Secondary Plant Compounds as Feeding Deterrents in the African Subterranean Termite *Schedorhinotermes lamanianus* Sjöstedt (Isoptera: Rhinotermitidae):

A Behavioural and Neurophysiological Approach

Inaugural-Dissertation
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vorgelegt von
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Für meine Eltern

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1 Introduction

In the tropic and sub-tropic regions, termites play an important ecological role (e.g. Harris 1966) and function as important ecosystem engineers (e.g. Black & Okwakol 1998, Jones et al. 1994). Due to their feeding habits as soil-, wood-, grass-, and litter-feeders and mound building activity (e.g. Bignell & Eggleton 2000, Lee & Wood 1971, Wood 1978) termites change structure and physicochemical properties of the soil (e.g. Jouquet et al. 2002, Lobry De Bruyn & Conacher 1990, Wood 1988, Wood & Sands 1978). Termites improve the availability of mineral N and P (e.g. Brown & Whitford 2003, Jones 1990, Ndiaye et al. 2003, 2004), and water for the surrounding vegetation (e.g. Donovan et al. 2001, Konaté et al. 1999) specifically in savannah and semi-arid ecosystems (e.g. Jouquet et al. 2005a,c). Furthermore, termites increase removal and mineralisation of leaf litter and dead plant material (e.g. Bignell & Eggleton 2000, Collins 1981, Schuurman 2005, Yamada 2005) and change the microbial composition in the surroundings of their nests (e.g. Fall et al. 2004, Jouquet et al. 2005b, Roose-Amsaleg et al. 2004).

Besides their ecological importance, some termite species have a high economic importance as pests of timber and buildings (Harris 1969, Su & Scheffrahn 1990, 2000). Of the worldwide 2,300 termite species, 183 are considered to be pests damaging buildings (Edwards & Mills 1986). Subterranean termites account for 80% (147) of the economically important species, mainly within the genera *Coptotermes*, *Odontotermes*, *Microcerotermes*, *Reticulitermes*, and *Heterotermes*. Among drywood termites, the genera *Cryptotermes*, *Incisitermes*, and *Kalotermes* are important (Su & Scheffrahn 2000). The worldwide costs due to damage caused by termites sum up to approximately 22 billion US$ annually (Fuchs et al. 2004). Therefore, high effort was put into the development of control methods in the last decades. Current control options include chemical and physical barriers, wood treatments, and population control by baits (reviewed in Su & Scheffrahn 2000). Nowadays commercially used chemical treatments as control agents against subterranean termites include soil barriers with organophosphates, pyrethroids, organochlorines or wood impregnations with chromated copper arsenates (CCA), coal tar creosote, pentachlorphenol, disodium octaborate tetrahydrate (DOT). As these control agents are also quite toxic or noxious compounds

to humans, recent research efforts were put into the investigation of naturally occurring wood preservatives against termites.

In general, insect herbivores like termites have to recognise suitable host plants. In order to understand in more depth the ecological and physiological processes involved in the recognition of suitable food sources it might be helpful using two approaches. On the one hand, it is important to investigate host plant characteristics and what kind of behavioural aspects of the herbivore (e.g. food choice, food consumption) are influenced by these characteristics under field and laboratory conditions. On the other hand, the neurophysiological mechanisms involved in the recognition of secondary compounds should be investigated to better understand proximate causes how e.g. plant-derived compounds mediate feeding behaviour in herbivores.

1.1 Host Plant Recognition and Feeding Behaviour

Different characteristics of potential host plants (e.g. form, colour, chemical compounds) might influence feeding behaviour in herbivores (Dethier 1982). Among the hundreds of chemical compounds produced by potential host plants, some secondary compounds might be toxic or noxious to herbivores. Pfeffer (1897) and Stahl (1888) already described at the end of the 19th century the *raison d'être* of these compounds as defence mechanism of plants (deterrents, repellents) against herbivores. Furthermore, number and structure variations of deterrent compounds in the plant kingdom are much higher than for phagostimulants (Schoonhoven et al. 1992). Thus, feeding deterrent compounds are most likely more important in host plant recognition than phagostimulants (Bernays & Chapman 1977, Jermy 1961, 1966). Host plant recognition processes were intensively investigated in agricultural pests focusing mainly on lepidopterous and coleopterous species (e.g. Blaney & Simmonds 1988, 1990, Messchendorp 1998). A number of specialised insects require in their food the presence of host specific chemicals like glucosinolates as token stimuli which stimulate feeding e.g. of several oligophagous insects occurring on cruciferous plants (e.g. Nielsen et al. 1979). But the same compounds can also inhibit feeding in other potential herbivores (e.g. Shields & Mitchell 1995). Furthermore, specialised insects may have a limited food range because they are deterred by a huge variety of allelochemicals occurring in non-host plants (Schoonhoven 1982).

Wood-feeding termite species are also restricted in their potential host plant range due to their exclusive feeding on wood (e.g. Wood 1978). As a counterstrategy many tropical tree species developed a resistance against wood-feeding termites during their evolution (e.g. Bavendamm 1955, Scheffrahn 1991). Several compounds functioning as feeding deterrents in termites have been isolated from these trees (e.g. neem tree *Azadirachta indica*: Delate & Grace 1995, Ishida et al. 1992, Paes et al. 2007, Serit et al. 1992; other tree species: Adams et al. 1988, Carter & De Camargo 1983, Grace et al. 1989, Reye-Shilpa et al. 1995, Shibutani et al. 2004). Additionally, also from non-woody plants antifeedant compounds against termites have been isolated (e.g. vetiver grass *Vetiveria zizanioides*: Maistrello et al. 2001a,b, 2003, Nix et al 2003, 2006, Zhu et al. 2001, 2003b, Indian patchouli *Pogostemon cablin*: Zhu et al. 2003a, dyer's woad *Isatis tinctoria*: Seifert & Unger 1994). Therefore, these plant-derived compounds might be a source of novel non-toxic wood preservatives and might offer new ways in termite control and management efforts. To assess the impact of these plant-derived compounds on feeding behaviour and tunnelling activity of subterranean termites, studies carried out so far used filter paper or wood choice and no-choice tests (e.g. Kawaguchi et al. 1989, Maistrello et al. 2003, Scheffrahn and Rust 1983, Serit et al. 1992) or tunnelling assays (e.g. Acda 2009, Maistrello et al. 2001a,b, Zhu et al. 2001). So far research efforts were mainly focused on subterranean termites of the genera *Coptotermes* and *Reticulitermes* as these species are the main pests in the southern United States and in Central Asia and Japan (e.g. Su & Scheffrahn 1990, Takahasi & Yoshimura 2002).

Subterranean termite species of another genus, *Schedorhinotermes*, have not been the subject of applied research though species of this genus cause remarkable damage to wooden constructions throughout Africa, Asia, and Australia (Snyder 1949). This might be due to their main geographical distribution in countries with generally very poor or poor developed economies. In the present study, the African subterranean termite *Schedorhinotermes lamanianus* Sjöstedt (Rhinotermitidae) was used as focus species. In its natural habitat this termite species does not utilise the tree species *Margaritaria discoidea* as food source although it is the second most abundant tree species (Mikus 2000, Mikus et al. 1997). The feeding deterrence is mediated by securinega-alkaloids in the bark and heartwood of this tree species (Mikus et al. 1998; Fehler 2000). Furthermore, more detailed research on feeding behaviour in *S. lamanianus* carried out by Reinhard and Kaib (1995) revealed that sensory cells on the antennae seem to play

an important role in food search. The authors described a characteristic behaviour of workers during food search which they called "rubbing-antennae" (Figure 1). Workers intensively rub the distal part of their antennae on the substrate indicating that receptors on the antennae might be involved in the recognition of suitable food sources.

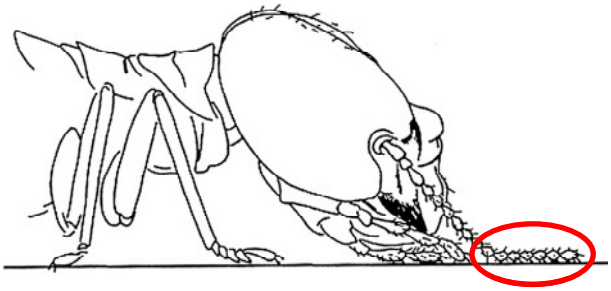

Figure 1: Position of the antennae of a *S. lamanianus* worker during food search (after Reinhard & Kaib 1995).

However, in contrast to lepidopterous or coleopterous species proximate causes specifically the physiological mechanisms involved in the recognition of secondary plant compounds to identify suitable food sources remain unclear in termites so far.

1.2 Host Plant Recognition and Chemosensory Input System

In general, the main physiological mechanism in host plant recognition based on secondary compounds is the direct detection of these compounds by herbivores via their sensory input system located on the antennae, mouth parts, or tarsae (e.g. Schoonhoven 1987, Schoonhoven et al. 1992, Schoonhoven & van Loon 2002, Städler et al. 1995). Several oligophagous herbivores recognising their host plants by using token stimuli possess specialised taste receptor cells responding to these stimuli. For instance, the cabbage butterflies *Pieris brassicae* and *P. rapae* possess taste receptor cells responding to glucosinolates produced by their cruciferous host plants (Schoonhoven 1987, Städler et al. 1995). The cinnabar moth *Tyria jacobaea* and rattlebox moth *Utetheisa ornatrix* possess taste receptors responding to host plant derived pyrrolizidine alkaloids (Bernays et al. 2003, 2004). Other herbivorous insect use the occurrence of feeding deterrent compounds in non-host plants to recognise palatable plants. Schoonhoven et al. (1992) proposed five mechanisms how feeding deterrents might mediate host plant recognition and feeding behaviour via the sensory input system. **(i)** Feeding deterrent neurons are specialised taste receptor cells inhibiting feeding behaviour. Thus plants which produce compounds stimulating these taste receptor cells are usually avoided by herbivores.

Feeding deterrent neurons are known in a variety of lepidopterous species e.g. in the silkworm *Bombyx mori* (Ishikawa 1966), the cabbage butterfly *Pieris brassicae* (Ma 1969, van Loon 1990) or the tobacco hornworm *Manduca sexta* (Glendinning et al. 2002). Other studies described deterrent receptor cells in non-lepidopterous species as the Colorado potato beetle *Leptinotarsa decemlineata* (Messchendorp et al. 1998) and the blowfly *Protophormia terranovae* (Liscia & Solari 2000). **(ii)** In some species, deterrents stimulate one or more taste neurons, which also respond to phagostimulants. In the monarch *Danaus plexippus* deterrent compounds evoke responses from three or more neurons per sensillum (Dethier 1980). The African cotton leafworm *Spodoptera littoralis* has at least one taste neuron responding to both phagostimulants and deterrents (Simmonds et al. 1990a,b). Total neural input, in the form of an across-fibre pattern, determines the resulting behavioural output, in the case considered here, the inhibition of feeding (Schoonhoven et al. 1992). **(iii)** Feeding deterrence by inhibition of phagostimulant neurons has also been reported in a variety of herbivorous species (e.g. Blaney 1981, Dethier 1987, Haskell & Schoonhoven 1969). In *Spodoptera littoralis*, "sugar" neurons are inhibited by the terpenoid azadirachtin (Simmonds & Blaney 1984). Inhibition of "sugar" neurons by deterrents has also been reported in *Leptinotarsa decemlineata* (Messchendorp et al. 1998) and *Protophormia terrannovae* (Liscia & Solari 2000). **(iv)** Temporal characteristics of neural input evoked by phagostimulants may be distorted after contact with a given deterrent compound. This can result in the inhibition of feeding. In *Leptinotarsa decemlineata*, response patterns are temporally less consistent for non-host stimuli compared to patterns evoked by host plant stimuli. This may be then interpreted by the CNS as "nonsense" thus no feeding occurs (Mitchell et al. 1990). Some compounds distort the functioning of taste receptors in a way that the "acceptance profile" needed by the CNS for initiating feeding behaviour is not evoked even in the presence of a potential host plant (Schoonhoven 1987). **(v)** Finally, some deterrent compounds elicit high impulse frequencies or "bursts" which results in an insensitivity of taste receptors to their normal stimuli. In *Manduca sexta* the glucose receptor responds vigorously to aristolochic acid but soon becomes insensitive to glucose (Frazier 1986). The terpenoid toosendanin evokes similar results in the fall armyworm *Spodoptera frugiperda* (Luo et al. 1989).

In termites, sensory input mechanisms involved in the recognition of plant-derived feeding deterrent compounds are rarely investigated so far. In general, only a few studies addressed the modalities of olfactory receptors (Abushama 1966, Floyd et al.

1976, Kaib et al. 1993, Ziesmann 1996, Ziesmann et al. 1992) and taste receptors (Mikus 2000, Mikus & Kaib 1997, 1998, Raina et al. 2003). Hardiess (2002) identified a potential feeding deterrent neuron in antennal taste sensilla of *Schedorhinotermes lamanianus* responding to securinega-alkaloids. Ohmura et al. (2006) showed in *Zootermopsis nevadensis* that the terpenoid azadirachtin reduces the neural response in taste sensilla on labial palps to phagostimulant extracts of akamatsu *Pinus densiflora*. However, a better understanding of the chemosensory input system may help to improve wood protection and termite control methods.

On the antennae of the termite *S. lamanianus* one can distinguish three types of so-called TP-sensilla (Wolfrum & Kaib 1988, Figure 2 A). Based on morphological criteria TP-sensilla represent hair-like sensory organs of insects baring taste receptor cells (Altner 1977). In this type of sensilla dendrites reach unbranched to the terminal pore which is the only access for molecules to penetrate (Figure 2 B). Furthermore, all three types bare a tubular body at the basis of the sensillum which belongs to a mechanoreceptor (Mikus 2000).

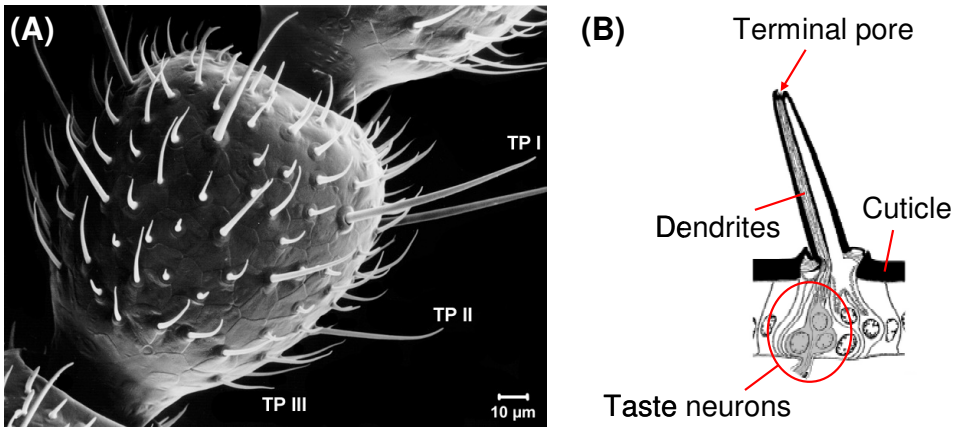

Figure 2: (A) Scanning electron microscopy picture of an antennal segment of a *S. lamanianus* worker with the 3 types of TP-sensilla indicated. **(B)** Schematical longitudinal section of a TP-sensillum (after Mikus 2000).

Four "water neurons", one with an additional salt receptor site, and two "glycine neurons" have been identified (Mikus 2000, Mikus & Kaib 1997, 1998). As already mentioned above Hardiess (2002) described an additional taste neuron in the TP II sensillum responding to the securinega-alkaloids securinine, phyllantine, and phyllantidine isolated from the tree *Margaritaria discoidea* (Fehler 2000).

Table 1 gives an overview about the morphological parameters and modalities for the different types of TP-sensilla in *S. lamanianus*. Therefore, *S. lamanianus* is an

appropriate model system to investigate the impact of secondary plant compounds on feeding behaviour and how this is mediated by the chemosensory input system.

Table 1: Morphological parameters of the three types of TP-sensilla in *S. lamanianus*. The nomenclature of gustatory neurons and their stimulus modality is given (after Mikus 2000; supplemented by data of Hardiess 2002).

Type	No. per Segment	Length [µm]	Position	Mechano-receptors	Gustatory Receptors	Stimulus Modality
TP I	6	76-83	distal	1	I/1	H_2O/NaCl
					I/2	H_2O
					I/3	Glycine
					I/4	?
					I/5	?
TP II	7	45-49	median	1	II/1	Glycine
					II/2	H_2O
					II/3	Securinega-Alkaloids*
					II/4	?
TP III	27	24-30	proximal	1	III/1	H_2O

* The modality of neuron II/3 given with securinega-alkaloids includes: securinine, phyllanthine, phyllantidine (after Hardiess 2002)

1.3 Aims of the Thesis

The investigation of proximate causes for feeding deterrence by secondary plant compounds in *S. lamanianus* was carried out in three steps.

1.) Feeding Behaviour:

The effects of secondary plant compounds on feeding behaviour in *S. lamanianus* were investigated using different food sources (filter paper, wood) and behavioural test designs (no-choice, single/multiple choice) to reflect the diverse approaches published in the literature. The following questions should be answered:

- Do alkaloids other than securinega-alkaloids known as feeding deterrents in other insect species influence food choice and quantitative food consumption in *S. lamanianus*?
- Do non-alkaloids known as feeding deterrents in other insect species also influence food choice and quantitative food consumption in *S. lamanianus*?

2.) Neural Input System for Deterrent Compounds:

The chemosensory input system for feeding deterrent plant compounds was investigated using the tip recording technique on TP II and TP I sensilla. The TP III sensilla were not investigated as this type bares only one single taste neuron responding exclusively to water. The following questions should be answered:

- Do alkaloids stimulate a taste neuron in the TP II sensillum?
- Do alkaloids stimulate the securinine-receptor (neuron II/3)?
- Do alkaloids stimulate different taste neurons in sensilla other than TP II on the flagellum of the antenna?
- Do non-alkaloids stimulate a taste neuron in the TP II sensillum?
- Do non-alkaloids stimulate the securinine-receptor (neuron II/3)?
- Do non-alkaloids stimulate different taste neurons in sensilla other than TP II on the flagellum of the antenna?

3.) Relationship between Neural Input and Behavioural Output:

- Is feeding inhibition related with the activity of neuron II/3?
- Is feeding inhibition related with the activity of different taste neurons other than neuron II/3?

2 Definitions and Abbreviations

Avoidance / preference describe the spatial orientation or distribution of gnawing termite workers under choice conditions.

Excitation / excitation level refers to the number of recorded spikes from the taste neuron in the first 200 ms during a chemical stimulation.

Feeding deterrent describes a secondary compound eliciting reduced quantitative food consumption on a non-toxic level under no-choice conditions.

Feeding deterrent receptor refers to taste neurons in insects responding to feeding deterrent plant compounds.

Gnawing describes a behaviour that workers show during food utilisation. In hypognathic head position a food particle is releases with the mandibles from the food source using jerky body movements.

Receptor / receptor site are used when the receptor site in the cell membrane of the taste neuron is referred to.

Repellent describes a secondary compound that (a) leads to an avoidance of certain feeding sites or (b) initiates termites to cover the food source with faeces.

Spike describes the sum of changes in the electrical potential, which occur at the formation of a transmitted action potential.

Compound / secondary compound describe plant-derived chemical substances of the secondary metabolism.

Taste neuron / taste receptor cell are used when the neuron itself is referred to.

TP-sensillum describes hair-like, gustatory-mechanosensitive organs of insects with a terminal pore at the tip.

Abbraviations of compound names:

Ana.....anabasine
Ajm .....ajmalicine
Are .....arecoline
Aza.....azadirachtin
Ber.....berberine
Bol.....boldine
Bru .....brucine
Caf.....caffeine
Chr .....chrysin
Col.....colchicine
Con.....coniine
Eme .....emetine
Har .....harmaline
Hyo.....hyoscyamine
Jug.....juglone
Lob.....lobeline
Lup.....lupanine
Nic.....nicotine
Nom .....nomilin
Noo.....nootkatone
Nos.....noscapine
Pap .....papaverine
Pil.....pilocarpine
Qui .....quinine
Sec.....securinine
Sin .....sinigrin
Sol .....solasodine
Spa .....sparteine
Str.....strychnine
Try.....tryptanthrin

Other abbraviations used:

SEM .....standard error of mean
.....number of ...

3 Materials and Methods

3.1 General

3.1.1 The Focus Species *Schedorhinotermes lamanianus* (Sjöstedt)

The subterranean termite *Schedorhinotermes lamanianus* is a common and widely distributed species of the tropical forests of Africa (Harris 1968, Brandl et al. 1996). The nests are commonly located subterranean but also occur in crutches or within tree trunks. *S. lamanianus* builds netlike galleries which connect on the one hand the nests with each other and on the other hand the nests and foraging sites. The foraging sites are commonly located on living trees where members of the worker caste feed on dead wood (Harris 1968, Renoux 1976). Gnawing workers show a typical gnawing behaviour and release small wood particles which are transported to the nest by other workers (Wassermann 1990). Only the worker caste is involved in food degradation and transport to the nest. They are protected and defended by minor soldiers (Kaib 1990).

3.1.2 Laboratory Colonies

Three colonies were collected in May 2001 in Gedi/Kenya and in October 1995 and December 2001 at the Shimba Hills National Reserve/Kenya respectively. Colonies were kept under constant climatic conditions (temperature: 26°C, humidity: 70-80%, dark-light cycle: 12/12 h) housed in covered stainless steel barrels (Ø 50 cm, height 50 cm). Each barrel was connected to a foraging arena (25x25 cm) consisting of a Perspex frame and two glass plates as bottom and lid which was filled with dried birch wood, and a drinking reservoir for water supply via silicon tubes (Figure 3). Additionally, the termites were fed *ad libitum* with moistened filter paper sheets every day.

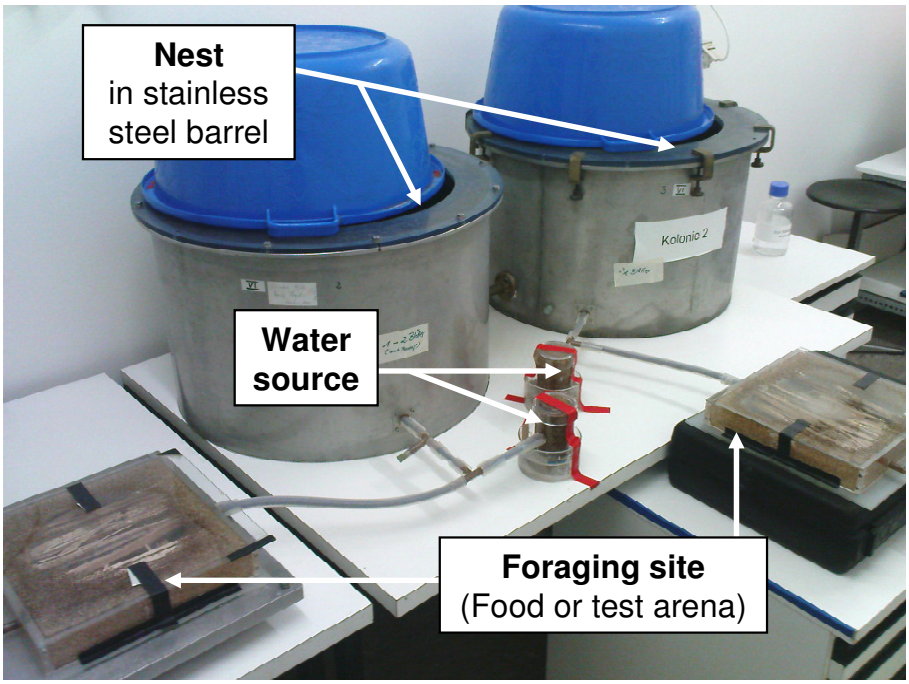

Figure 3: Laboratory colony with the indication of the nest, water source, and foraging sites. At the foraging sites normal food supply (wood) or feeding tests could be carried out.

3.2 Secondary Plant Compounds

In behavioural and electrophysiological investigations the following 24 alkaloids and 6 non-alkaloids were used in total (Figure 4 and 5). Some of these compounds were used in both behavioural and neurophysiological investigations or for technical reasons only in one of them (Table 2 and 3).

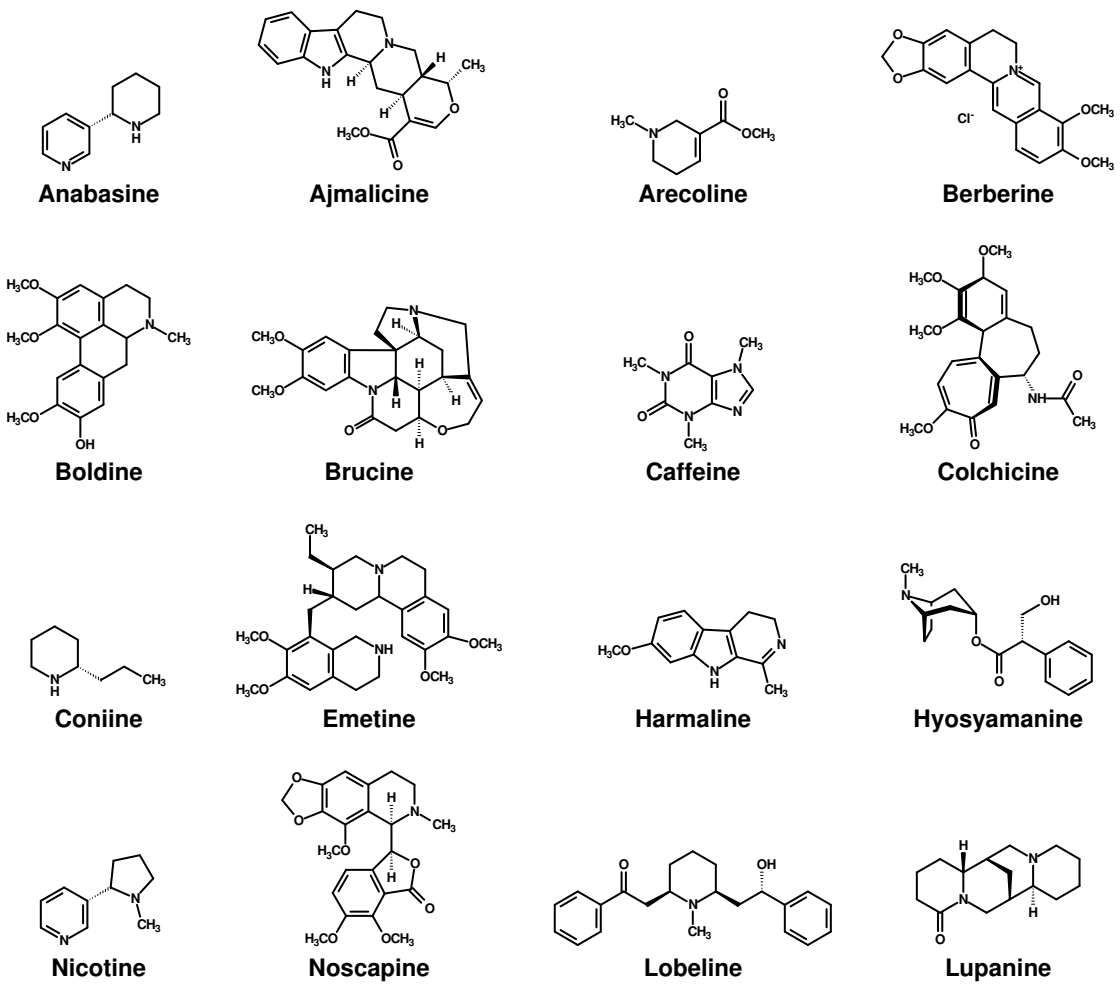

Figure 4: Chemical structures and names of alkaloids used in behavioural and/or neurophysiological investigation.

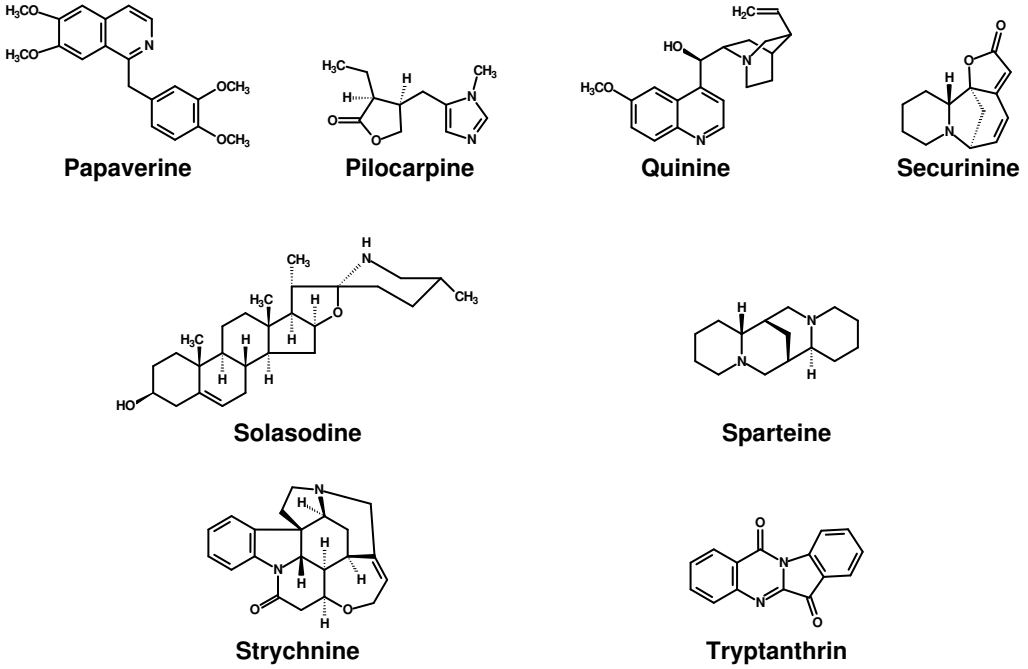

Figure 4 (continued)

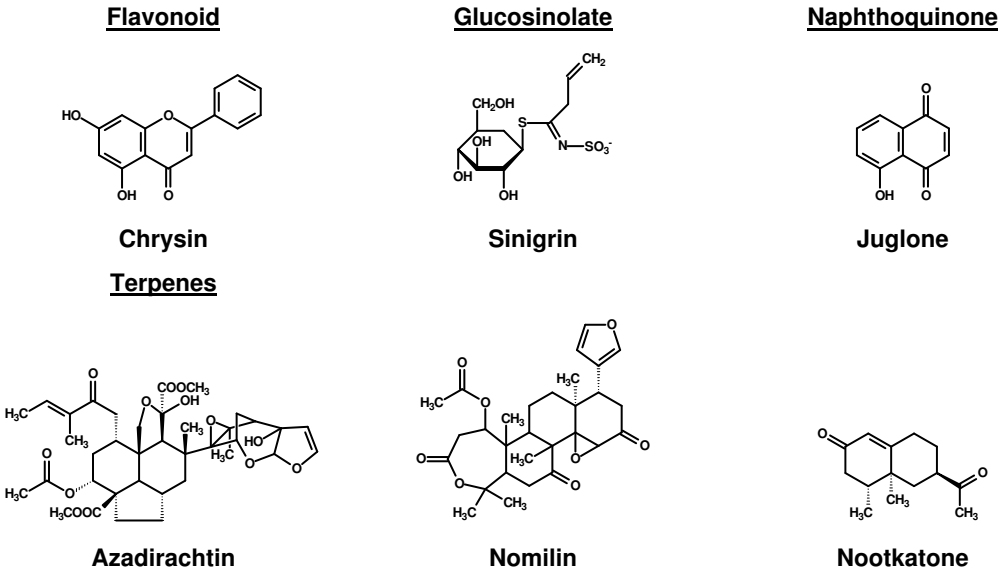

Figure 5: Chemical structures and names of non-alkaloids used in behavioural and/or neurophysiological investigations.

Table 2: List of alkaloids used in behavioural (BI) and/or neurophysiological investigations (NI) with the according solvent used. Abbreviations of names are given in brackets. Ethanol/Water means a 10% ethanol solution with a concentration of 1mM compound as stock solution.

Alkaloid	BI	Solvent BI	NI	Solvent NI
Anabasine (Ana)	–	–	+	Water
Ajmalicine (Ajm)	–	–	+	Ethanol/Water
Arecoline (Are)	–	–	+	Ethanol/Water
Berberine (Ber)	+	Methanol	+	Water
Boldine (Bol)	–	–	+	Ethanol/Water
Brucine (Bru)	–	–	+	Water
Caffeine (Caf)	+	Water	+	Water
Colchicine (Col)	+	Acetone	+	Water
Coniine (Con)	–	–	+	Water
Emetine (Eme)	–	–	+	Water
Harmaline (Har)	–	–	+	Ethanol/Water
Hyoscyamine (Hyo)	–	–	+	Ethanol/Water
Lobeline (Lob)	–	–	+	Water
Lupanine (Lup)	–	–	+	Water
Nicotine (Nic)	+	Chloroform	+	Water
Noscapine (Nos)	–	–	+	Ethanol/Water
Papaverine (Pap)	+	Water	+	Water
Pilocarpine (Pil)	–	–	+	Water
Quinine (Qui)	–	–	+	Ethanol/Water
Securinine (Sec)	+	Chloroform	+	Water
Solasodine (Sol)	–	–	+	Ethanol/Water
Sparteine (Spa)	–	–	+	Water
Strychnine (Str)	+	Chloroform	+	Water
Tryptanthrin (Try)	+	Acetone	+	Ethanol/Water

Table 3: List of non-alkaloids used in behavioural (BI) and/or neurophysiological investigations (NI) with the according solvent used. Abbreviations of names are given in brackets. Ethanol/Water means a 10% ethanol solution with a concentration of 1 mM compound as stock solution.

Non-Alkaloid	BI	Solvent BI	NI	Solvent NI
Azadirachtin (Aza)	+	Acetone	+	Ethanol/Water
Chrysin (Chr)	+	Acetone	–	–
Juglone (Jug)	+	Chloroform	+	Water
Nomilin (Nom)	+	Acetone	–	–
Nootkatone (Noo)	+	Acetone	+	Ethanol/Water
Sinigrin (Sin)	+	Water	+	Water

3.3 Behavioural Investigations

3.3.1 Filter Paper Choice Test

To determine whether secondary plant compounds influence food choice in the termite *S. lamanianus*, a filter paper choice test according to Reinhard and Kaib (1995) was used.

3.3.1.1 Experimental Design

Tests were carried out with workers in a foraging arena (10x10 cm) connected to the colony. Two filter paper semi-circles (Schleicher & Schuell™ (grade 0860), 2.5 cm diameter, 20 mg) were used for each test: one non-impregnated (= blank) and one impregnated semi-circle. The impregnated semi-circle was treated with 25 µl solution of a certain secondary plant compound (= test paper). After evaporation of the solvent, both semi-circles (blank, test paper) were placed in random order into the foraging arena and each was "glued" to the bottom with 10 µl water. They were additionally moistened with 25 µl water each. Four tests were carried out simultaneously in one foraging arena (Figure 6 A). In total 20 replicates were done (5 arenas á 4 tests).

The foraging arena was connected to the colony. After the first worker entered the arena, frequencies and spatial distribution of gnawing events by workers were recorded for 45 min using video techniques and subsequently analysed. For each test, the spatial distribution of the first 21 gnawing events was determined (Figure 6 B), and the semi-circle with the highest number of events was defined as "preferred". When a preferred semi-circle coincided with a test paper this semi-circle was called a "preferred test paper". In case it coincided with the blank paper it was called a "preferred blank paper".

All observations of termite behaviour were made under red light conditions. (Kaib & Ziesmann 1992, Reinhard & Kaib 1995)

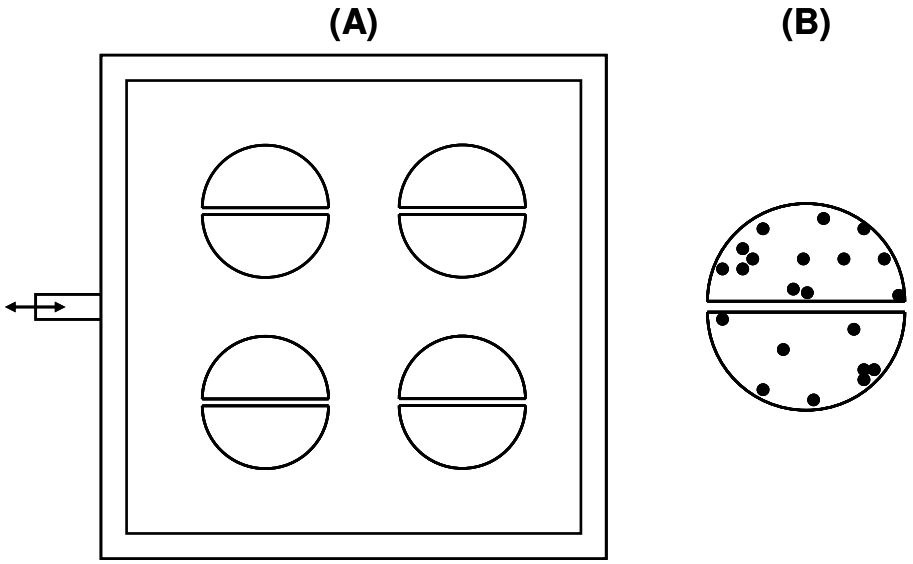

Figure 6: Experimental design of the filter paper choice test. (A) Test arena with 4 simultaneous tests. The double-headed arrow indicates the connection to the colony. (B) Example of one test with 21 gnawing events (●) in total. Spatial distribution and frequency of gnawing events in the given example: 13 events on the upper semi-circle and 8 events on the lower one respectively. Therefore the upper semi-circle would be defined as "preferred" (after Reinhard & Kaib 1995).

3.3.1.2 Data Analysis

Statistical analysis was carried out according to Zar (1974). The observed distribution in the frequencies of preferred blank and test filter papers was analysed using the sign-test. The significance level was set to $\alpha = 0.05$. A significant avoidance of treated semi-circles was shown by the termites if ≤5 preferred test papers were observed. The lowest applied amount of a certain compound eliciting a significant avoidance reaction by the termites was defined as the "compound specific threshold" (CST).

3.3.2 Filter Paper No-Choice Test

To determine whether secondary plant compounds also influence quantitative food consumption, a filter paper no-choice test (modified after Serit et al. 1992) was used. Termites could exclusively feed either on treated or untreated filter paper. Furthermore, the impact of secondary plant compounds on termite mortality was determined.

3.3.2.1 Experimental Design

For each test, standard plastic petri dishes (9 cm diameter) were used. Filter paper discs (Schleicher & Schuell™ (grade 0860), 2.5 cm diameter, 40 mg) were oven-dried for 24 h at 100°C and weighed (initial weight (IW)). The 3-fold CST (3CST) of each

secondary plant compound obtained from the filter paper choice test was applied to the filter paper discs (= test discs). Non-impregnated (= blank) discs were only treated with solvent. After evaporation of the solvent, one disc was placed in each petri dish. For each treatment 8 replicates were done. The starvation control consisted of a small ball of indigestible glass wool placed in the petri dish instead of filter paper. All filter paper discs and the glass wool balls were moistened with 50 µl water and 20 termite workers were placed into each petri dish. All prepared petri dishes were placed into a shaded Perspex box in a randomised order with moist filter paper at the bottom of the box for constant humidity conditions (Figure 7).

All paper discs and glass wool balls were re-moistened with 50 µl water every day for the whole duration of the experiment (7 days). The petri dishes were randomly re-arranged every day. The filter paper at the bottom of the Perspex box was also re-moistened every day. Additionally, dead termite workers were collected and counted every day. After 7 days, the filter paper discs were again oven-dried for 24 h at 100°C and weighed (final weight (FW)).

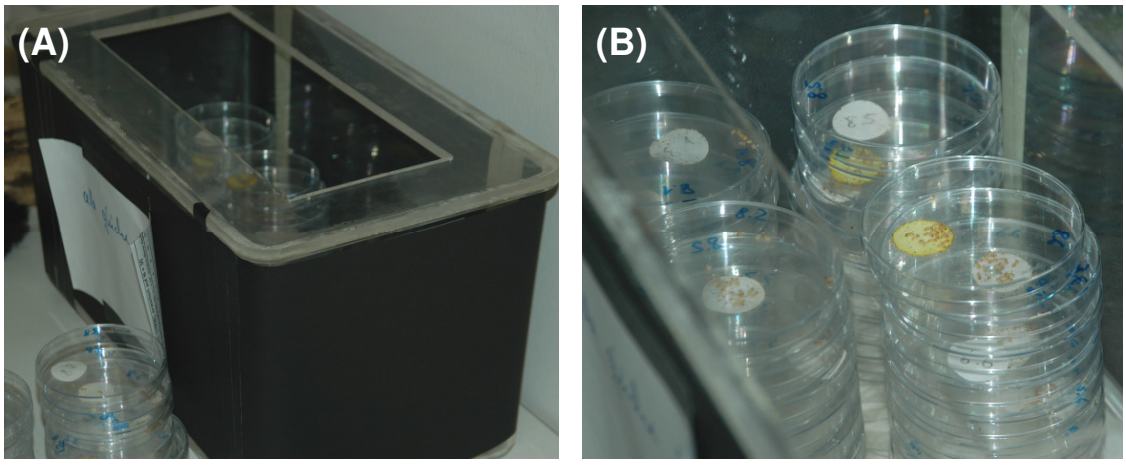

Figure 7: Experimental design of the filter paper no-choice test. **(A)** General setup with the shaded Perspex box. **(B)** Detailed view on the stacks of petri dishes inside the Perspex box. Each petri dish represents one test replicate.

3.3.2.2 Data Analysis

Food consumption (FC) was calculated as $FC = [1–FW/(IW+3CST)]\cdot 100\%$. Comparison of means of non-impreganted (= blank) and test discs was done using a generalised linear model for Gamma-distributed data with *log*-link function with the fixed factor "treatment" (15 level) as predictor variable. Mortality was calculated as the number of dead termites after 7 days. Means of starvation and blank control respectively, and test discs were compared using a generalised linear model for Gamma-

distributed data with *log*-link function with the fixed factor "treatment" (16 levels) as predictor variable. *Post-hoc* testing was done using the sequential step-down Bonferroni corrected Mann-Whitney U-test.

3.3.3 Wooden Cube Choice-Test I – Single Choice

A wooden cube choice test was used to determine whether it was possible to impregnate wooden cubes with secondary plant compounds preventing the utilization as food source

3.3.3.1 Experimental Design

3.3.3.1.1 Preparation of Compound Solutions

Each compound was dissolved in the appropriate solvent. The test concentration was obtained from the CSTs of the filter paper choice test. The CST was the lowest amount (µg) of a certain compound in 25 µl solution applied to the filter paper eliciting an avoidance reaction by the termites (see 3.3.1). As concentration the 5-fold CST (5CST) was used. To calculate the concentration of the impregnating solution from the CSTs the following equation was used: Conc [mg/ml] = (5CST [µg/25µl]·40)/1000. The factor 40 was used to calculate the amount (µg) in 1000 µl solution and the factor 1000 to convert the unit into mg/ml.

3.3.3.1.2 Impregnation of Wooden Cubes

Wooden cubes of 1x1x1 cm were oven-dried for 24 h at 100°C and weighed (IW). The test cubes were dipped into the compound solution for 5 min. To prevent solvent evaporation the solutions were kept on -22°C during this time. The non-impregnated (= blank) cubes were treated in the same procedure but only with solvent. Afterwards the cubes were oven-dried for 2h at 35°C to evaporate the solvent. For the impregnation with nootkatone the cubes were dried for 24 h at room temperature to evaporate the solvent. This was necessary as nootkatone has a very low melting point at 32-35°C.

3.3.3.1.3 Test Procedure

In total 16 cubes (8 blank, 8 test cubes) were randomly placed into one test arena (15x15 cm; Figure 8). For each compound, two test arenas were prepared and connected

to two different colonies. After 7 days the wooden cubes were oven-dried for 24 h at 100°C. Wooden cubes were cleaned from faeces using a tooth brush and the cubes were weighed (FW). The inter-test interval between two following tests was also 7 days. Termites had access to the normal foraging arena but with 50% reduced wood supply to prevent starvation of the colony but to ensure a sufficient foraging traffic into the test arena. The colonies were also fed with moistened filter paper each day. Therefore, the impact of secondary plant compounds under "normal" food supply conditions could be observed.

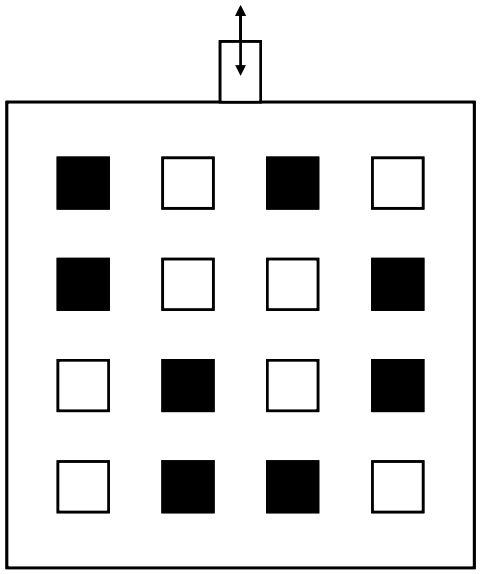

Figure 8: Experimental design of the wooden cube choice test I. 16 wooden cubes (8 controls + 8 impregnated cubes) were randomly placed in the arena. The double-headed arrow indicates the connection to the colony. Different colours indicate the different impregnation treatments: (□) non-impregnated (= blank) and (■) impregnated cubes.

3.3.3.2 Data Analysis

Food consumption (FC) was calculated as $FC = (1-FW/IW)\cdot 100\%$. Comparison of means of blank and test cubes for each compound was done using linear mixed-effects models with the fixed factor "treatment" (2 levels) and "colony-ID" as random factor. Significant effects were obtained using likelihood-ratio tests.

3.3.4 Wooden Cube Choice Test II – Multiple Choice

To determine the impact of different concentrations of secondary plant compounds on quantitative food consumption in *S. lamanianus* a second wooden cube choice test with a changed design (see below) was done.

3.3.4.1 Experimental Design

3.3.4.1.1 Preparation of Compound Solutions

Each compound was dissolved in the appropriate solvent to 4 different concentrations. The concentrations were obtained from the CSTs of the filter paper choice test. The CST was the lowest amount (µg) of a certain compound in 25 µl solution applied to the filter paper eliciting an avoidance reaction by the termites (see 3.3.1). The following 4 concentrations were used: 3-fold CST (3CST), 10-fold CST (10CST), 30-fold CST (30CST), and 100-fold CST (100CST; 80-fold CST (80CST) for chrysin and papaverine due to maximum saturation of the solution).

To calculate the concentrations of the impregnating solutions from the CSTs the following equation was used: Conc [mg/ml] = (3/10/30/100CST [µg/25µl]·40)/1000. The factor 40 was used to calculate the amount (µg) in 1000 µl solution and the factor 1000 to convert the unit into mg/ml.

3.3.4.1.2 Impregnation of Wooden Cubes

In total 1024 wooden cubes of 1x1x1 cm were oven-dried for 24 h at 100°C. The test cubes (in total 448) were dipped into the compound solution for 5 min. To prevent solvent evaporation the solutions were kept on -22°C during this time. The non-impregnated (= blank) cubes (in total 576) were treated in the same procedure but only with solvent. Afterwards the cubes were oven-dried for 2h at 35°C to evaporate the solvent. For the impregnation with nootkatone the cubes were dried for 24 h at room temperature to evaporate the solvent (see 3.3.3) All 1024 cubes were stored at -22°C until they were used in the tests.

3.3.4.1.3 Test Procedure

The test arena had the same design as the normal foraging arena (see 3.1.2) and was connected to the colony instead of the foraging arena. The cubes used in one test were weighed (initial weight, IW). In total 64 cubes (36 blank, and 28 test cubes) were randomly placed into one test arena. Of the 28 test cubes 4 cubes were impregnated with one of the 7 compounds (Figure 9). In each test run the same 7 compounds were used but in different concentrations. For each of the 28 combinations (7 compounds á 4 concentrations) 16 cubes were tested (in total 448). 4 cubes were used in 4 different

arenas. As far as possible, each compound with its 4 concentrations was tested in combination with each concentration of the other compounds. The 28 combinations were used in 16 different arranged test arenas. Two test arenas were simultaneously used in one test run.

After the test arena was connected to the colony, termites had access to it for 9 days since it lasted about two days until a sufficient foraging traffic was established. This meant a test duration of 7 days. During this time termites were fed with moistened filter paper directly on the nest. From the 4th day on an additional piece of wood (10.45±1.15 g) was available. Thus, the colony was kept on a constant "starvation" level. On the one hand termites could satisfy their needs but on the other hand it was ensured that an appropriate foraging traffic occurred into the test arena and was not outcompeted by the normal food supply.

After 9 days the wooden cubes were oven-dried for 24 h at 100°C. Cubes were cleaned from feaces using a tooth brush and the cubes were weighed (final weight, FW).

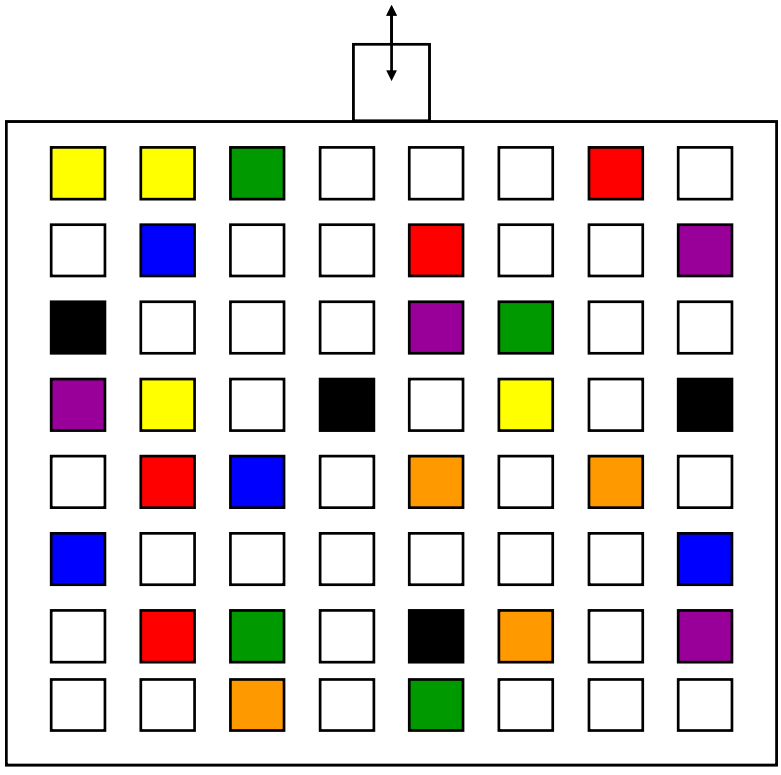

Figure 9: Example of the experimental design of the test arena in the wooden cube choice test II. 64 wooden cubes (36 non-impregnated (= blank) + 28 impregnated cubes) were randomly placed in the arena. The double-headed arrow indicates the connection to the colony. Different colours indicate the different impregnation treatments: (□) blank, (■) compound 1, (■) compound 2, (■) compound 3, (■) compound 4,(■) compound 5, (■) compound 6, and (■) compound 7.

3.3.4.2 Data Analysis

3.3.4.2.1 Food Consumption

3.3.4.2.1.1 Differences between Blank vs. *Treatment*

Food consumption (FC) was calculated as $FC = (1-FW/IW)\cdot 100\%$ for each wooden cube. Comparison between means of blank and test cubes for each concentration was done using linear mixed-effects models with the factor "treatment" (2 levels) as predictor variable and "arena-ID" and "replicate-ID" as random factors. Significant effects were obtained using likelihood ratio tests.

3.3.4.2.1.2 Differences between Concentrations

To determine the impact of increasing concentrations on food consumption a more detailed analysis was done. For each test arena, the mean FC of the 36 blank (FC_B) and 4 test cubes (FC_T) for each compound was calculated. From these data a feeding reduction index (FR) was calculated using the following equation: $FR = [1-FC_T/FC_B]\cdot 100\%$. The obtained FR's were plotted against the *log*-transformed concentrations and analysed using a linear Pearson regression.

3.4 Neurophysiological Investigations

All electrophysiological investigations were done using the tip-recording technique. This is the standard method for the registration of the activity of gustatory neurons in insects and was developed by Hodgson et al. (1955).

3.4.1 Tip-Recording Technique

3.4.1.1 General

3.4.1.1.1 Preparation of Termite Workers

Termite workers were taken from the colony and immobilized headfirst in a pointy plastic tube (Figure 10). To prevent termites from desiccation during the experiment the tube was closed with moistened filter paper. The antennae of the termite protruded from the tip of the plastic tube and were fixed with a double-sided duct tape. Hence, ventral sensilla of the first 7-8 antennal segments were available for neurophysiological investigations. To avoid desiccation of the antennae during the experiments a moist air stream (22°C, 70-85% relative humidity) was blown over the preparation.

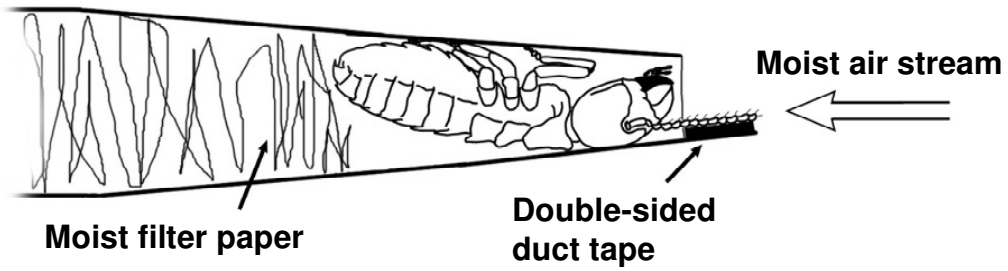

Figure 10: Preparation of a termite worker for the neurophysiological investigations (after Hardiess 2002).

3.4.1.1.2 Preparation of Stimulus Solutions

The usual solvent was water (bidest.) for all stimulus solutions. Some secondary compounds were first solved in 100% ethanol due to their lower solubility in water. But dilutions afterwards were done with water (bidest.) again with a resulting maximum ethanol content of 10% for the highest test concentration. All stimulus solutions were

prepared shortly before the first experiment and stored at -22°C for additional experiments.

3.4.1.1.3 Stimulation and Recording of Neuronal Signals

Spikes generated by neurons in the TP-sensilla were measured as the difference of the electrical potential between two glass electrodes (Figure 11). Glass electrodes were made from glass capillaries with filament (Fa. Hilgenberg, Malsfeld, Germany) using a pipette puller (Mod. 700C, David Kopf Instruments, Tujunga, USA). The resulting tip diameter was about 1 µm. The different electrode was made by cutting off the tip. The resulting diameter was about 20-30 µm.

The indifferent electrode (tip diameter: 1 µm) was filled with Ringer solution (8.5g/l NaCl, 0.42 g/l KCl, 0.2 g/l $NaHCO_3$, 0.24 g/l $CaCl_2$; Schlieper 1965) as electrolyte, and was inserted into the hemolymph lumen between the first two distal antennal segments. The different electrode was used simultaneously for stimulation and signal recording and therefore filled with the stimulus solution. Since stimulation and recording started and ended simultaneously, spontaneous activity of the neurons or activity after end of the stimulation could not be measured. For stimulation and recording, the different electrode could be put on the tip of the TP-sensillum under visual control (combined stereo microscope M3C, Fa. Wild, 400-fold magnification) using a micromanipulator (Fa. Leitz, Wetzlar, Germany). Indifferent and different electrodes were connected to an Ag-AgCl wire via a 3M KCl-agar (3-5%) bridge. The different electrode was connected to an impedance converter with capacitance neutralisation and pre-amplifier (input resistance 2 GΩ). Its signals were further amplified by a variable DC-Amplifier with low pass filter (2kHz, RC-characteristics, 24dB/octave). With the indifferent electrode the transepithelial and junction potential was measured and compensated in order to minimize the contact artefact at stimulus onset.

Chemical stimulation started with the recording of neural activity due to the combination of stimulation and recording. Shortly before stimulation/recording onset a small drop of the stimulus solution was squeezed out to renew the solution at the electrode tip. Together with the moist air stream over the preparation this procedure ensured that a defined concentration was used for stimulation. Single stimulations lasted for about 1 s. Inter-stimulus interval between repeated stimulation of the same sensillum was at least 10 min due to possible adaptations (Hardiess 2002). To prevent mechanical

stimulation of the sensillum it was paid attention that the sensillum was not bend during contact with the electrode.

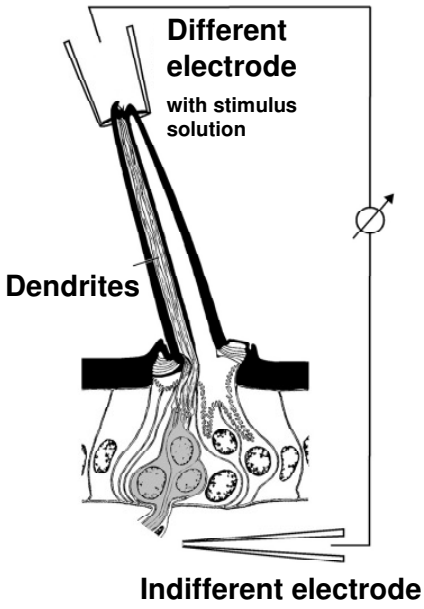

Figure 11: Scheme of a TP-sensillum with the position of the electrodes for the tip-recording technique.

3.4.1.1.4 Data Storage and Analysis

3.4.1.1.4.1 Data Storage

For a permanent storage, all signals were digitalised using an A/D-converter (resolution: 12 bit, sampling rate: 100 µs) and transferred to a computer (AT 80486 DX2-66). Digitalisation lasted 2 s and was started manually about 0.5 s before stimulation onset. Data recording was done using the programme SPIKE1 for GEM/3 (D. Piech, Datacentre, University of Regensburg, Germany). For data analysis and storage, data were saved on a harddisk.

3.4.1.1.4.2 Spike Discrimination and Attribution

Since one recording always records the activity of all neurons in one sensillum, the spikes had to be attributed to single neurons afterwards. In order to do this, the digitalised data were printed using the programme M1 (Dr. J. Gödde, Fa. Haag, Waldbrunn, Germany). The attribution of the recorded spikes to single neurons was done by visually comparing shape and temporal frequency of the spikes (Figure 12). It was further considered that the spike amplitude and shape of the same neuron could

change due to differences in the conductivity of the stimulus solution. Furthermore, a change of the spike amplitude and shape within one recording is also possible (Fujishiro et al. 1984, Hansen-Delkeskamp & Hansen 1995).

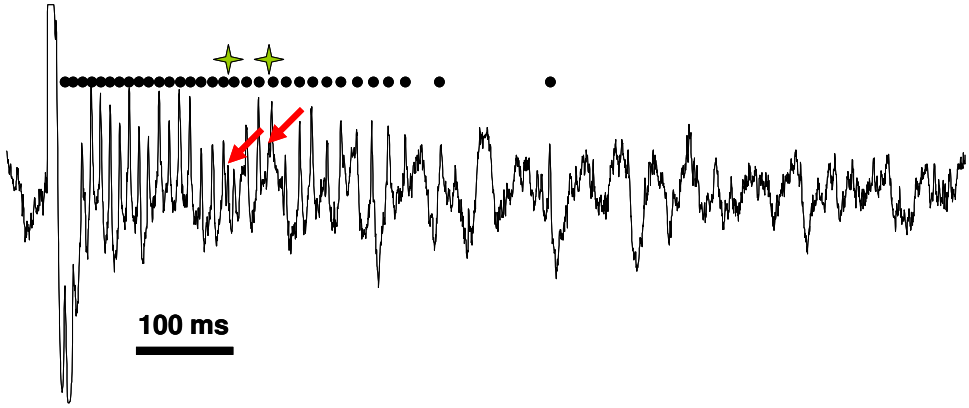

Figure 12: Attribution of spikes to the according neuron due to the temporal characteristics. Dots indicate spikes generated by neuron II/3 responding to a stimulation with 1 mM securinine. Asterisks and arrows indicate two additional spikes from another neuron.

3.4.1.1.4.3 Quantification of Neural Response

Since all neurons showed a more or less strong phasic response (Hardiess 2002, Mikus 2000), the number of spikes in the first 200 ms after stimulus onset was counted as measure for the excitation level of the neurons.

3.4.1.2 Experiments

3.4.1.2.1 Concentration-Response Relationship

Sensilla were repeatedly stimulated with increasing concentrations of each compound to determine the concentration-response relationship. The inter-stimulus interval was at least 10 min to minimise effects of adaptation of the neurons. Mean spikes frequencies in the first 200 ms after stimulus onset (+SEM) were plotted against concentrations on a logarithmic scale. The concentration evoking 50% of the maximum excitation (RC_{50}) was calculated from fitted regression models.

3.4.1.2.2 Cross-Adaptation between a Secondary Compound and Securinine

3.4.1.2.2.1 Experimental Design

Previous studies (Hardiess 2002) revealed that the stimulation of neuron II/3 with high concentrations of securinine ($\geq 10^{-3}$ M) leads to a long-lasting reduction of the excitation levels to subsequent stimuli.

In order to determine whether a secondary compound that positively stimulates a neuron in the TP II sensillum, stimulates the same neuron as securinine (neuron II/3), sequential stimulations with a concentration of 10^{-3} M were done (see scheme below). The inter-stimulus interval was 5 min.

Sequence	Stimulus		
	# 1	# 2	# 3
(I)	**Sec**	**X**	**Sec**
(II)	**Sec**		**Sec**
(III)	**X**	**X**	**Sec**
(IV)	**X**	**Sec**	**X**

Sec = securinine, X = secondary compound

3.4.1.2.2.2 Data Analysis

Comparisons of mean spikes frequencies in the first 200 ms after stimulus onset for securinine stimuli were done using linear mixed-effects models with one-way ANOVA for independent samples and paired *t*-tests for dependent samples as *post hoc* tests. The same procedure was used for comparisons between the different secondary plant compound stimuli.

For comparisons within each sequence, linear mixed-effects models with the fixed factor "stimulus" (3 levels) as predictor variable and "sensillum-ID" as random factor were used. *Post-hoc* testing was done using the same models with reduced data sets for pairwise comparisons ("stimulus": 2 levels). Significant effects were obtained using likelihood-ratio tests.

3.5 Statistics

All statistical tests used are given in the according section "Data Analysis" of each chapter for each experiment. All statistical analysis was done using R version 2.10.1 (R Development Core Team 2009). Mixed-effects models were fitted using the lme4-package with the Laplace approximation of the likelihood function (Bates 2005, Bates & Maechler 2009). *P*-values were calculated by likelihood-ratio tests based on changes in deviance (using maximum likelihood estimates) when each term was dropped from the full (main effects) model (Faraway 2006). Residuals were checked for normality using the Shapiro-Wilk test and visually checking Q-Q-plots. Homogeneity of variances was ensured using the Levene test and visually checking predicted value-residual-plots. The sign-test for analysing the filter paper choice test was done using SPSS™ version 13.0. All data are presented as mean+SEM unless otherwise given.

4 Results

4.1 Food Choice and Food Consumption

4.1.1 Threshold for Avoidance in a Single Choice Situation

In the filter paper choice test, all tested alkaloids and non-alkaloids had a negative impact on food choice in *S. lamanianus* workers at a compound specific threshold (CST; Table 4). Above this threshold, workers significantly preferred non-impregnated over impregnated filter papers thus showing a clear avoidance of impregnated filter papers (for further detail see Appendix).

The difference in the CST between the most effective alkaloid tryptanthrin and the less effective caffeine was a factor of 1,000 (Table 4). The difference in the CST between the most effective non-alkaloid juglone and the less effective sinigrin was a factor of about 3,000. Furthermore, juglone had a CST of one magnitude lower than tryptanthrin (Table 4).

Table 4: Compound specific threshold (CST) of different alkaloids (A) and non-alkaloids (NA) eliciting an avoidance reaction by *S. lamanianus* workers in the filter paper choice test. Securinine as reference compound is given in bold letters. The CST is given as amount per filter paper area, and amount per filter paper mass (20 mg) in [ppm] respectively.

Class	Compound	CST [µg/2.5 cm^2]	CST [ppm]
A	Tryptanthrin	0.3	15
A	Nicotine	1	50
A	Strychnine	3	150
A	**Securinine**	10	500
A	Colchicine	30	1500
A	Papaverine	30	1500
A	Berberine	30	1500
A	Caffeine	300	15000
NA	Juglone	0.03	1.5
NA	Azadirachtin	0.3	15
NA	Chrysin	1	50
NA	Nootkatone	1	50
NA	Nomilin	60	3000
NA	Sinigrin	100	5000

In contrast to all other tests, in the test with juglone termites needed longer to establish a constant foraging traffic into the test arena and at concentrations higher than 0.03 µg/2.5 cm^2 the test did not work anymore because termite workers refused to enter the test arena.

In the following, results of behavioural tests will be always given in the order according to Table 4.

4.1.2 Food Consumption

4.1.2.1 Quantitative Food Consumption on Filter Paper in a No-Choice Situation

In the filter paper no-choice test, all tested alkaloids (3CST) reduced filter paper consumption in *S. lamanianus* workers (Figure 13). Impregnated filter papers were significantly less fed on compared to non-impregnated filter papers ("Blank").

Apart from azadirachtin all non-alkaloids also reduced food consumption on impregnated filter papers (Figure 13).

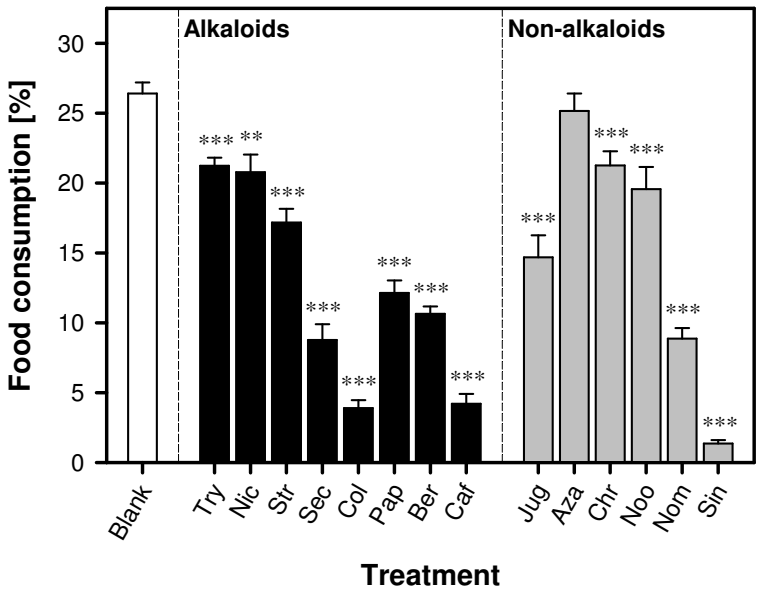

Figure 13: Food consumption (mean+SEM) after 7 days duration of the filter paper no-choice test. Statistics: GLM: "treatment": $\chi^2 = 817.86$; $DF = 14$; $P < 0.001$; *post-hoc:* Mann-Whitney U-test: ** $P \leq 0.01$; *** $P \leq 0.001$ *vs.* "Blank".

Furthermore, in this test mortality of termites was determined to exclude a feeding reduction effect due to toxicity of a certain compound and thus an increased mortality.

After starvation of 7 days, mortality was 24.4%. In comparison, mortality was significantly lower on the non-impregnated filter paper (Figure 14: "Blank"; 5.3%). Mortality of termites was lower compared to the starvation approach on filter papers impregnated with the alkaloids tryptanthrin (1.9%), nicotine (7.5%), strychnine (4.4%), papaverine (9.4%), and berberine (10.4%). Mortality on securinine impregnated filter papers was not different (22.5%). However, mortality was significantly increased on colchicine (95.6%) and

caffeine (81.2%) impregnated filter papers (Figure 14). Additionally, mortality was not different compared to blank filter papers in treatments with tryptanthrin, nicotine, strychnine, papaverine, and berberine. But mortality was increased for treatments with securinine, colchicine, and caffeine. Colchicine and caffeine treatments were the only ones where mortality was increased compared to both controls (starvation and blank). Therefore, these two alkaloids had toxic effects on termite workers.

For non-alkaloid treatments, mortality of termites was also significantly lower compared to the starvation approach on filter papers impregnated with juglone (13.1%), azadirachtin (11.2%; trend), chrysin (6.9%), and nootkatone (13.1%). Mortality on nomilin impregnated filter papers (25%) was not different. But on sinigrin impregnated filter papers mortality (50.6%) was significantly increased (Figure 14). Compared to blank filter papers mortality was not different in treatments with azadirachtin and chrysin. But mortality was increased in juglone, nootkatone, nomilin, and sinigrin treatments. Since only sinigrin showed a higher mortality compared to both controls (starvation and blank) it also had a toxic effect on termite workers as shown by the alkaloids colchicine and caffeine.

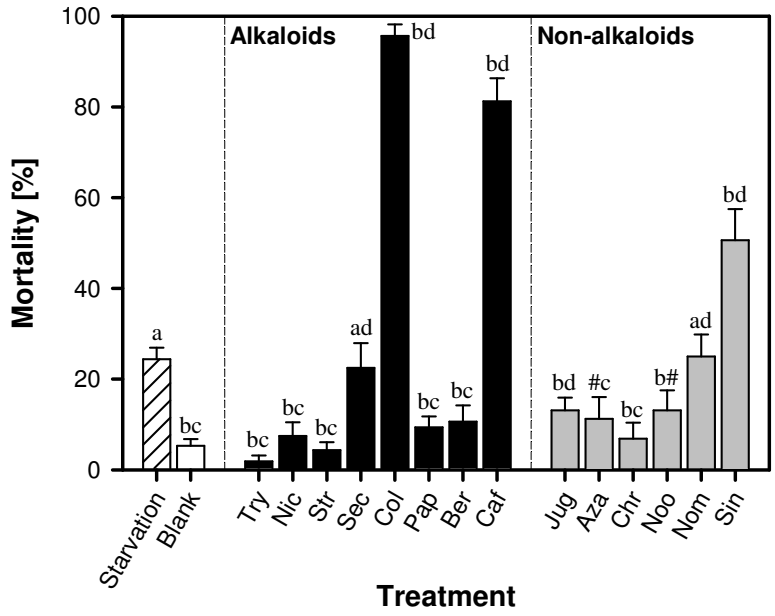

Figure 14: Mortality of termite workers (mean+SEM) after 7 days duration of the filter paper no-choice test. Statistics: GLM: "treatment": $\chi^2 = 3215.7$; $DF = 15$; $P < 0.001$; *post-hoc:* Mann-Whitney U-test: different letters indicate significant differences ($P < 0.05$) *vs.* "Starvation" (a,b); and *vs.* "Blank" (c,d); # $P < 0.1$.

Since the alkaloids colchicine and caffeine and the non-alkaloid sinigrin showed toxic effects in *S. lamanianus* workers, they were excluded from further behavioural investigations.

4.1.2.2 Quantitative Food Consumption on Wood in a Single Choice Situation

The alkaloids tryptanthrin, nicotine, strychnine, securinine, and papaverine reduced food consumption in *S. lamanianus* workers. Consumption was significantly lower on impregnated wooden cubes compared to non-impregnated (= blank) cubes (Figure 15, Table 5).

Out of the tested non-alkaloids, only chrysin caused a clear reduction in food consumption. Juglone and azadirachtin had no effect on food consumption in *S. lamaninaus* workers. There was no difference in food consumption on non-impregnated and impregnated wooden cubes (Figure 15, Table 5). Nootkatone had an inconclusive effect on food consumption in *S. lamanianus* workers (Figure 15, Table 5). In colony 1, nootkatone caused no feeding reduction (t = 0.067, DF = 14, P = 0.948). However, in colony 3, workers tended to feed less on impregnated wooden cubes compared to non-impregnated ones (t = 1.929, DF = 14, P = 0.074).

Furthermore, secondary compounds causing feeding reduction could be divided into several groups due to the effect size: a first group (strychnine) with a relatively low feeding reduction (≤33%), a second group (tryptanthrin, nicotine, chrysin) with a moderate feeding reduction (≤66%) and a third group (securinine, papaverine) with a high feeding reduction (>66%), (Table 5).

As already mentioned in chapter 4.1.1, in the test with juglone termite workers needed about two times longer to establish an appropriate foraging traffic into the test arena. The first signs of feeding on blank cubes were observed after 72-96 h test duration whilst for all other tested compounds this occurred already after 24-48 h.

Table 5: Linear mixed effects models with "colony-ID" as random factor for the food consumption on non-impregnated and impregnated wooden cubes (A = alkaloids, NA = non-alkaloids) in the wooden cube single choice test. For each secondary compound the χ^2- and *P*-values for the predictor variable "treatment", the feeding reduction index (FR) *vs.* blank wooden cubes (FR = $(1-FC_T/FC_B)\cdot 100\%$) and the effect size are given.

Class	Compound	"treatment"		FR [%]	Effect size[(a)]
		χ^2	*P*		
A	Tryptanthrin	17.48	**<0.001**	**35**	**++**
A	Nicotine	17.13	**<0.001**	**41**	**++**
A	Strychnine	5.18	**<0.05**	**25**	**+**
A	Securinine	32.77	**<0.001**	**76**	**+++**
A	Papaverine	46.29	**<0.001**	**72**	**+++**
NA	Juglone	0.21	0.65	-5	0
NA	Azadirachtin	0.01	0.92	1	0
NA	Chrysin	32.02	**<0.001**	**56**	**++**
NA	Nootkatone	2.05	*0.15*	22	(0)

[(a)] "0" no effect; "+" FR ≤ 33%; "++" FR ≤ 66%; "+++" FR > 66%

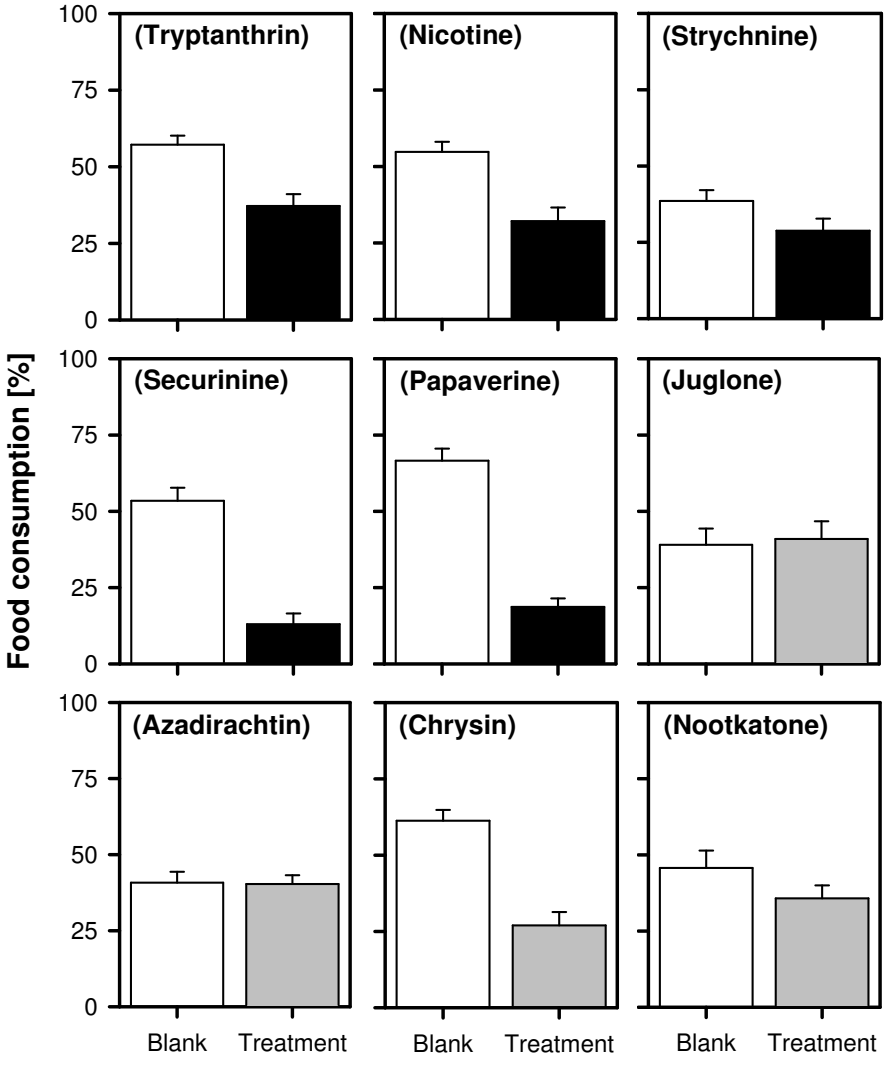

Figure 15: Food consumption (mean+SEM) after 7 days in the wooden cube choice test I on non-impregnated ("Blank" □ ; *N* = 16) and impregnated ("Treatment"; *N* = 16) wooden cubes (alkaloid ■ , non-alkaloid ▣). For statistics: see Table 5.

4.1.2.3 Quantitative Food Consumption on Wood in a Multiple Choice Situation

The alkaloids tryptanthrin, nicotine, strychnine, securinine, and papaverine caused significant lower food consumption on impregnated wooden cubes compared to non-impregnated ones (Figure 16 A). Furthermore the threshold for the occurrence of this feeding reduction was different for each alkaloid: strychnine, securinine (3CST) < nicotine, papaverine (10CST) < tryptanthrin (100CST).

The non-alkaloid chrysin also caused a significant feeding reduction on impregnated wooden cubes at concentrations equal or higher than 10CST (Figure 16 B). However, the impregnation with different concentrations of nootkatone did not lead to a feeding reduction. Feeding was even significantly increased on wooden cubes impregnated with 3CST and 30CST nootkatone (Figure 16 B).

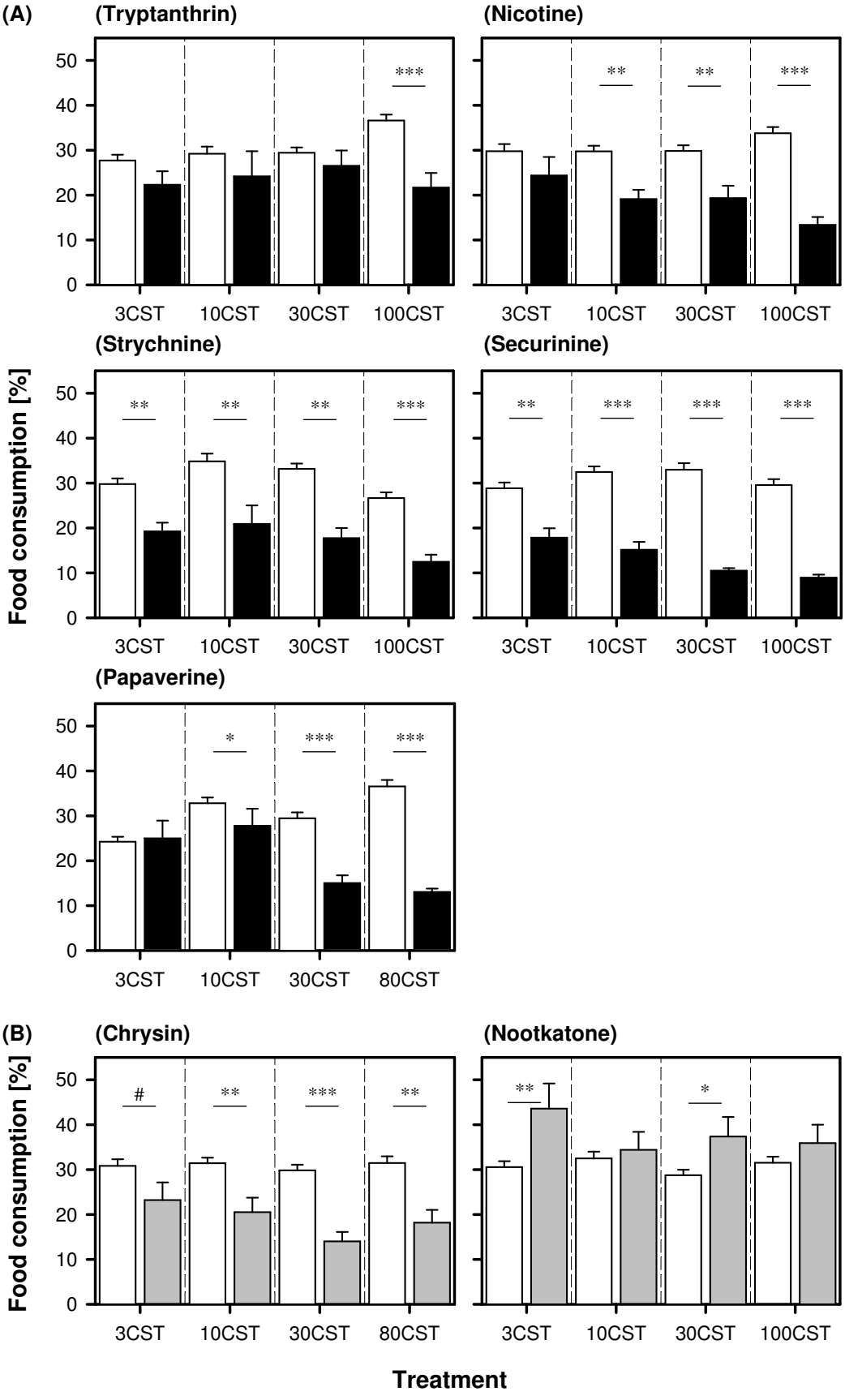

Figure 16: Food consumption (mean+SEM) on non-impregnated (□) and impregnated ((**A**) ■ alkaloid, (**B**) ▣ non-alkaloid) wooden cubes for the four different concentrations (given as n-fold CST) used in the wooden cube multiple choice test. Statistics: LMM with "arena-ID" and "replicate-ID" as random factors: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, # $P \leq 0.1$ *vs.* non-impregnated cubes.

Since food consumption on non-impregnated wooden cubes was highly variable, a feeding reduction index (FR; see 3.3.4) was calculated to test the influence of increasing concentrations on food consumption on non-impregnated and impregnated wooden cubes.

FR was independent of the concentration used for impregnation with the alkaloid tryptanthrin, and the non-alkaloids chrysin and nootkatone (Figure 17).

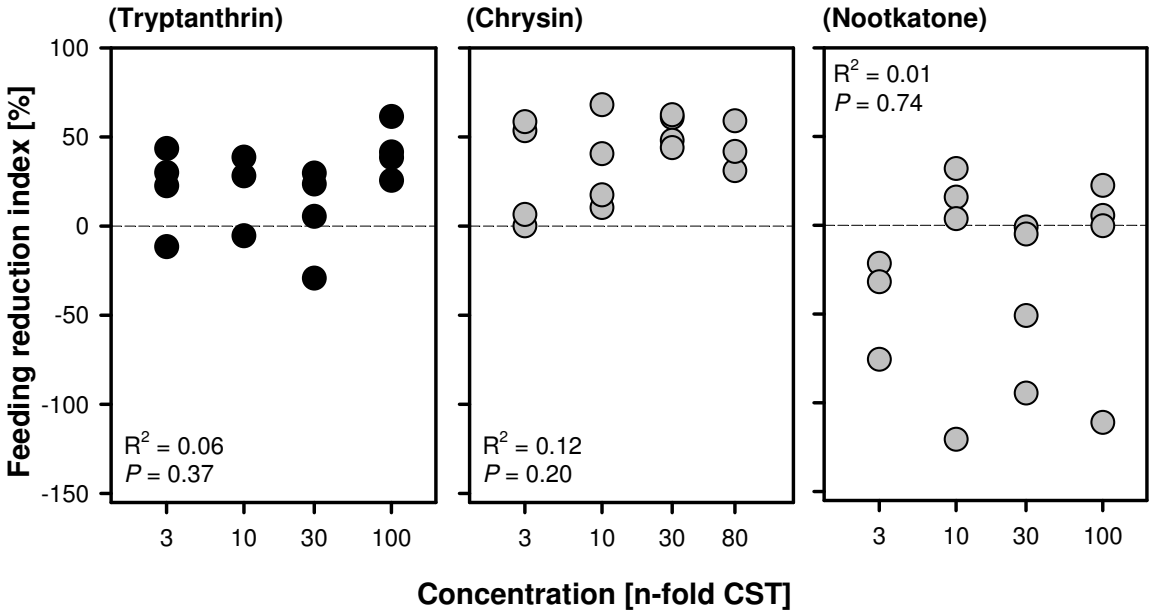

Figure 17: Relationship between concentration and feeding reduction index on impregnated wooden cubes in each individual test arena for the four concentrations used for impregnation with the alkaloid tryptanthrin (●), and non-alkaloids chrysin and nootkatone (⊙). Statistics: linear Pearson regression for *log*-transformed concentrations.

In contrast, FR positively correlated with the concentration used for impregnation with the alkaloids nicotine, strychnine, securinine, and papaverine. The higher the concentration was the higher was also the feeding reduction effect on impregnated wooden cubes compared to non-impregnated cubes (Figure 18). Thereby, the variable "concentration" explained 35-68% of the variance in FR (see R^2 in Figure 18).

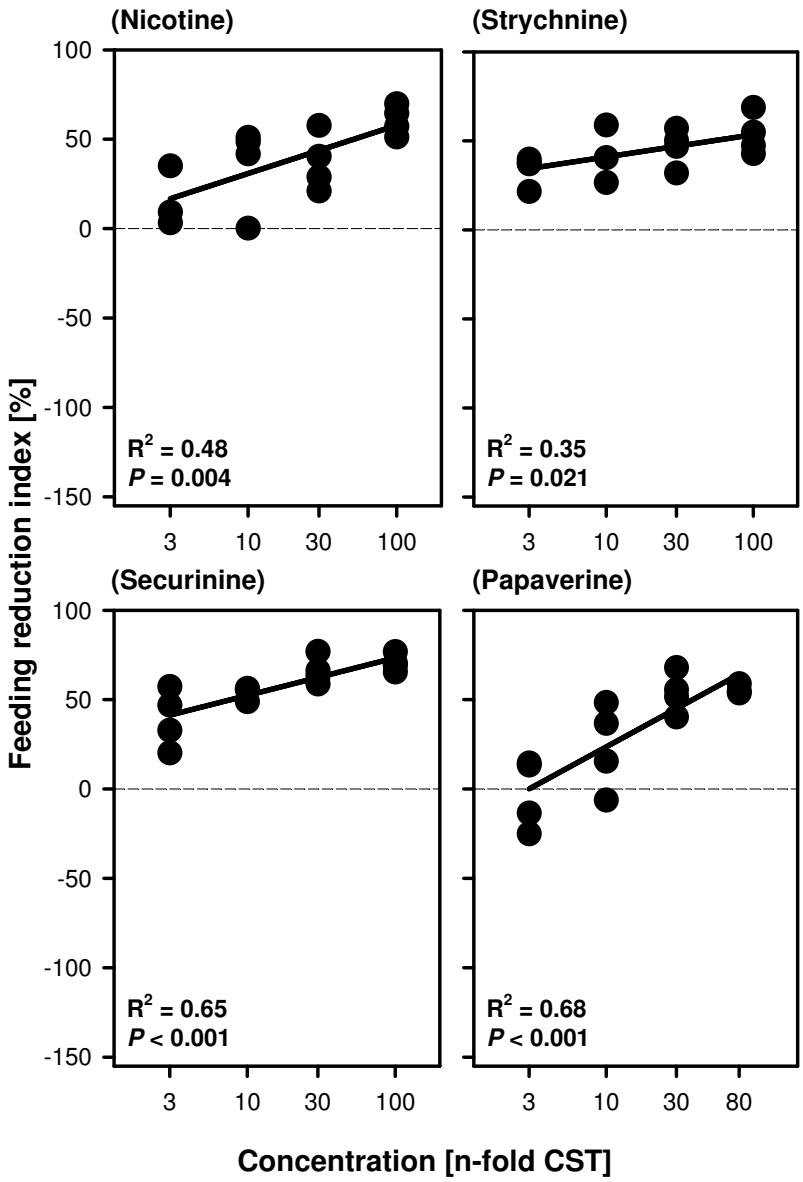

Figure 18: Relationship between concentration and feeding reduction index in each individual test arena for the four concentrations used for impregnation with the alkaloids nicotine, strychnine, securinine, and papaverine. Statistics: linear Pearson regression for *log*-transformed concentrations.

4.2 Neurophysiological Investigations

4.2.1 The TP II Sensillum and Alkaloids

4.2.1.1 Concentration-Reponse Relationships

4.2.1.1.1 Stimulation of the TP II Sensillum with Securinine

Previous investigations (Hardiess 2002) showed that securinega-alkaloids stimulate an excitation in one neuron of the TP II sensillum (neuron II/3). Therefore, in the present study securinine was used as a reference for all other tested compounds.

Repeated stimulations with increasing concentrations of securinine revealed a typical sigmoidal concentration-response relationship (Figure 19). The threshold concentration was about $3 \cdot 10^{-6}$ – 10^{-5} M. and the maximum excitation level of about 16 spikes/200 ms was reached at a concentration of 10^{-3} M.

The stimulation of "naive" (not previously stimulated) TP II sensilla (open triangles in Figure 19) with high concentrations of securinine showed higher maximum excitation levels with up to 19 spikes/200 ms compared to the concentration-response relationship obtained from repeated stimulations on the same sensilla.

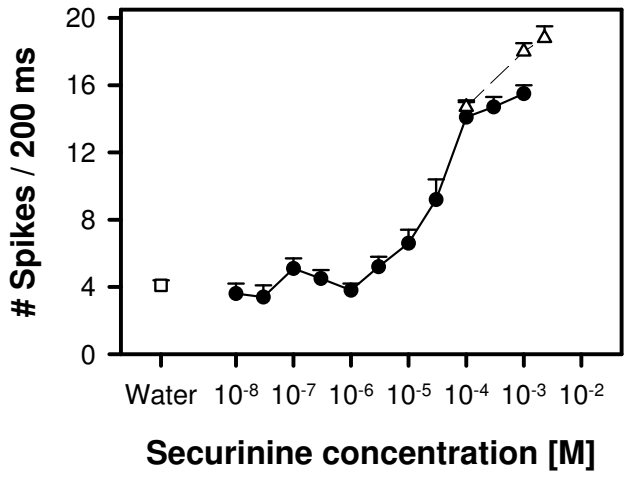

Figure 19: Concentration-response relationship for repeated stimulations of the TP II sensillum (●; N = 14-21) with increasing concentrations of securinine. Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. Excitation levels of naive neurons (10^{-4} M: N = 25; 10^{-3} M: N = 20; $2.3 \cdot 10^{-3}$ M: N = 8) are indicated by △.

4.2.1.1.2 Screening

23 additional alkaloids were screened for their effect to evoke excitations of taste neurons in TP II sensilla. Arecoline, berberine, brucine, coniine, hyocyamine, lobeline, lupanine, nicotine, pilocarpine, sparteine, and strychnine caused excitations of a taste neuron at different threshold concentrations. Spike shape and time characteristics were not distinguishable from excitations after stimulations with securinine. In contrast, anabasine, ajmalicine, boldine, caffeine, colchicine, emetine, harmaline, noscapine, papaverine, quinine, solasodine, and tryptanthrin did not stimulate any taste neuron (Table 6).

Table 6: List of the 24 screened alkaloids. It is given whether they act as feeding deterrent (FD; nt = not tested), whether they stimulate an excitation in one neuron of the TP II sensillum ("+" = excitation, "−" = no excitation), and the threshold concentration.

Alkaloid	Feeding Deterrence	Excitation	Threshold Range [M]
Anabasine (Ana)	nt	−	
Ajmalicine (Ajm)	nt	−	
Arecoline (Are)	nt	+	$10^{-4} – 3 \cdot 10^{-4}$
Berberine (Ber)	**FD**	+	$10^{-5} – 3 \cdot 10^{-5}$
Boldine (Bol)	nt	−	
Brucine (Bru)	nt	+	$3 \cdot 10^{-6} – 10^{-5}$
Caffeine (Caf)	**FD**	−	
Colchicine (Col)	**FD**	−	
Coniine (Con)	nt	+	$3 \cdot 10^{-6} – 10^{-5}$
Emetine (Eme)	nt	+	
Harmaline (Har)	nt	+	
Hyoscyamine (Hyo)	nt	−	$3 \cdot 10^{-6} – 10^{-5}$
Lobeline (Lob)	nt	+	$10^{-6} – 3 \cdot 10^{-6}$
Lupanine (Lup)	nt	−	$10^{-6} – 3 * 10^{-6}$
Nicotine (Nic)	**FD**	+	$3 \cdot 10^{-5} – 10^{-4}$
Noscapine (Nos)	nt	+	$3 \cdot 10^{-6} – 10^{-5}$
Papaverine (Pap)	**FD**	−	
Pilocarpine (Pil)	nt	+	$10^{-4} – 3 \cdot 10^{-4}$
Quinine (Qui)	nt	−	
Securinine (Sec)	**FD**	+	$3 \cdot 10^{-6} – 10^{-5}$
Solasodine (Sol)	nt	−	
Sparteine (Spa)	nt	+	$3 \cdot 10^{6} – 10^{-5}$
Strychnine (Str)	**FD**	+	$3 \cdot 10^{6} – 10^{-5}$
Tryptanthrin (Try)	**FD**	−	

4.2.1.1.3 Lower Responsiveness of the TP II Sensillum towards Arecoline, Berberine, Nicotine, and Pilocarpine Compared to Securinine

The measurement of the concentration-response relationships for arecoline, berberine, nicotine, and pilocarpine revealed a lower responsiveness towards these alkaloids. All concentration-response curves showed the typical sigmoidal progression. However, the characteristics of the concentration-response curves differed between these four alkaloids (Figure 20 I).

Arecoline and pilocarpine revealed a lower sensitivity caused by a shift of the threshold to higher concentration whilst the slope was similar to securinine (Figure 20 A II). The RC_{50} (concentration for 50% of maximum response calculated from the regression models, see Figure 20 II) for arecoline was $7.5 \cdot 10^{-4}$ M and for pilocarpine $4.8 \cdot 10^{-4}$ M compared to $3.2 \cdot 10^{-5}$ M for securinine. Therefore, the RC_{50} was around 1-1.5 magnitudes higher compared to securinine. The maximum excitation was reached for arecoline at 10^{-2} M and for pilocarpine at $3 \cdot 10^{-3}$ M. These were concentrations being about 0.5-1 magnitudes higher compared to securinine.

In contrast, berberine revealed a similar threshold as securinine but with a weaker slope (Figure 20 B II). The RC_{50} for berberine was $1.4 \cdot 10^{-4}$ M. This was around 0.5 magnitudes higher than for securinine. The maximum excitation was reached at 10^{-2} M, thus one magnitude higher compared to securinine.

Nicotine revealed also a lower sensitivity caused by a shift of the threshold to higher concentrations. The RC_{50} for nicotine was $1.3 \cdot 10^{-4}$ M. This was 0.5 magnitudes higher compared to securinine. However, the slope was much steeper compared to securinine (Figure 20 C II). Therefore, the maximum excitation was reached for both alkaloids at 10^{-3} M.

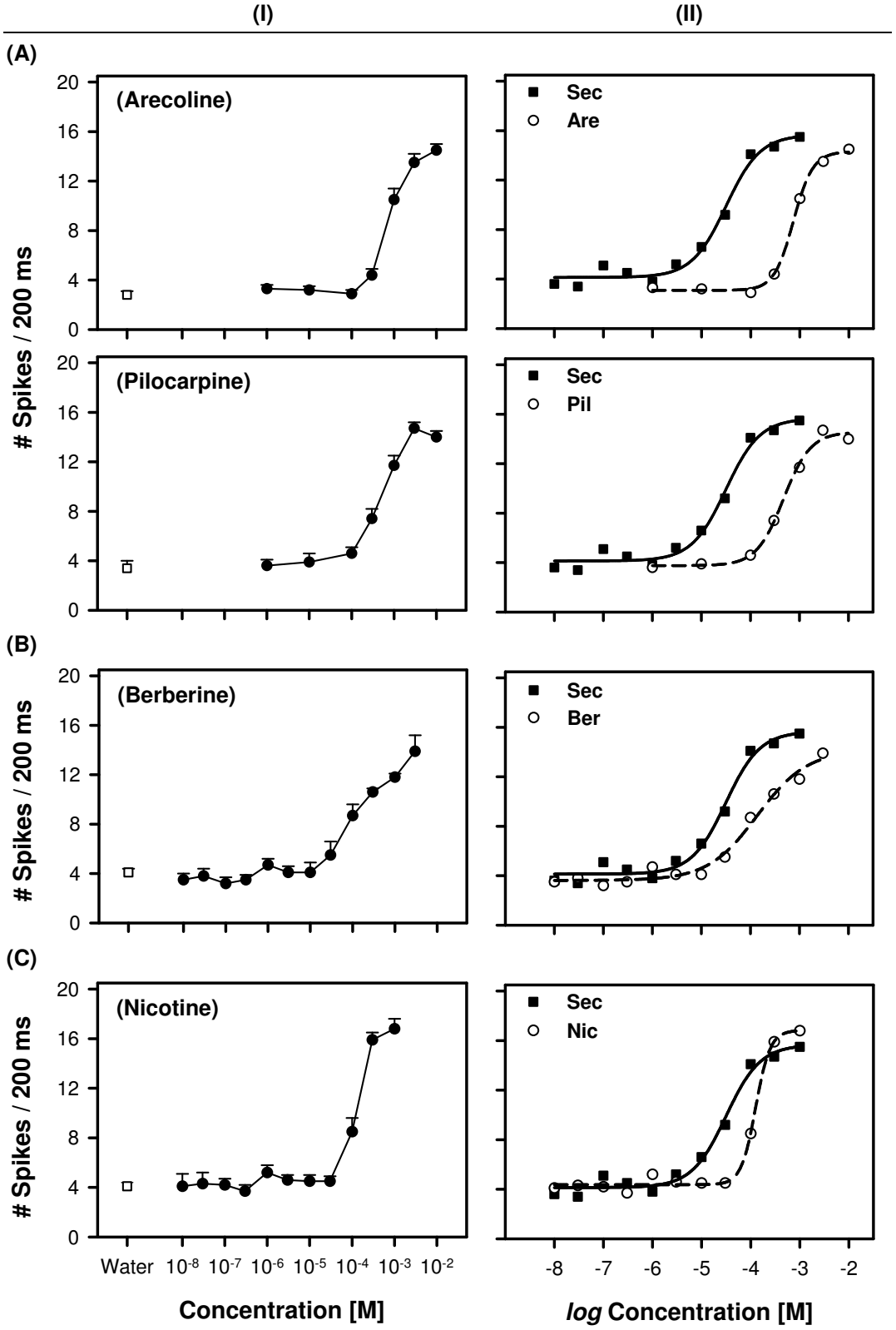

Figure 20: (I) Concentration-response relationships for repeated stimulations of the TP II sensillum (●) with increasing concentrations of (A) arecoline (N = 11-13) and pilocarpine (N = 7), (B) berberine (N = 8-14), and (C) nicotine (N = 8-14). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. **(II)** Fitted concentration-response curves for stimulation with (A) arecoline and pilocarpine, (B) berberine, and (C) nicotine (dashed line) and securinine (solid line).

4.2.1.1.4 Higher Responsiveness of the TP II Sensillum towards Brucine, Coniine, Hyoscyamine, Lobeline, Lupanine, Sparteine, and Strychnine Compared to Securinine

The measurement of the concentration-response relationships for brucine, coniine, hyoscyamine, lobeline, lupanine, sparteine, and strychnine revealed a higher responsiveness towards these seven alkaloids compared to securinine. Since the threshold was similar to securinine (see Table 6) the higher responsiveness was caused by a steeper slope of the concentration- response curves (Figure 21 II).

Brucine and sparteine showed a "switch-like" concentration-response curve. Within an increase of the concentration of 0.5 magnitudes, the excitation level "switched" from the minimum to the maximum level (Figure 21 A II). The RC_{50} of brucine ($3.9 \cdot 10^{-6}$ M) and sparteine ($3.8 \cdot 10^{-6}$ M) was about one magnitude lower compared to securinine ($3.2 \cdot 10^{-5}$ M). The maximum excitation for brucine and sparteine was reached at 10^{-5} and $3 \cdot 10^{-5}$ M. These were concentrations being two magnitudes lower than for securinine (10^{-3} M)

Lobeline and lupanine showed both a strong increase in the spike frequency with increasing concentrations, however the excitation level for the highest concentration dropped again about 4-6 spikes/200 ms compared to the preceding concentration (Figure 21 B II). The RC_{50} of lobeline ($3.3 \cdot 10^{-6}$ M) and lupanine ($5.7 \cdot 10^{-6}$ M) was about one magnitude lower compared to securinine. The maximum excitation for lobeline and lupanine was reached at 10^{-5} and $3 \cdot 10^{-5}$ M. These were concentrations being two magnitudes lower than for securinine.

Coniine, hyoscyamine, and strychnine showed steep sigmoidal concentration-response-relationships. The RC_{50} for coniine ($3.9 \cdot 10^{-6}$ M), hyoscyamine ($9.1 \cdot 10^{-6}$ M), and strychnine ($6.6 \cdot 10^{-6}$ M), was 0.5-1 magnitudes lower compared to securinine. The maximum excitation for coniine, hyoscyamine, and strychnine was reached between $3 \cdot 10^{-5}$ and 10^{-4} M at concentrations of about 1.5 magnitudes lower than securinine (Figure 21 C II).

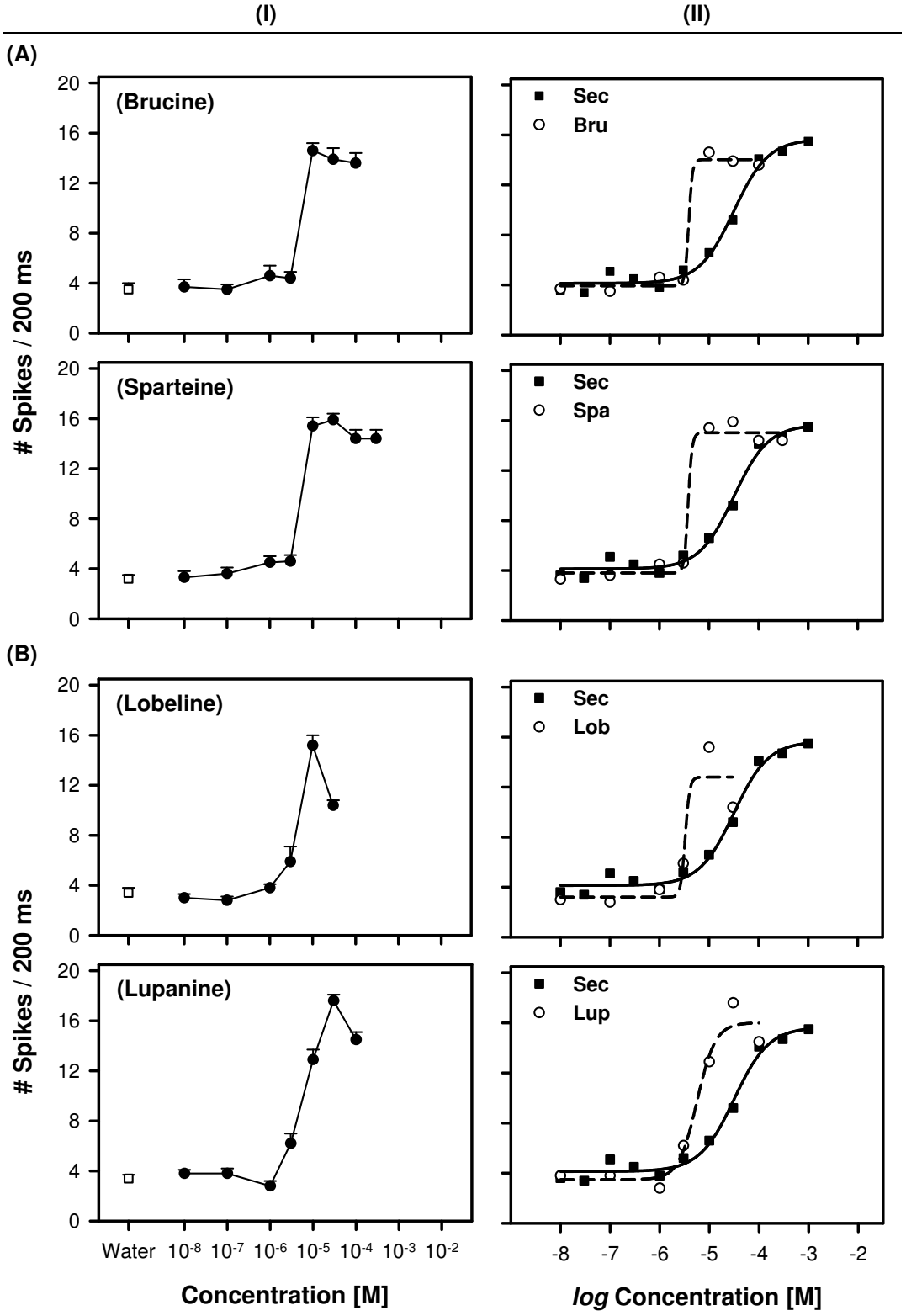

Figure 21: **(I)** Concentration-response relationships for repeated stimulations of the TP II sensillum (●) with increasing concentrations of **(A)** brucine (N = 8-10) and sparteine (N = 11-14), **(B)** lobeline (N = 11-15) and lupanine (N = 6-12), and **(C)** coniine (N = 6-7), hyoscyamine (N = 8-10), and strychnine (N = 21-27). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. **(II)** Fitted concentration-response curves for stimulation with **(A)** brucine and sparteine, **(B)** lobeline and lupanine, and **(C)** coniine, hyoscyamine, and strychnine (dashed line) and securinine (solid line).

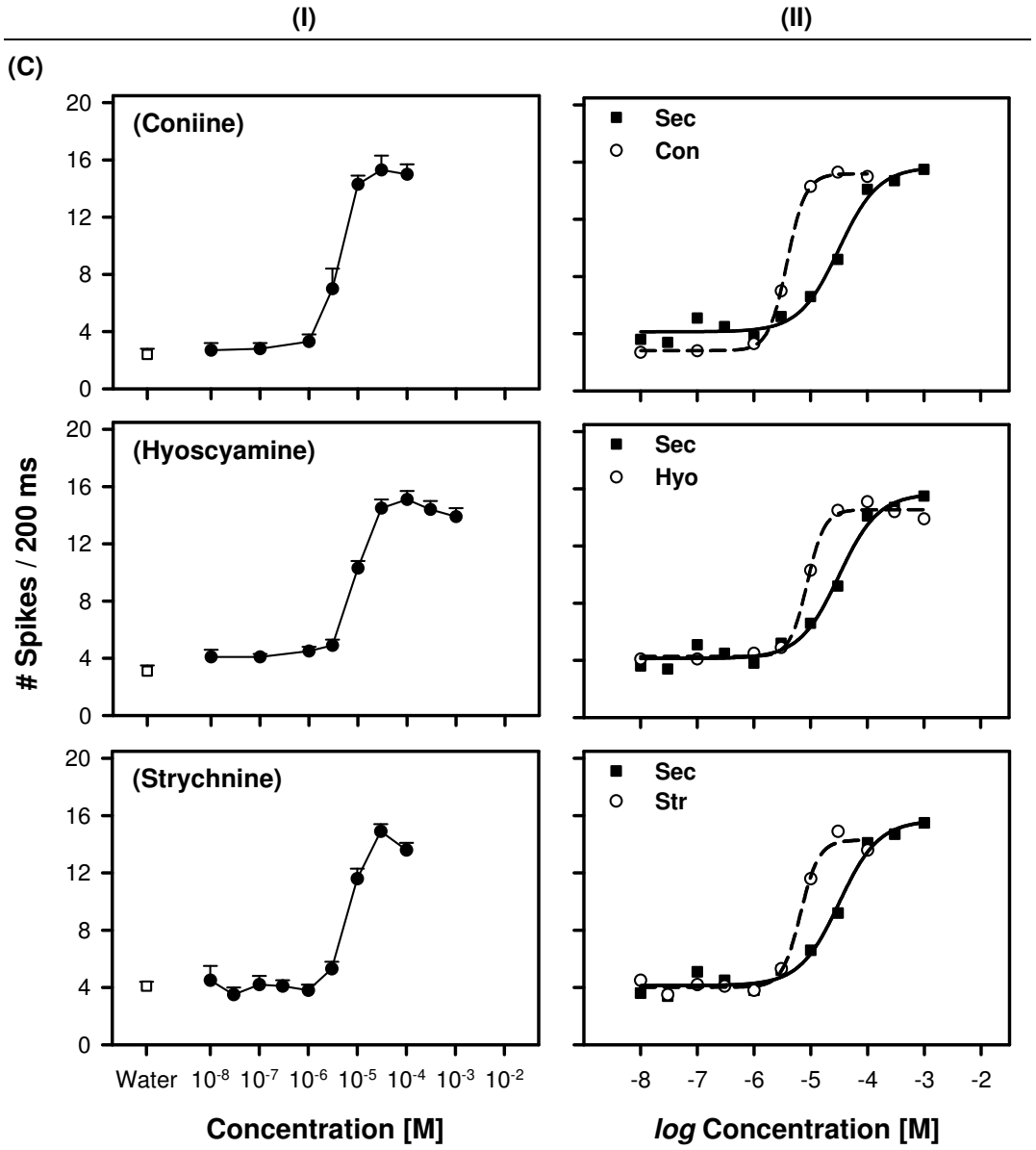

Figure 21 (continued)

4.2.1.1.5 No Responsiveness of the TP II Sensillum towards Caffeine, Colchicine, Papaverine, and Tryptanthrin

Since these four alkaloids acted as feeding deterrents, the concentration-response relationships for the stimulation of one neuron of the TP II sensillum were also determined.

For all excitations evoked by these four alkaloids maximum excitations were not distinguishable from the response to water alone. However, whilst the excitation level was constant for all caffeine and tryptanthrin concentrations (Figure 22 A), the excitation decreased with increasing colchicine and papaverine concentrations as typical for water receptors. No excitation could be recorded anymore at 10^{-3} M colchicine and $3 \cdot 10^{-3}$ M papaverine, respectively (Figure 22 B).

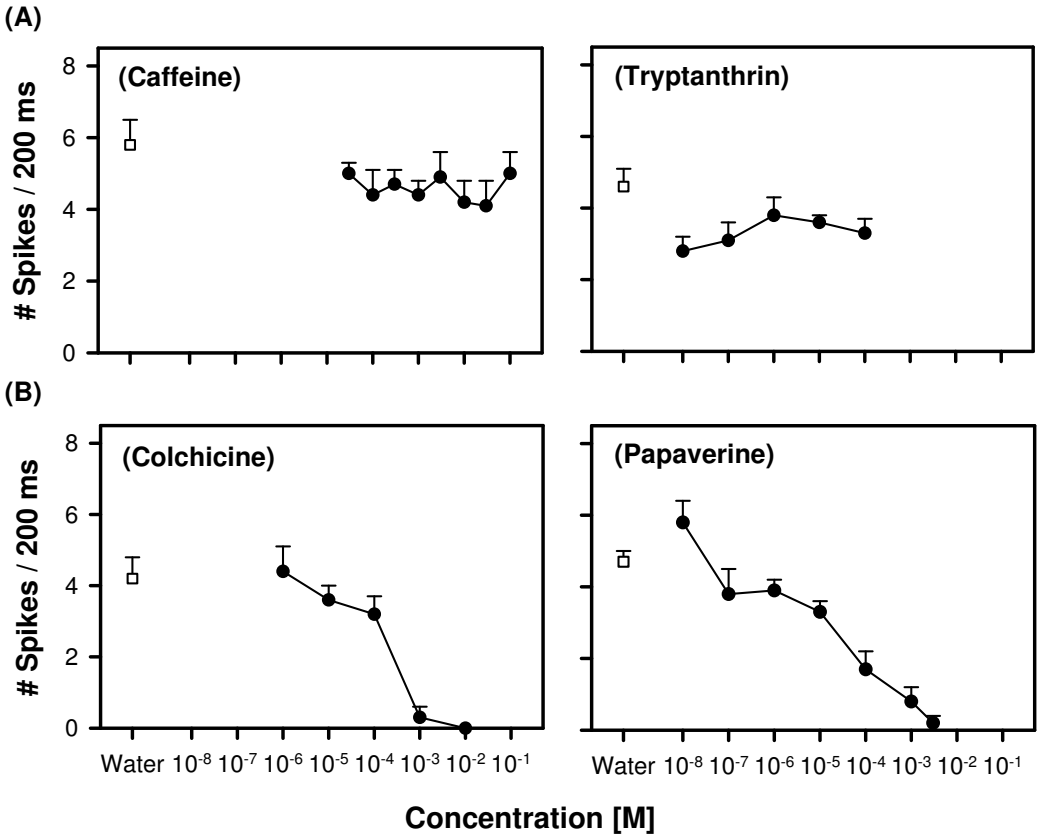

Figure 22: Concentration-response relationships for repeated stimulations of the TP II sensillum with increasing concentrations of **(A)** caffeine (*N* = 8-10), tryptanthrin (*N* = 6-8), and **(B)** colchicine (*N* = 9), papaverine (*N* = 4-9). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. The concentration-response relationship was recorded on the same sensilla.

4.2.1.2 Cross-Adaptation Tests at the TP II Sensillum

In order to test whether arecoline, berberine, brucine, coniine, hyoscyamine, lobeline, lupanine, nicotine, pilocarpine, strychnine, and sparteine stimulate the same neuron in the TP II sensillum as securinine cross-adaptation tests were carried out.

In a previous study, Hardiess (2002) showed that an initial securinine stimulus leads to a strong reduction of excitation levels to subsequent stimulations with securinine. The excitation level recovers slowly to the initial level over time (Figure 23).

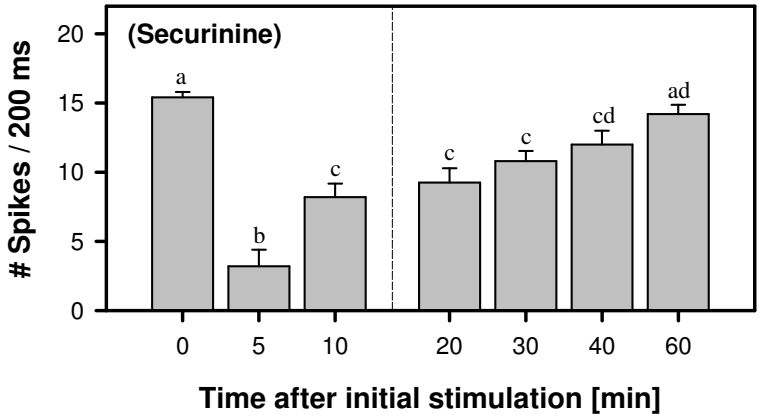

Figure 23: Response (mean+SEM) of neuron II/3 (N = 5) to repeated stimulations with 1 mM securinine. Different letters (a-d) mean significant differences (after Hardiess 2002).

If the tested alkaloids stimulate the same neuron in the TP II sensillum as securinine (neuron II/3 after Hardiess 2002) these alkaloids and securinine should inhibit each other in their response in a sequential stimulation procedure. Since Hardiess (2002) observed the strongest effects between the first three subsequent securinine stimuli (see separation by dashed line in Figure 23) sequential stimulations consisting of three subsequent stimuli with 5 min inter-stimulus intervals were used:

- **Sequence I (Sex-X-Sec)** was used to test whether an initial securinine stimulus inhibits the response to a subsequent stimulus with alkaloid X.
- **Sequence II (Sec-Sec)** with 10 min inter-stimulus interval was used as control to determine whether the response recovery in sequence I occurred in a normal fashion or whether the stimulation with alkaloid X had an additional inhibiting effect on the the response the subsequent securinine stimulus.
- **Sequence III (X-X-Sec)** was used to test whether an initial stimulation with alkaloid X inhibits the response to a subsequent stimulus with itself (self-adaptation) and

whether these two previous stimulations further have an inhibiting effect on the response to a subsequent stimulus with securinine.

- **Sequence IV (X-Sec-X)** was an inversed sequence I and was used to test whether an initial stimulation with alkaloid X inhibits the response to a subsequent securinine stimulus and whether this securinine stimulus could still inhibit the response to the subsequent stimulus with alkaloid X.

In total, three different cross-adaptation patterns could be distinguished.

4.2.1.2.1 Pattern A: "Berberine-Group"

A preceding stimulation with securinine always strongly inhibited the response to a subsequent berberine, arecoline, or pilocarpine stimulus (Figure 24 A-C: sequences I, IV). However, preceding stimulations with berberine, arecoline, or pilocarpine (Figure 24 A-C: sequences III, IV) did not inhibit the response to a subsequent securinine stimulus. The response was comparable to responses obtained after the first securinine stimulus in sequence I and II. In sequence I, the inhibition of the second response towards securinine was due to the first securinine stimulation since there was no difference compared to responses measured in sequence II (Figure 24 A-C).

In total, the strong inhibition of the response towards alkaloids of the "berberine-group" by securinine indicated that these alkaloids also stimulate neuron II/3 in the TP II sensillum.

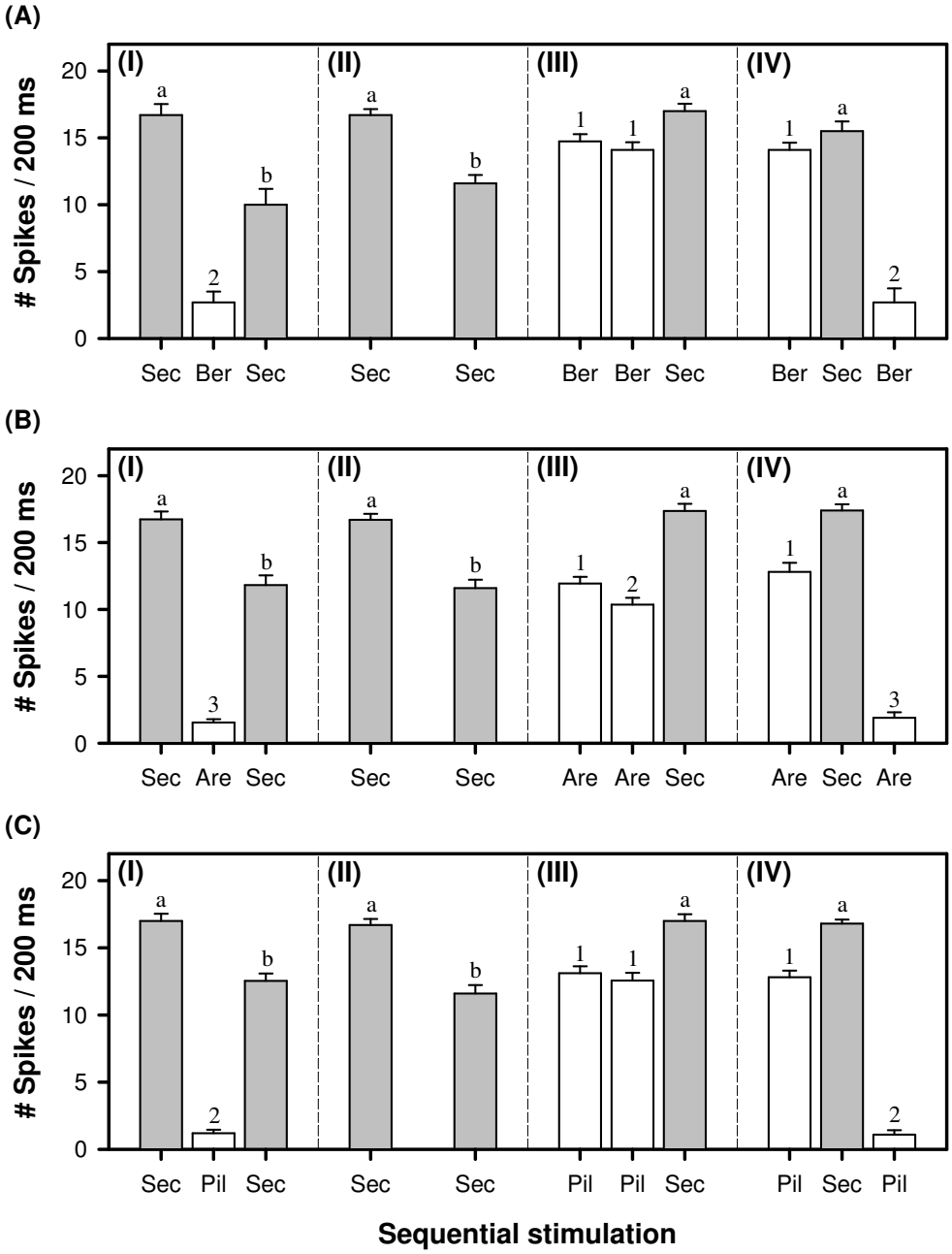

Figure 24: Response of the TP II sensillum to sequential stimulations with securinine (1mM; ▣) and alkaloids of the "berberine-group" (□): **(A)** berberine (1 mM), **(B)** arecoline (1mM), and **(C)** pilocarpine (1mM). Statistics: LMM with "sensillum-ID" as random factor: different letters (a,b) mean significant differences between responses to securinine; different numbers (1-3) mean significant differences between responses to the other alkaloids. For differences within each sequence I-IV see Table 7.

Table 7: Linear mixed-effects models with "sensillum-ID" as random factor for the effect of repeated stimuli with securinine and alkaloids of the "berberine-group" (berberine, arecoline, and pilocarpine) on the observed excitation levels within each sequence (I-IV) in cross-adaptation tests. For the predictor variable "stimulus" (3 levels) the number of measured sensilla (*N*), the χ^2-, *DF*-, *P*-value is given for each sequence. *Post hoc* testing within each sequence was done using the same models for a pairwise comparison of the different repeated stimuli. Different letters in brackets mean significant differences in the response to stimulations with the according alkaloid.

Sequence	**"stimulus"**				
	N	χ^2	***DF***	***P***	***Post hoc***
Berberine					
I	10	64.60	2	<0.001	Sec(a); Ber(b); Sec(c)
II	10	24.93	1	<0.001	
III	11	17.47	2	<0.001	Ber(a); Ber(a); Sec(b)
IV	10	96.49	2	<0.001	Ber(a); Sec(b); Ber(c)
Arecoline					
I	11	87.17	2	<0.001	Sec(a); Are(b); Sec(c)
II	10	24.93	1	<0.001	
III	14	71.82	2	<0.001	Are(a); Are(b); Sec(c)
IV	10	93.33	2	<0.001	Are(a); Sec(b); Are(c)
Pilocarpine					
I	15	123.85	2	<0.001	Sec(a); Pil(b); Sec(c)
II	10	24.93	1	<0.001	
III	9	31.68	2	<0.001	Pil(a); Pil(a); Sec(b)
IV	10	110.14	2	<0.001	Pil(a); Sec(b); Pil(c)

4.2.1.2.2 Pattern B: "Strychnine-Group"

A preceding stimulation with securinine always strongly inhibited the response to a subsequent strychnine, brucine, hyoscyamine, lobeline, or nicotine stimulus (Figure 25 A-E: sequences I, IV). Preceding stimulations with strychnine, brucine, hyoscyamine, lobeline, or nicotine (Figure 25 A-E: sequences III, IV) also inhibited the response to a subsequent securinine stimulus. However, this occurred to a lower extent compared to securinine in the *vice versa* situation. The response to a subsequent securinine stimulus was only inhibited at maximum to a level comparable with the response 10 min after a first securinine stimulus (sequence II). In sequence I, the inhibition of the second response towards securinine was due to the first securinine stimulation since there was no difference compared to the second response measured in sequence II (Figure 25 A-E).

In total, the strong inhibition of the response towards alkaloids of the "strychnine-group" by securinine in combination with the inhibition of the response towards securinine by these alkaloids strongly indicated that these alkaloids stimulate neuron II/3 in the TP II sensillum.

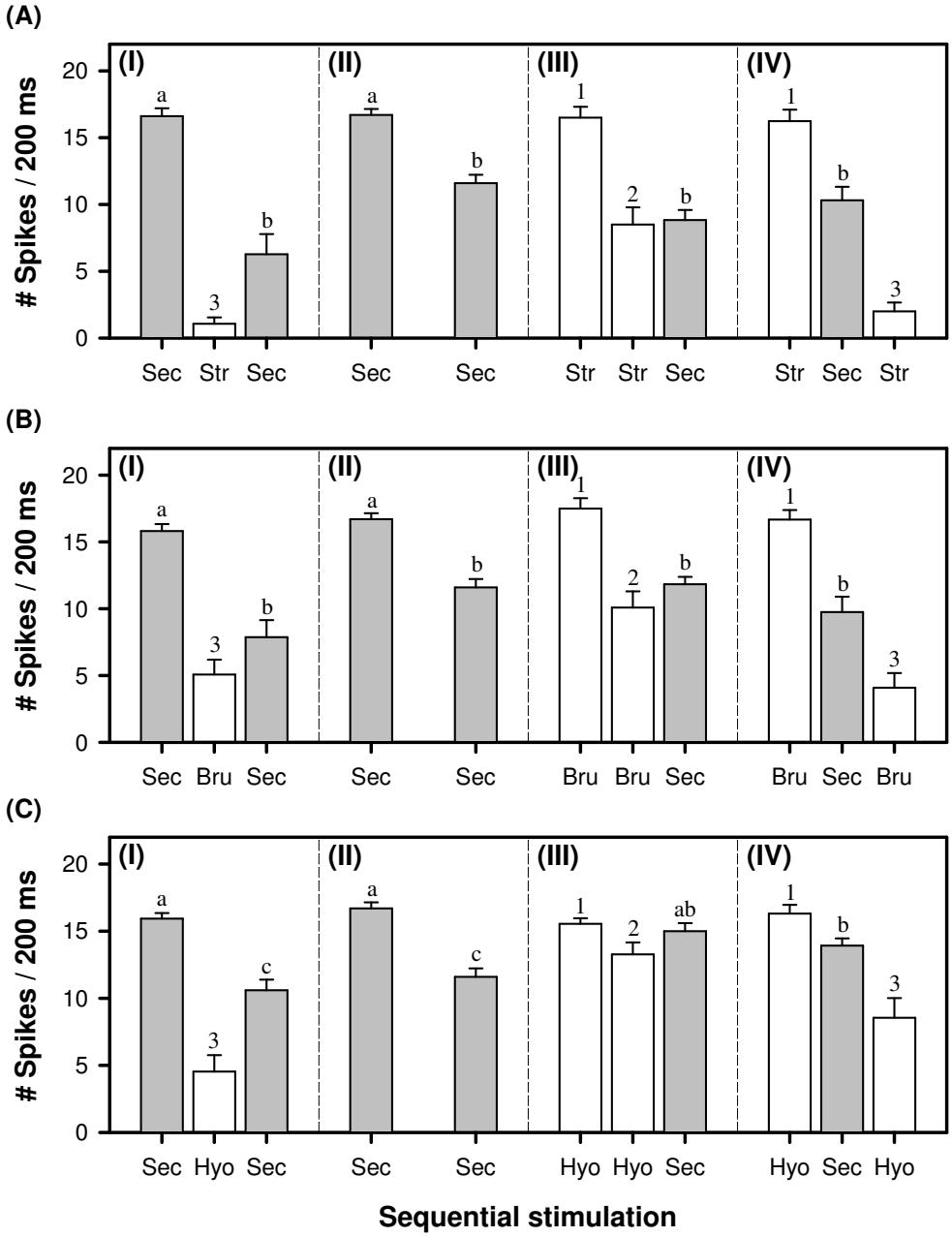

Figure 25: Response of the TP II sensillum to sequential stimulations with securinine (1mM; ▣) and alkaloids of the "strychnine-group" (□): **(A)** strychnine (1 mM), **(B)** brucine (1mM), **(C)** hyoscyamine (1mM), **(D)** lobeline, and **(E)** nicotine. Statistics: LMM with "sensillum-ID" as random factor: different letters (a-c) mean significant differences between responses to securinine; different numbers (1-3) mean significant differences between responses to the other alkaloids. For differences within each sequence I-IV see Table 8.

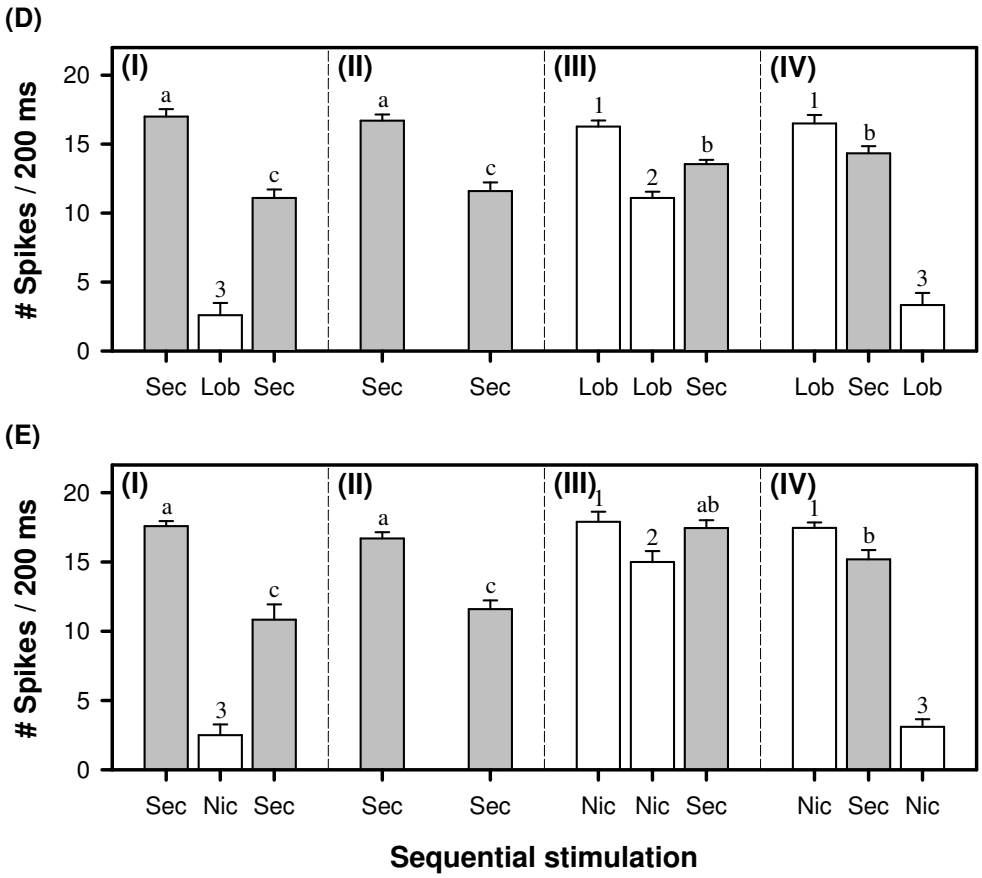

Figure 25 (continued)

Table 8: Linear mixed-effects models with "sensillum-ID" as random factor for the effect of repeated stimuli with securinine and alkaloids of the "strychnine-group" (strychnine, brucine, hyoscyamine, lobeline, and nicotine) on the observed excitation levels within each sequence (I-IV) in cross-adaptation tests. For the predictor variable "stimulus" (3 levels) the number of measured sensilla (*N*), the χ^2-, *DF*-, *P*-value is given for each sequence. *Post hoc* testing within each sequence was done using the same models for a pairwise comparison of the different repeated stimuli. Different letters in brackets mean significant differences in the response to stimulations with the according alkaloid.

Sequence	**"stimulus"**				
	N	χ^2	***DF***	***P***	***Post hoc***
Strychnine					
I	15	63.84	2	<0.001	Sec(a); Str(b); Sec(c)
II	10	24.93	1	<0.001	
III	12	30.31	2	<0.001	Str(a); Str(b); Sec(b)
IV	13	66.59	2	<0.001	Str(a); Sec(b); Str(c)
Brucine					
I	11	30.44	2	<0.001	Sec(a); Bru(b); Sec(c)
II	10	24.93	1	<0.001	
III	11	29.79	2	<0.001	Bru(a); Bru(b); Sec(c)
IV	10	18.20	2	<0.001	Bru(a); Sec(b); Bru(c)
Hyoscyamine					
I	14	55.99	2	<0.001	Sec(a); Hyo(b); Sec(c)
II	10	24.93	1	<0.001	
III	11	14.17	2	<0.001	Hyo(a); Hyo(b); Sec(a)
IV	13	27.27	2	<0.001	Hyo(a); Sec(b); Hyo(c)
Lobeline					
I	10	80.67	2	<0.001	Sec(a); Lob(b); Sec(c)
II	10	24.93	1	<0.001	
III	11	43.59	2	<0.001	Lob(a); Lob(b); Sec(c)
IV	13	74.24	2	<0.001	Lob(a); Sec(b); Lob(c)
Nicotine					
I	12	68.22	2	<0.001	Sec(a); Nic(b); Sec(c)
II	10	24.93	1	<0.001	
III	9	14.95	2	<0.001	Nic(a); Nic(b); Sec(a)
IV	10	83.75	2	<0.001	Nic(a); Sec(b); Nic(c)

4.2.1.2.3 Pattern C: “Coniine-Group”

A preceding stimulation with securinine always inhibited the response to a subsequent coniine, lupanine, or sparteine stimulius (Figure 26 A-C: sequences I, IV), however to a lesser extent compared to the "berberine-" and "strychnine-group". Preceding stimulations with coniine, lupanine, or sparteine (Figure 26 A-C: sequences III, IV) also inhibited the response to a subsequent securinine stimulus, but only to a level comparable to the response after a subsequent securinine stimulus 10 min after the initial securinine stimulus in sequence II.

In total, an initial stimulus either by securinine or an alkaloid of the "coniine-group" inhibited the response to subsequent stimuli. The second stimulus 5 min after the initial stimulus did not lead to a further inhibition of the response towards a third stimulus independent whether the second stimulus consisted of securinine or an alkaloid of the "coniine-group". This indicated that alkaloids of the "coniine-group" might stimulate a second receptor site at neuron II/3 or a second neuron in the TP II sensillum.

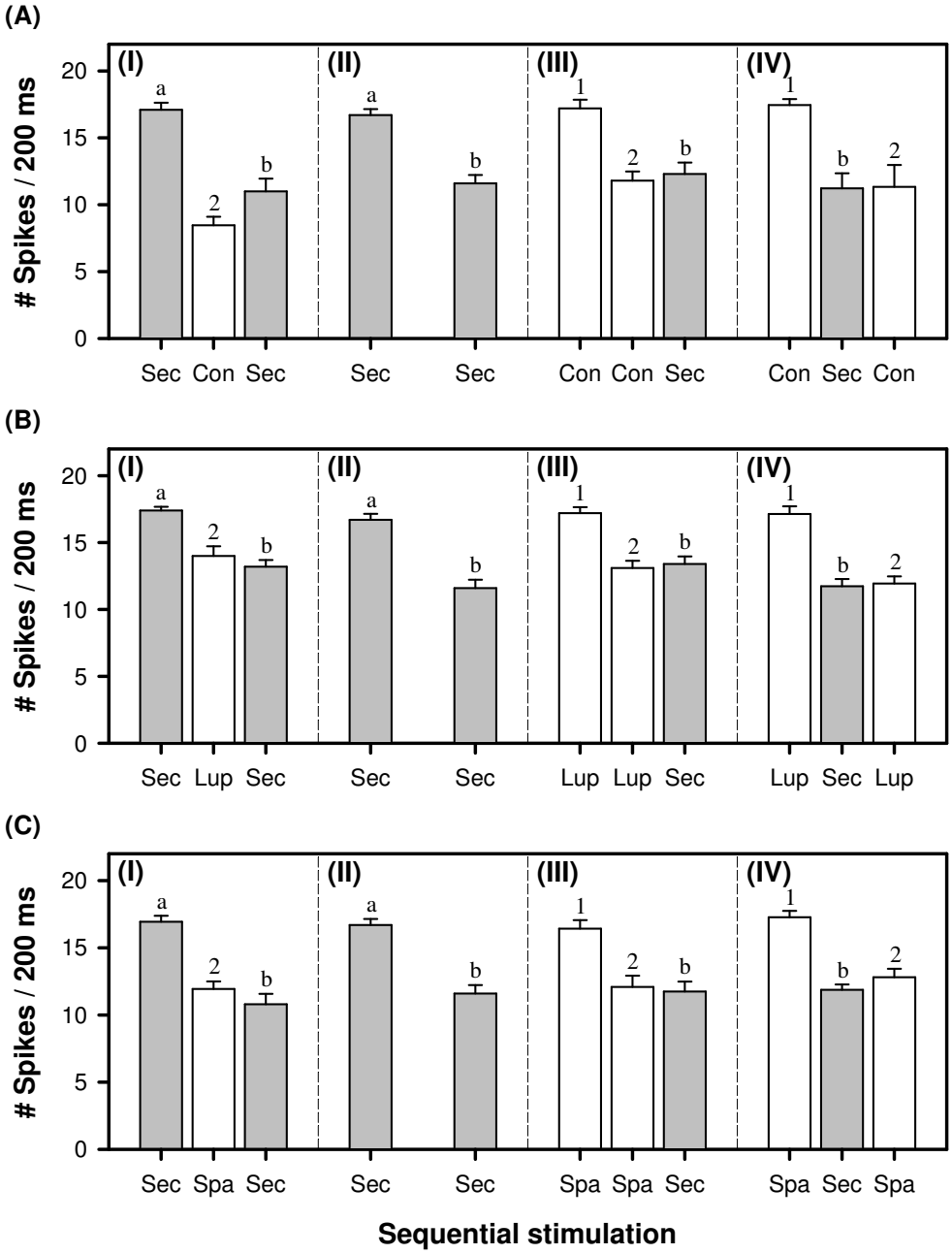

Figure 26: Response of the TP II sensillum to sequential stimulations with securinine (1mM; ▣) and alkaloids of the "coniine-group" (□): **(A)** coniine (1 mM), **(B)** lupanine (1mM), and **(C)** sparteine. Statistics: LMM with "sensillum-ID" as random factor: different letters (a, b) mean significant differences between responses to securinine: different numbers (1, 2) mean significant differences between responses to the other alkaloids. For differences within each sequence I-IV see Table 9.

Table 9: Linear mixed-effects models with "sensillum-ID" as random factor for the effect of repeated stimuli with securinine and alkaloids of the "coniine-group" (coniine, lupanine, and sparteine) on the observed excitation levels within each sequence (I-IV) in cross-adaptation tests. For the predictor variable "stimulus" (3 levels) the number of measured sensilla (*N*), the χ^2-, *DF*-, *P*-value is given for each sequence. *Post hoc* testing within each sequence was done using the same models for a pairwise comparison of the different repeated stimuli. Different letters in brackets mean significant differences in the response to stimulations with the according alkaloid.

Sequence	**"stimulus"**				
	N	χ^2	***DF***	***P***	***Post hoc***
Coniine					
I	11	47.62	2	<0.001	Sec(a); Con(b); Sec(c)
II	10	24.93	1	<0.001	
III	10	36.35	2	<0.001	Con(a); Con(b); Sec(b)
IV	9	20.21	2	<0.001	Con(a); Sec(b); Con(b)
Lupanine					
I	10	30.39	2	<0.001	Sec(a); Lup(b); Sec(b)
II	10	24.93	1	<0.001	
III	10	32.96	2	<0.001	Lup(a); Lup(b); Sec(b)
IV	15	65.21	2	<0.001	Lup(a); Sec(b); Lup(b)
Sparteine					
I	15	40.71	2	<0.001	Sec(a); Spa(b); Sec(b)
II	10	24.93	1	<0.001	
III	12	33.37	2	<0.001	Spa(a); Spa(b); Sec(b)
IV	15	67.47	2	<0.001	Spa(a); Sec(b); Spa(b)

4.2.2 TP I Sensillum and Feeding Deterrent Alkaloids

In the behavioural tests eight alkaloids were shown to deter feeding in *S. lamanianus* workers (see chapter 4.1). Four of these alkaloids (securinine, berberine, nicotine, strychnine) were shown to excite a neuron of the TP II sensillum, whilst the other four (caffeine, colchicine, papverine, tryptanthrin) did not (see chapter 4.2.1). Therefore, all eight alkaloids were investigated regarding the excitation of a neuron in the TP I sensillum.

4.2.2.1 Concentration-Response Relationships

4.2.2.1.1 No Responsiveness of the TP I Sensillum towards Securinine, Berberine, Nicotine, and Strychnine

For all stimulation with these four alkaloids maximum excitations were not distinguishable from the response to water alone.

Whilst the excitation level was constant for all applied nicotine concentrations, increasing securinine, strychnine, and berberine concentrations decreased the excitation level to various degrees as typical for water receptors. Securinine reduced the excitation to about 50% at a concentration of 10^{-3} M. Strychnine almost completely inhibited the excitation at a concentration of 10^{-3} M, whilst berberine completely inhibited the excitation at 10^{-4} M (Figure 27).

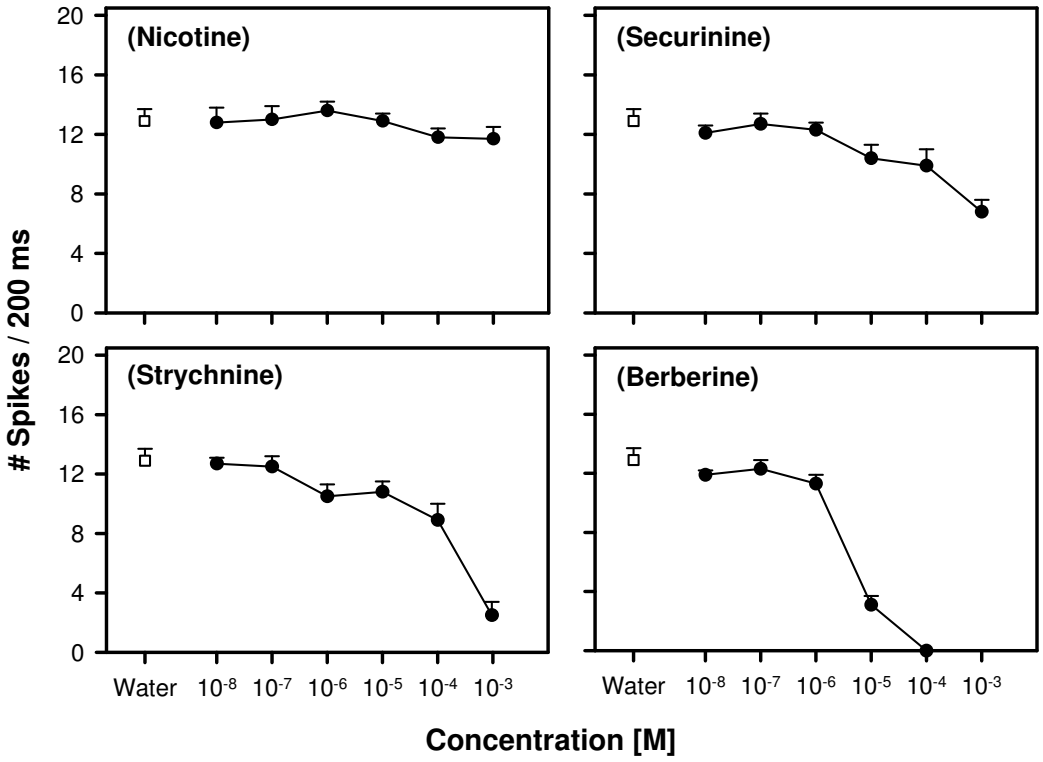

Figure 27: Concentration-response relationships for repeated stimulations of the TP I sensillum with increasing concentrations of nicotine (N = 11-20), securinine (N = 6), strychnine (N = 14-15), and berberine (N = 16). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given.

4.2.2.1.2 No Responsivness of the TP I Sensillum towards Caffeine, Colchicine, Papaverine, and Tryptanthrin

For all stimulations with these four alkaloids maximum excitations were not distinguishable from the response to water alone.

Whilst stimulations with caffeine, colchicine, and tryptanthrin resulted in a constant excitation for all applied concentration, the excitation level decreased with increasing papaverine concentrations as typical for water receptors. No excitation could be recorded anymore at $3 \cdot 10^{-3}$ M (Figure 28).

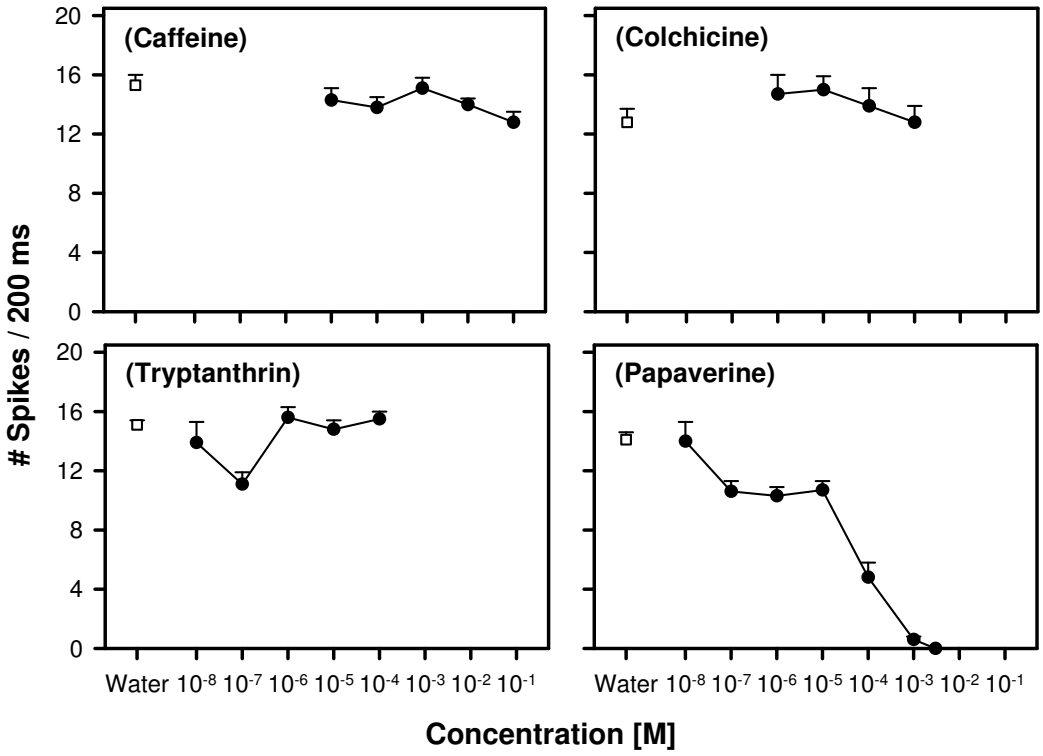

Figure 28: Concentration-response-relationships for the stimulation of the TP I sensillum with different concentrations of caffeine (N = 11-12), colchicine (N = 6-8), tryptanthrin (N = 10), and papaverine (N = 7-17). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. The concentration-response-relationship was recorded on the same sensilla.

4.2.3 TP II Sensillum and Feeding Deterrent Non-Alkaloids

4.2.3.1 Concentration-Response Relationships

4.2.3.1.1 No Responsivness of the TP II Sensillum towards Azadirachtin, Juglone, Nootkatone, and Sinigrine

Stimulations with all four tested non-alkaloids resulted in constant excitations that were not distinguishable from the response to water alone (Figure 29).

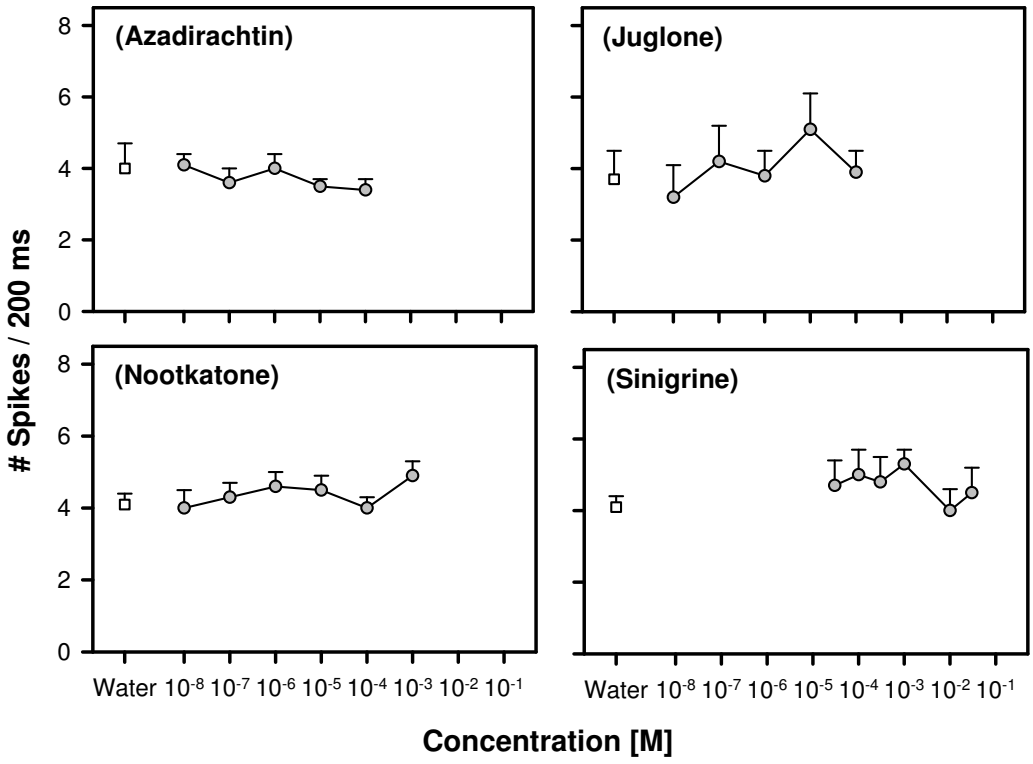

Figure 29: Concentration-response-relationship for the stimulation of the TP II sensillum with different concentrations of azadirachtin (*N* = 14-16), juglone (*N* = 6-7), nootkatone (*N* = 8-10), and sinigrine (*N* = 6-10). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. The concentration-response-relationship was recorded on the same sensilla.

4.2.4 TP I Sensillum and Feeding Deterrent Non-Alkaloids

4.2.4.1 Concentration-Response Relationships

4.2.4.1.1 No Responsivness of the TP I Sensillum towards Azadirachtin, Juglone, Nootkatone, and Sinigrine

Again stimulations with all four tested non-alkaloids resulted in constant excitations that were not distinguishable from the response to water alone (Figure 30).

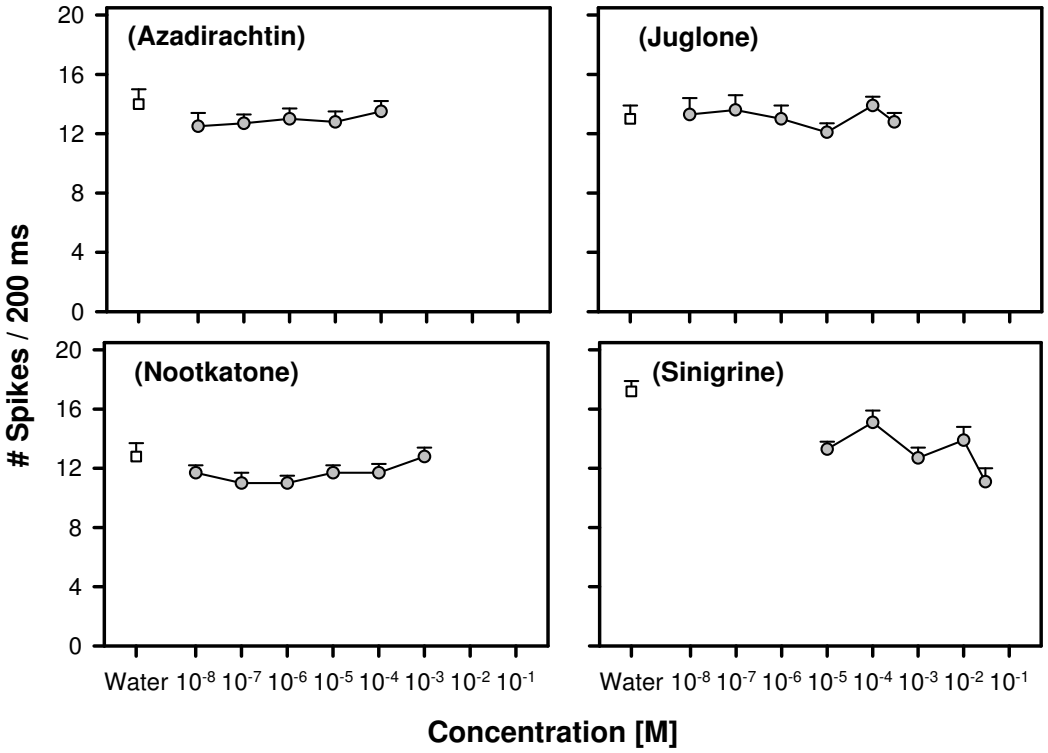

Figure 30: Concentration-response-relationship for the stimulation of the TP I sensillum with different concentrations of azadirachtin (N = 19-20), juglone (N = 5-7), nootkatone (N = 12-13), and sinigrine (N = 14). Mean spike frequencies (+SEM) in the first 200 ms after stimulus onset are given. The concentration-response-relationship was recorded on the same sensilla.

5 Discussion

Already in the 19th century Pfeffer (1897) and Stahl (1888) described the *raison d'être* of plant-derived secondary compounds as defence mechanism against herbivores. These compounds acting as feeding deterrents are usually avoided by herbivores (e.g. Fraenkel 1959, Schoonhoven 1982). Since all 2,300 termite species worldwide feed on plant material (e.g. Wood 1978) plant-derived feeding deterrent compounds might be a source for novel wood preservatives against economically important wood-feeding termites. Wood-feeding termites cause high damage on wooden constructions throughout the tropics, subtropics, and temperate regions (e.g. Fuchs et al. 2004, Su & Scheffrahn 2000). Additionally, these plant compounds might offer new ways in termite control and management. Recent research efforts have focused on the isolation of antitermitic compounds from various plant species (e.g. Adams et al. 1988, Seifert & Unger 1994, Zhu et al. 2001). So far, most studies focused only on the feeding behaviour in termites. In such studies, the authors investigated whether certain isolated plant compounds disrupt feeding (e.g. Scheffrahn and Rust 1983, Maistrello et al. 2003, Serit et al. 1992) or tunnelling activity of termites (e.g. Acda 2009, Maistrello et al. 2001, Zhu et al. 2001).

However, sensory input mechanisms particularly resulting in an aversion of a treated food source are rarely investigated so far in termites (only two studies: Hardiess 2002, Ohmura et al. 2006). Therefore, based on different behavioural tests towards an understanding of feeding deterrence in termites, the aim of the present study was to get a better insight into the sensory input mechanisms involved in the recognition of feeding deterrent compounds and how feeding behaviour is mediated by feeding deterrents.

The African subterranean termite *Schedorhinotermes lamanianus* was used as model organism as it is so far the best investigated termite species regarding the chemosensory inventory including the recognition of feeding deterrents (ultra-structure of antennal sensilla: Wolfrum & Kaib 1988, olfactory receptors: Kaib et al. 1993, Ziesmann 1996, Ziesmann et al. 1992; taste receptors: Hardiess 2002, Mikus 2000, Mikus & Kaib 1997, 1998)

5.1 Effects of Secondary Compounds on Feeding Behaviour

Two sets of behavioural experiments were done. Termites were exposed to potential feeding deterrent compounds either in choice or no-choice situations. Choice situations are

suited to obtain behavioural sensitivities or preferences towards these compounds while no-choice situations are better suited to reflect the natural condition (Schoonhoven 1982). Furthermore, filter paper and wood were used as food sources to reflect the different test approaches found in the literature and to get a better estimate of the feeding deterrent potential of the different compounds. The advantage of using filter paper as food source is that one can expose termites to defined amounts of secondary compounds. Using wood as food source provides information whether a certain compound has a high potential as wood preservative.

In the literature one can find many studies describing the phenomenon of feeding deterrence caused by plant-derived compounds in termites or other insect species. However, for the compounds used in the present study only a few studies on termites provide quantitative information for direct comparisons with e.g. threshold concentrations or feeding reduction. Available data for alkaloids and non-alkaloids are summarised in Table 10 and Table 11. Quantitative data obtained from studies on non-isopterous insects are summarised in Table 12 and Table 13.

5.1.1 Effects of Alkaloids on Feeding Behaviour

In the present study, the alkaloids berberine, nicotine, papaverine, securinine, strychnine, and tryptanthrin proved to be effective feeding deterrents in *S. lamaninaus* although these compounds seem to be non-toxic. These six alkaloids negatively influenced both food choice and food consumption on treated food sources under choice and no-choice conditions. This indicates that termites not only avoid unpalatable food sources but also feed less once they started feeding. Additionally, papaverine, securinine, strychnine, and tryptanthrin are contact repellents at higher concentrations. Caffeine and colchicine are rather strong toxicants than antifeedants in *S. lamanianus.*

Out of the tested alkaloids only securinine, berberine and tryptanthrin were known from previous studies as feeding deterrents in termites (Table 10). The compound specific threshold (CST) for securinine obtained in the present study is in agreement with Hardiess (2002). Securinine reduced also filter paper consumption under no-choice conditions on a non-toxic level. Mortality was only due to starvation. According to the definition given by Scheffrahn (1991) securinine clearly acted as feeding deterrent in *S. lamanianus*. Furthermore, securinine treatment resulted in high feeding reduction (FR) on wooden cubes which is concentration dependent. For high concentrations nearly no feeding could

be observed. Additionally, wooden cubes were often completely covered with faeces indicating securinine is not only a deterrent but based on Scheffrahn (1991) also a contact repellent in *S. lamanianus* workers. Termites use their faeces to isolate toxic or pathogenic material from the colony (Carter & De Carmago, 1983, Logan & Abood 1990, Pearce 1987). Overall, securinine has a high potential as wood protective as naturally observed in *Margaritaria discoidea* (Mikus & Kaib 1998, Mikus et al. 1997, 1998).

Kawaguchi et al. (1989) reported that berberine isolated from the bark of *Phellodendron amurense* acted as feeding deterrent against *Reticulitermes speratus*. In the present study, *S. lamanianus* seems to be more sensitive to berberine as similar mortality on even lower concentrations occurred (Table 10). But mortality of *S. lamanianus* workers was rather due to starvation than toxic effects as it was even lower compared to the starvation treatment. In non-isopterous insects, berberine can have detrimental effects on development and growth of larvae (Devitt et al. 1980) or can be photo-toxic (Philogène et al. 1984).

Table 10: Summary of quantitative data for effects of alkaloids on feeding behaviour in termites including *S. lamanianus* (italic letters) regarding threshold, feeding reduction (FR) and mortality on treated filter paper [FP] or wood [W], (nt = not tested).

Alkaloid	Species*	Threshold	FR	Mortality	Reference
Ber	*R.sp.*	nt	nt	10% [FP] (200 µg/cm^2)	Kawaguchi et al. 1989
	S.l.	*12 µg/cm^2*	*60% [FP] (5,000 ppm)*	*10% [FP] (40 µg/cm^2)*	*present study*
Caf	*S.l.*	*120 µg/cm^2*	*84% [FP] (50,000 ppm)*	*81% [FP] (50,000 ppm)*	*present study*
Col	*S.l.*	*12 µg/cm^2*	*85% [FP] (5,000 ppm)*	*96% [FP] (5,000 ppm)*	*present study*
Nic	*S.l.*	*0.4 µg/cm^2*	*21% [FP] (150 ppm)* *41-75% [W]*	*8% [FP] (150 ppm)*	*present study*
Pap	*S.l.*	*12 µg/cm^2*	*54% [FP] (5,000 ppm)* *72-75% [W]*	*9% [FP] (5,000 ppm)*	*present study*
Sec	*S.l.*	4 µg/cm^2	nt	nt	Hardiess 2002
	S.l.	*4 µg/cm^2*	*67% [FP] (1,500 ppm)* *76-80% [W]*	*23% [FP] (1,500 ppm)*	*present study*
Str	*S.l.*	*1.2 µg/cm^2*	*35% [FP] (500 ppm)* *25-75% [W]*	*4% [FP] (500 ppm)*	*present study*
Try	*R.s.*	nt	>97% [W]	0% [W]	Seifert & Unger 1994
	S.l.	*0.12 µg/cm^2*	*20% [FP] (50 ppm)* *35-70% [W]*	*2% [FP]*	*present study*

* *R.s. = Reticulitermes santonensis*, *R.sp. = Reticulitermes speratus, S.l. = Schedorhinotermes lamanianus*

Berberine was also described as feeding deterrent in non-isopterous insects (Table 11). *S. lamanianus* already avoided a treated food source at concentrations of only one forth of the concentration level needed in larvae of *Syntomis mogadorensis* (Lepidoptera; Table 11). This indicates a higher sensitivity of *S. lamanianus* towards berberine. In contrast, regarding feeding reduction (FR) much higher concentrations of berberine (factor

10-50) have to be applied in *S. lamanianus* to reach similar FR levels as in honey bees (*Apis mellifera*), the fall webworm (*Hyphantria cunea*) and the leaf beetle *Agelastica coerulea* (Table 11). In general, termites seem to be relatively insensitive to detrimental effects of berberine shown by the low mortality at even high concentrations (factor 170) compared to honey bees (Table 11).

Table 11: Summary of quantitative data for effects of alkaloids on feeding behaviour in non-isopterous insects compared to *S. lamanianus* (italic letters) regarding threshold, feeding reduction (FR) and mortality, (nt = not tested).

Alkaloid	Species*	Threshold	FR	Mortality	Reference
Ber	*A.m.*	nt	50% (100 ppm)	50% (30 ppm)	Detzel & Wink 1993
	A.c.	nt	58% (125 ppm)	nt	Park et al. 2000
	H.c.	nt	75% (500 ppm)	nt	Park et al. 2000
	S.m.	50 µg/cm^2	nt	nt	Wink & Schneider 1990
	S.l.	*12 µg/cm^2*	*60% (5,000 ppm)*	*10% (5,000 ppm)*	*present study*
Caf	*A.m.*	nt	50% (300 ppm)	50% (2,000 ppm)	Detzel & Wink 1993
	A.m.	nt	15% (150 ppm)	nt	Singaravelan et al. 2005
	P.r.	nt	37-53% (1,900 ppm)	nt	Blades & Mitchell 1986
	S.m.	5 µg/cm^2	nt	nt	Wink & Schneider 1990
	S.l.	*120 µg/cm^2*	*84% (50,000 ppm)*	*81% (50,000 ppm)*	*present study*
Col	*A.m.*	nt	50% (2,000 ppm)	50% (300 ppm)	Detzel & Wink 1993
	L.m.	nt	50% (10 ppm)	nt	Bernays & Chapman 1977
	S.m.	0.5 µg/cm^2	nt	nt	Wink & Schneider 1990
	S.l.	*12 µg/cm^2*	*85% (5,000 ppm)*	*96% (5,000 ppm)*	*present study*
Nic	*A.m.*	nt	50% (300 ppm)	50% (2,000 ppm)	Detzel & Wink 1993
	A.m.	nt	20% (5 ppm)	nt	Singaravelan et al. 2005
	L.m.		50% (20 ppm)		Bernays & Chapman 1977
	S.m.	5 µg/cm^2	nt	nt	Wink & Schneider 1990
	S.l.	*0.4 µg/cm^2*	*21% (150 ppm)*	*8% (150 ppm)*	*present study*
Pap	*P.r.*		61-86% (3,400 ppm)		Blades & Mitchell 1986
	S.m.	5 µg/cm^2			Wink & Schneider 1990
	S.l.	*12 µg/cm^2*	*54% (5,000 ppm)*	*9% (5,000 ppm)*	*present study*
Str	*A.m.*	nt	50% (200 ppm)	50% (2,000 ppm)	Detzel & Wink 1993
	P.r.	nt	41-55% (3,300 ppm)	nt	Blades & Mitchell 1986
	S.m.	50 µg/cm^2	nt	nt	Wink & Schneider 1990
	S.l.	*1.2 µg/cm^2*	*35% (500 ppm)*	*4% (500 ppm)*	*present study*

* *A.m.* = *Apis mellifera* (Hymenoptera), *A.c.* = *Agelastica coerulea* (Coleoptera), *H.c.* = *Hyphantria cunea* (Lepidoptera), *L.m. Locusta migratoria* (Orthoptera) *P.r.* = *Phormia regina* (Diptera), *S.m.* = *Syntomis mogadorensis* (Lepidoptera), *S.l.* = *Schedorhinotermes lamanianus*

As already mentioned, tryptanthrin was known as feeding deterrent in termites (*Reticulitermes santonensis*: Seifert & Unger 1994). Tryptanthrin is also a feeding deterrent in *S. lamanianus* with a threshold concentration (15 ppm) being 30-fold lower compared to securinine. Workers fed also less on tryptanthrin treated wood. In contrast to *Reticulitermes santonensis*, feeding reduction on wooden cubes was lower in the present

study whilst mortality was comparable (Table 10). Hence, *S. lamanianus* seems to be less susceptible to tryptanthrin in its feeding behaviour.

Remarkably, there were differences in observed feeding reduction levels caused by tryptanthrin between the two wooden choice tests in *S. lamanianus*. Under single-choice conditions, significant feeding reduction was already detectable for an impregnation solution of 0.06 mg/ml. In contrast, under multiple-choice conditions significant feeding reduction was only detectable for the highest (1.2 mg/ml) of the four concentrations used for impregnation. This difference (factor 20) might be due to the different test situations. Under multiple-choice conditions workers might change preferences due to the presence of other plant compounds used as wood treatments. Therefore, wooden cubes treated with low concentrations of trypthantrin might be still palatable in comparison to the stronger feeding deterrent treatments. This indicates that the impact of certain compounds can strongly depend on the conditions under which they are applied (Schoonhoven 1982). Seifert and Unger (1994) further showed that tryptanthrin has detrimental and toxic effects on intestine symbionts. It is likely that the same applies to *S. lamanianus.* Like all wood-feeding termites, *S. lamanianus* also depends on intestine symbionts e.g. for cellulose digestion (Breznak 2000). Similar to securinine, tryptanthrin was also a contact repellent in *S. lamanianus* as some wooden cubes were fully covered with faeces.

The present study provides first evidences that the alkaloids caffeine, colchicine, nicotine, papaverine, and strychnine affect feeding behaviour in termites (Table 10). These alkaloids were known as feeding deterrents in non-isopterous insects (Table 11). Only three of the five alkaloids, nicotine, papaverine, and strychnine, were feeding deterrents in the termite *S. lamanianus* similar to berberine, securinine, and tryptanthrin. They negatively influenced food choice and reduced food consumption at a non-toxic concentration level. The observed mortality was only due to starvation. These three alkaloids also reduced food consumption on impregnated wooden cubes in a similar fashion. All three alkaloids showed a positive concentration dependency of feeding reduction similar to securinine. Furthermore, likewise to securinine and tryptanthrin the two alkaloids papaverine and strychnine were contact repellents for *S. lamanianus* workers. Wooden cubes were also fully covered with faeces

Compared to non-isopterous insects, susceptibility of *S. lamanianus* in its feeding behaviour was lower to caffeine and colchicine, intermediate to nicotine, similar to papaverine, but higher to strychnine (Table 11). Similar to honey bees, high feeding

reduction caused by caffeine and colchicine was related to high mortality indicating toxic effects (Table 11).

Colchicine is a mitotic poison (Bullough 1949, Fankhauser & Humphrey 1952, Stroud 1952) most likely inhibiting the proliferation of intestine symbionts that are necessary for the digestion of cellulose (Breznak 2000, Breznak & Brune 1994). When intestine symbionts die termites can no longer properly digest their food and die from starving although they ingest food. However, since mortality is much higher compared to the starvation treatment in the no-choice test there have to be some direct toxic effects on the termites' itselves probably by detrimental effects on cell function. Caffeine must have also direct toxic effects on *S. lamanianus* workers as mortality was also strongly increased compared to the starvation treatment.

In contrast to the other alkaloids, nicotine and caffeine are also reported to be either phagostimulants or deterrents in honey bees depending on concentrations applied (Singaravelan et al. 2005). The two alkaloids occur naturally in the nectar of many plant species at very low concentrations functioning as feeding stimulants. But at higher concentrations that are still a factor of 30-100 lower compared to applied concentrations in *S. lamanianus* these alkaloids are feeding deterrents (Table 11). The locust *Locusta migratoria* also responds much more sensitive (factor 10-100 lower concentrations) in its feeding behaviour to the alkaloids colchicine and nicotine compared to *S.lamanianus* (Table 11). Janzen et al. (1977) found an increased mortality caused by caffeine, colchicine, nicotine, and strychnine in cowpea weevils (*Callosobruchus maculatus*). In larvae of the black blowfly *Phormia regina* (Calliphoridae) Green et al. (2002) observed a reduced weight gain on food treated with caffeine, nicotine, and strychnine concentrations >100 ppm.

5.1.2 Effects of Non-Alkaloids on Feeding Behaviour

In the present study, out of the tested non-alkaloids only the flavonoid chrysin proved to be an effective but non-toxic feeding deterrent for workers of *S. lamanianus* feeding on filter paper or wood as food source. Azadirachtin did not evoke long term feeding deterrent effects. Juglone seems to be an airborne repellent rather than a feeding deterrent in *S. lamanianus.* Nomilin and nootkatone were only feeding deterrent on filter paper as food source. Although sinigrin negatively influenced food choice and food consumption it was rather toxic than antifeedant.

Secondary compounds of the neem tree *Azadirachta indica* like e.g. the terpenoid azadirachtin are long known to be potentially feeding deterrent in insect (reviewed in Koul et al. 1990, Schmutterer, 1988, 1990). Azadirachtin negatively affects molting in insects (Aerts & Mordue 1997) and reduces larval digestion efficacy and relative growth rate (Nathan & Kalaivani 2005). Azadirachtin also negatively affects fecundity (Cowles 2004, Hussein et al. 2005). In contrast to non-isopterous insects, *S lamanianus* shows feeding reduction only at very high concentrations of azadirachtin (Table 13). Impregnations with 3-5-fold threshold concentrations of azadirachtin did not decrease food consumption on filter paper or wood over a longer period (7 days). This might be due to the low persistence of azadirachtin under "tropical conditions" (high temperature and humidity). Under these conditions it is only persistent for about 5-7 days (Koul et al. 1990, Schmutterer 1988, 1990, Serit 1992). Therefore, the applied amount most likely degraded below the effective threshold over time thus lacking feeding deterrent effects. In a previous screening test, azadirachtin also reduced feeding on filter papers but at much higher though still non-toxic concentrations (Table 12). Another reason for lacking effects might be habituation of termites as shown in larvae of the tobacco cutworm *Spodoptera litura* (Bomford & Isman 1996).

Within termites, *Reticulitermes speratus* and *Coptotermes formosanus* respond more sensitive in their feeding behaviour to azadirachtin. Both species show similar feeding reductions compared to *S. lamanianus* but at concentrations 7-150-fold lower than in *S. lamanianus*, although the observed threshold concentration for an avoidance of a treated food source was very low (15 ppm) in the present study (Table 12). Mortality in *S. lamanianus* was comparable to *Reticulitermes speratus* whilst *Coptotermes formosanus* seems to be more sensitive to detrimental effects of azadirachtin. Reasons for this difference might be that Grace and Yates (1992) did not use pure azadirachtin in their study. The authors used a mixture with other neem oil extracts which might have some additional detrimental effects explaining the high mortality on lower concentrations.

Nomilin has been already described as termite antifeedant (Serit et al. 1991, 1992). *Reticulitermes speratus* responds more sensitive in its feeding behaviour to nomilin compared to *S. lamanianus.* Similar feeding reductions were observed at concentrations differing by the factor 6 (Table 12). Mortality is comparable in *Reticulitermes speratus* and *S. lamanianus* (Table 12). In general, nomilin seems to have only weak detrimental effects on termites.

Nootkatone is a compound in essential oils and flavours of different plant species (e.g. Alaska yellow cedar *Chamaecyparis nootkatensis*: Kelsey et al. 2005, redblush grapefruit *Citrus paradisi*: Njoroge et al. 2005). Nootkatone was already known as feeding deterrent in termites. *Coptotermes formosanus* avoids tunnelling through treated sand at similar threshold concentrations where *S. lamanianus* avoided treated filter papers (Table 12). In *Coptotermes formosanus* sand treated with 100 ppm nootkatone was an effective tunnelling barrier for termites (Maistrello et al. 2001a,b). It is difficult to quantitatively compare feeding reduction between *Coptotermes formosanus* and *S. lamanianus* due to the great differences in applied concentrations (Table 12).

Whilst in *Coptotermes formosanus* nootkatone caused high feeding reduction on wood; it showed inconclusive effects regarding wood protection in *S. lamanianus*. Under single-choice conditions termites tended to feed less on nootkatone treated wood. However, under multiple-choice conditions they even fed more on nootkatone treated wood. One reason for these inconclusive effects might be that nootkatone evaporates over time as it has a very low melting point (32-35 °C). Additionally, nootkatone might need longer to adsorb properly to the wooden matrix than the other secondary compounds used in the present study. This might lead to a weaker impregnation with nootkatone. Furthermore, nootkatone treated wood might be still palatable for *S. lamanainus* in the presence of stronger feeding deterrents as discussed for the alkaloid tryptanthrin. Another reason might be the much lower concentrations (Table 12) and shorter impregnation time (5 min *vs.* 1 h) used in the present study compared to Maistrello et al. (2003). Hence, the effects of nootkatone in *S. lamanainus* should be re-evaluated in further studies.

The flavonoid chrysin was already described as wood preservative against the termite *Cryptotermes brevis* about 60 years ago by Wolcott (1953). In *S. lamanianus* the threshold was quite low compared to the other secondary compounds (50 ppm). Ohmura et al. 1999 and Shibutani et al. 2004 showed that taxifolin, a structurally similar flavonoid to chrysin, is also a feeding deterrent in termites. In *Reticulitermes sparatus* taxifolin causes a similar feeding reduction as chrysin in *S. lamanianus* but at a much higher concentration level (Table 12). Differences might be due to molecular differences or different sensitivities of the two termite species. In general, chrysin was a good wood preservative in *S. lamanianus* causing comparable feeding reduction like the alkaloids papaverine, securinine, and strychnine. Chrysin is also a contact repellent similar to the alkaloids papverine, securinine, strychnine, and tryptanthrin. Wooden cubes were also fully covered with

faeces. Different flavonoids in the wood of *Lonchocarpus castilloi* are feeding deterrent but non-toxic in *Cryptotermes brevis* (Reye-Shilpa et al. 1995) similar to the observed feeding deterrence of chrysin in *S. lamanianus*. In general, flavonoids can also reduce fecundity and increase mortality in termites (Boue & Raina 2003).

S. lamanianus is higly sensitive to the naphthoquinone juglone. At concentrations ≥1.5 ppm workers already avoided treated filter paper. Juglone also reduced food consumption on filter papers; however, it did not prevend wood to be fed on. Impregnated wooden cubes were equally consumed as non-impregnated ones by *S. lamanianus*. Carter et al. (1978) isolated the juglone derivate 7-methyljuglone from wood of common persimmon *Diospyros virginiana*. This compound caused high mortality in workers of *Reticulitermes flavipes* (Table 12). In contrast, juglone had no toxic effects in *S. lamanianus* at concentrations used in the present study. However, quantitative comparisons regarding mortality are difficult as applied concentrations were 40-fold higher in *Reticulitermes flavipes* than *S. lamanianus* (Table 12). Weissenberg et al. (1997) showed in the Mexican bean beetle *Epilachna varivestis* that juglone is an antifeedant. Larvae fed less on treated bean leaves compared with non-treated. The bark beetle *Scolytus multristriatus* does not feed on juglone treated food, too (Gilbert at al. 1967). However, both studies provided no quantitative information for direct comparisons with *S. lamanianus.* The western corn root worm *Diabrotica virgifera* and the Colorado potato beetle *Leptinotarsa decemlineata* respond less sensitive in their feeding behaviour to juglone compared to *S. lamanianus* (Table 13). Both coleopterous species showed similar feeding reductions at 250-350-fold higher concentrations as *S. lamanianus*. Furthermore, juglone seems to be an airborne repellent in *S. lamanianus.* Workers initially refused to enter the test arena in the filter paper choice test without any previous contact to the treated food source. In the wooden cube choice test, exploration of the new arena was delayed. It lasted two days (72-96 h) longer compared to the other treatments (24-48 h) until workers had established a stable foraging traffic into the test arena. This might explain why in the filter paper no-choice test reduced food consumption could be observed whilst in the wooden cube choice test this was not the case. In the first test, termites could not avoid the exposition to juglone and had no alternative food source. In the latter one, termites had an alternative food source and could "wait" until juglone evaporated below the threshold concentration. Hence, termites later fed equally on blank and treated wooden cubes.

Table 12: Summary of quantitative data for effects of non-alkaloids on feeding behaviour in termites including *S. lamanianus* (italic letters) regarding threshold, feeding reduction (FR) and mortality on treated filter paper [FP] or wood [W], (nt = not tested).

Non-Alkaloid	Species*	Threshold	FR	Mortality	Reference
Aza	*C.f.*	nt	69% [FP] (100 ppm)	37% [FP] (300 ppm)	Grace & Yates 1992
	R.sp.	nt	50% [FP] (2,000 ppm)	15% [FP] (6,800-13,600 ppm)	Serit et al. 1992
	S.l.	*0.12 µg/cm²*	*75% [FP] (15,000 ppm) 1% [W]*	*15% [FP] (15,000 ppm)*	*present study (data not presented)*
Chr	*R.sp.*	nt	10% (380 µg/cm²) **	nt	Shibutani et al. 2004
	S.l.	*0.4 µg/cm²*	*11% [FP] (1.2 µg/cm²) 56-60% [W]*	*7% [FP]*	*present study*
Jug	*R.f.*	nt	nt	100% (200 ppm) ***	Carter et al.1978
	S.l.	*0.012 µg/cm²*	*44% [FP] (5 ppm) -5% [W]*	*13% [FP] (5 ppm)*	*present study*
Nom	*R.sp.*	nt	50% [FP] (1,400 ppm)	25% [FP] (10,200 ppm)	Serit et al. 1992
	S.l.	*24 µg/cm²*	*60% [FP] (9,000 ppm)*	*25% [FP] (9,000 ppm)*	*present study*
Noo	*C.f.*	20-100 ppm	90% [W] (7,000 ppm)		Zhu et al. 2001; Maistrello et al. 2001a,b, 2003
	S.l.	*50 ppm*	*20% [FP] (150 ppm) -100-30% [W]*	*13% [FP] (150 ppm)*	*present study*
Sin	*S.l.*	*40 µg/cm²*	*95% [FP] (15,000 ppm)*	*51% [FP] (15,000 ppm)*	*present study*

* *R.f. = Reticulitermes flavipes, R.sp. = Reticulitermes speratus, C.f. = Coptotermes formosanus, S.l. = Schedorhinotermes lamanianus*
** taxifolin (structurally similar to chrysin) used
*** 7-methyljuglone used

Table 13: Summary of quantitative data for effects of non-alkaloids on feeding behaviour in non-isopterous insects compared to *S. lamanianus* (italic letters).

Non-Alkaloid	Species*	Threshold	FR	Mortality	Reference
Aza	*E.p.*	nt	62% (1 µg/cm²)	nt	Carpinella et al. 2002
	L.m.	nt	50% (100 ppm)	nt	Bernays & Chapman 1977
	S.e.	nt	57% (0.25 µg/cm²)	nt	Carpinella et al. 2002
	S.li.	nt	50-75% (1.3 ng/cm²)	nt	Bomford & Isman 1996
	S.li	nt	67% (0.015 µg/cm²)	nt	Koul et al. 2004
	S.li.	nt	54% (1 ppm)	nt	Nathan & Kalaivani 2005
	S.l.	*0.12 µg/cm²*	*75% (120 µg/cm² 15,000 ppm)*	*15% (15,000 ppm)*	*present study (data not presented)*
Jug	*D.v.*	nt	50% (14 µg/cm²)	nt	Mullin et al. 1997
	L.d.	nt	50% (10 µg/cm²)	nt	Mullin et al. 1997
	S.l.	*0.012 µg/cm²*	*44% (0.04 µg/cm²)*	*13% (5 ppm)*	*present study*
Sin	*M.c.*	nt	95% (2,000 ppm ppm)	nt	Schields & Mitchell 1995a
	T.n.	nt	90% (2,000 ppm)	nt	Shields & Mitchell 1995a
	S.l.	*40 µg/cm²*	*95% (15,000 ppm)*	*51% (15,000 ppm)*	*present study*

* *A.m. = Apis mellifera* (Hymenoptera), *A.c. = Agelastica coerulea* (Coleoptera), *D.v. = Diabrotica virgifera* (Coleoptera), *E.p. = Epilachna paenulata* (Coleoptera), *L.d. = Leptinotarsa decemlineata* (Coleoptera), *L.m. = Locusta migratoria* (Orthoptera), *M.c. = Mamestra configurata* (Lepidoptera), *S.e. = Spodoptera eridania* (Lepidoptera), *S.li. = Spodoptera litura* (Lepidoptera), *T.n. = Trichoplusia ni* (Lepidoptera), *S.l. = Schedorhinotermes lamanianus*

Similar to the alkaloids nicotine and caffeine, the glucosinolate sinigrin is also reported to be either a phagostimulant or feeding deterrent depending on the insect species concerned. In the cabbage aphid *Brevicoryne brassicae* and the pea aphid *Acyrthosiphon pisum*, sinigrin increases phloem sap consumption (Gabrys & Tjallingii 2002). In larvae of the diamondback moth *Plutella xylostella*, sinigrin also stimulates feeding (van Loon et al. 2002). In contrast, sinigrin is a feeding deterrent in the monarch butterfly *Danaus plexippus* (Vickerman & de Boer 2002). The present study is the first description of sinigrin affecting feeding behaviour in termites. In *S. lamanianus* sinigrin functions rather as strong toxicant than feeding deterrent. Compared to the two moth species *Mamestra configurata* and *Trichoplusia ni*, *S. lamanianus* shows similar feeding reduction but at 7-fold higher concentrations (Table 13) But, similar to the alkaloids caffeine and colchicine, sinigrin also increased mortality in *S. lamanianus* indicating that it had some toxic effects. Sinigrin and its hydrolysis products have toxic effects on microorganisms (Brabban & Edwards 1995). Therefore, it is likely that sinigrin might have similar detrimental effects as colchicine on intestine symbionts in termites.

5.2 Neural Input for Feeding Deterrents

The detection of feeding deterrent compounds is crucial for many insect herbivores in host plant recognition (Glendinning et al. 2006). Some insect species (mainly lepidoptera: reviewed by Schoonhoven et al. 1992, Schoonhoven & van Loon 2002) possess taste receptor cells responding to feeding deterrent compounds at low concentrations. For example, Ishikawa (1966) described a "bitter receptor" in the silkworm *Bombyx mori* responding to alkaloids and phenolics. The tobacco hornworm *Manduca sexta* has four "deterrent neurons" responding to various bitter compounds (Glendinning et al. 2002). Larvae of *Pieris brassicae* and *P. rapae* have two feeding deterrent neurons responding to a broad spectrum of secondary plant compounds (Ma 1969, van Loon 1990). The blowfly *Protophormia terranovae* has a deterrent neuron responding to various alkaloids (Liscia & Solari 2000). Larvae of the Colorado potato beetle *Leptinotarsa decemlineata* posses a neuron responding to the feeding deterrents drimane and sinigrin (Messchendorp et al. 1998).

5.2.1 Recognition of Alkaloids

Since alkaloids provided the most consistent behavioural results in the present study the main focus in the neurophysiological investigations was on alkaloids. Hardiess (2002)

already described a taste neuron in the TP II sensillum (neuron II/3) on the antennae of *S. lamanianus* responding to securinega-alkaloids. Therefore, securinine was used as a reference substance. Additionally to the eight alkaloids (including securinine) tested in relation to the behavioural physiology, 16 further alkaloids were used for the neurophysiological investigations. These alkaloids were also known from the literature as feeding deterrents in non-isopterous insects (e.g. Castells & Berenbaum 2006, Güntner et al. 2000, Wink & Schneider 1990).

Out of the in total 24 alkaloids tested, only twelve alkaloids namely securinine (reference), arecoline, berberine, brucine, coniine, hyoscyamine, lobeline, lupanine, nicotine, pilocarpine, sparteine and strychnine stimulated a concentration-dependent excitation in a taste neuron in the TP II sensillum. Spike shape and time characteristics for all stimulations were not distinguishable from excitations to stimulations with securinine. This provides strong evidence that these alkaloids stimulated only taste neuron II/3 as securinine does. There was no apparent structure-activity relationship which could explain why certain alkaloids stimulate taste neuron II/3 whilst others did not. However, this seems to be a widespread phenomenon for insect feeding deterrent receptors. Many insect feeding deterrent receptors respond to a broad spectrum of chemically unrelated compounds (e.g. Schoonhoven 1987, van Loon 1990). The receptor cells even respond to compounds that the insect species concerned cannot have experienced in its recent evolution (Bernays & Chapman 1987, Schoonhoven 1981).

5.2.1.1 TP II Stimulating Alkaloids

5.2.1.1.1 Concentration-Response Relationships

All TP II stimulating alkaloids showed the typical sigmoidal concentration-response relationship as described for many taste receptor cells (e.g. Wieczorek 1976, Mitchell & Gregory 1979, Peterson et al. 1993, Städler 1994, van Loon & Schoonhoven 1999). The concentration-response relationship for securinine obtained in the present study was in agreement with Hardiess (2002). The threshold concentration was also about $1\text{-}3\cdot10^{-5}$ M. This is within the typical concentration range (10^{-8}-10^{-5} M) for deterrent receptors in insects (e.g. Hollister et al. 2001, Messchendorp et al. 1998, van Loon & Schoonhoven 1999). Similar to Hardiess (2002) adaptation caused by repeated stimulations with securinine could be observed. Naive neurons showed higher excitation levels compared to previously stimulated neurons. Furthermore, in comparison to securinine two major types

of concentration-response relationships could be observed in the present study. The first group of compounds (arecoline, berberine, pilocarpine) showed concentration-response relationships similar to that after stimulations with securinine but with either thresholds at higher alkaloid concentrations (arecoline, pilocarpine) or weaker slopes (berberine). This indicates that workers of *S. lamanianus* are less sensitive towards these three feeding deterrent alkaloids. Compared to securinine, the second group of compounds (coniine, brucine, hyoscyamine, lobeline, lupanine, strychnine, sparteine) had concentration-response curves with a similar threshold but a much steeper slope. This indicates that workers of *S. lamanianus* show a higher responsiveness towards these feeding deterrent alkaloids. Some alkaloids (e.g. brucine, sparteine) showed very steep sigmoidal curves with a "jump" of the excitation level from the minimum to the maximum within a very narrow concentration range. For these alkaloids the stimulated taste neuron acted like a "switch" between an "on-off"-state. These findings suggest that *S. lamanianus* can only sense the presence of these compounds. For compounds with weaker slopes, termites might also be able to measure concentrations of these compounds. This is supported by the findings that for the alkaloids nicotine, securinine, and strychnine intermediate excitation levels could be recorded and that termites also showed concentration-dependent feeding reduction for these compounds (see wooden cube multiple choice test).

The observed decreases in excitation levels at high concentrations of lobeline and lupanine were probably either to longer lasting self-adaptation occurring at this high concentration (see also cross-adaptation tests, sequence III) or detrimental effects on the taste neuron itself. Detrimental effects at high concentrations of feeding deterrent compound might possible as many of these secondary compounds can also directly interact with cell membranes disrupting normal membrane structure and cell function (Schoonhoven et al. 1992).

5.2.1.1.2 Cross-Adaptations at TP II Sensilla

The stimulation of TP II sensilla with non-securinine alkaloids showed excitations of a taste neuron with spike shape and time characteristics not distinguishable from excitations after stimulations with securinine. This indicates that only one of the four taste neurons (II/3) was stimulated. Another approach to test whether the same neuron is stimulated by different compounds is using cross-adaptation tests. When molecules are recognised by the same receptor they tend quite often to cross-adapt or cross-enhance (Froloff et al. 1998).

Chapman et al. (1991) used cross-adaptation experiments to show that different feeding deterrents stimulate the same neuron in a taste sensillum of the grasshopper *Schistocerca americana.* Liscia et al. (2004) used the same method in the blowfly *Protophormia terranovae.* Furthermore, cross-adaptation can be explained by both molecules binding to the same receptor site or modulating the same transduction process used by different receptor sites in one receptor cell (Froloff et al. 1998). Hence, existing cross-adaption between two different compounds is a strong indicator that these compounds stimulate the same neuron. In *S. lamanianus* the stimulation with securinine (≥1 mM) evokes long-lasting self-adaptation of neuron II/3 (Hardiess 2002). Therefore, a preceding securinine stimulus should decrease the excitation to a subsequent stimulation with other alkaloids if neuron II/3 is again stimulated.

Alkaloids could be divided into three major groups according to the observed cross-adaptation patterns. Both, the responsiveness to alkaloids of the "berberine-group" (arecoline, berberine, pilocarpine) and "strychnine-group" (brucine, hyoscyamine, lobeline, nicotine, strychnine) was strongly inhibited (80-90%) by a preceding securinine stimulus. The three alkaloids of the "berberine-group" did not inhibit the responsiveness to subsequent securinine stimuli. Furthermore, no (berberine, pilocarpine) or only a slight self-adaptation (arecoline) could be observed. In contrast, the five alkaloids of the "strychnine-group" were able to decrease the excitation to subsequent securinine stimuli but to a lower extent as securinine does in the *vice versa* situation. Different degrees of self-adaptation could be observed in all five alkaloids. Both cross-adaptation patterns might be explained by lower binding capacities compared to securinine at the same receptor site.

Molecules binding stronger to the receptor site have also increased half-lives for the stimulus/receptor complexes. After an initial stimulation receptor sites are blocked for the binding of a new molecule and thus cannot be activated again. Competitive inhibition at the receptor site between different compounds is then possible (Frohloff et al. 1998). For securinine, half-life of the stimulus/receptor complexes seems to be relatively long as self-adaption evokes a long-lasting decrease of the excitation level for subsequent stimuli (Hardiess 2002). In contrast, the half-lives of the stimulus/receptor complexes for "berberine-group" alkaloids seem to be relatively short as no or only very low self-adaptation occurred. Hence, the proposed membrane model (Figure 31) illustrates, that after the stimulation of naive neurons with securinine (adapting stimulus, Figure 31 A:▽)

most receptor sites might still be blocked (Figure 31 B) due to the stronger binding of securinine. Thus arecoline, berberine and pilocarpine (▼) are not able to competitively replace bound securinine or to bind to an appropriate number of free receptor sites (Figure 31 C) to evoke excitations similar to the stimulation of naive neurons (first stimulus in sequence III, IV). A similar scenario applies to alkaloids of the "strychnine-group" (Figure 31 A-B, D:▼).

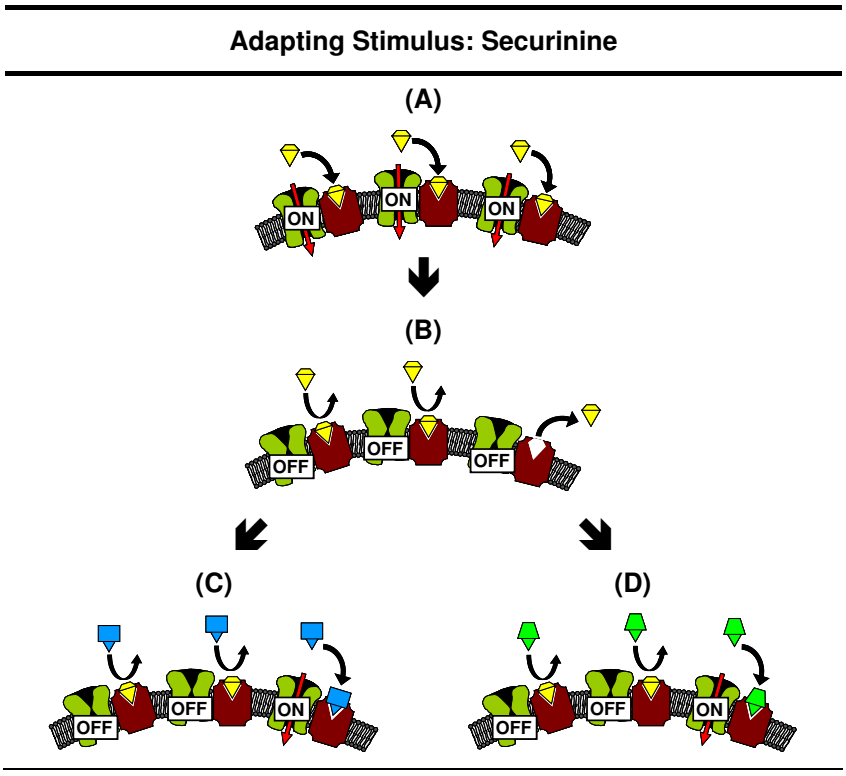

Figure 31: Membrane model for cross-adaptations with the adapting stimulus by securinine. A-C: Subsequent stimulus with "berberine-group" alkaloids. A-B, D: Subsequent stimulus with "strychnine-group" alkaloids. ON: ion channel open. OFF: ion channel closed. The greater the number of open channels is the higher is the excitation of the neuron. Further descriptions see text.

In the *vice versa* scenario, the proposed membrane model (Figure 32) predicts that securinine is either able to bind to free receptor sites (Figure 32 A-C) or competitively replace bound arecoline, berberine, and pilocarpine molecules (Figure 32 A, D-E) due to its stronger binding capacity. Therefore, securinine evokes a normal excitation of neuron II/3. In contrast, the half-lives of the stimulus/receptor cmplexes for "strychnine-group" alkaloids seem to be longer than for alkaloids of the "berberine-group" as significant self-adaptation (sequence III) occurred. In this case, securinine might only be able either to bind to a lower number of free receptor sites (Figure 32 F-H) or can competitively replace to a lower extent bound brucine, hyoscyamine, lobeline, nicotine, and strychnine molecules resulting in a decreased excitation of neuron II/3 (Figure 32 F, I-J).

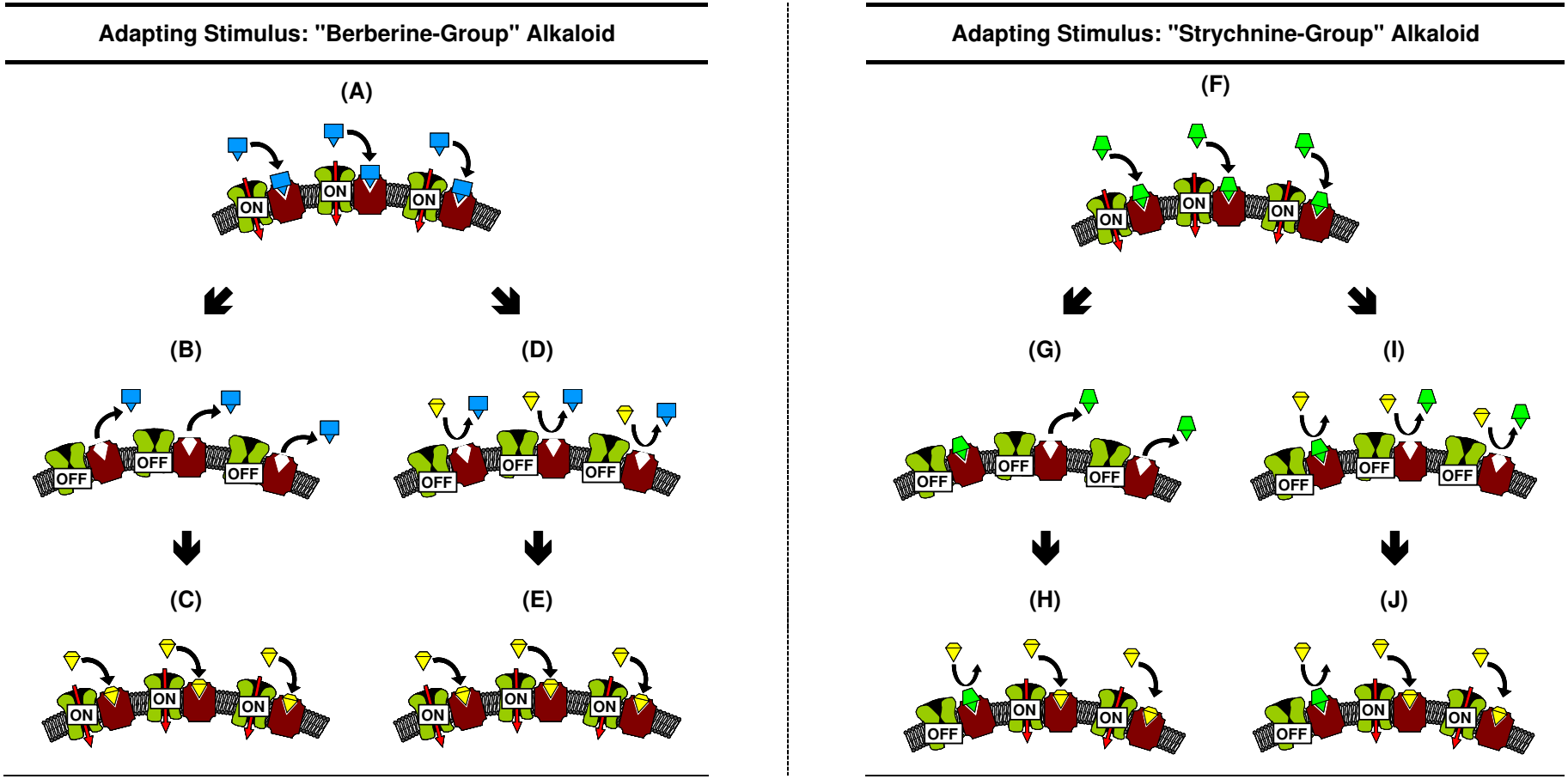

Figure 32: Membrane model for cross-adaptations. A-E: Adapting stimulus with "berberine-group" alkaloids and subsequent stimulus with securinine. F-J: Adapting stimulus with "strychnine-group" alkaloids and subsequent stimulus with securinine. ON: ion channel open. OFF: ion channel closed. The greater the number of open channels is the higher is the excitation of the neuron. Further descriptions see text.

In contrast to the former two groups, adaptation caused by securinine was lower in the "coniine-group". The responsiveness to coniine, lupanine, sparteine was inhibited by a preceding securinine stimulus but to a lower extent compared to the former two groups. This pattern might be due to similar binding capacities of these alkaloids and securinine. At least for securinine and the lupine-alkaloids lupanine and sparteine there are structural similarities of the ring-system (Figure 33 rings A-C, Seifert, pers. communication) supporting the assumption of similar binding capacities.

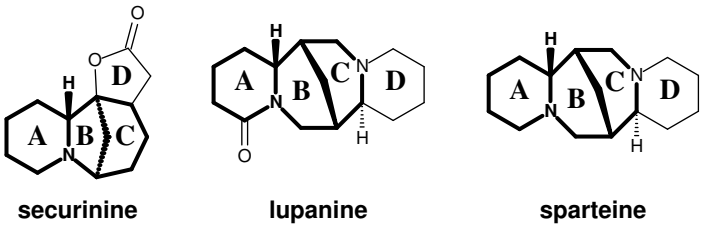

Figure 33: Comparison between chemical strucutures of securinine and lupine-alkaloids. Similar ring-structures (A-C) are indicated in bold.

Therefore, coniine, lupanine, and sparteine (▼) might be able to competitively replace bound securinine to a higher extent at the receptor site evoking higher excitations after an adapting securinine stimulus compared to the "berberine-" and "strychnine-group" (Figure 34 A-C; D-F).

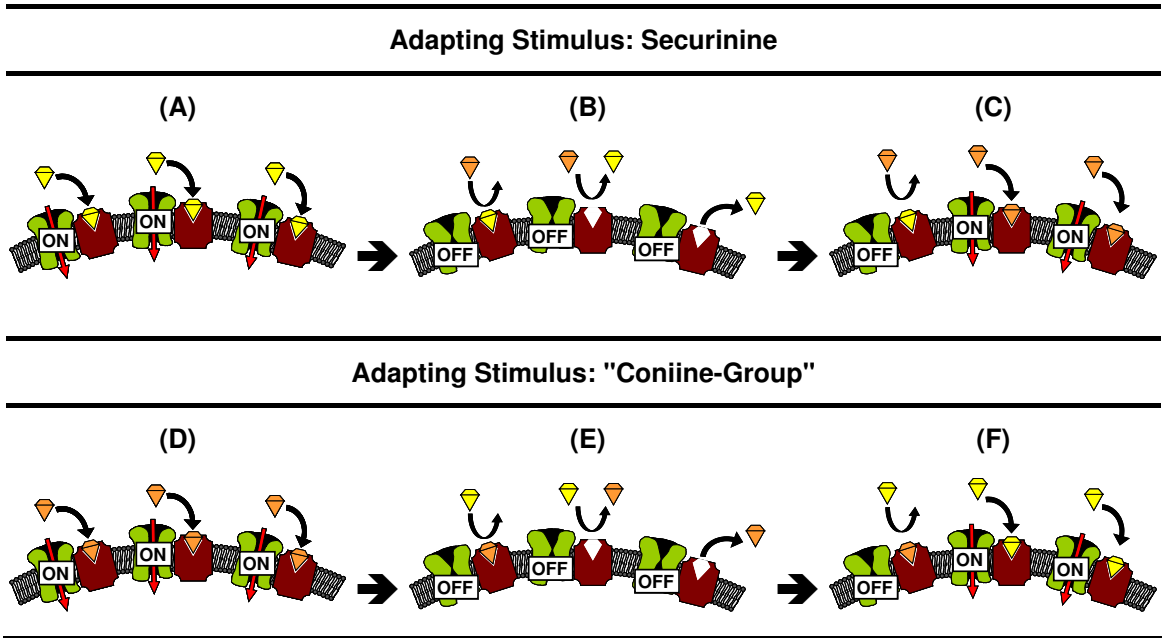

Figure 34: Membrane model for cross-adaptations between securinine and alkaloids of the "coniine-group". A-C: adapting stimulus with securinine. D-H: adapting stimulus with alkaloids of the "coniine-group". ON: ion channel open. OFF: ion channel closed. The greater the number of open channels is the higher is the excitation of the neuron. Further descriptions see text.

The lower adaptation caused by securinine might also indicate two different receptor sites for securinine ("Sec"-site) and "coniine group" alkaloids ("Con"-site). After an adapting securinine-stimulus when the "Sec"-site is inhibited, alkaloids of the "coniine group" might by-pass the "Sec"-receptor using the "Con"-site. Two receptor sites for different modalities can be either located in one neuron or in two different neurons. Multiple receptor sites in the membrane of one neuron have been described in insect taste receptor cells. Mikus (2000) already described in *S. lamanianus* that neuron I/1 harbours one receptor site for water and one receptor site for sodium cations. Furthermore, e.g. insect "sugar cells" possess up to four different receptor sites: a furanose site, a pyranose site, a D-galactose site, and 4-nitrophenyl-α-glucoside site for the perception of different sugars (Schoonhoven et al. 1992). But, cross-adaptation alone does not exclude the possibility of two different neurons responding to securinine and "coniine-group" alkaloids. However, spike shape and time characteristics of excitations after stimulations with "coniine-group" alkaloids were not distinguishable from excitation after securinine stimulations. Together with these two other criteria, the cross-adaption pattern strongly suggests that "coniine group" alkaloids also stimulate neuron II/3 as securinine does.

Different degrees of cross-adaptation can also depend on the transduction pathway a taste compound activates in the taste receptor cell. Cross-adaptation might be stronger for taste compounds activating the IP_3-pathway as the intracellular calcium pool is limited and needs longer to be replenished. In contrast, cross-adaption might be lower for taste compounds activating the cAMP-pathway as much larger quantities of calcium are available from the extracellular pool (Frohloff et al. 1998). Taste transduction mechanisms are well investigated in vertebrates (e.g. Kinnamon & Margolskee 1996, Spielman et al. 1991). For the perception of bitter compounds several modes of action have been proposed: **i)** bitter compounds may either inactivate K^+-channels (Cummings & Kinnamon 1992) or activate Na^+-channels (Tsunenari et al. 1999). **ii)** Bitter compounds may directly interfere with the membrane changing the membrane potential (Naito et al. 1993). **iii)** Bitter compounds may also bind to a receptor and increase intracellular Ca^{2+} concentration via an increase of IP_3 (Yan et al. 2001) or cAMP/cGMP concentrations (Rosenzweig et al. 1999). **iv)** Bitter compounds may be transported into the cytosol and activate directly G-protein coupled pathways (Peri et al. 2000), or **v)** decrease cGMP concentration thus activating cyclic-nucleotide inhibited ion channels (Ruiz-Avila et al. 2002).

Overall, the obtained cross-adaption patterns in *S. lamanianus* provide strong evidence that all TP II stimulating alkaloids stimulate only one taste neuron, the securinega-alkaloid-sensitive neuron (II/3).

5.2.1.2 TP II Non-Stimulating Alkaloids

The feeding deterrent alkaloids papaverine and tryptanthrin did not stimulate a taste neuron in the TP II sensillum. Maximum excitation levels were not distinguishable from the response to water alone. This indicates that only the "water cell" (neuron II/2: Mikus 2000) was stimulated by the water used as solvent for the stimulus compounds. The same applied for stimulations with the toxicants caffeine and colchicine. Furthermore, increasing colchicine and papaverine concentrations led to decreasing excitations and total inhibition as typical for the inhibition of water receptor cells by alkali metal cations (e.g. Goshima et al. 1997, Schnuch 1996) or high concentrations of non-electrolytes (e.g. Evans and Mellon 1962, Rees 1970). Mikus (2000) showed that the "water cell" in the TP II sensillum of *S. lamanianus* can be inhibited by increasing KCl and NaCl concentrations with a total inhibition at concentrations ≥100 mM. This is within the typical concentration range (100-1000 mM) for the total inhibition of insect "water cells" by univalent cations (e.g. Evans & Mellon 1962, Messchendorp et al. 1998, Schnuch & Hansen 1992). Mikus (2000) did not test bivalent cations (e.g. Ca^{2+}) at neuron II/2, but described an additional "water cell" in the TP I sensillum (neuron I/1) which is already totally inhibited at low concentrations of Ca^{2+} (1 mM). In contrast, colchicine and papaverine totally inhibited the "water cell" in TP II at 10 and 3 mM respectively, which is similar to the concentration range for bivalent cations but much lower than for univalent cations. Hence, colchicine and papaverine might use a similar mechanism as bivalent cations. Another possible mechanism could be that both alkaloids directly affect the dendritic membrane disrupting normal cell function (Schoonhoven et al. 1992).

5.2.1.3 Stimulation of TP I Sensilla

None of the six feeding deterrent alkaloids berberine, nicotine, papaverine, securinine, strychnine, and tryptanthrin elicited a concentration-dependent excitation of a taste neuron in TP I sensilla of *S. lamanianus.* Spike shape and maximum excitation levels were not distinguishable from the response to water alone. This indicates that only a water receptor cell (neuron I/1: Mikus 2000) was stimulated by the water used as solvent. The same also applied for the two toxic alkaloids caffeine and colchicine.

Furthermore, increasing concentrations of berberine, papaverine, securinine, and strychnine inhibited the response of neuron I/1. Concentration-response relationships for this inhibition were similar to the inhibition by calcium cations described by Mikus (2000). Neuron I/1 was totally inhibited by these four alkaloids at concentrations between 0.1-10 mM. Berberine is a univalent quaternary ammonium cation (Ber^+Cl^-) thus seems to act as an electrolyte whilst the other alkaloids may act as non-electrolytes. Since univalent cations and non-electrolytes usually inhibit insect "water cells" at much higher concentrations different mechanisms might be possible. Because of the total inhibition at a similar concentration range as calcium cations (1 mM), the four alkaloids might use a similar mechanism as bivalent cations. On the other hand, these compounds might directly affect the dendritic membrane disrupting normal cell function as already discussed for effects of colchcine and papaverine on neuron II/2.

5.2.1.4 Relationship between Neural Input and Behavioural Output

A possible correlation between neural input via neuron II/3 and behavioural output was analysed for the four feeding deterrent and neuron II/3 stimulating alkaloids berberine, nicotine, securinine, and strychnine. The concentrations (RC_{50}) evoking half-maximum response of neuron II/3 were compared. The neural input seems to be well correlated with the behavioural output. The RC_{50}-value of berberine was 4-fold higher compared to securinine. In the filter paper choice test *S. lamaninaus* also needed a 3-fold higher concentration of berberine compared to securinine to respond with an avoidance of the treated food source. In the case of strychnine, the RC_{50}-value was 5-fold lower compared to securinine. In the filter paper choice test strychnine elicited an avoidance of the treated food source at a 3-fold lower concentration than securinine. These results strongly suggest that chemosensory differences are reflected in the behavioural differences as predicted by the brain functioning model in insects proposed by Blom 1978a,b; and Schoonhoven & Blom 1988. In *S. lamanianus* feeding behaviour seems to be directly influenced by the activity of neuron II/3.

Kester et al. (2002) also showed in larvae of two *Manduca* species that chemosensory differences are reflected in feeding behaviour. Both, *Manduca sexta* and *M. quinquemaculata* are deterred in their feeding by nicotine. However, larvae of *M. sexta* showed a more vigorous response of their deterrent neuron to nicotine than *M. quinquemaculata*. This correlates well with the feeding behaviour. Larvae of *M. sexta* only

accepted feeding sites with low nicotine concentrations whilst *M. quinquemaculata* also accepted feeding sites with higher nicotine concentrations.

In contrast, in *S. lamanianus* behavioural output for nicotine can not be explained by neural input. The RC_{50}-value for nicotine was 4-fold higher than for securinine. Apparently, the neural input signal seems to be weaker for nicotine compared to securinine. In contrast, *S. lamaninaus* avoided treated food sources at nicotine concentrations 10-fold lower compared to securinine. Such a discrepancy between behavioural and chemosensory sensitivities was also observed by Messchendorp et al. (1998) in the Colorado potato beetle *Leptinotarsa decemlineata.* The authors tested the glucosinolate sinigrin and the terpenoid drimane. Sinigrin had a higher behavioural threshold than drimane although the chemosensory thresholds of the deterrent cell were *vice versa*. The authors explained this apparent discrepancy by the fact that drimane additionally inhibited the "sugar cell". This could lead to a stronger feeding deterrence signal in the CNS for drimane than sinigrin. A similar phenomenon was described in the blowfly *Protophormia terranovae* by Liscia and Solari (2000). The deterrent amiloride had a 10-fold higher behavioural threshold than quinine whilst the chemosensory thresholds were *vice versa*. Also in this case the authors explained this apparent discrepancy with the inhibition of the "sugar cell" by quinine resulting in a stronger feeding deterrence signal in the CNS. According to the brain functioning model in insects the information from receptor cells sensitive to feeding deterrents or phagostimulants are subtracted algebraically. Inhibition of phagostimulant neurons increases the role of the deterrence signal in the CNS (Blom 1978a, Schoohoven & Blom 1988). Water is considered as a phagostimulant in termites (Mikus 2000). The inhibition of water receptors might be a different mode of action increasing the deterrence signal in the CNS of termites.

However, in *S. lamanianus* nicotine had no additional effect on water receptors. Therefore, the apparent discrepancy for nicotine in *S. lamanianus* might be explained by other scenarios. **(i)** *S. lamanianus* might possess a second sensory input system for feeding deterrent compounds on the mouth parts. Taste sensilla on the mouth parts e.g. labial palps also seem to play a key role as they are also in close contact to the substrate during food search (Reinhard & Kaib 1995, Figure 1 in chapter 1.1). More than one deterrent receptor in different taste sensilla was described in *Manduca sexta* (Glendinning et al. 2002) and *Pieris brassicae* and *P. rapae* (Ma 1969, van Loon 1990). **(ii)** Nicotine might also have

additional effects on phagostimulant taste receptors on the mouth parts increasing the deterrence signal in the CNS compared to securinine.

Though papaverine clearly is a non-toxic feeding deterrent in *S. lamanianus* this alkaloid did not stimulate neuron II/3 or any other neuron in TP II or TP I sensilla. Hence, other neurons are likely to be involved in the perception of papaverine. Two scenarios might be relevant: One possible mechanism might be the inhibition of a phagostimulant neuron (Schoonhoven et al 1992). As mentioned above, water is a feeding stimulant in termites. Hence, the inhibition of the two "water cells" II/2 and I/1 may evoke feeding deterrence of papaverine. Higher salt concentrations also inhibit the activity of the water receptor cells and workers of *S. lamanianus* avoid higher salt concentrations in choice tests. (Mikus 2000).

On the other hand, further feeding deterrent neurons on the mouth parts could be involved in the feeding deterrence by papaverine as discussed for nicotine. This would also apply for the feeding deterrence of tryptanthrin. This alkaloid is clearly feeding deterrent and non-toxic in *S. lamaninaus* even at very low concentrations. But it did not stimulate neuron II/3 or inhibited the activity of water receptor cells. According to Schoonhoven et al. (1992) other possible mechanisms might be **(i)** the stimulation of broad spectrum taste neurons, **(ii)** the distortion of the sensory code or **(iii)** causing irregular impulse patterns ("bursts") in other taste neurons.

The lacking stimulation of any taste neuron by caffeine and colchicine may explain the high mortality both alkaloids caused in *S.lamanianus*. Most likely *S. lamaninaus* does not sense these two alkaloids at all. That might be the reason why termites were poisoned by both alkaloids in the filter paper no-choice test. The observed avoidance evoked by colchicine treated semi-circles in the filter paper choice test might be due to the inhibition of one water receptor cell (neuron II/2) by colchicine as this test was done with moist filter paper semi-circles. On blank filter papers a stimulation of neuron II/2 was possible increasing the likelihood that workers stayed there. Caffeine may have some unspecific effects at the high concentrations needed to elicit avoidance of a treated food source.

5.2.2 Recognition of Non-Alkaloids

5.2.2.1 Relationship between Neural Input and Behavioural Output

The non-alkaloids azadirachtin, juglone, nootkatone, and sinigrin did not stimulate neuron II/3 nor any other taste neuron in TP II or TP I sensilla in a concentration dependent manner. Evoked maximum excitation levels were not distinguishable from the response to water alone. This indicates that only the "water cells" II/2 and I/1 were stimulated. Furthermore, no inhibition of these "water cells" could be observed. Chrysin and nomilin could not be tested as these compounds were not soluble in appropriate amounts neither in water nor 10% ethanol.

Chrysin, nomilin and partly azadirachtin and nootkatone were non-toxic feeding deterrent compounds in *S. lamanianus.* As discussed for the alkaloids nicotine, papaverine, and tryptanthrin, a second sensory input system for feeding deterrents (mouth parts) can also be proposed. Furthermore these compounds may act via the inhibition of phagostimulant taste neurons e.g. on the mouth parts. Ohmura et al. (2006) showed in the termite *Zootermopsis nevadensis* that azadirachtin inhibited the response to a phagostimulant in taste hairs of the labial palps. Other scenarios might also involve additional mechanisms, which have been already discussed for the alkaloids papaverine and tryptanthrin.

The findings that juglone did not stimulate any taste neuron on the antennae but termites refused to enter the test arenas without any previous substrate contact indicates that juglone might be detected rather by olfactory than taste neurons. Abushama (1966) and Floyd et al. (1976) showed that repellent odours evoke excitations of antennal olfactory receptor cells in the termites *Zootermopsis angusticollis* and *Reticulitermes lucifugus*, respectively. Hence, juglone needs further investigation regarding olfactory receptors.

Sinigrin stimulates deterrent neurons in the Colorado potato beetle *Leptinotarsa decemlineata* (Messchendorp et al. 1998) and in the two moth species *Mamestra configurata* and *Trichoplusia ni* (Schields & Mitchell 1995a,b). However, it did not stimulate any taste neuron in TP II or TP I sensilla of *S. lamanianus.* This might explain the increased mortality in the filter paper no-choice test. *S. lamanianus* may not sense sinigrin at all as already discussed for the alkaloids caffeine and colchicine.

5.2.3 Relevance of Neuron II/3 in the Termite *S. lamanianus*

The activity of neuron II/3 negatively influences feeding behaviour in *S. lamanianus* leading to the avoidance of unpalatable food sources or reduced food consumption. Furthermore, as neuron II/3 only responded to various alkaloids it is rather a "specialist deterrent receptor" than a "general deterrent receptor" according to van Loon and Schoonhoven (1999). The authors demonstrated that larvae of *Pieris brassicae* possess two kinds of "deterrent receptors". Besides their "general deterrent receptor" responding to a broad spectrum of secondary compounds, larvae have also a "specialist deterrent receptor" responding exclusively to cardenolids. Furthermore, the broad sensitivity of deterrent receptors in insects seems to be part of a general sensitivity to particular classes of compounds to which these receptors respond. Hence, natural selection may increase or reduce sensitivity to classes of compounds, but not to individual chemicals (Schoonhoven et al. 1992). This might have led in *S. lamaninaus* to the development of an *"*alkaloid cell*"*. Since neuron II/3 did not respond to all alkaloids it seems likely that the "alkaloid cell" might be adapted to certain groups of alkaloids abundant as secondary compounds in plants being frequent in the termites' environment.

5.3 Conclusion

In termites, feeding inhibition by secondary plant compounds is a very complex process. Including neurophysiological investigations into studies about feeding deterrence in termites might be a helpful approach for the improvement and development of wood preservatives and novel termite control and management systems. The results of the present study suggest systematic investigations of the modality and specificity of taste receptor cells in termites to get a better overview of the chemosensory inventory in this ecologically and economically important group of insect pests. Systematic neurophysiological investigations of the chemosensory inventory in termites would provide information how the neural input system triggers the behavioural output e.g. in terms of food choice and food consumption. Information on structure-activity relationships could increase the efficiency in the search for antitermitic secondary compounds in plants.

6 Summary

In the present study, the influence of plant-derived secondary compounds on feeding behaviour in the subterranean termite *Schedorhinotermes lamanianus* was investigated. Furthermore, the chemosensory input system responsible for the perception of these compounds was investigated using electrophysiological methods.

The obtained results provide evidence that in *S. lamanianus* a variety of structurally diverse secondary plant compounds (alkaloids and non-alkaloids) other than securinega-alkaloids influence feeding behaviour. These compounds evoke an avoidance of food sources or lower food consumption under choice conditions even at lower concentrations obtained for securinega-alkaloids. Furthermore, these compounds also reduce feeding under no-choice conditions. Termites seem to ingest less food even when they started feeding and no alternative food source is available. Therefore these compounds act as repellents and feeding deterrents in *S. lamanianus* depending on the test conditions under which they are applied.

Furthermore, the present study provides strong evidence that different proximate mechanisms explain feeding inhibition in *S. lamanianus*: 1) Twelve structurally very diverse alkaloids, including feeding deterrent alkaloids in *S. lamanianus*, stimulated the taste neuron II/3 in TP II sensilla on antennae of this termite species. Non-alkaloids did not stimulate neuron II/3. Therefore, this neuron II/3 is an "alkaloid cell" negatively influencing feeding behaviour in this termite. 2) Feeding inhibition seems also to be influenced by the inhibition of phagostimulant taste neurons ("water cells") on antennae. 3) A second sensory input system for the perception feeding deterrent plant-derived secondary compounds seems to be evident as some tested compounds (alkaloids and non-alkaloids) are clear antifeedants in *S. lamanianus* but do not influence feeding behaviour by the former two mechanisms.

Hence, in termites feeding inhibition by secondary plant compounds is a very complex process which needs further investigation. Including neurophysiological investigations of the chemosensory input system seems to be a promising approach to better understand feeding inhibition in termites which may lead to improved wood protection and termite management.

7 Zusammenfassung

In der vorliegenden Arbeit wurde der Einfluß pflanzlicher Sekundärstoffe auf das Fraßverhalten der Termite *Schedorhinotermes lamanianus* untersucht. Weiterhin wurde das sensorische Eingangsystem zur Wahrnehmung dieser Substanzen mittels elektrophysiologischer Methoden untersucht.

Die Ergebnisse der vorliegenden Studie zeigen, daß eine Reihe strukturell sehr unterschiedliche Sekundärstoffe (Alkaloide und Nicht-Alkaloide) das Fraßverhalten von *S. lamanianus* beeinflussen. Unter Wahlbedingungen zwischen behandelten und unbehandelten Futterquellen meiden Termitenarbeiter behandelte Futterquellen oder zeigen reduzierte Nahrungsaufnahme. Termitenarbeiter zeigen auch eine reduzierte Nahrungsaufnahme, wenn sie ausschließlich auf einer behandelten Futterquelle fressen können. Daher fungieren diese pflanzlichen Sekundärstoffe als Schreckstoffe (Repellenzien) oder Fraßhemmer für *S. lamanianus* abhängig von den Testbedingungen, unter denen diese Substanzen appliziert werden.

Die Untersuchungen zum sensorischen Eingang zeigen, daß bei *S. lamanianus* verschiedene proximate Ursachen die Fraßinhibition durch pflanzliche Sekundärstoffe erklären: 1) Zwölf der getesteten strukturell sehr unterschiedlichen Alkaloide erregen den Geschmacksrezeptor II/3 im TP II Sensillum auf den Antennen von *S. lamanianus*. Die getesteten Nicht-Alkaloide erregten diesen Rezeptor nicht. Daher stellt dieser Geschmacksrezeptor einen "Alkaloid-Rezeptor" dar, dessen Aktivität das Fraßverhalten von *S. lamanianus* negativ beeinflußt. 2) Die Ergebnisse deuten weiterhin darauf hin, daß die Hemmung des Fraßverhaltens von *S. lamanianus* auch durch Hemmung fraßstimulierender Rezeptoren erfolgt. Die Aktivität von Wasserrezeptoren auf den Antennen wirkt fraßfördernd bei Termiten. Fraßhemmende Alkaloide, welche den Geschmacksrezeptor II/3 nicht erregten, hemmten aber Wasserrezeptoren auf den Antennen von *S. lamanianus.* 3) Einige der fraßhemmenden Sekundärstoffe, sowohl Alkaloide als auch Nicht-Alkaloide, erregten weder den Geschmacksrezeptor II/3 noch hemmten sie Wasserrezeptoren. Daher wird postuliert, daß es mindestens ein zweites sensorisches Eingangssystem z.B. auf den Mundwerkzeugen zur Wahrnehmung dieser Substanzen geben muß.

Die Beeinflussung des Fraßverhaltens durch pflanzliche Sekundärstoffe ist ein sehr komplexer Prozeß bei Termiten, der weitere intensive Forschung benötigt.

Neurophysiologische Untersuchungen des chemosensorischen Eingangssystems scheinen ein vielversprechender Ansatz zu sein, um ein besseres Verständnis der Fraßhemmung durch pflanzliche Sekundärstoffe bei Termiten zu erhalten. Dies könnte zur Verbesserung und Entwicklung geeigneter Holzschutzmittel und biologischer Kontroll- und Managementsysteme gegen Termiten beitragen.

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9 Appendix

Supplementary Data of the Filter Paper Choice Test

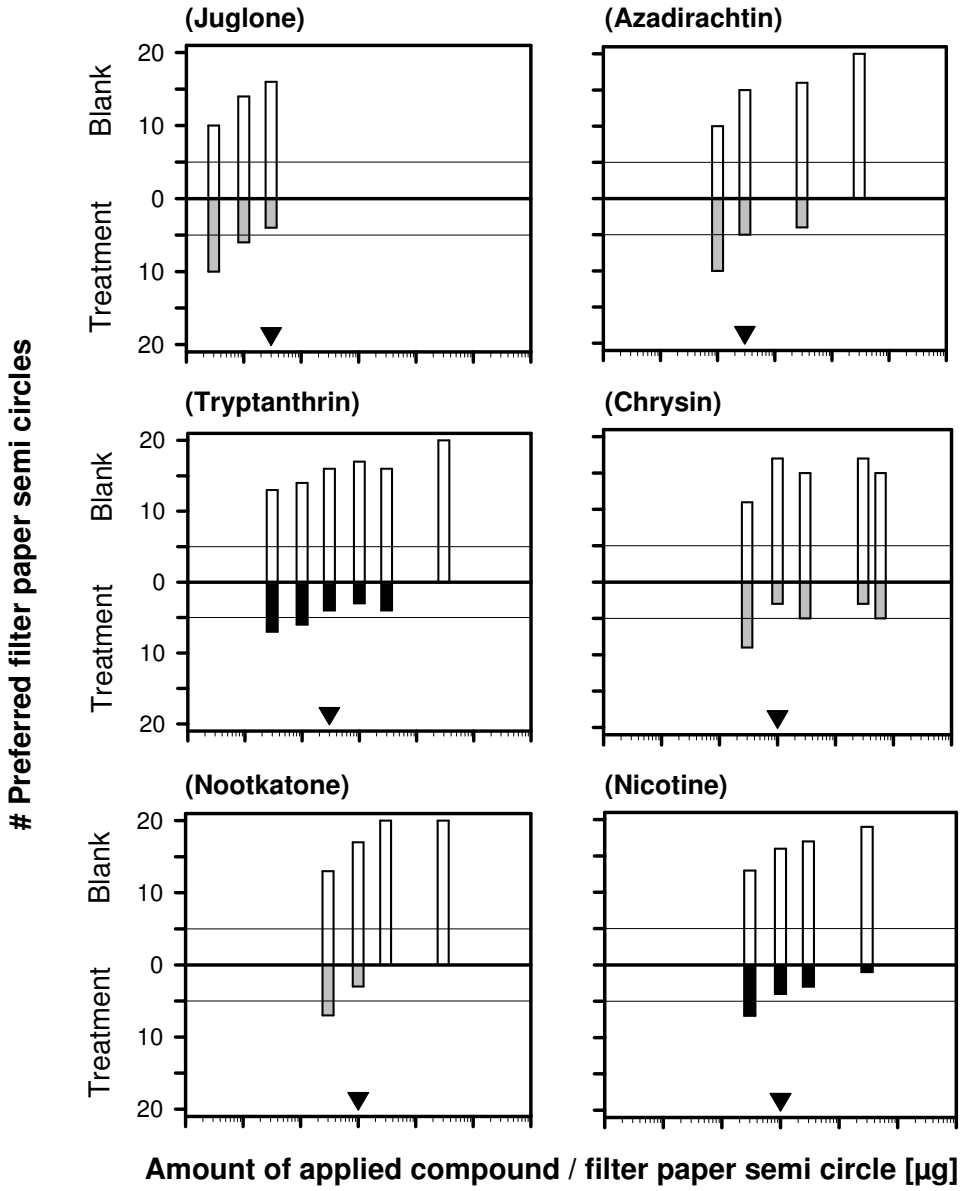

Figure A - I: Number (#) of preferred non-impregnated ("Blank" □) and impregnated ("Treatment") filter paper semi circles for different non-alkaloids (□) and alkaloids (■) in the filter paper choice test. Significant differences were obtained using the sign-test. The upper hairline indicates for $P \leq 0.05$ the critical border for a significant preference of the impregnated semi circles (# "Blank" ≤ 5) and the lower one for a significant avoidance of the impregnated semi circles respectively (# "Treatment" ≤ 5). The compound specific threshold (CST) is indicated by ▼.

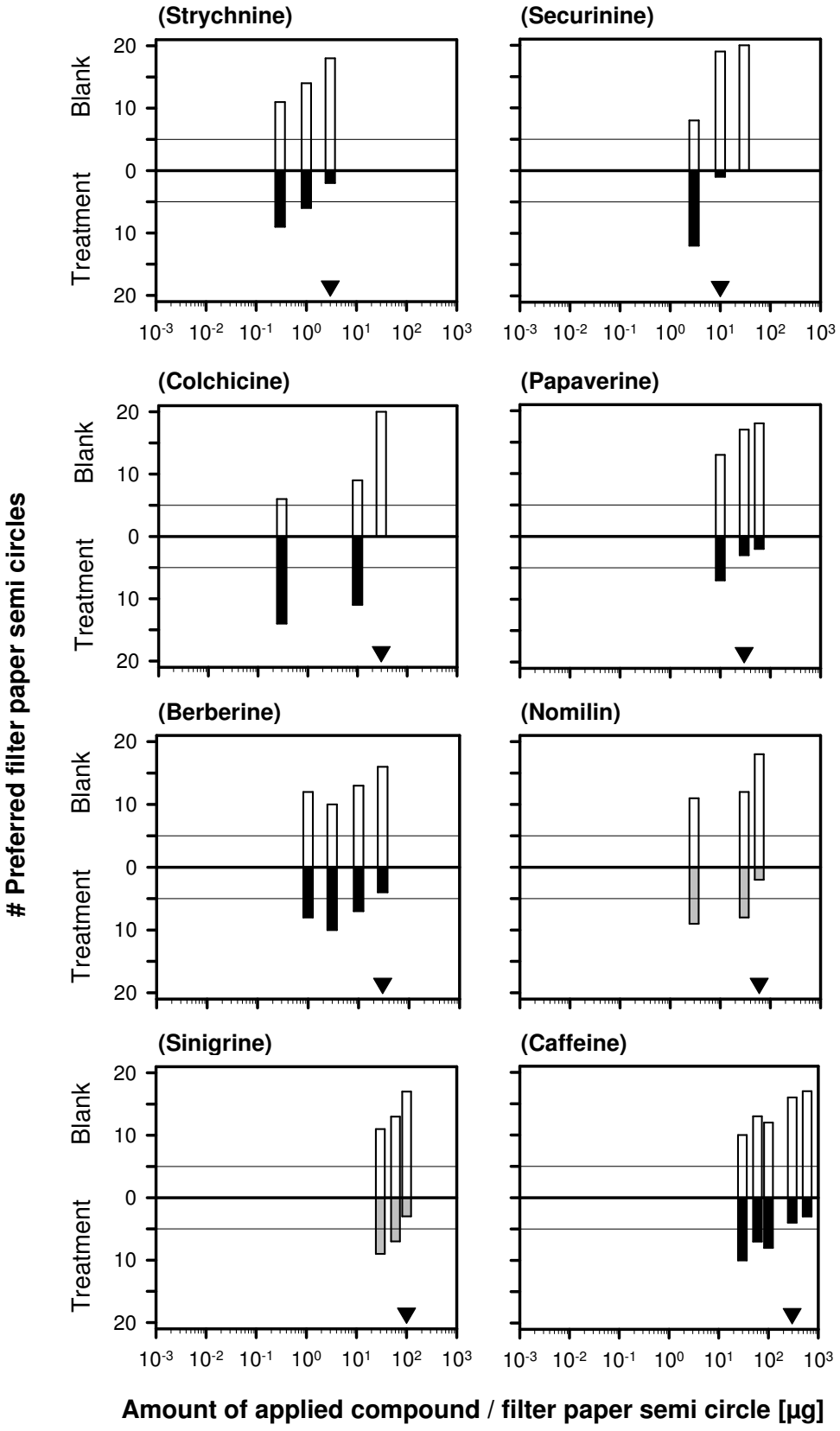

Figure A - I (continued)

Erklärung

Hiermit erkläre ich, daß ich die Arbeit selbständig verfaßt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Ferner erkläre ich, daß ich anderweitig mit oder ohne Erfolg nicht versucht habe, diese Dissertation einzureichen. Ich habe keine gleichartige Doktorprüfung an einer anderen Hochschule endgültig nicht bestanden.

Bayreuth, den _____ _____

Stefan Groß