±0.04)	29.70	<0.001	***
Stage 3	Areoles-Peristome > Veins-Peristome	0.79 (±0.04)	17.99	<0.001	***
Stage 3	Areoles-Peristome > Veins-Tube	1.22 (±0.04)	27.65	<0.001	***
Stage 3	Areoles-Peristome > Peristome-Tube	0.24 (±0.05)	4.51	<0.001	***
Stage 3	Areoles-Tube > Veins-Peristome	-0.52 (±0.04)	-11.73	<0.001	***
Stage 3	Areoles-Tube > Veins-Tube	-0.09 (±0.04)	-1.99	0.7044	

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Stage 3	Areoles-Tube > Peristome-Tube	-1.07 (±0.05)	-19.79	<0.001	***
Stage 3	Veins-Peristome > Veins-Tube	0.43 (±0.04)	9.71	<0.001	***
Stage 3	Veins-Peristome > Peristome-Tube	-0.55 (±0.05)	-10.20	<0.001	***
Stage 3	Veins-Tube > Peristome-Tube	-0.98 (±0.05)	-18.13	<0.001	***
Stage 4	Areoles-Veins > Areoles-Peristome	-0.59 (±0.07)	-8.67	<0.001	***
Stage 4	Areoles-Veins > Areoles-Tube	0.3 (±0.07)	4.43	<0.001	***
Stage 4	Areoles-Veins > Veins-Peristome	0.22 (±0.07)	3.24	0.0178	*
Stage 4	Areoles-Veins > Veins-Tube	0.3 (±0.07)	4.53	<0.001	***
Stage 4	Areoles-Veins > Peristome-Tube	-0.54 (±0.09)	-6.15	<0.001	***
Stage 4	Areoles-Peristome > Areoles-Tube	0.88 (±0.08)	11.34	<0.001	***
Stage 4	Areoles-Peristome > Veins-Peristome	0.81 (±0.08)	10.23	<0.001	***
Stage 4	Areoles-Peristome > Veins-Tube	0.89 (±0.08)	11.42	<0.001	***
Stage 4	Areoles-Peristome > Peristome-Tube	0.05 (±0.1)	0.51	1.0000	
Stage 4	Areoles-Tube > Veins-Peristome	-0.07 (±0.08)	-0.96	1.0000	
Stage 4	Areoles-Tube > Veins-Tube	0.01 (±0.08)	0.08	1.0000	
Stage 4	Areoles-Tube > Peristome-Tube	-0.84 (±0.1)	-8.72	<0.001	***
Stage 4	Veins-Peristome > Veins-Tube	0.08 (±0.08)	1.04	1.0000	
Stage 4	Veins-Peristome > Peristome-Tube	-0.76 (±0.1)	-7.86	<0.001	***
Stage 4	Veins-Tube > Peristome-Tube	-0.84 (±0.1)	-8.78	<0.001	***
Stage 1	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.43 (±0.39)	1.08	1.0000	
Stage 1	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.92 (±0.39)	2.32	0.2077	
Stage 1	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.87 (±0.39)	2.22	0.2539	
Stage 1	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	0.49 (±0.39)	1.24	1.0000	
Stage 1	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.45 (±0.39)	1.14	1.0000	
Stage 1	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.04 (±0.39)	-0.10	1.0000	
Stage 2	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.49 (±0.23)	2.15	0.2875	
Stage 2	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.38 (±0.23)	1.68	0.6793	
Stage 2	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.03 (±0.23)	0.13	1.0000	
Stage 2	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.11 (±0.23)	-0.47	1.0000	
Stage 2	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	-0.46 (±0.23)	-2.02	0.3661	
Stage 2	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	-0.35 (±0.23)	-1.55	0.8467	
Stage 3	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.33 (±0.23)	1.45	1.0000	
Stage 3	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	-0.23 (±0.23)	-1.00	1.0000	
Stage 3	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.4 (±0.23)	1.74	0.6168	
Stage 3	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.56 (±0.23)	-2.45	0.1632	
Stage 3	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.07 (±0.23)	0.29	1.0000	
Stage 3	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.62 (±0.23)	2.73	0.0920	
Stage 4	<i>S. x leucophylla</i> > <i>S. x Juthatip soper</i>	0.81 (±0.39)	2.06	0.3403	
Stage 4	<i>S. x leucophylla</i> > <i>S. x mitchelliana</i>	0.74 (±0.4)	1.87	0.4885	
Stage 4	<i>S. x leucophylla</i> > <i>S. purpurea</i>	0.89 (±0.39)	2.25	0.2391	
Stage 4	<i>S. x Juthatip soper</i> > <i>S. x mitchelliana</i>	-0.08 (±0.4)	-0.19	1.0000	
Stage 4	<i>S. x Juthatip soper</i> > <i>S. purpurea</i>	0.07 (±0.39)	0.19	1.0000	
Stage 4	<i>S. x mitchelliana</i> > <i>S. purpurea</i>	0.15 (±0.4)	0.38	1.0000	
Areoles-Veins	Stage 1 > (2, 3, 4)	-0.57 (±0.16)	-3.53	0.0106	*
Areoles-Veins	Stage 2 > (1, 3, 4)	0.47 (±0.16)	2.95	0.0378	*

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Areoles-Veins	Stage 3 > (1, 2, 4)	-0.02 (± 0.12)	-0.19	1.0000
Areoles-Veins	Stage 4 > (1, 2, 3)	0.12 (± 0.16)	0.75	1.0000
Areoles-Peristome	Stage 1 > (2, 3, 4)	-0.49 (± 0.17)	-2.95	0.0332 *
Areoles-Peristome	Stage 2 > (1, 3, 4)	-0.07 (± 0.16)	-0.43	1.0000
Areoles-Peristome	Stage 3 > (1, 2, 4)	0.43 (± 0.12)	3.59	0.0080 **
Areoles-Peristome	Stage 4 > (1, 2, 3)	0.13 (± 0.17)	0.76	1.0000
Areoles-Tube	Stage 1 > (2, 3, 4)	-0.27 (± 0.17)	-1.61	0.4971
Areoles-Tube	Stage 2 > (1, 3, 4)	-0.22 (± 0.16)	-1.34	0.7884
Areoles-Tube	Stage 3 > (1, 2, 4)	0.11 (± 0.12)	0.92	1.0000
Areoles-Tube	Stage 4 > (1, 2, 3)	0.37 (± 0.17)	2.25	0.1474
Veins-Peristome	Stage 1 > (2, 3, 4)	-0.03 (± 0.17)	-0.19	1.0000
Veins-Peristome	Stage 2 > (1, 3, 4)	-0.17 (± 0.16)	-1.06	1.0000
Veins-Peristome	Stage 3 > (1, 2, 4)	0.27 (± 0.12)	2.21	0.1590
Veins-Peristome	Stage 4 > (1, 2, 3)	-0.06 (± 0.17)	-0.39	1.0000
Veins-Tube	Stage 1 > (2, 3, 4)	-0.57 (± 0.16)	-3.43	0.0115 *
Veins-Tube	Stage 2 > (1, 3, 4)	0.22 (± 0.16)	1.36	0.7661
Veins-Tube	Stage 3 > (1, 2, 4)	0.11 (± 0.12)	0.88	1.0000
Veins-Tube	Stage 4 > (1, 2, 3)	0.24 (± 0.17)	1.46	0.6456
Peristome-Tube	Stage 1 > (2, 3, 4)	-0.59 (± 0.18)	-3.37	0.0104 *
Peristome-Tube	Stage 2 > (1, 3, 4)	-0.42 (± 0.17)	-2.50	0.0861
Peristome-Tube	Stage 3 > (1, 2, 4)	0.53 (± 0.13)	4.15	0.0015 **
Peristome-Tube	Stage 4 > (1, 2, 3)	0.48 (± 0.18)	2.71	0.0481 *
<i>S. x leucophylla</i>	Stage 1 > (2, 3, 4)	-0.18 (± 0.31)	-0.60	1.0000
<i>S. x leucophylla</i>	Stage 2 > (1, 3, 4)	-0.23 (± 0.24)	-0.97	1.0000
<i>S. x leucophylla</i>	Stage 3 > (1, 2, 4)	-0.1 (± 0.22)	-0.47	1.0000
<i>S. x leucophylla</i>	Stage 4 > (1, 2, 3)	0.52 (± 0.31)	1.70	0.4383
<i>S. x Juthatip soper</i>	Stage 1 > (2, 3, 4)	-0.06 (± 0.31)	-0.21	1.0000
<i>S. x Juthatip soper</i>	Stage 2 > (1, 3, 4)	-0.2 (± 0.24)	-0.83	1.0000
<i>S. x Juthatip soper</i>	Stage 3 > (1, 2, 4)	0.14 (± 0.22)	0.66	1.0000
<i>S. x Juthatip soper</i>	Stage 4 > (1, 2, 3)	0.12 (± 0.31)	0.40	1.0000
<i>S. x mitchelliana</i>	Stage 1 > (2, 3, 4)	-0.8 (± 0.31)	-2.62	0.0771
<i>S. x mitchelliana</i>	Stage 2 > (1, 3, 4)	-0.14 (± 0.24)	-0.59	1.0000
<i>S. x mitchelliana</i>	Stage 3 > (1, 2, 4)	0.8 (± 0.22)	3.68	0.0088 **
<i>S. x mitchelliana</i>	Stage 4 > (1, 2, 3)	0.14 (± 0.31)	0.46	1.0000
<i>S. purpurea</i>	Stage 1 > (2, 3, 4)	-0.62 (± 0.31)	-2.03	0.2419
<i>S. purpurea</i>	Stage 2 > (1, 3, 4)	0.46 (± 0.24)	1.87	0.3220
<i>S. purpurea</i>	Stage 3 > (1, 2, 4)	0.1 (± 0.22)	0.46	1.0000
<i>S. purpurea</i>	Stage 4 > (1, 2, 3)	0.07 (± 0.31)	0.22	1.0000

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Table S5. Factors of variation in the number of prey individuals, flying Hymenoptera individuals and crawling Hymenoptera individuals. Only the best models are presented. “Chisq” values refer to type III Wald chi-square tests. Symbols describe various levels of p-values *: p<0.05, **: p<0.01, and ***: p<0.001. Contrasts are detailed in Table 3.

Dependent variable	Explanatory variables	Chisq	Df	p-value
Number of prey individuals	Intercept	33.58	1	<0.001 ***
	Plant taxon	40.80	3	<0.001 ***
	Pitcher stage	86.04	3	<0.001 ***
	Aperture width	9.55	1	0.0020 **
	Pitcher length	45.67	1	<0.001 ***
Number of flying Hymenoptera individuals	Season	32.67	1	<0.001 ***
	Pitcher length	39.14	1	<0.001 ***
	Areoles-Tube colour contrast	16.77	1	<0.001 ***
	Areoles brightness contrast	7.83	1	0.0051 **
Number of crawling Hymenoptera individuals	Areoles-Peristome colour contrast	36.87	1	<0.001 ***
	Intercept	1.70	1	0.1925
	Plant taxon	80.69	3	<0.001 ***
	Pitcher stage	15.12	3	0.0017 **

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**Capture efficiency of *Sarracenia* pitcher plants
towards the Asian hornet, *Vespa velutina*, under
laboratory conditions: contribution of lid visual
signals, nectar and pitcher shape**

Résumé

Bien que les insectes puissent influencer l'évolution et la diversification des plantes carnivores, chez les sarracénies, aucune tentative n'a été faite pour vérifier si certaines caractéristiques de piégeage sont plus adaptées pour piéger des proies spécifiques. Pourtant, les sarracénies présentent des différences dans la morphologie de leurs pièges et dans la taille et les guildes de proies piégées, par exemple *S. leucophylla* piège de nombreux taxa volants de grande taille et *S. purpurea* principalement des fourmis. Nos objectifs étaient de (1) mesurer l'efficacité de piégeage de quatre espèces, dont *S. purpurea* et trois de ses hybrides formant un gradient de parenté avec *S. leucophylla*, vis-à-vis d'un grand insecte volant : le frelon asiatique, *Vespa velutina*, une espèce envahissante en Europe et de (2) identifier les traits impliqués dans sa capture. Pour cela 50 frelons ont été introduits dans une cage contenant trois plantes de 8 urnes chacune pour chaque espèce et trois plantes factices de 8 urnes également. Pour chaque plante, nous avons mesuré les taux de visite du frelon, les événements de piégeage et d'évasion au cours du temps. A la fin, nous avons mesuré les taux de capture finaux, les caractéristiques de l'opercule, les caractéristiques morphologiques des urnes et observé les microstructures sur la surface interne des urnes. 80% des frelons ont été trouvés morts à l'intérieur des urnes, la plupart dans *S. X leucophylla*, aucun dans *S. purpurea* et les plantes factices. *S. X leucophylla* avait les taux de fréquentation les plus élevés et *S. purpurea* et les plantes factices les plus bas. Les deux espèces les plus proches de *S. leucophylla* étaient les seules à différer des plantes factices en termes de taux de capture, *S. X leucophylla* s'avérant la plus efficace contre le frelon. La probabilité de visite dépendait significativement de l'espèce et de la zone de l'urne considérée, l'opercule étant la zone la plus visitée. Les visites de l'opercule étaient favorisées par la production de nectar, la couleur blanche dominante de sa partie extérieure et la couleur rouge dominante de sa partie intérieure. La probabilité de capture dépendait également de l'espèce et s'est avérée augmenter avec la longueur de la cavité de l'urne et diminuer avec la largeur de son orifice et la largeur de son aile. Les taux de captures par rapport aux visites étaient inférieurs à 10 % et variaient significativement selon l'espèce : ils étaient les plus élevés pour *S. X leucophylla* et les plus bas pour *S. purpurea*, qui s'est démarquée également par l'absence de paroi glissante avec des micro-pointes dirigées vers le bas, tout en présentant un taux d'évasions du frelon de 100 %.

Ces résultats mettent en évidence les adaptations structurelles présumées de *S. leucophylla* dans la capture de grandes proies volantes et fournissent des informations sur les traits d'attraction et de piégeage à considérer dans le but de développer un piège biomimétique contre le frelon asiatique.

**Capture efficiency of *Sarracenia* pitcher plants
towards the Asian hornet, *Vespa velutina*, under
laboratory conditions: contribution of lid visual
signals, nectar and pitcher shape**

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Abstract

Although insects may shape the evolution and diversification of carnivorous plants, in *Sarracenia* pitcher plants, no attempt has been made to test whether certain trapping features may be more adapted to trap specific prey. Yet, *Sarracenia* pitcher plants display differences in trap morphology and prey guilds and sizes, e.g. *S. leucophylla* traps many large flying taxa and *S. purpurea* primarily ants. Our objectives were (1) to measure the trapping efficiency of four taxa, including *S. purpurea* and three of its hybrids forming a kinship gradient with *S. leucophylla*, towards a large flying insect: the Asian hornet, *Vespa velutina*, an invasive species in Europe and (2) to find out the traits involved in its capture. Fifty hornets were introduced into a cage containing three 8-pitcher plants for each taxon and three 8-pitcher dummy plants. For each pitcher, we measured visit rates, trapping and escape events over time. At the end, we measured final capture rates, lid characteristics, morphological features of pitchers and checked for microstructures on pitcher inner surface. 80% of the hornets were found dead inside pitchers, most in *S. X leucophylla*, none in *S. purpurea* and dummy plants. The former had the highest visitation rates, the two latter the lowest. The two taxa closest to *S. leucophylla* were the only taxa to differ from control plants in terms of capture rates, with *S. X leucophylla* being the most effective taxon against the hornet. Visit probability was significantly dependent on taxon and pitcher area, with lid the most visited area. Lid visits were enhanced by nectar production, and upper sides dominated by white colour and inner sides dominated by red colour. Capture probability was also dependent on taxon and increased with cavity length and decreased with orifice width and wing width. The capture-to-visit rates were less than 10% and varied significantly by taxon being highest for *S. X leucophylla* and lowest for *S. purpurea*, which also singularized by the absence of a slippery inner surface of downward pointing tips, while showing an escape-to-fall rate of 100%. These results highlight the presumably structural adaptations of *S. leucophylla* to the capture of large flying prey and provide information on the attraction and trapping devices to be considered with the goal of developing a biomimetic trap against the Asian hornet.

Introduction

Among the angiosperms, carnivory has evolved at least 9 times independently (Givnish, 2015). The ability to derive nutrients from arthropods, which they capture, allows carnivorous plants to supplement their nutrient supply and survive in the very poor wetland soils where they typically grow (Juniper *et al.*, 1989; Ellison & Adamec, 2018). Carnivorous plants have evolved very performing attraction systems that often mimics that of flowers including visual cues (Moran *et al.*, 1999; Schaefer & Ruxton, 2008), olfactory cues (Jaffé *et al.*, 1995; Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010) and nectar secretions (Dress *et al.*, 1997; Bennett & Ellison, 2009). Once attracted, arthropods are then trapped by an arsenal of leaf traits adapted to their capture. Carnivorous plants have evolved foliar traps of very diverse shapes and trapping mechanisms, such as flypaper traps, snap traps or pitcher traps, some of which are similar although they have arisen independently during evolution, exhibiting remarkable examples of evolutionary convergence. (Albert *et al.*, 1992). The *Sarraceniaceae* of America and the *Nepenthaceae* of Asia, for example, are two distinct families of carnivorous plants that share pitcher-shaped leaves functioning as pitfall traps (Cresswell, 1993; Bhattarai & Horner, 2009; Gaume & Di Giusto, 2009; Chin *et al.*, 2010; Moran *et al.*, 2012; Gaume *et al.*, 2016; Thorogood *et al.*, 2018; Sheridan *et al.*, 2021). Pitchers are filled with a digestive liquid that contains various enzymes as well as inquiline organisms allowing digestion of prey fallen inside (Juniper *et al.*, 1989; Hatano & Hamada, 2008; Adlassnig *et al.*, 2011). In addition, pitchers often present structural adaptations, such as viscoelastic mucilage (Gaume & Forterre, 2007; Bonhomme *et al.*, 2011; Bazile *et al.*, 2015) or microstructures forming slippery surfaces such as downward-pointing cells often covered by a layer of epicuticular wax (Gaume *et al.*, 2002; Gorb *et al.*, 2005; Poppinga *et al.*, 2010), which reduce their adhesion and cause them to fall or be retained in the trap. Some studies even suspected the interplay of alkaloids, that could numb insects and cause them to lose balance and fall into the trap (Mody *et al.*, 1976; Ratsirarson & Silander Jr, 1996; Hotti *et al.*, 2017). These different features are often combined to form whole trapping syndromes, which target specific guilds of animals and/or characterise specific diets (Juniper *et al.*, 1989; Pavlovič *et al.*, 2007; Gaume *et al.*, 2016).

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In *Nepenthes*, numerous studies have shown the role of specific foliar traits in the capture of specific guilds of prey or even specific food resources. Regarding the last point, the most conspicuous examples are to be found in *Nepenthes lowii*, *N. rajah* and *N. macrophylla*, characterised by concave lids and pitchers of very large orifices adapted to the 'capture' of tree shrew faeces for nutritional benefit (Chin et al. 2010). In more typical (arthropod-trapping) *Nepenthes*, the capture of flies is favoured by viscoelastic fluids (Bonhomme *et al.*, 2011; Bazile *et al.*, 2015) or translucent tissues in the form of fenestrations acting as light traps (Moran *et al.*, 2012), while slippery waxy surface, nectar production and fluid acidity altogether increase the capture of ants (Bonhomme *et al.*, 2011; Gaume *et al.*, 2016). Capture of flying and flower-visiting insects is correlated with the presence of sweet odours and morphological features such as pitcher conicity or large aperture width (Gaume *et al.*, 2016). This specialisation to specific prey could be explained by an adaptation to particularly abundant type of fauna in the plants' immediate environment, or by a niche partitioning in response to interspecific competition for trophic resources (Gibson, 1983; Gonzales *et al.*, 1991). Darwin (1875) hypothesised from the observation of the Venus flytrap, *Dionaea*, that it would be far more advantageous for the carnivorous plant to specialize on large prey because of their higher nutrient return. Based on this hypothesis, Gibson & Waller (2009) even explained the emergence of snap-traps from sticky traps by the evolutionary advantage of trapping larger insects, which are rarer but nutritionally more interesting for the plant.

In *Sarracenia*, insect prey has received little attention, although it can play a role in the evolution and diversification of these pitcher plants. *Sarracenia* species have been shown to compete for trophic resource (Gibson, 1983) and to display some niche segregation by certain authors (Gibson, 1983; Folkerts, 1992) but not enough according to others, who rather consider them as passive traps with a random trapping (Ellison & Gotelli, 2009). Prey partitioning between species, when it was shown, has most often been ascribed to differences in pitcher morphology (Gibson, 1983; Stephens *et al.*, 2015). According to the results of a recent study focused on the volatile compound and prey analyses of these pitcher plants, odours are even supposed to play a primary role in prey segregation (Dupont et al., 2022b, submitted). Prey composition can indeed vary between *Sarracenia* species. In *Sarracenia minor*, the two small-leaf and long-leaf (var. *okefenokeensis*) forms were shown to capture mostly ants for the former and flying insects for the latter. The majority of the many prey items captured by *okefenokeensis*

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(e.g., Lepidoptera, Coleoptera, and Diptera) were reported to be larger than the predominantly ants captured by *S. minor* sensu stricto, suggesting that « these plants captured not only a higher number of prey, but also a larger biomass of prey » (Stephens *et al.*, 2015). Gibson showed that the long-leaved *S. leucophylla* was able to capture a broad range of insects including many large flying prey (Gibson, 1983; Gibson, 1991) in contrast to, for example, *S. purpurea*, which was mostly reported to capture predominantly ants (Heard, 1998; Newell & Nastase, 1998). He also showed that the maximum prey size was positively correlated with the width of the pitcher orifice for a sample including different *Sarracenia* species (Gibson 1991).

In this study, we aimed to test which traits of these pitcher plants are involved in the capture of large flying prey. To achieve this objective, we considered on the one hand four *Sarracenia* taxa, including *S. purpurea* and three of its hybrids forming a relatedness gradient with *S. leucophylla*, and on the other hand the Asian hornet, *Vespa velutina*, chosen because of its large size (worker-size mean: ~ 25 mm, Rome & Villemant (2017)) and because it is an invasive and bee predatory species in Europe (Monceau *et al.*, 2013; Requier *et al.*, 2019), against which no effective traps have yet been developed (Lioy *et al.*, 2020; Sanchez & Arias, 2021). We sought to answer the following questions. Are *Sarracenia* pitchers more than just pitfall traps? Do *Sarracenia* species differ in their attraction and capture efficiency of large flying prey, such as the Asian hornet? Which traits among attractive and trapping ones are most involved in its capture?

Material and methods

Plant taxa and growing conditions

We considered four *Sarracenia* taxa differing in visual aspect (Dupont *et al.* 2022a, submitted), odour production (Dupont *et al.* 2022b, submitted), and leaf shape (Fig. 1B). All the studied plants were horticultural specimens obtained from the carnivorous plant nursery “Nature and Paysages” (Peyrusse-Massas, Gers, France) at the same juvenile stage and replanted in the beginning of April 2016 in the experimental station of AMAP (Montpellier, France). Two of the taxa were natural species: *S. purpurea* subsp. *venosa* and *S. X mitchelliana* = *S. purpurea* X *S. leucophylla*. The two others were horticultural

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hybrids: *S. X Juthatip soper* = (*S. leucophylla* X *S. purpurea*) X *S. leucophylla* and *S. X leucophylla* = *S. leucophylla* X *S. X Juthatip soper*. The plants of the four taxa were grown outdoors in individual pots in a sunny place. The pots were filled with a peat mixture consisting of 2/3 blond peat and 1/3 sand on a background of clay balls. They were regularly watered with demineralized water to match their natural moist and mineral-poor environment. The plants were pruned one week before the observation to leave 8 mature pitchers on each of them. In addition to the 4 plant taxa, control plants were used, each consisting of 8 conical, green plastic dummy pitchers planted in a pot identical to that of the pitcher plants. Each of them were filled with 25ml of water to reach a level somehow comparable to the level of the digestive fluid in pitchers of *Sarracenia* plants.

Studied hornets

Vespa velutina nigrithorax workers were caught one day before observation by sweeping in front of four beehives at the study site of Lavalette (Montpellier, France), in mid-November 2019. Once captured, each hornet was placed individually in a transparent polyethylene box with small holes to allow air circulation. The hornets were then brought to a breeding chamber at ambient temperature and fed with water and candy (honey and sugar mixture) *ad libitum*.

Experimental set-up

The experimental set-up consisted in a glass cage topped with a metal net (total dimension of 120x60x80 cm, see Fig. 1A) containing three pots of each plants (including control plants) placed randomly on the floor. There were thus 3 plants per species, for a total of 15 plants. At the beginning of the experiment, 30 hornet workers were introduced into the cage. Thirty minutes after the introduction of all the hornets, all the plants were observed for 10 min by three observers. Observation was repeated 90 min after the hornets were introduced and then every 30 min for a total of 5 hours. During the observation time, for each pitcher, the following events were recorded: number of hornets visiting the pitcher (i.e. hornets landing on the pitcher), number of visits to the operculum (Fig. 1C), number of visits to the peristome, i.e. the pitcher rim circling the aperture width (Fig. 1C), number of visits to the pitcher body (Fig. 1C), number of falls into the pitcher cavity, and number of escapes. The experiment was repeated two days

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after, when there were no more living hornets in the cage, under the same conditions, with the same plants, using 20 additional hornets. During the observation, video recordings of different observed events were carried out.

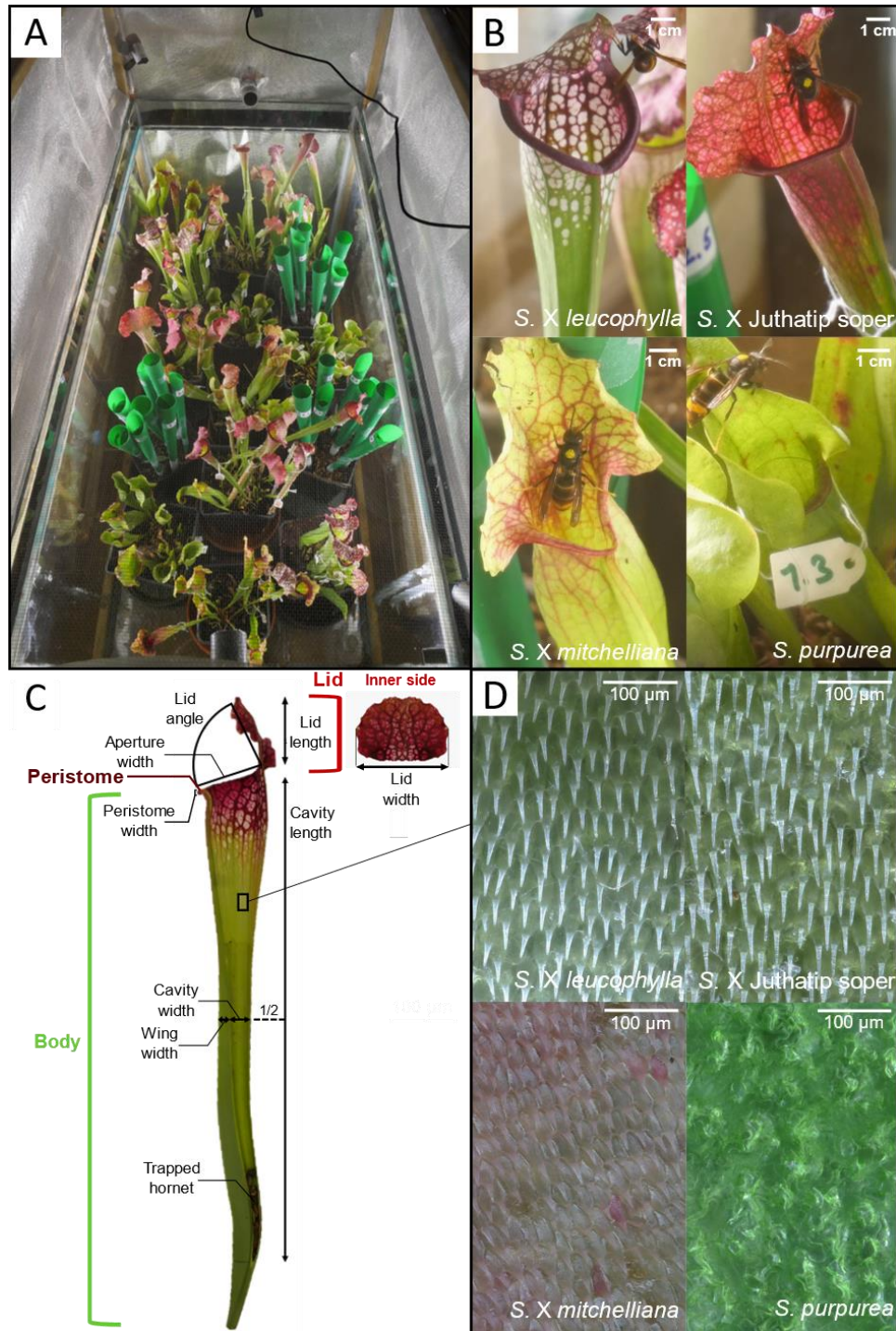


Figure 1. Photographs of experimental set-up, hornets and plants. (A) Experimental set-up showing plants in the cage. (B) Hornet visiting the nectar-secreting lid of a pitcher in the four *Sarracenia* taxa used in the experiment. (C) Longitudinal section of a pitcher of *S. X leucophylla* and the morphological variables that have been measured. (D) Light microscope image (Keyence VHX7000) of the upper inner pitcher area of the four *Sarracenia* taxa used in the experiment.

Pitcher measurements and hornet-capture analysis

Two days after the experiment, after having checked that all 50 hornets were died, the pitchers were cut out. The number of hornets trapped in each pitcher was counted and pitchers were measured for cavity length, cavity width (at mid-height of the cavity), wing width (at mid-height of the cavity), aperture width, peristome width, lid length, lid maximal width, and lid opening angle (see Fig. 1C). For each pitcher, we noted whether the lid was nectar-producing or not and we also noted the dominant colour for both the inner and the outer sides of the lid. In addition, a rectangular portion of the pitcher was excised with a razor blade in the upper third of the pitcher for all four taxa, and observed and photographed under a Keyence (VHX7000) light microscope using a stacking method to recompose the images and obtain a sharp image over a large depth of field.

Statistical analysis

Hornet attraction success and contribution of lid features

First, we investigated whether the number of hornet visitors per plant varied among the four different taxa and the control plants. We used a linear mixed model testing as explanatory fixed variables, the factors ‘plant taxon’ and ‘day of observation’, and the quantitative variable ‘time of observation’, as well as all two-way and three-way interactions, and as a random variable, ‘plant identity’. Because the number of introduced hornets differed between the two observation days, the number of visitors per plant was corrected by the initial number of hornets introduced on the observation day, and we considered the proportion of plant visitors (number of plant-visiting hornets / initial number of hornets) as the dependant variable. The proportions were square-root transformed to achieve normality of residuals.

Second, we investigated whether the total number of visits (cumulative visit numbers for the 180 minutes of observations) per pitcher varied among the three different pitcher areas (lid, peristome and body) of the four plant taxa. We used a general linear mixed model with a Poisson distribution and a log-link function testing ‘plant taxon’, ‘pitcher area’ and their interactions as explanatory fixed variables. We also considered ‘plant identity’ as a random variable.

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Third, in order to get a general view of how the plant taxa differed in the traits displayed by their pitcher lids, we performed a Multiple Component Analysis (MCA) considering the categorical variables of species, nectar and lid colors and using the “mca” function of the R package “FactoMineR”. The binary variables ‘Visit’ and ‘Capture’ corresponding to the occurrences of hornet visit and capture respectively were not used to build the projection but were put as supplementary variables to know their distances to the factorial axes and the plant variables.

We then investigated whether the total number of hornet visits to the pitcher lid can be explained by its nectar production and the dominant colour on each of its sides, using a mixed model testing for a random ‘Plant’ effect and for the effects of fixed variables, i.e. the binary (0/1) ‘Nectar’ variable, the ‘Dominant colour of the outer side of the lid’ (Green, Red, White) and the ‘Dominant colour of the inner side of the lid’ (Red, Not-red). The inner side of the lid was most frequently dominated by red and its colour was thus separated into only two categories (Red, Not-red) to keep the sampling balanced and limit the number of freedom degrees accounted for by the model.

Hornet capture success and contribution of pitcher morphological traits

To investigate whether the plant taxa differed in their capture rates, we performed a poisson regression model, using as dependant variable for each plant the ‘number of hornets that had been successfully trapped’ and as explanatory variable the ‘plant taxon’. To explore how the plant taxa differed in the morphology of their pitchers and to find out which morphological traits contributed most to their discrimination, we first used a Linear Discriminant Analysis (LDA) using the “lda” function of the R package “MASS”. The significance of discrimination in morphology among taxa was assessed by Multivariate Analysis of Variance (MANOVA) using the “manova” function and Wilks method. We then used mixed multiple logistic regression models with a binomial distribution and a logit function and explored which morphological variables best explained the probability for a pitcher to capture hornets. We also considered the variable ‘plant identity’ as a random variable.

Relative contributions of attraction, capture and retention abilities in the plant trapping success

Our goal was to compare the capture and retention ability of the different taxa independantly of their attraction ability in order to assess their own contributions in the plant trapping success. Considering

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the proportions of observed visits for each taxon, we first tested, using a multinomial test (four probabilities for the four taxa) and the “multinomial.test” function from the R package “EMT”, whether the 4 taxa differed in their ability to make hornets fall inside pitchers based on the numbers of observed falls. We then tested using a multinomial test whether the 4 taxa differed in their ability to retain fallen hornets based on the number of observed escapes and considering the probabilities of observed falls for each taxon. We finally tested their ability to trap hornets considering their attraction probabilities and based on the final numbers of captures.

For all linear models, backward selection and Type III tests were carried out and we selected the best model with the lowest AIC (Akaike criterium). Mixed models were estimated with the functions “lmer” and “glmer” from the R package “lme4” (Bates D *et al.*, 2015). For each significant factor, post-hoc tests were carried out between any two factor levels, when necessary, using the function “contrast” from the R package “emmeans” (Russell VL, 2020). P-values were adjusted for multiple comparisons using the Holm’s method. Statistics were performed using the software R version 4.0.3 (R Core Team, 2020). Model assumptions were checked by plotting residuals against fitted values and normal QQ plots of the residuals.

Results

Interspecific variation in short-distance attraction of hornets

The proportion of hornet visitors to plants varied according to the plant taxon (Table 1A, Fig. 2), *S. X leucophylla* was the most visited taxon, being together with *S. X Juthatip soper*, significantly more visited than the control plants (Table 1B). *S. X leucophylla* was more visited than *S. X mitchelliana* and *S. purpurea* while *S. X Juthatip soper* was more visited than *S. purpurea*, which received the least visitors (Table 1B). The proportion of visitors changed according to the day of observation (Table 1A), it was lower on the second day (Table 1B). The proportion of visitors also decreased over time (Table 1B, Fig. 2) and this decrease was even greater on the second day of observation (Table 1B). Hornets visited some parts of the pitchers more often than others (Table 2A). For all taxa, the area with the greatest number

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of visits during the total duration of the observation (cumulated times of each observation) was the lid (Fig. 3A). We noticed a concentration of visits on its inner side, where hornets were observed to feed for a long time on nectar (Movie S1). The peristome, on the other hand, was the least visited of the three areas (Fig. 3A). The four taxa differed both in nectar production and dominant colour of their lids (Fig. 1, S1). While *S. purpurea* was the least nectar-productive taxon with a mostly green lid, *S. X leucophylla* was the most nectar-productive taxon with a lid often dominated by a white colour. *S. Juthatip soper* is a taxon, which is rather nectar-productive and with a lid dominated by red colour, while *S. x mitchelliana* displayed an intermediate profile between *S. purpurea* and *S. X Juthatip soper*. Outer and inner lid colours, as well as nectar production explained significant parts of the variance of the total number of hornet visits to the lid, in addition to plant identity (Table 2B). However, the dominant colour of the outer lid was by far the factor that explained most of the variance in hornet visits (Table 2B) and also most probably in hornet captures (correlation shown in Fig. S1). Lid visits were about three times most numerous when the dominant colour of the outer lid was white than when it was another colour and they were the least numerous when it was green (Fig. 3B). Moreover, hornet visits were more numerous when the dominant colour of the inner lid was red than when it was white or green (i.e. not red, Fig. 3C). They were also more numerous when the lid produced nectar than when it did not (Fig. 3D).

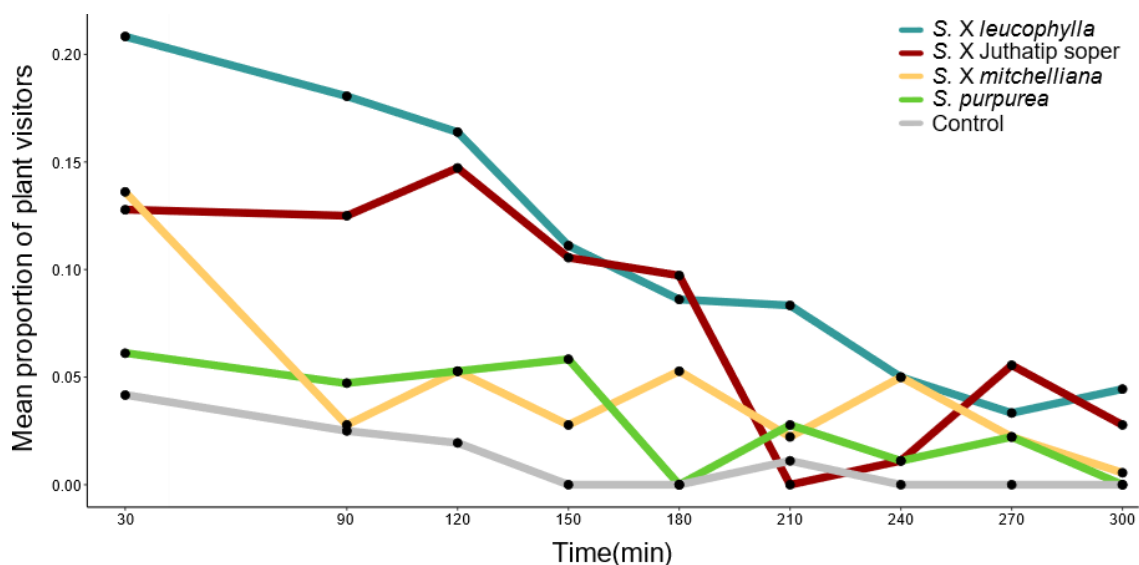


Figure 2. Hornet visits to plants over time. Mean proportion of plant visitors (i.e. number of hornet visitors / initial number of hornets) as a decreasing function of time (with increasing capture events) for each taxon and control. The standard deviations are not shown for reasons of clarity.

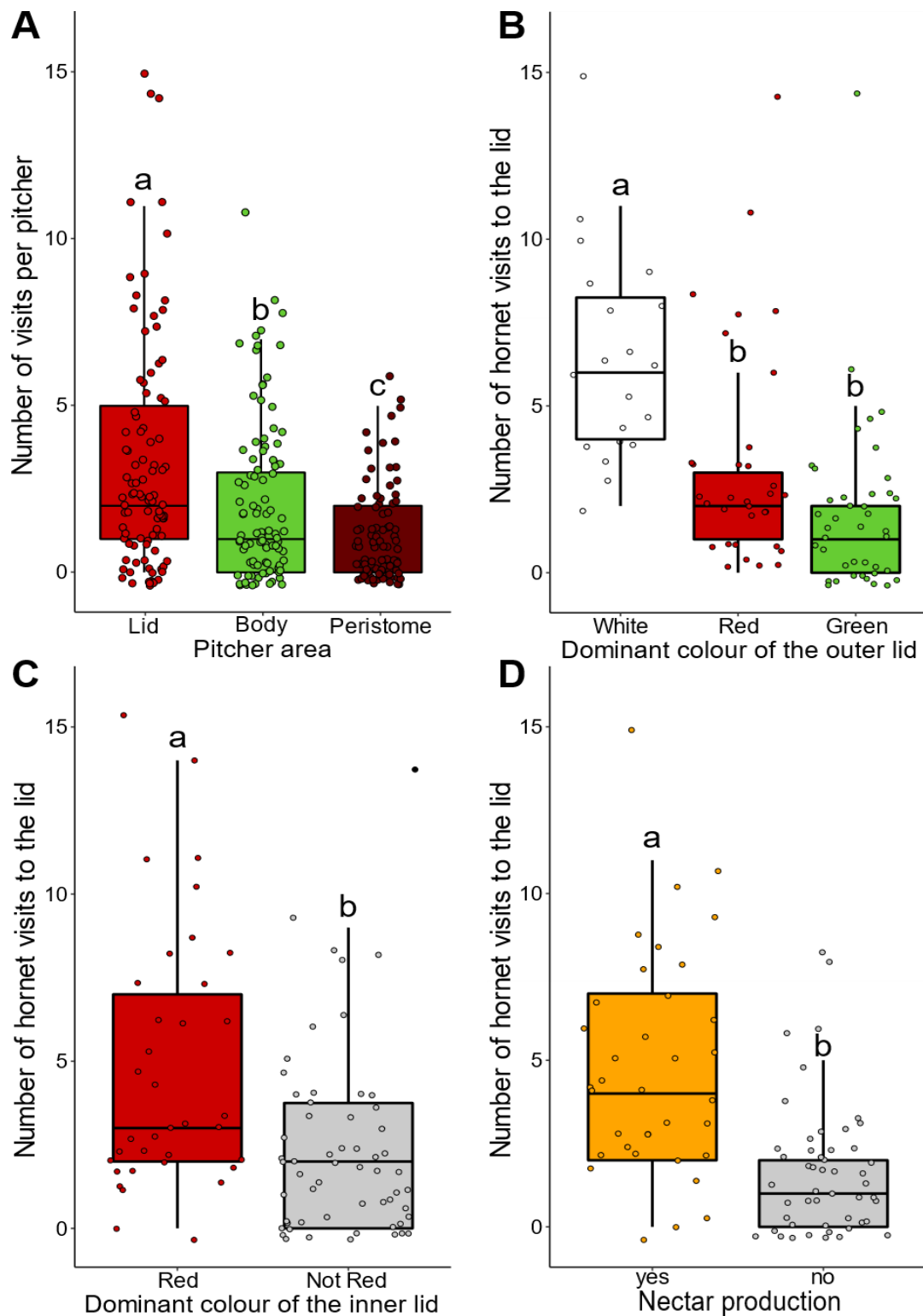


Figure 3. Hornet visits to pitchers and lids. (A) Boxplot of the total number of visits per pitcher during the cumulated time of observation for the four plant taxa according to the pitcher area. The different letters indicate significant different means between areas once the interspecific differences were considered. Boxplots of the total number of visits to the lid, according to its dominant outer colour (B), dominant inner colour (C) and status of nectar production (D). The different letters indicate significant different means between the compared categories once the variance due to the other categories were accounted for.

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Table 1. Factors influencing hornet visits to plants (A) Factors of variation of the proportion of plant visitors, i.e. number of hornet visitors / initial number of hornets (sqrt-transformed) according to a GLM mixed model. The random variable, ‘Plant identity’, was retained in the model and is presented in italics. “Chisq” values refer to type-III Wald tests for mixed models. (B) Estimates of the model coefficients and results of the pairwise comparisons between factor levels of the explanatory variables using the Holm’s correction for multiple comparisons. Different letters show statistically significant differences in means between factor levels.

Dependant variable:			Proportion of visitors (sqrt-transformed)							
(A)	Explanatory variables	Chisq	Df		(B)	Explanatory variables	Factor level	Estimate (±SE)	Df	Contrasts
	Intercept	144.91	1	<0.001 ***	Plant taxon	<i>S. X leucophylla</i>		0.24 (±0.02)	10	a
	Plant taxon	53.47	4	<0.001 ***		<i>S. X Juthatip soper</i>		0.19 (±0.02)	10	ab
	Time	14.31	1	<0.001 ***		<i>S. X mitchelliana</i>		0.13 (±0.02)	10	bc
	Day	24.87	1	<0.001 ***		<i>S. purpurea</i>		0.09 (±0.02)	10	c
	Time*Day	4.89	1	0.0271 *		Control		0.04 (±0.02)	10	c
	<i>Plant identity</i>				Day	Day 1		0.18 (±0.01)	35	a'
						Day 2		0.09 (±0.01)	35	b'
					Time*Day	Time*Day 1		-0.05 (±0.01)	252	a''
						Time*Day 2		-0.09 (±0.02)	252	b''

Table 2. Factors influencing hornet visits to pitchers and pitcher lids. (A) Factors of variation of the total number of hornet visits per pitcher during the cumulated time of observation according to a mixed Poisson regression model. Contrasts between areas are shown in Fig. 3A. (B) Factors of variation of the total number of hornet visits to the lid during the cumulated time of observation according to a mixed Poisson regression model. The random variable, ‘Plant identity’, was retained in the model and is presented in italics. “Chisq” values refer to type-III Wald tests for mixed models. Contrasts are shown in Fig. 3B, C, D.

Dependant variable:	(A) Number of hornet visits to pitcher			(B) Number of hornet visits to lid			
Explanatory variables	Chisq	Df	p-value	Explanatory variables	Chisq	Df	p-value
Intercept	53.70	1	<0.001 ***	Intercept	14.66	1	< 0.001 ***
Plant taxon	29.28	3	<0.001 ***	Nectar	5.10	1	0.0239 *
Area	104.20	2	<0.001 ***	Lid outer colour	9.87	2	0.0072 **
<i>Plant identity</i>				Lid inner colour	4.94	1	0.0263 *
				<i>Plant identity</i>			

Interspecific variation in the hornet capture efficiency

At the end of the experiment, 40 out of the 50 hornets, i.e. 80% of them were found dead in the pitchers while the remaining 10 hornets were found dead inside the cage. Among the hornets that were found dead inside pitchers, 27 were found in 14 pitchers of *S. X leucophylla*, 8 in four pitchers of *S. X Juthatip soper*, 5 in two pitchers of *S. X mitchelliana*, zero in *S. purpurea* and zero in control plants. The number of hornets captured by the plants significantly varied according to the plant taxon (Poisson regression model, $\chi^2 = 60.98$, $df = 4$, $P < 0.001$). Only *S. X leucophylla* and *S. X Juthatip soper* significantly differed from control plants in their capture rates (Fig. 4). The numbers of hornets found trapped upside-down in the pitchers were 13, 0, 1 and 0 in *S. X leucophylla*, *S. X Juthatip soper*, *S. X mitchelliana*, and *S. purpurea*, respectively. According to a logistic regression, the proportion of hornets captured upside-down in plants having trapped hornet was significantly dependant on *Sarracenia* taxa ($\chi^2 = 9.4$, $Df = 2$, $P = 0.009$), reaching 45% in *S. leucophylla*, 12% in *S. X mitchelliana* and 0% in *S. X Juthatip soper*. Most of the hornet falls occurred from the lid (e.g. Movie S2). The fall-to-visit rates were weak for all four taxa (2.1%, 2.0%, 7.6% and 3.2% for *S. X leucophylla*, *S. X Juthatip soper*, *S. X mitchelliana* and *S. purpurea*, respectively). The numbers of falls did not reflect the numbers of visits to the plant taxon (multinomial test, $P=0.04$) meaning that, independently of their attraction ability, some plant taxa were more efficient at causing hornets to fall into the trap, as seems to be the case for *S. X mitchelliana* (Fig. 5). By contrast, the escape-to-fall rates were quite high for all four taxa (57.1%, 25.0%, 66.7% and 100% for *S. X leucophylla*, *S. X Juthatip soper*, *S. X mitchelliana* and *S. purpurea*, respectively). But, the taxon-related difference in the propensity of hornets to escape from the pitcher after falling was not significant, the numbers of escapes reflecting somehow the numbers of falls in this small sample of events (Fig. 5). However, according to our own observations and also to the movie S3 taken as an example, the hornets were observed to make several attempts to get out from the trap of *S. X leucophylla* and *S. X Juthatip soper*, sliding many times before possibly succeeding (Movie S4), in contrast to *S. X mitchelliana* and especially *S. purpurea*, where escapes were far easier and faster. When they fell head first into the trap of *S. X leucophylla*, they were unable to turn around to try to escape (Movie S5). As for the final rates of capture, they did not reach 10% of the visits for any of the taxa considered (8.3%, 3.9%, 4.2% and 0% regarding species ranked in the same order as above). The final capture numbers

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did not reflect the visit numbers of the plant taxon (multinomial test, $P=0.03$, Fig. 5), meaning that some taxa, *S. X leucophylla* in particular, were actually more effective at trapping prey than others, such as *S. purpurea*, which was the least effective of all.

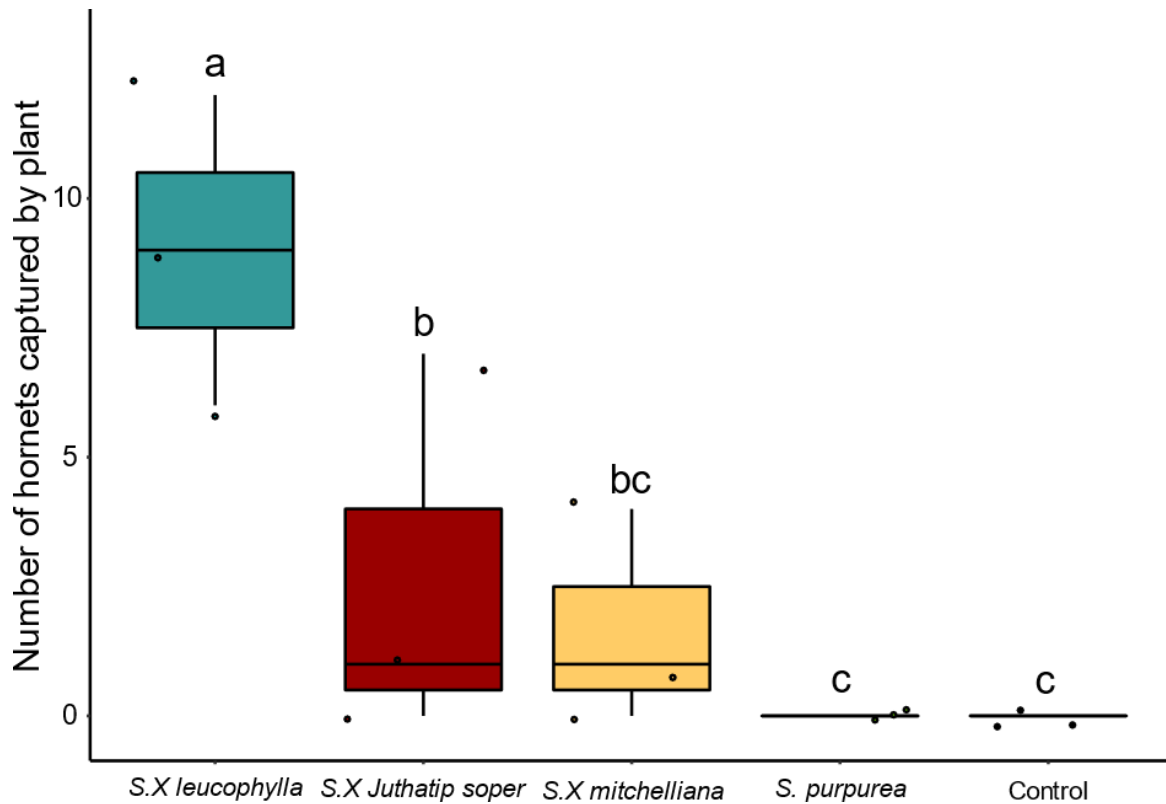


Figure 4. Trapping success of plants. Boxplot of the number of hornets captured by plants of the different *Sarracenia* taxa. The different letters indicate significant different means in the number of captures between plant taxa according to the Poisson regression analysis and pairwise comparisons using the Holm's correction for multiple comparisons.

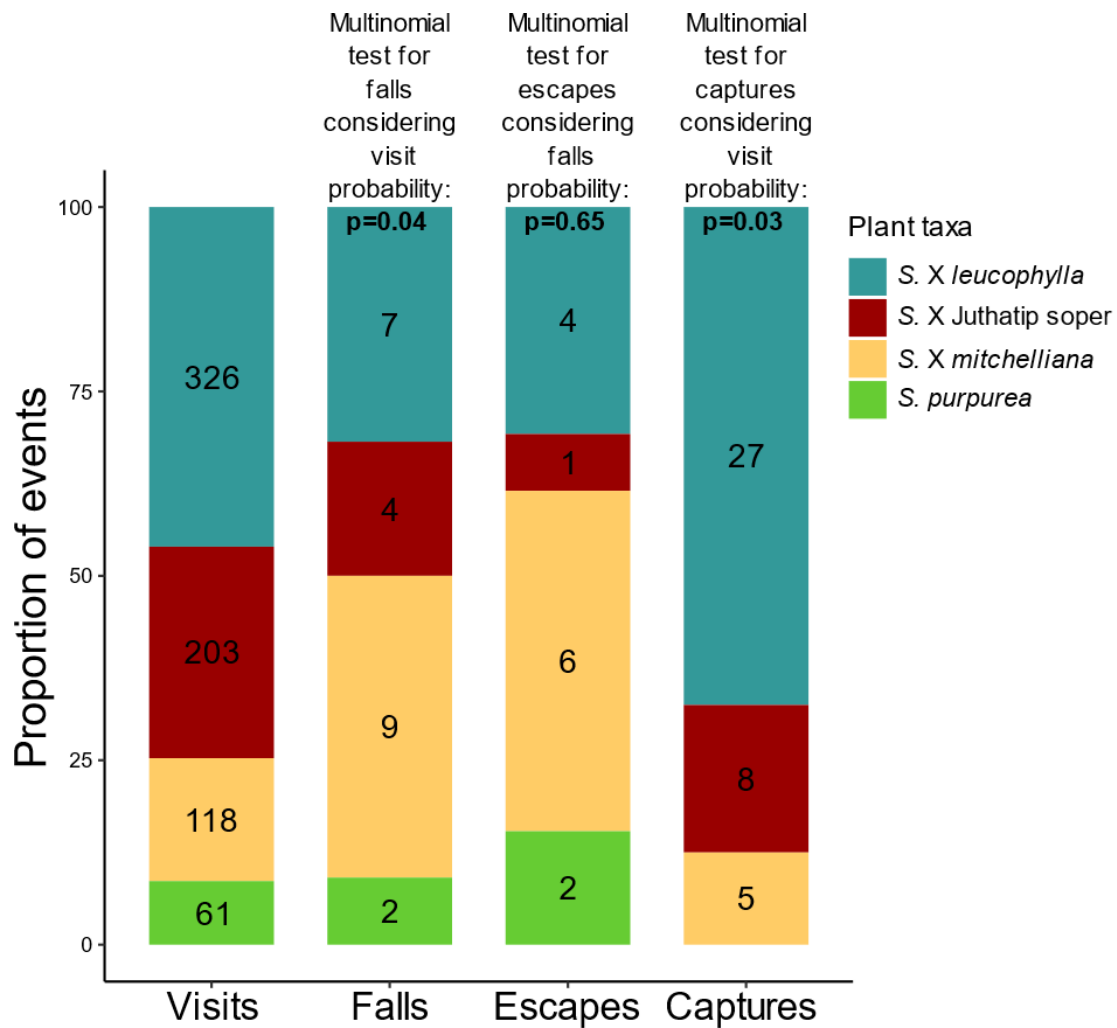


Figure 5. Contributions of attraction, capture and retention abilities in the plant trapping success. Proportions of the cumulated events observed for the 24 pitchers of the four *Sarracenia* taxa. Visit, fall and escape events were counted for each pitcher during observation time while capture events refer to final capture occurrences at the end of the experiment.

Pitcher morphological variation and correlation with hornet capture

According to the LDA and the first two factorial axes, the four taxa were discriminated by their morphological traits (Fig. 6A). Axis 1 explained most of the variation (90%) in pitcher morphology and was strongly negatively correlated with cavity length on the one hand, and positively correlated with cavity width and lid width and, to a lesser extent, lid opening angle and wing width on the other hand (Fig. 6B). Axis 2 was correlated positively with lid length (Fig. 6B). The two axes separated taxa into three groups: *S. X leucophylla* and *S. X Juthatip soper* with long but narrow pitcher cavities and lids, relatively small lid opening angles and small wings in the one side, *S. purpurea* with large lids and wide opening angles and short but wide cavities and wings in the other side, and *S. X mitchelliana*

intermediate to the two previous groups in length and width of these morphological traits but with longer lids (Fig. 6). Under the light microscope, *S. X leucophylla*, *S. X Juthatip soper* and *S. X mitchelliana* displayed downward-pointing cells on the inner surface of their pitchers. In contrast, *S. purpurea* did not show such structures (Fig. 1D).

Pitchers that trapped hornets were for the most in the left side of axis 1 associated to the long and narrow-leaved taxa (Fig. 6). When considering morphology of pitchers in the analyses, we found that cavity length, aperture width and wing width explained most of the hornet capture success of plants (Table 3). The probability of trapping hornets significantly increased with cavity length and decreased with cavity width, aperture width and wing width (Table 3).

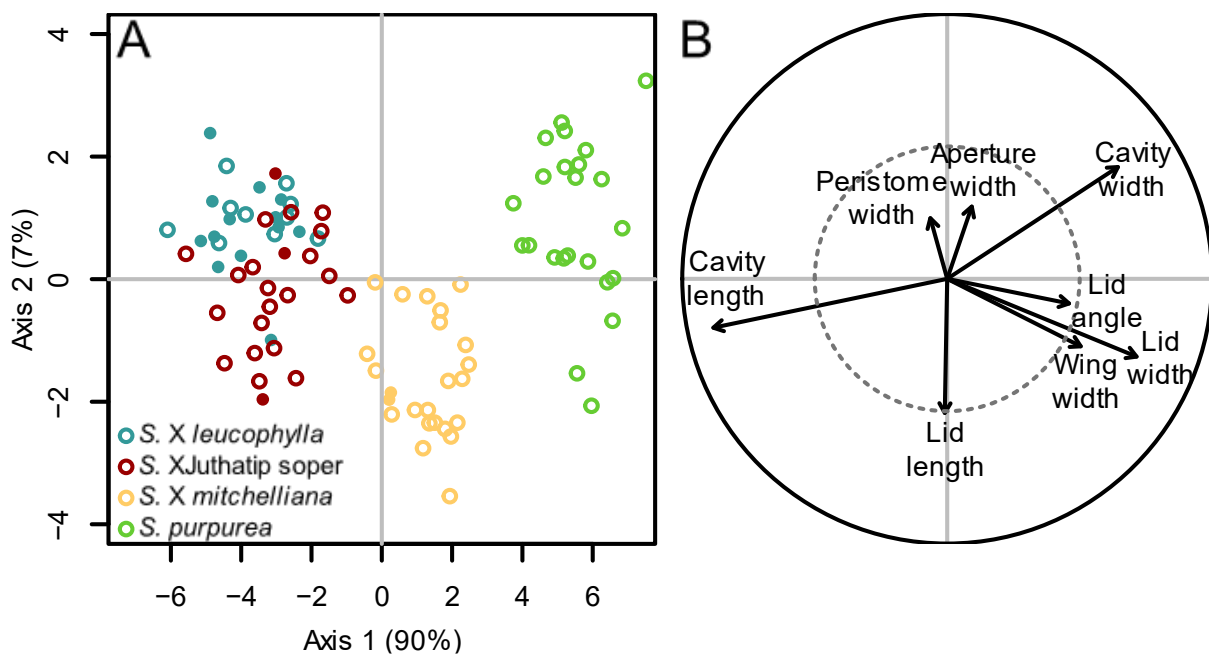


Figure 6. Discriminant analysis among plant taxa according to morphological quantitative variables pertaining to pitchers. (A) Representation of the pitchers on the first two factorial axes. Pitchers that have trapped hornets are represented by a solid circle. (B) Representation of the morphological variables on the correlation circle of the first two factorial axes. The significance of discrimination in morphology among taxa was assessed by a MANOVA ($F= 27.65$, $df=3$, $p < 0.001$).

Table 3. Plant morphological variables influencing hornet capture. Effects of the pitcher morphological variables retained in the best logistic mixed regression model to explain the probability of hornet capture. “Chisq” values refer to type-III Wald tests

Explanatory variables	Hornet capture			
	Estimate (±S.E.)	Chisq	Df	p-value
Intercept	0.40 (±2.33)	0.03	1	0.8652
Cavity length	0.05 (±0.02)	8.52	1	0.0035 **
Aperture width	-0.31 (±0.13)	6.24	1	0.0125 *
Wing width	-0.45 (±0.16)	8.09	1	0.0044 **
<i>Plant identity</i>				

Discussion

Our results, based on observation of direct interactions between Asian hornets and four *Sarracenia* taxa, show a high capture success of hornets in these carnivorous pitcher plants under enclosure conditions. But only the two taxa closest to *S. leucophylla* trap this large flying prey effectively and do significantly better than simple dummy pitfall traps. Such a high trapping rate is due to a combination of attraction and capture traits, which we will attempt to disentangle in order to assess their respective contributions. We will also highlight the traits that are most successful in trapping these large flying insects, which may make *S. leucophylla* a species particularly adapted to the capture of such prey.

Rapid and efficient capture of hornets by the hybrids closest to *S. leucophylla*.

Even if the results are expected to be less striking under freedom conditions in the field, the trapping of *Sarracenia* pitcher plants towards the Asian hornet in laboratory is a proven success. In total, 80% of the hornets were found dead inside pitchers 2 days after the end of our observations. Moreover, the proportion of visitors decreases significantly with the time of observation, reaching already 10% of the initial number after only five hours of observation. Since we did not observe a loss of interest in the plants by the hornets over time, this testifies of the rapidity of hornet capture. However, hornet capture varied between *Sarracenia* taxa. Only *S. X leucophylla* with more than half of the hornets caught and *S. X Juthatip soper* differ significantly from the control plants in terms of trapping success, suggesting that

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they -and probably also its close relative, *S. leucophylla*- are better equipped than the other two to trap flying prey such as the Asian hornet. This questions the vision of a passive and random trapping system that has been proposed for *Sarracenia* pitcher plants based on a literature review (Ellison & Gotelli, 2009). Indeed, as *S. purpurea* does not differ from control pitfall plants in its poor capture success and as it is the most studied taxon (Wolfe, 1981; Cresswell, 1991; Cresswell, 1993; Dress *et al.*, 1997; Heard, 1998; Newell & Nastase, 1998; Deppe *et al.*, 1999; Ellison & Gotelli, 2002; Bott *et al.*, 2008; Milne, 2010; Luciano & Newell, 2017), one can wonder if this general vision of “passive pitfall traps” is not biased. What makes the two pitcher plants closely related to *S. leucophylla* so successful and what explains the differences observed in capture success with the other two?

Attraction: the central role of lid as a colourful banner and nectariferous area.

First of all, attraction seems to play a major role in the trapping system of the carnivorous plants. This is supported by the very high number of hornet visits to the pitchers and the long duration of these visits. Attraction signals are diverse among carnivorous pitcher plants (Ellison and Adamec 2018) but in this experiment, visual signals might play a predominant role over olfactory ones because the odour emanation from the different taxa are likely to mix in the cage. We show that the lid, which received most of the visits, concentrates most of the attractive signals.

Indeed, the dominance of a white colour on the outer side of the lid is associated with a higher number of visits. White areoles are a particularity of the two hybrids, *S. X leucophylla* and *S. X Juthatip soper*, probably inherited from their close natural relative, *S. leucophylla*. These white areas are strongly suspected to be attractive signals for flying hymenopterans because of the high contrasts against the green background and the pitcher body they produce for the vision system of these insects (Dupont *et al.* (2022). In hooded pitcher plants where the pitchers form an upper dome bearing an almost vertical orifice (Moran *et al.*, 2012; McGregor *et al.*, 2016), such white semi-transparent fenestrations have been shown to function as light traps in the inner side of the hood, enticing prey going further into the trap once in its vicinity. In our study, the white fenestrations seem to play a major role -but from the outer side- in the initial and longer distance attraction of insects, which supports earlier results found with flies (Schaefer & Ruxton, 2014).

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The white colour is less frequently dominant on the inner side of the lid; but a more frequently dominant red colour, especially in *S. X Juthatip soper* and *S. X leucophylla*, is associated with a higher number of hornet visits. At first look, this suggests that red coloration is also attractive to insects, as often suggested for carnivorous plants (Juniper *et al.*, 1989) and as proposed by Schaefer & Ruxton (2008) who found that *Nepenthes ventricosa* with red pitchers captured significantly more flying insects than green ones, the same trend having been observed with artificially painted pitchers. However, such a positive relationship between red coloration and capture rate is not always supported (Green & Horner, 2007; Bennett & Ellison, 2009; Bhattarai & Horner, 2009). These latter studies instead proposed the interplay of other factors such as nectar production. Extrafloral nectar and red coloration are indeed often associated (Bennett & Ellison, 2009; Gaume *et al.*, 2016) and can thereby be easily confounded as proximate factors, in part because high light intensity, which is a crucial parameter for the growth of carnivorous plants (Givnish *et al.*, 1984), is known to enhance the production of both extrafloral nectar (Jones & Koptur, 2015) and anthocyanins in these plants (Yoon *et al.*, 2019) and other plants as a protection against UV/oxidative stress (Winkel-Shirley, 2002). The inner side of the lid is a zone of concentration of nectary glands (Adams & Smith, 1977) and is also the area of the lid the most visited by hornets, which we observed to feed on nectar. The sugar collected from nectar is indeed an essential fuel for them (Richter, 2000; Perrard *et al.*, 2009). Moreover, nectar production is found to be significantly associated with the number of hornet visits. Nectar, mostly produced by the inner side of the lid is therefore essential for keeping insects in the vicinity of traps, increasing their chances of being trapped. Yet, in our statistical analysis, the red coloration of the inner lid still significantly explains hornet visits when nectar production is accounted for, suggesting a real and additional role of red colour in the attraction of hornets. Even if hymenopterans like hornets are trichromatic and do not detect red colour, they usually use brightness to detect red (Martínez-Harms *et al.*, 2010). Therefore, although only measures of spectrophotometry would provide final proof, we suggest that brightness and/or colour contrasts of the inner lid against the green pitcher body (or against the outer lid when white) may serve as an attractive signal for insects. Other contrasting patterns between the upper part (especially the peristome) of the pitcher and the body part are known to serve as visual guides for insects in *Nepenthes* pitcher plants (Moran *et al.*, 1999). Moreover, the contrast between red and other plant colours is more

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often evoked to explain animal attraction in plant-animal interactions than the red colour itself (Schmidt *et al.*, 2004).

In summary, *S. X leucophylla* and *S. X Juthatip* soper are suggested to be more attractive towards hornets because of their highly nectariferous lid, whose both inner and outer sides contrast in colour with the pitcher body. The particular low attractiveness of green traps of *S. purpurea* in this study is explained by the absence of conspicuous contrasting patterns and a low nectar production, the species being known to produce more sugar-concentrated nectar at night (Deppe *et al.*, 1999).

The difference in height of the 4 *Sarracenia* taxa may also contribute to explain the higher visitation rates of the two longer-leaved plants, which may be more conspicuous to the hornet. This is supported by the strong correlation observed between the capture rate and the cavity length of the pitcher, which also reflects pitcher size. Such an apparency hypothesis proposed for flying insects has also been raised in a context of prey-pollinator conflict in a carnivorous sundew to explain stronger attraction of pollinators to flowers erected at a higher distance than traps (El-Sayed *et al.*, 2016).

Capture and retention: the role of slippery surfaces and downward-pointing microstructures

The initial fall occurs mostly from the pubescent surface of the lid (Adams & Smith, 1977), which causes insects to lose their footing, as we observed in our videos. Such a pubescent surface, which is indeed expected to reduce insect adhesion by reducing the points of contact with insect pad, is also observed in *Heliamphora* (Sarraceniaceae), causing the fall of insects into the trap particularly when it is wetted by rain (Bauer *et al.*, 2013). We can suppose that such aquaplaning events also occur when the surface is wetted by nectar secretions. The higher fall/visit ratio observed in *S. X mitchelliana* might be explained by the presence in this species of a larger lid, which offers a larger pubescent and nectar area where hornets are supposed to linger more easily, increasing their chance of being trapped.

The low fall-to-visit ratio, as well as the observation of several escapes, show that capture is not systematic. Systematic capture of prey could prove counterproductive for the pitcher plant. Firstly, the capture of too many prey items could cause the pitcher to decay if digestion is not rapid enough (personal

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observation CD, LG). Secondly, from an evolutionary point of view, if a proportion of visitors are spared, this allows the recruitment of nestmates and thus ensures a less important but more reliable and sustainable food source for the plant in the long term, as well as the possibility for selection to shape attractive traits. These plants were indeed considered by certain authors to perform mutual rather than deceptive relationships with the insect communities in their habitats because they produce extrafloral nectar to insects, ants in particular, while trapping only a weak portion of the nectar visitors (Joel, 1988).

Once fallen into the trap, the hornets experience much difficulty in climbing up the pitcher wall where they were observed struggling and sliding, especially in *S. X leucophylla* and *S. X Juthatip soper*. The images from microscope showing the presence in these two taxa of a dense carpet of downward-pointing tips in the upper inner wall of the pitcher shed light on the incapacity of hornets to adhere to the surface. Slippery surfaces are common in pitcher plants and downward-pointing cells encountered also in *Nepenthes* and *Cephalotus* genera are good examples of evolutionary convergences (Poppinga *et al.*, 2010; Thorogood *et al.*, 2018). The microroughness caused by such downward-pointing tips, that are described as cuticular folds by Poppinga and collaborators, may reduce the surface contact area with insect pads (Poppinga *et al.*, 2010). We suppose that the surface anisotropy caused by the orientation of these folds can also prevent claw encroaching, as it was experimentally shown in *Nepenthes* (Gaume *et al.*, 2002; Gorb & Gorb, 2011). In addition to these structures, Poppinga *et al.* (2010) observed wax platelets in *S. leucophylla*, but not in *S. purpurea*. The wax observed in different pitcher plant genera was shown to reduce insect adhesion through the contamination of their pads by Gaume *et al.* (2004). We did not use scanning electron microscopy as the authors did and therefore did not observe any waxy layer in our taxa. However, both the absence of wax reported for *S. purpurea* and the absence of downward-pointing tips, which we report here, may easily explain the 100% escape rates in this species, and also partly the significant differences among taxa of the capture-to-visit ratios.

The traits that matters for the capture of large flying prey

The differences of capture-to-visit ratios among taxa imply differences in their ability of capture or retention *sensu stricto* (i.e. not trapping, which also involves attraction) of Asian hornets.

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With regard to retention, the trapping success of the pitchers, which increases with the length of the cavity and decreases with the width of the orifice (a variable correlated with the width of the cavity), can easily be explained if we consider that the hornet is a large flying prey. Indeed, for this type of insect, the narrower the trap, the more difficult it is for the insect to use its wings to fly and escape. However, if the trap is narrow enough, the large flying insect, which is then forced to use its legs, can instead stride between the walls of the trap and take support on either side of the walls to get up. But it will be hindered if the walls are slippery. Thus, it can be predicted that taxa more specialized in trapping large flying prey will combine at least the three characteristics: wide traps, narrow traps, and slippery traps, all of which together maximize the retention success of such prey. *S. X leucophylla*, which is known to trap comparatively more large flying insects than some of its congeners (Gibson, 1983; Gibson, 1991), indeed bears long, narrow pitchers with both of downward-pointing tips and wax. The same is true for *S. flava*, *S. alata*, and *S. rubra* (Poppinga *et al.*, 2010), which are also taxa with long, narrow leaves but whose prey spectrum is though unknown. However, the positive correlation obtained by Gibson (1991) between orifice width and maximum prey size is not, at first glance, in agreement with our results. Indeed, it should be considered that the width of the orifice should not be too narrow to allow the entry of the insect in the trap. The relationship between trapping probability and orifice width should therefore be quadratic, increasing up to a maximum and decreasing after a threshold. In our sample, most pitchers were likely to have an orifice width greater than this threshold since quadratic regression was not retained in our model selection. In contrast, the relationship obtained by Gibson is more easily explained if we consider that his sample conversely includes many pitchers with very small orifice sizes (including 6mm wide pitchers!) but also the maximum prey size, meaning that he tested more for selection than retention of prey.

With respect to prey capture, a notable difference between *S. X leucophylla* and the other taxa in the study is that it captures a higher proportion of head-first fallen hornets, presumably because this taxon has the pitchers with the smallest cavity widths thus preventing any possibility for the fallen hornet to turn around and escape. Similarly, Bazile *et al.* (2015) showed on the genus *Nepenthes* that for a fly, falling dorsally on the wings reduces its probability of escaping from the viscoelastic digestive fluid. They also speculate that the position of nectar glands near the aperture (Adams & Smith, 1977), as in

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Sarracenia, may cause insects to bend over before falling as observed by (Juniper *et al.*, 1989), thereby increasing their probability of being captured upside down. It may be noted that *S. X leucophylla* has a smaller lid opening angle than *S. X mitchelliana* and *S. purpurea*. Whether a lower angle might favour head-first captures remains to be demonstrated.

Finally, the fact that capture probability is negatively correlated with the maximal width of the wing is consistent since the wider the wings, the lower the investment in carnivorous traits (Ellison & Gotelli, 2002; Farnsworth & Ellison, 2008).

In conclusion, we were able to disentangle the traits involved in trapping in *Sarracenia* pitcher plants. The colour and nectariferous features of the lid must play a central role in attraction, the morphology of the pitcher must play a key role in prey retention, while its microtopography has more to do with insect slip and fall. As for traits involved in the capture of large flying prey, we will retain the presence of white areoles as visual signals, and trapping characters such as long and narrow pitchers combined with a zone of downward pointing tips covered by wax. Therefore, our study shed light on the traits under selection, which help understanding morphological evolution and diversification in these pitcher plants. From a less fundamental perspective, in a context where the invasive species, *Vespa velutina*, poses a threat to European honey bees (Perrard *et al.*, 2009), and because its trapping remains a failure (Rojas-Nossa *et al.*, 2018), drawing inspiration from the visual, morphological and trap-surface traits of these pitcher plants could help for the development of a more effective and ecologically-friendly trap.

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Supporting information

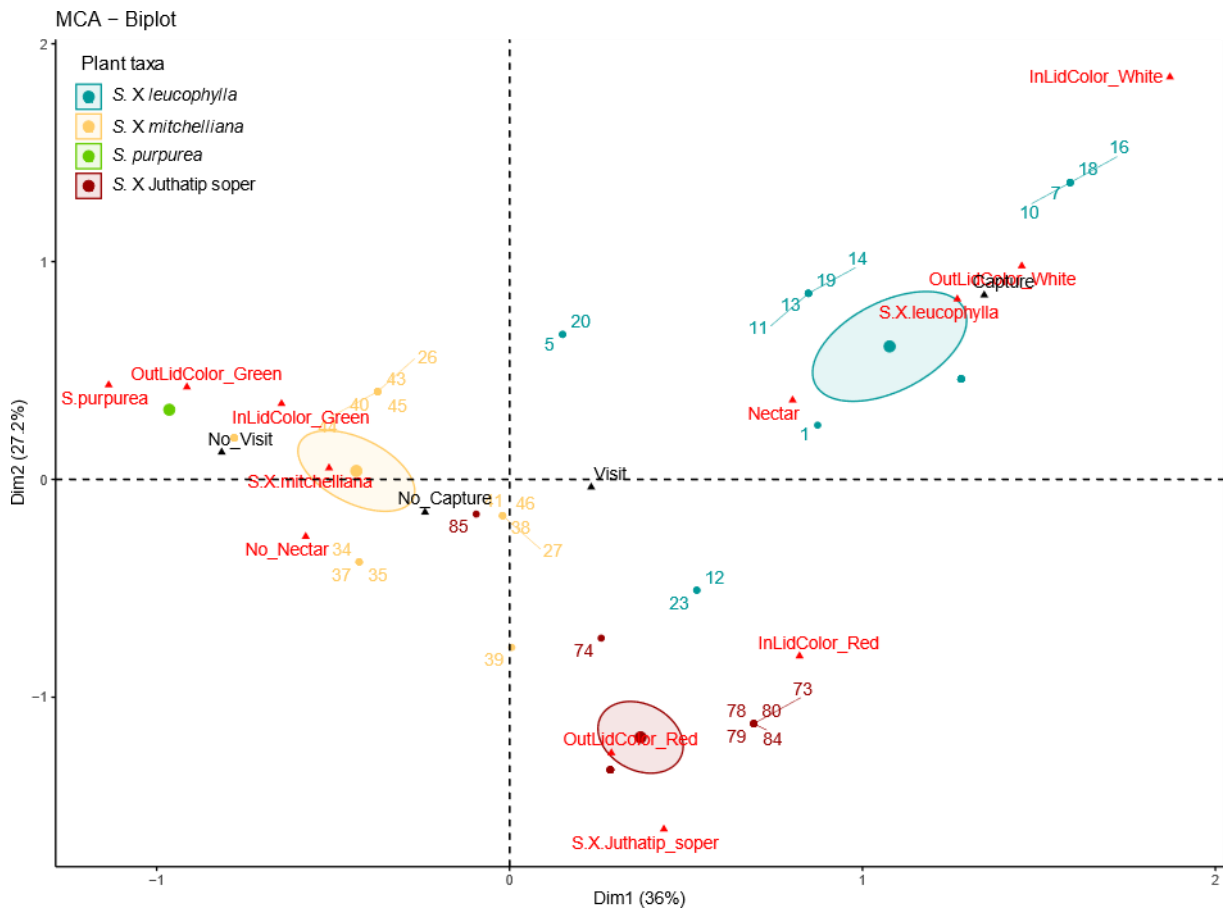


Figure S1. Results of the MCA analysis of the lid characteristics of all taxa. A. Map of pitcher individuals and variables pertaining to taxa, nectar production and dominant outer and inner lid colours on the first two factorial axes. The supplementary variables ‘Capture’ and ‘Visit’ are shown on the map but were not used in the calculation of distances of individuals. Pitcher individuals were grouped by taxon and confidence ellipses around taxa are shown.

Movie S1. A *Vespa velutina* feeds on nectar on the lid of a *S. X mitchelliana* pitcher

Movie S2. A *Vespa velutina* falls into the pitcher of *S. X leucophylla*

Movie S3. A *Vespa velutina* loses his footing while trying to get out of a pitcher of *S. X Juthatip soper*

Movie S4. A *Vespa velutina* escapes a pitcher of *S. X Juthatip soper*

Movie S5. A *Vespa velutina* trapped head first in a *S. X leucophylla* pitcher fails to turn around

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Table S1. Correlation between morphological parameters in all taxa. Correlation table pertaining to morphological variables (cavity length, cavity width, aperture width, peristome width, lid length, lid angle, lid width, and wing width). Correlation coefficients between each pair of variables, estimated using the Pearson method, are presented with their associated P-values, *: P<0.05, **: P<0.01, and ***: P<0.001.

	lid length	lid angle	cavity length	cavity width	peristome width	lid width	wing width
aperture width	0.60 ***	-0.01	0.15	0.40 ***	0.59 ***	0.53 ***	0.17
lid height		0.02	0.29 **	0.15	0.43 ***	0.61 ***	0.15
lid angle			-0.30 **	0.36 ***	-0.07	0.39 ***	0.08
cavity length				-0.49 ***	0.18	-0.37 ***	-0.27 **
cavity width					0.29 **	0.40 ***	0.17
peristome width						0.25 *	0.0001
lid width							0.44 ***

Chapitre 4

**Olfactory attraction of the Asian hornet,
Vespa velutina, to *Sarracenia* pitcher plants**

Résumé

Bien que le frelon asiatique continue d'envahir l'Europe, menaçant de plus en plus les abeilles domestiques, aucun piège efficace n'a encore été trouvé contre lui, les appâts actuellement utilisés n'étant ni assez attractifs ni sélectifs. Comme les sarracénies carnivores émettent des odeurs pour attirer leurs proies et que certaines espèces cultivées en Europe se sont révélées capables de capturer le frelon asiatique dans des expériences en laboratoire et sur le terrain, nous avons testé l'hypothèse que leurs odeurs étaient attractives pour le frelon asiatique. Nous avons d'abord analysé les odeurs de 32 plantes appartenant à quatre espèces de sarracénies. Nous avons ensuite testé les plantes en olfactométrie pour évaluer l'attractivité de leurs odeurs pour le frelon asiatique en plus de celles des abeilles et du miel en étudiant le parcours des frelons à travers le labyrinthe formé par la boîte de départ et l'olfactomètre en forme de Y. Un total de 406 tests a été réalisé et les données ont été analysées avec un modèle de Markov. Enfin, nous avons testé l'efficacité de capture des plantes sur le terrain en analysant le contenu de 223 urnes. Selon nos résultats, les odeurs des plantes présentaient différentes combinaisons de terpènes, benzénoïdes et dérivés d'acides gras selon l'espèce, deux espèces ayant un profil olfactif plus similaire entre elles. Les odeurs de ces deux mêmes espèces, *S. X mitchelliana* et *S. X Juthatip soper*, se sont avérées attirer significativement le frelon asiatique en olfactométrie, tout comme les odeurs de miel et d'abeille qui suscitaient le plus d'attraction. Les odeurs de *S. purpurea*, le taxon le moins odorant, n'étaient pas attractives et celles de *S. X leucophylla*, le plus odorant, ont provoqué les taux de sortie (de la boîte de départ) les plus élevés du frelon mais ensuite aucun déplacement en direction de la source d'odeur. Cependant c'est *S. X leucophylla* qui avec *S. X Juthatip soper*, a été la plus efficace pour piéger les frelons, avec un succès de capture des urnes entre 50% et 100% selon les saisons et les années. *S. purpurea* n'a piégé aucun frelon et *S. X mitchelliana*, le taxon le plus attractif en olfactométrie, n'a pas piégé beaucoup de frelons non plus probablement à cause de la forme de ses urnes dont il a été démontré précédemment qu'elles étaient inefficaces pour les retenir.

Nos résultats sont donc encourageants pour la recherche d'un appât pour piéger les frelons. Nous discutons des COVs possiblement candidats pour de futurs tests et utilisations.

Olfactory attraction of the Asian hornet, *Vespa velutina*, to *Sarracenia* pitcher plants

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Abstract

Although the Asian hornet continues to invade Europe, threatening more and more honeybees, no effective trap has yet been found against it, as the current baits used are neither effective nor selective. As carnivorous pitcher plants emit odours to attract their prey and as certain *Sarracenia* taxa grown in Europe have been shown to capture the Asian hornet in both control and field experiments, we tested the hypothesis that their odours were attractive to the Asian hornet. We first analysed the odours of 32 plants belonging to four *Sarracenia* taxa. We then tested the plants in olfactometry to assess their attractiveness to the Asian hornet in addition to those of bees and honey by studying the path of the insects through a maze made of a starting box and an Y-shaped olfactometer. A total of 406 bioassays were performed and the data were analysed with a Markov model. Finally, we tested the capture efficiency of the plants in the field by analysing the contents of 223 pitchers. Plants were shown to have different combinations of terpenes, benzenoids and fatty acid derivatives depending on taxon, with two taxa having a more similar odour profile between them. The odours of these same two taxa, *S. X mitchelliana* and *S. X Juthatip soper*, were found to significantly attract the Asian hornet in olfactometry, in addition to the odours of honey and bees, which elicited the most attraction. The odours of *S. purpurea*, the least odoriferous taxon, were not attractive and those of *S. X leucophylla*, the most odoriferous, were found to elicit the highest exit rates (from the starting box) of hornets but no further approaches to the source. But it was the taxon with *S. X Juthatip soper*, the most effective in trapping hornets, with a pitcher capture success between 50% and 100% depending on seasons and years. *S. purpurea* did not trap any hornet. *S. X mitchelliana*, the most attractive taxon in olfactometry, did not trap many hornets probably because of the shape of its pitchers, which has been previously shown to be ineffective at retaining them. Our results are thus promising for the search of a bait for hornet biocontrol. We discuss of candidate COVs for future testing and use.

Introduction

Biological invasions, often caused by humans, are one of the greatest threats to biodiversity and have a significant impact on native species communities and the ecological services they provide (Chapin *et al.*, 2000; Bellard *et al.*, 2016). Eusocial insects, especially social wasps, are among the most successful invaders worldwide owing to their great adaptability and ability to exploit local resources (Cervo *et al.*, 2000; Lozada & D'Adamo, 2009). They use both visual and olfactory cues to locate their food sources (Wang *et al.*, 2014). Additionally, beyond their innate preferences, a high learning capacity allows them to quickly detect attractive *stimuli* emitted by potential food sources (McPheron & Mills, 2007a; Gong *et al.*, 2019).

The yellow-legged Asian hornet, *Vespa velutina nigrithorax* de Buysson, accidentally introduced in France in 2004 (Villemant *et al.*, 2006), is one of these invasive eusocial wasps. It has rapidly colonised other European countries such as Spain, Portugal, Belgium, Italy, the United Kingdom, The Netherlands and Germany (<https://frelonasiatique.mnhn.fr/biologie/#CarteProgression>). As many vespid wasps, hornets forage in search of both proteins and carbohydrates (Edwards, 1980). In the European invaded areas, this hunter mainly attacks domestic honeybees, *Apis mellifera*, (Rome *et al.*, 2021) because they represent high concentration of defenceless prey (Arca *et al.*, 2014; Dong *et al.*, 2018). The Asian hornet not only applies direct predation pressure on bee colonies (Monceau *et al.*, 2014), but, in hover flight in front of the hives, it also causes them stress (Leza *et al.*, 2019) and foraging paralysis (Requier *et al.*, 2019), which leads sometimes to their total dismemberment (Requier *et al.*, 2019). Bees provide vital ecosystem services and play a key role in the maintenance of biodiversity (Biesmeijer *et al.*, 2006; Hung *et al.*, 2018) and in agricultural production (Gallai *et al.*, 2009). Since Asian hornets also threaten a diversity of other pollinators (Perrard *et al.*, 2009), as important as honeybees (Rader *et al.*, 2016), and disrupt pollination processes (Leza *et al.*, 2019; Rojas-Nossa & Calviño-Cancela, 2020), finding sustainable control strategies is a European matter of concern for both ecosystems and agriculture.

Among ecologically-friendly solutions, biocontrol seems promising (Laurino *et al.*, 2020), but the trapping systems so far tested still lack selective and efficient baits (Lioy *et al.*, 2020; Sanchez &

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Arias, 2021). Studies in search of baits are multiplying but they all focus on insect-derived cues and have not produced a satisfactory solution yet. For example, Asian hornet workers have been shown to be strongly attracted to the odours of bees, bee pheromones and hives products such as honey (Couto *et al.*, 2014). Some pheromones of the hornet itself have been identified (Cheng *et al.*, 2022) in the purpose of finding a pheromone-based trap. In comparison, little is known about the hornet attraction to plant volatiles.

However, the Asian hornet is involved in multiple interactions with a diversity of flowering plants (Ueno, 2015). An increasing body of evidences further call for studies devoted to plant cues. For instance, Chen *et al.* (2018) have shown that seed dispersal of *Stemosa tuberosa* by *Vespa velutina* was promoted by attractive cuticular hydrocarbon chemicals. Furthermore, other species of hornets and wasps involved in orchid pollination have been shown to be attracted to green leaf volatiles or bee alarm pheromones emitted by orchid flowers (Brodmann *et al.*, 2008; Brodmann *et al.*, 2009).

Generalist carnivorous plants have very performing visual and olfactory attraction systems, which often mimic flowers (Joel, 1988; Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010; Kreuzwieser *et al.*, 2014; Dupont *et al.*, 2022a) and/or exploit the innate or learned responses of insects to particular volatiles (Ruxton & Schaefer, 2011). This strategy helps them catch insects to supplement their nitrogen intake since they usually grow in very poor soils (Ellison *et al.*, 2003). *Sarracenia* pitcher plants, native to North America, are known to capture a lot of hymenopterans, mainly ants in some species (Heard, 1998; Newell & Nastase, 1998), many flying hymenopterans in other ones (Gibson, 1983). *Sarracenia flava* introduced in European fens has been observed to trap the European hornet, *Vespa crabro* but not bees (Adlassnig *et al.*, 2010). In March 2015, two horticultural hybrids have been reported in the bog of a French botanical garden to trap lots of Asian hornets among other Vespidae and *Diptera* (Meurgey & Perrocheau, 2015), while in fall the capture rate of these hybrids were reported to be less effective (Wycke *et al.*, 2018). However, the two taxa were mixed in those analyses, preventing any interspecific comparison. In a laboratory experiment, Dupont *et al.* (2022b) carried out a comparative analysis on four *Sarracenia* taxa and reported a high attraction and trapping success of *Vespa velutina* by taxa genetically close to *S. leucophylla*. Therefore, we raise the hypothesis that attractive olfactory cues in some *Sarracenia* pitcher plants are involved in the capture of the Asian hornet.

To test this hypothesis, we first analysed the odour of plants belonging to four natural and horticultural *Sarracenia* taxa. We then carried out olfactory experiments on workers of *Vespa velutina* by testing as *stimuli* the plant odours of the four taxa as well as those of bees and honey taken as positive controls because they were known to elicit attraction of *V. velutina* (Couto *et al.*, 2014). In addition to the simple analysis of insects' decision-making behaviour in the form of binary choices as usually done (Di Giusto *et al.*, 2010; Zhang, 2018; Proffit *et al.*, 2020), we also modeled the successive behavioural events of hornets in the olfactometer using an original and relevant method though rarely used in chemical ecology, a Markov chain model (Ricard & Davison, 2007; Davison & Ricard, 2011). We based this model on the real-time records of a digital tool that we specifically designed for insect-behaviour records in olfactometry. This kind of analysis considers all the information available (timing of events, duration, successive displacements, air turbulence zones in the tube) and allowed us to better account for the complex behaviour of hornets in their olfactory interaction with the plants. Finally, to assess the long-distance attraction to the hornet of these pitcher plants, we also compared their trapping success in the field for 3 years throughout the period of bee predation by the hornet at a site with hives.

Materials and method

Studied species, tested *stimuli* and sampling periods

Vespa velutina nigrithorax workers were caught in front of beehives at two study sites (Lavalette and Baillarguet, Montpellier, France) by sweeping between September and November from 2016 to 2019 and also in May-June in 2018 and 2019. Once captured, each hornet was put into an individual polyethylene box pierced with 5mm-diameter holes for air flux. Then the boxes were brought to a breeding chamber at a mean temperature of 23.30 ± 1.9 °C. The hornets were fed with water and candy (honey and sugar mixture) *ad libitum*.

All the studied plants were horticultural specimens obtained from the carnivorous plant nursery "Nature and Paysages" (Peyrusse-Massas, Gers, France) at the same juvenile stage and replanted in the beginning of April 2016 in the experimental station of AMAP and Lavalette (Montpellier, France). Two of the taxa were natural species: *S. purpurea* subsp. *venosa* and *S. X mitchelliana* = *S. purpurea* X *S.*

leucophylla. The two others were horticultural hybrids: *S. X Juthatip soper* = (*S. leucophylla* X *S. X mitchelliana*) and *S. X leucophylla* = *S. leucophylla* X *S. X Juthatip soper*. The plants of the four taxa were grown outdoors in a sunny place at AMAP and in front of hives at Lavalette, at about 12m distance of them in peat mixtures consisting of 2/3 blond peat and 1/3 sand on a background of clay balls. They were regularly watered with demineralized water to match their natural moist and mineral-poor environment.

The odours of 8 plants per taxon were collected by the headspace technique during 8 sessions in 2016 (4 taxa per session for 4 sessions, 1 taxon per session for 4 other sessions, 6 sessions in summer [end of August], 2 in autumn [Beginning of October]) and analysed by GCMS using the procedure detailed in (Dupont *et al.*, 2022a). There were only 7 samples for *S. X mitchelliana* because one chromatoprobe filter was broken when sampled. The *Sarracenia* taxa were tested in olfactometry each of the 4 years (about 10 plants per taxon), bees were tested in 2017 and 2018 only, honey in 2018 and 2019 only. *Apis mellifera* bees tested as *stimulus* were caught in front of beehives at Lavalette site by sweeping and kept in individual chambers in the same conditions as the hornet. Only one bee was tested each time and the bee was renewed as soon as it showed signs of fatigue (total of 8 bees). Honey tested as *stimulus* was harvested from the hives and 1 cl was tested each time.

Y-tube olfactometer setup

Bioassays were carried out using a Y-tube olfactometer (total length of the glass tube: 36 cm, diameter: 4 cm, inner angle: 50°, Fig. 1). To avoid any visual bias, the Y-tube was placed in an off-white Styrodur® polystyrene box and illuminated by a central overhanging lamp. An air flow of 40 ml/min was sent to both arms of the Y-tube olfactometer using a pump. The air was previously cleaned of any atmospheric pollutants by passing it through a 1000 mL vial filled with activated charcoal, and humidified by passing it through a vial filled with water. Two glass chambers, were each connected to one of the olfactometer arms (via El or Er, Fig. 1) with Teflon® tubes of the same lengths. The chambers used were glass vials when the *stimulus* tested was a bee or honey, or they were odour- and -leak less Nalophan bags for plant *stimuli*. Both chambers at each side of the olfactometer were systematically of the same nature. The flow rate of 40ml/min was selected because it was the rate that minimized the turbulence events in the Y-arm

junction area as we observed in previous smoke tests using smoke pens composed of paraffin and stearic acid; however, it did not completely suppress the reflux phenomenon in the control arm.

Insect olfactory bioassay

To test the attractiveness of a *stimulus*, in one arm of the Y-tube, clean air was sent through the chamber containing the *stimulus* sample, while clean air alone was sent as control through the empty chamber of the other arm. An opaque individual box containing a hornet was connected to the end of the Y-tube (Fig. 1). Control tests were performed with only clean air being passed into each arm of the Y-tube. Once the air flow was turned on, the box was opened to allow the hornet access to the olfactometer and its behaviour was recorded during five minutes. A digital tool, programmed with Access software, was specifically designed to record each of the transition events (triggered by observers), i.e. the insect movements between the different compartments of the olfactometer including the starting box (Fig. 1) and their associated timings. During each 5-min session, all the successive transition events were thus recorded. To avoid bias due to any preference of insects for one side of the Y-tube, the position of the tested *stimulus* and control were reverted every five runs. Moreover, we also *a posteriori* tested for a side preference and there was no preference of the hornet for a specific side. After each trial, the Y-tube was replaced with a new one and cleaned using 90% ethanol. A hornet individual was never tested twice on the same type of *stimulus*. For each test, the hornet's previous number of tests, length of captivity and time of day at the time of testing were also recorded. A total of 406 bioassays were thus carried out on 268 different individuals with an average of 1.51 bioassays per hornet.

Field capture of hornets by the pitcher plants

As the lifespan of a pitcher is 1.5-2 months depending on the plant species (unpublished results) and as for such time laps the head capsules of hymenopterans were not digested by the plant in comparison to the soft parts (pers. observ.), we haphazardly selected mature pitchers of each species on different individual plants each two months 3 times per year, i.e. in the middle-end of August, middle-end of October and middle-end of December, which correspond to three two-month trapping-periods for

pitchers: July-August, September-October, November-December, covering the whole season of hornet foraging activity in front of beehives. We thus collected a total of 223 pitchers for three consecutive years (2016, 2017, 2018). After collection, each pitcher was opened to check whether they had trapped Asian hornets or not.

Statistical analyses

Analysis of plant odour profiles

We tested whether the odour quantities released by pitcher plants differed according to taxon and season considering the variability imputable to sampling session as a random variable in a general linear mixed model. The quantities of Volatile Organic Compounds (VOCs) were square-root-transformed prior to analysis to achieve normality. We also tested whether the plant taxa differed in their relative quantities of VOCs using a non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarities of relative proportions (of VOCs), using the “metaMDS” function from the *r* package “Vegan”. The significance of differences in scent and prey patterns among taxa was assessed by permutational multivariate analyses of variance (PERMANOVA) with 999 random permutations using the “adonis” function from the *r* package “Vegan” and pairwise comparisons, whose P-values were adjusted for multiple comparisons using the Holm’s method.

Analysis of *stimulus* attraction using logistic regression models

We explored how the *stimulus* differed in their attraction towards the Asian hornet and which other factors influenced this attraction using mixed logistic regression models with a binomial distribution and a logit link function. We thus tested whether the probabilities for the hornet (1) to get out of the starting box, (2) to choose the arm with *stimulus* first when it went out of the box, and (3) to touch the arm tip with *stimulus* first when it made a choice, were dependent on the type of *stimulus*, the identity of the hornet (taken as random variable), its previous number of tests, its duration of captivity, the season and the time of the day (morning, afternoon).

As none of the above factors except *stimulus* significantly influenced these probabilities, for each *stimulus*, we performed a binomial test to know whether the probability to choose the arm (or touch

the arm tip) with *stimulus* first was significantly different from a random choice, using the function “binom.test” and a probability of 0.5.

For each *stimulus*, we also tested whether the number of visits to the arm or touches of the arm tip were significantly higher in the presence of the *stimulus* odour than in its absence, using a Wilcoxon signed rank test adapted for paired data sets. We could not use the more powerful Student T-test adapted for paired data sets because the difference between the two values of the paired data were not normally distributed. Using a Poisson regression model, we also tested for an effect of *stimulus* presence and type of *stimulus* on the number of visits to the arm or touches of the arm tip.

We also explored whether the *stimuli* elicited different exit times using a survival regression model, assimilating the occurrence time of an exit to a survival time, which was right-censored since the observation time was limited to 300 seconds. We used a survival regression model using function “survreg” from the R package “survival” (Therneau, 2015) testing the factors ‘*stimulus*’, ‘previous number of tests’, ‘duration of captivity’, ‘season’, and ‘time of the day’ as explanatory variables of the occurrence of an exit and the duration time until it occurred. We also used the function “survfit” from the R package “survival” and Kaplan–Meier plot to visualize the survival curves.

Overall analysis of attraction of *stimuli* using a Markov model

We investigated whether the *stimuli* differed in their overall attractiveness using a Markovian model. The olfactometry device was considered as a set of 6 compartments connected to each other as shown in Fig. 1. Once in a compartment, the insect had the possibility to move freely from this compartment to any connected compartment. For the special case of the compartments E_l and E_r into which the hornets could not enter, we considered that each time the insect touched the inner walls of such a compartment with its antennae, there was a round trip from its compartment to that compartment, which was thus recorded as two transitions events. The complete trajectory of an insect is the succession of compartments it has visited. This trajectory is divided in elementary events that are the transitions between two successive compartments. These transitions are supposed independent from each other (Markov' hypothesis). The question was thus to model the choice by the insect of the next compartment knowing its current position. To do this, we defined transition probabilities between compartments based

on a characterization of the compartments of destination by a positive coefficient that we called “Attractiveness”. From a given compartment, the transition probability to a connected compartment was calculated as the ratio of the “Attractiveness” of this compartment to the sum of the “Attractiveness” of all the connected compartments. For example, if the hornet was in compartment T connected to compartments L, R and B, the probability of joining the compartment L was:

$$p_L = \frac{A_L}{A_L + A_R + A_B}$$

The “attractiveness” was not necessarily the same for each compartment of the olfactometer. Its value was assessed from the characteristics of the experimental device. Here we considered the concentration of the *stimulus* in the different compartments. To approximate the phenomenon of reflux observed during the smoke tests, we considered the following concentrations: 100% for the arm and the tip of the arm where the *stimulus* odour was emitted, 50% for the opposite arm, 0% for the tip of the opposite arm and 50% for the tunnel and the starting box. More generally, if x is the concentration of the stimulus within a given compartment, the attractiveness is modelled by $A(x) = \exp(a \cdot x)$ where “ a ” is a coefficient to estimate. If $a > 0$, the *stimulus* is attractive; if $a < 0$, it is repulsive. The objective was to test the effect of the different *stimuli* on the attraction of the hornet, based on the observed trajectories of the hornet and the Markovian chain modelling the transition probabilities at each step. The model postulates that the transition events are conditionally independent from each other. This is Markov' property: knowing the present, the past does not bring information about the future. However, information about the past can be used by introducing dependent variables concerning the history in the modelling of attractiveness.

Analysis of field captures

To test whether the plant taxa differed in their capture success of the Asian hornet in the field, we used a logistic regression and a logit function testing as explanatory variables of the capture success, the factors ‘plant taxa, ‘year’ and ‘trapping period’ of the pitchers when they were collected.

For all linear and survival models, backward selection and Type III tests were performed and we selected the best model with the lowest AIC (Akaike's Information Criterion). Mixed models were estimated

with the function “lmer” from the R package “lme4” (Bates *et al.*, 2015). When necessary, post-hoc tests were performed between air control and other *stimuli* using the function “contrast” from the R package “emmeans” (Russell VL, 2020). P-values were adjusted for multiple comparisons using the Holm’s method. Statistics were performed using the software R version 4.0.3 (R Core Team, 2020). Model assumption was checked by plotting residuals against fitted values and normal QQ plot of the residuals.

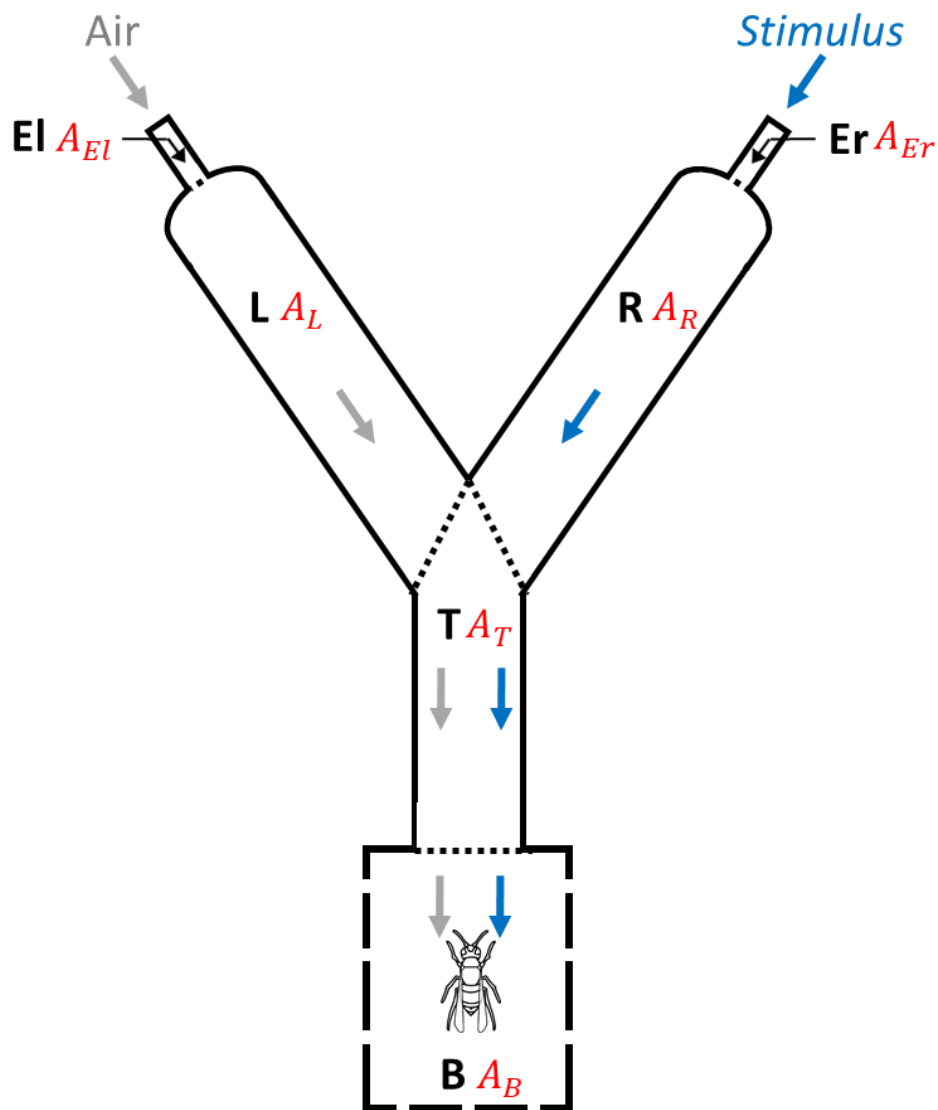


Figure. 1 Olfactometry setup, top view. Dotted lines mark the spatial transition between the different compartments of the olfactometer: starting box (**B**), tunnel (**T**), right arm (**R**), left arm (**L**), right arm tip (**Er**) and left arm tip (**El**). Each time a hornet crossed one of these boundaries, a transition event was recorded. In this example, the *stimulus* was placed in the right arm; we switched sides for the *stimulus* every fifth test. Arrows show the main directions of the air flow.

Results

Plant odours

In the end of summer and autumn, the *Sarracenia* pitcher plants released scents containing mainly terpenes, which made up three quarters of the odour bouquets, with also fatty acid derivatives and benzenoids. The *Sarracenia* pitcher plants emitted significantly different amounts of odours according to taxa (Fig. 2A, Table 1). *S. X leucophylla* emitted the highest quantity of VOCs, followed by *S. X. Juthatip soper* and *S. X mitchelliana*, which did not differ significantly from each other in their VOC release. *S. purpurea* released the lowest quantity of VOCs. *S. X. leucophylla* differed from the other ones in emitting more monoterpenes, while *S. purpurea* differed from the other ones in releasing less sesquiterpenes. Therefore, the plant taxa also differed in their relative quantities of VOCs (Fig. 2B) as further shown by the NMDS analysis (Fig. 3, Table 2), which separated the pitcher plants into three groups: *S. X leucophylla* on one side, *S. purpurea* on the other side, and *S. X Juthatip soper* and *S. X mitcheliana* in an intermediate group, which means that their VOC profiles resemble more to each other than to the VOC profiles of the other two ($F = 6.72$, $P < 0.0001$, Table 2). In particular, *S. X Juthatip soper* and *S. X mitcheliana* differentiated from the other two in releasing more (E)- β -Bergamotene (mean_*S.X.Juth.* = 10.5 ± 6 , mean_*S.X.mitch.* = 7.3 ± 6.5) than *S. purpurea* (mean = 0.07 ± 0.2) and *S. X leucophylla* (mean = 0). Altogether with *S. X leucophylla*, they also emitted more (E)- β -Ocimene, β -Caryophyllene, α -Ylangene and Myrcene than *S. purpurea* (Fig. 2). There was a seasonal effect in the releases of monoterpenes and benzenoids only (Table 1), the quantities of which were far lower in autumn than in summer (lsmeans \pm SE for summer/fall for monoterpenes: $7.87 \pm 0.78 / 2.71 \pm 1.32$, and for benzenoids: $2.53 \pm 0.41 / 0.38 \pm 0.70$).

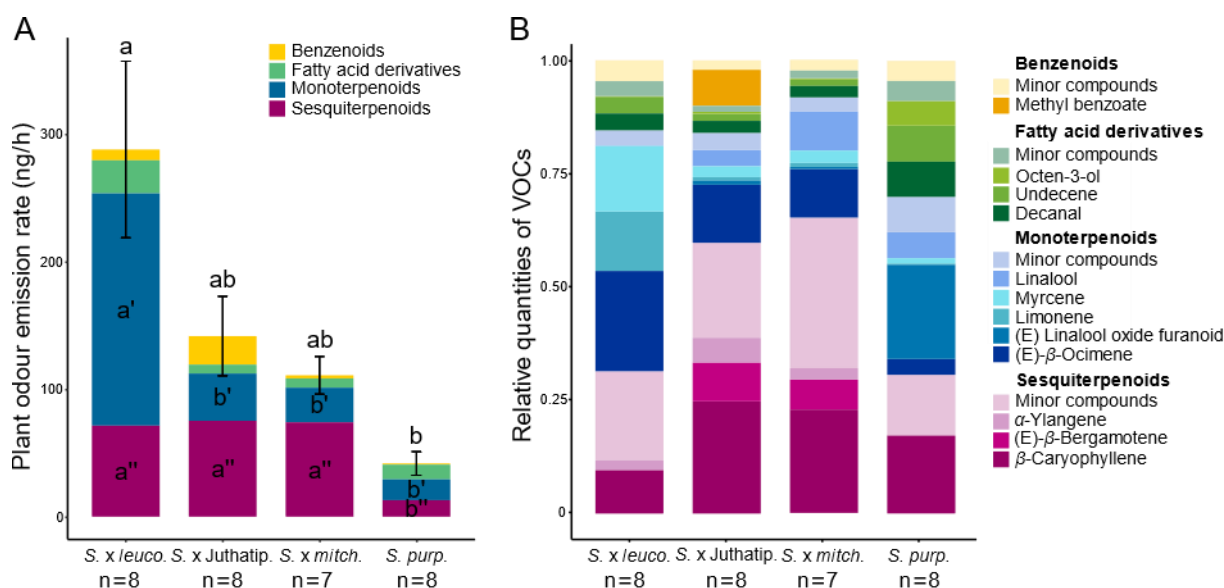


Figure 2. Odour profiles of entire plants of the different *Sarracenia* taxa. A. Emission rates in terms of absolute quantities of total VOCs and of VOCs of different chemical classes. Mean values are presented with their associated standard errors. Different letters show statistically different means among plant taxa according to the mixed linear models considering also the effects of season and the random variable “Sampling session” and using pairwise tests with Holm’s correction for multiple comparisons. B. Relative quantities of VOCs emitted in the scents. Only major VOCs representing more than 5% of the quantity of the odour bouquet were detailed.

Table 1. Factors influencing VOC emission rates from pitcher plants. Result of the mixed model testing an effect of plant taxon and season on total emission rates and emission rates of different VOC chemical classes.

Emission rate of	total VOCs		Benzenoids		Fatty acid derivatives		Monoterpenoids		Sesquiterpenoids						
	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value			
Explanatory variables															
Intercept	88,44	1	<0.001 ***	1,20	1	0,2738	19,35	1	<0.001 ***	22,99	1	<0.001 ***	83,03	1	<0.001 ***
Plant taxa	17,37	3	<0.001 ***	10,41	3	0,0154 *	1,37	3	0,7122	27,98	3	<0.001 ***	26,82	3	<0.001 ***
Season				7,06	1	0,0079 **				11,36	1	<0.001 ***			

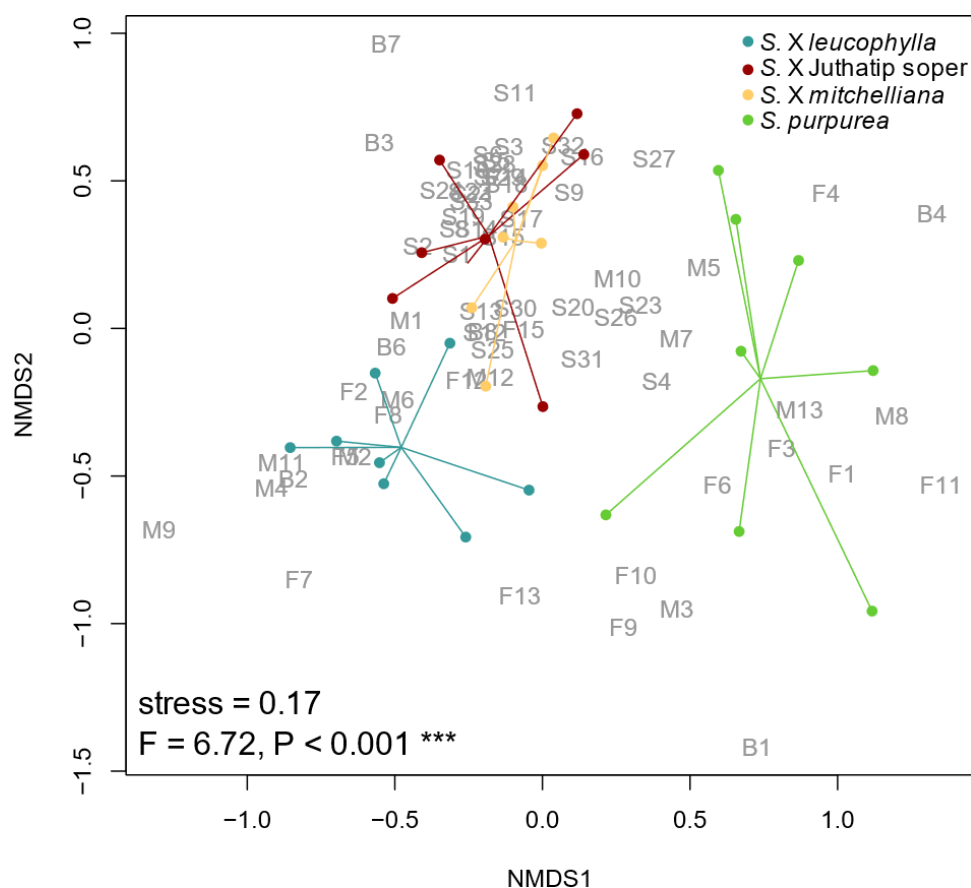


Figure 3. Similarity analyses of plant odour composition of the *Sarracenia* taxa. Distribution of *Sarracenia* plants according to the similarity of their odour in terms of relative amounts of VOCs emitted. The non-metric multi-dimensional scaling (NMDS) based on Bray-Curtis dissimilarity index showed a globally significant difference between *Sarracenia* taxa according to permutational multivariate analyses of variance ($P < 0.001$, see S2 for pairwise comparisons). The stress value was given to evaluate the quality of the ordination fit to the observed data. The VOCs are referred as Fi, Bi, Mi, Si, where i is the COV number and F, B, M and S refer to Fatty acid derivatives, Benzenoids, Monoterpenes and Sesquiterpenes, respectively.

Table 2. Post-hoc tests for taxa similarity analyses in plant odour composition. Results of the pairwise comparisons between taxa associated to the permutational multivariate analyses of variance on the relative amounts of VOCs emitted by plants (Fig. 3). P-values were adjusted for multiple comparisons with the Holm’s correction, *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$.

Differences in VOC composition of the plant odour bouquet			
Pairwise factors	F	Df	adjusted P-value
<i>S. X leucophylla</i> vs <i>S. X Juthatip soper</i>	7.47	1	0.006 **
<i>S. X leucophylla</i> vs <i>S. X mitchelliana</i>	7.36	1	0.006 **
<i>S. X leucophylla</i> vs <i>S. purpurea</i>	8.98	1	0.006 **
<i>S. X Juthatip soper</i> vs <i>S. X mitchelliana</i>	1.75	1	0.083
<i>S. X Juthatip soper</i> vs <i>S. purpurea</i>	7.27	1	0.006 **
<i>S. X mitchelliana</i> vs <i>S. purpurea</i>	6.34	1	0.006 **

Hornet olfactory attraction

The probability of first exit, arm choice and arm tip choice all depended on the type of *stimulus* being tested (Table 3). The proportion of hornets that went out of the starting box was higher for any *stimulus* than for clean air taken as control (Fig. 4A). *S. X leucophylla* *stimulus* elicited the exit of almost all hornets (97%), *S. purpurea* half of them, clean air only a quarter of them, while the other *stimuli* elicited between 64 and 70% of exits. According to the survival curves, the probability of hornet exit was significantly higher with any *stimulus* than with clean air and it was by far highest with the odour *stimulus* of *S. X leucophylla* (Fig. 4B). The predicted times of exit were accordingly highest for clean air and lowest for *S. X leucophylla* than for the other *stimuli* (Fig. 4C). The survival curves also showed a quicker exit of hornets when the *stimulus* was the odour of *S. X leucophylla* or *S. X mitchelliana* than when it was another *stimulus* (Fig. 4B).

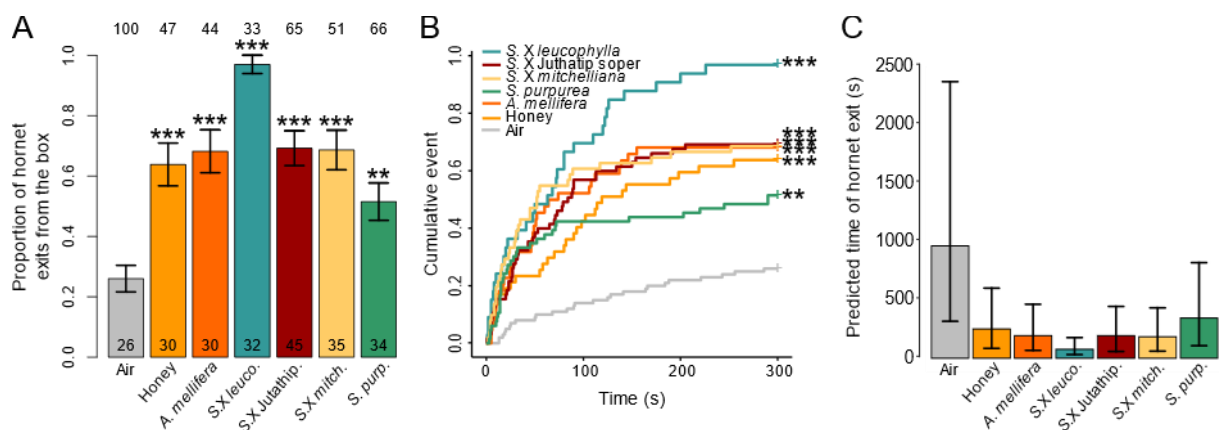


Figure 4. Probability and time of hornet exit according to *stimulus*. A. Mean (\pm SE) proportion of hornet exit from the starting box for each *stimulus* according to post-hoc pairwise tests of a logistic regression. B. Kaplan-Meier estimates of hornet exit probability over the 5min-time of observation for each *stimulus*. Mean (\pm CI) time of hornet exit for each *stimulus* predicted by the survival model based on right-censored data.

In control experiments, at first choice, the two arms with clean air were chosen equally often (Binomial test: $P=0.5235$), or were touched at their tips equally often (Binomial test: $P=1.0000$), attesting to the absence of side bias in the olfactometer. In contrast, the arms with the honey *stimulus* and the *S. X mitchelliana* *stimulus* were chosen first by hornets more often than the control arms with clean air (Fig. 5A), while the arms with the honey or bee *stimulus* were touched at their tip first more

often than the control arms (Fig. 5B). On the other hand, the number of touches in the arms with any type of *stimulus* was not higher than in the arms without *stimulus* (Table 4A, Fig. 5C). However, the number of touches of the arm tips significantly varied with the presence and type of *stimulus* (Table 4B). According to Wilcoxon signed rank tests for paired design, this number of touches was significantly higher in the arms with *stimulus* when the *stimulus* was honey or bee than in the associated control arms and it was marginally higher in the arms with the odour of *S. X Juthatip soper* than in the associated control arms (Fig. 5D). The number of touches in the arms with *stimulus* was highest for the bee odour and lowest for the *S. X leucophylla* odour (Fig. 5C). According to the Markovian model and the assumed concentrations in each compartment, the odours of bee, honey and the two *Sarracenia*, *S. X mitchelliana* and *S. X juthatip soper*, elicited significant attraction of hornets (Table 5).

Table 3. Effect of the type of *stimulus* on the probability of hornets to get out of the starting box according to (A) a logistic regression model and (B) a survival model, and on the probability to (C) choose the arm with *stimulus* first and (D) touch the arm tip with *stimulus* first (D) according to logistic regression models. “Chisq” values refer to type-III Wald tests.

Dependent variable: occurrence or not of	(A) getting out of the starting box			(B) getting out of the starting box			(C) choosing the arm with <i>stimulus</i> first			(D) touching the arm tip with <i>stimulus</i> first		
	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value	Chisq	Df	p-value
Explanatory variables												
<i>Stimulus</i> type	79.27	6	<0.001***	114.86	6	<0.001***	11.56	5	0.0413 *	14.55	5	0.0125*

Table 4. Effects of *stimulus* presence and type of *stimulus* on (A) the number of hornet passages in the arm and (B) the number of hornet touches of the arm tip in the olfactometer, according to a multiple Poisson regression model. “Chisq” values refer to type-III Wald tests.

Dependent variables	(A) Number of passages in the arm			(B) Number of touches of the arm tip		
	Chisq	Df	p-value	Chisq	Df	p-value
Explanatory variables						
<i>Stimulus</i> type	41.18	5	<0.001 ***	6.07	5	0.2994
<i>Stimulus</i> presence	0.24	1	0.6266	21.12	1	<0.001 ***
<i>St. type</i> : <i>St. presence</i>	3.05	5	0.6922	17.37	5	0.0038 **

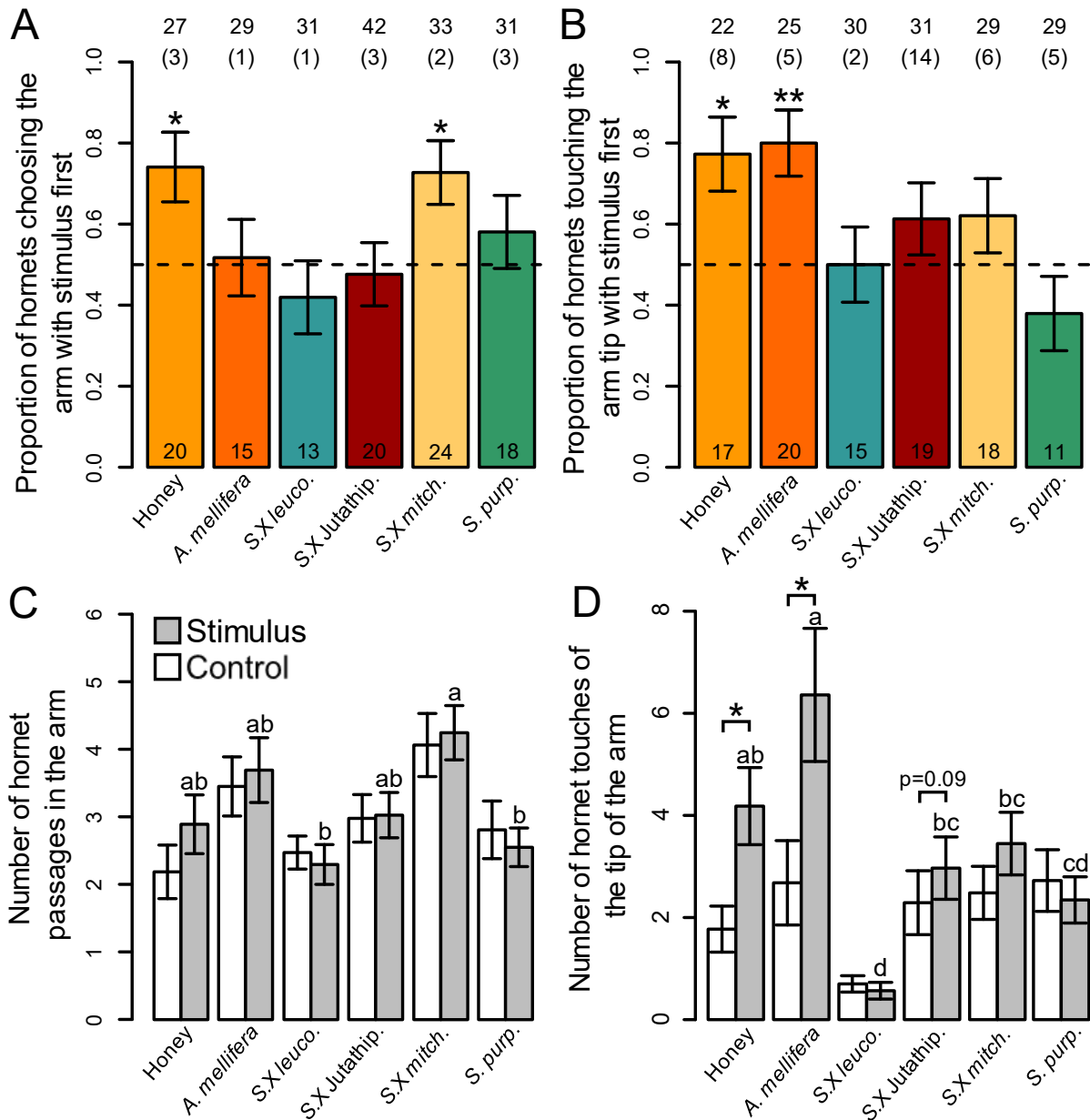


Figure 5. Hornet attraction to different *stimuli* according to choice (A, B) and motivation (C, D) behaviours. Mean (\pm SE) proportion of hornets, which (A) chose the arm with *stimulus* first, (B) touched the arm tip with *stimulus* first, for different types of *stimulus*. We compared these proportions with those obtained under the hypothesis of a random choice using binomial tests with a probability of 0.5. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$. The numbers of successes and total numbers of tests are shown. Hornets which did not make any choice (or did not make any touch) are indicated in brackets. Mean (\pm SE) number of hornet passages (C) and tip touches (D) compared among *stimuli* and between arms of the olfactometer (grey: with *stimulus*, white: without *stimulus*). The pairwise comparisons between arms were done using Wilcoxon signed rank tests designed for paired samples and the degrees of significance of the tests are given using * symbols. Different letters show statistically different ($P < 0.05$) means among *stimulus* types according to the Poisson multiple regressions and using pairwise tests with Holm's correction for multiple comparisons.

Table 5. Olfactory attractiveness estimated by the Markovian model for the different *stimuli* tested on the Asian hornet *: P<0.05, **: P<0.01, and ***: P<0.001.

Attractiveness			
<i>Stimulus</i> type	Estimate (±SE)	z-value	p-value
<i>A. mellifera</i>	0.78 (± 0.16)	4.96	<0.001 ***
Honey	0.66 (± 0.19)	3.40	<0.001 ***
<i>S. X mitchelliana</i>	0.45 (± 0.15)	3.01	0.0026 **
<i>S. X Juthatip soper</i>	0.33 (± 0.16)	2.11	0.0346 *
<i>S. X leucophylla</i>	0.13 (± 0.28)	0.47	0.6366
<i>S. purpurea</i>	0.11 (± 0.18)	0.62	0.5332

Hornet capture in the field

The proportion of pitchers that have trapped hornets depended on the plant taxon, the trapping period and the year (Table 6). *S. X leucophylla* and *S. X Juthatip soper* were the taxa with the highest hornet trapping success of 56% (36 out of 64 and 34 out of 61 pitchers, respectively) for the three years and three trapping periods, followed by *S. X mitchelliana* (7 out of 61 pitchers) with a trapping success of 11.5%, while the trapping success of *S. purpurea* was zero whatever the trapping period and year of sampling. The proportion of pitchers that have trapped hornets increased with the years of observation amounting to 100% for *S. X leucophylla* and *S. X Juthatip soper* and 20% for *S. X mitchelliana* in the trapping period of September-October in 2018. In all years, there was a peak in hornet trapping success in the trapping period of September-October while the trapping success was lowest in the trapping period of July-August, as shown by the predicted mean values in Fig. 6.

Table 6. Factors influencing the hornet trapping success by pitchers of *Sarracenia* carnivorous plants according to a logistic regression. “Chisq” values refer to type-III Wald tests

Dependent variable: Hornet trapping success of pitchers			
Explanatory variables	Chisq	Df	p-value
Plant taxon	87.96	3	<0.001 ***
Year	32.61	2	<0.001 ***
Trapping period	23.86	2	<0.001 ***

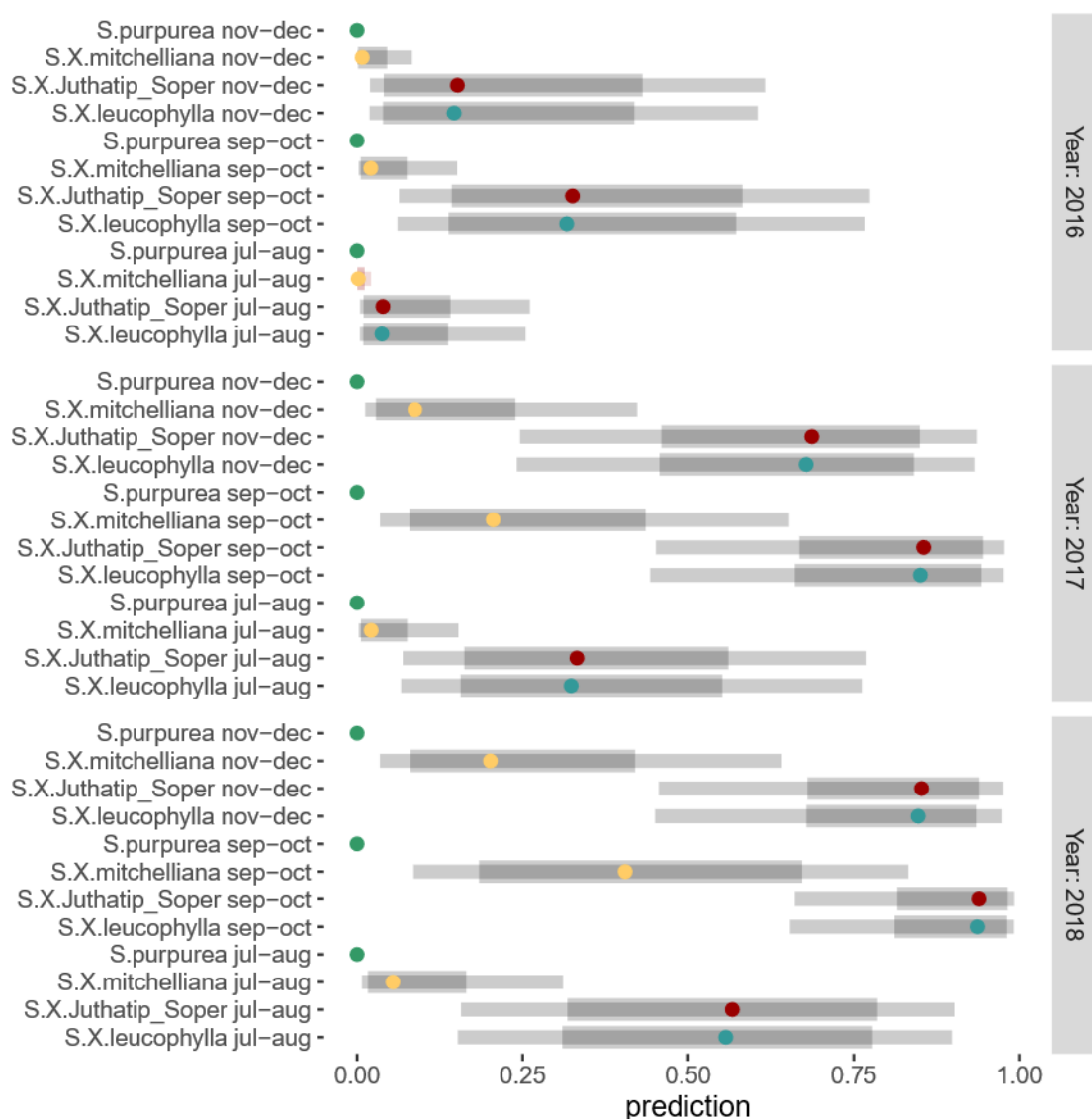


Figure 6. Predicted mean (\pm prediction intervals and confident intervals) proportions of pitchers that have captured hornets for the four plant taxa for each year and trapping period according to the logistic regression.

Discussion

The characterization of the odour profiles of four *Sarracenia* pitcher plants in late summer and fall shows that the four species release odour bouquets containing terpenes, fatty acid derivatives and benzenoids. The plants of *S. X leucophylla* were the most odoriferous, with an odour dominated by monoterpenes and those of *S. pupurea* the least odoriferous, while the plants of *S. X Juthatip soper* and *S. X mitchelliana*, intermediate in their emission rates, present quite similar odour compositions

dominated by sesquiterpenes. The insect bioassays show an attraction of the Asian hornet to the odours of *S. X mitchelliana* and *S. X Juthatip soper* only, but less marked than to the odours of honey and bees themselves. *S. X leucophylla* odors elicit the greatest rate of hornet exits, but hornets, once out of the box, do not move forward much more to seek the source of the *stimulus* as they do with the other *stimuli*. Our three-year field survey shows efficient (and increasing over time) success in capturing Asian hornets only for *S. X leucophylla* and *S.X Juthatip soper*, the two longer and narrower-leaved taxa, with a peak in capture during September-October pitcher trapping. We will discuss these results in light of our multi-approach analysis of hornet behaviour in the olfactometer and previous results on plant retention capacity. We will also propose the most promising avenues of research to identify the volatile organic compounds most likely to be used in the fight against the Asian hornet.

Hornet attraction to the tested odours

Hornets went out of the starting box more often and faster in the presence of an odour of any *stimulus* than with air controls. This response to all the tested *stimuli* is not surprising in such an opportunist species with a protein-diversified diet (Rome *et al.*, 2021) and with an extensive foraging activity on plants in search of carbohydrates (Matsuura & Yamane, 1990; Ueno, 2015). The neuroanatomical observation in this species of a complex antennal lobe with numerous *glomeruli* (Couto *et al.*, 2016) also supports the hypothesis that *Vespa velutina* is able to recognize a wide array of odour cues. These positive responses may also mean that all of the *stimuli* tested were rather attractive or at least not repulsive to the hornet since insects rarely leave their initial box and/or rarely walk/fly into the wind when repelled by an odour (Steck *et al.*, 2012; Stelinski & Tiwari, 2013).

Hornet attraction to honey and bees was unambiguous since the two *stimuli* repeatedly triggered positive response in the hornet choice and motivation analyses. Honey seems to be the most attractive signal since whatever the criterion considered, its positive effect stands out significantly. This is consistent with the results of Couto *et al.* (2014) who showed a strong attraction of Asian hornets to hive products, such as honey. The odour of honey elicits behavioural attraction in hornets because honey is a rich source of carbohydrates and an essential resource for them (Edwards, 1980), but as it is stored by bees, it also signals the presence of these highly coveted prey, whose protein supply is of crucial

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importance for the growth of their larvae (Perrard et al. 2009). Indeed, despite its generalist and opportunistic nature (Rome *et al.*, 2021), *Vespa velutina* shows a preference for honey bees in both native regions and invaded areas (Van der Vecht, 1957; Perrard *et al.*, 2009; Monceau *et al.*, 2013). As reported by Couto *et al.* (2014), social wasps are sometimes more influenced by signals associated with -but not directly emanating from- their prey (Cornelius, 1993; McPherson & Mills, 2007b). As a matter of fact, in our bioassays, the odour signal of the bee itself reveals to be weaker than that of honey since contrary to the case of honey, the hornet did not systematically choose the arm with the bee at its first choice. Its motivation seems to become high only when approaching the *stimulus* source since the bee was the *stimulus* that elicited the highest proportion of hornets touching the arm tip first on its side and the highest number of touches of the arm tip on its side.

Our study shows a comparatively weaker but significant attraction of hornets to the odours of the two *Sarracenia* taxa, *S. X mitchelliana* and *S. Juthatip soper*, but *a priori* no attraction to the odour of *S. X leucophylla* or *S. purpurea*. These results are consistent with the odour analyses of these pitcher plants since these first two taxa together form a distinct cluster and are most similar to each other but different from the other *Sarracenia* taxa in terms of emission rate and VOC composition. These results are however only partly consistent with field results since *S. X mitchelliana*, which is the most attractive species has only a weak trapping success in the field contrary to *S. X Juthatip soper*, which has high trapping success. But this is easily explained considering that the pitchers of the two taxa do not have the same shapes and retention abilities (Dupont *et al.*, 2022b). In a laboratory experiment, Dupont et al. showed that Asian hornet capture success was higher with longer and narrower traps, like those of *S. X Juthatip soper* and *S. X leucophylla*. In contrast, hornets would escape more easily from smaller pitchers with larger orifices such as those of *S. X mitchelliana* and *S. purpurea*. In line with this hypothesis, in their respective native distribution areas, the natural taxon *S. leucophylla* has a high retention success (Gibson, 1991) while *S. purpurea* shows very low retention capacity (Newell & Nastase, 1998). Furthermore, there is little doubt that *S. purpurea* is not attractive since this species does not capture any hornets in the field and is the least odoriferous species, with an odour that elicits the fewest exits and *stimulus* choice rate from the hornet. Conversely, the contrasting results obtained for *S. X leucophylla* question the attractiveness of the latter taxon to the Asian hornet. On the one hand, there is a body of

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evidences that *S. X leucophylla* is attractive to the Asian hornet. (1) It is the most odoriferous of the four taxa. (2) It releases a flower-like odour particularly rich in monoterpenes, whose emission rate at the pitcher scale is strongly correlated with the number of wasp prey, according to results obtained in summer (Dupont *et al.*, 2022a). (3) Its efficiency in capturing Asian hornets in the field is high, with *S. X leucophylla* and *S. Juthatip* soper even showing the highest capture rates of the four taxa in each year and period of trapping. (4) Its odour elicits the fastest and most numerous exits of hornets from their boxes. On the other hand, hornets show no preference for the arm with *stimulus* when the *stimulus* is the odour of *S. X leucophylla* and even display the lowest number of touches of the *stimulus* arm tips, as if the odour was repellent. Interestingly, for this taxon, the number of touches of the control arm tip is also very low suggesting that the odour is so strong that it backs up into the control arm, probably due to turbulence at the junction of the Y tube, as we observed with smoke tests. We hypothesize that the high concentration of odour in *S. X leucophylla* plants may trigger a rapid exit of the hornet, but, once out of the box and once the odour has diffused sufficiently into the olfactometer, the hornet does not try to go much further and find the source of the odour, as if the signal was too strong. It is known that the olfactory preference for an odorant can change with its concentration (Semmelhack & Wang, 2009). Therefore, the concentration of the odorant bouquet, which would have been olfactory attractive to the hornet when it was more diluted (at the very beginning of the experiment or in the field) could have become too high in the olfactometer and thus cause the signal to shift towards a repulsive effect. Alternatively, there is maybe some repulsive VOCs in the odour bouquet of *S. X leucophylla*, which are only detected when the hornets move sufficiently close to the source (are out of the starting box). Several hypotheses have been raised to explain the contrasted behavioural responses of insects based on the composition of attractants and repellents in the odour blend, such as the response-ratio hypothesis and the repellent-threshold hypothesis (Verschut *et al.*, 2019). Moreover, compared to honey or bee odours, the compounds diversity of these *Sarracenia* is very high (Dupont *et al.*, 2022a). It is thus likely that a difference of only a few compounds between taxa could induce quite different responses from the hornet.

A Markov model to consider the complexity of attraction behaviour in a Y-tube device

Using olfactometry to understand the attraction behaviour of an insect toward a plant odour only depicts part of the complex behaviour associated with decision making under natural conditions (Ballhorn & Kautz (2013). Similarly, focusing only on first binary choices, as many studies do (Brodmann *et al.*, 2008; Di Giusto *et al.*, 2010; Zhang, 2018) can further lead to short interpretations. For example, if we had not considered hornet exit time for the *S. X leucophylla stimulus*, we would have rejected without hesitation the hypothesis that it is attractive, attractiveness which in fact turn out to be likely at long range given the high concentration of VOCs released by this taxon and its high capture capacity in the field. Focusing only on the first binary choices, we would also have rejected the hypothesis of attractiveness of bee odour and that of *S. X Juthatip soper*. However, it was worth considering the number of arm tip contacts that could measure hornet motivation behaviour at shorter distances, as they were found to be significantly and marginally higher in the presence of *stimuli* for bees and *S. X Juthatip soper* respectively, highlighting their previously unnoticed attractive power.

In our study, the number of hornet passages never differ between the arm with and the arm without *stimulus* regardless of the nature of the tested *stimulus*. Hornets may have a tendency to go back and forth in an attempt to escape the device rather than actually seeking the source of an attractive odour. In general, captured and stressed Hymenoptera attempt to return directly to their nest after capture (Turchi & Derijard, 2018). However, the number of passages can also measure the overall excitability of the hornet, as insects generally show more activity when there is olfactory interest (Steck *et al.*, 2012). In this case, given the results of the Poisson regression analysis, the *stimulus S. X mitchelliana* would elicit significantly higher hornet arousal than any other *stimulus*, while *S. X leucophylla* and *S. purpurea* would elicit significantly lower hornet arousal than any other *stimulus*, which is consistent with the other results obtained. The lack of difference in the number of passages for the two arms, therefore, may instead reflect turbulence events in the air flow at the Y junction, which leads to partial reflux of the odour into the control arm and thus to signal interference in the first part of the arm.

Therefore, using a method that considers the bias of the device and incorporate all available information, such as the Markovian model we proposed, should yield results more in line with the reality of the attraction. In particular, our model considering the transition probabilities from one compartment

to the other, allows to highlight the attractiveness of *S. X Juthatip soper*, which is much less obvious using the other simpler attractiveness criteria. Finally, in our study we did not consider the residence time in each compartment as Davidson and Ricard did in their model, which presents a different network architecture than ours but the general principles are the same (Davison & Ricard, 2011). Consideration of this information in the future would certainly help to test and portray the overall behaviour of hornets towards attractive odours in a very accurate manner.

Candidate VOCs for a bait inspired from the pitcher plants

The Asian hornets, which have been caught in front of beehives, were certainly not naïve to the odours of bees and honey and this may explain their particularly high attraction to these two *stimuli*. Indeed, social wasps, as many other social hymenopterans (Giurfa & Sandoz, 2012), possess efficient memory, as well as high associative learning abilities (El-Sayed *et al.*, 2018) and this is notably the case for the Asian hornet (Gong *et al.*, 2019). In contrast, they are expected to be naïve to the odours of *Sarracenia* pitcher plants native to the Americas, which they are assumed to have never encountered before. Therefore, the olfactory attraction of the Asian hornet to certain *Sarracenia* taxa, demonstrated in these bioassays, although not outstanding, seems promising for the investigation of candidate VOCs with a view to seeking a bait for its biocontrol. The plants themselves cannot be used directly in biocontrol because, although they show some specificity to certain guilds of insect prey depending on the taxon (Dupont *et al.*, 2022a), like most carnivorous plants they are rather generalist, and thus not very selective (Wycke *et al.*, 2018; Dupont *et al.*, 2022a). The only major VOC that differentiates the odours of the two attractive taxa, *S. X mitchelliana* and *S. X Juthatip soper*, from the other two is (E)- β -Bergamotene. Unlike its associated isomer, α -Bergamotene, which is released by a number of plants, maize in particular, to attract herbivore predators in case of herbivory (Schnee *et al.*, 2006), this molecule is less frequently encountered. (E)- β -Bergamotene is known to be the male pheromone of a parasitic wasp (Cônsoли *et al.*, 2002) and it is found in the essential oils of root and flower of a tropical plant (Blanchard Boué *et al.* 2018). But nothing is known about its possible attractiveness to *Vespa velutina*. Considering that *S. X leucophylla* is also a plant of interest for hornet control, four molecules would additionally deserve to be tested in olfactometry: the typical floral compounds, (E)- β -Ocimene, Myrcene, β -

Caryophyllene (Knudsen *et al.*, 2006) and α -Ylangene, since they are shared in large amounts among the three hornet-attracting *Sarracenia*. (E)- β -Ocimene is a volatile pheromone of *Apis mellifera* brood, which expresses brood nutritional requirements to workers prompting them to seek new food resources (Maisonasse *et al.*, 2010). This molecule has been shown to be attractive to the Asian hornet by Couto *et al.* (Couto *et al.*, 2014). However, from the perspective of developing a selective trap for hornets, it is not a good VOC candidate since it is expected to attract bees as well. Myrcene is released in large quantity from the leaves and flowers of *Hedera helix* and *Heracleum sphondylium*, known to be visited by many vespid species for nectar rewards and also shown to elicit their response in electroantennography (Lukas *et al.*, 2020). Thus, Myrcene is also likely to be attractive to the Asian hornet. (E)- β -Caryophyllene is released as an indirect defence response against herbivores by old varieties of Maize and attracts parasitic wasps (Kollner *et al.*, 2008). Such herbivore-induced plant volatiles are known to attract plant bodyguards, as social wasps can be (Mumm & Dicke, 2010). As for α -Ylangene, it is present in some grape berries, giving them a ‘pepper’ aroma (Parker *et al.*, 2007), and vespids are known to be fond of grape berries and to forage on grapes in the fall (Madden *et al.*, 2017).

In conclusion, it is relevant to draw inspiration from the olfactory signals of *Sarracenia* pitcher plants to develop an effective bait against the Asian hornet. In the future, olfactory attraction experiments should be conducted on the specific VOCs most likely to be responsible for its attraction, which should be tested, in olfactometry and electroantennography, at different concentrations to obtain hornet dose-response curves. In addition, given that pitcher plant taxa exhibit very different visual cues that may influence their attraction and capture (Dupont *et al.*, 2022c) and that the Asian hornet is known to locate its food by both olfactory and visual cues (Wang *et al.*, 2014), it would make sense to test the combination of olfactory and visual cues in the future.

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Chapitre 4

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DISCUSSION

Dans la section suivante, nous apporterons un éclairage plus général sur ces travaux que celui qui a été développé au sein de chaque chapitre. Dans un premier temps nous synthétiserons les résultats obtenus lors des différentes expérimentations afin de mettre en évidence les performances d'attraction et de capture comparées des pièges des différents taxa de sarracénies vis-à-vis des insectes proies en général et du frelon en particulier. Ensuite, dans une seconde partie, nous mettrons en lumière les résultats nouveaux obtenus sur les signaux des sarracénies et discuterons de l'apport de notre travail dans la compréhension des mécanismes complexes d'attraction chez les sarracénies. Dans une troisième partie, nous évaluerons les signaux et les traits les plus efficaces dans l'attraction et la capture du frelon asiatique. Enfin, dans une dernière partie, nous proposerons des pistes de recherche pour le développement d'un appât attractif pour le frelon inspiré du piège des sarracénies.

1. Synthèse des résultats obtenus sur les performances du piège des différentes espèces

Le travail effectué nous permet d'abord de synthétiser les caractéristiques propres à chaque espèce de sarracénie, résumées dans le tableau 1.

Sarracenia purpurea forme de petites urnes avec une large ouverture qui capturent peu, principalement des fourmis et des diptères et aucun frelon asiatique. Elle est de couleur verte et rouge avec peu de contrastes. L'urne et la plante émettent une quantité moyenne à faible d'odeurs dominées par des dérivés d'acides gras et des terpènes et ne suscite pas d'attraction en olfactométrie pour le frelon qui la visite peu en laboratoire. Les capacités de rétention de l'urne vis-à-vis du frelon sont nulles.

Sarracenia X leucophylla forme de longues urnes étroites qui capturent beaucoup, principalement des hyménoptères volants et des diptères mais peu de fourmis et fréquemment du frelon asiatique.

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Le haut de l'urne est de couleur blanche et rouge avec des alvéoles blanches très contrastées en couleur et en clarté aux yeux des hyménoptères volants, elle émet une quantité importante d'odeurs dominées par des monoterpènes et suscite beaucoup de visites du frelon en laboratoire mais un comportement mitigé (tout puis rien) en olfactométrie. Les capacités de rétention de l'urne vis-à-vis du frelon sont moyennes.

Sarracenia X Juthatip soper forme de longues urnes étroites qui capturent beaucoup, principalement des hyménoptères volants et des fourmis et aussi fréquemment du frelon asiatique, le haut de son urne est de couleur rouge et blanche/rose avec des alvéoles très contrastées en couleur. L'urne émet une quantité faible d'odeurs dominées par des monoterpènes, des dérivés d'acide gras et des aromatiques tandis que la plante émet une quantité moyenne d'odeur dominée par les sesquiterpènes et suscite une attraction en olfactométrie pour le frelon qui la visite beaucoup en laboratoire. Les capacités de rétention de l'urne vis-à-vis du frelon sont fortes.

Enfin, *Sarracenia X mitchelliana* forme des urnes de taille moyenne avec une large ouverture qui capturent peu, principalement des fourmis et des diptères et assez peu fréquemment du frelon asiatique. Elle est de couleur rouge-orangé et verte avec peu de contrastes alvéolaires. Son urne émet une quantité moyenne d'odeurs dominées par des dérivés d'acides gras et des terpènes tandis que la plante émet une odeur dominée par des sesquiterpènes et suscite une attraction forte en olfactométrie pour le frelon qui la visite au départ beaucoup puis rapidement de moins en moins dans l'expérience en laboratoire. Les capacités de rétention de l'urne vis-à-vis du frelon sont plutôt faibles.

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Tableau 1. Synthèse des caractéristiques des 4 espèces de sarracénies étudiées au cours de cette thèse concernant leur capacité de capture, d'attraction, et la nature de leurs signaux et de leurs pièges.

Caractéristiques	<i>S. X leucophylla</i>	<i>S. X Juttahtip soper</i>	<i>S. X mitchelliana</i>	<i>S. purpurea</i>
Quantité des captures	grande	grande	faible	faible
Nature des captures	hyménoptères volants +++ diptères ++ lépidoptères ++ fourmis +	hyménoptères volants +++ diptères + lépidoptères + fourmis ++	hyménoptères volants + diptères ++ lépidoptères + fourmis +++	hyménoptères volants + diptères ++ lépidoptères + fourmis +++
Taille et forme du piège	longue et étroite	longue et étroite	moyenne et large	petite et large
Couleurs dominantes de l'opercule	blanc +++ rouge ++ vert +	blanc ++ rouge +++ vert +	rouge +++ vert ++	rouge + vert +++
Contrastes des alvéoles sur un fond vert	couleur +++ clarté +++	couleur +++ clarté +	couleur + clarté +	couleur + clarté +
Quantité d'odeur des urnes	+++	+	++	++
Quantité d'odeur des plantes	+++	++	++	+
Nature des odeurs des urnes	aromatiques + dérivés d'acides gras ++ monoterpènes +++ sesquiterpènes ++	aromatiques ++ dérivés d'acides gras ++ monoterpènes +++ sesquiterpènes +	aromatiques + dérivés d'acides gras +++ monoterpènes ++ sesquiterpènes ++	aromatiques + dérivés d'acides gras +++ monoterpènes ++ sesquiterpènes +
Nature des odeurs des plantes	aromatiques + dérivés d'acides gras + monoterpènes +++ sesquiterpènes ++	aromatiques ++ dérivés d'acides gras + monoterpènes ++ sesquiterpènes +++	aromatiques + dérivés d'acides gras + monoterpènes ++ sesquiterpènes +++	aromatiques + dérivés d'acides gras ++ monoterpènes +++ sesquiterpènes ++
Attractivité des odeurs pour le frelon	(+++/--)?	++	++	nulle
Capture du frelon sur le terrain	++	++	+	nulle
Capture du frelon en laboratoire	+++	++	+	nulle

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Taux de visites des frelons en laboratoire	+++	++	++ _{naïfs} / + _{expérimentés}	+
Taux de chute des frelons en laboratoire	++	++	+++	+
Taux de rétention du frelon en laboratoire	43%	75%	33%	0%

2. Les signaux des sarracénies responsables de l'attraction des insectes

Alors que chez les sarracénies, le rôle des odeurs dans l'attraction a été peu étudié (Jürgens *et al.*, 2009) et celui des signaux visuels restait encore très controversé (Newell & Nastase, 1998; Green & Horner, 2007; Schaefer & Ruxton, 2008; Bennett & Ellison, 2009), notre travail apporte des évidences de l'implication de ces deux types de signaux dans les stratégies de capture chez ces plantes à urnes.

a. Des signaux généralistes avec des particularités taxonomiques

Nous avons montré l'aspect généraliste du signal olfactif des sarracénies avec l'émission d'une grande diversité de composés connus pour être attractifs pour de nombreux insectes. Nous avons aussi montré la ressemblance du signal olfactif des pièges foliaires de certaines espèces comme *S. X leucophylla* et *S. X Juthatip* soper avec des odeurs typiques de fleurs. Ce signal généraliste est probablement adaptatif pour ces plantes car il permet d'attirer toutes sortes de proies comme le montre l'analyse des captures. Pour des plantes carnivores dont la survie dépend de la capacité à capturer des arthropodes, source la plus importante de nutriments dans leur milieu appauvri (Schulze *et al.*, 1997), la capacité d'attirer n'importe quelle proie en toute circonstance paraît donc essentielle.

Cependant ce caractère généraliste ne doit pas nous faire considérer les sarracénies comme de vulgaires pièges passifs capturant au hasard leurs proies sans réelle mise en place de stratégies

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spécifiques d'attraction, comme le suggèrent certains auteurs (Ellison & Gotelli, 2009). En effet, notre étude a également mis en avant des différences dans les traits d'attraction entre les espèces qui ciblent des types de proies particuliers, suggérant que ces plantes sélectionnent leurs proies et que les odeurs et les couleurs jouent un rôle initial dans cette sélection. Ainsi, nous avons montré des différences interspécifiques de composition et de nature biochimique des bouquets d'odeurs des sarracénies ainsi que dans leurs signaux visuels en lien avec des différences interspécifiques de composition de leurs captures. Cela ne remet cependant pas en cause le rôle de la morphologie, qui a été jusqu'ici le plus souvent considérée pour expliquer les variations dans les proies capturées (Gibson, 1983; Stephens *et al.*, 2015; Sheridan *et al.*, 2021). De fait, nous avons aussi montré que les urnes les plus grandes sont en général celles qui capturent le plus de proies volantes et que la forme et la taille de l'urne peuvent jouer directement sur les capacités de capture et de rétention. La morphologie agit en deuxième lieu par rapport aux signaux mais joue un rôle important de filtrage qui s'observe sur la composition finale des captures.

b. Faisceaux de traits et syndromes de piégeage

Notre étude suggère que certains traits jouent en fait probablement de concert avec des effets de synergie sur les captures en formant de véritables syndromes de carnivorie ciblant des taxa particuliers, comme cela a été montré chez les Népentes par exemple (Pavlovič *et al.*, 2007; Gaume *et al.*, 2016). En effet nous avons montré au final que ce sont des une combinaisons de traits qui semblent le mieux expliquer la composition des captures. Par exemple, les grandes urnes avec des alvéoles blanches et des odeurs florales semble associées à la capture d'hyménoptères volants tandis que les petites urnes produisant plus de dérivés d'acides gras sont associées à une capture plus importante de fourmis. L'avantage de ces signaux spécifiques pour ces plantes qui ont à priori intérêt à généraliser leurs captures, reste encore à éclaircir mais bien que l'aspect généraliste garantis une efficacité du piège en toute condition, la spécificité du signal pourrait, elle, réduire la compétition parfois forte des espèces entre elles (Gibson, 1983) en permettant aux urnes de cibler des proies

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particulières. La pression de compétition pourrait ainsi favoriser l'évolution vers 'un déplacement de caractère' dans le signal comme l'ont originellement défini par Brown et Wilson (Brown & Wilson, 1956). Certes cette hypothèse évolutive ne s'applique pas à nos hybrides horticoles qui ont été sélectionnés par l'homme. Mais le gradient de parenté que forment les 3 hybrides entre *S. purpurea* et *S. leucophylla*, deux espèces naturelles totalement différentes dans leurs traits foliaires et leur habitudes alimentaires, et que l'on trouve en sympatrie dans leurs aires d'origine (Folkert 1992), et le fait que les signaux soient en partie héréditaires comme en témoignent Sheridan (1997) sur l'aspect visuel et nos résultats (chapitres 1 et 2), laissent penser que les spécificités observées des hybrides reflètent en quelque sorte celles de leurs parents naturels. Ainsi les différences interspécifiques mises en évidence dans notre étude aident, malgré tout, à comprendre les traits sous sélection qui pourraient exister en conditions naturelles. Une telle étude, si elle était réalisée sur les espèces naturelles dans leurs habitats d'origine, aiderait incontestablement à mieux comprendre l'évolution et la diversification biochimique et morphologique chez ces espèces carnivores.

Pour finir, d'autres études restent à mener pour appuyer nos observations basées en partie sur une approche corrélationnelle. Des expérimentations par la suite permettraient de démontrer le lien de cause à effet des signaux sur les captures. Aussi, afin de toujours mieux décomposer la part imputable aux signaux et celle imputable à la morphologie dans le piégeage des proies, il serait intéressant de considérer d'autres espèces, par exemple une espèce grande et étroite ne présentant pas d'alvéoles blanches comme *S. alata*. Dans ce même objectif, il sera judicieux d'observer l'impact direct des signaux sur les visiteurs, en effet si la capture et les visites sont probablement assez corrélées pour les espèces de sarracénie avec de grande capacité de rétention, c'est sûrement moins le cas pour les autres, comme *S. purpurea*.

c. Des signaux olfactifs différents entre l'urne et la plante entière

Notre étude a mis en avant chez ces plantes carnivores des différences de signal olfactif selon l'échelle considérée. En effet les urnes et les plantes entières semblent différer quelque peu dans leurs

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bouquets d'odeurs. Dans le chapitre 4, nous avons montré par exemple une ressemblance forte entre les profils d'odeurs des plantes de *S. X mitchelliana* et *S. X Juthatip soper* qui formaient un même cluster avec une forte émission commune de sesquiterpènes tandis que dans le chapitre 1, nous avons montré, malgré la forte variabilité du signal, une ressemblance plus importante entre les profils d'odeur des urnes de *S. X mitchelliana* et *S. purpurea*, toutes deux émettant plus de dérivés d'acide gras. D'une manière générale, toutes les plantes semblaient se différencier des urnes par une moindre émission de dérivés d'acide gras (**Figure 12**). Ces différences peuvent s'expliquer par la saison d'échantillonnage ou par la méthodologie employée ou par la saison d'échantillonnage. D'une part contrairement aux urnes toutes échantillonnées en été, une partie des échantillons des plantes ont été prélevés en automne, une saison où la part de monoterpènes et d'aromatiques était plus faible (chapitre 4). Cependant, si on compare le profil d'odeurs des urnes et des plantes seulement en été (**Figure 12**), on remarque que ces différences subsistent. D'autre part, lors des prélèvements d'odeurs, les chromatoprobes utilisés sur les urnes étaient placés au plus près de leur ouverture, cette proximité a pu induire le prélèvement plus important de composés typiquement émis par les feuilles comme les « green leaf volatiles », principalement des dérivés d'acide gras (Matsui, 2006). Il n'est pas impossible non plus qu'une part des dérivés d'acides gras émis par les urnes viennent des odeurs délivrées par les insectes proies prisonniers ou morts dans les urnes. Par ailleurs ces différences plantes/urnes peuvent aussi s'expliquer par la forte variation du signal à l'échelle de l'urne. Il a par exemple été montré des différences de production de nectar et de coloration chez les sarracénies avec l'âge (Fish & Hall, 1978; Horner *et al.*, 2012). Dans le chapitre 2, nous montrons également une évolution du signal visuel en fonction de l'âge des urnes. Il est probable que des paramètres physiologiques comme l'âge ou encore la nutrition de l'urne influe aussi sur ses émissions de COVs (Jürgens *et al.*, 2009). Chez la *Dionaea*, une autre plante carnivore avec des pièges à mâchoires, il a été démontré que la plante émet une plus grande quantité plus de terpènes quand « elle a faim » (Kreuzwieser *et al.*, 2014). A l'échelle de la plante, qui comporte des urnes d'âges différents, cette variation est probablement atténuée. Il serait intéressant de tester l'attractivité olfactive des urnes seules en plus de celle des plantes pour savoir si

les différences de signal à différentes échelles influent sur l'attraction. A l'échelle de la plante, c'est une attractivité à longue distance qui pourrait se jouer, alors qu'à l'échelle de l'urne c'est peut-être une attractivité à courte distance. Ainsi on pourrait supposer que le signal à longue distance attirerait l'insecte sur la plante, et que le signal de l'urne à courte distance renseignerait sur son état physiologique la rendant (selon son état de digestion par exemple) attractive ou non pour l'insecte qui se tournerait alors vers une autre urne de la même plante plus en demande.

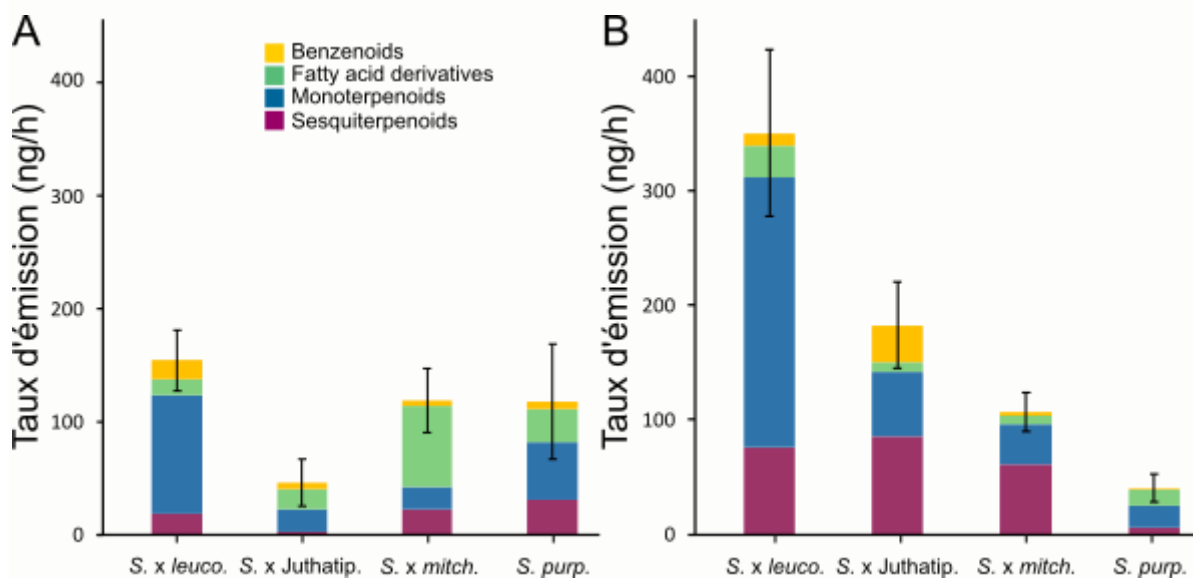


Figure 12. Profils olfactifs des quatre espèces de sarracénies en termes de taux d'émission en été pour leurs urnes (A) et leurs plantes entières (B). Les valeurs moyennes sont présentées avec leurs erreurs standard associées.

3. Les signaux et autres traits les plus efficaces dans l'attraction et la capture du frelon

Les pièges et appâts fabriqués ou commercialisés actuellement pour lutter contre le frelon asiatique capturent entre 1 et 5 % de frelons asiatiques en termes de nombre de spécimens (Figure 13 A, B, C). En l'état, d'après nos propres observations sur une même période d'échantillonnage en automne (Figure 13 D, E, F, G), il s'avère que le frelon asiatique constitue respectivement 2%, 5% et 9% des proies en nombre d'individus de *S. X mitchelliana*, *S. X leucophylla* et *S. X Juthatip soper*, tandis que *S.*

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purpurea n'en capturerait aucun. Les urnes des espèces de sarracénies montrent donc en moyenne une sélectivité semblable aux pièges commercialisés. Il est cependant à noter que *S. Juthatip soper* a une sélectivité deux à quatre fois plus élevée envers le frelon et les guêpes en général (19% des proies) que les autres types de pièges. En comparaison, l'étude de Wycke et ses collègues montrait une part globale inférieure à 5% des captures de frelons asiatiques pour les espèces de *S. X Juthatip soper* et *Sarracenia evendine* dont les parts respectives n'ont cependant pas été différenciées (Wycke *et al.*, 2018). Comme ces auteurs cependant, nous constatons que l'on ne peut utiliser directement ces plantes pour un contrôle biologique du frelon à cause de leur faible sélectivité et du nombre important d'autres insectes sauvages qu'elles capturent. Néanmoins, notre idée préalable était de trouver dans les leurres de ces plantes les molécules et les traits visuels les plus attractifs afin de les exploiter dans l'élaboration d'un piège artificiel. Nos résultats démontrent ainsi l'existence de certains traits efficaces chez les sarracénies dont on pourrait s'inspirer pour la confection d'un piège plus adapté.

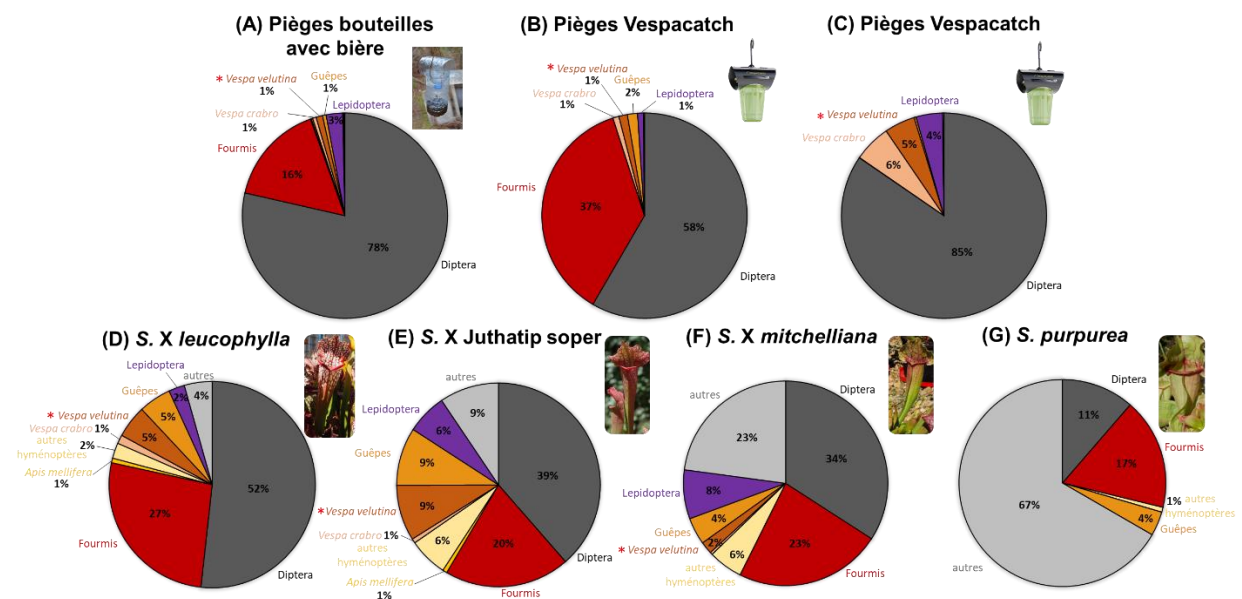


Figure 13. Proportions relatives du nombre de spécimens des principaux groupes d'arthropodes capturés par les pièges en automne pour (A) les pièges bouteilles classiques appâtés à la bière (N = 882 spécimens) d'après Lioy *et al.* (2020) (B) les pièges et appâts VespaCatch® (N = 4955) produits par Vétopharma d'après Lioy *et al.* (2020) (C) les pièges et appâts VespaCatch® (N = 2317) produits par Vétopharma d'après Sanchez & Arias (2021) et (D,E,F,G) les pièges des 4 espèces de sarracénies, *S. X leucophylla* (N = 2197), *S. X Juthatip soper* (N = 858), *S. X mitchelliana* (N = 258) et *S. purpurea* (N = 132) d'après les identifications réalisées sur 3 années consécutives 2016, 2017 et 2018 sur le site de Lavalette, Montpellier, France. Les groupes constituant moins de 1% des captures ne sont pas indiqués.

a. Les odeurs les plus attractives pour le frelon et les composés d'intérêt

Les plantes d'au moins deux espèces de sarracénie, *S. X mitchelliana* et *S. X Juthatip soper*, semblent ainsi présenter des signaux olfactifs exploitables pour attirer le frelon. Certes, d'après nos expériences, l'attraction olfactive de ces plantes reste inférieure à celles du miel et de l'abeille, mais étant donné que les individus testés étaient capturés directement devant les ruches, il est probable que cette plus forte attraction pour l'abeille et le miel résulte de l'apprentissage préalable par le frelon de ces signaux de la ruche et de leur association avec une source de nourriture. Couto *et al.* (2014) dans leur expérience font le même constat et ils notent d'ailleurs que leurs ouvrières n'étaient étrangement que très peu attirées par la viande ou le poisson, des ressources alimentaires pourtant habituellement attractives pour *V. velutina* (Spradbery, 1973; Richter, 2000). *V. velutina* est en effet un insecte qui a une mémoire importante et de fortes capacités d'apprentissage (Gong *et al.*, 2019) qu'il utilise en associant à ses sources de nourriture leurs odeurs et couleurs comme signaux à retenir, tout comme le font les abeilles (Giurfa & Sandoz, 2012). Dans notre expérience, comme les frelons n'ont pas été au préalable en contact avec ces sarracénies, l'attraction observée en olfactométrie ne résulte pas d'une précédente expérience d'association et démontre l'existence de signaux olfactifs dont l'attractivité est 'innée' pour le frelon. Le taux de visites somme toute limité observé en laboratoire sur les urnes de *S. X mitchelliana* semble démentir cette attraction innée pour les odeurs de la plante. Mais si on regarde plus en détail le nombre de visites dans les tous premiers temps d'observation chez cette espèce, on s'aperçoit qu'il est important pendant la première session d'observation à $t=30\text{min}$ et qu'il chute seulement pour cette espèce -et non pour les autres- aux deuxième temps d'observation à $t=90\text{min}$ (**Figure 14**). Or *S. X mitchelliana* est l'espèce pour laquelle il y a le plus d'évènements de glissades et de chutes dans l'urne avec un taux également conséquent d'évasions (le frelon s'échappe les 2/3 du temps). Il est donc probable que le frelon soit au départ naïvement attiré par l'odeur de cette espèce mais qu'il ait rapidement appris à l'associer comme un piège plutôt que comme une récompense à cause des faibles performances de son piège, revêtant un caractère beaucoup moins fatal que celui de sa congénère, *S. X juthatip soper*, qui exhibe un plus fort taux de rétention (75%).

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Cela nous renseigne donc sur l'importance de considérer les fortes qualités d'apprentissage chez ce frelon et de confectionner des pièges implacables pour éviter qu'ils soient irrémédiablement contournés par le frelon après une première mauvaise expérience.

Ces résultats d'attraction du frelon par *S. X mitchelliana* et *S. X Juthatip soper* sont donc encourageants. Quant aux odeurs très concentrées de *S. X leucophylla*, elles pourraient susciter une attraction à distance et une répulsion à proximité, d'après les comportements « tout puis rien » observés en olfactométrie. Les forts taux de visites en laboratoire et de capture de frelons en laboratoire et sur le terrain observé chez cette espèce suggèrent cependant que son odeur n'est probablement jamais aussi concentrée et donc potentiellement répulsive que dans l'olfactomètre. Cette espèce ne doit donc pas être écartée dans notre recherche d'un appât mais nécessite des tests plus poussés. Ces résultats nous suggèrent que la concentration des composés est un facteur fondamental dans l'attraction et que la confection d'un appât doit nécessiter des courbes doses-réponses et des tests non seulement en olfactométrie mais aussi sur le terrain.

Couto et ses collègues reportent que le nombre de glomérules dans le lobe antennaire des ouvrières de frelon est de 265 (Couto *et al.*, 2016), ce qui est au-dessus de la moyenne car dans la plupart des espèces d'insectes, ce nombre varie plutôt entre 40 et 160 (Hansson & Anton 2000). Ce nombre reflète les capacités de discrimination des odeurs de l'espèce et doit être mis en relation avec la complexité de l'environnement olfactif de celle-ci (Hansson & Anton, 2000; Kelber *et al.*, 2009). Il est donc probable que le frelon asiatique, espèce hautement opportuniste, soit capable d'enregistrer un nombre important d'informations neuronales et de reconnaître des combinaisons de signaux olfactifs complexes comme les mélanges des effluves de sarracénies.

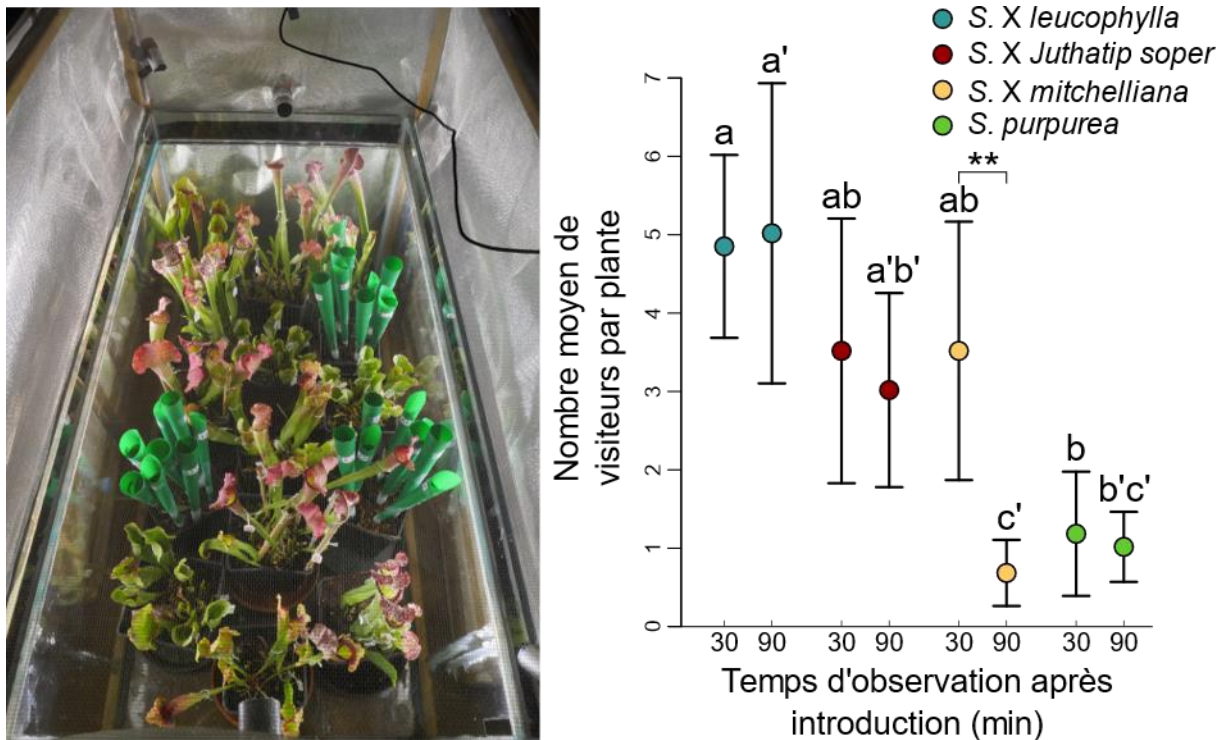


Figure 14. Nombre moyen (\pm SE) de frelons visiteurs observés par plante par session de 10min pour chaque espèce dans le dispositif du chapitre 3 en début d'expérience, c'ad 30 minutes et 90 minutes après leur introduction dans la cage. Les lettres différentes indiquent des différences de moyennes significatives (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) entre les espèces. La significativité des tests a été obtenue via un modèle linéaire généralisé avec distribution de Poisson prenant en compte l'effet de l'espèce, du timing de l'observation, de la date en effet fixe et de l'identité de la plante en facteur aléatoire. L'interaction entre l'espèce et le timing d'observation a également été retenue.

La comparaison des bouquets des différentes espèces de sarracénie permet de mettre en avant les composés potentiellement responsables de l'attraction et sur lesquels il faudrait focaliser notre attention par la suite. Par exemple le (E)- β -Bergamotène, le (E)- β -Ocimène, l' α -Ylangène et le Myrcène sont retrouvés en plus grandes quantités dans les mélanges des plantes attractives pour le frelon. Certains de ces composés comme le (E)- β -Bergamotène sont déjà connus pour être des 'HIPV (*herbivory-induced plant volatiles*)' (Schnee *et al.*, 2006), des composés typique de fleurs comme le (E)- β -Ocimène et le Myrcène (Knudsen *et al.*, 2006) ou encore des molécules émises par les abeilles comme le (E)- β -Ocimène (Maisonasse *et al.*, 2010) tous susceptibles de pouvoir attirer le frelon ou les guêpes en général comme discuté dans le dernier chapitre. Un savant mélange de ces COVs pourrait donc éventuellement aboutir à un signal plus attractif pour le frelon mais étant donné la fréquence de ces composés observés dans les effluves de fleurs des Angiospermes (Knudsen *et al.*, 1993), si on veut

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s'orienter vers un piège spécifique, il serait peut-être plus judicieux de se tourner vers le α -Ylangene, qui est un composé plus rare, retrouvé dans les effluves de certaines variétés de raisins (Parker *et al.*, 2007) dont on sait que les frelons sont friands (Madden *et al.*, 2017). Si on s'intéresse aux composés émis par les urnes, les pièges des deux espèces attractives, *S. X Juthatip soper* et *S. X mitchelliana*, semblent émettre des dérivés d'acides gras en grandes quantités (cf chapitre 1), parfois typiques des *green leaf volatiles* émis en cas d'herbivorie. Or, les *green leaf volatiles* sont particulièrement susceptibles d'attirer les frelons. Par exemple, les fleurs d'orchidée du genre *Epipactis* émettent des 'green leaf volatiles' suggérant la présence de chenilles herbivores attaquant la plante pour attirer leurs pollinisateurs principaux, des guêpes prédatrices, et restent peu attractives pour les abeilles domestiques (Brodmann *et al.*, 2008).

b. Les couleurs et les contrastes : une composante du piège à ne pas négliger

Dans la recherche d'appât attractif, l'aspect visuel est bien souvent délaissé. Par défaut les pièges utilisés sur les guêpes sont en général de couleur jaune (**Figure 7B**) mais rien ne semble vraiment prouver l'efficacité de l'utilisation d'une telle couleur. Papas et ses collègues ont étudié l'efficacité d'un même piège coloré en rose, en blanc ou en jaune sur la capture de vespides et ont montré que le rose était celui ayant capturé le plus grand nombre d'individus (Papas *et al.*, 2021), la majeure partie de leur capture était d'ailleurs constituée de frelons *V. orientalis*. Sorvari de son côté a montré que la coloration jaune des pièges n'améliorait pas leur efficacité pour la capture des guêpes des genres *Vespula* et *Dolichovespula* (Sorvari, 2019). Il est donc essentiel d'approfondir nos connaissances sur le rôle des signaux visuels dans la préférence du frelon et notamment d'étudier ces signaux tels qu'ils sont véritablement perçus par le frelon. Avec cette façon de procéder, nous avons ainsi montré que pour le système visuel du frelon, les alvéoles blanches des sarracénies constituaient un signal puissant. Il serait donc primordial de s'inspirer de l'aspect visuel de ces plantes. Les signaux visuels très peu considérés pour le moment, semblent pourtant exploités par le frelon dans la recherche de nourriture comme l'ont montré Wang et co-auteurs (Wang *et al.*, 2014).

c. S'inspirer de la forme et des propriétés de surface du piège des sarracénies

Rojas-Nossa et ses collègues ont testé différents types de pièges et ont montré des performances de captures du frelon différentes selon les pièges indépendamment de l'appât utilisé (Rojas-Nossa *et al.*, 2018). Ils n'ont néanmoins pas pu expliquer quels paramètres contribuaient à ces différences parmi par exemple la position de l'entrée (au-dessous ou au-dessus) ou la taille du trou qui change hypothétiquement le taux d'évaporation de l'appât et donc la quantité de signal olfactif. Notre étude de la morphologie des urnes à travers les différents chapitres a aussi montré l'importance de la taille et de la forme du piège dans l'efficacité de capture du frelon. Aucune donnée n'existe sur les pièges actuels concernant leur capacité de rétention et leur capacité à piéger ce qu'ils attirent. Dans tous les cas, l'étude du piège des sarracénies ouvre aussi de nouvelles pistes pour l'élaboration de pièges plus efficaces dans leur rétention et leur attraction, comme par exemple l'utilité de favoriser un piégeage tête la première du frelon. Le positionnement de l'appât et la forme du piège doivent ainsi être calculés avec précision pour optimiser le succès de piégeage.

Notre travail a également mis en évidence des différences inter-spécifiques dans les microstructures des urnes et leurs propriétés de surface. Dans la nature, les microstructures biologiques sont le résultat de millions d'années d'évolution et confèrent aux organismes des propriétés uniques, qui méritent d'être exploitées pour des applications biomimétiques. Un exemple très célèbre est celui de l'effet lotus', un phénomène de superhydrophobie causé par les microstructures des feuilles du lotus, *Nelumbo sp.* (Shirtcliffe *et al.*, 2009). Ces microstructures ont été étudiées pour leurs propriétés auto-nettoyantes, et ont inspiré la confection de divers matériaux dans de nombreux domaines (Latthe *et al.*, 2014). En vue de l'amélioration des pièges actuels, il serait pertinent de s'inspirer des pièges développés par ces plantes carnivores et notamment de l'agencement des microstructures qui tapissent la paroi de leurs urnes, responsables des propriétés glissantes particulièrement efficaces sur les insectes. Les propriétés lubrifiantes des urnes de Népentes ont d'ailleurs déjà inspiré la confection d'un matériau déperlant, spongieux rempli de fluide

lubrifiant (Wong *et al.*, 2011) avec des propriétés encore plus efficaces que les microstructures du lotus pour repousser des liquides complexes comme les hydrocarbures, le pétrole brut ou encore le sang.

4. Conclusion et perspectives pour le développement d'un piège biomimétique

Pour conclure, ce travail a permis non seulement de mieux comprendre le fonctionnement des mécanismes d'attraction chez les plantes carnivores du genre *Sarracenia* mais il a également apporté des connaissances nouvelles sur la communication visuelle et olfactive du frelon avec les plantes. Ainsi nous montrons et c'est chose nouvelle que ces plantes carnivores diffèrent dans leurs leurres olfactifs à l'échelle inter-spécifique et intra-spécifique (signaux urne/plante). Nos résultats, même s'ils sont en partie issus d'observations sur des hybrides horticoles, suggèrent que dans leurs milieux naturels, ces plantes pourraient avoir développé de véritables stratégies d'attraction qui pourraient expliquer leur spécialisation partielle vers des guildes d'insectes particulières. Nous montrons aussi que les signaux visuels semblent tenir une place importante dans le comportement de recherche de nourriture chez le frelon. Ceci est plutôt nouveau, car selon les quelques connaissances dont on dispose sur les guêpes, lorsqu'elles sont entraînées sur les odeurs et les couleurs, elles montreraient une préférence pour l'odeur plutôt que la couleur (McPheron & Mills, 2007). Enfin, même si notre quête est loin d'avoir abouti, ce travail présente des résultats encourageants pour la poursuite des recherches d'un appât efficace inspiré de ces plantes, considérant par ailleurs qu'il n'y a toujours pas d'appât efficace proposé dans le cadre de la lutte biologique contre cet envahisseur. Nous proposons dans ce qui suit des pistes pour la poursuite de ces recherches concernant l'appât.

a. Affiner la recherche de COVs

Nous avons proposé de tester un certain nombre de composés les plus susceptibles d'être attractifs. Pour affiner notre compréhension de l'effet complexe des signaux olfactifs sur le frelon, il s'agira de tester en olfactométrie ces composés spécifiques seuls ou en mélange. Tester ces composés

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de cette manière, nous permettra de faire un 'tri' entre de potentiels effets attractifs et répulsifs et de mettre en évidence de potentiels effets synergiques provoqués par les mélanges. Il a par exemple été montré dans la relation très spécifique entre *Ficus carica* et son pollinisateur, *Blastophaga psenes*, que seul un mélange avec une proportion bien précise de quatre VOCs est attractif pour l'insecte au contraire des composés testés seuls ou d'un tout autre mélange (Proffit *et al.*, 2020). Démontrer le caractère répulsif d'un COV ou d'une combinaison de COVs serait aussi intéressant. Beaucoup d'études ont en effet cherché à identifier des composés répulsifs pour les guêpes (Zhang *et al.*, 2013; Boevé *et al.*, 2014) dans le but de les repousser de l'humain. Il serait également envisageable d'utiliser des répulsifs pour éloigner les frelons des ruches ou même de les utiliser dans le cadre d'une stratégie de 'push pull' en combinaison avec les substances attractives. La stratégie 'push-pull' implique la manipulation comportementale des insectes nuisibles par l'intégration de *stimuli* qui rendent la ressource protégée peu attrayante ('push') tout en les attirant vers une source attractive ('pull') où les nuisibles sont ensuite éliminés (Cook *et al.*, 2007). Tester les composés individuels en olfactométrie aurait également l'avantage de tester différentes concentrations susceptibles de modifier l'attractivité. Cela permettrait peut-être d'expliquer pourquoi l'observation de répulsion olfactive observée chez *S. X leucophylla* très émettrice vis-à-vis du frelon ne reflète pas les observations de terrain. Tester ces composés sur d'autres insectes pollinisateurs comme l'abeille ou la syrphé ou encore le frelon européen pourrait aussi permettre d'affiner la sélectivité de l'appât. Certes le filtrage des autres insectes, plus petits, pourra se faire mécaniquement en utilisant des pièges avec une structure et une maille adaptée au frelon asiatique comme le piège à nasse (**Figure 7C**), mais pour une utilisation locale à proximité des ruches, un appât ne perturbant pas les abeilles serait la meilleure option.

La réponse antennaire est un second critère pertinent pour la sélection de composés candidats puisque qu'elle témoigne de ce qu'un insecte est capable de sentir ou non. Cette réponse antennaire peut être mesurée comme une réponse électroantennographique, c'est-à-dire une mesure du signal électrique de l'antenne représentant la somme des réponses de tous les neurones des récepteurs olfactifs logés dans les sensilles de l'antenne (Schneider & Kaissling, 1957). Ce dispositif

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d'électroantennographie peut notamment être utilisé avec un dispositif de chromatographie en phase gazeuse couplée à un détecteur à ionisation de flamme (GC-FID) pour identifier les composés d'un bouquet d'odeur complexe élicitant spécifiquement une réponse de l'antenne (**Figure 15**). Ce travail a été entamé sur les bouquets d'odeurs de *S. X mitchelliana* et *S. X Juthatip soper*, avec des tests sur les ouvrières et les fondatrices de *V. velutina* mais aussi sur les ouvrières d'*A. mellifera*. Ces résultats mettent en avant certains composés de sarracénies détectés par les ouvrières de frelon mais pas par les ouvrières d'abeilles (**Figure 15B, C**). Même si cette réponse antennaire n'informe en rien sur l'aspect attractif ou répulsif des composés, elle permet de sélectionner des composés qui induisent une réponse du frelon mais pas de l'abeille et affine le choix en permettant de ne pas tester des composés non perçus par le frelon. L'utilisation de l'électroantennographie permettra également via l'établissement de courbes doses-réponses de connaître les seuils de détectabilité des composés, ce qui en vue de la production d'un piège sera essentiel pour estimer la concentration nécessaire pour une diffusion suffisante de l'odeur.

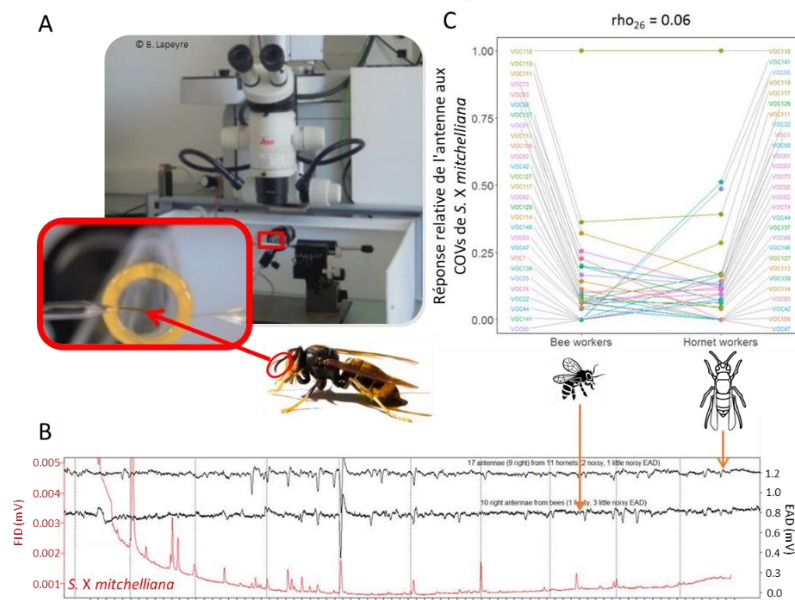


Figure 15. d'après M.-J. Holveck. **(A)** Dispositif d'électroantennographie de la PACE, CEFE, Montpellier, France ; **(B)** Enregistrement simultané des réponses antennaires d'ouvrières de *V. velutina nigrithorax* (noir haut) et d'*A. mellifera* (noir bas) aux composés volatils émis par *S. X mitchelliana* (GC-FID des profils d'odeurs en rouge). Les composés (pics rouges) suscitant une détection électro-antennaire (EAD) sont révélés par la dépolarisation du signal moyenné de plusieurs antennes (pics noirs). Les lignes pointillées montrent la position des n-alcane (de C7 à C16) ; **(C)** Réponse antennaire non corrélée aux composés volatils émis par *S. X mitchelliana* entre les ouvrières de frelon et d'abeille.

b. Combiner les signaux

Chez le frelon asiatique, il a été montré par Wang et co-auteurs que ce frelon utilisait à la fois les signaux visuels et olfactifs pour localiser les abeilles, préférant se diriger vers un leurre qui ressemble et sent comme une abeille plutôt que vers un leurre uniquement basé sur l'odeur (Wang *et al.*, 2014). Il sera essentiel de tester une combinaison de COVs et de couleurs différentes étant donné que le signal visuel est susceptible d'amplifier l'attractivité de l'appât olfactif (**Figure 16**).

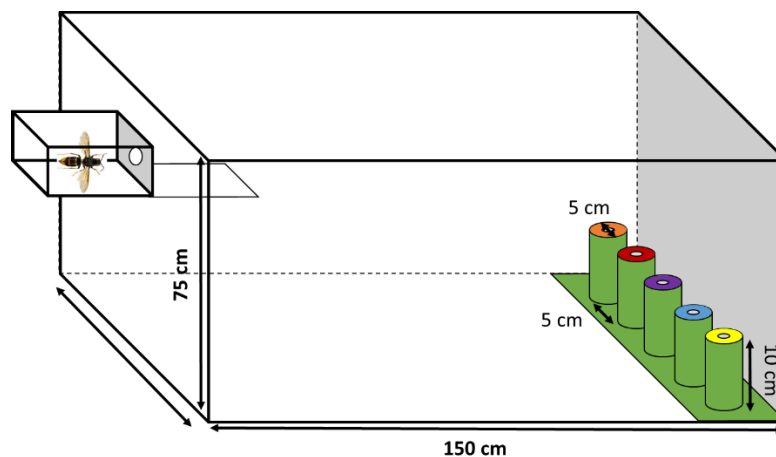


Figure 16 : Protocole expérimental conçu pour tester l'apport des couleurs dans le choix d'une odeur. Le signal olfactif est émis par les boîtes cylindriques vertes parées de couleurs différentes au niveau de leurs ouvertures. Des boîtes vertes avec et sans odeur pourront être utilisées comme témoins.

c. Tester sur le terrain

Enfin il sera essentiel de poursuivre les études par la suite directement sur le terrain. Les études sur les hyménoptères mettent de plus en plus en avant l'importance, pour leurs capacités à discriminer et apprendre des odeurs, du contexte environnemental dans lequel les insectes se trouvent. L'implication de l'apprentissage associatif des insectes sociaux en condition naturelles dans leur comportement de choix (El-Sayed *et al.*, 2018). Ils ont montré chez deux populations de guêpes sociales, *Vespula vulgaris*, une localisée dans les plages forestières de Nouvelle-Zélande où le miellat produit par des homoptères était abondant et l'autre localisée dans une zone sub-urbaine du même pays, que les premières étaient attirées par les odeurs de miellat alors que les secondes n'étaient pas attirées par ces odeurs. Ces guêpes présentaient un profil ADN et des réponses électro-antennaires en toute part similaire. Cette

Discussion

étude laisse supposer que dans le cadre du développement d'un piège attractif, l'attraction observée en laboratoire est susceptible de changer quelque peu sur le terrain selon l'endroit où l'on se situe. Finalement, bien que ce travail ne représente qu'une première étape dans la production de l'appât tant recherché, il fournit des bases solides et prometteuses qui nous rapprochent toujours plus d'une solution efficace à un problème européen majeur.

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