

CHAPTER 5

DISCUSSION

5.1 Discussion of used methodology

The general concept of used methodology was based on six applications of NeemAzal-T/S with a time interval of two weeks. The moistening of tree trunks with the active compound and assessment of effects, describes the chosen principle of application used in this research. The timing of initial application was dependent on pest phenology and actual pest occurrence.

Field visits with visual tree observation, visual leaf analysis and HPLC-MS analysis for Azadirachtin detection were the basis for extraction of results. In a best case scenario would the detailed lab analysis have confirmed and strengthened results from visual observations, what actually happened to some extent. But often did findings from lab-analysis not proof the expected results. This was not the consequence of poorly applied methodology, but derived out of the general frame, which had been chosen to widespread. Intentioned are the tree species, their spread location, their age and size, the diversity of target pests and parameters observed, the fact that plants transform active compounds into metabolites which are partly unknown and therefore not to be measured and all this in a real outdoor scenario with all its unpredictable and unseen influences.

The consequence of multiple leaf collections from three different canopy layers, deriving from three different tree species yielded in an extended database to be sorted, analyzed and summarized. This mass on data did not deepen the findings as much as it intensified the workload. For detection of Azadirachtin A content, only a few leaves were needed, and due to expensive HPLC-MS analysis and highest concentrations expected in the lower tree canopy, only few analyses had been conducted anyway.

Further research is needed to learn about penetration features of substances into the different kinds of tree-barks and to qualify and quantify the required parameters. For instance, does the bark of Oak trees features qualifications for better penetration and absorption of substances as Sycamore and Horse chestnut barks do? Or are these differences in Azadirachtin A content of the different tree species caused by other circumstances, such as wind-drift, degradation of active ingredient through sun light or tree specific metabolites? These reasons for no active ingredient found in the other tree species are still hidden and unknown.

Knowledge about the penetration of substances through the different kinds of tree barks in general, in the case of this research the active compound of NeemAzal-T/S, is not documented to that extent, that significant assertions about the needed parameters for successful application can be made. To really proof the penetration into the plant tissue after outside application, a research setting with elimination of all other possible factors influencing this analysis, should be generated on different bark species and ages.

To correlate leaf mines directly to their corresponding larvae and their diameter of head capsule was a necessary step, which unfortunately did not gain in significant degrees, since effects of NeemAzal-T/S seemed to be overrun by the mass occurrence of HCL on the Horse chestnut trees. On Sycamore and Oak, were the silent absence of SLB and the early OPM nest removal unexpected incidents not foreseen in the overall action plan, but did not change the methodology used as such, only influenced the amount of analyzed data.

The correlation of total leaf area to total leaf mine area on Horse chestnut, based on the general weight of the used 80g/m² paper sheets, was a time intensive analysis as well, but did not gain expected results, since NeemAzal-T/S effects were not displayed. The principal structure of this analysis was clear and comprehensible, but the percentages of leaf mines might have been too small for the accuracy of measured leaf weight and calculated leaf area for effects to be documented.

The methodology as such was qualified, but a smaller scope of research setting with reduced influences, would have improved significance of results. Even though each of the components used in this research proofed its qualification, their implementation in concert did not show expected results. The final evaluation concerning the potential of this spot stem application of NeemAzal-T/S for pest control need therefore be postponed.

5.2 Principles resulting from research

Trees are living organisms and act according to their specific physiology. In the case of this research, the tested trees grew outside in the setting of an urban environment with all the stresses and unmeasured parameters involved. This already gives a variety of possible combinations of parameters influencing the effects observed.

To better back-track effects to their actual cause, a smaller scale of research outline is recommended. For instance, research conducted in greenhouses does assure elimination of effects influenced by weather conditions. The use of smaller trees would reduce costs of applied compound and alleviate execution of applied methodology, e. g. leaf collection and infestation evaluations.

In the case of testing efficacy of new approaches and methods, even if single components proved their validity already, the variety of combinations should be limited to assure accurate interpretation of results.

5.3 Hypotheses and initial assumption

The verification of systemic distribution of NeemAzal-T/S after spot stem application had been formulated as part of the hypotheses. Even though Azadirachtin A, the main active ingredient of NeemAzal-T/S, had been detected in the two treated Oak trees 46 and 47, the amounts were low and should only be considered as appraisal values. The proof of systemic distribution had not been recorded in any other case.

Therefore, systemic distribution, accumulation in tree leaves and effectiveness of NeemAzal-T/S against target pest is not assured under given conditions. The same

is true for the repellent, feeding deterrent and lethal effects on pests, since no significantly reduced leaf mine area and smaller larvae head capsule were observed.

The observation of effects of NeemAzal-T/S towards other insects and beneficials happened only to a small extent and did not deliver significant and qualified data to be presented.

The result of not approved effects of NeemAzal-T/S does effectuate the suggested wider applications of this method as well. For this to happen, further research would need to consolidate the findings of the effectiveness of this application method, first.

5.4 Results in contrast with used methods and materials

The data collected for formulation of results did not always follow predicted trends to match stated hypotheses, as it is observed quite frequently in cases of experimental research designs. But still are the single components used in this research sound and valid to be tested for application within a real world scenario.

Even though the quality of data collection for visual leaf analysis improved during the process, but, for example the HCL larvae not measured and attached to its original leaf mine, led to data losses especially for leaf collection day May 25th. If, as well as how these data would have influenced existing results, has to be questioned, but in general should all data obtained be processed and extracted in the same manner, to assure objective display of findings.

Analyzing the results of the total leaf surface assessment on Horse chestnut gives reason for further considerations concerning the total leaf mine area estimated in the visual observations during the field trips. At the end of July, the visual assessment

estimated a 40% leaf mine coverage of total leaf surface, whereas the analysis done through leaf weight converted into leaf size displayed a leaf mine coverage of only about 7,5 % max.

Not the claim that one of the two results would represent the exact situation, but the fact that both values are way apart and that visual observations seem to be influenced by strong personal evaluations, astounds.

Even though the results describing this trend were expected to show more unity, the fact, that with the visual observations, average trends related to the whole tree canopy were recorded, whereas with the converted leaf weights, specific leaves were measured. These variety found in the results may be partly explained by these different circumstances, which accompanied the process of their extraction.

Considering the results of HPLC-MS analysis for Azadirachtin A detection, different procedures as well as physiological preconditions influenced the analyses, and are therefore on display. Detection of traces of a substance distributed within a living organism, in which the cannels between places of initial deposition and collection for analysis are known only to their most parts, comprises variation during interpretation of results.

This describes the situation of Azadirachtin content being recorded in Oak leaves after three NeemAza-T/S applications, whereas in Sycamore and Horse chestnut, even after five applications, no active ingredients were detected. Since general distribution of this substance within the tree has been documented after tree trunk injections and even stem applications on various occasions, the bark as ending and protective tissue need to be considered and scrutinized in more detail.

The situation of penetrated substance in the case of the Oak trees on one hand, and the cases of no active ingredients found at Sycamore and Horse chestnut, on the other hand, is an interesting finding, which demands further research for qualified assertions.

5.5 Results in contrast with related research

The search for alternative methods of pest control, especially for inhabited areas, has increased in recent years. Even though innovative ideas of pesticide application methods have been suggested, the problem of no formulations being registered, avoided their adoption so far. The increase of public interest and sentiment concerning these issues of urban pest control is mirrored for example in the official writing of the federal government of Germany (printed matter 16/9981 from July 15th, 2008), where questions about strategies for HCL control were debated as an answer to questions of congressmen and political parties.

The attempt of this research in contributing to this search for alternative methods of pest control, displays some of the problems involved, as well. Since research hypothesis has not been documented as stated, possible causes and conditions leading to this assessment are to be evaluated.

The findings of SCHENKE *et al.*, (2009) described a delayed detection of applied substance in Horse chestnut tree leaves, compared to other tree species. The mentioned research suggested differences in plant physiology and differences in the age of plants as possible causes for this phenomenon. Tree bark structure and bark thickness changes within tree life and together with the tree stem diameter influences the absorption and distribution of applied substances within the tree system.

These physiological differences of Horse chestnut trees, compared with Oak and Sycamore, as it describes the research situation, may give hints towards an explanation of findings, since no Azadirachtin A contents were recorded in Horse chestnut as well as Sycamore. Detailed research on each of the tree species concerning their physiological properties influencing distribution and concentration of active compounds, need to be conducted for formulation of tree specific application methods and substances.

Another cause may be founded in the degree of photodegradability of the active ingredient used in this research. As it has been proven (DUREJA and JOHNSON, 2000), that Azadirachtin A is photodegradable to a considerable extent, this thesis need to evaluate their findings in comparison to this fact.

For evaluation of photodegradability, amongst other things, measurements of UV radiation amounts from the sun light would have been necessary. Since this was not the case, the collected and displayed weather data (see Appendix B) may provide further hints towards a better understanding of research findings.

On none of the application days, rain has been documented during the process, indicating none to low cloud coverage in general, which allows high UV radiation from the sun to be present. This as well is displayed in the many hours of length of sunshine, especially for the month of April and May.

Especially the exposed location of Horse chestnut trees as well as the Sycamore trees, with their perforated and widely spaced canopy allowed a high amount of UV light to be present at the tree stems, and therefore contributed towards photodegradation of applied active ingredient.

These examples of possible late and alleviated occurrence of effects, especially on Horse chestnut, and the photodegradability of the used active ingredient Azadirachtin A, are describing verified findings of other researchers. The same causes behind those findings may have influenced the results of this research as well.

Another observation deals with the later phases of HCL larva and pupa. The observed occurrence of parasitic and pathogenic organisms mainly in the later phases of HCL larval as well as pupal stages are in accordance to the findings of KAETHNER in 1990. Even though the active ingredient Azadirachtin A was not detected analytically in Horse chestnut leaves, traces of this substance may already effected the vitality of this late HCL stages. Occurring numbers were too low for significance to be displayed, but again this may be a small building block, indicating the actual happening of the systemic distribution of NeemAzal-T/S within the trees after spot stem application.

5.6 Possible practical application

The aim of using research findings for a wider application had not been achieved. The results, and any assertion made towards their relevance and further extraction for practical application is therefore not secured. Theoretical evidence of combined methodology need to be confirmed first, before practical applications can be developed.

5.7 Concept suggestions / further research ideas

Since systemic distribution of active ingredient after spot stem application has been documented at various occasions (e.g. SCHENKE *et al.*, 2008), methodology and scale of research need to be reconsidered for future investigations. The testing of systemic distribution of active compound on small trees with a young and thin bark layer in a controlled atmosphere, promises results without interference of parameters out of scope.

The photodegradability of Azadirachtin A, as discussed earlier, is a parameter influencing the results, which has not been quantified. Alternating application times during the day, or even at night, may be another option for discovering findings in this matter.