Sex, Wine and Chemical Communication in Grapevine Moth *Lobesia botrana*

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Abstract

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The grapevine moth, *Lobesia botrana*, is a destructive pest of grapevine. Deregulation of many of the insecticides used in the control of this pest along with public demand of residue-free products, have augmented the interest for innovative tools in pest management. Behaviour-modifying semiochemicals can be used for environmentally safe insect management and are a promising alternative to insecticides. The aim of this thesis was (1) to reinvestigate the female sex pheromone and (2) to identify behaviourally active host plant compounds.

In the flight tunnel, *L. botrana* females were attracted to green grapes and shoots of grapevine *Vitis vinifera*. Females were also attracted to headspace collections from grape, showing that they rely on olfactory cues during host search. Analysis of the volatiles of *V. vinifera* headspace disclosed high variation in the odour profiles both between varieties of the same species and different phenological stages of the same variety. A synthetic mimic of grape volatiles, consisting of the compounds eliciting the strongest antennal responses, was as attractive as the host-plant to mated *L. botrana* females in the wind tunnel. The essence of this attractant was boiled down to a blend of three ubiquitous terpenes. The results show that attraction to grape is encoded by a ratio-specific blend of at least three compounds. Grapevine moth is a generalist herbivore. Females were attracted also to its native host, *Daphne gnidium*. The compounds from *D. gnidium* which elicited antennal activity attracted more females than the corresponding blend of *V. vinifera* compounds. In addition, more females were attracted to the compounds co-occurring in *Daphne* and *Vitis*, than to the compounds which occurred in *Vitis* only. Attraction to widely occuring plant volatiles may thus in part account for a generalist feeding habit.

Analysis of female sex pheromone glands showed the presence of three previously unidentified compounds. Two of these compounds significantly enhanced the male response to the main pheromone compound. Behavioural studies showed that mating disruption dispensers, applied in the vineyard the previous season emitted pheromone and attracted *L. botrana* males. This suggests that aged dispensers contribute to the mating disruption effect. An additional study concerned the disorientation technique, a new pheromone-based insect control method. This technique uses a reduced pheromone application rate and its efficacy against grapevine moth was comparable to mating disruption.

Keywords: Host plant volatiles, sex pheromone, *Lobesia botrana, Vitis vinifera*, grapevine, *Daphne gnidium*, behaviour, wind tunnel, chemical analysis, electrophysiology.

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Appendix

Papers I-VII

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Tasin, M., Anfora, G., Ioriatti, C., Carlin, S., De Cristofaro, A., Schmidt, S., Bengtsson, M., Versini, G., and Witzgall, P., 2004. Antennal and behavioural response of *Lobesia botrana* to volatiles from grapevine. *Journal of Chemical Ecology* 31, 77-87.

II. Tasin, M., Bäckman A.-C., Bengtsson, M.,Varela, N., Ioriatti, C., and Witzgall, P., 2005. Wind tunnel attraction of grapevine moth *Lobesia botrana* to natural and artificial grapevine headspace. Submitted.

III. Tasin, M., Bäckman, A.-C., Bengtsson, M., Ioriatti, C., Francke, W., Witzgall, P., 2005. Essential host plant cues in the grapevine moth. Submitted.

IV. Tasin, M., Anfora, G., Carlin, S., Witzgall, P., Ioriatti, C., and Bäckman, A.-C., 2005. Host recognition in grapevine moth (*Lobesia botrana)*, a generalist herbivore. Submitted.

V. Witzgall, P., Tasin, M., Buser, H.C., Wegner-Kiß, G., Marco Mancebón, V.S., Ioriatti, C., Bäckman A.-C., Bengtsson, M., Lehmann, L., Francke, W., 2004. New pheromone components in the grapevine moth *Lobesia botrana*. *Journal of Chemical Ecology.* In press.

VI. Anfora, G., Tasin, M., Bäckman, A.-C., De Cristofaro, A., Witzgall, P., Ioriatti, C., 2005. Attractiveness of year-old polyethylene Isonet sex pheromone dispensers for *Lobesia botrana*. *Entomologia Experimentalis et Applicata.* In press.

VII. Tasin, M., Anfora, G., Angeli, G., Baldessari, M., De Cristofaro, A., Germinara, G. S., Rama, F., Vitagliano, S., Ioriatti, C., 2005. Control of grapevine moth *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae), by disorientation. *Bulletin IOBC wprs.*, 28. In press.

Introduction

The insect studied: the grapevine moth

The grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae) (Figure 1) is the most damaging pest of grapevine throughout the European wine-growing area as well as adjacent Mediterranean countries in North Africa and Asia Minor (Bovey, 1966; Gabel & Roehrich, 1995). Up to four generations can occur throughout the growing season (Marchesini & Dalla Montà, 2004). The larvae inflict damage to flower buds and to grape berries. Bacteria and fungi, especially grey mould *Botrytis cinerea*, develop rapidly on the grapes damaged by the larvae and deteriorate entire grape bunches (Fermaud & Giboulot, 1992).

Figure 1. The grapevine moth *Lobesia botrana* (Photo by Marco Tasin).

Life history and host-range

Depending on regional and climatic conditions, adults of the grapevine moth emerge from the overwintering sites from the middle of March to the end of May. After mating, the females search for suitable plants for egg-laying. Females are selective regarding the phenological stage of grapevine plants, mostly laying eggs on flower buds but not on flowering clusters (Thiéry & Gabel, 2000). The newly hatched larvae feed on the clusters until pupation. Fully developed pupae cocoon inside the bunches. The eclosion of new adults occurs from May to July. Larvae of this generation use green berries as food source. Later on, one or two more generations can follow depending on regional climate.

The grapevine moth is a polyphagous insect and can develop on plants belonging to different families. So far, more than 40 plant species have been reported to be attacked by this moth (Ben-yehuda *et al.*, 1993; Moleas, 1988; Stoeva, 1982). Beside grapevine, occurrence of the grapevine moth on *Tanacetum vulgare* (Asteraceae) (Gabel, 1992; Gabel & Thiery, 1994, 1995; Gabel *et al.*, 1992) and *Daphne gnidium* (Tymeliaceae) (Nuzzaci & Triggiani, 1982; Thiery & Moreau, 2005) has been the object of investigation.

Current management

In Europe grapes are grown on 4 million hectares and *L. botrana* is controlled by means of broad-spectrum neurotoxic insecticides, insect growth regulators and biological insecticides (Boselli & Scannavini, 2001). Sprays are usually aimed at the second and third generations, as injuries caused by the first generation are not considered to be relevant for the crop value. Due to favourable climatic conditions in 2003, *L. botrana* developed a fourth generation in Northern Italy (Marchesini & Dalla Montà, 2004). This required a further pesticide application near the harvesting time which is potentially harmful for the consumers.

The use of insecticides will be limited due to deregistration of the most toxic compounds and due to stringent regulatory requirements for the registration of new active ingredients. Several of the most frequently used compounds are being deregulated in Europe. In addition, health aspects and environmental issues have challenged the whole agricultural world to move towards environmentally friendly techniques. Both consumers' and growers' associations are conscious of the problems connected with pesticide residues and are disposed to effort changes (Varner *et al.*, 2001).

Semiochemicals

Semiochemicals are substances carrying information between organisms. Allelochemicals are semiochemicals mediating communication between organisms belonging to different species and are classified as kairomones, allomones and synomones. Kairomones convey an adaptive advantage for the receiver but are disadvantageous for the emitter. In the case of allomones, the situation is opposite. When both emitter and receiver benefit from a signal, the compound is named synomone. Pheromones are semiochemicals enabling communication within the same species (Wyatt, 2003).

Pheromones

Insects use pheromones to communicate. Compounds released to attract mates are termed sex pheromones. In Lepidopteran insects, sex pheromones are low molecular weight compounds most commonly emitted by the female. The male follows the odour trace upwind to find the calling female (Baker & Vickers, 1996; Haynes & Baker, 1989; Witzgall, 1996; Wyatt, 2003).

Sex pheromones have been identified from most economically important insect pests (Cardé & Minks, 1996; El-Sayed, 2005; Witzgall, Bengtsson & Tóth, 2005). Pheromone based monitoring methods are nowadays applied against most lepidopteran pests. Pheromone-mediated mating disruption is used to control several species (Cardé & Minks, 1995; Cross, 2005).

Sex pheromones of the grapevine moth

The sex pheromone of *L. botrana* has been investigated for three decades. Roelofs et al. (1973) and Buser et al. (1974) identified (*E*,*Z*)-7,9-dodecadienyl acetate (*E*7,*Z*9-12:Ac) as the main compound. Further studies contributed to the identification of several other related compounds (Arn *et al.*, 1988; El-Sayed *et al.*, 1999). El Sayed *et al.* (1999) optimized synthetic blends in the wind tunnel and concluded that none of these blends could mimic the female pheromone gland extract. This indicated that the sex pheromone of the grapevine moth was not completely identified.

Pest management with pheromones

Detection and monitoring of the grapevine moth is achieved by using traps lured with sex pheromone (Cardé & Minks, 1995; Ridgway, Silverstein & Inscoe, 1990). This information is used to optimize the timing of insecticide applications. By aerial dissemination of synthetic pheromone, olfactory communication between sexes and mate-finding can be disrupted (Charmillot & Pasquier, 2000; Varner *et al.*, 2001). This method, termed mating disruption, is species-specific and non-target organisms are not affected. One important drawback is that only male behaviour is affected, not the behaviour of gravid females.

Sex pheromone of the grapevine moth is also used for population control in European vineyards. Nowadays, pheromone-based disruption of mating is used on ca. 90 000 ha in Europe. In the last few years this technique has been increasingly applied throughout

Europe. This has allowed to completely or partially replace insecticides, which have represented the main control strategy until present (Charmillot & Pasquier, 2001; Varner *et al.*, 2001). However, the mating disruption technique against *L. botrana* is not efficient at high population densities (Arn, 1996; Charmillot & Pasquier, 2001; Gordon *et al.*, 2005; Louis & Schirra, 2001; Schmitz *et al.*, 1997; Schmitz, Roehrich & Stockel, 1995; Varner *et al.*, 2001). Moreover, for an effective application of the mating disruption technique, constant monitoring of moth populations with the help of pheromone traps and constant larval assessments are necessary. This allows to timely plan supplementary chemical treatments which eventually will be needed during the season (Moschos *et al.*, 2004).

The efficiency of mating disruption programs depends also on the size of the treated area. Area-wide applications are usually successful because the immigration of moths from the surrounding areas is minimized and the pheromone drift caused by the wind is limited (Ioriatti *et al.*, 2004).

Disorientation, a new pheromone-based control technique, has recently been developed for the control of the grapevine moth in isolated and small vineyards. In this case, the mechanism acting on the mating behaviour is the competition between natural and synthetic sources of pheromone, also termed false-trail following (Bartell, 1982). Dispensers are loaded with a reduced amount of pheromone and are placed in the vineyard in a much higher number comapared to mating disruption.

Potential of volatile plant substances in insect management

Plant use volatile compounds to overcome the peculiar constraints resulting from their stationary way of life. These metabolites are emitted in a huge variety, suggesting they may provide a detailed language for communication. Although primary and secondary compounds are not always clearly separated in plant metabolism, a large number of plant compounds are not directly related to plant growth and reproduction (Metcalf & Metcalf, 1992). From a chemical point of view, volatile compounds found in plants can be classified as terpenoids, phenyl-propanoids, alcohols, esters, aldehydes, acids, hydrocarbons and nitrogen- and sulphur-compounds (Knudsen, Tollsten & Bergström, 1993).

Host-finding in insect herbivores is largely guided by volatile phytochemicals (Bernays & Chapman, 1994; Visser, 1986). Host plant selection seems to be driven by both presence of attractants in host plants and repellents in non-host plants. Insects use host volatile cues to locate suitable food sources and reproduction sites (Schoonhoven, Jermy & van Loon, 1998). Plant volatiles can therefore be used to manipulate the behaviour of egg-laying females and thus to enhance pheromone-based control methods (Ansebo *et al.*, 2004; Hartlieb & Rembold, 1996; Light *et al.*, 2001; Phelan & Baker, 1987; Phelan *et al.*, 1991).

Volatiles from *Vitis vinifera* have been studied, especially with respect to aromas of flowers and ripe fruit (Buchbauer *et al.*, 1995; Buchbauer *et al.*, 1993; Darriet *et al.*, 2002; Dourtoglou *et al.*, 1994; Schreier, Drawert & Junker, 1976; Welch, Johnston & Hunter, 1982). Different generations of grapevine moth are confronted with different volatiles released from grapevine during phenological development. However, very little attention has been paid to the chemical communication between grapevine and *L. botrana*. A solvent extract from flowering clusters of cv. Müller-Thurgau have been shown to inhibit oviposition in grapevine moth females (Thiéry & Gabel, 2000). Although infestations of *L. botrana* are reported on more than 40 species of plants, only a few studies have focused on the identification of behaviourally active plant volatiles (Gabel, 1992; Katerinopoulos *et al.*, 2005).

Methods

In this thesis, several methods were used to study the chemical communication in *L. botrana*.

Sources of Semiochemicals

Calling females

Calling females were used as a reference pheromone source for the wind tunnel and field studies. Females grapevine moths were observed to start calling immediately after the beginning of dusk and lasted until the end of the dark phase. Pheromone glands for chemical and electrophysiological analysis were extracted during the first hour of calling.

In comparison to gland extraction, collection of the effluvia emitted by a calling female allows quantification of the female release. Collection of effluvia from calling females of *L. botrana* was done using solid-phase microextraction (SPME) in static air. Volatiles were adsorbed from the surrounding air on a fibre coated with a solid sorbent. The SPME fibre was then directly inserted into the GC for thermal desorption and analysis (Frerot, Malosse & Cain, 1997).

Synthetic pheromone

Synthetic sex pheromone was used in wind tunnel bioassays, in field trapping and in mating disruption and disorientation dispensers. In the wind tunnel, male attraction to synthetic compounds identified in the pheromone gland were compared with attraction to calling females. Pheromone compounds were formulated alone or in blends. They were delivered into the wind tunnel by means of a sprayer or rubber septa. Polyethylene tube dispensers applied in the field for mating disruption experiments were also used in wind tunnel bioassays.

Plants and plant headspace

Branches and green grape clusters of *Vitis vinifera* were used as host attractants in the wind tunnel. They were freshly picked and inserted into a glass cylinder covered by a net in order to not provide visual cues for the test insect.

Volatile plant compounds were collected by means of headspace techniques. Plants or plant parts were placed in a glass chamber. Released volatiles were collected onto a trap of adsorbent material. After the end of the collective entrapment, the trap was eluted with a solvent (Agelopoulos & Pickett, 1998; Bengtsson *et al.*, 2001). The elution was then submitted either to chemical analysis, to electroantennographic-detection and/or to behavioural assays. Plant volatiles were also collected using SPME.

Synthetic plant compounds

Volatile plant compounds which consistently (n>3) elicited an antennal response were identified by means of gas chromatography coupled to mass spectrometry (GC-MS). The system used for collection of plant headspace was calibrated with synthetic compounds in order to reconstruct the blend emitted by the plant. Their recovery rate on a SuperQ trap ranged from 35% (methyl salicylate) to 83% (*E*,*E*-α-farnesene) (Figure 2). Synthetic plant compounds used in wind tunnel were delivered from a sprayer (Figure 3B), at known constant rates (El-Sayed, Gödde. & Arn, 1999b; Gödde, Arn & El-Sayed, 1999).

Antennal recordings

Insect response to pheromones and to plant volatiles was studied using the insect antenna as a sensor. Measurements obtained from the entire antenna are termed electroantennograms (EAG). For EAG recordings, a freshly cut antenna is suspended between two electrodes and connected to an amplifier. When a stimulus is applied, a depolarisation occurs over the antenna and the response can be measured. The antennal response is dose-dependent with an increasing change in EAG amplitude with increasing amount of stimulus.

Splitting the outlet of a gas chromatograph between an antenna and a flame ionisation detector (FID) allows a simultaneous recording of both chemical and physiological information. GC-EAD, gas chromatographic-electroantennographic detection, provides us with information about the antennal response to each compound in an extract, indicating potentially bioactive substances (Arn, Städler & Rauscher, 1975).

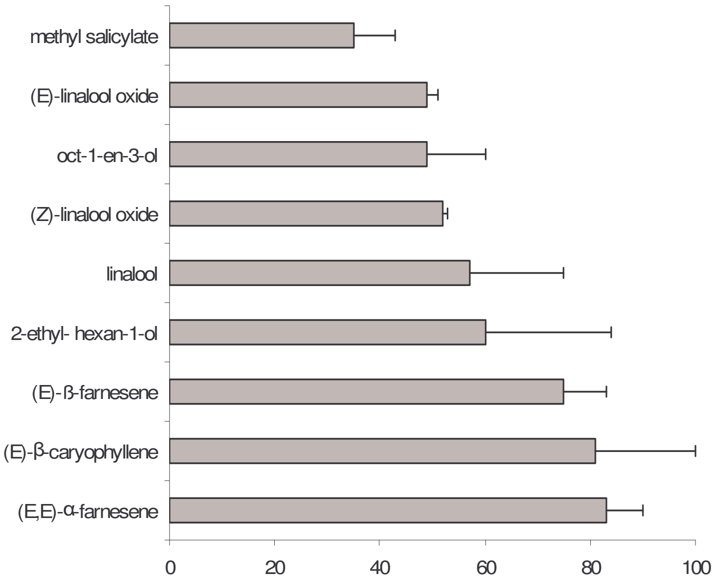

Figure 2. Recovery of synthetic volatiles released from glass capillaries. The measured recovery during headspace collection was used to formulate a corrected blend (termed "synthetic grape mimic").

Wind tunnel

The electrophysiological technique gives information about the antennal detection of plant compounds. However, bioassays are required to reveal the behavioural role of the compounds showing antennal activity. Both pheromone (calling females and synthetic pheromones) and plant stimuli (plant parts, plant headspace collections and synthetic mixtures of volatiles) were tested in a wind tunnel similar to the one described by Arn (1990) (Figure 3A).

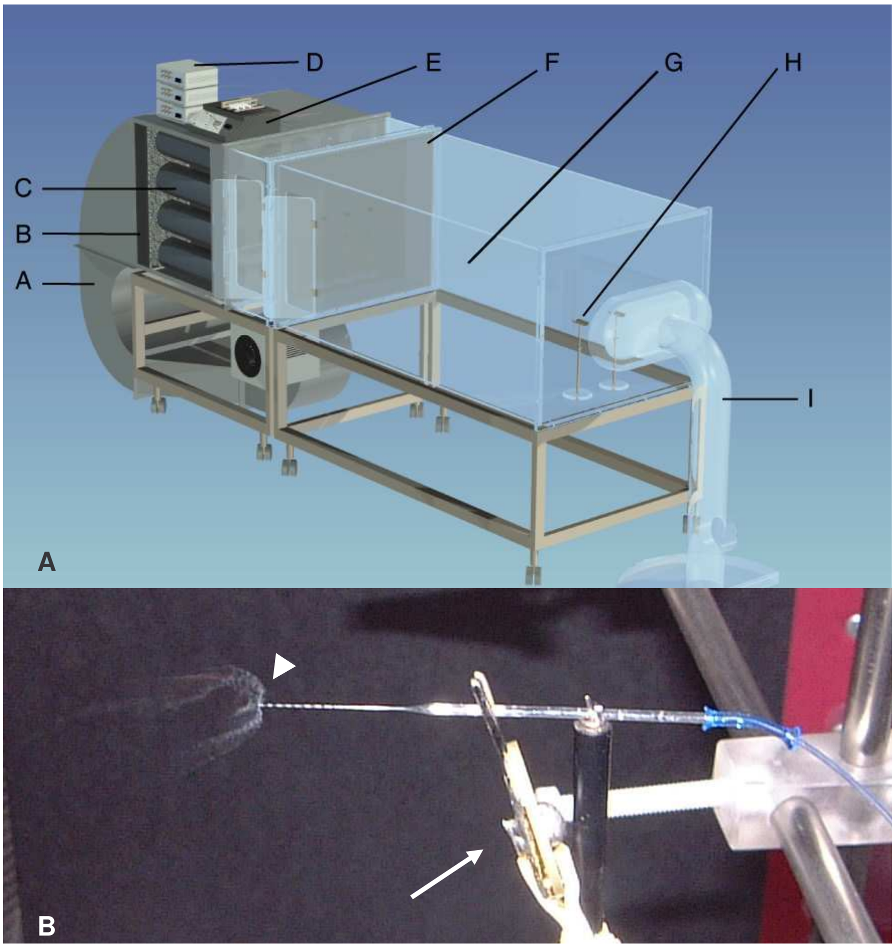

Figure 3. A: wind tunnel for behavioural studies A: air inlet; B: dust filter; C: charcoal filters; D: function generator; E, high-precision pump; F, airflow screen; G, flight section; H, insect holding platform; I, exhaust. (Drawing by Jörgen Lantz).
B: sprayer for the quantitative application of chemical stimuli. The device uses a ultrasound piezo-electric disc (arrow) to disperse a solution of volatile chemicals as an aerosol. A motor-driven syringe controls the rate at which the solution is released from a glass capillary. Vibration of the capillary disperses the released solution into microdroplets that evaporate completely within a few centimeters from its tip (arrowhead; photo by Marco Tasin).

Field tests

Field trapping

Field trapping experiments with synthetic sex pheromone compounds and with virgin females were done to verify the results of wind tunnel studies. In field trapping, factors such as trap design and trap placement are known to affect the outcome of the bioassay. Field experiments were carried out in *L. botrana* infested areas in +Foggia and Trento (Italy). Synthetic lures were prepared by formulating compounds on rubber septa. Standard tetra traps (Arn, Rauscher & Schmid, 1979) were baited with septa and placed 2 m above ground in grapevine plants.

Mating disruption and disorientation

Mating disruption experiments were made to study the possibility to reduce the number of the dispensers per hectare. We hypothesized that dispensers exposed in the field during 12 months emit pheromone in quantities large enough to compete with calling females. If so, the dispensers applied in earlier treatments could contribute and enhance the disruption efficacy. To verify this hypothesis comparisons of pheromone release rates between calling *L. botrana* females and field aged dispensers were made. Dispensers were collected in the field after different periods of exposure. Their pheromone content and release along with laboratory and field attractivity were tested by chemical analysis and behavioural bioassays.

A two-year experiment was set up in the field to compare the efficacy of disorientation with traditional mating disruption and with conventional chemical treatment. The mode of action of disorientation was investigated in the field using traps lured with pheromone and virgin females. For this purpose, males marked with fluorescent powder were released into the vineyards.

Results

Identification of the grapevine moth sex pheromone (Paper V)

The sex pheromone of the grapevine moth *L. botrana* has been reported to contain several 12-acetate derivatives as active components (Arn *et al.*, 1988; El-Sayed *et al.*, 1999). Analysis of the pheromone gland showed the presence of three new pheromone-related compounds, (*E*)-7-dodecenyl acetate (*E*7-12:Ac) and the (*E*,*E*)- and (*Z*,*E*)-isomers of 7,9,11-dodecatrienyl acetate (Figure 4). Two of these compounds, *E*7-12:Ac and (*Z*)-7,(*E*)-9,11-dodecatrienyl acetate, significantly enhanced male attraction to the main pheromone compound.

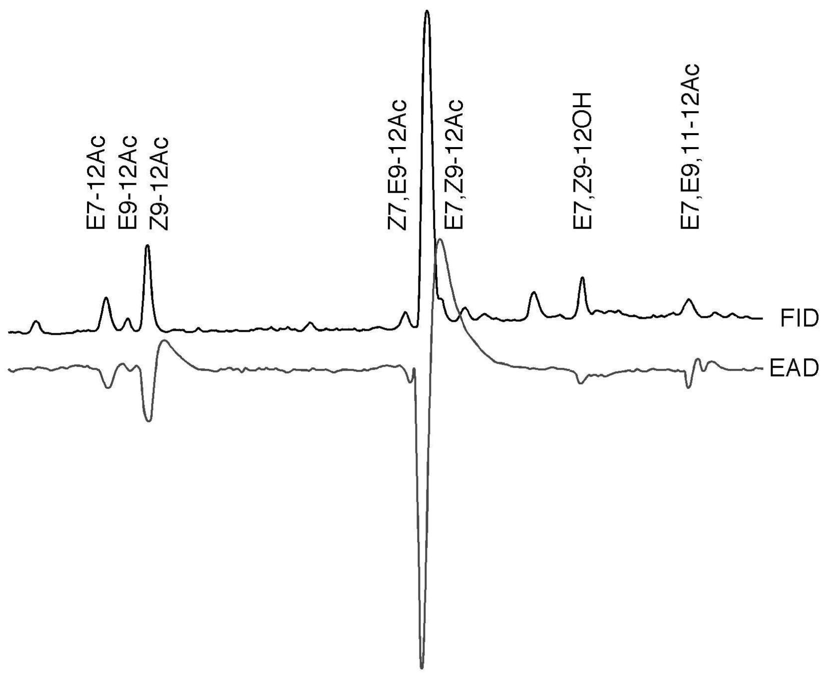

Figure 4. Analysis of pheromone gland extracts of grapevine moth *L. botrana* females from Northern Italy (N = 5) by GC-EAD. FID: response of the flame ionisation detector; EAD: response of a *L. botrana* male antenna.

Comparison of sex pheromone sources in mating disruption and disorientation (Paper VI and VII)

Females

Female sex pheromone glands contained 0.7 ng of *E*7,*Z*9-12Ac, released at a rate of 0.3±0.1 ng/hr. In the wind tunnel calling females were the most attractive source for the males compared to several synthetic sources (Table 1). Traps baited with 100 µg synthetic pheromone on rubber septa, both in pheromone treated and conventional vineyards were more attractive than virgin females (Table 2).

Rubber septa

Male grapevine moths flew upwind in higher number to rubber septum loaded with 100 µg synthetic sex pheromone than to sprayed pheromone or to a confusion dispenser exposed in the field during 2003 in the wind tunnel. However, the number of males landing at the source did not differ between the dispensers aged in the field during 2002 and 2003 and the rubber septa (Table 1).

Table 1. Attraction of virgin males *L. botrana* to E7,Z9-12:Ac released from different sources.

Pheromone source	Emission rate (ng/hr)		Wind tunnel [h]		Trap captures [g,h]	
	E7,Z9-12:Ac	Z9-12:Ac	% Oriented flight [e]	% Landing [f]	2003	2004
Calling females [a]	0.3±0.1 [i]	- [m]	90 c	83 c	-	-
Rubber septa [b]	- [l]	0	60 b	20 b	7.7±5.6 b	15.2±8.7 b
MDPD[c] 2002	6320±119 [i]	8320±140 [i]	60 b	20 b	44.0±13.5 c	-
MDPD[c] 2003	13.2±0.15 [i]	50.7±0.28 [i]	23 a	5 b	-	26.0±23.1 b
Sprayer[d]	0.6	-	23 a	15 b	-	-
	6	-	38 ab	23 b	-	-
	60	-	33 ab	23 b	-	-
Blank	-	-	0	0	0 a	0 a

[a] virgin females (n=2), 2-3 d old placed into glass tubes
[b] loaded with 100 µg of E7,Z9-12:Ac
[c] mating disruption dispenser after 12 months of exposure in the vineyard
[d] pheromone evaporator (see text for details)
[e] males flying upwind over 100 cm (n=40)
[f] males touching the pheromone source, 150 cm from the release point (n=40)
[g] males catches in delta traps (n=5)
[h] mean ± STD, values in the same column followed by the same letter are not statistically different (ANOVA, Tukey's test; df=6, F=17.008, p<0.001 for oriented flight; df=6, F=22.206, p<0.001 for touching; df=23, F=15.220, p<0.0001 for trap captures of 2003; df=2, F=9.300, p<0.05 for trap captures of 2004)
[i] collected by SPME
[l] not measured
[m] not detected

Polyethylene tubes

Dispensers used for mating disruption in 2002 and 2003 caught *L. botrana* males when they were used as baits in field traps the subsequent year. While one-year-old dispensers from 2003 were as attractive as the monitoring lures (rubber septa), one-year-old dispensers from 2002 caught a higher number of males in field trapping test. This difference was due to a lower release rate from the dispenser of year 2003. In the wind tunnel male attraction to one-year-old dispensers from 2002 was not different from attraction to a rubber septum. However, significantly fewer males were attracted to dispensers from 2003 (Table 1).

Table 2. Trap catches of marked *L. botrana* males released in the field. Means followed by the same letter are not significantly different (GLM; virgin females: $F_{11,2}=18.4$; $p<0.001$; rubber septa: $F_{11,2}=27.5$; $p<0.001$)

Pheromone source[c]	**Control**	**Disorientation**[a]	**Mating disruption**[b]
Virgin females	4.3±3.6 b	0 a	0 a
Rubber septa	21.8±12.0 b	0.8±1.0 a	0.3±0.5 a

[a] 20 g/ha of E7,Z9-12Ac (2000 dispensers/ha)
[b] 86 g/ha of E7,Z9-12Ac (500 dispensers/ha)
[c] n=4

Chemical and electrophysiological analysis of volatiles emitted by grapevine *Vitis vinifera* (Paper I and II)

Compounds released by grapevine cv. "Chardonnay" were collected and identified for five different phenological stages. Hydrocarbons were characteristic for partly flowering clusters, and many of these disappeared during full flowering. Flower clusters at full bloom released many terpenoids. Green grapes contained fewer terpenoids, and instead several alcohols, aldehydes, esters, and aromatic compounds emerged. Compared to green fruits, branches with leaves emitted a higher amount of terpenoids, but both esters and alcohols were released at lower quantities.

GC-EAD analysis of Chardonnay extracts showed a number of odorants detected by the female antennae. Antennae of grapevine moth females responded to a broad variety of compounds. These included the most abundant compounds identified in the plant headspace, such as α-farnesene, 4,8-dimethyl-1,(*E*)3,7-nonatriene, (*E*)-β-farnesene, β-caryophyllene and (*Z*)-3-hexenyl acetate, but the antennae also responded to compounds that were present in much smaller amounts, for example limonene, (±)-linalool, and heneicosane. Many alcohols, ketones, and hydrocarbons with straight carbon chains elicited antennal responses. In contrast, only a few cyclic compounds such as methyl salicylate, limonene, and caryophyllene elicited antennal responses. GC-EAD analysis were also done with headspace collections from green berries of cv. Casana (Figure 5 C). Several of the compounds eliciting an antennal activity in Chardonnay volatile collections were absent in Casana, and vice versa. The most active compounds (mV*10^4/ng) collected from both varieties were oct-1-en-3-ol, 4,8-dimethyl-1,(*E*)3,7-nonatriene and (*E*,*E*)-α-farnesene.

Attraction of the grapevine moth to grapevine volatile compounds (Paper II and III)

In a wind tunnel bioassay, mated females were attracted by upwind flight to the oviposition substrate green grape berries of *Vitis vinifera* (Figure 5A). A headspace collection from these berries, released from a sprayer, attracted females in the wind tunnel (Figure 5B).

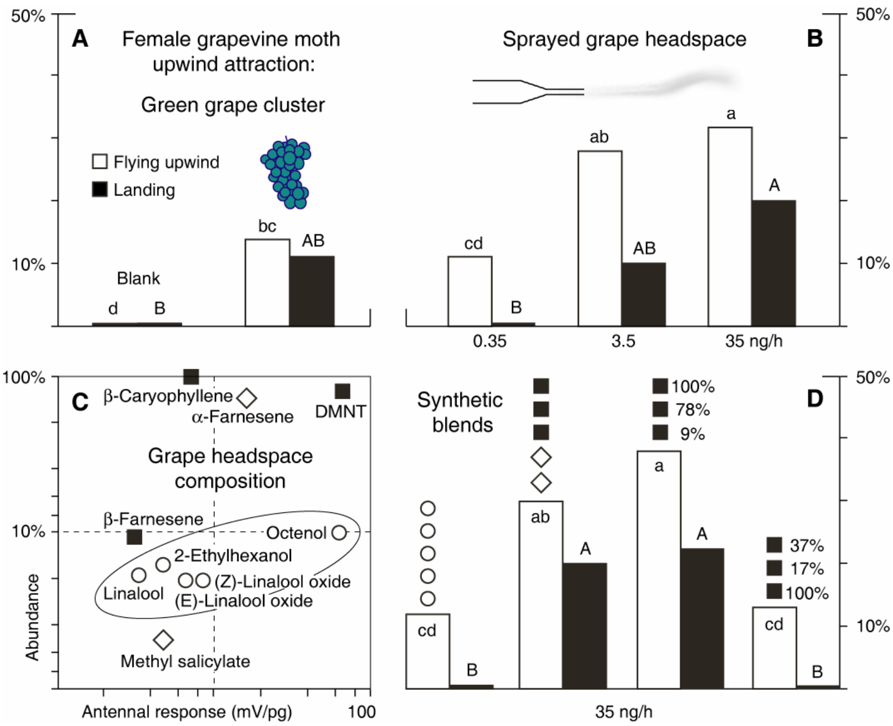

Figure 5. Wind tunnel attraction of *L. botrana* females to grape (A) and grape headspace (B). Identified grape volatiles eliciting antennal response (C) were formulated as synthetic blends for wind tunnel bioassay (D). Bars capped with the same letter did not significantly differ at P<0.05 (ANOVA, Duncan's test)

This demonstrated that volatile cues mediate attraction of grapevine moth females to grape berries, and in addition, that headspace collections capture the essence of this odour signal. A synthetic grape mimic was then formulated, consisting of the ten compounds eliciting the strongest antennal response (see "Grapevine mimic" in Figure 7 for composition). The compound ratio in this mimic blend was corrected according to the collection efficiency of individual compounds. Subsequent wind tunnel tests showed that female attraction to this synthetic 10-component blend was not significantly different from attraction to grape berries or to filter extracts of headspace collection from the same berries. At a release rate of 3.5 ng ß-caryophyllene/hr, 18% of the females approached headspace filter extracts compared to 8% to the 10-component blend. At a higher dosage of grape mimic (35 ng/hr of ß-caryophyllene) 20% of the moths approached the source. In comparison, 100 g of green berries, which released ß-caryophyllene at a rate of ca. 4.7 ng/h, attracted 10% of the females by upwind flight followed by landing. Subsequent wind tunnel experiments were done to clarify the effect of different combinations of compounds on the attraction of female moths. The 10 component blend was split into 2 groups (Figure 5D). Moths responded to a blend of four common terpenoids and methyl salicylate with 30% of upwind flight and 20% of landing. In contrast, only 13% flew towards and none landed at the second blend, containing typical grape volatiles. This shows that the first group of five compounds may hold a more reliable signal for host-location. A blend containing only three of those five volatiles, (4,8-dimethyl-1,(*E*)3,7-nonatriene, (*E*)-β-caryophyllene and (*E*)-β-farnesene), was as attractive as the grape mimic. However, the blend proportion was critical: the same three compounds, released at the proportion found in apple headspace (37:17:100), did not elicit upwind flights to the source.

Antennal and behavioural activity of volatile compounds emitted by *Daphne gnidium* (Paper IV)

GC-EAD analysis of headspace collections from open flowers showed fifteen compounds eliciting antennal responses (Figure 6). These compounds were subsequently identified using GC coupled to mass spectrometry. Compounds in both leaves and flowers were (±)-linalool, ethyl benzoate, (*E*)-β-caryophyllene, (*E*,*E*)-α-farnesene, methyl salicylate, benzothiazole and (*Z*)-3-hexenyl benzoate. Compounds emitted only by flowers were (*E*) and (*Z*) isomers of linalool oxide furanoids and pyranoids. Three EAD-active compounds were not identified.

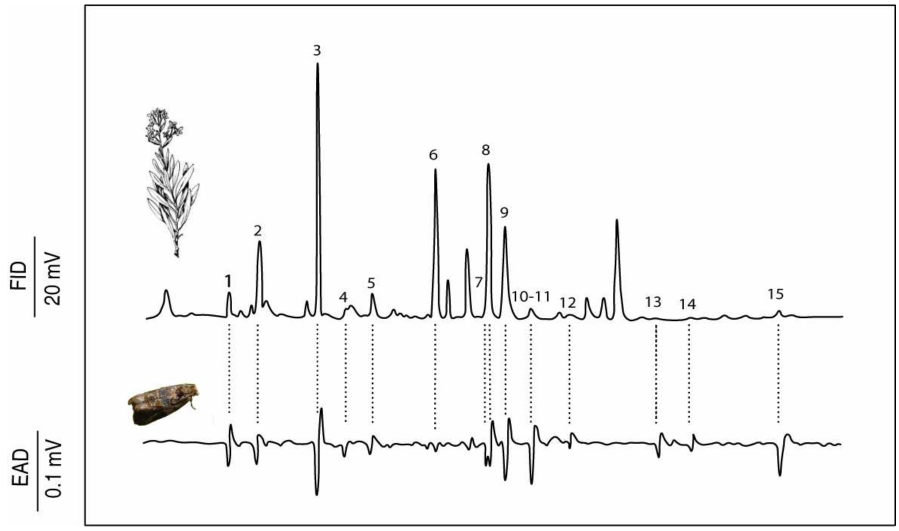

Figure 6. Simultaneously recorded gas-chromatographic-electroantennodetection analysis of volatiles collected from full blossoming flowers and leaves of *Daphne gnidium*. The upper trace represents FID response and the lower the antennal responses (EAD) of female *Lobesia botrana*. Activity was found to the following compounds: 1) (*E*)-linalool oxide furanoids, 2) (*Z*)-linalool oxide furanoids, 3) (±)-linalool, 4) unidentified, 5) (*E*)-β-caryophyllene, 6) ethyl benzoate, 7) (*E*,*E*)-α-farnesene, 8) (*E*)-linalool oxide pyranoid, 9) (*Z*)-linalool oxide pyranoid, 10) methyl salicylate, 11) unidentified, 12) unidentified, 13) benzothiazole, 14) unidentified, 15) (*Z*)-3-hexenyl benzoate.

Grapevine moth females responded to a blend with compounds found in both *D. gnidium* and *V. vinifera* with 30% of oriented flight and 20% of landing at the source (Figure 7). When a synthetic mixture containing the specific volatiles emitted by flowers of *D. gnidium* were presented to the moths, only 15% oriented in the plume and 7.5% landed at the source. The addition of the specific volatiles released by the Daphne flowers to the common blend increased the response to 55% of oriented flight and 32.5% of landing at the source (Figure 7). This blend was then compared with a Daphne mimic in which the ratio among compounds were as in Daphne flower headspace. No significant difference was found in both orienting and landing (see Paper IV for details).

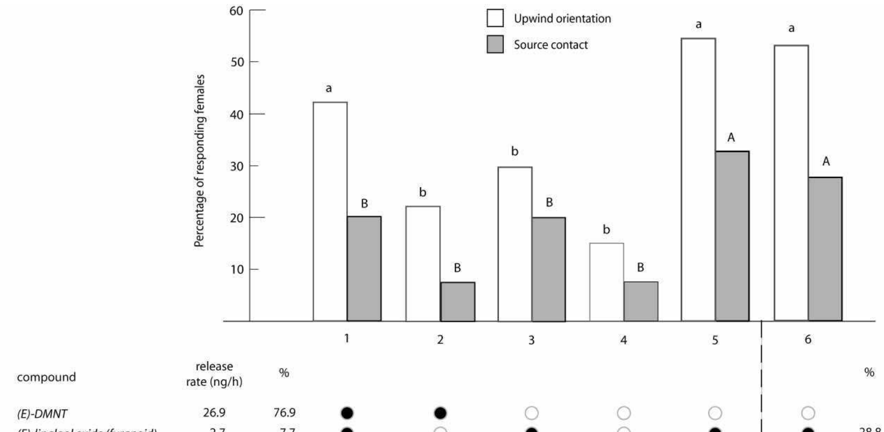

Figure 7. Attraction of mated *L. botrana* females in the wind tunnel to synthetic blends of plant volatiles. Females were scored for locking on to the odour plume (white bars) and making source contact (grey bars). Bars capped with the same letter are not statistically different (GLM; source contact $F_{23,5}$=6.3; p=0.003; locking on the plume $F_{23,5}$=10.72; p<0.001). No female upwind attraction was recorded to blank stimulus.

Concluding remarks

What was found concerning the sex pheromone…

Two new behaviourally active compounds were found in the sex pheromone of the grapevine moth. One of them is a triply unsaturated acetate, which has not been reported earlier among tortricid moths. The other is a monounsaturated 12-acetate, with a potential use in mating disruption.

It was also found that one-year-old pheromone dispensers contained and emitted a quantity of pheromone much higher than a calling female and could therefore still disrupt the sexual communication of the moth in the field.

In the plots where the disorientation method was used, released males were not able to locate traps baited with calling females and traps baited with synthetic pheromone. This indicates that the disorientation method efficiently disrupted the communication between sexes.

Pheromone disruption of mating has been used in European wine districts for two decades. Nevertheless, the size of the treated areas is still very limited in comparison to conventional methods. Field and wind tunnel studies presented in this thesis show that the number of dispensers per hectare used in mating disruption can be lowered depending on the climatic conditions of the previous season. Consequently, a reduction in dispenser application rate in areas where mating disruption has previously been applied may contribute to the economy of this safe technique for the control of the grapevine moth. In addition, the disorientation method shows potential for managing grapevine moth in small and isolated vineyards.

…and concerning the host plant volatiles

L. botrana females were attracted to grapevine volatiles and these cues may serve to locate the host from a distance. The odour blends that attract females to grape have been characterized in the wind tunnel. Redundancy has been observed for many blend combinations and this may be related to the generalist feeding habit of grapevine moth. However, some compounds play a central role for attraction. This was demonstrated trough a subtractive bioassay. The attractive essence of a complex plant headspace mimics composed of ten volatiles was boiled down to a blend of three compounds. The ubiquitous character of the three components of the blend confirms the hypothesis that plant odour specificity is achieved by a particular ratio between volatiles generally distributed among plant species .

Despite a lower extent, females also responded to a mixture of non-generic volatiles, indicating a possible synergistic effect of these specific compounds on the attraction to the more generic ones.

In addition to attraction to grapevine, *L. botrana* females were also attracted to volatiles emitted by *Daphne gnidium*. A higher number of females responded to an eleven-component blend of *D. gnidium* compounds than to a ten-component blend of *V. vinifera*. When the ratio among the eleven daphne volatiles was changed, there was no difference in response. This could indicate that the ratio among compounds is not an important factor when the blend reaches high level of completeness. Some volatiles released by flax-leaved daphne are not found in grapevine and could thereby represent a possibility for the manipulation of female behaviour in vineyards.

This thesis presents evidence for the role of volatile signals in host recognition and host finding by the female grapevine moth. It thus confirms the role of plant volatiles in plant-insect relationships, and takes a step beyond by identifying the chemicals which encode this interaction. The volatiles which trigger female attraction to larval food plants have been studied by chemical analysis, antennal recordings, and behavioural studies. Grapevine moth responds strongly to nanogram amounts of these volatiles and it has been shown for the first time that females are almost as sensitive to plant volatiles as the males are to female-produced sex pheromone. The results make it now possible to develop plant compounds for behavioural manipulation of egg-laying females. Such tools could be used in complement with the mating disruption method for sustainable and environmentally safe control of grapevine moth. This work also provides a knowledge-base for breeding of new grape varieties which are less susceptible to the grapevine moth.

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