Identification of pollinator-attracting semiochemicals of wasp-pollinated *Epipactis* species

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Zusammenfassung

Einige der in Europa vorkommenden *Epipactis* Arten gelten als Wespenblumen, da sie hauptsächlich von Wespen, darunter auch *Vespula germanica*, bestäubt werden. Untersuchungen der Vergangenheit beschäftigten sich meist mit der Frage nach den Bestäubern oder der optischen Attraktivität der Blüten. Jüngst wurden auch Vermutungen über einen Zusammenhang zwischen der Bestäubung durch Wespen und der möglichen Anlockung durch spezifische Duftstoffe geäußert.

Um festzustellen welche Reizqualitäten für bestäubende Wespen besonders attraktiv sind, wurden Biotests mit Arbeiterinnen von *V. germanica* im Freiland unternommen. Abwechselnd wurden den Wespen ganze Infloreszenzen, Blüten ohne Duft, der natürliche Duft und der Duftstoff-Extrakt von *E. helleborine* angeboten.

Bei den beiden Wespen bestäubten Arten *E. helleborine* und *E. purpurata* identifizierte ich mit Hilfe von chemischen Analysen (GC, GC-MS), Elektroantennographie und Verhaltensexperimenten wespenanlockende Duftstoffe in Blütenextrakten. Zum Vergleich wurde auch das Blütenbouquet der durch Hummeln bestäubten Art *E. atrorubens* untersucht. Mit dem gekoppelten Verfahren der Gaschromatographie und der Elektroantennographie (GC-EAD) konnten bislang 20 Verbindungen gefunden werden, die Rezeptorpotentiale in den Antennen von *V. germanica* auslösen.

Mittels Gaschromatographie gekoppelt mit der Massenspektrometrie wurden 7 GC-EAD aktive Verbindungen identifiziert. Die bereits identfizierten Hauptkomponenten waren bei allen drei Arten Aldehyde (Heptanal, Pentadecanal, Hexadecanal and Vanillin), Alkohole (Pentadecanol, Hexadecanol) und Phenole (2-Methoxy-4-methylphenol)

Ein Vergleich der Duftstoff-Bouquets mittels multivariater Statistik (Hauptkomponentenanalyse und Diskriminanzanalyse) ergab eine größere Ähnlichkeit der Duftbouquets bei den Wespen bestäubten Arten. Das Duftbouquet von *E. atrorubens* zeichnete sich durch einen besonders hohen Vanillin-Anteil aus.

Weiters wurde untersucht, ob die *V. germanica* Arbeiterinnen bei Spontan-Wahltests Präferenzen für die Duftstoffe der einzelnen Orchideen zeigen. Dazu wurden Pentan-Extrakte der *Epipactis* Arten und eine synthetische Mischung aus 7 der bereits identifizierten Komponenten des natürlichen Duftes von *E. helleborine* bei einem sogenannten Y-Rohr Test angeboten.

Als Ergebnis der chemischen Analysen zeigen die untersuchten *Epipactis* Arten signifikante Unterschiede in den relativen Anteilen an Heptanal, Pentadecanol sowie Pentadecanal und Hexadecanal. Letztere Komponente unterscheidet sich bei den wespenbestäubten Arten nicht signifikant, ist bei *E. atrorubens* jedoch in der kleinsten Menge vorhanden.

Der multivariate Vergleich mittels Diskriminanzanalyse zeigt signifikante Unterschiede zwischen allen *Epipactis* Arten. Die erste Diskriminanzfunktion ist in der Lage *E. atrorubens* von den wespenbestäubten Arten zu trennen, die einen Cluster bilden. Dabei weist der Factor score 1 einen hoch signifikanten Unterschied zwischen der Gruppe der von Wespen bestäubten Arten und der von Hummeln bestäubten *E. atrorubens* aus. Hauptsächlich Heptanal und 2-Methoxy-4-methylphenol tragen zur Diskriminanzfunktion bei.

Die Ergebnisse der Biotests zeigen eine höhere Attraktivität der olfaktorischen gegenüber den optischen Reizen. Jedoch bedarf es beider Reizqualitäten, damit ein Verhalten ausgelöst wird, dass eine gesicherte Bestäubung garantiert. Der Blütenduft hingegen ist signifikant weniger attraktiv für die Wespen als die Kombination aus Extrakt und optischen Reizen. Duftstoff-Extrakte in Kombination mit optischen Reizen waren sogar attraktiver als die natürliche Infloreszenz. Weiters zeigt sich eine Infloreszenz nicht signifikant attraktiver als der Blütenduft, bringt aber deutlich mehr Wespen dazu die Pflanze zu berühren. Erklärbar ist dies durch eine höhere Konzentration und damit verbundenen höheren Attraktivität der Duftstoffe im Pentan-Extrakt. Ausschließlich visuelle Reize von *E. helleborine haben* die geringste Attraktivität für *V. germanica* Arbeiterinnen.

In den Olfaktometer Tests zeigt sich eine klare Präferenz der Wespen für Duftstoffe der wespenbestäubten Arten, wogegen *E. atrorubens* weniger attraktiv ist. Extrakte von *E. helleborine* und *E. purpurata* sind signifikant attraktiver als das Lösungsmittel. Auch der Extrakt von *E. atrorubens* wird gegenüber dem Lösungsmittel bevorzugt gewählt. Die Extrakte der wespenbestäubten Arten sind attraktiver als Extrakte von *E. atrorubens* und werden annähernd gleich oft gewählt.

Die Wirkungsweise der Anlockung von Wespen zur Bestäubung von *E. helleborine* und *E. purpurata* kann wie folgt interpretiert werden: 1) *Epipactis* Blüten ahmen sogenannte green-leaf-volatiles nach. Diese Hypothese wurde aber aufgrund der Unähnlichkeit der gefundenen Substanzen mit typischen green-leaf-volatiles abgelehnt. 2) Der Gehalt an Alkoholen und Aldehyden, legt aber den Verdacht nahe, dass wespenbestäubte Arten möglicherweise zuckerhaltige Futterquellen z. B. gärendes Obst imitieren. Wespenbestäubte *Epipactis* Arten werden daher als Nektar belohnende und Futterquellen imitierende Orchideen diskutiert.

Identification of pollinator-attracting semiochemicals of wasp-pollinated *Epipactis* species.

Abstract :

Some of the in Europe occurring *Epipactis* species are so called “Wasp-flowers”, because they get pollinated by social wasps like *Vespula germanica*. Investigations in the past treated mainly the question of the pollinators and the optical attractiveness of the blossoms. Recently, assumptions were made that *Epipactis* flowers lure their pollinators by specific scents.

To find out which cues have a high attractiveness for pollinating wasps, workers of *V. germanica* were tested in bioassays. In various tests they were offered whole infloresences, blossoms without scent, natural scent and blossom extracts of *E. helleborine*.
Pentane blossom-extracts of *E. helleborine* and *E. purpurata* were collected and the wasp attracting volatiles were identified by means of chemical analyses combined with electrophysiological recordings (GC-EAD) and behavioural experiments. Using GC-EAD, 20 compounds triggered receptor potentials in the antennas of *V. germanica* workers. Seven main components were identified as aldehydes (heptanal, pentadecanal, hexadecanal and vanillin), alcohols (pentadecanol, hexadecanol) and phenols (2-methoxy-4-methylphenol).
I used multivariate statistics (principal component analyses, discriminant function analyses) to compare the scents of the wasp pollinated species with the bumble-bee-pollinated species. The bouquets of the wasp-pollinated species were more similar to each other in the main components than to *E. atrorubens*. The latter is distinguished by a high content of vanillin in the blossom-extract.
Furthermore wasps were tested in spontaneous choice tests with y-tubes.

To test preferences for fragrances of certain *Epipactis* species pentane-extracts of flowers and a blend of 7 already identified components were offered in dual choice experiments.
As a result of the chemical analyses the investigated *Epipactis* species show significant differences in their relative amounts of heptanal, pentadecanol, pentadecanl and hexadecanal. The latter is not significantly different between the wasp-pollinated species and occurs in smallest amounts in *E. atrorubens*.

The multivariate comparison via DFA show a significant difference between all *Epipactis* species. The first discriminant function allows to separate *E. atrorubens* from the wasp-pollinated species which form a cluster. Factor score 1 indicates a high significant difference between the wasp-pollinated *Epipactis* and the bumble-bee-pollinated *E. atrorubens*. Mainly heptanal and 2-methoxy-4-methylphenol contributed most weight to the disriminance function.

The behavioural experiments show, that optical cues in combination with flower extract is more attractive than the inflorescence alone. But a combination of visual and olfactorial cues may be important to elicit in the wasps the whole range of behavioural patterns that are necessary to guarantee pollination. The flower scent however is significant less attractive to wasps than a combination of extract and optical cues. Further an inflorescence is not significant more attractive than blossom scent, but elicit a behaviour of wasps to pounce the plants significant more often. This circumstance is explainable by a higher concentration and higher attractiveness of scent in the pentane extracts. Just visual cues have the lowest attractivity for *V. germanica* workers.
IThe olfactometer tests showed a clear preference of wasps for scent of wasp-pollinated species, whereas *E. atrorubens* is less attractive. Extracts of *E. helleborine* and *E. purpurata* are significant more often chosen than the solvent. Also the extracts of *E. atrorubens* are preferred chosen over the solvent. The extracts of wasp-pollinated species are more attractive and chosen approximately same often.

The effects of attraction of wasps for the pollination of *E. helleborine* and *E. purpurata* are discussed as follows: 1) *Epipactis* flowers mimic scent of so-called green-leaf-volatiles (GLV). However, I could not found typical green-leaf-volatiles in the floral scent of the investigated *Epipactis* species. Therefore, an alternative hypothesis arises. The content of alcohols and aldehydes suggests, that wasp-pollinated *Epipactis* species possibly imitate sugar containing food sources (e.g. fermenting fruit). Wasp-pollinated *Epipactis* species are therefore discussed as nectar-rewarding and food-source-imitating orchid species.

Keywords: Pollination by wasps, floral scent, *Epipactis helleborine*, *Epipactis purpurata*, *Epipactis atrorubens*, *Vespula germanica*, Yellow jackets

Introduction

Wasp pollinated flowers sensu strictu are plants which have evolved recognizable adaptations to the visit and the pollination by *Vespidae* (KEPPERT 2001). The adaptations are of morphological, physiological and phenological nature, although wasp pollinated flowers are also visited by other insects (KEPPERT 2001). In literature some plants repeatedly have been treated as wasp flowers: *Scrophularia nodosa*, *S. umbrosa* (Scrophulariaceae), *Symphoricarpos* sp. (Caprifoliaceae), *Cotoneaster* sp. (Rosaceae) and the orchids *Epipactis helleborine* and *E. purpurata* (Orchidaceae) (MÜLLER 1873,1881; KNUTH 1899; KIRCHNER 1911; WERTH 1943, 1956; SCHREMMER 1962; KUGLER 1970; FAEGRI & V.D. PIJL 1979; PROCTOR ET AL. 1996). Typical for such flowers is the possession of „reddish-brown“ (MÜLLER 1881) „dirty purple“ (WERTH 1956) or „dirty brown, violet or dismal“ (SCHREMMER 1962) coloration of the inflorescence, further having „relative small, mostly bulbous blossoms with broad entrance“ (SCHREMMER 1962)

„bulbous widened, nectar-rich juice holder“ (MÜLLER 1881) and „plentiful and open presented honey“ (MÜLLER 1873). All these characters are cited elsewhere (LOEW 1895; KIRCHNER 1911; CAMMERLOHER 1931; KNOLL 1956; FAEGRI & V.D. PIJL 1979; HESS 1990; HARBONE 1995, PROCTOR ET AL. 1996). According to WIEFELSPÜTZ (1970) wasps are attracted by „dull brown, dirty-red to washed yellow-green colours“. Wasps cannot discriminate between) red and black due to their different spectrum of the visual sense, which gives them a spatial impression with high stimulus value (SCHREMMER 1941). KUGLER (1970) described stronger UV-reflexions of the hypochile of *Epipactis helleborine*, whereas ROSEN & BARTHLOTT (1991) concern the blossoms of *E. helleborine* and *purpurata* as neutral in comparison to their surrounding natural habitat, also UV absorbing patterns do not occur.

All wasp flowers have similarities of the nectar in common: BAKER & BAKER (1983a,b, 1990) discovered the dominance of sucrose sugar and a high content of amino acids (BAKER & BAKER 1973, 1983b,1986

Despite the frequency of descriptions of so-called wasp pollinated flowers almost nothing is known about adaptations to pollinators and which signals are really responsible for the attractiveness of the blossoms. In case of *Epipactis* the pollinating wasps are thought to be attracted by visual cues only (WIEFELSPÜTZ 1970). No data exist about floral scent that may attract wasp pollinators. KEPPERT (2001) observed behavioural patterns of wasps while visiting flowers and suggested that floral scent is involved in wasp attraction.

The genus *Epipactis* is part of the large family of the Orchidaeceae, a worldwide distributed and species rich plant family (20.000 species; BUTTLER 1986). Most species can be found in the rain forests of the tropic regions (BUTTLER 1986). The interdependency between orchids and pollinators is such that the evolution of one may influence that of the other (in V.D. CINGEL 1995). Several strategies to lure reliable pollinators to a flower in order to transfer the pollinia from one plant to another are realised and show a broad spectrum of possibilities. Nectar collecting birds like hummingbirds are attracted by bright and vivid colours and flower forms (V.D.PIJL & DODSON 1969). The fragrance for long-distance attraction and optical signals in the closeness of blossoms guides insects and (sometimes) tactile stimuli are important to bring them into the position for pollinia-uptake. Various syndromes of orchid-pollinator relationship occur. The spectrum of pollinators ranges from beetles at *Prasophyllum odoratum* (COLEMAN 1933), flies e.g. at *Phragmopedium* partially (V.D.PIJL & DODSON 1969), bees e.g. at *Orchis militaris* (VÖTH 1999), wasps at *Epipactis spp.*, moths at *Platanthera* (DARWIN 1877*)* to butterflies at *Nigritella nigra* (VÖTH 1999). Sometimes the pollinators get rewarded with nectar, an easy and cheap produceable flower-product. Males of some tropical Euglossine bees collect fragrances of certain orchids in special, hairy or swollen tarsal structures, possibly to use in attraction of mates (WILLIAMS AND WHITTEN 1983). Other orchids do not offer reward and cheat their pollinators. Orchids have evolved different types of floral deception. *Traunsteinera globosa* is a food deceptive species that mimics the food rewarding *Scabiosa columbaria* (Dipsacaceae) which occurs in the same habitat and blooms synchronously (V.D. CINGEL 1995). *Cephalanthera rubra* flowers are believed to mimic the pollen producing sexual organs in the food rewarding *Campanula* flowers (NILSSON 1983) and there is a similarity of the fragrances of bellflowers and *Cephalanthera* flowers (reviewed in V.D. CINGEL 1995). *Serapias* offers shelter to solitary bees by imitating nest holes and *Ophrys helenae* mimics a nest hole, but does not offer shelter (V.D. CINGEL 1995). Sexually deceptive orchids represent another flower type with great variance richness (Kullenberg 1961) The flowers of the mainly mediterranean genus *Ophrys* imitate females of a potential pollinator by shape, colour and odour (DAFNI 1984; AYASSE ET AL. 2000, 2001, 2003; SCHIESTL ET AL. 1999) and tactile stimuli given by the hairy areas determines the orientation of the males, imitating the dorsal hairs of the females. This pollination syndrome of pseudocopulation guarantees an efficient pollen transfer and because of the strong *Ophrys*-pollinator-relationship also the separation of co-occuring species (BUTTLER 1986; KULLENBERG 1961,1973; KULLENBERG & BERGSTRÖM 1976; PAULUS & GACK 1990, 1994). An overview of pollination syndromes in orchid flowers relation to exploited insect drives is given by V.D. CINGEL (1995). Beside these mechanisms of allogamy, some orchids are self-pollinated. Optional autogamy is reported from those species where the pollinators do not occur in the edges of the distribution area. For example *Ophrys apifera* is autogamous in the north of Europe (BUTTLER 1986). An extreme of autogamy is the so called cleistogamy, where the orchid gets pollinated within the bud before it opens. Many *Epipactis* species as well as some other orchids e.g. *Nigritella* are obligatory apomictic (TEPPNER & KLEIN 1990).

The genus *Epipactis* is distributed throughout Africa, Europe and North America (IVRI & DAFNI 1976; JUDD 1971). The species have a rather short column structure and the spurless labellum is divided in epi- and hypochile, in which the latter is concave and nectariferous (V.D. CINGEL 1995). The number of species is unclear so far: BUTTLER (1986) names 14 species, BURNS-BALOGH ET AL. (1987) 22 species, v.d. Cingel (1995) gave a table with 20 species and 2 further subspecies. Descriptions of new species were given by Baumann & Baumann (1988) and Robatsch (1993). In Austria there are 12 species (Vöth 1999). The genus includes allogamous and autogamous (even cleistogamous) species. The prior are more widespread and show more uniformity over the whole area of distribution, the latter are more localised and have rather disjunct distributions. Reasons therefore are discussed in v.d. Cingel (1995). Autogamic Austrian species can be characterized by lacking of rostellum and/ or rostellum gland (*E. albensis, E. greuteri, E. leptochila, E. muelleri*) and the ending of the stigma very close to the anthers (*E. greuteri*). If the rostellum gland dries up, the anthers are able to move forward laterally (*E: pontica, E. voethii*). Through swelling already in bud-condition, pollinia swing out of the antheres and pollen falls onto the stigma (*E. albensis*). Pouring of pollinia also occurs in *E. leptochila* during bad weather periods and supports cleistogamy (Vöth 1999). The insect-pollinated *Epipactis* species always produce nectar as a reward for pollinators. *E. palustris* has been investigated by Darwin (1888). He described the small connection between hypo- and epichile as a kind of trampoline which would flick the pollinator (the main pollinator *Apis mellifera*) and move it upwards against the antheres. Müller (1988) recognized an up and down movement of the labellum, caused by the bee. If the pollinator presses itself deeper into the flower, the labellum presses back when the bee grasps the upper parts of the flower with the four front legs and catapults itself like a trampolin-athlete, visiting flower after flower with the same experience. A similar mechanism occurs in the Israelien *E. veratrifolia* (=*consimilis*). Ivri & Dafni (1976) observed several Syrphid flies as pollinators. Territorial males of this flies defend orchid plants against other males and try to copulate with visiting females. From time to time a fly lands on the epichile to drink some nectar, and bends the labellum under its weight. By entering the hypochile, the epichile suddenly returns to the original position and flicks the fly against the stigma (similar as in *E. palustris*). With the trial to escape backwards, the pollinia are adhered at the dorsal part of the insect and carried away. Female flies are also pollinator of this species: they are first hovering in front of the flowers. After landing on the labellum they try to lay eggs beside black-spotty and bulbous structures of the epichile or at other parts of the plant. During or after this behaviour the orchid gets pollinated. Since the larvae of Syrphid flies are carnivorous and feed on aphids, the female's behaviour is interpreted by IVRI & DAFNI (1976) as an aphid-mimicry of the *Epipactis.* The egg laying behaviour is probably triggered by visual and fragrance stimuli. *E. atrorubens,* a rather earlier flowering species, was found to be pollinated by *Bombus* species, honey-bees and sometimes wasps (VÖTH 1999; MÜLLER 1988). Characteristic is the reddish colour of the blossom and the strong fragrance of vanilla. Due to the lack of the deepness of a spur and its non-typical construction for bumble-bees, KUGLER (1970) postulated the nectar not drawn up, but licked off the hypochile. For this reason the pollinia not always adhered at the tip area of the bumble-bee's proboscis. The remaining three Austrian *Epipactis* species are more or less wasp pollinated. Beside wasps, *E. distans* is pollinated by ants and beetles (see VÖTH 1999).

Although *E. helleborine* is treated as a „typical“ wasp-flower it has a broad observed spectrum of visitors: beside honeybees (MÜLLER 1988; WERTH 1956; V.D. CINGEL 1995), bumble-bees (WERTH 1952¸1956; SCHREMMER 1961;

WIEFELSPÜTZ 1970; VÖTH 1982; V.D. CINGEL 1995) and wild bees (JUDD 1979; VÖTH 1982; MÜLLER 1988; V.D. CINGEL 1995) syrphid flies (WERTH 1952; WIEFELSPÜTZ 1970; VÖTH 1982; MÜLLER 1988) were reported. A similar situation can be found in *E. purpurata.* Beside wasps, flowers are visited by wild bees and syrphid flies (VÖTH 1982; MÜLLER 1988). Observations of peculiar intoxications of wasps visiting *Epipactis* flowers are described by LØJTNANT (1974) VÖTH (1982), BURNS-BALOGH ET AL. (1987) and MÜLLER (1988). Consequently the nectar of *E. helleborine* and *E. purpurata* (MÜLLER 1988; EHLERS & OLESEN 1997) were investigated on the occurrence of alcohol, bacteria and fungus. Actually trace amounts of alcohol could be detected.

KEPPERT (2001) confirmed that *E. helleborine* and *E. purpurata* are wasp-flowers due to some, here summarized facts: several blossoms are opened synchronously over a few days and present nectar, which is rich on sucrose sugar and amino acids. The relatively long flowering period extends from July-August in *E. helleborine* to August-September in *E. purpurata* and is synchronous with a high population of wasps. The inconspicuous blossoms have a recognizable vanilla-like scent and show whitish-green to pink colours in contrast to a dark centre. The wasps approach the blossoms easy on a landing-platform (labellum) and get positioned via a ventral groove. The syndrome of wasp-flowers was coarsely sketched (MÜLLER 18731881; KNUTH 1899; KIRCHNER 1911; WERTH 1943, 1956; SCHREMMER 1962; KUGLER 1970; FAEGRI & V.D. PIJL 1979; PROCTOR ET AL. 1996; KEPPERT 2001), but many questions concerning the importance of optical versus olfactorial cues are still left open.

The goal of the investigations was to identify pollinator attracting scent in the wasp-pollinated species *Epipactis helleborine* and *Epipactis purpurata* and to compare it with the volatiles of the bumble-bee-pollinated species *Epipactis atrorubens* using gas chromatography (GC) combined with electroantennographic-detection (GC-EAD), behavioural tests and chemical analyses. The following questions should be answered:

1. Do *E. helleborine* flowers attract wasps by visual, olfactory cues or both?
2. Which chemical compounds of *Epipactis* flowers can be detected by wasps?
3. Do wasps discriminate between wasp-pollinated *Epipactis* species and bumble bee-pollinated flowers?
4. Are there differences in scent between wasp-pollinated and bumble bee-pollinated *Epipactis* species?

Collection of orchids and wasps

In Summer 2000 and 2001 *E. helleborine*, *E. atrorubens* and *E. purpurata* flowers were collected at Schlagl, Preiner Gscheid and Wang (Lower Austria, *E. helleborine* and *E. atrorubens*), Jubiläumswarte and Höhenstraße (Vienna, *E. purpurata*), in Switzerland (coll. Paulus, *E. purpurata*) and and Slovenia, Visic Paß (coll. Ayasse, *E. atrorubens*). *Vespula germanica* workers were caught with an insect-net on the roof of the Institute of Zoology and used for biotests and GC-EAD analysis.

Sampling of floral scent

Blossoms of several orchid plants were cut off with a pentane-cleaned pair of scissors and directly transferred into vials with pure pentane. These „pools“ were stored for 24 hours at room temperature (25°C) in darkness. Afterwards the blossoms were removed, the extracts were concentrated in a water bath at a temperature of 40°C. The concentrated samples (30 µl/ blossom equivalent) were then transferred into small glass tubes with a teflon-seal and stored in a freezer at –25°C for later use (GC, GC-EAD, and behavioural experiments).

Electrophysiology (GC-EAD)

GC-analyses were performed with a Hewlett Packard 6890 gas chromatograph (GC) equipped with a DB-5 column (length 30 m, 0.32 mm id, 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA). One microliter of each sample was injected splitless at 50°C followed by opening the split valve after 1 min and programming to 310°C at a rate of 10°C/ min. Helium was used as the carrier gas. A GC effluent splitter (press-fit-connection, split ratio 1:1) was used and the outlet was added to purified and humidified air stream, directed towards an excised antenna of a worker of *Vespula germanica*. The tip of the excised wasp-antenna was cut off and mounted between two glass electrodes filled with Ringer-solution (SCHIESTL ET AL. 2000). The electrodes were connected to grounded Ag-AgCl wires and via interface box to a signal acquisition interface board (IDAC, Syntech, Hilversum) for signal transferring to a PC. Both signals, EAD and FID were recorded simultaneously. GC-EAD-analyses were performed with extracts of *E. helleborine* and *E. atrorubens*, respectively. To get meaningful data only reproduceable peaks of odour compounds in several runs of EAD's were accepted and labelled as „active“.

Chemical analysis

Blossoms solvent extracts were analysed on a gas chromatograph Hewlett Packard 6890 gas chromatograph (GC) equipped with a DB-5 column (length 30 m, 0.32 mm id, 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA). One micro litre of each sample was injected split less at 50°C followed by opening the split valve after 1 min and programming to 310°C at a rate of 10°C/ min. Helium was used as the carrier gas. For quantitative analysis, n-hexadecane was added as an internal standard. Structure elucidation of individual compounds was based on gas chromatography-mass-spectrometry (GC-MS) analysis (VG70/ 250 SE instrument, Vacuum Generators, Manchester, England, linked to a HP 5890 equipped with a DB5 capillary column (30 m x 0.32 mm internal diameter) gas chromatograph was operated split less at 120°C for 30 sec, followed by programmed increase to 280°C at 4°C/min); mass spectra were compared with those reported in the literature (MC LAFFERTY & STAUFFER 1989) and gas chromatographic retention times (co-injection) with those of authentic reference samples.

Bioassays

Various bioassays were performed in July and August 2001 at the Institute of Zoology. In a first bioassay the importance of visual versus olfactorial signals of *E. helleborine* flowers were examined in field experiments. All tests were made under sunny conditions and temperatures about 26-29°C at the roof terrace of the Institute of Zoology where the abundance of *Vespula germanica* workers was high. Each test lasted 20 min and was performed at least 10 times. Two types of behavioural patterns of wasps were recorded: approach: a wasp moved towards an inflorescence at a distance of 0-20 cm; pouncing: a wasp pounced an offered plant. The following test series were performed: 1) An inflorescence of *E. helleborine* watered in a glass tube was presented to the wasps. 2) To test the olfactorial cues a plant of *E. helleborine* was covered by a cardboard cylinder (ca.

10 cm id). Pre cleaned air (200 ml/min), passed a charcoal filter, was directed into a glass chamber, containing 3 blossoms of *E. helleborine*. The floral odour was directed just in front of the cylinder. 3) To test the importance of visual cues a plant of *E. helleborine* was covered with an acrylic glass cylinder. 4) To test, if blossom extract contains pollinator-attracting compounds a plant of *E. helleborine* was covered with an acrylic glass cylinder. Pre cleaned air (200 ml/min), passed a charcoal filter, was directed into a glass chamber containing a filter paper (ca. 2 x 4 cm) impregnated with a pentane extract of 3 *E. helleborine* blossom-equivalents (total volume: 100 µl). The extract was refreshed after each test.

y-tube-tests

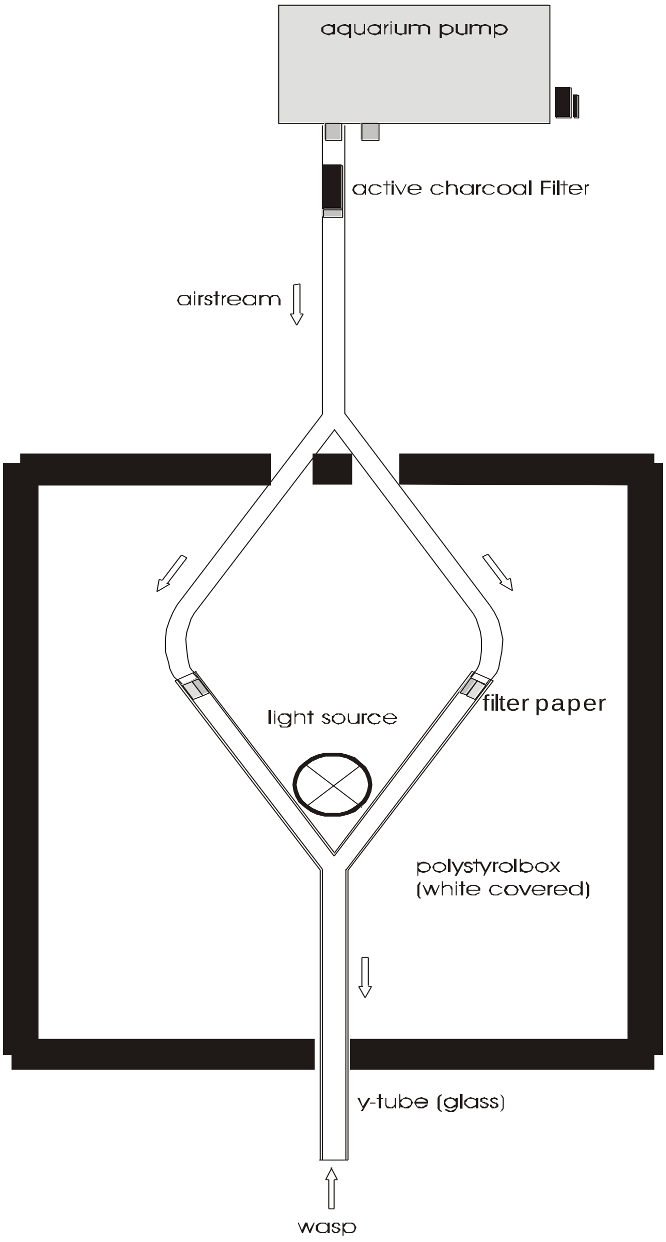

Fig. 1: Diagram of the olfactometer used for preference tests with V. germanica workers.

I used adapted y-tube-test developed by BERTSCHY ET AL. (1997) to investigate odour preferences of *Vespula germanica* workers. Wasps were caught and kept in a plastic box (30x30x30 cm) at room temperature and in darkness for 15 min prior to the tests. The olfactometer consisted of a precleaned y-shaped glass tube horizontally mounted in a white-coloured polystyrol-box (ca.35x20 cm, Fig.1). A 20 W neon lamp (20 x 10 cm) was positioned directly above the crossing of the y-tubes arms. At the end of each shorter arm (7 mm id) a piece of v-shaped folded filter paper (ca. 7x16 mm) impregnated with odour samples or solvent was inserted before each test. An additional function of the filter paper was to prevent the wasps to leave the glass tube and to contaminate the air stream-system. Pre cleaned (active charcoal filter, Supelco) air was directed into each arm of the y-tube. The airstream was controlled with a flow-meter (Supelco) before each test. After measuring the air flow, a wasp was taken out of the cage and put in a Drosophila tube. Either 1 µl of the test-mixtures (each 1 µl= 1/ 30 blossom equivalent) or of the solvent was added onto the filter paper. To avoid side specific preferences of the wasps, after each test the shenk with the odour-impregnated filter paper was changed. After 20 s the wasp was placed into the tube and crawled towards the light source. Pentane-extracts of *E. helleborine*, *E. purpurata*, *E. atrorubens* were tested against each other and against a solvent control, a synthetic mix of 7 GC-EAD active compounds of *E*. *helleborine* (1 µl= 1/ 30 blossom equivalent) was tested against hexane (solvent of the mix) and a natural extract of *E. helleborine.* One of both glass shenks were counted as “chosen”, if the wasps touched the filter paper at the end of the tube. Each test group was repeated 45 times.

Statistics

A principial component analysis (PCA) based on the relative proportions of 7 electrophysiologically active components was performed on a set of 19 pentane extracts of the blossom. The resulting principal components with an eigenvalue above one were used to test for differences in odour bouquets by means of a discriminant function analysis (DFA). I compared odour samples of *E. helleborine*, *E. purpurata* and *E. atrorubens* from different populations (see orchids) using calculated factor scores for each case on the principal axes (SPSS 1997). The standardized discriminant function coefficients were used to assess the importance of individual compounds. We considered a compound to have a high factor loading if the loading was above 0.5 (AYASSE ET AL. 2000).

I compared the total numbers of the behaviours approach and pouncing by a Mann Whitney U-test and an ANOVA, followed by a Tanhame as posteriory test was used to discriminate between different test groups. To test for differences between observed and expected frequencies for various test groups we used a χ^2-test (LORENZ, 1996).

Electrophysiologically active compounds in flower extracts

In GC-EAD analysis with flower extracts of. *E. helleborine* and *E. atrorubens* we registrated 20 compounds triggering receptor potentials in the *Vespula germanica* worker antennas (Fig. 2). By GC-MS analysis we found out that all substances occur in both flower extracts. However, not all compounds showed EAD signals in both flower samples.

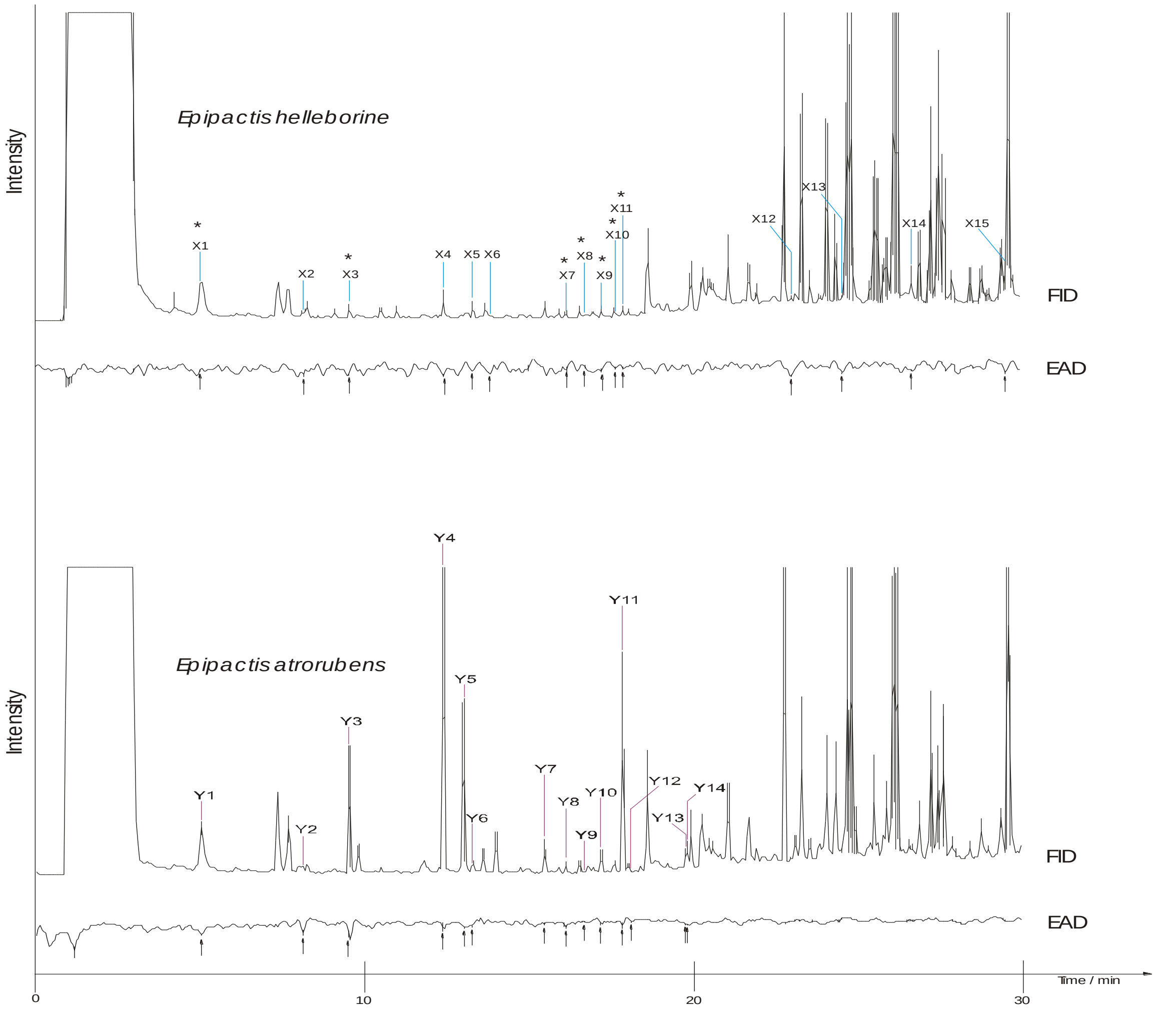

Fig. 2: GC-EAD analysis of *E. helleborine* (above) and E. atrorubens (below) flower extracts performed with antennas of *Vespula germanica* workers. Numbered peaks correspond to compounds that elicit EAD responses. A * indicates substances used for a synthetic mixture of *E. helleborine*.

Seven of 15 compounds (see Tab.1) in the flower extracts of *E. helleborine* were identified by GC-MS. These compounds belong to 3 different chemical compound classes: aldehydes (heptanal, pentadecanal, hexadecanal and vanillin), alcohols (pentadecanol, hexadecanol) and phenols (2-methoxy-4-methylphenol).

Table. 1: List of GC-EAD active components. Peak sequence in increasing retention times.

X1 = Y1 = heptanal
X2 = Y2 = unknown
X3 = Y3 = 2-methoxy-4methylphenol
X4 = Y4 = vanillin
? = Y5 = 4-methoxy-3-methoxybenzylalcohol
X5 = Y6 = 3,4-dimethoxybenzylalcohol
X6 = ? = unknown
? = Y7 = 3,4-dimethoxybenzylacetat
X7 = Y8 = pentadecanal
X8 = Y9 = pentadecanol
X9 = Y10 = hexadecanal
X10 = ? = unknown
X11 = Y11 = hexadecanol
? = Y12 = unknown
? = Y13 = unknown
? = Y14 = unknown
X12 = ? = unknown
X13 = ? = unknown
X14 = ? = unknown
X15 = ? = unknown

Species-dependent patterns of volatile compounds

In a comparison of the floral scent of three *Epipactis* species, I found significant differences in both the relative proportions (Fig. 3) and the total amounts (Tab. 2). In the relative amounts there are no significant differences for vanillin (ANOVA, p = 0.359) and hexadecanol (ANOVA, p = 0.370). All 3 species differed significantly in the relative amounts of heptanal and pentadecanol (Fig. 3). The remaining 3 EAD-active compounds showed non significant smaller amounts of 2-methoxy-4-methylphenole (ANOVA, LSD, p = 0.068), pentadecanal (ANOVA, p = 0.705) and hexadecanal (ANOVA, LSD, p = 0.407) in the wasp pollinated species. Differences between E. *atrorubens* and E. *purpurata* are significant lower relative amounts of 2-methoxy-4-methylphenole (ANOVA, LSD, p = 0.006), pentadecanal (ANOVA, LSD, p = 0.003) and hexadecanal (ANOVA, LSD, p = 0.039).

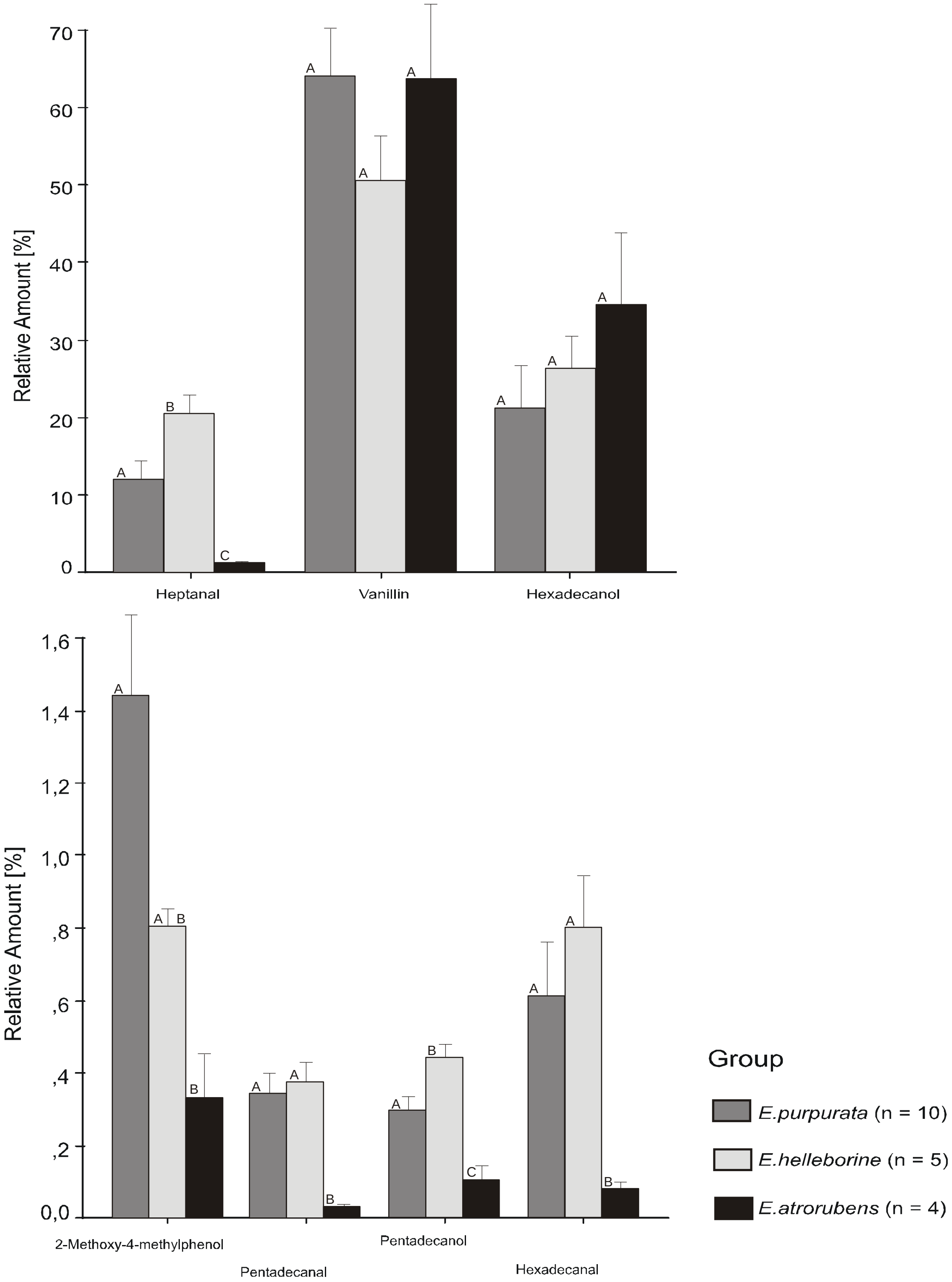

Fig 3: Comparison of the mean relative proportions (+SE) of GC-EAD active compounds in the floral scent of *E. helleborine, E. purpurata* and *E. atrorubens.* Different letters indicate significant differences (ANOVA, LSD, $p<0.05$).

The extract *E. helleborine* contain significant more pentadecanal (ANOVA, LSD, p = 0.003) and hexadecanal (ANOVA, LSD, p = 0.016) than those of *E. atrorubens*. The relative proportions of vanillin are similar high in *E. purpurata* and *E. atrorubens* (ANOVA, LSD, p= 0.971 but not significant different to E. helleborine (ANOVA, LSD, p = 0.176). There is more pentadecanol in extracts of *E. purpurata* (ANOVA, LSD, p = 0.026) than in those of *E. helleborine* and significant more than in extracts of *E. atrorubens* (ANOVA, LSD, p = 0.008). The relative amounts of hexadecanal are not significant different in the wasp pollinated species (ANOVA, LSD, p = 0.407). But *E. atrorubens* has the lowest relative amount of this GC-EAD active compound, less than *E. purpurata* (ANOVA, LSD, p = 0.039); *E. helleborine* contains the biggest amount (ANOVA, LSD, p = 0.016) (see Fig. 3).

A comparison of the absolut amounts of active compounds is shown in Tab.2.

Table 2. Absolute amounts (µg) of GC-EAD active compounds in *E. helleborine*, *E. purpurata* and *E. atrorubens*. Significant differences ANOVA (p<0.05) indicated by *, significant differences LSD (p<0.05) indicated by different letters.

	E. helleborine		***E. purpurata***		***E. atrorubens***		
active compound (µg)	Mean	SD	Mean	SD	Mean	SD	LSD (p< 0.05)
heptanal	1,424	0,358	0,954	0,400	1,060	0,235	A B A
2-methoxy-4-methoxylphenol *	0,058	0,020	0,118	0,040	0,323	0,146	A A B
vanillin *	3,808	2,152	7,636	7,795	47,466	57,902	A B B
pentadecanal	0,025	0,004	0,028	0,010	0,027	0,004	A A A
pentadecanol *	0,030	0,006	0,026	0,010	0,100	0,032	A A B
hexadecanal	0,055	0,015	0,045	0,028	0,078	0,017	A A B
hexadecanol *	1,859	0,701	1,891	1,425	32,340	6,510	A A B

A multivariate comparison with a DFA performed with factor scores of a PCA showed a significant difference between all investigated *Epipactis* species (DFA, Chi²= 15.62, df= 4, $p<0.05$). Discriminant function 1 was able to separate *E. atrorubens* from both wasp-pollinated species, that perform a separate cluster (Fig. 5). Factor score 1 show a high significant difference between the group of wasp pollinated *Epipactis* species and *E. atrorubens* which is pollinated by bumble bees (Fig.4).

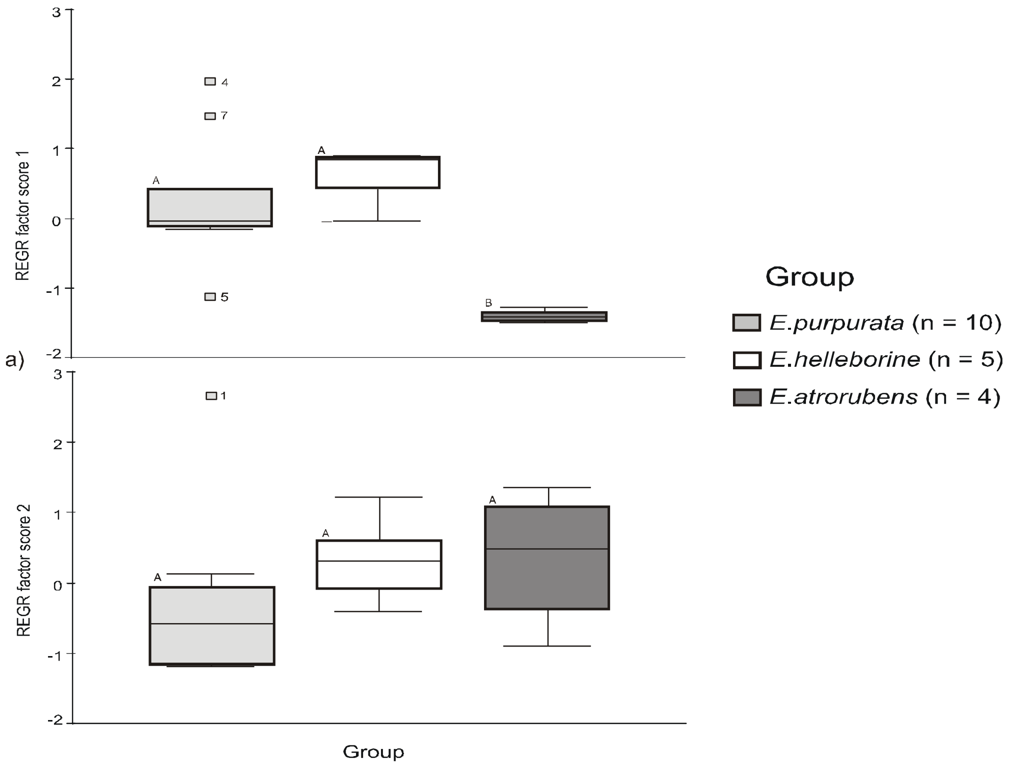

Fig. 4: Boxplots showing values of calculated factor scores of a PCA for *E. helleborine, E. pupurata* and *E. atrorubens*. (n = 19, different letters indicate significant differences (ANOVA, Bonferroni, p <0.01).

Mainly heptanal (58,47%) and 2-methoxy-4-methoxylphenol (25,48%) contributed most weight to the discriminant function and explained 83.95% of the total variance. There was an overlapping of both wasp-pollinated species as can be seen in Fig. 5.

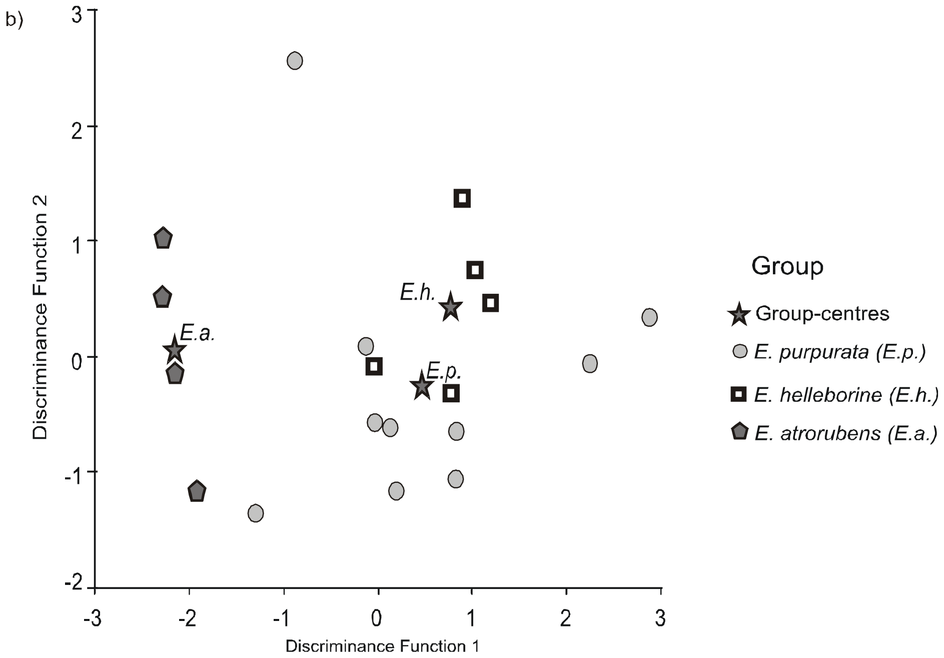

Fig. 5: Comparison of the pollinator attracting scent of 3 *Epipactis* species (n=19). I used the relative proportions of 7 GC-EAD active compounds for a PCA. The DFA was performed with principal components with an Eigenvalue above one. There was an sign. Differences in the floral scent of wasp pollinated species and bumble-bee pollinated species (DFA 1: chi^2= 15.62, DF= 4, p<0.01, DFA2: chi^2= 1.47, DF= 1, p> 0.05. 93.8% variance was explained by the first DF.

Importance of visual and olfactorial cues to wasp pollinators

Visual cues in combination with blossom extract of *E. helleborine* were more attractive for wasps then the inflorescence alone (Fig. 6). In the first case, significantly more males approached an inflorescence (U-test, p=0.003) and pounced against the test set (U-test, p=0.0012). Scent of blossoms were significantly less attractive for wasps than the combination of visual cues and a blossom extract (U-test, p<0.001 for approach), although males did not pounce more often against the test set (U-test, p=1).

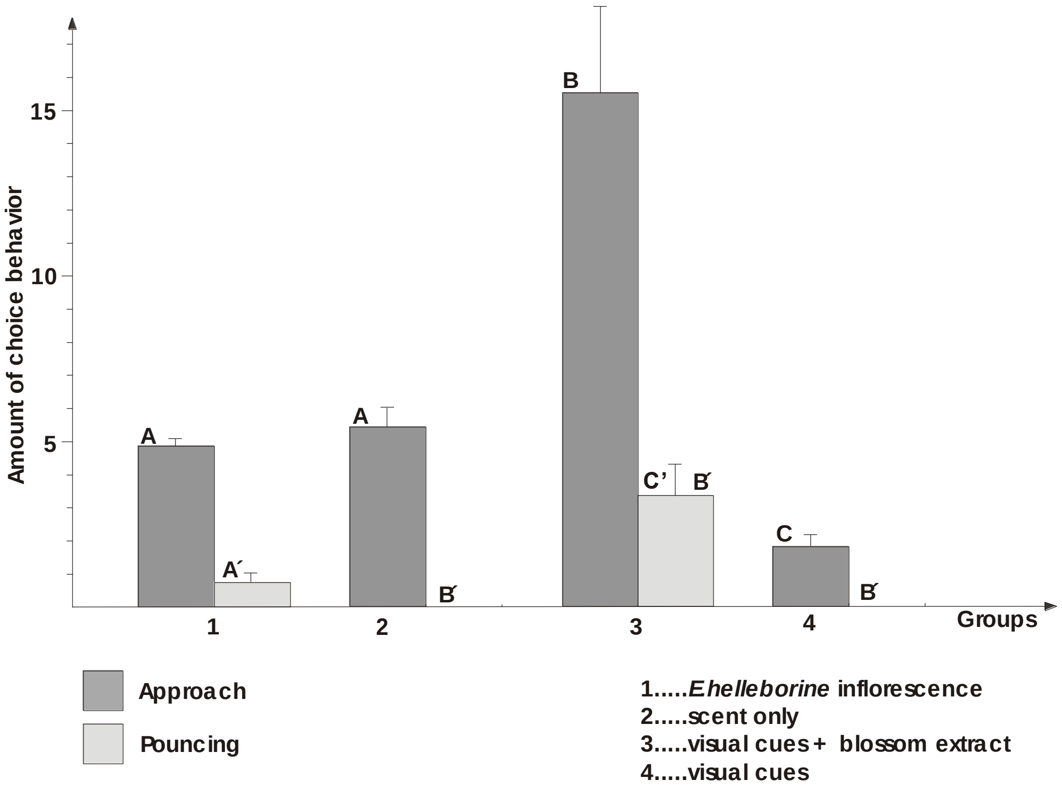

Fig. 6: Importance of visual and olfactorial cues of an *E. helleborine* inflorescence for *Vespula germanica* workers. Stimuli were offered single and in combination. (Mann-Whitney U-test with Bonferroni correction, significant differences ($p < 0.0083$) indicated by different letters.

Therefore, an inflorescence of *E. helleborine* was not significantly more attractive than floral scent alone (U-test, p=0.702), but elicited significantly more wasps to pounce the plant (U-test, p=0.015). Visual cues showed the lowest attractiveness for *Vespula germanica* workers (Fig. 6).

Olfactometer tests

In Y-tube experiments, *Vespula germanica* workers showed a clear preference for the floral scent emitted by wasp-pollinated *Epipactis* species (Fig. 7), whereas the mainly bumblebee pollinated *E. atrorubens* was less attractive. The blossom extracts of *E. helleborine* (chi²-test, chi²= 9.823; p<0,05) and of *E. purpurata* (chi²-test, chi²= 8.049; p<0,05) were significant more attractive than the control, indicating the importance of olfactorial cues. The synthetic mixture of *E. helleborine* was non-significantly more attractive than solvent (chi^{2}–test, chi^{2}= 3.746; p<0,05) or the solvent extract of *E. helleborine* (chi^{2}–test, chi^{2}=1.793; p<0,05). When the wasps had to choose between *E. atrorubens* scent and solvent they preferred the floral scent (chi^{2}–test, chi^{2}=2.68, p<0,05). In a direct comparison, the blossom extracts of wasp-pollinated species were slightly more attractive to the wasps than the extract of the bumblebee-pollinated *E. atrorubens*. Extracts of *E. helleborine* and *E. purpurata* attracted almost the same number of wasps (Fig. 7).

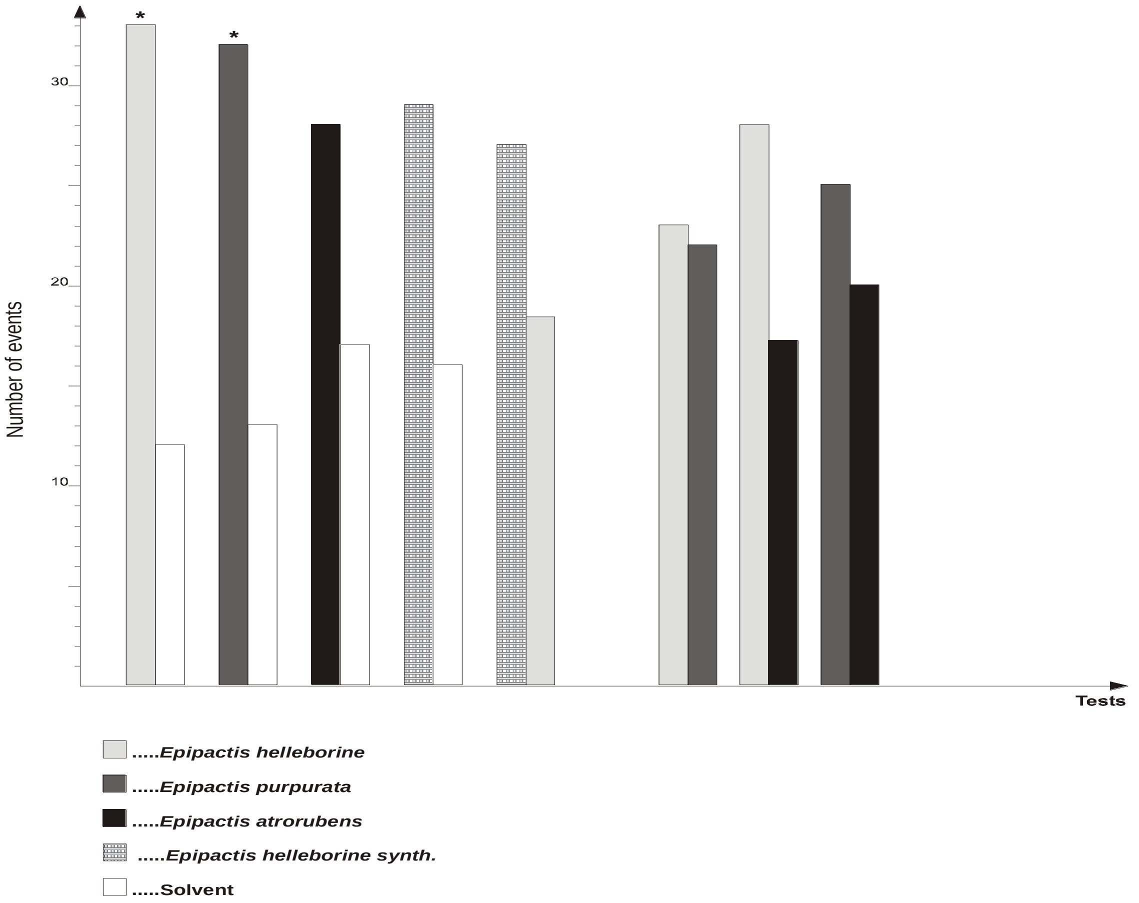

Fig. 7: Preferences of *Vespula germanica* workers for blossom extracts of *E. helleborine*, *E. purpurata*, *E. atrorubens* and a synthetic mixture of 7 GC-EAD compounds of *E. helleborine. Dual choice* tests were performed with Y-tubes (*chi^{2}-test, significant differences $p < 0.05$).

Importance of visual versus olfactorial cues in pollinator attraction by *Epipactis* flowers

E. helleborine and *E. purpurata* settle in semi-shade to shade areas, often dark coniferous forests with a shortage of pollinators (DELFORGE 1984; V. D. CINGEL 1995). Wasp pollinators are obviously attracted from a distance by the flower´s fragrance. An evidence therefore is the typical chemotactically mediated searching behaviour that can be observed in *V. germanica* workers (KEPPERT 2001). The wasps approach the flowers in closer and closer swinging movements. The results of my behavioural experiments support the superior importance of olfactorial cues for attracting wasps from a distance. The scent of the whole plant and also scent offered without visual cues attracted the wasps to approach my experimental setup (Fig. 6). However, the wasps never showed pouncing behaviour, if visual cues or olfactorial cues were singly offered. Therefore, a combination of visual and olfactorial cues may be important to elicit in the wasps the whole range of behavioural patterns that are necessary to guarantee pollination. The superior importance of olfactorial versus visual cues is supported by the finding that blossom extracts in combination with optical cues were even more attractive to the wasps as compared to an *E. helleborine* inflorescence.

While offering blossom extracts to the wasps often 2-4 individuals approached the experimental setup simultaneously. A reason for the stronger reactions of the wasps while offering blossom extracts in stead of an inflorescence might be a higher concentration of the pollinator attracting compounds in the first case.

By offering blossom extracts all the volatiles included in a flower tissue are presented in high concentrations during a relatively short time period while an intact inflorescence normally evaporates smaller amounts per time period.

V.D. PIJL & DODSON (1969) estimate, that about 8,000 species of orchids are deceptive and do not reward pollinators with nectar and pollen like *Epipactis* flowers do. The scale of attractive odours is enormous and develops its effect over distance and often at night and is therefore a basis for the specificity of visitors (V.D. PIJL & DODSON 1969). Beside the species rich group of Hymenoptera, butterflies and flies are attracted by the fragrances of orchids (V.D. PIJL & DODSON 1969; KAISER 1993, V.D. CINGEL 1995).

Another factor that may influence the attractiveness of flowers might be the age of the plants: older blossoms have stronger scent than younger ones and there are observations that older flowers are more attractive to pollinators (VÖTH 1982) due to the higher concentration of fragrance (KEPPERT 2001).
Furthermore, in many orchids there is a measurable difference in sensitivity of pollinators to certain colours (V.D. PIJL & DODSON 1969). For wasp pollinators visiting *Epipactis* flowers, the colour of the inflorescence or the contrast of bright and dark patterns in the blossom may play an important role (Vöth 1982). The blossoms of *Epipactis* flowers get a more or less rosy colour when they become older, while the inner side of the hypochile is dark red. VÖTH (1982) assumed that a strong contrast of the hypochile to all other parts of the plant is important in wasp attraction (VÖTH 1982).

In my experiments visual cues were least attractive for wasps. Therefore, visual cues do probably only have a function for short distance attraction and guide the wasps to single blossoms (KEPPERT 2001) while volatile fragrances emitted by *E. helleborine* flowers attract pollinators from longer distances.

Chemistry of the pollinator attracting substances

Floral fragrances produced by plants contain several types of chemical substances. There are terpenoids, aromatics and aminoid compounds or hydrocarbons (ROUBIK 1989) and often there are monoterpens (WILLIAM & WHITTEN 1983; PHEM-DELEGUE ET AL. 1986). The odours of bees, which are important pollinators, often resembles fragrances of flowers. A fact that is interpreted as mimicry of some pollinators odours by the flower (ROUBIK 1989). Typical monoterpenes of orchids include substances like geraniol, myrcene, merol, cineole, menthol, limonene, camphor and geranial (ROUBIK 1989). There are 2-18 aromatic compounds including methyl salicylate, eugenol, methyl cinnamonate and vanillin in a single orchid fragrance (ROUBIK 1989). Floral fragrances are primary attractants in the relationship between plants and their pollinators (ROUBIK 1989), and the enormous diversity of odours in flowers is probably a consequence of attracting animal pollinators (KAISER 1993).

The in this survey treated wasp pollinated orchid species *E. helleborine, E. purpurata* and *E. atrorubens* are not exceptions. Part of the compounds they produce are probably important for the attraction of wasps. However, they may have also another meaning in plant-animal-relationships (see below).

Three from 7 in *Epipactis* flowers identified GC-EAD active compounds that do have a function to attract wasps are aldehydes, a group of chemical compounds that are produced by most plants. They can have various functions. Plant cuticular waxes consist of long chain aldehydes that probably have a function to control transpiration (RIEDERER & SCHREIBER 1995). However, aldehydes do also have a function in insect – plant interactions. Heptanal was identified in the blossom extracts of *Epipactis* flowers and is a known plant volatile (HEDIN ET AL. 1976, LIGHT ET AL. 1988) that is perceived by the vine weevil *Otiorhynchus sulcatus* (VAN TOL & VISSER 2002). Furthermore, heptanal is described as a so-called green leaf volatile (GLV). VAN TOL & VISSER (2002) suspect, that GLV-composition is an important criterion on host-plant selection. HUBER ET AL. (2000) identified 25 volatile compounds in the barks of different trees, heptanal among them, which were electrophysiologically-active for bark beetles. Alternatively, aldehydes do also have a function as fungicides. Heptanal was identified as volatile organic compound (VCO) produced by *Trichoderma* spp. inhibiting wood decay fungi (WHEATLEY ET AL. 1997). The effectiveness of the inhibitory function of heptanal was confirmed by HUMPHRIS ET AL. (2001).

Hexadecanol is a major component of floral fragrance of *Rosa hybrida* detected with headspace technique by KIM ET AL. (2000). The function of this component in the blossoms fragrance is hitherto unknown.

Vanillin Is a common compound which occurs in the scent of many plants and also in orchids. The European species *Nigritella nigra* and some species of e.g. the neotropical orchid genus *Cattleya* also contain vanillin (KAISER 1993). The function of vanillin in e.g. the bee genus *Xylocopa* is an chemical attractant or marker (Roubik 1989). Because of the widespread occurrence of this compound more functions are thinkable,

Do wasp pollinated *Epipactis* species use similar volatile bouquets?

The assumption of Vöth (1999) that *E. atrorubens* lures insect pollinators with a colourful perianth and a strong vanilla-like scent was confirmed by the results of the chemical analyses performed in the investigated *Epipactis* species that produce high amounts of vanillin. The total amount of vanillin was similar in *E. helleborine* and *E. purpurata*, whereas the bumble-bee-pollinated species is clearly distinguishable by the higher total amount of vanillin and in the composition of the active compounds although the same chemical substances are produced by all investigated *Epipactis* species .

Important in the discrimination between wasp and bumble-bee pollinated *Epipactis* species is the contribution of the single active compounds to the standardized discriminant function coefficient The first discriminant function explained 93,8% of the total variance. Because their standardized discriminant function coefficients were highest for PC1, heptanal, pentadecanal, pentadecanol, hexadecanal and 2-methoxy-4-methylphenol contributed most to the discrimination between wasp and bumble-bee pollinated *Epipactis* species.

The predicted probability of a correct classification to a species on basis of the discriminant function is 80% for *E. purpurata* and only 20% for *E helleborine*, while 100% of all *E.atrorubens* individuals were correctly classified. Interstingly the misclassified individuals of *E. helleborine* showed a more similar odour bouquet to *E. purpurata*. As a consequence is *E. atrorubens* clearly separated from the wasp pollinated species, while the odour bouquets of the wasp pollinated species are overlapping.

A closer look on the results of a comparison of absolute amounts of active components showed a high correspondence between *E. helleborine* and *E. purpurata*. The latter is distinguishable by its higher amount of heptanal and has a little higher amount of vanillin (the scent of *E. purpurata* was already described as vanilla-like by VÖTH (1999)) than *E. helleborine*. The bouquets of *E. helleborine* and *E. purpurata* are similar and evolved maybe as an adaption to wasp-pollination in *Epipactis*.

How do *Epipactis* flowers achieve to attract social wasps?

My investigations clearly showed that the wasp-pollinated *Epipactis* species use a common odour bouquet to attract wasp pollinators. The attractiveness of the blossoms is increased by visual cues, i.e. the contrast of a reddish centre to the surrounding lighter parts of the flower. But what is the motivation for wasps to visit such flowers? How do the flowers manage to attract wasps as pollinators?

During their whole life, adult *Vespula* workers feed on the carbon hydrate rich nectar of flowers to cover their needs of energy. However, in summer and later in the year they take sugar from various sources like fruits, lemonades and sweets. To cover their needs for amino acids, proteins and other nutrition, they interact in a certain manner with larvae in the colony (trophallaxis). Thereby workers stimulate with their antennae the larvae to produce a secret of the labial glands which contains sugar, amino acids, proteins and enzymes (BRIAN & BRIAN 1952, MASCHWITZ 1966 and others). Wasp larvae are fed with meat that is hunted by the workers. They catch mainly other insects and preferably feed the larvae with before chewed meat of muscles from the thorax (SPRADBERRY 1973). The red centre of the blossoms of *E. helleborine* and *E. purpurata* resembles in colour meat or rotten fruit, the first being the food-source for larvae and the latter being a food-source for workers of *V. germanica*. But the results of our tests indicate, that visual cues of *E. helleborine* and *E. purpurata* are less important than fragrances. Alcohols are normally produced during fermentation by rotten fruit. Therefore, the alcohols I found among the GC-EAD active compounds might have a function to mimic rotten fruits in order to attract wasps. Actually VÖTH (1999) described that the scent of the blossoms of *E. helleborine* resembles fermenting fruit. The yeast *Saccharomyces cerevisiae* produces higher alcohols like benzyl alcohol, heptanol, octanol, decanol and Z-3-hexanol during fermentation (VALERO ET AL. 2001). Pear aged 24h or a combination of isobutanol and 0.5% acetic acid were found to be highly attractive to several species of wasp including *V. germanica* (DAY & JEANNE 2001)

However, the active substances we have found are alcohols with a higher chain-length. The question arises whether *Epipactis* flowers lure wasps imitating fragrance of sugar-rich and fermented fruits and rewards the pollinator with some nectar as compensation? In that case the reddish centre of the blossoms maybe associated with the colour of rotten and on the ground laying fruit. Further bioassays with the alcohols I found should be performed in order to investigate their possible function in wasp attraction.

An alternative hypothesis is that *Epipactis* flowers produce so called green-leaf-volatiles (GLV) to attract wasps preying for caterpillars. Wasps are predators hunting for potential prey by inspecting the surrounding of their nesting sites. During such search-flights they may be attracted by plant-volatiles, so called green-leaf- volatiles (GLV). These characteristic substances are released after mechanical damage of green plant tissue and are mostly saturated and monounsaturated six-carbon aldehydes like hexanal, (E)-2-hexenal, (E)- and (Z)-3-hexenal and corresponding alcohols formed by enzymatic degradation of fatty acids (HATANAKA ET AL. 1995, GALLIARD ET AL. 1977). On one hand the antimicrobial activity of this compounds might serve as wound disinfectants (SCHÜTZ ET AL. 1997), on the other hand they attract herbivores towards their host plant and do have a function as synomones (QUIROZ ET AL. 1998 , reviewed in PARÉ & TUMLINSON 1999).

Indeed *E. helleborine* flowers produce aldehydes (for example heptanal, pentadecanal, hexadecanal). However, the aldehydes so far described with a function as GLV (see e.g. HATANAKA ET AL. 1995, GALLIARD ET AL. 1977) differ with the exception of heptanal from the ones I could identify. Therefore, it is unclear so far, if *Epipactis* flowers produce aldehydes to mimic GLV.

The identification of pheromones of stink bugs and the attractiveness to the *V. germanica* relative *V. maculifrons* by ALDRICH ET AL. (1986) reveals a 12% portion of benzyl alcohol in the female pheromone. An Investigation on the attractiveness of parts of the aggregation pheromone of *V. maculifrons* (α-terpineol and linalool) in combination with (E)-2-hexenal (GLV) resulted in the hypothesis, that the wasps use this damaged-leaf-odour as a general kairomone to find insects feeding on leaves (ALDRICH ET AL. 1985). If this circumstance is also valid for *V. germanica* wasps is unclear at the moment and has to be investigated.

In conclusion I suggest that wasp pollinated *E.* flowers attract their pollinators with scent produced in the blossom and guide them with optical cues at a closer distance. The hitherto known EAD active components seem to imitate food sources like rotten, fermenting fruits. The presence of alcohols, aldehydes and benzyl alcohol supports this hypothesis. The alternative hypothesis that flowers mimic GLV has to be ruled out for the moment because of the different structure of these components. *E. helleborine* is therefore a food-rewarding and food source imitating orchid species. Further investigation should clarify the role of benzyl alcohol. EAD's and further bioassays should be performed to find its function in wasp attraction. The role of benzyl alcohol as a fermentation product and in the pheromone of potential prey is especially interesting.

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