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**Reasons for and effects of changes in the parasitoid complex of the horse chestnut leafminer, *Cameraria ohridella* (Lepidoptera Gracillariidae)**

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## ABSTRACT

The parasitism of the horse chestnut leafminer remains at a low level in Europe since many years. As a consequence, horse chestnuts in regions with more than 20 years of *C. ohridella* infestation still suffer from heavy leafminer attack. However, results of our investigations show changes in the parasitoid complex over the years, reflecting three different strategies exemplified by three parasitoid species. (1) The abundance of the most common parasitoid, *Minotetrastrichus frontalis*, seems to remain at the same low level, indicating that the parasitoid does not actively search for special leafminer hosts but rather encounter them by chance. The relative impact of these species decreases as other parasitoids gain importance. (2) Other species, like *Pediobius saulius*, seem to undergo a slow adaptation process. Their relative importance in the parasitoid complex increases at many locations infested for a long time. In the long run, parasitism of these species may reach levels exhibiting a controlling effect on the leafminer's populations. (3) A third group of species seems to follow the spread of its new host and invade new regions. Starting in the year 2000, the originally eastern European species *Cirrospilus talitzkii* was recorded from *C. ohridella* in countries where it has never been found before on any other leafminer. Meanwhile, the species can be found as a regular element of the parasitoid complex of the horse chestnut leafminer in many south and central European countries.

To the present, these changes in the parasitoid complex have no significant impact on overall parasitism of *C. ohridella*. However, there are measurable effects, like an increasing rate of pupal parasitism due to the action of *P. saulius*. In the long run, changes in the parasitoid spectrum of the horse chestnut leafminer may increase the impact of parasitism and contribute substantially to the control of the invasive species in future.

## INTRODUCTION

The spread of the horse chestnut leafminer in Central Europe started in Austria, probably as a consequence of accidental introduction, and led to mass outbreaks of this pest in the following years. Due to the epidemic population densities of the leafminer and the strong public concern for the health of the popular horse chestnut trees in urban environments, plant protection measures were started immediately throughout Austria and some neighbouring countries. One argument for these measures, including chemical treatments, was the fact that the control of *C. ohridella* by natural enemies was very poor. Indeed, mass outbreaks of the pest never decreased extensively at any place in Europe by means of natural control mechanisms (Grabenweger, 2004). In particular, chalcidoid parasitoids, which are often responsible for a substantial part of the leafminer mortality, have no impact on the population dynamics of *C. ohridella*.

Several reasons for the failure of the leafminer control by parasitoids have been proposed. There is evidence that leafminer parasitoids search for suitable host trees rather than for particular host insects (Askew & Shaw, 1974). Since white flowering horse chestnuts are a pontomediterranean tree species, they are poor in herbivorous insects and therefore parasitoids may not be attracted to the trees in general. Furthermore, the toxic substances of *A. hippocastanum* consumed by the leafminer larvae may protect them against natural enemy attack. In addition, the phenology of the parasitoids is not synchronised with the development of the leafminer in springtime (Grabenweger, 2004), which hampers parasitism in the first generation.

Further measures for the protection of horse chestnuts depend on the ability of native natural enemies like parasitoids to increase their impact on the invasive leafminer. Up to now, distinct trends towards a more effective parasitism have not been proved. The preliminary results of this study show that there are measurable changes in the parasitoid complex of the horse chestnut leafminer. These changes reflect the host selection strategies, searching behavior and spread of certain parasitoid species. In the long run, some of these changes may have stimulating effects on the parasitism of the horse chestnut leafminer and increase the impact of parasitoids in the control of *C. ohridella*.

## MATERIALS AND METHODS

Infested horse-chestnut leaves were collected at more than 70 locations all over Europe for a period of three years. To guarantee comparability of the samples, leaves were picked when the majority of the moths were in the pupal stage. Three samples a year were collected following the moth's trivoltine development. Mined leaves were randomly picked from the lower branches of the trees and the contents of an average of 500 mines were examined for signs of parasitism under a dissecting microscope. Mines containing parasitoids were isolated and the juvenile parasitoids were kept in glass vials until they completed development.

Mines with dead leafmining stages and emerged or dead parasitoids were counted as parasitised. The percentage of parasitized mines in relation to the total number of mines dissected at each location was chosen as a measure for the parasitism level.

To measure a density dependent response of the parasitoids to their host populations, we estimated the infestation levels of the leafminers on horse chestnut trees by visual rating as outlined in Gilbert & Gregoire (2003).

Monitoring the development of *C. ohridella* parasitism on a large timescale was not feasible within a four years project. To measure changes in parasitism as a function of time, we compared locations where *C. ohridella* has only arrived recently with locations infested earlier. This gave us the opportunity to investigate changes within a time period of at least 15 years.

## RESULTS

*Minotetrastichus frontalis* was the most abundant parasitoid of larval stages of *C. ohridella* in most of the examined locations. However, parasitism of this species is negatively correlated with leafminer abundances on horse chestnut trees, showing a negative response of the parasitoid species to its host's densities (fig. 1). In addition, larval parasitism was not increasing with time (explanatory value of linear regression model  $r^2 = 0.005$ ,  $p = 0.612$ ). *Pediobius saulius* is the main pupal parasitoid in the leafminer's complex. It is the most abundant or at least a dominant species at many places where the leafminer was already present for years. A correlation of parasitism through this species and leafminer abundances did neither show negative nor positive trends (fig. 2). In contrast to the above mentioned results, pupal parasitism shows a weak, but still measurable trend to increase with time, mainly due to *P. saulius* (explanatory value of linear regression model  $r^2 = 0.107$ ,  $p = 0.012$ ).

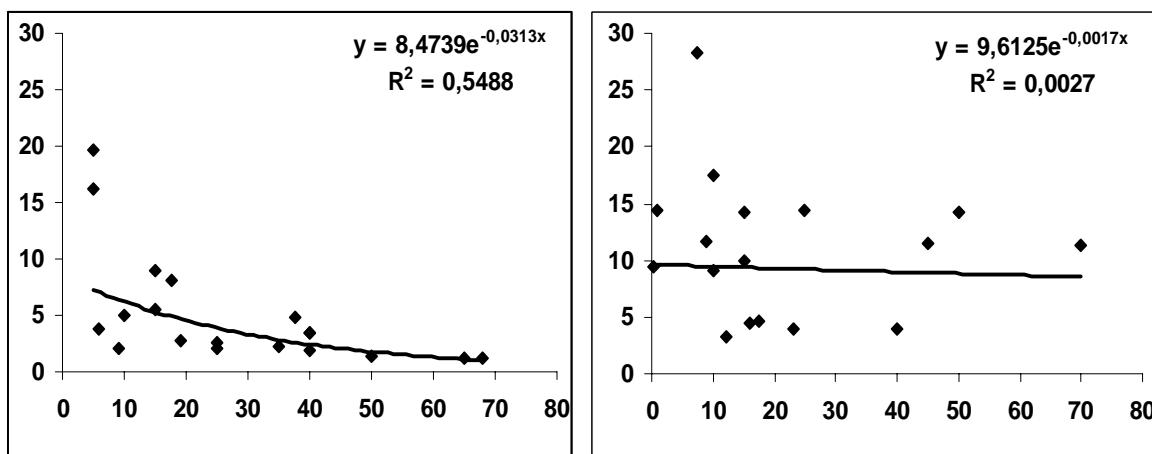


Fig. 1: Parasitism rate of *M. frontalis* correlated to leafminer host density  
(abscissae = mean percentage of infested leaf-area, ordinatae = percentage parasitism rate)

Fig. 2: Parasitism rate of *P. saulius* correlated to leafminer host density  
(abscissae = mean percentage of infested leaf-area, ordinatae = percentage parasitism rate)

*Cirrospilus talitzkii* was first found on *C. ohridella* in Bulgaria in 2001. In 2002, the species was found on the horse chestnut leafminer in Greece and at the same time recorded by Radeghieri et al. (2002) from Italy. In 2003, it was already found in Austria and in the south of France. In the last examination of the parasitoid complex of *C. ohridella* in Vienna (spring 2004), *C. talitzkii* already accounted for 1-3% of all species collected, ranging among the 5 most common parasitoid species in the complex of the horse chestnut leafminer.

## DISCUSSION

*M. frontalis* is one of the most common and polyphagous parasitoids of various groups of leafminers. The probability of this extremely polyphagous species to encounter a suitable host just by chance is high. Therefore, *M. frontalis* is able to integrate new insects into its host spectrum quickly, and in fact the species was among the first parasitoids reared from *C. ohridella* in Europe. On the other hand, *M. frontalis* will not focus its searching behavior on a particular host species and it does not show a positive density dependent response on the leafminer's increasing populations. This lack of adaptation will probably inhibit an increase of the parasitoid's relative impact on the pest insect as a function of time.

Although *P. saulius* is present in the whole area invaded by *C. ohridella*, it is recorded mainly from regions where the leafminer is already present for approximately 10 years. After this comparably long time for adaptation, however, the species gains quickly in relative importance and soon dominates the parasitoid complex. There is a weak, yet significant evidence that parasitism through this species increases with time. As the relative impact of the species is growing, it may have a controlling effect on its host's populations in future.

At first sight, records of *C. talitzkii* on *C. ohridella* in Europe are somewhat similar to that of *P. saulius*. However, the distribution of the former does not reflect a slow adaptation process but a recent spread of the species as a consequence of the host's invasion. Once established in a new area, it is most likely that the generalist *C. talitzkii* will move from *C. ohridella* to other hosts, too. At present, its impact on the horse chestnut leafminer as well as on other naturally occurring hosts in the newly colonized regions is unclear.

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