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Approaches for a control strategy and biology of *Pentatoma rufipes* L. in organic fruit growing in Southern Germany

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Abstract

The red legged stinkbug Pentatoma rufipes *L. has developed into a serious apple pest in 2020 and 2021 in several regions of Southern Germany. Direct control measures show a certain efficacy but not high enough for a really successful control. Albeit for the combination of Neudosan® NEU and Trifolio®-S-forte moderate side effects on beneficials are to be expected, Spruzit® NEU and the mixture of Kantaro® and Neudosan® NEU will cause higher damage. Furthermore, the mixture with Kantaro® and Neudosan® NEU has to be considered toxic to bees (B1), following the current legislation in Germany. A really successful and sustainable strategy for the control of the red legged stinkbug has still to be developped. The first trial to release* T. cultratus *as a biocontrol agent shows promising results.*

Keywords: *Pentatoma rufipes, Trissolcus cultratus*, Neudosan® NEU, Kantaro®

Introduction

The red legged stink bug *Pentatoma rufipes* L. (Hemiptera, Pentatomidae) is common in Europe and was sometimes also found in the tree crown. It was considered an indifferent species until the first decade of this century when considerable damage in pear orchards was observed (Koenig, 2014) and all over Europe (Kehrli, P., Pasquier, D. 2012**;** Peusens, G., Beliën, T. 2012 and Powell, G. 2020). In 2017, in the frame of a BOELN-project, a monitoring was started for the occurrence and the damage potential of this species in apple orchards. In 2019, fruit damage in apple orchards that could be attributed to the occurrence of nymphs of *P. rufipes* in spring, was observed the first time in Germany (Al karrat et al., 2020). In the years 2020 and 2021 the monitoring of the biology and the occurrence of *P. rufipes* in correlation with the fruit damage in organic apple orchards was continued. Furthermore, the efficacy of the mixture of Neudosan® NEU with Trifolio®-S-forte that in previous trials (Al karrat et al., 2020) proved best was compared with the efficacy of a product based on pyrethrum and the mixture of Neudosan® NEU and Kantaro® (Maltodextrin). The parasitation of the eggs of the stink bug in the field was monitored and with the egg parasitoid reared from these eggs a first field trial was conducted to evaluate the potential of this parasitoid for a biological control of *P. rufipes.*

Material and Methods

*Monitoring of the biology of P. rufipes***:** Two years monitoring (2020–2021) of *P. rufipes* was carried out in an organic apple orchard at Lake Constance from March to October. A monitoring programme using beating trap samples (100 trees per sample, 1 branch/ tree) at regular timings during the growing season was performed. Falling insects were collected in a tray, counted and identified (life stage L1, L2, L3, L4, L5 and adult).

*Monitoring of fruit damage***:** The study was conducted over 3 years (2019–2021) in 13-15 organic apple orchards in the Lake Constance province, where *P. rufipes* was observed. The number of nymphs was determined by beating trap samples in spring. To determine the fruit damage, 500 fruits in each orchard were randomly taken in June before thinning and in August before harvest and externally inspected.

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Field trials: in 2020 and 2021 field trials were executed at different timings during the season (before blossoming and after harvest) in organic apple orchards with high infestation to test and compare the efficacy of several organic products. The rows in all plots were sprayed only one time and in direction of the driving. All experiments were conducted in a randomized complete block design. To determine the effect of these products on the occurrence of the 2nd instar nymphs of *P. rufipes* beating trap samples (50 branches per replicate = 100 branches per treatment and orchard) were taken immediately before the spraying and after one day. In the both years the application was done by the farmer with a modulation of the sprayer for 800 L water per ha.
<u>Trial 1: Before blossom</u>**:** The plots (6 rows * 33 trees, 2 plots per treatment) were arranged in two orchards. I: Early spring of 2020, two treatments (Mixture of Neudosan 2% and TS-forte 1% and Spruzit® NEU 4,6 l/ha) were applied. In 2020 and 2021 the experiments were repeated in the same orchards.
<u>Trial 2: After harvest</u>: In 2020 the experiment in trial I was repeated in the same orchards using only the mixture of Neudosan 2% and TS- forte 1%. In 2021 the experiment was conducted in two other orchards in October 2021 to determine the effect of the mixture of Neudosan 1% and TS- Forte 1% on the bug in comparison with the effect of Spruzit® NEU 4,6 l/ha and the effect of mixture of Neudosan® Neu (2%) and Kantaro (2,5%) on the overwintering stage of *P. rufipes* (2nd instar nymphs). The plots were designed as in trial I, only in trial II the single plots had more trees (55 trees per plot and treatment).
Laboratory trials: The efficacy against the second nymphal stage of *P. rufipes* of five different treatments was tested: **1.**- Neudosan®NEU 2%, **2**- Trifolio-S-Forte 1%, **3**- Neudosan®NEU 2% and Trifolio-S- forte 1%, **4**- Kantaro® (Maltodextrin) 2.5 % and **5**- mixture of Neudosan® Neu (2%) and Kantaro® (2,5%). Five 2nd instar nymphs of *P. rufipes* were placed in a Petri dish 9 cm containing a piece of an organic apple fruit. Nymphs were treated with the five treatments. For each treatment, five Petri dishes, with 5 nymphs each were tested. The number of dead *P. rufipes* was assessed after 2, 4, 24, 48, and 72 hours. In all experiments, the efficacy of each insecticide was calculated in relation to an untreated control treated with pure water and calculated as corrected mortality (%) according to Schneider- Orelli (Püntener, 1981).
Egg parasitoids: Surveys of the egg parasitoids of *P. rufipes* were conducted in the last three years in apple orchards at the Lake Constance, where the red-legged shieldbug is most commonly found. The egg masses usually found on the underside of leaves were collected each year from August to October. The collected egg clusters were each put into a Petri dish (9 cm), on a filter paper with absorbent cotton ball. The egg masses were reared in climatic chamber (24 ±1°C, 65% RH, 16:8 L: D), and checked daily until the parasitoids or nymphs emerged. Parasitization rate was calculated for each year.
I: Identification of the parasitoids: The collected parasitoid species was identified using SEM Micrographs by examining relevant body organs, such as head, antennae, thorax and abdomen. The first adult parasitoids emerged from parasitized bugs were kindly identified by Prof. Tortorici, then in our lab identified using morphologically traits following Talamas et al. (2017). Molecular analysis followed the method described by Tortorici et al. (2019) and the results were confirmed by rDNA-ITS2 sequences (GenBank Accession No. MN613474, MN613473).
II: Rearing of parasitoids: Frozen eggs of *Halyomorpha halys* were found to be suitable hosts for propagation of the parasitoid *Trissolcus cultratus* Mayr. The parasitoid colony has been maintained since 2019 using these frozen eggs under laboratory conditions at 24 ± 1 ˚C and 65 ± 5% RH, with a 16:8 L:D photoperiod. In 2020 and 2021 the *T. cultratus* population was revitalized in autumn by allowing them to parasitize native *P. rufipes* host egg.

III: Field release of *T. cultratus* for biological control*:* A trial to control *P. rufipes* by releasing the egg parasitoid *T. cultratus*, was conducted in an orchard in the Lake Constance region. The experiment was carried out in organic apple orchards with high infestation of *P. rufipes* where no parasitized eggs were found in the previous years. However, parasitized eggs were found in orchards in the region of Lake Constance. The orchards were divided across the rows into two plots each plot consisted in6 rows x 33 trees. In the first plot *T. cultratus* was released whereas the second plot served as untreated control. Between the two plots a buffer zone of ca. 60 m was not assessed. Release points were alternately chosen at each third tree along the two middle rows. For each release point a cardboard roll was prepared: approximately 50-55 parasitized eggs (10 days old eggs shortly before hatch of parasitoids). were glued with honey on a piece of paper and put inside the cardboard. A drop of honey was laid as an adult food source inside the roll. Release dates were as follows: (29.7., 5.8., 13.8., 20.8., 27.8., 2.9., 10.9., and 17.9.), and around 1000 parasitoids were released per date. To determine the efficacy of the parasitoid, the naturally laid eggs of *P. rufipes* were collected in August and September from treated plot and control and the parasitisation was monitored. To monitor the effect on the number of nymphs, beating trap samples (50 trees per sample and plot, 1 branch/ tree) were conducted at the end of September in the rows where the parasitoids were released, and in the control.

Results

Monitoring of the biology: As already observed in previous studies (Al karrat et al., 2020), *P. rufipes* performs one generation per year and overwinters as nymph in the 2nd nymphal stage. In spring, with the first warm days, they become active again and start their development by piercing into buds, leaves and later also into the young fruits. Adults appear in summer and in late s summer mating takes place and eggs are laid at the underside of the leaves of the fruit trees. The date when the eggs hatch and the first young nymphs appears was rather variable in our observations: In 2019 and 2020, at mid-September the eggs hatched and the first young nymphs were found. However, in 2021 the first nymphs were found only in the first days of October. In 2017, the first nymphs appeared even at mid October.

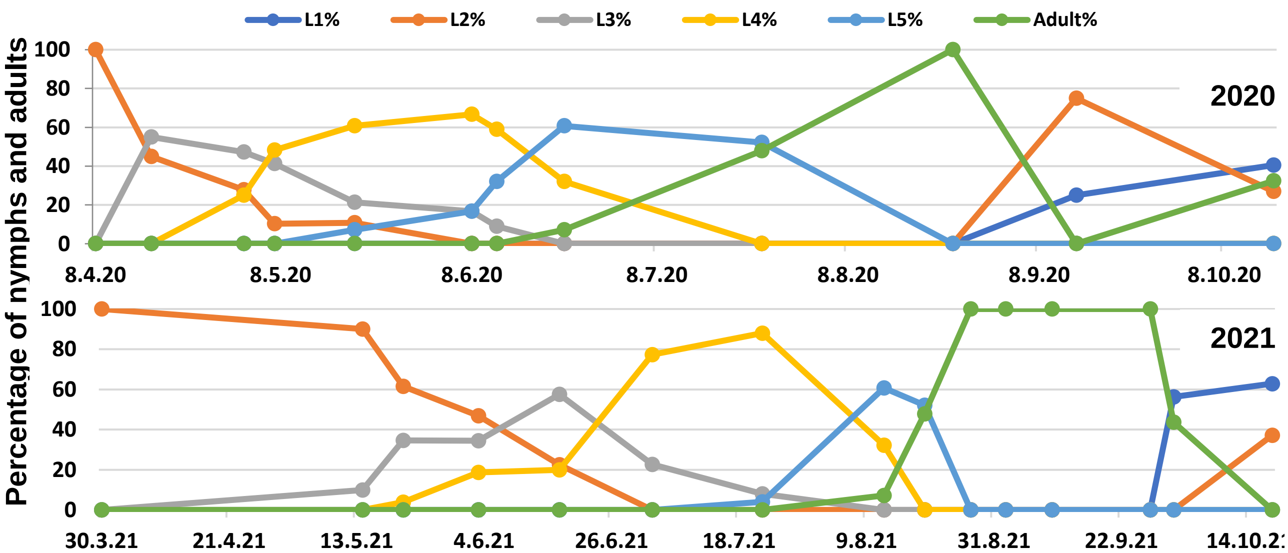

Figure1. Biology of *P. rufipes* in years 2020-2021 under field conditions.

Monitoring of the fruit damage: During the past three years of the monitoring of fruit damage, it was observed that the damage could be attributed to the occurrence of of red-legged shieldbug nymphs in spring in some orchards. The variety Santana seems to be very attractive for the bug. In 2020 and 2021, high damage has been observed on this variety in 4 orchards with higher populations of the stink bug, whereas the damage before thinning was more than 12% and reached up to 16% in 2021 in August before harvest. Higher fruit damage has also been observed on the variety Topaz, when the bug population was more than 15 nymphs in the beating trap sample (100 beats). On the late variety Braeburn, however, the fruit damage was 7% with a bug population of 27 nymphs in spring. Normally, early varieties as Santana and also Elstar (experience from practice) are more concerned, however, also in later varieties as Breaburn or Jonagold higher populations occur and can produce considerable damage (Tab. 1).

Table 1: Number of nymphs in the beating trap samples (100 beats) in spring and fruit damage at the assessments before thinning (June) and before harvest (August) in the years 2020 and 2021.

Variety/year	2020			2021		
	Nymphs in beating trap (2.4.)	Total damage (26.6.)	Total damage (12.8/2.9.)	Nymphs in beating trap (31.3.)	Total damage (26.6.)	Total damage (8.8./1.9.)
Topaz	**60**	8.8	12.6	**14**	3.4	6.6
Topaz	**8**	0.6	3.2	**37**	9.8	15.8
Galant	**0**	1.4	1.8	**0**	1	1.6
Topaz	**3**	2.2	1.6	**11**	3.6	4.6
Santana	**2**	1	1.2	**9**	3.2	2.2
Topaz	**2**	2.2	0.8	**18**	7.8	4
Topaz	**8**	3.8	2.6	**32**	9.2	3.8
Santana	**61**	7.4	13.2	**48**	13.2	12
Braeburn	**13**	2	2.2	**27**	7.2	3.2
Topaz	**12**	1	3	**28**	7.8	6
Topaz	**2**	0.2	1	**0**	0.4	0.4
Topaz	**5**	0.2	1.6	**1**	0.2	0.6
Jonagold	**65**	10.6	10.8	**43**	14.2	8.8
Jonagold	**3**	0.8	0.6	**9**	3.4	2.8
Santana	**38**	6	11.2	**47**	12.6	16.2
Santana	**106**	14.6	5.4	**46**	13	12.6
Santana	--	-	-	**62**	13.2	11.8

Field trials: In the spring of the year 2020 and before blossom, the mixture of Neudosan® NEU 2% and Trifolio S-forte® 1% resulted in the highest efficacy (~95 % efficacy by Henderson and Tilton) compared with the efficacy of a singular application of Spruzit® NEU 4.6 l/ha, while the highest efficacy of this mixture was lower after harvest in the same year (Fig.2).

The trial in spring 2021 (Fig.3) showed the same results as the previous trials for the mixture of Neudosan® Neu 2% and Trifolio S-forte® 1% which reduced the number of bugs, the efficacy ranged between 52-83%.

In the trial in autumn 2021, due to the promising laboratory results (Fig. 5), also the mixture of Neudosan® NEU 2% and Kantaro® 2.5% was tested. The efficacy of this mixture was with 71 % about 10 % higher than the efficacy of the combination of Neudosan® NEU and Trifolio®-S-forte (Fig. 4).

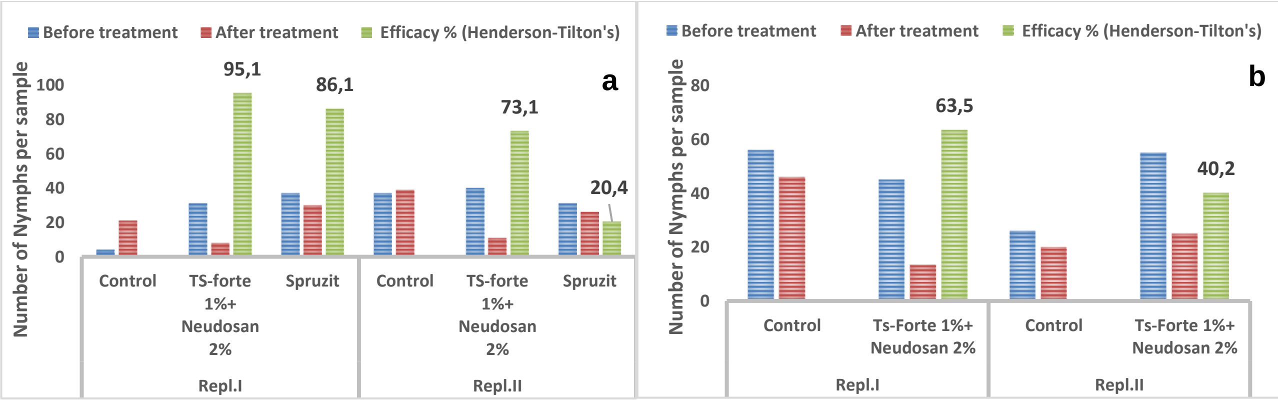

Fig. 2: Number of nymphs of *P. rufipes* found before and after the application (a: at 8.4.2020 and b: at 14.9.2020) of Spruzit® NEU 4.6 l/ha and the mixture of Neudosan® NEU 2 % and Trifolio-S-forte® 1%. The efficacy (Henderson & Tilton) is indicated above the bars.

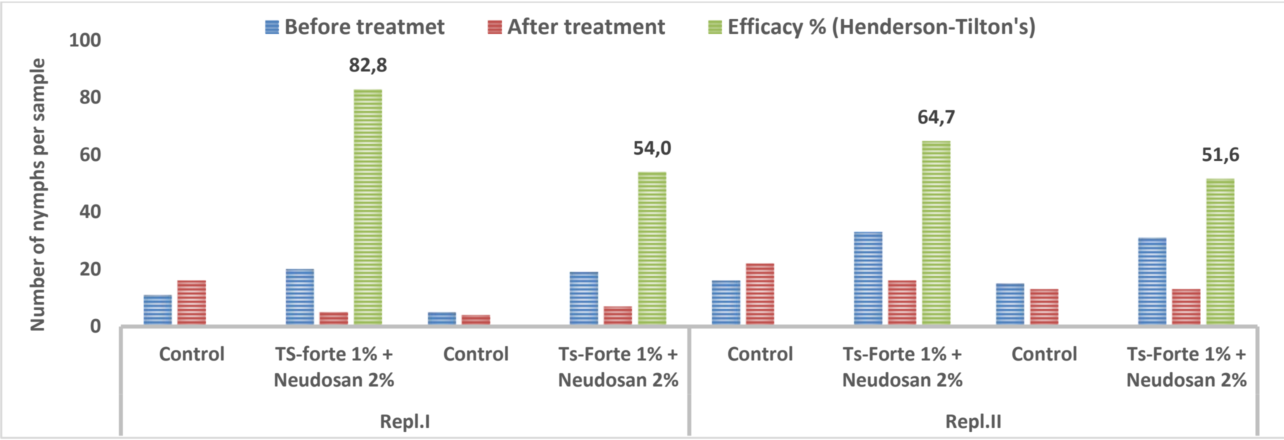

Fig. 3: Number of nymphs of *P. rufipes* found before and after the application at 1.4.2021 1 of Spruzit®NEU 4.6 l/ha and the mixture of Neudosan® NEU 2 % and Trifolio®-S-forte 1%. The efficacy (Henderson & Tilton) in % is indicated above the bars.

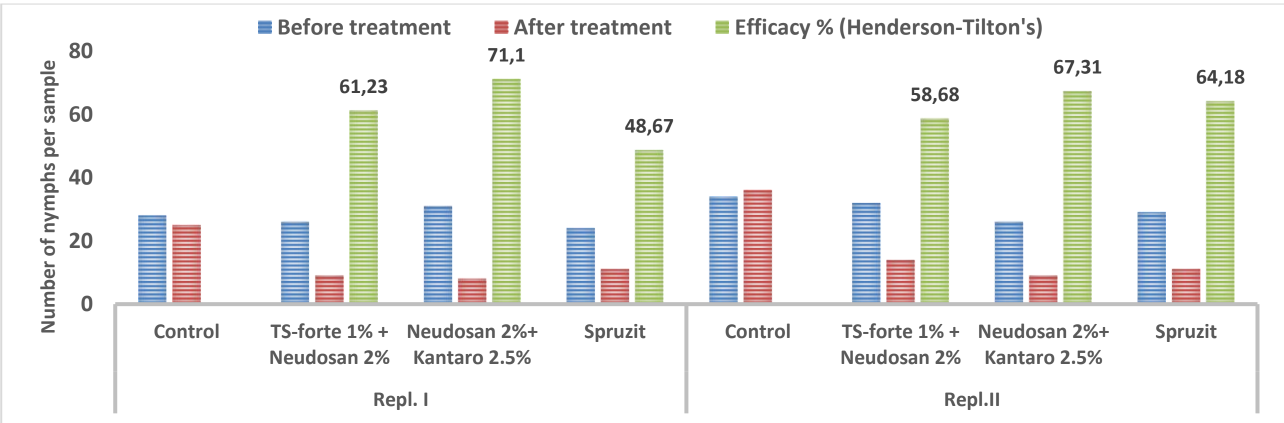

Fig. 4: Number of nymphs of *P. rufipes* found before and after the application **at 30.9.2021** of the different products as follows: 1. Mixture of Neudosan® 2 % and Trifolio S-forte® 1%, 2: Mixture of Neudosan® 2 % and Kantaro® 2.5% and 3: Spruzit® NEU 4.6 l/ha. The efficacy Henderson & Tilton) in % is indicated above the bars.

Laboratory test: The combination of Neudosan® NEU 2% and Kantaro® 2.5% was the best and in this test it killed more than 90 % of the nymphs within 3 days, exerting the highest efficacy (Fig. 5). The mortality in the treatments with the other compounds was lower but for Neudosan® NEU was about 80 %. However, for the treatement with Neudosan® NEU alone and its combination with Trifolio®-S-forte the initial mortality was higher than for the combination with Kantaro®.

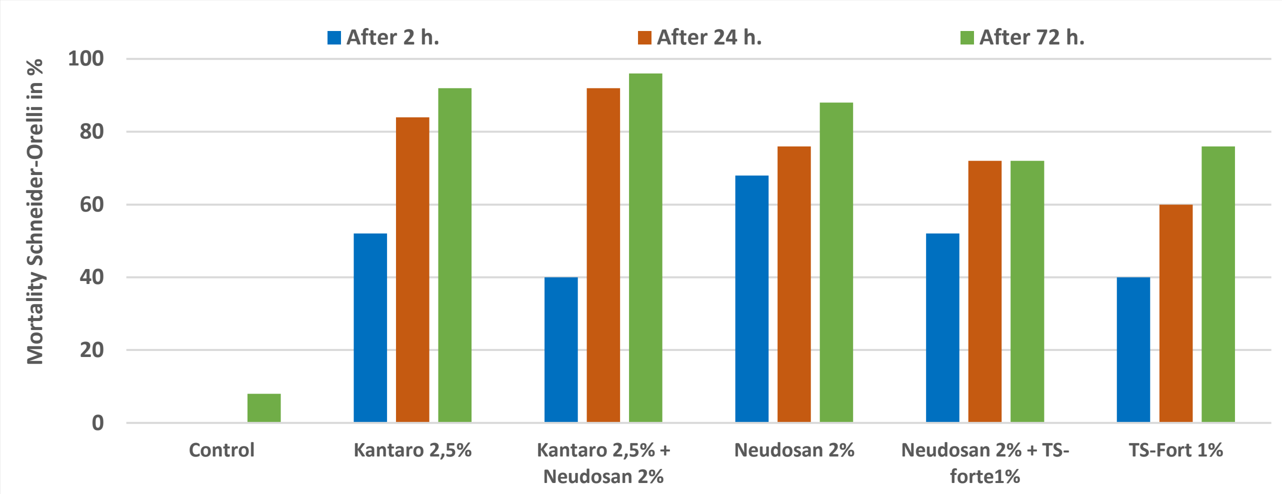

Fig. 5: Corrected mortality of second nymphal instar *P. rufipes* after 2, 48 and 72 h when exposed to different compounds on the second nymphal stage of *P. rufipes* in the laboratory.

Parasitization of stinkbug eggs in the field

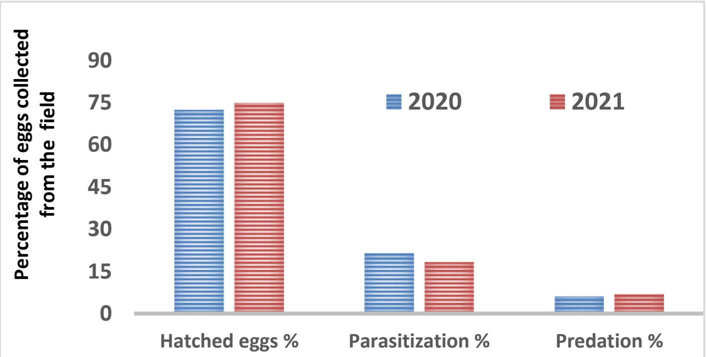

Fig.6: Proportion of hatched parasitoids, parasitized and predated eggs of *P. rufipes* collected in 2020 and 2021 from the field.

All parasitoids hatched from the eggs collected in the field were identified as *T. cultratus.* The parasitization rate of the collected eggs was about 20 % in both years 2020 and 2021 (Fig.6). Predation played a minor role.

Field trial for the release of T. cultratus

The parasitoid colony has been maintained under laboratory conditions (24 ± 1 ˚C, 65 ± 5% RH, 16: 8 L: D) since 2019 at the laboratory of the University of Hohenheim, Institute of Phytomedicine, using frozen eggs of *Halyomorpha halys* as host. The frozen eggs were obtained from Katz Biotech AG.

In the plot where *T. cultratus* was released the parasitation rate of the eggs was above 90 % in both repetitions whether in the control plots no parasitization was found (Fig. 7a). The efficacy (Abbott, 1925) in reduction of the number of nymphs hatched was about 90 % in the first plot and 70 % in the second plot (Fig. 7b).

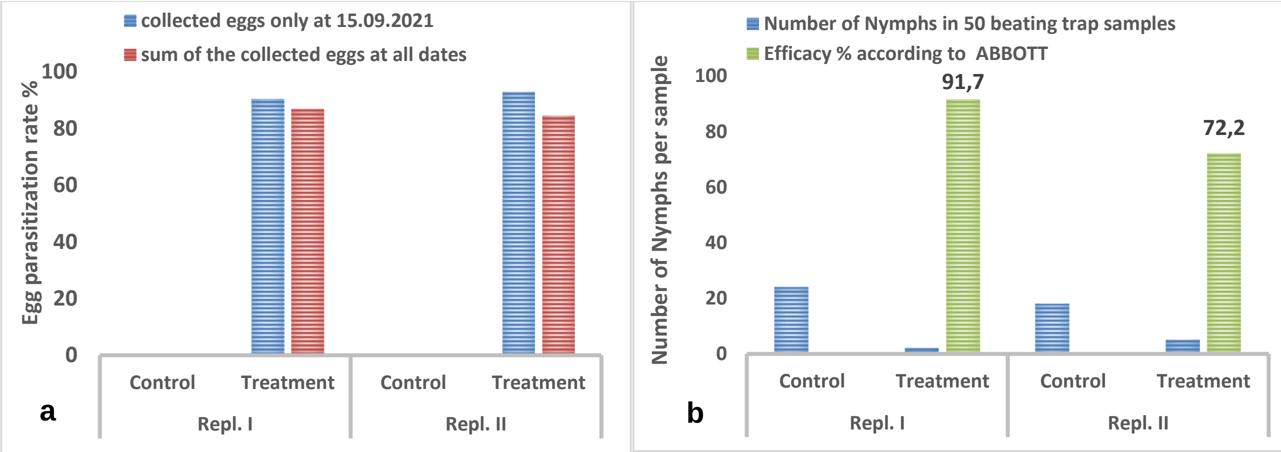

Fig. 7: a: Parasitization rate of *P. rufipes* eggs in the plots with release of *T. cultratus,* **b**: Number of nymphs of *P. rufipes* found in control and treatments after release the parasitoids. (Efficacy % calculated according to ABBOTT).

Discussion

The monitoring data of the years 2020 and 2021 show that the red legged stink bug that in 2019 caused first time damage in apple (Al karrat et al., 2020) is changing into a serious apple pest in 2020 and 2021 in several regions of Southern Germany. In Rhineland-Palatinate, until now, the damage is limited to pear orchards (Zimmer, 2022, oral communication) whether in the Neckar valley also some fruit damage in apples was observed in practice. Some special preference for the variety Santana seem to appear but damage was observed also in other varieties. It is still too early to establish a definitive threshold for the occurrence of damage based on the number of larvae found in spring. However, it is already clear that in each case this will be lower than 10 nymphs in a 100 beats-beating trap sample.

Direct control measures show a certain efficacy but not high enough for a successful control. The efficacy of the mixture of Kantaro® and Neudosan® NEU revealed in the last trial in autumn 2021 has to be confirmed in further trials. Whereas for the combination of Neudosan® NEU and Trifolio®- S-forte moderate side effects on beneficials are to be expected, Spruzit® NEU and the mixture of Kantaro® and Neudosan® NEU will entail higher damage on beneficials. Furthermore, the mixture with Kantaro® and Neudosan® NEU has to be considered toxic to bees (B1) following the current legislation in Germany. In spring, for organic farms it will be rather difficult for organic orchards to be conform to this request. Whether the 10 % increase of efficacy for this mixture in comparison to the mixture with Trifolio® -S-forte are worth the trouble must be evaluated further. Considering the side effects, application in autumn seems more attractive. However, it is advisable to wait until all nymphs are hatched and then in some years the first nymphs are already in the overwintering places and will not be compromised by the application. Furthermore, the most important natural antagonist, the egg parasitoid *T. cultratus*, hatches in autumn together with the nymphs and, thus, will probably be compromised by such treatments.

A really successful and sustainable strategy to control the red legged stinkbug has still to be developed. The first trial to release *T. cultratus* as a biocontrol agent shows promising results. Further research has to be done to establish a production of this parasitoid and to design the best strategy for a release in combination with other measures. However, the data collected monitoring the biology over several years show that the oviposition period of *P. rufipes* is rather variable in time. For a successful release of the egg parasitoid it will be necessary to find clear criteria to determine the date of the peak of oviposition.

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