Abstract

The larvae of *Tomostethus nigritus* F. (Hym.: Tenthredinidae) began causing severe defoliation on ash along avenues and tree lines in Zagreb, Croatia since 1997. The phenomenon of population outbreaks in periurban and urban environments is known but poorly documented in the literature; the fact that it has not yet been recorded in Croatian forests has spurred authors to investigate further into this natural event. Populations achieved outbreak levels in the second year after defoliation was first recorded. Although only *Fraxinus excelsior* L. was attacked; palatability tests indicate that larvae show no preference among several ash species, larval development *in situ* and their dispersion in the field suggest thatphenology of the host tree was the dominant factor in host selection. Based on foliage availability, *F. excelsior* proved to be the most suitable species for females during their oviposition period. Detailed study of all developmental stages, especially the late larval and prepupal stages, confirmed earlier published knowledge on this sawfly but provided also some new facts and practical solutions that can be utilized for monitoring and managing this species. Parasitoid fauna have also been studied and, despite the relatively small impact that was recorded from laboratory rearings, it is assumed that parasitoids and floods are the most important natural controlling factors affecting this species in forests. Based on the spread and intensity of the recent outbreak and its similarity to European outbreaks, we presume that environmental factors, mainly the impact of late fall and early spring floods, are the most important trigger mechanism involved in this phenomenon.

Introduction

In terms of their ecological and economic value, the lowland forests of pedunculate oak (*Quercus robur* L.), narrow leaved ash (*Fraxinus angustifolia* Vahl), and several other hardwood species are the most valuable components of forests in Croatia. Based on the tree species composition and forest structure, these forests are natural or very close to natural as is the fauna that thrives in them. Common ash (*Fraxinus excelsior* L.) grows on drier and higher sites, and along with *F. pennsylvanica* Marshall is commonly planted as an amenity tree. Periodic outbreaks of common forest pests are well monitored in the area so the first dramatic signs of ash defoliating caused by *T. nigritus* aroused interest among forest entomologists who were concerned about the threat it could pose to these valuable tree species.

After the initial taxonomic problem due to absence of adult stages, it was identified as *T. nigritus* F. (Hym.: Tenthredinidae). It was first detected on *F. excelsior* planted along an avenue in Zagreb, Croatia in 1997 and has not appeared in forest stands as was the case in some European countries (Liška 2000, Holuša pers. comm.). Mrkva (1965) reported that it completely defoliated up to 50 ha of mixed or pure ash stands while Liška (2000) and Liška and Holuša (2001) reported that *T. nigritus* defoliated almost 800 ha of mixed and pure ash stands during the years 1999 and 2000. However, Austara (1991) and Hrašovec and Diminić (2000) documented that this species occurs in urban areas. In a recent faunal review of Croatian sawflies Perović and Leiner (1991) include this species in the checklist with available loci occurring in only a part of the country. As noted in some older European works (Escherich 1942, Mrkva 1965) *T. nigritus* is a widely distributed Euroasian species which occurs at very low densities and occasionally reaches higher population levels.

Our research on this species was stimulated by the occurrence of new outbreak loci in urban areas and by some reports that a similar pest was causing serious damage on ash species in forest and...
periurban stands in northern Italy (Stergulc, pers. comm.) The main goal of our research was to devise monitoring techniques, possibly predict population trends, determine why outbreaks were initiated in urban habitats, and to test available suppression methods in the urban environment.

**Material and Methods**

The biology and development of the sawfly was studied both in the laboratory and in the field. In order to monitor closely some details of embryonic, larval and prepupal development, freshly laid eggs, larvae and cocooned eonymphs and pronymphs were brought to the laboratory and reared under various ambient conditions.

In the laboratory, palatability tests were conducted whereby young leaves of three ash species, *F. excelsior*, *F. angustifolia* and *F. pennsylvanica* were offered to young larvae of *T. nigritus* in *petri* dishes (20 larvae/dish); leaves were changed daily. A total of 100 larvae were reared on each tree species. The same trial was conducted in larger insectariums where 100 larvae were reared collectively on foliage of each of the three ash species. *F. angustifolia* was included in this experiment because of the relative importance of this species; *F. pennsylvanica* was chosen because it was obviously not being attacked in the area of massive outbreak, whereas *F. excelsior*, the most severely attacked ash species in the field, served as a control.

Laboratory and field trials were conducted on various larval stages using applications of four chemical pesticides (two IGR products, a pyrethroid, and neem oil) in order to determine the most appropriate method of suppressing this pest in urban and possibly forest environments.

The incidence of parasitism and species involved were checked in the field and by rearing various developmental stages but mainly the cocooned eonymphs or pronymphs. One hundred earthen cocoons were collected during February 2002 and placed in glass vials. After parasitoids emerged, the rest of the apparently non-parasitized cocoons were dissected to determine the occurrence of other possible mortality causing factors. Also, insect nets were used to capture the massive swarming of parasitoids that occurred in the ash crowns during the peak of larval feeding.

**Results**

Most of the findings of Mrkva (1965) regarding the biology and development of *T. nigritus* were verified by our research. There are expected differences regarding phenology since in our climate, the adults started swarming much earlier and larval feeding peaked and was completed by the beginning of May throughout our period of monitoring. This is approximately one month earlier than what was reported in central Europe. In Croatia we consider that *T. nigritus* is a monophagous species because *F. excelsior* is the only species of ash with which it is synchronous phenologically; the perfectly conditioned leaves of *F. excelsior* are attractive to the swarm of *T. nigritus* and to the females which oviposit on the margins of newly emerged foliage. Both visual and chemical clues could be involved in the selection of oviposition sites by female sawflies.

We made observations on larval development and conducted morphometric studies to determine the number of larval instars. Color of the larva changes through its development. First instar larva, whitish or almost hyaline, during eclosion emerges from the swollen chamber on the leaf margin, and molts soon after into the second larval instar which has a greenish cast as the gut fills with leaf chloroplasts. Later instars become more light green with even light coloured ventral parts. The most conspicuous change happens after the last larval molt when larva changes to an olive green color just before descending from the tree and prior to spinning its cocoon. Dynamics of defoliation is typical and quite dramatic as the developing larvae consume proportionately more foliage; what appears initially as a moderate episode of defoliation becomes total defoliation over a period of several days. Under these conditions, a large number of larvae are often unable to complete their development and die due to starvation. It is interesting to note that even under these conditions, larvae would not disperse and feed upon the foliage of nearby *F. pennsylvanica*. 
A plot of the measurements of head capsule width and larval length indicate that there are five distinctive larval instars (Fig. 1). In this research we did not distinguish between male and female developing larvae as Mrkva did (1965), nor are we certain that he included the measurement of the last descending instar in his study. The head capsule widths for the 5th larval instar in Mrkva (1965) vary between 1.87 and 2.06 mm which is clearly larger than our results for the last feeding instar, therefore there is a need for additional measurements in order to clarify the description of larval instars.

The last larval instar observed (in this study it was referred to as the 5th larval instar), olive green in colour, stops feeding, descends the trunk and enters the soil. Larvae don’t go too deep into the soil which, in our study site was a compacted managed grassland. As described by Mrkva (1965), they concentrate in the vicinity of the root collar and form shiny, black parchment-like cocoons covered on the outside with earth. They transform into enonymphs and most of them later become pronymphs. Pupae were found by mid March and adult emergence began in late March and early April. The period of massive swarming varied from year to year but usually peaked in mid April.

Palatability tests indicated that there were no preferences among the three ash species tested and that all larvae reared in both series of tests devoured the foliage and completed their development successfully. It should be noted however that because of difficulties in obtaining the first instar larva, only second and partially third larval instars were collected and fed. Mortality of reared larvae could not be observed.

Beginning in 1998, laboratory and field trials were conducted in order to determine the best possible way of suppressing *T. nigritus* populations with chemical pesticides. All tests conducted under laboratory conditions gave satisfactory results and results differed only in the time lag between the application of the products and first symptoms of larval intoxication. Four commercially available chemicals were tested: NOMOLT SC (a.s.teflubenzuron 150 g/l), BONUS SC (a.s.alphacypermethrin/teflubenzuron 40/120 g/l), SOxJA (neem oil) and FASTAC SC (a.s.alphacypermethrin 100g/l) which was used as the control. Based on operational success, market availability, and legislative restrictions on the use of chemical treatments in urban areas, we selected NOMOLT SC for field use and since 1998 it has been applied annually. The success of the applications has varied. It was influenced by the efficiency of the equipment, in terms of good crown coverage and droplet distribution, and correct timing of application. In the context of this research the applications are considered as additional impact factors which obscured the otherwise more natural dynamics of the population.

On February 21, 2002, 100 cocoons were collected in the field and placed individually into glass vials. After a period of three month, they were dissected and provided the following results: 57 cocoons were empty of filled with earth, 19 cocoons were parasitized, 8 contained live enonymphs or pronymphs and 16 contained dead and dried enonymphs (due to fungi and other unidentified
causes). The parasitism rate, calculated on the basis of 43 cocoons bearing either dead or live sawfly or parasitoids was 44%. All emerged parasitoids belong to the Ichneumonoidea and are not yet identified. Taxonomic identification of the parasitoids collected in earlier years (netted during swarming in the crowns) is still in the progress, however preliminary results revealed that two ichneumonids, *Synoecetes* sp. Förster and *Ctenopelma* sp. Holmgren were most numerous; less numerous were other ichneumonids belonging to the subfamily Ctenopelmatinae and three species of braconids.

**Discussion**

Information on the biology of *T. nigritus* combined with the results of the palatability tests so far, support our suggestion that this sawfly can be regarded as a monophagous pest of *F. excelsior* in Croatia. For this research, it was important to determine if *T. nigritus* larvae feed equally well on *F. angustifolia*. Narrow leaved ash is an important tree species in valuable lowland forests in Croatia and the threat of defoliation by *T. nigritus* could pose a serious threat. Though larvae reared in the laboratory fed well on all three ash species the situation was markedly different in the field. The most important determining factor based on our observations was the synchrony in phenology of the insect with that of its candidate host plant. Successful oviposition was critically dependant on the availability of emerging foliage. At the time of adult eclosion and later swarming, only the *F. excelsior* trees provided foliage at the required development stage. Even within the same tree species, there was a significant variation in individual phenology which resulted in variation in the oviposition rates and subsequent defoliation. It is suggested that the pronounced variation in tree phenology within the same ash species is caused by the induced effect of the prior years defoliation. We observed that trees which were totally defoliated in previous years leafed later in the following year. Other ash trees that occurred in proximity to the attacked *F. excelsior* trees were left almost untouched. Although we are not sure of the different scenarios that may exist in other locations, in the area where the outbreak took place during the past 6 years, only *F. excelsior* was affected. It is still possible that *T. nigritus* could successfully thrive on *F. angustifolia* at other locations but in that case some other suppressive factors might be involved in keeping the insect population at low levels.

Regarding the spatial and temporal distribution of larval stages and postembryonic development, it is of practical interest to note that it is quite common to have almost all larval instars present at the same locality at the same time. This is due to the intraspecies diversity in tree phenology. This complicates the timing of application of suppression tactics and is the most important factor that contributes to inconsistent results of the treatments. Early appearance of the egg laying females and insufficient leaf area available for spray deposits also contribute to application problems. Among the chemicals tested, both IGR compounds appeared to be effective however the treatments had little impact on the ongoing outbreak and complicated interpretation of the research data. Investigation of the parasitoid community was expected to reveal some clues into the regulation of *T. nigritus* specifically what causes the decline in populations. However the parasitism rates were not as high as was reported by Mrkva (1965). He reported parasitism rates as high as 80% and stated that even a 20% rate was considered as adequate to cause the population decline and eliminate the need for spraying. The parasitism rate obtained in our monitored population was less than 50% and this level did not seem to have any effect on sawfly populations. It’s very possible that repetitive chemical treatments had detrimental effects on the parasitoid community. At the same time, ineffective treatments only partially reduced *T. nigritus* populations which might have contributed to the continuation of the outbreak itself (Speight and Wainhouse 1989).

Although flooding was not a regulating factor in our study site, it has been documented to be an important factor by other authors (Mrkva 1965, Liška 2000, Liška and Holuša 2001). In all described cases of *T. nigritus* outbreaks occurring in forests, one thing was common—they were al semi-natural or natural forest types located in riparian zones. In the undisturbed and periodically flooded forests of this type, it is normal to have at least two flood seasons—late fall to winter and in the spring. Species of *Fraxinus* are naturally accustomed to this excessive surface water and thrive well in these conditions. It is also known that the floods can have a negative impact on some populations of forest arthropods that occur in the soil or litter including some serious forest pests (Schowalter 2000). Excessive moisture in the soil during a portion of the year could heighten the impact of pathogenic
fungi thus enhancing the mortality of arthropod populations. Presumably, the high levels of flood water during adult emergence in spring could also serve as a source of mortality; all of these parameters might have a significant role in regulating some other pest populations in these forests (Hrašovec 1993). Competitive adaptation is a mechanism that enables insect populations to escape the niche during the flood period through its adaptive biology (as is the case with another tenthredinid sawfly, Apethymus filiformis Klug). Absence of floods in these habitats might alter the existing equilibrium and, in the case of T. nigritus populations, could be the triggering mechanism for outbreaks.

In conclusion we emphasize that we lack considerable knowledge about population dynamics of this sawfly, especially the role of natural enemies. The fact that it rarely (if ever) occurs in Croatian forest habitats and thrives obscurely at low population levels, hinders the pace of research. Additional studies of the dynamics of the natural populations will be needed in order for us to assess the potential threat of this sawfly on Croatian forests.

Acknowledgments

We would like to thank Franjo Perović, Croatian Natural History Museum in Zagreb, for his help during taxonomic validation of the T. nigritus, Csaba Thuroczy, Systematic Parasitoid Laboratory in Koszeg, for his help in parasitoid identification, Jaroslav Holuša, Forestry and Game Management Research Institute in Jíloviště-Strnady, on supplying the valuable literature references, and Suzana Videc, for her valued contribution in the field and laboratory work.

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