CHAPTER 2

LITERATURE REVIEW

2.1 Biology of tree species in interaction with their insect pests

The description of the tree species examined in this thesis is meant to provide a general background of the biology, growing conditions, properties and uses of those species, describing the interactions and stages of the considered insect pest. Each of the species are well known and widely distributed urban tree species which enhances their pest problems to a larger scale of consideration.

2.1.1 Oak tree with Oak processionary moth

The Oak trees considered are of the species *Quercus robur* L., which are native to most of Europe, Anatolia, Caucasus and parts of North Africa. *Q. robur* is a large deciduous tree reaching heights of 25–35 m in average and circumferences exceeding other tree species reaching four meters to exceptional twelve meters. The species is known for its hard and durable timber and counts as one of the most common and important deciduous forest species with attributes for many desired uses. Construction, furniture, toys, firewood with high calorific value and even for the barrels of the “Barrique wine”, a special treat, this high quality timber is expedient. The hard and durable wood allows the trees to reach ages up to around 1000 years, even in Europe’s temperate and humid climate.

The leaves are lobed, nearly sessile and very distinct. *Q. robur* is also valued for its importance to insects and other wildlife. Numerous insects live on the leaves, the fine bark of the twigs, the nutritious buds and fruits, which are called acorns and shown in Figure 2.1a-c.

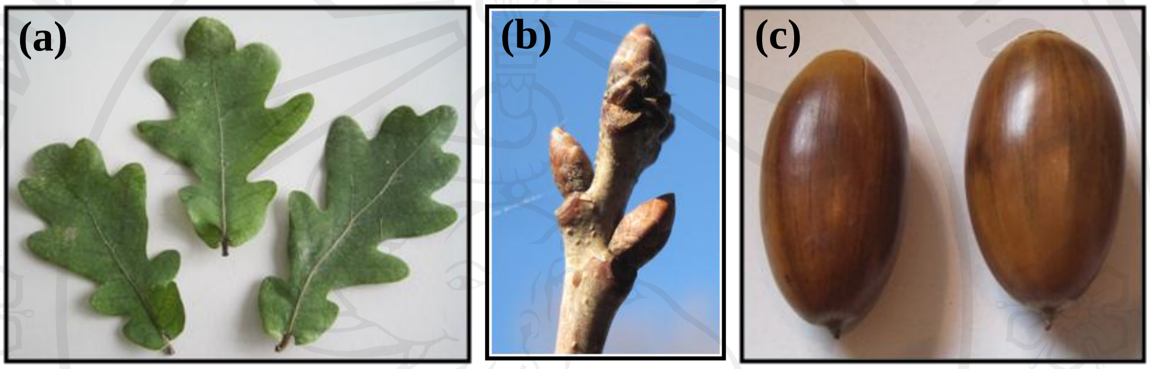

Figure 2.1: Oak tree leaves (a), Oak tree buds (b) and Oak tree seeds (c)

With more than 400 species, *Quercus ssp.* supports the highest biodiversity of insect herbivores of any European plant genus. The acorns ripen in the autumn and are known to be a favored food source for many wild animals, often small mammals and birds. Jays and squirrels are known to assure distribution of the acorns, and in doing so propagate this species. Besides growing *Q. robur* in temperate forests as high value timber, the cultivation as an ornamental tree is also a common practice in most continents. A wide number of cultivars are grown in gardens, parks, alleys, boulevards, arboreta and botanical gardens.

The Oak processionary moth (OPM) *(Thaumetopoea processionea)*, called after it´s habit to march head to tail building long lines (processions), was first mentioned in Germany over 175 years ago, disappeared for a while before reentering after a mass outbreak in Austria in the 1920s.

Increased occurrence in the mid 90s and later, especially in 2003, OPM became negatively famous, after warm dry summers assured their expansion and many human victims got terrified, having had firsthand experience with the effects of their poisonous hairs.

These hairs are contaminated with a toxin called thaumetopoein or closely related compounds, causing epidemic caterpillar dermatitis manifested as allergic skin reactions, serious eye infections and, if inhaled bronchial irritations like pharyngitis and respiratory distress, including asthma or anaphylaxis. Caterpillar with poisonous hairs and moving habit is shown in Figure 2.2a-c.

Figure 2.2: Single Oak processionary moth (OPM) (a) and in groups (b, c)

Global warming and climate change are sited to be the driving factors to help propagate these thermopile insects. Now well established in most parts of Germany, OPM generally used to be native to central and southern Europe and considers oak trees as their principal host.

OPM hibernates during the winter as egg batches attached on the twigs, from where during late March and onwards the larvae emerges and start to feed on the leaves (see Fig. 2.3a). These first stage larvae are the primary targets for insecticide applications, if observed in time. Larvae development takes 9 to 12 weeks depending on weather conditions and passes through six instars during their feeding activity while getting progressively bigger.

Preventive action should be done from instar 1 to 3, as feeding activity and mobility is high and goes along with susceptibility to insecticides.

Larvae habit changes during instar 4 to 6, where larvae gets more sedentary and therefore protected in their silken nests, develop their poisonous hairs and eventually moult to the pupal stage. In this stage most larvae has returned from the branches and twigs and settled in the nest (see Fig 2.3b), which opens abatement opportunities again.

Figure 2.3: OPM feeding pattern on Oak leaves (a) and OPM nest with larvae(b)

Manual removal and destroying of larvae and their nests are then main control measures, requiring trained and skilled professionals accompanied with special equipment. Flaming, vacuuming and conglutinating before hand-picking of OPM nests are the most frequently used methods. Even done with much care and use of protective equipment, adhering hairs and cast skins may inevitably contact the operators on uncovered skin areas. These manual methods of OPM population reduction are as effective as cost intensive.

Emergence of adult OPM moths happens from mid July to early September, were males as strong flyers can be caught and monitored in female pheromone baited traps.

2.1.2 Sycamore tree with Sycamore lace bug

The Sycamore trees considered are of the species *Platanus x hispanica*, which is a synonym for *Platanus x acerifolia*, thought to be hybrids of *P. orientalis* and *P. occidentalis*. With heights up to 50 m, canopy diameters of about 35 m and massive trunks and branches the deciduous Sycamore tree impresses with quite remarkable sizes. Together with its *Acer* (Maple) shape looking leaves it is the bark, falling of the trunk of older trees in yellowish-green-brown plates every year, which distinguishes this species clearly from others.

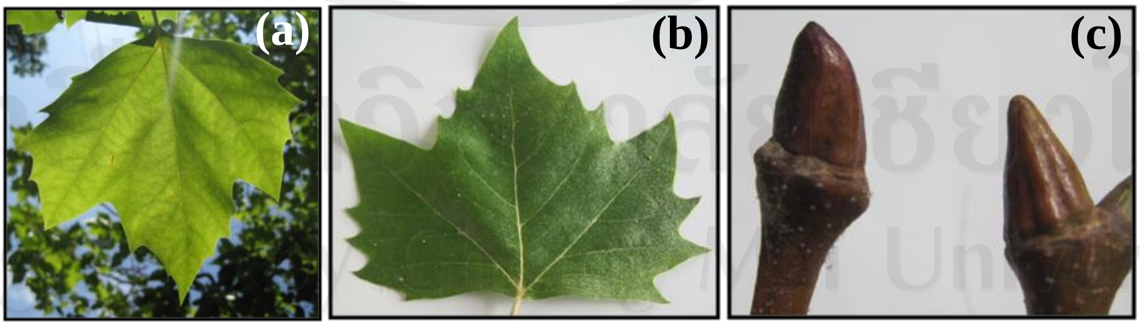

Figure 2.4: Sycamore tree leaf (a, b) and Sycamore tree buds

The flowers and later fruits of the Sycamore trees are aggregated and brown and grow in dense, globular and unisex capsules of about 3 cm. Birds like finches and squirrels appreciate those as nutritious food source.

Properties like high tolerance against salt, wind, atmospheric pollution and root compaction, while standing strong winter frost made this species a highly recommended and widespread urban tree species. When first introduced, not too many pathogens were known to be associated with this species, which contributed to the current situation, that Sycamore trees can now be found quite commonly in most cities throughout the temperate regions of the world.

However, there are also a number of problems affiliated with this species, notably the short stiff hairs shed by young leaves and dispersing seeds which may cause breathing difficulties, especially for people with asthma.

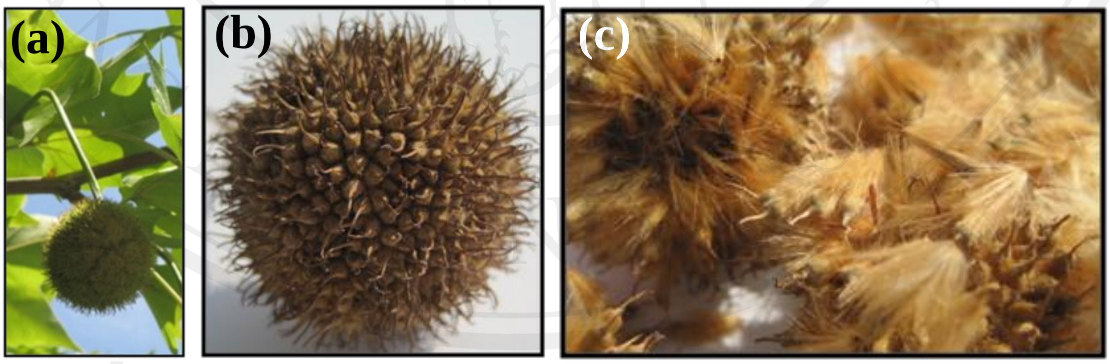

Figure 2.5: Aggregated Sycamore seeds (a, b) and dispersed seeds (c)

Problematic as well are the large and durable leaves, which rot slowly and create disposal problems and often the enormous growth, which reaches its limitation in a space zoned urban environment.

Counteracting these problems alternative approaches of pruning techniques, called pollarding, drastically reduces the tree canopy on a frequent basis. This distinct and Mediterranean looking shaped tree found its advocates, but comes along with high management costs.

A common pest on Sycamore is the leaf mining moth *Phyllonorycter platani* (STAUDINGER). Frequently observed in central Europe is the fungus *Apiognomonia veneta*, SACC and SPEG. (Syn. *Gnomonia platani*) which causes anthracnose on *P.x hispanica* and more common in southern Europe, the fungus *Ceratocystis fimbriata* ELLIS and HALST, causes Sycamore-cancer.

In recent years the threat of a fungus named Masaria (*Splanchnonema platani*, BARR) causing strong and healthy looking branches to break off, scatters doubt on the broad use of this species by endangering human health and property walking or driving below.

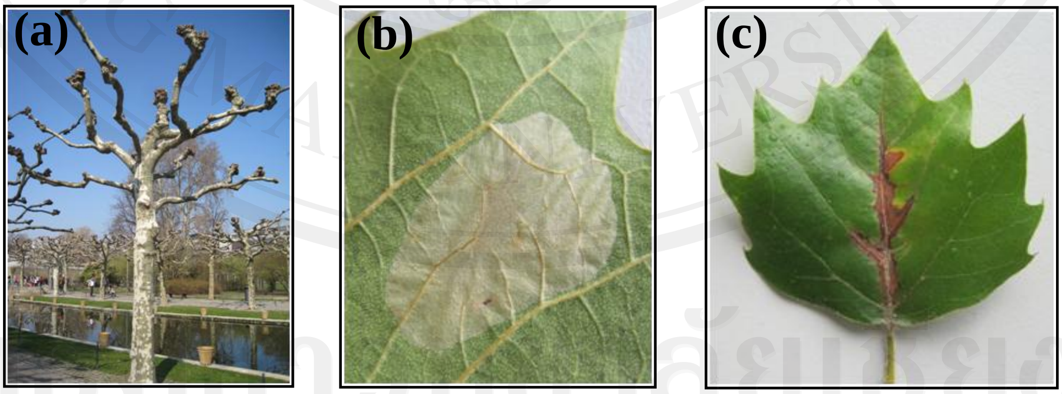

Figure 2.6: Pollarded Sycamore tree (a), leaf mine of *A. platani* (b) and *A. venata* (c) on Sycamore leaves

In the 1960s the Sycamore lace bug (SLB) (*C. ciliata*) was introduced in southern Europe, presumably via international goods shipped into Italian ports coming from North America and Canada. In only a few decades the insect spread across borders into neighboring countries reaching central Europe, were it still expands and now is well established.

The SLB is parasitic and monophagous on Sycamore trees, sucking sap from the leave cells. The bug is a neozoon with invasive tendencies. SLB are relatively small in size reaching 3 to 4 mm while showing a mesh-like patterning, which is characteristically found on bugs of the tingidae family. Adult bugs as well as up to three generations per year feed on plant sap from the lower side of the leaves.

The white stippling of the leaves caused through the sucking SLB may eventually progress into chlorotic foliage and cause premature senescence of leaves. Accompanied with severe SLB infestation, defoliation of trees may occur in late summer.

Usually SLB hybernate under loose plates of the bark, or in nearby cracks. SLB are extremely cold tolerant, hemimetabolous capable flyers and through wind transportation quite mobile (MACELJSKI, 1986). After mating, oviposition happens on leaf surface, whereas eggs are placed in patches along the leaf veins. The first three instars remain as a group on the place of oviposition, before scattering in the later instars.

Native antagonists are not yet established in adequate numbers to regulate the massive occurrence of SLB, even more as they use their setae to repel enemies with special exudates.

Public opinion suggests, that these exudates are capable of causing irreversible marks on the varnish of cars and itchiness accompanied with skin irritation on humans. Generally it is the mass appearance of SLB with the effect of bugs being bushed in people´s hair-do, ears, drinks and meals, which annoys the majority of the displeased crowd.

Amongst their natural predators are kissing bugs, mites, spiders, and some entomopathogenic fungi which do reduce their population some, but not yet to any desirable degree.

The introduction of foreign predators and antagonists is controversially discussed, since as it is for the SLB, no natural regulation mechanisms will work to effectively control population density of this newly introduced neozoon.

Two fungi are associated with SLB in North Italy, which are *Ceratocystis fimbriata* and *Gnomonia platani*, which, in combination with the lace bug, may cause decline and death of the trees. It is suspected that the lace bugs may serve as vectors for these fungi (MACELJSKI, 1986). Considering the practical impact of occasional late-season defoliation caused by the sucking insects, has on otherwise healthy sycamore trees no lasting effects and therefore only aesthetic character.

2.1.3 Horse chestnut tree with Horse chestnut leafminer

The tree species considered is of the species *Aesculus hippocastanum*, a nowadays widely cultivated species, originated in southeast Europe, including places like northern Greece, Albania, Macedonia, Serbia and Bulgaria. The large deciduous tree reaches up to 35 m in height and usually divides into several secondary trunks after a few meters. That's why the canopy shape generally appears domed from a distance, with pendulous outer branches and upward twisted tips. With minor importance as a forestry species, *A. hippocastanum* is a popular ornamental tree with the characteristic of twisted trunk growth, shown in Figure 2.7a and b.

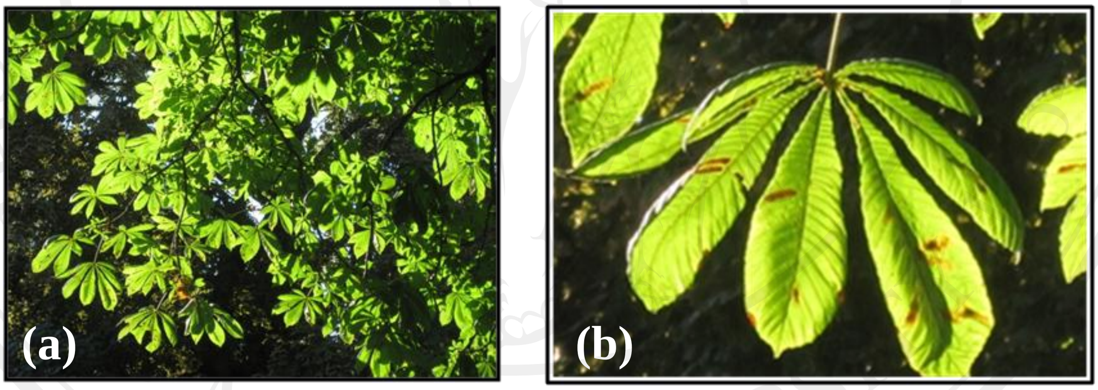

Figure 2.7: General leaf setting (a) and single leaf shape (b) of Horse chestnut

The distinct leaves are palmately compound in shape, showing 5 to 7 leaflets each and spread up to 60 cm across. In blossom the tree lightens a bright white shower of 20 to 50 flowers per panicle, on which the fruit develops in autumn.

Those shiny nut-brown Horse chestnuts are also called conkers, measuring 2 to 4 cm in diameter. Shoots of the Horse chestnut trees are stout and have a sticky and shiny reddish-brown bud, shown in Figure 2.8a-c.

Figure 2.8: Horse chestnut buds (a), seed capsule (b) and seeds (c)

Successful cultivation is possible in a wide range of climatic conditions, tied to the requirement of decent temperatures in the summers. Plant pathogens and pests are often the ones determining the end of a life cycle of this species.

It may be bacteria causing bleeding canker, fungi like guignardia leaf blotch *(Guignardia aesculi,* PECK STEWARD*)*, or *Armillaria mellea,* (VAHL) P. KUMM, and *Ganoderma* ssp., which are fungi causing root or wood rot, *Phytophthora* ssp., which are as aggressive as destructive plant pathogens, or scale insects like *Pulvinaria regalis,* CANARD or the Horse-chestnut leaf miners, (*C. ohridella*) who weakens and in consortium with other primary and secondary pathogens may finally finish up with those trees at their location.

The Horse-chestnut leaf miner (HCL) (*C. ohridella*) (Figure 2.9a, b), was first noticed in Macedonia in 1984, even though already collected and pressed in herbaria sheets by T. HELDREICH in 1879 and described as a new species by DESCHKA and DIMIC in 1986, and has now spread over mainland Europe. *C. ohridella* is of the order of Lepidoptera (butterflies) and the family of Gracillariidae (mining moths).

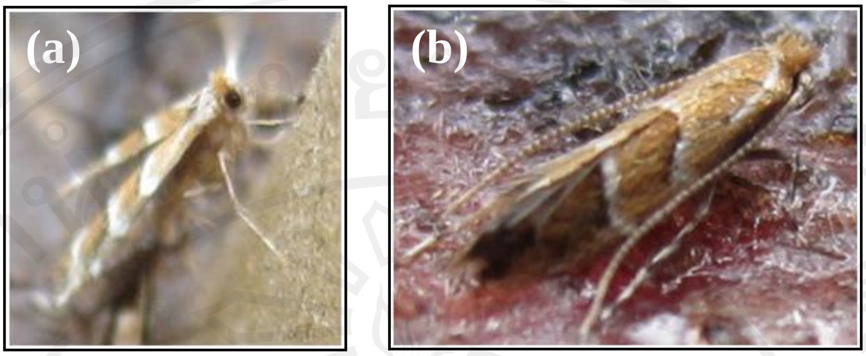

Figure 2.9: Horse chestnut leafminer moth (a, b)

HCL invades the white flower Horse chestnut trees and is apparent mainly through the constantly growing leaf mines (Figure 2.10a-c), which in years of high population density, may eventually fill up the whole leaf surface. This causes significant damage to the summer foliage, whereas the infestation develops from the lower to the upper canopy. The leaves turn brown, start to wilt and fall of the tree starting in late summer.

Figure 2.10: First instar leaf mine (a), older and growing leaf mine (b) and final stage leaf mines (c) of HCL covering high percentages of leaf surface

This so called "late summer browning" and foliage lost of Horse chestnut trees does not necessarily damage the trees permanently, but may affect tree performance and vitality, which in consequence, may attract secondary plant pathogens. HCL has now become a major problem on Horse chestnut trees in urban areas.

The larvae hibernate in the fallen leaves, hatches in the spring and flies for around three weeks in the form of imagines. The moth itself measures around 5 mm and lays eggs (approx. 30) singly on the upper surface of the leaves, from where the 1st instar hatches after two to three weeks.

The larval stages, described by five feeding phases or five instars, in which the mine is progressively getting bigger, and two prepupal phases, in which the larvae spins their pupal nest before the final pupal phase. Dependent on circumstances like the weather conditions the pupa may hibernate in the mine or finish development of one cycle and hatch as adult moth after around two weeks.

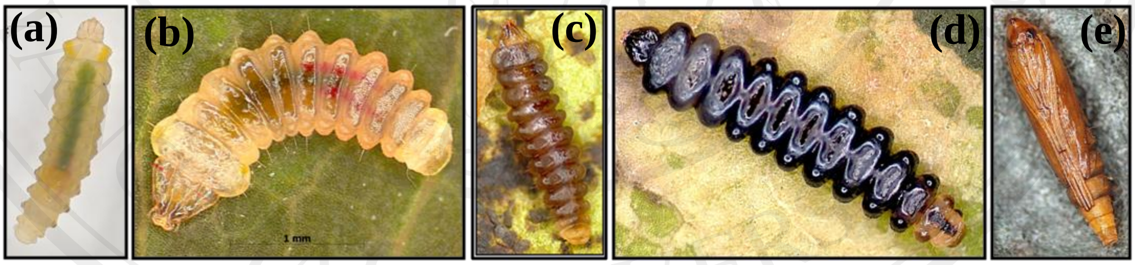

Figure 2.11: First instar larva of HCL (a), later instars of HCL larvae (b, c), fifth and last instar of HCL (d), and pupal stage of HCL (e)

Up to three generations per year of HCL, dependent on weather conditions, are commonly observed in central Europe. Consequences of HCL infestation are leaf damage through the mines, early leaf fall, stress symtomes like distress flowering in autumn, reduced augmentation, low seed weights, formation of secondary canopy and occurence of secondary pathogens, (LTZ, 2010).

Observed most commonly on *A. hippocastanum*, HCL may also occur on *Aesculus pavia*, *Acer platanoides* and *Acer pseudoplatanus*. More or less resistant varieties are the red-flowering Horse chestnut trees, e.g. *Aesculus x carnea ´Briotii´*.

Quite similar to the leaf mines of HCL which appear as dead brown tissue patches are the signs of the fungus *Guignardia aesculi*, called Guignardia leaf blotch. It is distinct from HCL by its conspicuous yellow band, more laminar appearance and the tendency of trespassing leaf veins more commonly.

De-icing salt causes also a similar syndrome, but appears on the leaf edge and is a man-made problem along roadsides.

The pupae of HCL may be mistaken with pupae of *Phyllonorycter* ssp., but HCL does not have a cremaster, but strong lateral spines at the first five abdominal sections, which the *Phyllonorycter* ssp. do not.

Amongst the most effective predators of HCL are the tits, namely blue tits (*Parus caeruleus*, L.), great tits (*Parus major*, L.) and marsh tits (*Parus palustris*, L.), which all feed on the larvae. Another possible feeder on HCL is the southern oak bushcricket (*Meconema meridionale*, COSTA), but altogether are the antagonists not yet established well enough, to really capably regulate peaking HCL population levels.

In recent years several approaches to control HCL have been under observation. Different chemical formulations showed effectiveness, but eradicated HCL antagonists as well while being cost intensive.

Furthermore there is no accreditation for such formulations yet available. Researchers count on establishment of effective antagonists in the future to control HCL.

The substitution of *A. hippocastanum* with plantings of other species of the *Aesculus* family, which are not infested are recommended, to reduce pest population and infestation levels of HCL. Early leaf fall of *A. hippocastanum* caused through HCL and their mining activity can be counteracted with leaf collection and displacement of leaves, which assures significantly reduced pest population the following year. If done as soon as leaf fall appears, it may even reduce autumn leaf damage, allowing energy reserves to be stored within the tree.

The precondition for this to happen is accurate leaf removal during the year (BBA, 2003). Composting of fallen leaves may not be recommendable, since the needed temperature of min. 40°C may not be reached fast enough to kill HCL.

The control of HCL with synthetically insecticides is not possible, since no formulations are currently registered for public use. The whole tree application to these often tall and spreading trees is very time-consuming and costly (LTZ, 2010).

Therefore the clearing of all Horse chestnut leaves combined with their burning to eliminate all pupae is the most practicable method nowadays, to reduce pest infestation levels of HCL. But again, the effort expressed in time, labor and finance to do so, may once again exceed the budget of most administration authorities.

2.2 Nature and origin of the used Neem compound

2.2.1 The Neem tree

Azadirachta indica, (A. JUSS), is a well known species in India and neighboring countries for more than 2,000 years. Even though the tree has been used in Ayurvedic and Unani medicine for a long time, which is expressed in its Sanskrit name Arishtha, meaning "reliever of sickness", no wider publicity and convincing experiments have been performed to reveal the hidden capacity of this species untill quite recent. In "The Neem Tree", does SCHMUTTERER H. compile a 30year research on the multiple talents of this species published in 1995. "Neem – a tree for solving global problems!?"

Figure 2.12: Adult Neem tree (a), re-sprout growth (b) and leaves of Neem (c)

The Neem tree is considered as a multipurpose tree, and is next to its various medicinal uses, highly appreciated for its durable lumber, good quality firewood, enormous ecological amplitude and re-sprouting capacity (Schmutterer, 1995).

The Neem tree (*A. indica*), is a member of the mahogany family *(Meliaceae)* and the provenance of the seed kernels of which the product NeemAzal-T/S is derived. Presumably originated in India and Pakistan it is a tree of tropical and semi-tropical regions with a high degree of drought tolerance, growing in various soils and now introduced and established mainly in dry areas throughout the tropics and sub-tropics. Neem trees are popular for their shading capacity which explains the common plantations along many road sides.

The Neem tree is described as a fast growing species reaching heights of up to 25 m, evergreen if not under too much drought stress and with a habitus showing a roundish but dense canopy (SCHMUTTERER, 1995).

The olive like looking drupe-fruit of the Neem tree has a smooth yellow-greenish skin, and is elongated oval to round. Usually one, seldom up to three of the brown and elongated seeds are enclosed in the white and hard inner shell, which is surrounded by the mesocarp, an up to 5 mm thick fibrous bitter-sweet pulp (Figure 2.13a and b).

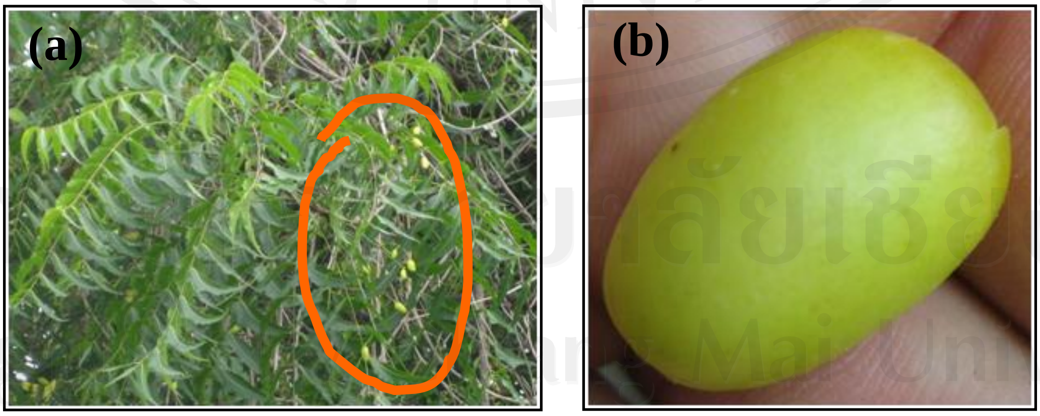

Figure 2.13: Dispersed fruiting panicle (a) and single Neem fruit (b)

During the past decades the biological activity of Neem extracts has been investigated intensively, and several international Neem conferences have been held (SCHMUTTERER *et al*., 1981; SCHMUTTERER and ASCHER, 1984, 1987), covering mainly the application of crude extracts in laboratory and field tests.

In general, extracts of Neem fruit, seeds, seed kernels, twigs, stem bark, and root bark have been shown to possess insect antifeedant, insecticidal, insect growth disrupting, nematicidal, fungicidal (JACOBSON, 1989; RAND-HAWA and PARMAR, 1993; SCHMUTTERER *et al*., 1981; SCHMUTTERER and ASCHER, 1984, 1987), bactericidal (ARA *et al*., 1989), anti-inflammatory (DHAWAN and PATNAIK, 1993; FUJIWARA *et al*., 1982, 1984), antitumor (FUJIWARA *et al*., 1982, 1984), immunostimulating (VAN DER NAT *et al*., 1987, 1991), and other (RANDHAWA and PARMAR, 1993) activities.

Since the early investigations by SIDDIQUI (1942) more than 100 compounds have been isolated from various parts of the Neem tree and several reviews on constituents of Neem (CHAMPAGNE *et al*., 1992; DEVAKUMAR and SUKH DEV, 1993; JONES *et al*., 1989; KOUL *et al*., 1990; LEE *et al*., 1991; SIDDIQUI *et al*., 1988; TAYLOR, 1984; WARTHEN, 1979, 1989) have been published to date.

2.2.2 The active ingredient

Azadirachtin is a secondary metabolite present in Neem seeds. This chemical compound belongs to the limonoid group and its complex molecular structure is described as a highly oxidized tetranortriterpenoid.

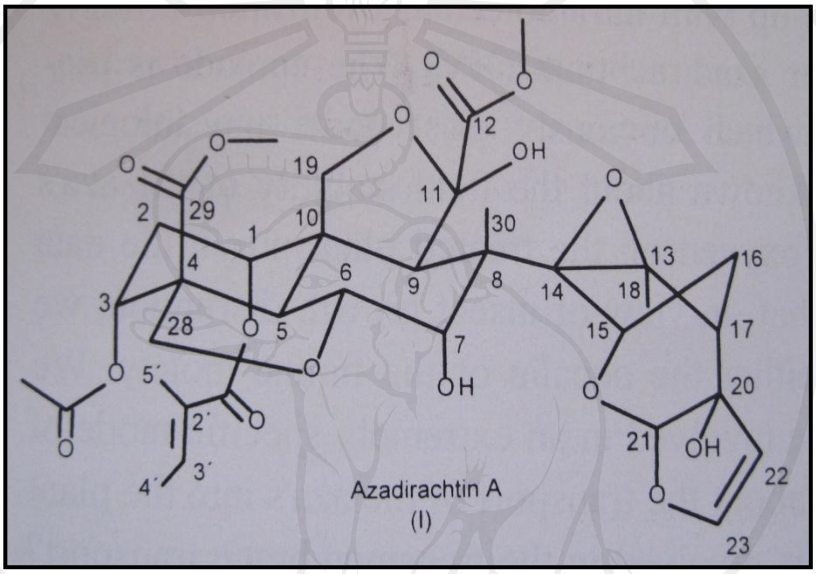

Figure 2.14: Structural formula of Azadirachtin A

Azadirachtin, obtained from the seeds kernels of *A. indica*, has proven to be one of the most promising plant ingredients for integrated pest management (IPM) at present time. Its nature and effects on insects are described as a phago- and oviposition deterrent, repellent, antifeedant, growth-retardant, molt-inhibitor and sterilant, while disturbing vital physiological processes in insects.

Considering toxicological and ecological aspects, it is of special importance that azadirachtin and related compounds from *A. indica* have a very low toxicity or are practically non-toxic to warm-blooded organisms including man.

Usually 20 to 50 g of active principle is sufficient to treat one hectare of area to achieve a satisfactory reduction in pest population. Furthermore it has been recorded that the compounds have only weak or none-consequential side effects against pest antagonists or other non-targets, such as pollinators (SCHMUTTERER, 1995).

The Neem tree *(A. indica)* is today widely distributed throughout tropical and subtropical Asia, Africa, Australia, and Central and South America (SCHMUTTERER 1990). This means that it has adapted to different climatic and edaphic conditions. Efforts to select certain elite types by measuring the content of azadirachtin (aza A), the main active ingredient, by means of high performance liquid chromatography (HPLC) have shown that the content of aza A varies greatly among different trees and regions or countries (ERMEL *et al*., 1984, 1987; BENGE, 1989; KETKAR and KETKAR, 1993). It is not yet clearly understood what factors – internal and/or external – influence the quantity of active ingredient(s) concentrated in Neem seed kernels (NSKs) (MURRAY 2004).

For toxicological and eco-toxicological reasons, the concentration control of the residue studies in water, in the soil and in plant material are important. In this regard, the half-life-time of a compound plays a significant role.

The half-life-time of Azadirachtin A in water is dependent on soil pH and soil temperature and displayed in the following table. (Tab. 2.1)

Table 2.1: Half-life-time of Azadirachtin A in relation to soil pH and temperature

pH	Temperature (°C)	half-life-time (d)
4	22	36,4
	20	49,9
	18	68,8
7	20	19,5
8	20	4,4

To reflect concentration levels of residue in plants, a division into a leafy plant material category and a fruity plant material category is advisable. The decrease of Azadirachtin A in leaves and in fruits is presented in following diagram, using tomato leaves and tomato fruits as testing objects. (Tab. 2.2)

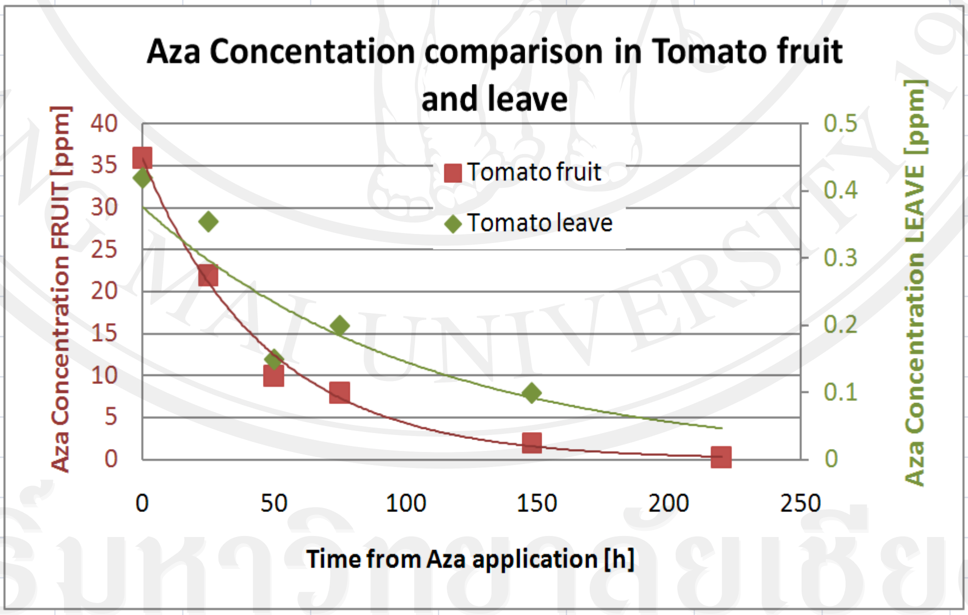

Figure 2.15: Decrease of Azadirachtin A concentration in tomato fruits and leaves

The table indicates a half-life-time of Azadirachtin A content for the leaves of approximately 1 day and for the fruits of approximately 3 days, after treatment with

5% NeemAzal-T/S. Not only the half-life-time in leaves and in the fruits shows remarkable differences, also the concentrations of Azadirachtin A directly after treatment differ as well. Generally do leafy plant materials show a higher initial concentration of Azadirachtin A (approx. 3mg Aza A per kg) whereas the fruits generally show an initial concentration below 0,1mg Aza A per kg.

For estimation of compound residues in plant material for consumption, these results play an important role and help define waiting periods before the plants are suitable for human consumption. The authorities recommend a waiting period of 8 to 9 days after treatment for leafy plant material and 1 to 3 days after treatment for fruity plant material to reach a concentration level of compound residue of less than 0,01mg per kg before the material should be consumed (RUCH and KLEEBERG, 2000).

Environmental aspects of the Neem ingredients generally are described as environmentally friendly and not harmful. Due to the fast degradation of Azadirachtin A in water and soil, is there no hazard to aquatic organisms, no danger to groundwater and no influence on important microorganisms in the soil. This as a concluding summary of an extended research on environmental aspects of Neem compounds leads to the assumption, that NeemAzal-T/S, operating with Azadirachtin A as its main active ingredient, may be regarded as an environmentally safe active compound for pest control (KLEEBERG and ZEBITZ, 1997).

2.2.3 The Neem compound

NeemAzal-T/S was produced to combat free-living sucking and biting insects and mites. Next to the naturally occurring compounds of *A. indica,* plant oils and tensides are further ingredients in this product that act as protective agents.

Previous research already showed, how the active agent Azadirachtin affects the ecdysteroid-synthesis of insects and therefore influences their moulting processes (SIEBER and REMBOLD, 1983; ADEL and SEHNAL, 1999).

NeemAzal-T/S has accreditation for combating aphids, thrips, leaf-miner flies, white flies and other pathogenic insects in greenhouses as well as free ranging. Accreditation also exists for winter moth, ermine moth, potato beetle, rosy apple aphid and is approved for ecological farming, not harmful for bees, preserves most of the beneficial organisms and has no water pollution restrictions. With adherence to recommended instructions, no harm to bees (*Apis ssp*.), predatory mites, e.g. (*Typhlodromus pyri,* SCHEUTEN*)*, ground beetles, e.g. *(Poecilius cupreus*, L.*)*, ladybeetle (*Coccinella septempunctata*, L.*)* and certain wasps, e.g. *(Aphidius rhopalosiphi*, PEREZ*)* is documented. On populations of the flower fly (*Episyrphus balteatus,* DE GEER*)*, harmful effects were documented.

NeemAzal-T/S contains NeemAzal, the pure active agent derived from the seed kernels of *A. indica*. After application of the Neem compound, the active agent penetrates into the leaves and is transported part-systemic within the plant, where it accumulates in the insects induced through their biting and sucking activity.

The mode of action of the agent causes the insects to stop ingestion which also eliminates the plant damaging activity. Active agent is 1% of Azadirachtin A (10g/l) congruent to max 4% of NeemAzal, which is the natural Neem seed kernel extract. Effects observed are rather abatement of population development than immediate elimination of insect pest.

Beneficial organisms are only affected conservatively by active agents, helping to combat pest population even more. NeemAzal-T/S is approved by the federal office for consumer protection and food security accordant of § 18a of the plant protection law of Germany.

Figure 2.16: Production process of NeemAzal-T/S from seed to product

Following instruction are given from the producer (Trifolio-M GmbH) concerning the use and application of the Neem compound:

Always dilute compound in required amount of water, stir mixture thoroughly, use mixture within one day, generally mixtures of 0.3% to 0.5 % are recommended, assure moistening of all plant parts with the spray mixture, respect waiting time before crop consumption. Read instructions and respect general protection measures. Durability of Neem compound is given for two years, if stored dry and cool. The Neem compound NeemAzal-T/S is available in containers to 1 liter, 2,5 liter, 5 liter and 25 liter.

Research on the pine processionary moth *(Thaumetopoea. pityocampa,* DENIS and SCHIFFERMÜLLER) showed significant inhibition on larval growth and reduced feeding activity obtained with applications of NeemAzal-T/S suspended in distilled water at concentration levels of 0.3, 0.5 and 1% (UNAL and AKKUZU, 2009).

Research on OPM treated with the insecticide NeemAzal-T/S has been conducted under laboratory conditions. The treatment has been carried out with 0,3%, 0,5% and 1,0% solution and showed good results on the first 4 instars. Retardation of growth was an obvious effect of the experiment, and may be a direct consequence of the reduced food ingestion.

Other researchers point out, that growth reduction derive from the intake of the toxic compounds (e.g. Azadirachtin) (e.g. FAGOONEE, 1984, SCHLÜTER and SCHULZ, 1984, WILPS, 1987, BREUER and DE LOOF, 1998).

It is also documented, that most OPM larvae treated with NeemAzal-T/S, showed moulting disruption.

Especially for insects with hemimetabolous development, molting disruption is the most obvious sign of toxicity. This may appear either between larval instars or at the ultimate molt to adult stage.

Disruption of the larval-pupal molt in lepidopterans is frequently reported and clearly indicates effectiveness of the active ingredient Azadirachtin A (SCHMUTTERER et al. 1983, TANZUBIL and MCCAFFERY, 1990, KOUL and ISMAN, 1991). Disturbance of metamorphosis on lepidopterans after treatment with Azadirachtin containing components are observed as well (e.g. SCHMUTTERER *et.al*., 1983, TANZUBIL and MCCAFFERY, 1990, KOUL and ISMAN, 1991).

Other research verified that the impact of direct sun light through UV radiation leads to a faster degradation of the active agent Azadirachtin (DUREJA 2000). The application of Neem compounds in the field should therefore happen in the shade, to avoid this early degradation. In lab research a half-life period of 48 minutes were measured, when Azadirachtin was irradiated through a glass surface with UV light of 254nm (DUREJA and JOHNSON, 2000). Further research was also able to verify those findings (KOLEV, 2011).

Another important prerequisite for effective application of Neem compounds in practical experience is the ability of not repelling the target organisms. This has been verified for several Neem compounds in lab analysis as well as in field research (KOLEV, 2011).

2.3 Related research

2.3.1 Further Neem applications

All parts of the Neem tree are known to have medicinal properties and are used in cases of malady or for special occasions. Neem preparations have been used to treat blood disorders, hepatitis, eye diseases, cancer, ulcers, constipation, diabetes, indigestion, sleeplessness, stomach ache, boils, burns, cholera, gingivitis, malaria, measles, nausea, snakebite, rheumatism and syphilis (JACOBSON, 1989). Numerous formulations are used as antiseptics, astringents, emollients, febrifuges, anodynes, diuretics, parasiticides, pediculicides, purgatives, sedatives, stomachics and tonics (DUKE and WAYNE, 1981). Neem products with these reported activities are available commercially (KOUL *et al*., 1990; JACOBSON, 1989).

Acaricidal and insecticidal effects of patented Neem seed extracts have been proven when diluted in solutions at different concentration level. It has been shown that a broad range of pests and parasites, such as house dust mites, poultry mites, harvest mites, *Ixodes* and *Rhipicephalus* ticks, cat fleas (adults, larvae), bed bugs (all stages), head lice and mallophaga, cockroaches (genera *Blatta*, *Blattella*, *Gomphadorhina*), raptor bugs (*Triatoma*), and even postharvest pests (*Tenebrio molitor*) might be controlled with this extract, which is available as Tre-san® (against house dust mites) and MiteStop® (against mites, ticks, insects of any kind) to be diluted in water or as Wash Away Louse® or Picksan LouseStop® being diluted in a shampoo. Tests on skin compatibility proved that there are no skin irritations during or after use. However, some target species are less sensitive (beetles, *Triatoma* stages, fly maggots), while the specimens of the other species cited above were successfully killed even at low concentrations of the extract (SCHMAHL *et al*., 2010).

The effect of *Melia azedarach* (L.) extract on the activity of NADPH-cytochrome c reductase and cholinesterase was studied in *Spodoptera frugiperda* (J. E. Smith). Larvae were fed an artificial diet containing fruit extract and their midgut was used for enzyme determination. As compared to the control sample, consumption of the extract containing diet resulted in a 31% inhibition of the cholinesterase activity and a 34% activation of the NADPH-cytochrome c reductase activity. In the cockroach *Leucophaea maderae*, the effect on reductase activity was even more pronounced (43%) (BREUER *et al*., 2003).

Absorption of Dimethoat into leaves of *Acer platanoides* L., *Tilia vulgaris "Palida"* L. and Horse chestnut trees has been researched after tree bandages were applied to the tree stems. The findings recorded a delayed detection of substance in the Horse chestnut leaves, compared to the ones of *A. platanoides* and *T. vulgaris*. Whereas Dimethoat was recorded within one day in *A. platanoides* and *T. vulgaris*, it took 41 days to detect the same substance within the Horse chestnut leaves (SCHENKE *et al*., 2009).

2.3.2 Pesticide application methods on trees

Although a wide variety of pesticides and application methods are known and available to control pest populations, their high costs and sometimes only marginal efficacy while stressing the public sentiment are well founded reasons for not applying them in all their possible operational ranges. The following list of application methods is meant to give a general overview and to briefly name their pros and cons.

- Spraying, often executed as a foliar spray, is an application method owning accreditation for various insect pests and pesticides. The foliar sprays usually achieve efficient results, but are cost intensive due to the high costs of auto-lifts or even helicopters and are also time consuming. The direct application onto the foliar surface guarantees efficiency, since active compounds are in direct contact with insect food sources. But this application method as such is not sensitive to public sentiment, due to the fuming of spray film, which often spreads, transported via wind, over large areas, which frustrates the use of this application method, especially in urban areas
- Tree trunk injection, is a more recent method of pesticide application and has been tested for example on pomaceous fruit to combat fire blight. It is still in the examination phase and has no accreditation for general use in Germany yet.

To achieve efficient results, high concentrations and amounts of the active components need to be injected into the tree trunk, to be transported and distributed via the sap flow. Even though lasting effects were documented, there was no guarantee for equal distribution within the tree canopy. Equal canopy distribution of pesticide requires high amounts of drill holes in the trunk, which are wounds and opening gates for secondary plant pathogens. Clefts, cambial necrosis and other negative consequences scatter doubt onto the idea, that tree trunk injection will be the method of choice for a wide range of future pesticide applications on trees.

- Tree patches or bandages, impregnated with the active compound are another method which found its niche of application, but also has no accreditation for public use yet. Active compounds penetrated through the bark and distributed within the tree systemically may effect the pest population to some extent, but so far no lasting effects are documented. In the case of marching insects, which climb up the trunk and trespass the tree patches, effects of active compounds are able to operate directly. This works fine with younger trees and smooth bark without any cracks and niches, but not in cases of older trees with cracked and fissured bark, allowing the insects to bypass the bandage tape. The mode of action of this method is not selective to the target pest and annoys public sentiment due to long retention time of patches on the tree trunk.

- Soil injection counts as a recent method with no public accreditation for Germany yet. The active compound injected into the soil, assimilated by the roots and distributed within the tree through the sap flow is the general mode of action of the application method. Due to the fact, that for efficient results higher doses of active compounds are needed than allowed makes the use of this method quite problematic. The high doses of residue endanger soil fauna, groundwater and pollute the substrate, in which all plants need to dwell.

Further research in this area needs to be done to fully reveal the potential of the soil injection method and its appropriate application range.

- Pheromone traps, baited with a pest specific lure for achieving mating disruption works usually quite well, to monitor actual pest population density. The pheromone traps are highly species-specific, inexpensive and easy to implement, but are strongly limited in their capacity to control pest population densities. Effective control of pest population through pheromone traps seems manageable in a limited area, a precondition we find in greenhouses, but not in open spaces, conditions in which we find our urban trees living.

- Spot stem application on tree trunks is the pesticide application method of choice for this research work. Pesticide application happens with a back pack sprayer from the ground, moistening only the first few meters of the tree trunk. Penetration of active compound through the bark into the tree and transportation via plant sap for distribution within the tree canopy is mode of action of this application method.

For effectively controlling pest populations with this method, higher doses of active compound are needed, as well as it is with the soil and tree trunk injection methods. Since costs and public sentiments of this application method are at well acceptable levels, it is the effectiveness of this new application approach, which was assessed in this research.