

Short Paper

## New insights into *Eriosoma lanigerum* (Hausm.), the Woolly Apple Aphid (WAA), through adaptation of spring migration research methods

Neue Erkenntnisse zur Apfelblutlaus, *Eriosoma lanigerum* (Hausm.) (WAA) durch die Adaption von Untersuchungsmethoden zum Migrationsverhalten im Frühjahr

Nuove conoscenze sull'afide lanigero del melo (WAA), *Eriosoma lanigerum* (Hausm.), adattando i metodi di indagine sulla biologia della migrazione primaverile

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### ABSTRACT

Current survey methods for monitoring *Eriosoma lanigerum* Hausmann, the woolly apple aphid (WAA), in apple orchards are often labor-intensive, imprecise, and inadequate for addressing specific monitoring challenges. To improve this, we initiated the development of innovative approaches to investigate the spring migration and to monitor the colonization of individual observational trees. This study presents the preliminary findings from field trials conducted in 2021 and 2022. We evaluated the effectiveness of repeated, simplified surveys of easily observable parameters (e.g. WAA visual spring colonization % on shoots) on selected tree groups. When combined with additional field observations, these simplified methods enabled risk assessments of WAA infestation distribution at group or plot level, and offered a potential for near-term orchard-wide infestation predictions. Notably, a reduced, non-destructive shoot monitoring protocol on a limited number of observational trees yielded sufficient information on colony dynamics across larger orchard sections for the early spring period. However, attempts to extrapolate from current migration intensity to future shoot infestation levels proved unsuccessful. Surprisingly, we documented previously unreported migratory behavior in WAA instars believed to be non-migratory. Despite this, our initial results are promising and support the feasibility of simplifying WAA monitoring methods without compromising accuracy. Some findings, however, warrant further validation.

### KEYWORDS

*Eriosoma lanigerum*, Woolly Apple Aphid (WAA); Methods, *Aphelinus mali*

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## INTRODUCTION

During the past 5 years, *Eriosoma lanigerum*, commonly known as the woolly apple aphid (WAA) has increasingly become a significant pest in South Tyrolean apple production. The negative impact of this pest has risen due to various abiotic factors and a reduced portfolio of synthetic insecticides, with limited effective alternatives available. This situation has heightened the challenges faced by field technicians and advisory services in managing WAA infestations.

The apple canopy recolonization phased by WAA in spring is accompanied by the migration of the offspring of the overwintering N1 individuals. From this observation it can be deduced, among other things, that the colonization of the stem parts and fruiting shoots in spring starts from the rootstock collar or from the root itself. Using WAA-resistant or tolerant rootstocks could mitigate the problem [1]. For practical reasons, the planned plantation including WAA-resistant/susceptible rootstock/cv combinations was set up as a randomized block of 5474 trees, using 24 rows of an existing *Fuji* variety quarter with many years of WAA infestation. Between 2018 and 2020, we initiated first preparatory activities to establish the experimental model apple orchard in this field. Until 2024, we used the orchard (2.2 ha of different cv *Fuji* clones grafted on *M9* planted since) to test different alternative WAA management products. The orchard has been under severe attack conditions from WAA and only a minimum of insecticide treatments have been performed during the period (2020-2024). The field studies concerned preliminary investigations on application methods used to treat with entomopathogenic agents in the different compartments soil, wood and shoots.

This project gave us the opportunity to adapt and refine existing WAA survey methods during the years 2021-2022, testing and implementing them in the present field stud-

ies [2]. Our approach was to experiment the utility of performing multiple surveys on easily observable parameters (see the Methods part), which, due to their frequent repetition on selected groups of trees, together with other observations, enable a risk assessment for a WAA infestation distribution on tree groups or plots or help to predict it for the entire orchard for the near future.

An initial part of the investigation started in 2020, from which we present the results related to two seasons, 2021 and 2022. A second part of investigations, with similar intentions had been conducted during 2023 and 2024 in a second field because the field (used in 2021 and 2022) in the same orchard had been cleared. Not all the data we recorded have been discussed properly because the datasets were too small for statistical analysis. Although part of the data that we collected have been used are mentioned or graphically displayed only. We continued our research after finishing the present investigations in the second field. A datasets for two years are included in a repository, to which we refer in the results part [3].

## GOALS OF THIS STUDY

The standard evaluation protocols for WAA [2] are time intensive and frequently do not always provide consistent information, depending on tree's vigour or environmental conditions. The idea was to combine the results of different simplified investigation methods (e.g., spring migration and shoot colonization), to create a "risk map" representing the spatial variation of WAA distribution in single apple plantings, extrapolating them to a defined area and predicting the actual development in the canopy (e.g., a section or a plot inside the orchard).

## METHODS

Sticky bands [4] were fitted on single observation trees, to investigate the annual upward and downward trunk migration of the first nymphal WAA instar (N1) mounting the lower

of the two sticky bands at a minimum height of 40 cm from the ground above the grafting point between the rootstock and the cv (KIKU 8 *Fuji*<sup>TM</sup>).

The observation trees were not distributed equally (equidistant) inside the plots and the respective two double rows. In both the combined rows (45 and 46) and (47 and 48), we chose a definitive number of trees per plot and per row in spring 2021. In the southern plots 1 or 2, we worked on 8 observation trees; in the northern plots 3 or 4, we investigated 7 trees during the two years (Fig. 1). The total number of trees inside the field (field 1) to which we refer was 387, a part of which was organized in the four plots (Fig. 1). We used parallel series of observation trees per exposure period in the rows 45 and 47, alternating them with an equal number of trees in the rows 46 and 48. Alternating the "treatment thesis" on the selected observation trees between "sticky" and "non-sticky" periods (alternating repeatedly between different rows) allows free migration for WAA, antagonists and ants, preventing due restriction.

At the end of every exposure period (lasting from 2 to 18 days) the transparent sticky bands were collected in the field and mounted on a paper support, then covered with a transparent PE layer. The paper sheet was transferred for a minimum of 4 hours to a -80 °C refrigerator to stop the activity of the collected organisms. We used inert paper bands to cover the sticky base during non-capture (non-sticky) periods which were substituted with the transparent sticky band at the beginning of the next period of exposure. Regarding the original methodology [4], we used the ready-made double-sided sticky bands commonly used as insect migration barriers for different purposes (Stocker Leimring Art. 45118).

We counted the total number of N1 instars present in the selected area and extrapolated the numbers to the total diameter of the observation trees measured at the height of the mounting point. The results of

Tab. 1: The values for both years and the respective rows and plots show the mean for the spring season downward and upward N1 migration, and the mean catches of *A. mali*.

Parameter	Year	Row/row	Plot	Mean N1 downward/d	Mean N1 upward/d	Mean <i>A. mali</i> /d
spring mean	2021	45/46	1	279.76	585.04	103.96
spring mean	2021	45/46	3	366.42	773.14	126.80
spring mean	2021	47/48	2	157.29	243.38	103.91
spring mean	2021	47/48	4	184.75	860.77	217.16
spring mean	2022	45/46	1	370.41	450.25	136.28
spring mean	2022	45/46	3	516.56	384.08	205.81
spring mean	2022	47/48	2	194.83	242.40	90.15
spring mean	2022	47/48	4	298.67	338.33	147.44

single observation tree counts are reported for the respective rows of the related plot, providing mean values for every exposure period.

Instead of using traditional yellow traps to capture *A. mali* [4], we counted the parasitoid adults in selected areas on the sticky bands used for the WAA migration investigations. We counted the number of adult individuals on each of the sticky bands in a restricted area of 8 cm<sup>2</sup> each. We summed up the total number of *A. mali* individuals caught during the period as the arithmetic mean per day.

The “migration” data are shown as the mean daily values of each upward and downward migrating WAA instars per plot, and one of the double rows, as well as *A. mali* counts on both the sticky bands. The calendar data are related to the final data of the exposure period during which the bands were activated.

Weather conditions relevant to the development and thresholds for the migration and activity behaviour of both *E. lanigerum* and *A. mali* were recorded by the local weather station at the 2 m level.

We recorded the visual observations on shoots on observation trees at different moments (mean values per n observation tree per plot) and the related mean values for casually chosen trees per plot for the destructive sampling method. In several instances we choose the shoots to be inspected randomly during spring season, counting the number of actual fully developed

leaves (internodes from the base), recording the respective internodes occupied by WAA colonies based on an overview of 10 shoots per observation trees. We considered the presence or absence of WAA settlements on the most distal internodes on the shoots but not the strength of colonies on the internodes itself. Destructive shoot sampling was performed at random on trees in the respective plots containing the observation trees, excluding them from further sampling. The sampling was restricted to the upper part of the tree from 1.5 m from the ground. Simplifying the official sampling protocols [2], after eliminating part of the leaves, the collected shoots were divided into smaller unities of 5-6 internodes, preserving the leaf stalks at the internode. We collected the shoots to record the degree of presence of WAA instars after dissecting them in the lab (colonies/settlements) per internodes. We also recorded any sign of parasitism, such as mummified nymphal instars, with or without hatching holes present on the internodes. Like the visually inspected shoots, the collected and lab-examined shoots were randomly chosen on casually selected trees in the four plots. The trees partly surrounded the visually inspected observation trees (see above) of the respective plots. The shoots were chosen at the top of the fruitlets. Counting the total number of internodes, we reported the number of internodes with presence of a WAA colony, with or without signs of

parasitization (a sample consisted of 100-200 shoots per plot and double rows), to the total number of internodes per shoot sample.

We compared the percentage presence of WAA on randomly collected shoots from several apple trees in the four plots near the observation trees with the percentage data of the visual settlement recordings on observation trees shoots.

We further attempted to correlate trunk upward migration in each single plot for the entire spring season with the respective degree of shoot colonization (percentage internodes) on the observation trees and in a second step, with the degree of infestation on randomly selected and sampled trees in the plots.

In this article we omitted the original idea of presence/absence surveys referring to ants or WAA predators. Instead, we showed the records on activities of the (unexpected) migration of “darkened” N4 *E. lanigerum* nymphae. The data are displayed as percentage activity (presence/absence: >1 / =0) referring to recordings on each of the upper sticky band of the observation trees present in the single plots at different moments.

## RESULTS 2021 AND 2022

In 2021 and 2022 we conducted surveys for 219 and 238 days respectively, starting on 25.03.2021 and 29.03.2022 on 30 observation trees, organized into 4 rows and 4 plots

Tab. 2: The values for both years, the respective rows and plots show the spring season maximum for downward and upward N1 migration, and maximum *A. mali* catches.

Parameter	Year	Row/row	Plot	Max. N1 downward/d	Max. N1 upward/d	Max. <i>A. mali</i> /d
spring max	2021	45/46	1	1603.39	3014.60	553.57
spring max	2021	45/46	3	2367.00	4824.15	780.29
spring max	2021	47/48	2	957.57	1277.22	803.77
spring max	2021	47/48	4	632.16	6887.85	1185.07
spring max	2022	45/46	1	1018.84	1634.05	492.62
spring max	2022	45/46	3	1981.40	2015.20	1150.77
spring max	2022	47/48	2	680.14	1249.35	270.84
spring max	2022	47/48	4	967.92	1347.89	390.57

(two plots in each double row; two double rows) (Fig. 1). From here on we refer to this period (until November) as “season”. We arranged the data in different ways considering the four single plots as the smallest units for which we achieved prediction values for the canopy colonization of shoots for the spring season period only (see definition below). For this reason, we also arranged the “spring season” colonization data on shoots in the same way, expressing the percentage of WAA settlement on the internodes present at the single data point for both methods (see below). We considered the meteorological records for a better understanding of the seasonal phenology of WAA and *A. mali* (Fig. 2).

The sticky bands series exposed during the first survey periods of 2021 and 2022, which included the absolute onset of the spring upward migration, were stopped at 01.04.2021 (2021) and 04.04.2022 (2022). As shown for 2021 and 2022 (Fig. 3), the first relevant increase of the upward trend after the start of the upward migration of the N1 instars in spring occurred between the 03.05.2021 and 12.05.2021 and 26.04.2022 and 09.05.2022. The downward migration data for both seasons (2021 and 2022) are shown in figure 4.

The primary natural antagonist, *A. mali*, was surveyed using the modified observation technique referred to the sticky band method used to investigate the WAA N1 migra-

tion. The results for *A. mali* are expressed as mean catches per day referred to the various periods displayed (related to the end data of the period and the single plots) in the figure below (Fig. 5). *A. mali* flight was first recorded from the 25.03.2021 with a remarkable increase on 30.06.2021 to 229 *A. mali*/d. In 2022 the *A. mali* flight started on a similar date (29.03.2022) increasing slowly but significantly earlier during the flowering period. We observed values of 65.5 *A. mali* on 14.04.2022 and the season peak of 560 *A. mali*/d on 28.06.2022.

#### POPULATION PARAMETERS REFERRING TO N1 MIGRATION ON STEM

The annual upward spring season migration maximum occurred on 26.05.2022. In 2021 the maximum was observed on the 30.06.2021 (Fig. 3). For both years we have to consider the spring season maxima also as the maxima for the entire season. The “fall” migration activity was investigated in the context of the overwintering biology of WAA and will be discussed separately (article currently in preparation).

Setting lower limit values for the end of the spring season upward migration (at <10 N1/d) helped us to define objective but individual endpoints for the spring season migration for each of the two years. The endpoint data indicates a prolonged lasting spring upward movement in 2021 (with a higher year maxima vs.

2022) which started to decrease on 15.07.2021 after 116 days, ending by definition on the 26.07.2021 and in 2022 after the upward maxima at 26.05.2022 with a significant decrease until 13.06.2022, ending after 102 days on 13.07.2022. We set the conditions for providing a second population development parameter, at which, by definition, the switch occurred from the prevalent upward spring migration to a permanent downward migration. We defined this point for the spring seasons (2021 and 2022) considering the data, when the conditions where (N1/d downward migration > N1/d upward migration) (see discussion part). Considering the mean migration of all the observation trees, 99.5% of the entire cumulative season’s upward migration (100% at the end of the year) per year was observed during the spring season period, as defined, occurring until the 26.07.2021 in 2021. In 2022, 92.3% of the total annual upward migration was reached at the 13.07.2022. The respective relative downward migration on both dates was 97.05% in 2021 and 95.71% in 2022.

Considering only the “spring season” migration data of 2021 (116 days; from 25.03.2021 on) and 2022 (102 days; from 29.03.2022 onwards), we noted that the strength of the upward migration of N1 instars appeared different between rows and the related plots, but was not significantly different (Tab. 1) and [3]. The differences were not significant at the 0.05 level for year, row or plot. This accounts

for the mean spring season and the maximum spring season upward migration values (Tab. 1 and Tab. 2) [3]. The downward movement in the plots and the two paired rows 45/46 and 47/48 differed significantly between the two years ( $F = 17.274$ ;  $p = 0.025$ ) and was higher in 2022 vs. 2021 (Tab. 1, Tab. 2). Significant differences in downward movement could be observed between single plots, especially in 2022 ( $F = 23.561$ ;  $p = 0.014$ ) for the mean values. For the statistics consult the repository part [3] which reports the comparisons for mean and maximum values for both years between plots and rows. The *A. mali* presence is reported for the different years, rows and plots; results are reported (Tab. 1, Tab. 2) and discussed in the last part.

#### VARIABLE EXPOSURE PERIODS FOR STICKY BANDS ON OBSERVATION TREES

Table 3 and table 4 report the mean upward migration of N1 per day in classes, showing a difference between the two years. As mentioned, we adopted variable numbers of days of exposure to the sticky bands on the observation trees during the entire season related to the strength of the upward migration. The mean exposure period for 2021 was 8.2 days with a minimum of 2 and a maximum of 18 days (Tab. 3). In 2022 the mean exposure period was 8.5 with a minimum of 4 days and a maximum of 16 days (Tab. 4).

#### WAA % INCIDENCE ON SHOOTS

During the first survey periods in May in both years an equal number of internodes had already evolved: 11.14 internodes per shoot (17.05.2021) and 11.35 internodes/shoot (16.05.2022).

We recorded WAA development on shoots applying two different methods. The results of the spring season development survey on shoots are displayed for the destructive method reporting the relative percentage of active WAA internode

Tab. 3: The exposition period of the upper and lower sticky band on the testimonial trees in the year 2021. At the peak moments of the upward migration the number of days of exposition have been reduced.

mean N1 upward/d	<3	3-5	6-8	9-11	12-14	15-18
0-600		4	4	4	4	1
600-1200		1	1			
1200-1800		1				
6600-7200	1					

presence related to both years (Tab. 5, Tab. 6). The season total is set to 100%. The percentage shoot colonization was different in both years. Here, we present mean and maximum data referred to the spring season period of both years for the single plots for both methods (Fig. 6, Fig. 7). We added a final datapoint accounting for the remaining fall season periods (2021 and 2022). The fall data referring to the later period are currently in preparation for a specific article.

Both methods provided reliable information on the mean and maximum percentage incidence of WAA on shoots (Fig. 6, Fig. 7) during spring season, the incidences we measured were not significantly different ( $p > 0.05$ ). For the statistics and the raw data, we refer to the data repository [3]. We noticed differences between both years for the mean and maximum values, independently of the method applied (Fig. 6, Fig. 7). In 2021 the mean percentage incidence of WAA on shoots was higher compared to 2022 (21.68% vs. 16.68%;  $F = 6.31$ ;  $p < 0.03$ ) (Fig. 6). Considering the maximum value of percentage WAA incidence on internodes recorded by both methods, we observed a similar situation to that of the mean values, with a 2021 maximum value of 62.67% and a lower value, indicating a lower presence in 2022, which numbered 39.8% of internodes settled by WAA ( $F = 9.44$ ;  $p < 0.012$ ) (Fig. 7).

The statistics and the absolute percentage data on the mean and maximum shoots colonization percentage investigated with the destructive method are reported in the repository part [3]. The respective data concerning the spring season

period surveys done with the non-destructive method on the observational trees are also reported.

Considering only the destructive method, in 2021 the percentage spring season maximum on internodes peaked at 07.07.2021 (61.33% of internode with WAA presence) resulting as the higher percentage of both years. After this peak the presence of WAA colonies on the shoot dropped vs. 20.07.2021 [3]. From this data, we learn that in 2021, WAA presence increased notably from 01.06.2021 to 24.06.2021, rising from 4.59% to 26.9% compared to the increase prior to the 2021 maximum (Tab. 5). In 2022 the relative spring season percentage and the maximum of internodes occupied by WAA was different in amount. An alternative shape of the curve was observed, with a constant plateau (>20%) lasting from 08.06.2022 (23.06%) to the maximum at 01.07.2022 (26.07%), with a constant % rate of occupied internodes [3].

Due to the lack of significant differences between the upward WAA migration activity in the different plots in spring (Tab. 1 and Tab. 2), we couldn't relate migration with any parameter obtained from the percentage of internode/shoot colonization surveys. For a graphical visualization of the distribution of migration intensity in the single plots vs. the distribution of the shoot percentage WAA settlement (investigated by destructive method), we refer to the repository part [3] which reports a graphical comparison (% field distribution) of both parameters for the mean and maximum values for both years.

Tab. 4: The exposition period of the upper and lower sticky band on the testimonial trees in the year 2022. At the peak moments of the upward migration the number of days of exposition have been reduced.

Mean N1 upward/d	3-5	6-8	9-11	12-14	15-17
0-600	2	5	5	1	2
600-1200		2			
1200-1800		1			

### A. MALI ACTIVITY ON SHOOTS

The percentage of internodes with the presence of WAA instars parasitized by *A. mali* was recorded until the end of the spring season period, displaying the shoot colonization for both years. The *A. mali* presence on the internodes was recorded just by the destructive investigation protocol. To complete the comparison, we included in this analysis also the samples collected after the end of the so called “spring season” period. We showed the mean values for the spring season in graph format only (Fig. 8) and the maximum (Fig. 9) data, comprehensive of the WAA percentage internode presence on the shoots, also including the *A. mali* activity percentage and calculating the basic statistics [3]. Comparing the mean and the maximum for *A. mali* activity on internodes for the spring season, we noticed significantly different values for minimum and maximum for both years regarding shoot colonization (2021 vs. 2022 mean  $F = 122.9$ ;  $p < 0.001$ ; maximum  $F = 281.7$ ;  $p < 0.001$ ).

To permit the comparison, the spring season data are displayed as a relative WAA percentage distribution of shoot colonization for spring seasons 2021 and 2022 (Tab. 5, Tab. 6). The *A. mali* presence data on shoot internodes are shown in (Tab. 7 and Tab. 8). The percentage incidences at the different data are relative to the spring season. The spring season sum is set at 100%. The raw percentage data are reported in the repository [3]. In 2021, the percentage maximum of parasitized WAA internodes peaked at 20.07.2021, accounting for 88.74% of internodes with intact mummies or mummies with an exit

hole [3]. At this moment the actual percentage relative to the entire season (set at 100%) varied between 35.72 (plot 1) and 47.29% (plot 3) (minima and maxima). In this year, we observed internodes with increasing presence of different parasitized morphae over a period of 26 days, from the first appearance of *A. mali* parasitism on shoots from the 24.06.2021, to the maximum of internodes occupied by WAA instars on shoots at 20.07.21 (Tab. 7).

In 2022, the percentage maximum of internodes with parasitized WAA instars was observed on 17.06.2022 (21.96%) [3]. The percentage relative to the spring season (100%) ranged between 26.02% in plot 1 and 44.28% in plot 2 (minima and maxima). We observed parasitism on internodes earlier compared to the previous year, for a sampling period of 23 days from 08.06.2022 to 01.07.2022 (Tab. 8), after which the active WAA development on shoots decreased.

### “DARKENED” ERIOSOMA NYMPHAE MIGRATION

Starting from the 2020 surveys (data not shown), we observed some unexpectedly downwards migrating nymphal instars on the inert paper bands (Fig. 10, Fig. 11). For this reason, after examining “regularly” sticky bands and counting N1 instars, we started to record the presence/absence of dark-coloured N4 nymphs on the upper board of the upper sticky band (Fig. 12).

We estimated the presence (incidence  $\geq 1$  of N4 individuals) of the aforementioned downward-migrating WAA instars on the up-

per sticky bands for every observation tree investigated during both the 2021 and 2022 seasons. However, we could not find any similar activity in the opposite migration direction (data not shown).

This behaviour started in single plots from 20.05.2021, with a continuous increase of the incidence until the end of the 2021 season. In 2022, the activity of these particular instars on the upper board of the upper sticky band were first observed on 14.04.2022 on single observation trees, lasting until the end of the season. Figure 13 shows the cumulative incidence of downward migration vs. the upper band of the darkened N4 instars, expressed as a percentage incidence value ranging from 0 to 100, as well as the relative amount of cumulative *A. mali* flight, expressed as the relative incidence percentage for the two years.

## DISCUSSION

### SEASONAL DYNAMICS OF WWA MIGRATION AND TEMPERATURE

We recorded the “seasonal” N1 migration data in both directions over 219 days (2021) and 238 days (2022), including the *A. mali* activity due extra surveys on both sticky bands (Fig. 3, Fig. 4, Fig. 5).

The migration data and the *A. mali* flight activity recorded on the observation trees are displayed as seasonal dynamics graphs for both years. Migration should be discussed alongside the actual temperature values and developmental limitations [5]. The meteorological data recordings are shown day by day for both years (Fig. 2), with minimum and maximum values.

The intersection of the minima and maxima graphs with the dashed lower horizontal line indicates the minimum temperature required for WAA development (5.2 °C) [5]. This temperature is present the entire year (referring to the daily maxima) and from April to November for the daily minima during the season in both years (Fig. 2). Instead of applying glue to the bands [6] [7], we used prefabricated double-sided sticky bands that had previously-

been tested at low temperatures, including periods with night frost (autumn 2020).

During the first period of significant upward migration (01.04.2021), we observed 8.9 hours/day exceeding the 15 °C threshold [7]. In 2022 (04.04.2022) during the beginning of the upward migration, we recorded a similar condition at an earlier date (8.3 hours/day) prior to the beginning of the spring migration.

In both years we noticed a substantial change in crawler behaviour between 15.07.2021 and 26.07.2021, and 13.06.2022 and 21.06.2022. During these intervals the prevalent upward migration direction changed to a predominantly downward migration.

In 2022, the upper WAA temperature development threshold, with no further development [5] was reached for the first time on 13.06.2022, when the temperature exceeded 32 °C for a minimum of 2 h/day for one week. Thus, we can assume that temperatures in 2022 had a decisive influence on the behaviour changes observed. On the contrary, in 2021, the 32 °C threshold was trespassed three weeks prior to 15.07.2021 and 26.07.2021 from 21.06.2021 to 30.06.2021, which is also indicative of spring season maxima. For this reason, we have to conclude that, despite temperature, other un-

known factors acting in 2021 were absent or acted later in 2022, inducing a later change in migration behaviour. Considering the limited number of years studied, we cannot conclude that temperature peaks or thermoperiodic cues, exceeding known thresholds, are the main factors influencing changes in migration behaviour. More detailed work at different phenological locations is necessary to prove these assumptions. As noted in the results, most of the downward and upward migration occurred at the same time. Downward migration appeared intense when upward migration was also significantly intense (Fig. 14, Fig. 15). These findings are in accordance with results from former investigations [6] [8].

#### WAS ADAPTING THE STICKY BANDS METHOD ADVANTAGEOUS?

We adapted the “original” sticky band method [8] to improve the accuracy of the surveys of WAA migration behaviour (Fig. 16). We mounted (Fig. 17) the sticky bands on a white “sticky” support at the top of the grafting point (Fig. 18). Typically, the exposure periods of the sticky bands on observation trees involve continuous exposure of multiple series for a fixed period of 7-14 days [4] [6] [7] [8] [9]. Each series ends with the dismantling of the sticky bands prior to fixing the new

series with renovated sticky bands.

In our work, we changed the protocols [3] [6] [7] [8] [9], first using a number of observation trees set out in 4 rows and conducting the sticky band method alternating two active rows with two inactive rows for a variable number of days (Fig. 1). We adopted a variable number of intermittent days during which one half of the observation trees were paper-banded with non-adhesive paper bands, while the other half was properly banded with sticky bands. This approach was thought to eliminate artefacts derived from a permanent isolation of ants and predatory arthropods in the upper or lower part of the tree.

To determine the exposure period, we observed the actual intensity of the migration by surveying a part of the observation trees in the field during the first days after we started a new series. Inspecting on the amount of N1 instars covering the lower sticky band, we decided how many more days a series should continue. We reduced the number of exposure days of sticky bands at the peak moments of the upward migration, successfully preventing the total covering of the sticky area and the overrun of the lower band by the upward migrating WAA instars in a few days, preventing the contamination of the upper band.

Despite the short covering period by the sticky bands, we were able to give an accurate description of the population dynamics of the WAA migration and the adult *A. mali* activity in the individual plots during the season (Fig. 3, Fig. 4). We observed absolute higher upward migration numbers compared to the values cited in the literature. We noticed this also for the downward direction. During both years, we recorded peak spring maxima of 1,000 to 4,000 N1 individuals/tree/d for groups of observation trees, which exceeded the known field maxima of several authors [4] [6] [7] [8] with the highest weekly values of 2,000 [9].

From other findings, the amounts of the migrating N1 population observed in our work are also of prac-

Tab. 5: Values for the respective rows and plots recorded by destructive surveys on trees in the plots during “spring season” show the relative WAA presence, in terms of percentage, on internodes on shoots at several days, including the surveys in late summer 2021.

Rows (double)	45/46	45/46	47/48	47/48
plot	1	3	2	4
12.05.2021	0.00	0.00	0.00	0.00
18.05.2021	1.65	1.28	1.22	1.76
26.05.2021	2.45	1.20	1.45	176
01.06.2021	5.03	6.07	3.41	3.84
24.06.2021	26.38	33.81	22.80	24.60
07.07.2021	30.35	31.16	34.14	36.88
20.07.2021	21.94	24.95	28.26	27.95
31.08.2021	12.20	1.54	8.72	3.20
Total spring season	100%	100%	100%	100%

tical interest for pesticide testing. The absolute numbers we observed in some of the plots in 2021 were unexpectedly high. These results could explain why sometimes the effectiveness of the most effective products against WAA is limited to only some of the trees treated.

Starting from the values we recorded, we should mention that we did not expose the standard yellow traps for the *A. mali* flight. In our experience, counting *A. mali* on both sticky bands has simplified the procedure by recording all the targets (WAA N1 and *A. mali* adults) on the same surface, exposed for identical periods in the same area, e.g., the surroundings of trees or tree groups. Other authors have referred to data from surveys in which a limited number of yellow traps was exposed inside experimental orchards [4].

We have to consider that our countings of *A. mali* individuals on the same sticky bands used for N1 migration surveillance, connects the local *A. mali* adult flight micro-activity and WAA N1 migration data more effectively at the level of a single observation tree or plot. Specific investigations are needed to effectively compare our approach to *A. mali* surveillance with the standard yellow trap catch method. We have to study whether factors acting within single plots (e.g., locally

restricted WAA presence) affect the development of *A. mali* behaviour in the restricted area.

Finally, we have to mention that in some cases (N1 daily migration > 2,000/day/tree) only a reduced amount of *A. mali* individuals could be caught on the sticky bands, because N1 individuals covered completely the bands and reduced the sticky surface completely.

#### CANOPY COLONIZATION BY WAA AND *A. MALI* RECORDED ON SHOOTS DURING SPRING SEASON

The destructive sampling of a fixed number of trees in the plots (each containing a defined number of observation trees that were not destructive sampled) and the visual inspections of the selected observation trees themselves, were not done at the exact same time intervals. Despite this, the single data at the onset of shoot colonization and the mean and maximum data recorded by applying the two different methods (adapted destructive samplings and visual observations on observation trees) can be directly compared for the two spring season periods of 2021 and 2022.

We simplified the standardized destructive method [2] reducing quantitative counting to qualitative recordings referred to internodes. This explains why the percentage of WAA

presence on internodes on shoots collected from different plots or observed on observation trees are quite similar, with no statistically different values (Fig. 6, Fig. 7).

Our findings also account for the first visual surveys in May, performed directly on randomly selected shoots from observation trees during the first upward migration period, and data from sampled and dissected shoots from the trees surrounding the observation trees. We compared the percentage values recorded on the sampled and dissected shoots' internodes with the visually observed data. In 2021, starting from 12.05. onwards, the WAA presence on internodes (visually recorded) increased from 0 to 4% until 18.05.2021. The destructive method reported that 2.76% of the internodes were infected by WAA colonies. In 2022, the visual surveys on shoots started at 29.04.2022 with an earlier visually observed increase (10.05.2022) with 6.76% of the internode occupied by active colonies. The destructive sampling reported a 10.5% increase of internode presence. The visually collected data on internode presence were quite similar and indicative of surveys regarding the early part or the start of the colonization. For the data, please refer to [3]; for the % internode WAA presence recorded by both methods.

Comparing the results of the present field investigations on spring season shoot colonization at plot level for both years showed that, repeatedly visually inspecting shoots on a limited number of selected observational trees in years with strong "pressure" could provide sufficient information regarding the mean and maximum colonization status of the shoots of all the trees in the plots surrounding the observation trees [3]. This agrees with the improved fit of the WAA settlement percentage in the spring season of 2021 (Fig. 6), when the absolute mean and maximum percentage values recorded by sampling and dissecting shoot specimens from the plots exceed

Tab. 6: Values for the respective rows and plots recorded by destructive surveys on trees in the plots during "spring season" show the relative WAA presence, in terms of percentage, on internodes on shoots at several days, including the surveys in late summer 2022.

Rows (double)	45/46	45/46	47/48	47/48
plot	1	3	2	4
29.04.2022	0.00	0.00	0.00	0.00
09.05.2022	14.54	3.47	8.58	5.06
18.05.2022	11.26	20.25	18.40	12.23
26.05.2022	13.90	11.46	16.73	12.18
08.06.2022	18.76	21.19	14.23	19.31
17.06.2022	15.50	22.76	25.98	16.32
01.07.2022	26.04	20.88	16.09	18.10
04.08.2022	0.00	0.00	0.00	16.79
Total spring season	100%	100%	100%	100%



the 2022 values. In 2022 (with an inferior mean and maximum presence of WAA on shoots regarding 2021) during spring season, visual inspections on shoots on the observation trees failed to provide similar accurate data on the seasonal activity of WAA settlement, considering mean and maximum values per plot, compared to the previous year. When we visually inspected the shoots on observation trees, we overestimated the mean and maximum values compared to the recordings obtained by dissecting and counting specimens from the destructive sampling. Probably in 2022, longer periods without relevant precipitations meant that WAA colonies maintained intact the waxy layers around the settlements on shoots for a long period and were considered active.

At the beginning of the surveys, in 2021, we selected the 30 observation trees in the four plots within 4 rows with the idea that they could reflect the actual general constitution of tree vigour and other agronomic characteristics of all the trees present in the entire field area. We further assumed, that investigating accurately the observation trees could also provide a valuable information about the WAA presence by extrapolating some parameters for all the trees included in plots inside the 2,000 m<sup>2</sup> area.

The lower mean and maximum percentage of WAA presence on the shoots during the spring season of 2022 and a lower upward migration activity in 2022 and the opposite situation in 2021 seemed to support our initial goals to obtain information on the canopy WAA colonization process considering the amount and the dynamic of the spring migration. But our intent, to predict the WAA settlement intensity on shoots starting from the population parameters of the upward migration dynamics at the plot level was unsuccessful.

Although we expected to find some, we did not obtain any remarkable differences in the percentage distribution of the upward migration on the observation trees between the 4

different plots during the two years that could be related to a different percentage distribution of the incidence of WAA settlements on the internodes in the investigated plots, as determined by both methods. For *A. mali* % distribution see below. For this reason, we omitted correlation and regression approaches, instead representing the data on migration and shoot colonization distribution on the different plots graphically in the repository part [3]. Similar investigations were conducted in a second field in the same orchard to improve some of the methods. The data from this fieldwork (2023 and 2024) are currently been prepared for publication.

As noticed, we observed differences regarding the development of WAA percentage colonies on internodes between the two years. We observed a higher absolute percentage of WAA presence on shoots at the peak moment, as well as a different shape of the spring season development curve, with a shorter lasting period of activity in 2021 vs. 2022. Prior to the maximum peak in spring season of 2021 (61.33% internodes with WAA on 07.07.2021), we had recorded just 40-50% of the total spring season WAA activity on internodes.

In 2022, the spring season peak with a lower maximum value of 26.7% of internodes with WAA activity (01.07.2022) occurred one week earlier compared to 2021. In 2021,

a minimum of 20% internode settlement lasted during the 3 intervals from 08.06.2022 to 01.07.2022 and not increasing after this data. High temperatures at these two moments (07.07.2021 and 01.07.2022) have had their negative impact on the WAA development on shoots.

#### A. MALI FLIGHT COVERING THE N1 MIGRATION AND THE SETTLING OF WAA ON SHOOTS

In both years the minimal temperature condition for *A. mali* development [10] (> 8,3 °C) and *A. mali* hatching (> 12 °C) [11] were reached starting from February and March. Flights started and increased differently in both years, without any pre-flowering flight activity in 2021 despite the fact that flight and development temperature conditions were already present. Under similar circumstances regarding temperatures, we observed that the flight of *A. mali* in 2022 started earlier.

We must argue that the different *A. mali* flight periods and the different intensity of *A. mali* spring flight activity have been acting differently on the developing WAA population on shoots and the degree of *A. mali* parasitism on shoots in all compartments during spring season in 2021 and 2022. We must suppose that in 2021, the N1 spring season migration (recorded on observation trees) and, to the same extent, also the

Tab. 7: Mean values for the respective rows and plots recorded by destructive surveys on trees in the plots during "spring season" show the relative *A. mali* presence (percentage) on internodes including the surveys in late summer 2021.

Rows (double)	45/46	45/46	47/48	47/48
Plot	1	3	2	4
12.05.2021	0.00	0.00	0.00	0.00
18.05.2021	0.00	0.00	0.00	0.00
26.05.2021	0.00	0.00	0.00	0.00
01.06.2021	0.00	0.00	0.00	0.00
24.06.2021	10.45	14.16	8.67	8.86
07.07.2021	16.86	18.71	15.98	18.50
20.07.2021	35.72	47.29	42.89	36.86
31.08.2021	36.97	19.84	32.46	35.78
Total spring season	100.00	100.00	100.00	100.00

WAA colonization process of WAA instars on shoots (Tab. 5) and in other suitable tree compartments was probably poorly covered by any significant adult *A. mali* flight activity prior to the 30.06.2021 (Fig. 5). In 2022, we had a different situation regarding the *A. mali* flight start and shape of the activity displayed by the curve until the spring flight maximum (Fig. 5). The entire *A. mali* flight period matched the WAA spring season migration activity period (Fig. 3, Fig. 4) and the period during which active WAA settlement was observed on shoots in 2022 (Tab. 6). Considering the two experimental years, we had two opposite situations of the seasonal *A. mali* flight: a good fit, with an early flight onset, covering the N1 migration period in 2022 and a delayed *A. mali* hatch and flight in spring 2021.

*A. mali* parasitization presence on shoot internodes started late on 24.06.2021 and peaked late at 20.07.2021 (maximum 88.48% of internodes) [3] (relative % data, Tab. 7). After this date a decrease in active WAA development on shoots was observed (Tab. 5). The flight intensity observed in 2021 increased just one week earlier to the 24.06.2021 date. From this moment on, the flight lasted until the first decade of August, when active WAA colonies on shoots had dropped but WAA colonies on internodes were already present at a low level. In 2022, we observed an earlier first appearance (08.06.2022) of parasitized morphae on the shoots and an earlier parasitic instar maximum peak (17.06.2022 lasting to 01.07.2022) [3] (relative % data, Tab. 8). The spring season parasitism maximum percentage on internodes (maximum 25.5% of internodes) in 2022 was inferior compared to 2021. After the WAA peak we noticed on shoots on the 01.07.2022, we started to observe a general gain of active WAA development vs. the August sampling date in 2022 (Tab. 6). During this period temperatures higher than 32 °C lasted during the third and fourth week of July, inhibiting WAA development and indirectly also *A. mali* activities on the shoots.

Tab. 8: Mean values for the respective rows and plots recorded by destructive surveys on trees in the plots during “spring season” show the relative *A. mali* presence (percentage) on internodes including the surveys in late summer 2022.

Rows (double)	45/46	45/46	47/48	47/48
Plot	1	3	2	4
29.04.2022	0.00	0.00	0.00	0.00
09.05.2022	0.00	0.00	0.00	0.00
18.05.2022	0.00	0.00	0.00	0.00
26.05.2022	0.00	0.00	0.00	0.00
08.06.2022	20.87	20.77	19.91	33.19
17.06.2022	26.02	36.42	44.28	28.84
01.07.2022	39.27	31.43	27.62	24.95
04.08.2022	13.85	11.38	8.19	13.02
Total spring season	100.00	100.00	100.00	100.00

Our seasonal *A. mali* flight recordings are in agreement with observations and results from field data collected in our region or under comparable circumstances. Baldessari et al. 2018 [11] reported similar situations with an early beginning of WAA migration activity and a delayed hatching and flight activity of *A. mali* in higher fruit growing locations. The reasons for the unsynchronized phenology of prey and parasitoid are probably due to the amount of overwintering WAA instars, the actual incidence of their degree of parasitization prior to overwintering, and their overwintering success. In agreement with Baldessari et al. [11], we have to conclude that part or all of this factors had probably led to higher pressure in 2021 without a proper regulation of the WAA colonies developing on shoots and other plant tissues, like cuts or wounds.

We started first specific investigations on overwintering efficacy and outcome in spring from the winter 2021/2022 in the current experimental field. The same investigations were conducted in the second field for the overwintering periods 2023/2024 and 2024/2025. These data will be discussed together with the autumn development in an article that is still in progress.

In accordance with our general attempts to address the percentage distribution of the different WAA parameters for the rows and plots, we

tested the presence of differences for the mean *A. mali* presence distribution between the four plots inside the field. We did not observe such differences between the mean and the maximum spring season values for the factor year, rows and plots ( $p > 0.05$ ). Comparing the field distribution of the parameters, the percentage WAA internode presence and *A. mali* parasitism on internodes confirmed that a local higher incidence of the prey (WAA) in the single plots leads to a higher activity of the antagonist *A. mali* in the same plot. Because of the limited data of only two years, we omitted correlation and regression approaches. The *A. mali* distribution inside the field on shoots and the WAA presence using the destructive method is showed in figure 19 and figure 20.

#### UNEXPECTED BEHAVIOR OF WAA NYMPHAL INSTARS

In this part of the discussion, we refer to the darkened N4 WAA instars moving downwards unexpectedly during the intermittent no-catch periods, as opposed to the upper board of the upper sticky band crawling under the upper paper band (Fig. 10a).

From our direct observations in the field, we should argue that the darkened N4 nymphae (Fig. 10b) left the WAA colonies downwards, starting from the upper part of the canopy (e.g. the trunk and the

shoots) rather than the lower part of the trunk. Since we discovered them under the paper-banded stripes during the intermittent periods (Fig. 10a), the most reliable explanation is that they had already been parasitized before leaving the colonies actively (Fig. 11).

The downward migration of this particular instar (estimated as a fourth nymphal instar by size) was first noticed on 14.04.2022 on single observation trees, lasting until the end of the season. In 2021, the shape of the cumulative seasonal activity was similar as in 2022, but the first appearance wasn't observed prior to the 20.05.2021, increasing significantly after this date, with a presence exceeding 60% of the observation trees in all the plots by the 15.07.2021. Compared to 2021, a higher incidence (over 80% of observation trees) was observed earlier from 09.05.2022 onwards, and lasted until the end of the season. We must suppose that, in 2022 the early part of the "darkened" N4 population (e.g. in April/May 2022) consisted of WAA instars that have been parasitized prior to the 2021/2022 overwintering period. By contrast, in 2021, we have to assume that the "darkened" N4 population activity noticed on the observation trees only from the 15.07.2021 on, was initiated by WAA virginopare, who had developed and had been parasitized by *A. mali* in the same year (2021) (Fig. 13).

Referring to the "darkened" N4 instars as probably parasitized mobile instars (the future mummified WAA instars with an developing *A. mali* instar inside) (Fig. 21), we have to argue that this behaviour is induced either by the growing *A. mali* larva inside its host *E. lanigerum*, or by a more complex interaction between WAA and its parasite [12]. So far, this behaviour has not yet been described in any of the research papers for the WAA/*A. mali* system [4] [5] [9], but is generally known as a paradigmatic example for different insect-parasite systems [13]. Behavioural manipulation of host organisms is a widely observed phe-

nomenon among parasitoids, particularly regarding changes in the spatial distribution of parasitized versus unparasitized hosts. It is known that particularly in aphid/parasitoid systems such host-manipulations are part of the strategies of the parasitoid at the end of its reproductive process in the host [13].

Even though we cannot currently explain the exact nature of the biotic and abiotic factors that are inducing this behaviour at different intensities during the season, there could be some severe implications. The data from investigations related to the percentage of parasitized WAA instars on shoots or other upper plant parts could be biased, leading to an incorrect estimation of the incidence of WAA and *A. mali*, due to portions of the population (the migrating N4 instar) actively leaving the colonies on the shoots (Fig. 21). A second, similar important question is whether this atypic nymphal instar contributes to the overwintering population of *A. mali* and as a consequence regulates the WAA population with more efficacy during the early-spring colonization process.

More data are required for a more accurate explanation of the phenology of the appearance of this particular type of nymphal instar during the entire season period. This includes investigations in apple orchards, in different location inside the South Tyrol apple growing areas. The investigation in the second field (2023 and 2024 data is in preparation) in the same orchard has actually finished and was focused particularly on the possibility to automatize the visual survey processes applying A.I. technology. For this reason, no specific investigation has been carried out regarding this phenomenon. However, during the 2023 and 2024 investigations, we still recorded the appearance of this particular *E. lanigerum* morphae as activity, confirming our findings from one field of the 2021 and 2022 surveys.

## SUMMARY

The primary objective of this investigation was to adapt methods/protocols ensuring effective reduced time-intensive surveillance of seasonal WAA (*Eriosoma lanigerum*; Hausm.) occurring in canopy infestations. We also provided some interesting phenological data of both the WAA population and the main antagonist *A. mali*. We observed a first spring/summer activity migration in both upward and downward directions from the beginning of April, with a "peak" (15.07.2021 and 13.06.2022) and a second point considered as the summer minimum on 26.07. in 2021, and 13.07. in 2022. At the latter two data points, the upward migration reached a value near to a zero for the first time of the respective year, following continuous upward migration that started in April and a spring season peak. Referring the actual temperature recordings to the dates when the population parameter values were observed should help us to explain part of the population dynamics referred to the migration data. More data and observations are needed to refine the initial findings. A positive result regarding our methodical research goals was, that simplifying survey methods on shoots applying a reduced protocol of non-destructive observations, limited to a set number of observation trees, could provide equally basic information about the colonies' dynamics in spring for a major number of trees inside selected areas of the orchard. Furthermore, adapting the migration protocols provided us with consistent data focusing on the peak moments by limiting the observation periods to a minimum of days near to the migration maxima. However, our attempt to extrapolate future shoot infestation levels for a group of trees based on actual migration intensity was unsuccessful. Our intent to correlate trunk migration dynamics and shoot WAA settlement showed that migration data observed on the trunk of selected trees could only theoretically predict the current and future dynamics of WAA settlement on the shoots of trees near to the observation trees.

The initial and the increased WAA internode presence on shoots during spring season by N1 was in line to the upward migration on the trunk but absolutely not exclusively dependent on it. There was a delay between the beginning of the N1 upward migration in early May, the upward migration maximum data and the onset and significant increase of WAA presence on internodes on shoots in mid-May and the maximum on shoots. We have to suppose that the settlement process on the growing shoot depends on

other factors other than the intensity of the upward-migrating N1 population.

Considering the evidence that the phenomena of the population of N4 darkened, supposedly parasitized WAA instars, lasted differently in terms of length and intensity during different periods in both years, should lead us to further considerations and more accurate research. Despite the upward and downward migration of the N1 instars during the spring season, the instars to which we refer migrated exclusively

downward. Extending research to other locations to study whether the phenomenon was exclusively constrained to this field or to a specific ecotype inside a *A. mali* population must be the next step. The impact of this variable behaviour could interfere differently with standardized observation about WAA settlement and *A. mali* presence on shoot colonies. This means that quantitative data, as well as qualitative presence/absence percentage activities, on the appearance of this particular morphae should be recorded.

## ZUSAMMENFASSUNG

Die derzeit verfügbaren Erhebungsmethoden zur Überwachung der Apfelblutlaus, *Eriosoma lanigerum* Hausmann, in Apfelanlagen sind häufig arbeitsintensiv, ungenau und für bestimmte Fragestellungen ungeeignet. Zur Verbesserung wurden innovative Ansätze getestet, jene zur Überwachung der Frühjahrswanderung sowie jene der Überwachung der Besiedelung der Baumkrone. Diese Studie stellt erste Ergebnisse aus Feldversuchen der Jahre 2021 und 2022 vor. Untersucht wurde der Nutzen wiederholter, vereinfachter Erhebungen leicht beobachtbarer Parameter an ausgewählten Baumgruppen. In Kombination mit weiteren Feldbeobachtungen sollten diese Methoden eine Risikobewertung der Befallsverteilung auf Gruppen- oder Parzellenebene sowie Prognosen für den kurzfristigen Befall auf Bestandesebene ermöglichen. Erste Ergebnisse haben gezeigt, dass ein reduziertes, nicht-destruktives Erhebungsprotokoll an einer begrenzten Anzahl von Referenzbäumen ausreichen kann, um Aussagen zur Besiedelungsdynamik im Frühjahr auf Bereiche der Anlage zu übertragen. Die Versuche, anhand der Migrationsstärke auf den zukünftigen Befall von Trieben zu schließen, verliefen hingegen erfolglos. Erstmals wurde ein bislang unbekanntes Wanderverhalten von Apfelblutlaus-Nymphen dokumentiert, welche bisher als nicht migrierend galten. Trotz dieser Einschränkungen sind die ersten Ergebnisse vielversprechend und belegen die Möglichkeit, die Feldarbeit bei der WAA-Überwachung zu vereinfachen, ohne an Genauigkeit zu verlieren. Einige Befunde bedürfen noch einer weiteren Bestätigung.

## RIASSUNTO

I metodi di indagine attualmente disponibili per il monitoraggio dell'afide lanigero, *Eriosoma lanigerum* Hausmann, nei meleti sono spesso dispendiosi in termini di manodopera, imprecisi e inadatti a determinate problematiche. Per migliorare l'efficienza, sono stati testati approcci innovativi per il monitoraggio della migrazione primaverile e della colonizzazione della chioma degli alberi. In questo studio si presentano i primi risultati di prove in campo effettuate nel 2021 e nel 2022. È stata studiata l'efficacia di indagini ripetute e semplificate su parametri facilmente osservabili su gruppi selezionati di alberi. In combinazione con ulteriori osservazioni sul campo, questi metodi dovrebbero consentire una valutazione del rischio della distribuzione dell'infestazione a livello di gruppo o di appezzamento, nonché previsioni di infestazione a breve termine a livello di impianto. I primi risultati hanno dimostrato che un protocollo di indagine ridotto e non distruttivo su un numero limitato di alberi di riferimento può essere sufficiente per estrapolare le informazioni sulle dinamiche di colonizzazione in primavera all'impianto. I tentativi di trarre conclusioni sulla futura infestazione dei germogli sulla base dell'intensità di migrazione, invece, non hanno avuto successo. Per la prima volta è stato documentato un comportamento migratorio delle ninfe dell'afide lanigero finora sconosciuto, che in precedenza erano state considerate non migratorie. Nonostante alcune incertezze, i risultati iniziali sono promettenti e dimostrano la possibilità di semplificare il lavoro sul campo nel monitoraggio del WAA senza perdere in accuratezza. Alcuni risultati richiedono di una ulteriore conferma.

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**ANNEX: FIGURES**

row/chamber	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
45	orange	green	green	green	green	yellow	orange	green	green	green	green	yellow	orange	orange	green	orange
46	orange	green	green	orange	green	green	orange	orange	green	green	green	green	green	orange	orange	orange
47	orange	green	green	green	green	green	green	orange	green	green	green	green	orange	orange	green	green
48	orange	green	green	orange	green	green	green	green	green	green	orange	green	orange	green	orange	green

Fig. 1: The rows (45,46,47,48) and the “chambers” 1-4 and 13-16 forming 4 plots (1,2,3,4) with a respective number of observational trees inside. Two rows 45/46, 47/48 are organized to form four single plots. The series had been performed alternatively in the rows 45 and 47 or 46 and 48.

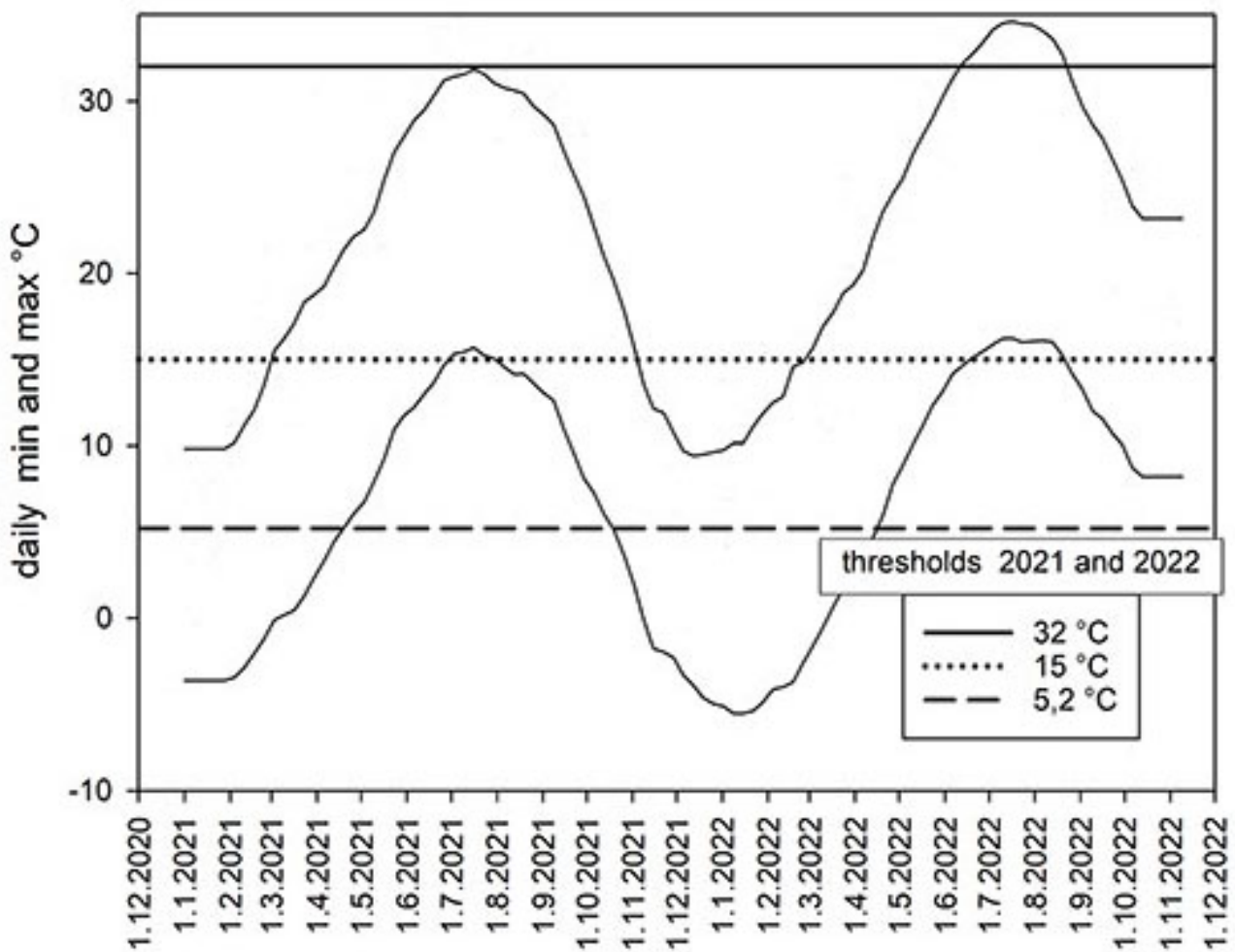


Fig. 2: The meteorological conditions reporting the daily minima, the mean values and the maximum values during the two seasons! The periods of the season during which the temperature exceeded 5.2 °C 15 °C or 32 °C are indicated.

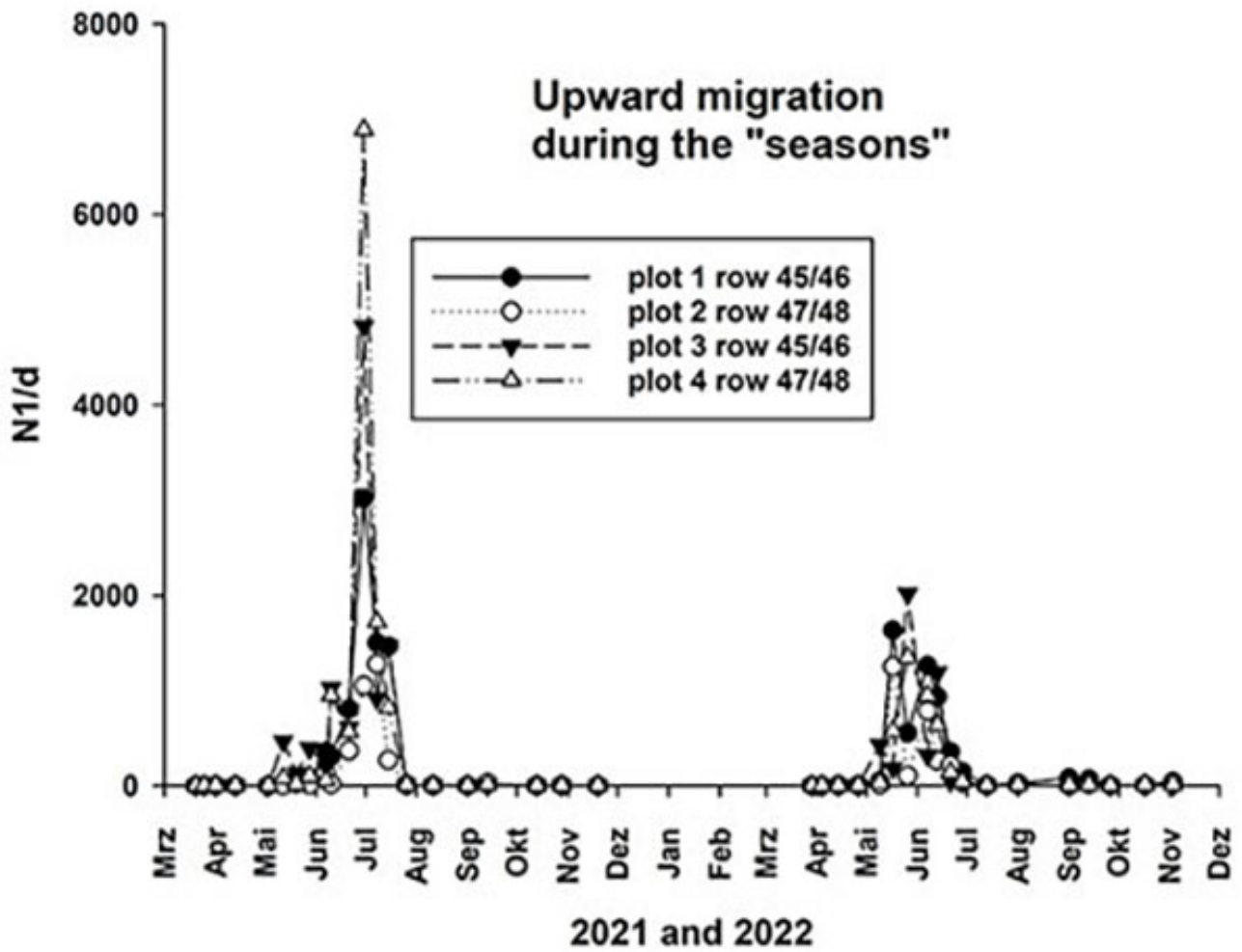


Fig. 3: The N1 migration expressed as nymphae/d collected on the testimonial trees in the different plots during the entire season (lower sticky band).

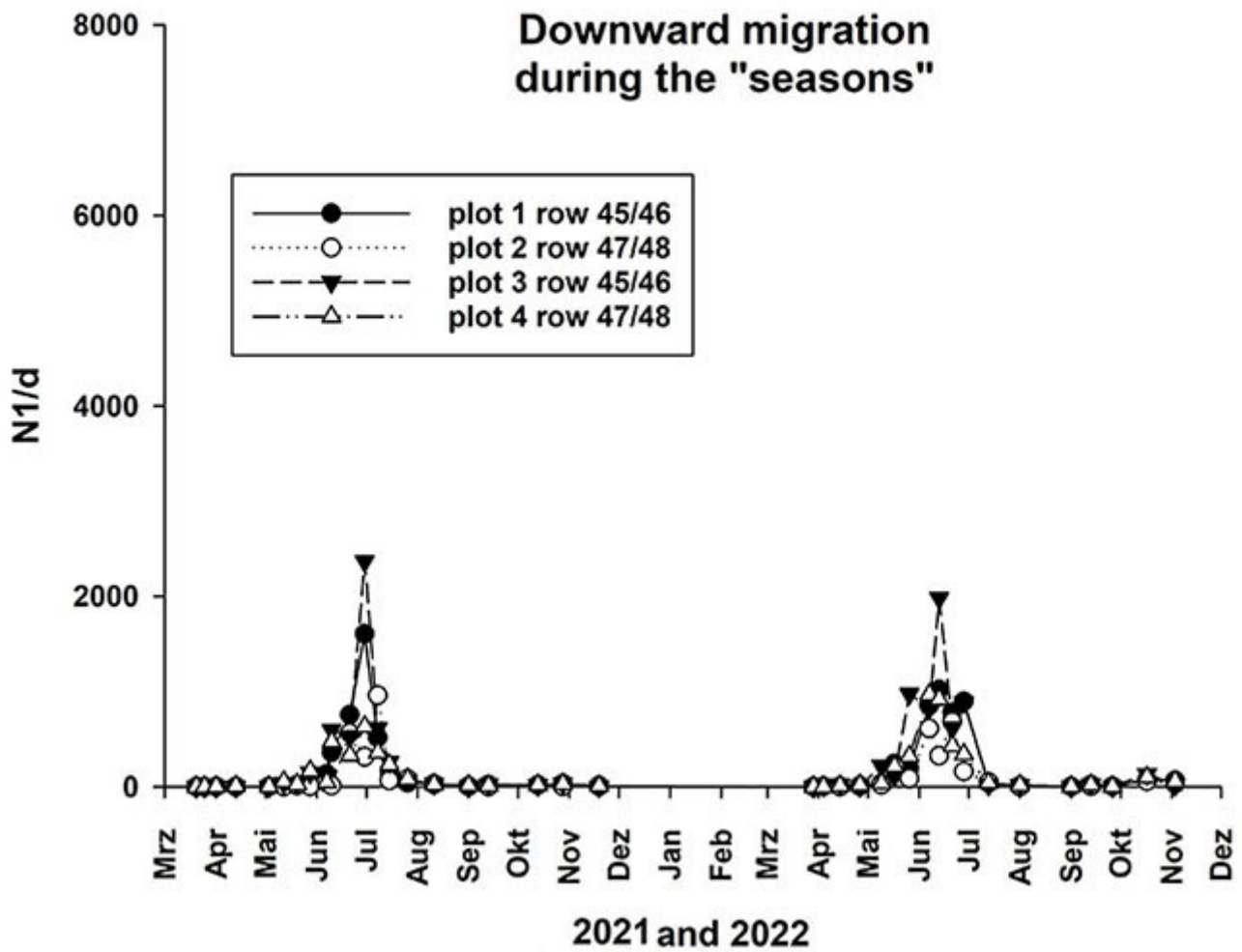


Fig. 4: The N1 migration expressed as nymphae/d collected on the testimonial trees in the different plots during the entire season (upper sticky band).



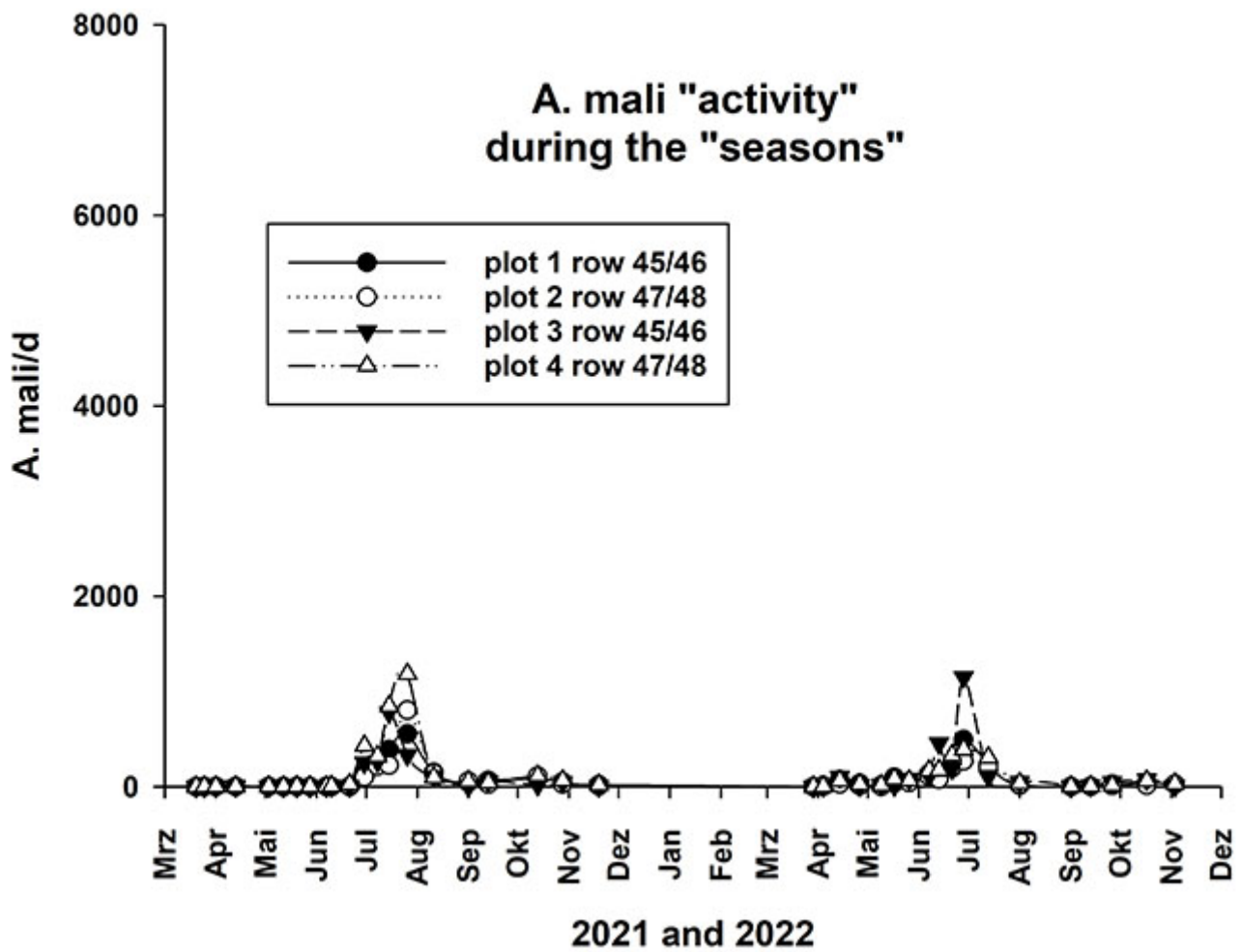


Fig. 5: The *A. mali* flight expressed as adults/d collected on the testimonial trees in the different plots during the entire season (mean number of lower and upper sticky band).

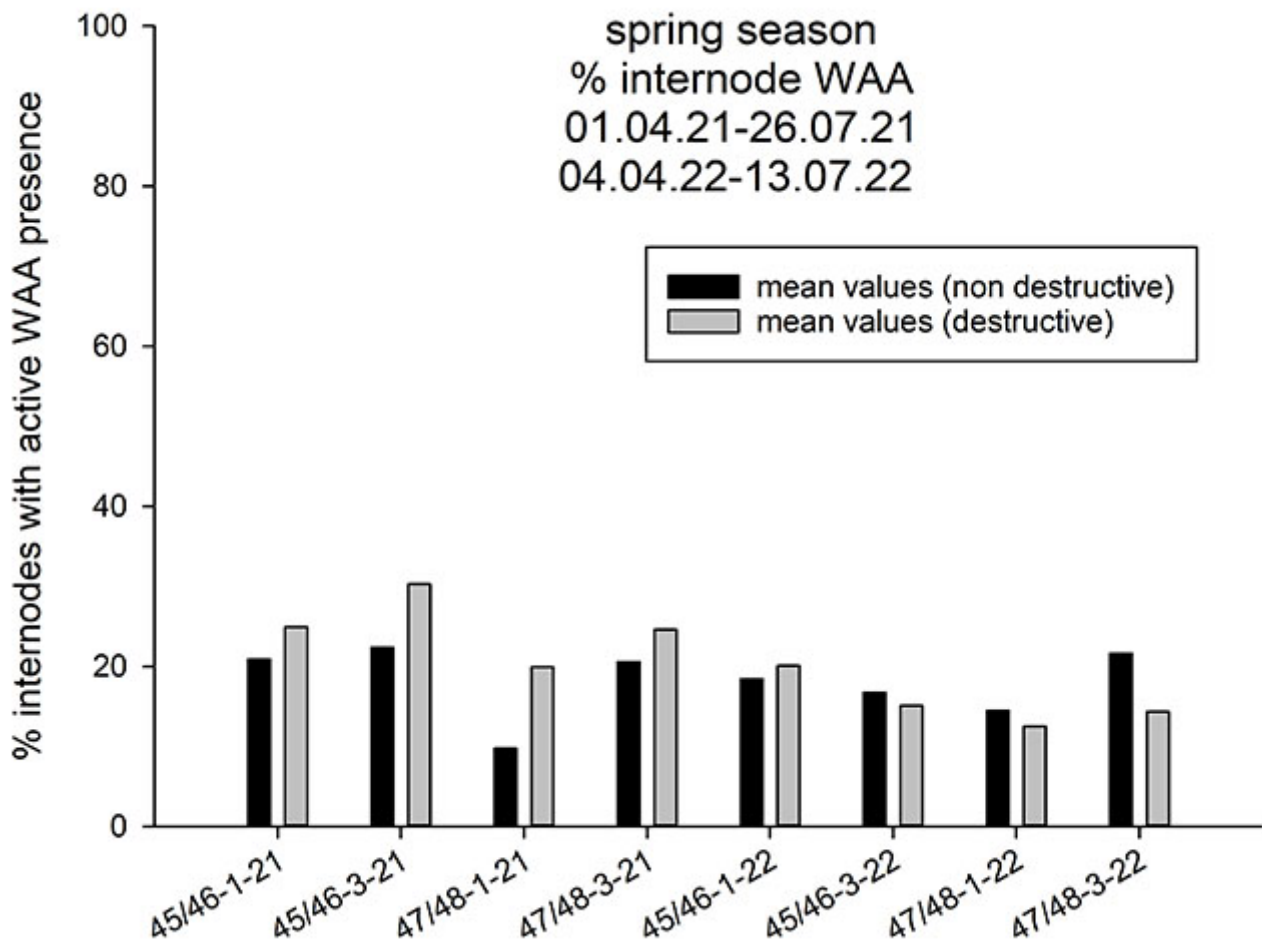


Fig. 6: The WAA presence on shoots on observation trees for both years 2021 and 2022 expressed as mean values of the respective rows and plots recorded by visual surveys (non destructive). The mean % values obtained by destructive surveys on trees in the plots during "spring season" are also displayed.

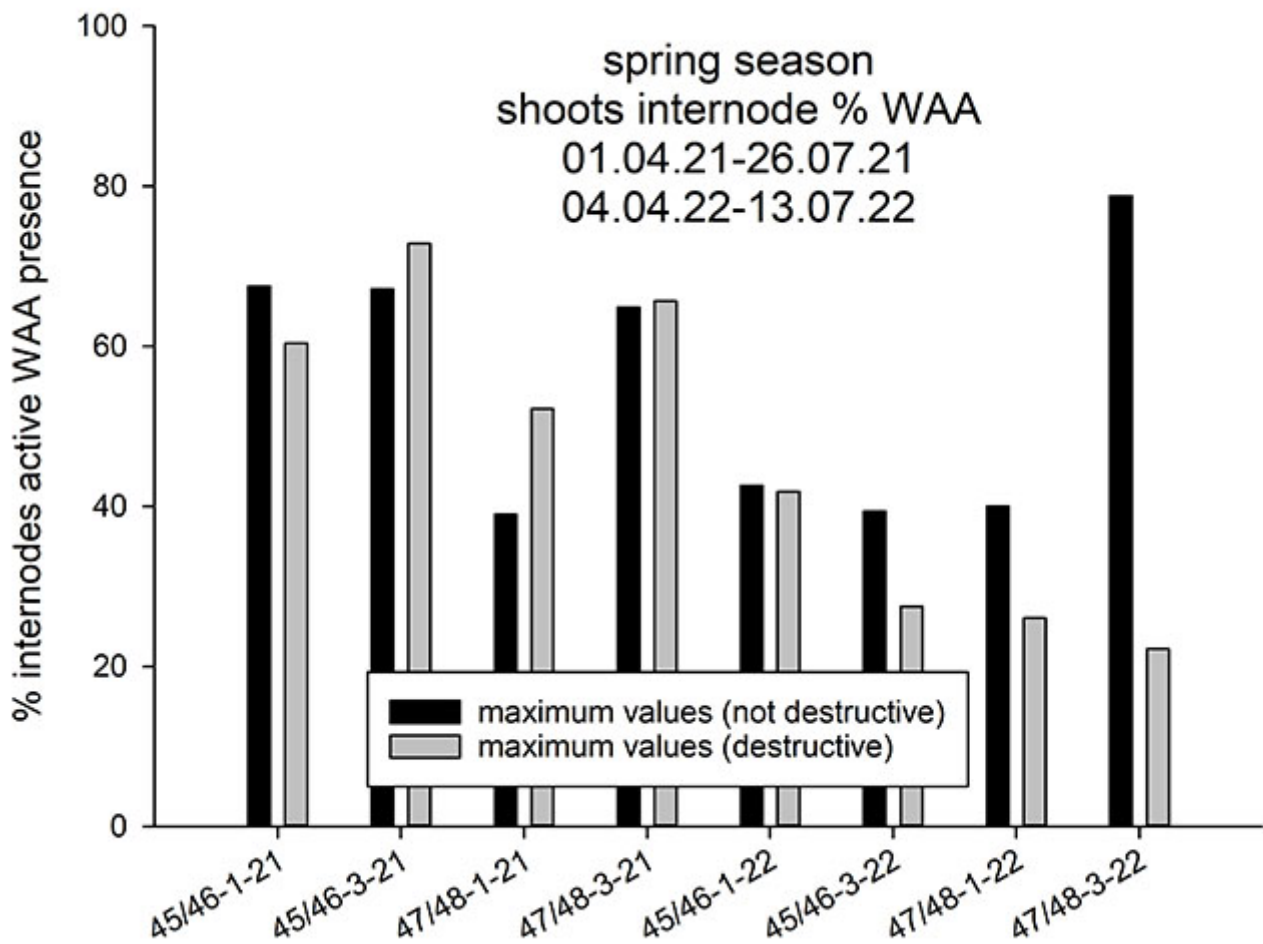


Fig. 7: The WAA presence on shoots (maximum) on trees in both years 2021 and 2022 expressed as maximum values of the respective rows and plots recorded by visual surveys (non destructive). The maximum % values obtained by destructive surveys on trees in the plots during "spring season" are also displayed.

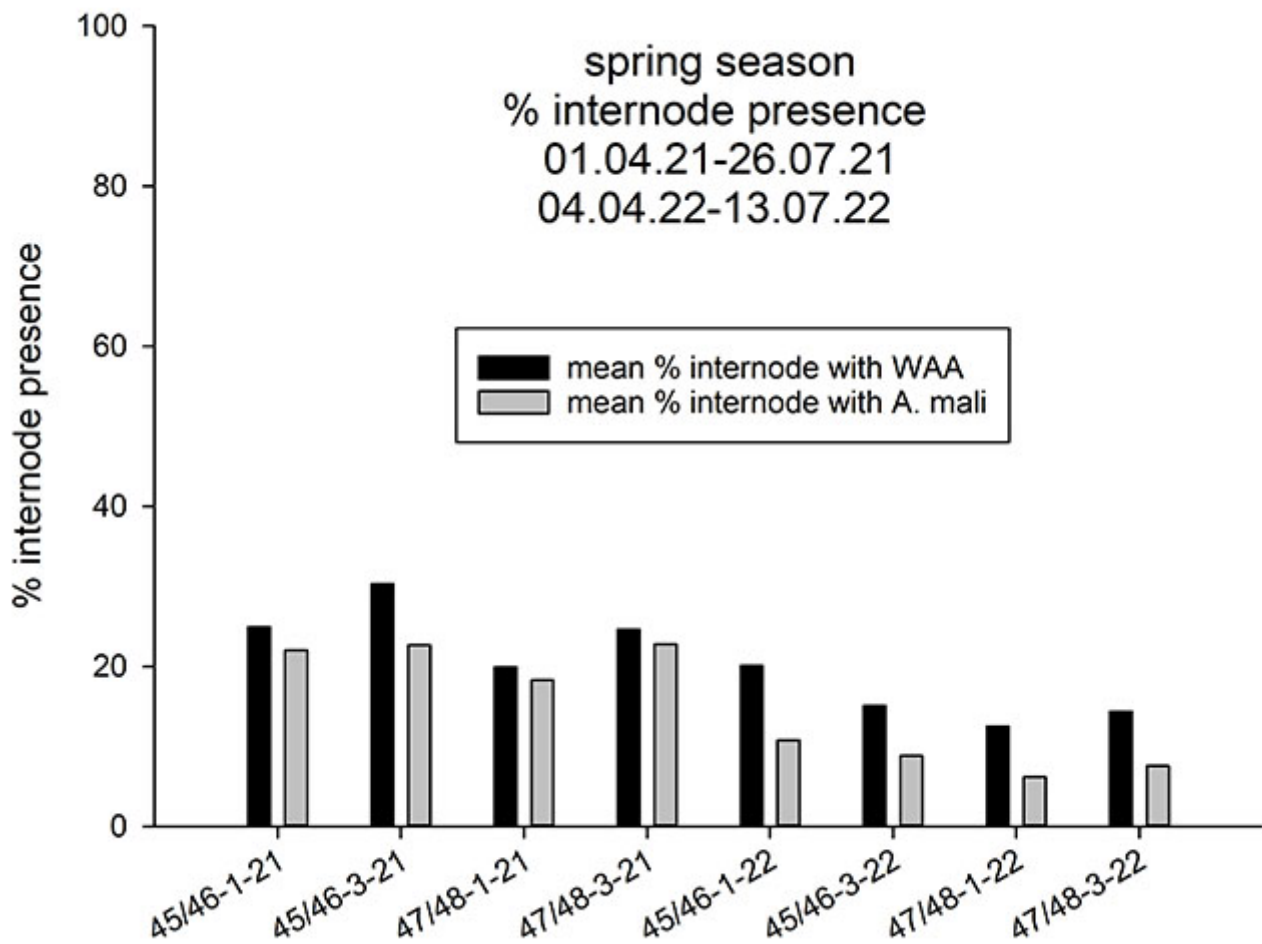


Fig. 8: The mean % WAA presence on internodes of shoots of trees for 2021 and 2022 expressed as values for the respective rows and plots recorded by destructive surveys on trees in the plots during "spring season". *A. mali* % incidence on internodes is displayed for the single plots.

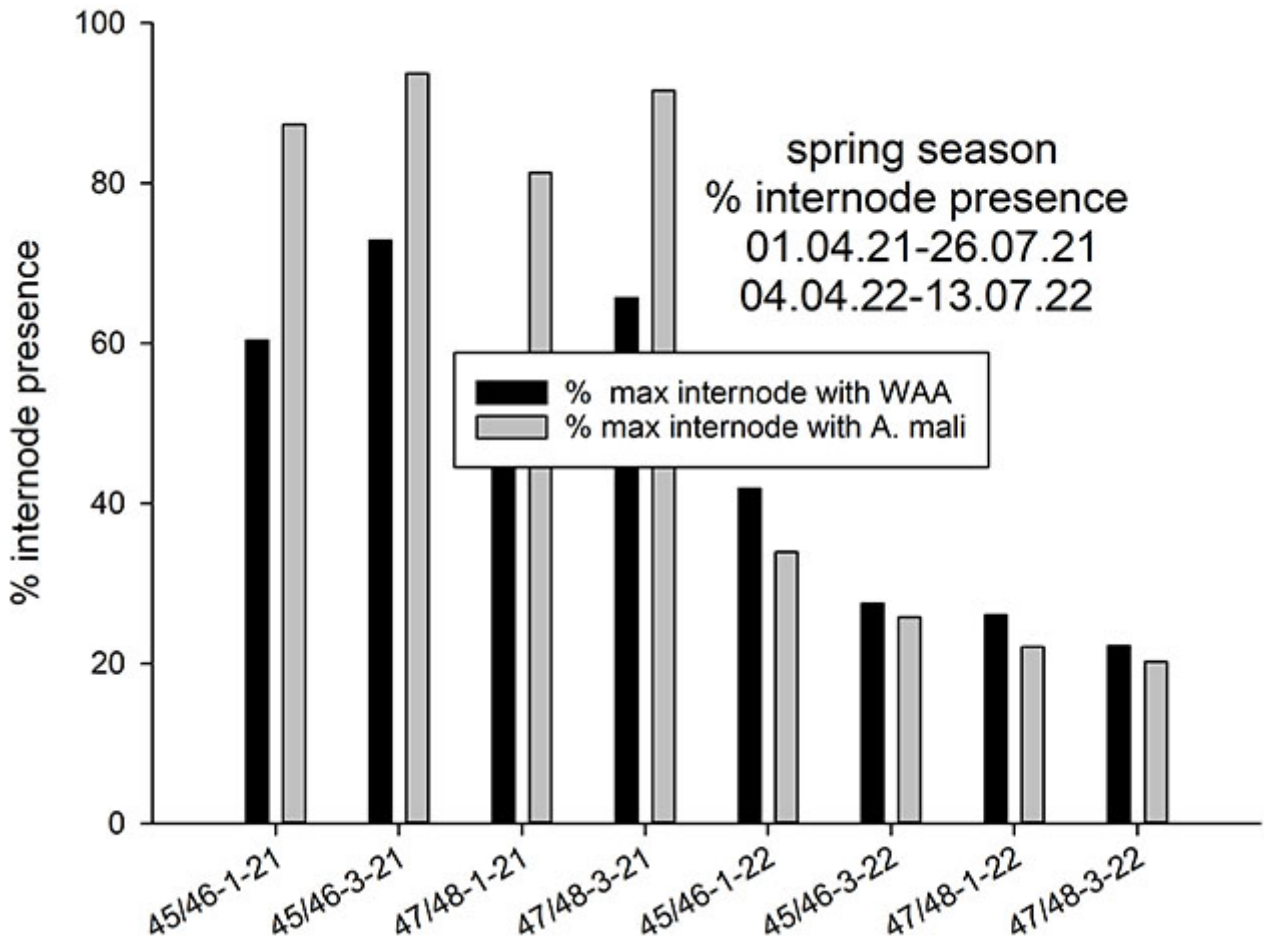


Fig. 9: The maximum % WAA presence on internodes of shoots of trees for 2021 and 2022 expressed as % values for the respective rows and plots recorded by destructive surveys on trees in the plots during “spring season”. *A. mali* % incidence on internodes is displayed for the single plots.



Fig. 10: The remains of the darkened N4 instars on the non-sticky paper band (sticky side inside) mounted on the testimonial trees during the intermittent periods permitting free migration on the surface (10b).



Fig. 11: Migrating “darkened” N4 instars still secreting waxy materials on the upper part off the upper sticky band on the testimonial trees during the activated periods.



Fig. 12: Darkened, WAA instars at the top of an actually activated observational tree; the sticky base is covered with the double sided sticky transparent band.

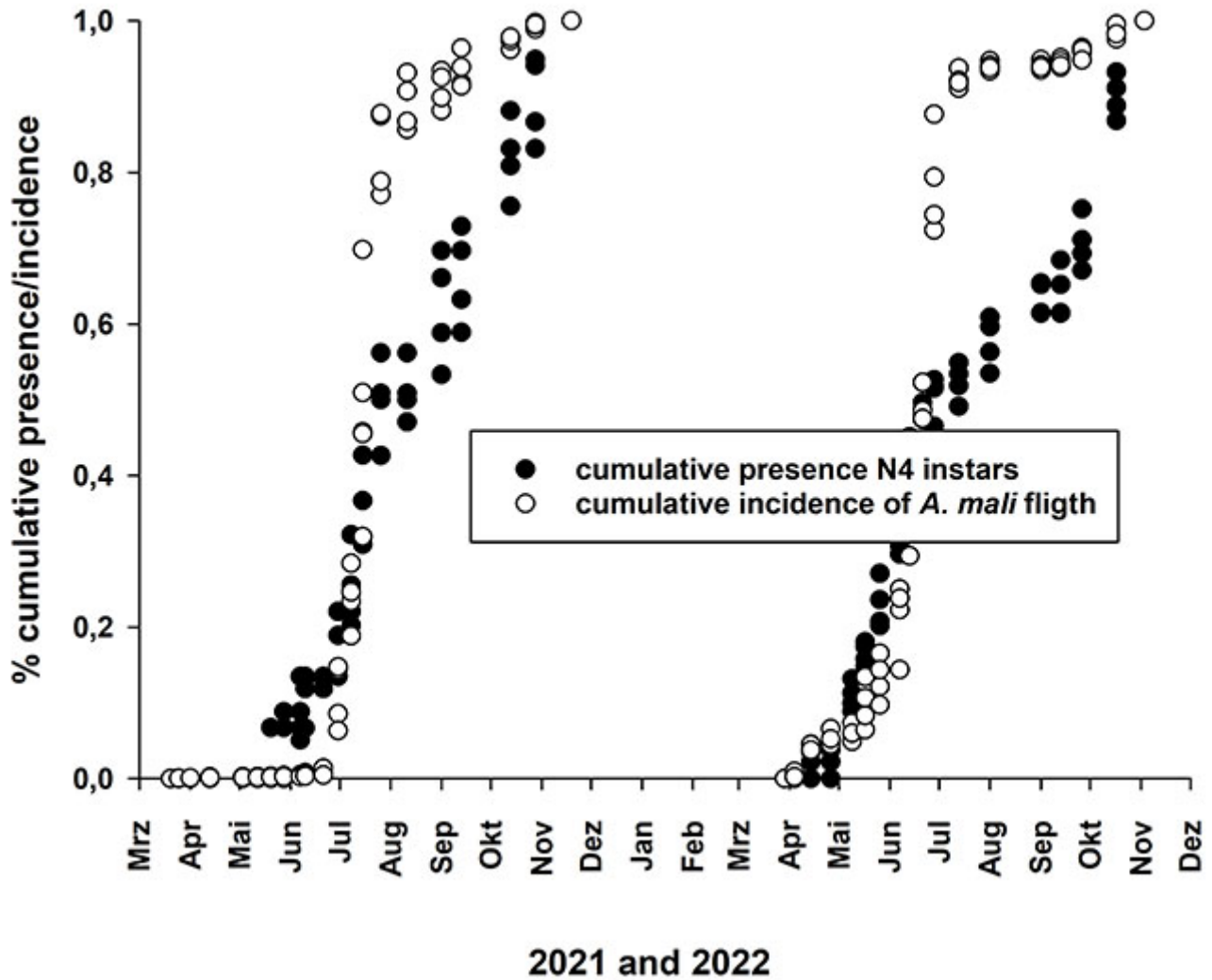


Fig. 13: The seasonal dynamic of the cumulative %age of N4 instars activity and the *A. mali* flight (cumulative annual ) for 2021 and 2022.



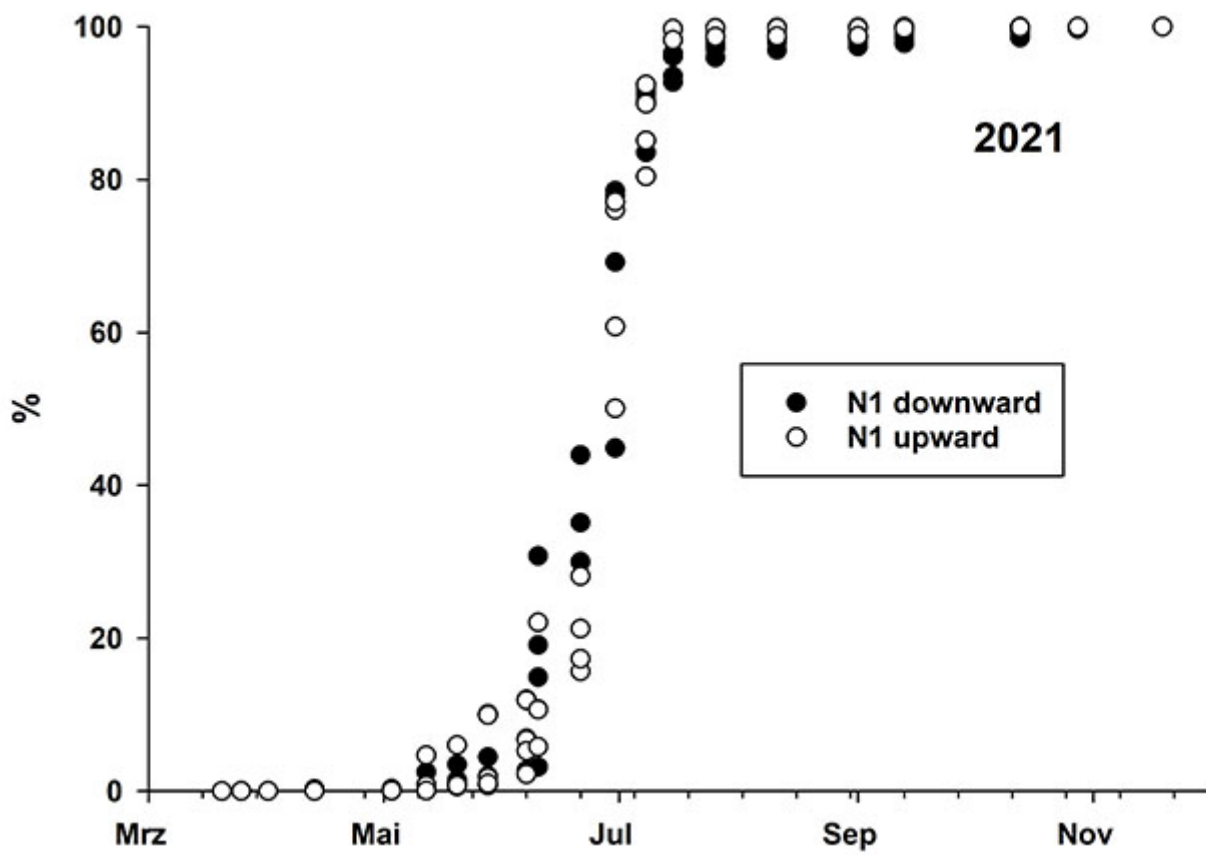


Fig. 14: The N1 migration expressed as the cumulative % (nymphae/d) for 2021, collected on the observation trees in the different plots during the entire season (upper and lower sticky band).

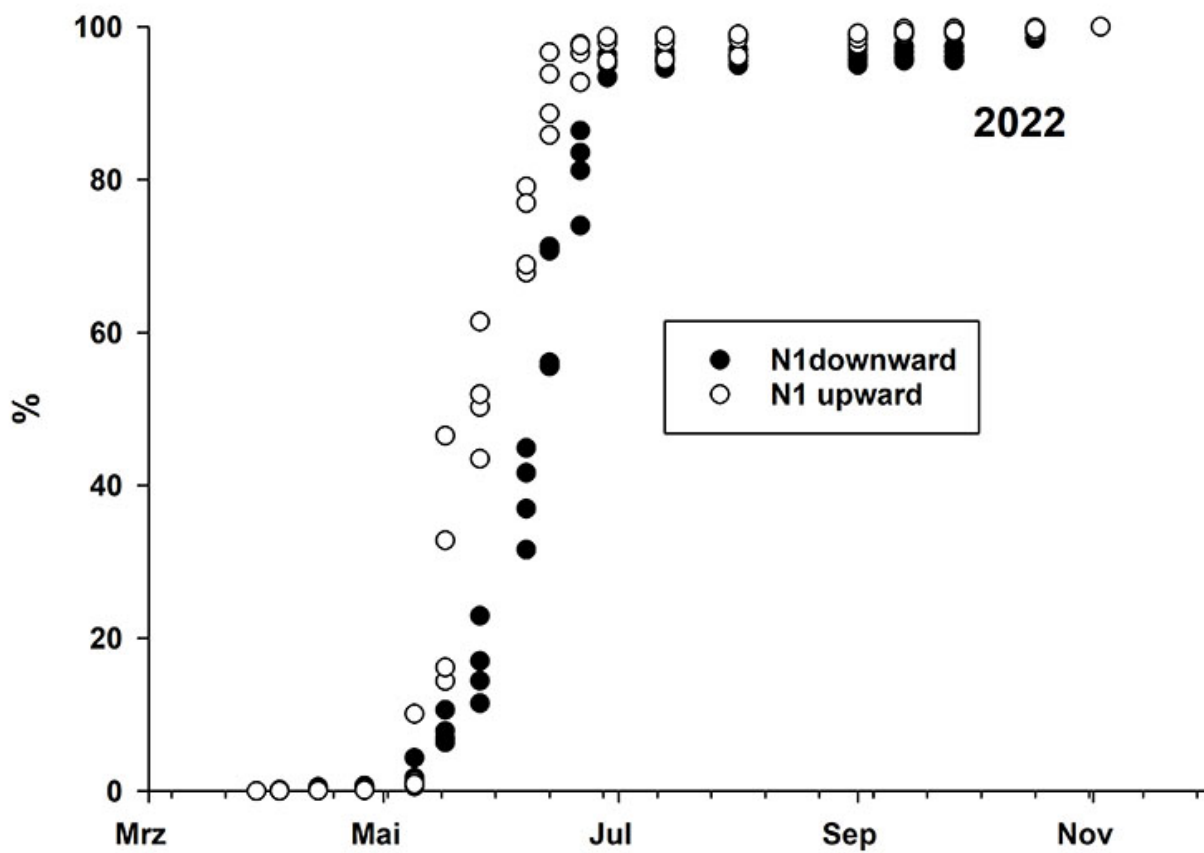


Fig. 15: The N1 migration expressed as the cumulative % (nymphae/d) for 2022, collected on the observation trees in the different plots during the entire season (upper and lower sticky band).



Fig. 16: The activated status with both sticky bands mounted on the white sticky base.



Fig. 17: The mounting process, applying the “double sticky band” on the upper white sticky base.



Fig. 18: The sticky bands mounted on the lower part of the tree above the grafting point of the observation tree.

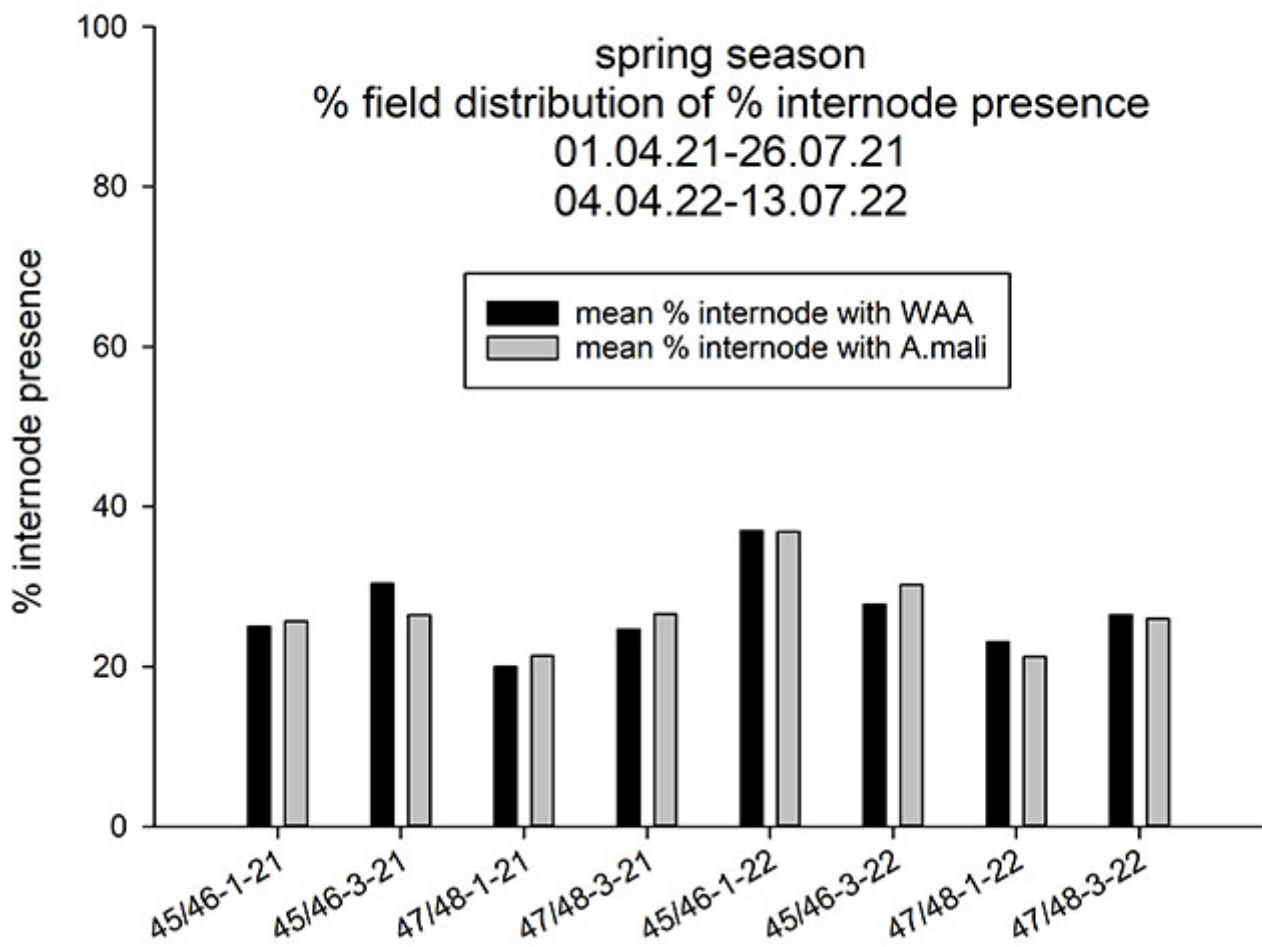


Fig. 19: Comparison of the % distribution of the mean WAA shoot colonization referred to the plots and the incidence of parasitism by *A. mali* per plot on the respective trees for each of the years 2021 and 2022.

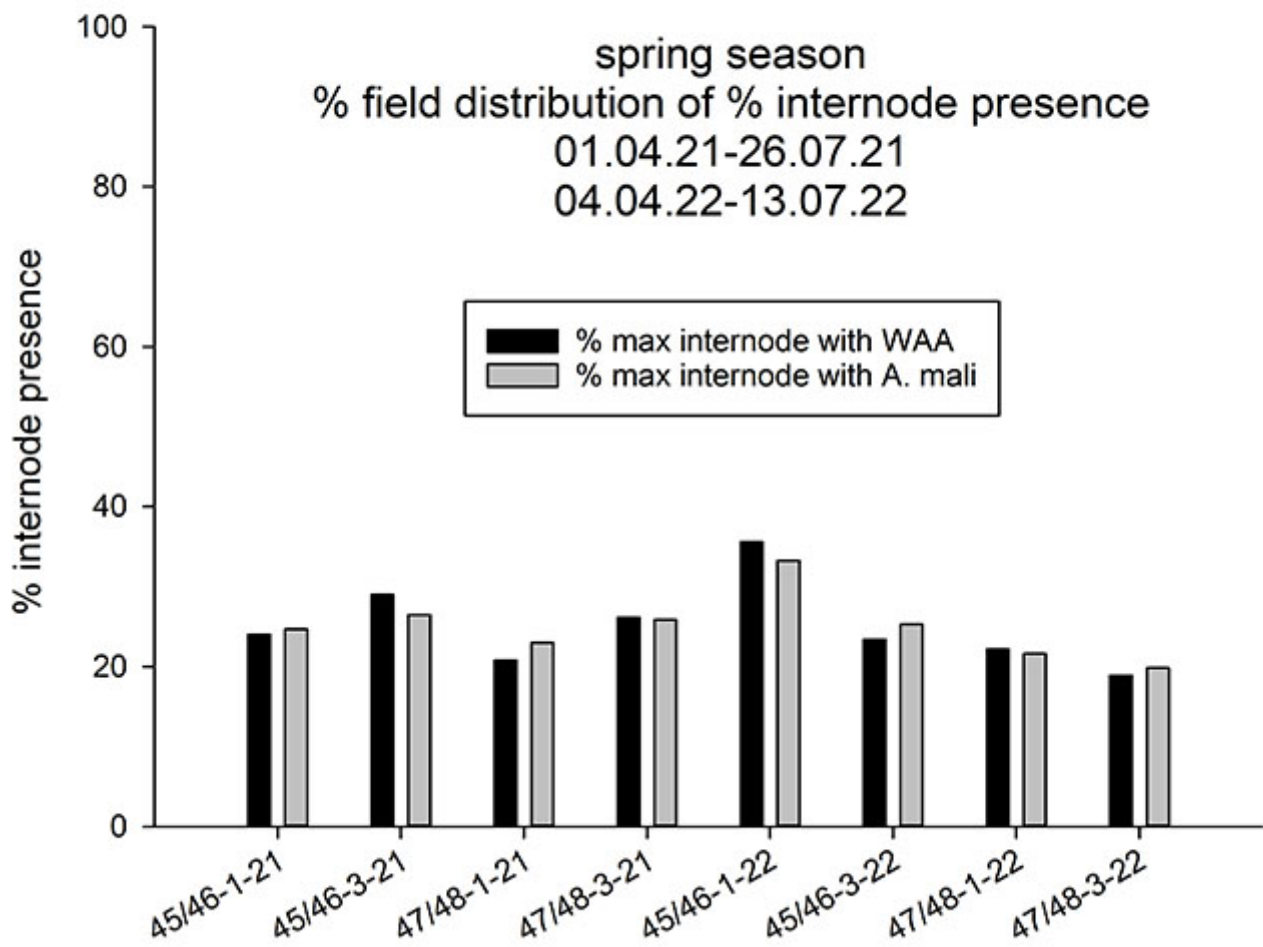


Fig. 20: Comparison of the % distribution of the maximum WAA shoot colonization referred to the plots and the incidence of parasitism by *A. mali* per plot on the respective trees for each of the years 2021 and 2022.



Fig. 21: As a result of the darkened, WAA instar migration, “mummies” accumulated at the top of an actually non activated observational tree, showing the typical exit holes.