Research Article

Received: 21 June 2022 Revised: 20 March 2023 Accepted article published: 19 April 2023 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7504

Effectiveness of field-exposed attract-and-kill devices against the adults of *Popillia japonica* (Coleoptera: Scarabaeidae): a study on duration, form and storage

Francesco Paoli,[a*] Immacolata Iovinella,[a] Francesco Barbieri,[a] Chiara Sciandra,[a] Giuseppino Sabbatini Peverieri,[a] Giuseppe Mazza,[a] Giulia Torrini,[a] Gian Paolo Barzanti,[a] Claudia Benvenuti,[a] Agostino Strangi,[a] Giovanni Bosio,[b] Emiliano Mori,[c,d] Pio Federico Roversi[a] and Leonardo Marianelli[a]

Abstract

BACKGROUND: The Japanese beetle *Popillia japonica* Newman is an insect pest native to Japan that has spread into North America, the Azores and, recently, into continental Europe. Here, we present a study assessing the effectiveness of a long-lasting insecticide-treated net (LLIN), assembled in semiochemical-baited attract-and-kill devices (A&Ks) as a low environmental impact means to control *P. japonica* in the field. We compared the attractiveness of three different forms of A&Ks that were left outdoors throughout the summer, and the residence time of *P. japonica* landing on them. Moreover, we performed a preliminary study testing the effectiveness of new LLINs after storage. Collected data also allowed us to investigate the beetles' diel flight patterns in relation to meteorological conditions.

RESULTS: Killing effectiveness of the field-exposed A&Ks declined steadily over the flight season (from 100% to 37.5%) associated with a decrease in residues of α-cypermethrin, the active ingredient in the LLINs. The different A&K forms (pyramidal, octahedral and ellipsoidal) attracted similar numbers of beetles. Individual beetles' residence time ranged from 75 to 95 s and differed slightly between A&K forms. Effectiveness of LLINs decreased by ≈30% after 1 year storage. Based on numbers landing on A&Ks, the beetles' flight activity peaked about 14:30 h and was inversely correlated with relative humidity.

CONCLUSION: This study indicates that semiochemical-baited A&Ks are effective for controlling *P. japonica* in the field. Because of active ingredient decay, the LLINs should be replaced after 30–40 days of field exposure to ensure that the A&Ks remain fully functional.

Supporting information may be found in the online version of this article.

Keywords: activity rhythm; Japanese beetle; insecticidal net; insect trap; pest management

1 INTRODUCTION

The Japanese beetle, *Popillia japonica* Newman, is a scarab beetle native to Japan that was accidentally introduced into the United States more than 100 years ago.[1] To date, it also is present in other countries such as Canada, the Azores, Switzerland and Italy, where it is steadily spreading and where attempts of eradication appear difficult.[2] Owing to its status as an agricultural pest, *P. japonica* is listed among the EU priority pests.[3] Strategies to control or curtail the spread of *P. japonica* are urgently needed. In recent years, campaigns were conducted in Italy to identify natural biological control agents for this pest. During these studies new species/strains of parasitic and entomopathogenic nematodes (*Heterorhabditis*

* Correspondence to: F Paoli, CREA Research Centre for Plant Protection and Certification, 50125 Florence, Italy. E-mail: francesco.paoli@crea.gov.it

a CREA Research Centre for Plant Protection and Certification, Florence, Italy

b Settore Fitosanitario e Servizi Tecnico-scientifici, Torino, Italy

c Consiglio Nazionale delle Ricerche, Istituto di Ricerca sugli Ecosistemi Terrestri, Sesto Fiorentino, Italy

d National Biodiversity Future Center, Palermo, 90133, Italy

bacteriophora POP16) and fungi (*Metarhizium robertsii* 17/T02) were isolated and tested.[4–9] In Italy, fields infested by *P. japonica* were treated with entomopathogenic nematodes and/or fungi as a part of an integrated pest management (IPM) programme, with variable results.[10–13] To limit the spread of this beetle, additional sustainable strategies need to be used. To date, control of adults, which are gregarious and defoliate a wide range of plant species, has mostly relied on chemical insecticides.[1,2]

A promising technique with low environmental impact that has been used in recent years by the regional Plant Protection Services in Italy is the use of *attract and kill* devices, hereafter referred to as A&Ks.[14] Such devices are made of long-lasting insecticide-treated nets (hereafter referred to as LLINs) baited with the standard Japanese beetle dual semiochemical lure.[15] The LLINs usually are provided with a pyrethroid as active ingredient, such as α-cypermethrin or deltamethrin, in different concentrations and with different release techniques (impregnated or coated fibers). The adults are attracted by the lure and then grasp and walk on the LLINs of the A&K devices. Following a few seconds of tarsal contact (≥5 s), the insect becomes paralyzed and eventually, depending on the insecticidal concentration and duration of contact, dies. In laboratory experiments[16] the effectiveness of LLINs against *P. japonica* suggested that their use in A&Ks could be a useful pest management tactic in the field.

Compared to mass trapping, which is more effective for monitoring purposes rather than for control in places where the pest is established,[17] the A&K method does not require continuous emptying of traps during the flight peak period, which in areas with severe infestations needs to be done more than twice per day. On the contrary, beetles that have tarsal contact with the LLINs often do not become paralyzed until 10–30 min thereafter,[16] allowing them time to fly away before dying. With standard *P. japonica* traps, the odor of collected decaying dead beetles may reduce subsequent captures,[18] so having the time for LLIN-exposed beetles to fly away before death may help preserve the lure's attractiveness.

Because LLINs were originally produced for different purposes (e.g. indoor use against mosquitoes and use in forests against bark beetles), no data are available on the duration of the effectiveness of A&K against *P. japonica* under a prolonged sun exposure in the field. Indeed, pyrethroids are known to decay under UV ray exposure,[19] decreasing their killing effectiveness. Because it is important to assess how long LLINs can be used before replacement, we evaluated the effectiveness of field-exposed A&K over time by tracking the beetle mortality and pyrethroid decay over time. We also investigated whether different forms of A&Ks may influence the number of attracted adults or their mean residence time on the LLIN. In addition, we performed a pilot experiment on the effectiveness of LLINs after storage. Finally, the meteorological data along with the number of walking beetles on different A&Ks allowed us to define the daily activity rhythm of *P. japonica* adults, information that is relevant to the application of control measures against this pest.

2 MATERIALS AND METHODS

2.1 Proof of concept of the A&K set-up

Two different forms of A&K were tested for 7 days to evaluate their attractiveness to *P. japonica*. The first A&K consisted of a tripod from the Trinet® (BASF™, Ludwigshafen a. R., Germany) template (hereafter referred to as 'Trinet'), which had an *open setup* with a lure freely accessible to insects (upper and lower parts of the A&K were not covered by the net) [Fig. 1(A)]. The second A&K was housed by an umbrella frame to support the LLIN (hereafter referred to as 'Umbrella'), with a *closed setup* having a lure unattainable from insects (the LLIN covered all the frame) [Fig. 1(B)]. In both cases the dark green Storanet® LLIN (1.57 mg α-cypermethrin/g fiber) by BASF™ covered the device. The A&Ks were baited with the standard floral (phenethyl propionate + eugenol + geraniol, 3:7:3) and sex pheromone, (R,Z)-5-(1-decenyl) dihydro-2(3*H*)-furanone dual lure produced by Pherocon® (TRÉCÉ Inc., Adair, OK, USA). In summer 2018, one Trinet and one Umbrella were set up in an infested zone near 'Villa Picchetta' (Cameri, northern Italy N 45° 30′ 51″, E 8° 41′ 47″). The A&K devices were checked on 7 days between 26 June and 2 August 2018. The A&Ks were placed ≈50 m apart and their positions were routinely exchanged every 60 min to exclude possible biases from environmental factors such as wind direction and proximity to food plants. The number of *P. japonica* walking on the A&K was counted every 60 min between 10:00 h and 17:00 h.

Beetle counts were compared between the two devices using the Mann–Whitney U-test. Data for this trial and subsequent ones are presented as means ± standard error (SE).

2.2 Effectiveness of field-exposed LLINs throughout the flight period and related pyrethroid content decay

These experiments aimed to evaluate the duration of effectiveness of LLINs under field conditions over the flight period of *P. japonica*. In June 2019, in an area near the above-mentioned Villa Picchetta, four fields with similar agronomic management, 500 m distant from each other, were selected to conduct a field test with A&Ks. In each of the selected fields, three Umbrella A&Ks covered by Storanet® LLINs and baited with the aforementioned standard dual lure were deployed on 3 June. Another A&K with a noninsecticidal net was placed in each field as a control.

A brand new LLIN, just unwrapped from the packaging, was tested in June (T0) to confirm the laboratory results obtained in 2018.[16] Twenty *P. japonica* adults (10 males, 10 females) were allowed to walk on Storanet® for ≈90 s which is the mean residence time of *P. japonica* on an A&K.[16]

To test how long LLINs were effective upon field exposure, one A&K was removed from each field at the beginning of July (T1), August (T2) and September (T3), corresponding to 29, 60 and 91 days of exposure in the field, and the LLIN was separated from the device. On the same day, at a different location, ≈100 *P. japonica* (50 males, 50 females) were hand-picked from the field and divided into five groups of 20 individuals. The beetles from four groups were allowed to walk on a single piece of the field-exposed LLIN (one net per each tested group per test time) for *c.* 90 s, while a control group was allowed to walk on a net without insecticide for the same period. All tests were conducted outdoors on sunny days between 10:00 h and 14:00 h, a period for which laboratory tests already had been carried out,[16] when *P. japonica* adults are actively feeding in the field.[20] The groups of treated and untreated insects were transferred to the laboratory of CREA-DC (Florence), kept in plastic cages in an insect-rearing room at 26 °C with a 16 h: 8 h, light:dark photoperiod, and fed with fresh hazelnut leaves replaced every 2 days together with a moistened cotton swab. The beetles' health (alive, paralyzed, dead) then was monitored for 13 consecutive days. To assess the mortality, data from groups of insects that received the same treatment were pooled before analysis. Chi-square contingency tests followed by pairwise comparisons (significance level $P < 0.05$) were

Figure 1. *Attract-and-Kill* forms used in the proof-of-concept trial. A: Trinet® form. B: Umbrella form.

performed. The effectiveness of field-exposed LLINs was assessed using Henderson–Tilton's formula.[21]

All of the field-exposed LLINs were sealed in plastic bags and stored in the dark at room temperature (RT) after their use as A&Ks in the previous experiment. In October 2019, three 10 cm × 10 cm pieces of each of the field-exposed LLINs collected at T1, T2 and T3 were cut and sent to BASF™ laboratories for a residual analysis of the α-cypermethrin content. The analysis was performed following the protocol reported in Rolf *et al.*,[22] whose method was based on CIPAC 454/LN/M/3.[23] To analyze the pyrethroid decay the one-way Welch's ANOVA was performed followed by the Tamhane *post hoc* test using SPSS STATISTICS 20.

Correlation between LLIN effectiveness, as determined by Henderson–Tilton's index, and mean pyrethroid content was assessed.

2.3 Effectiveness of new LLINs after storage

A pilot test on the persistence of insecticidal effectiveness of LLINs on *P. japonica* was performed on the Storanet®, stored in the dark at RT (*c.* 15–25 °C winter–summer). To compare the effectiveness of the LLIN tested in June 2019, we conducted new tests in September 2019 and in June 2020 with LLINs coming from the same batch used in June 2019. In September 2019 and June 2020, 20 field-collected adult *P. japonica* per period (10 males, 10 females) were allowed to walk on the LLINs for *c.* 90 s, whereas the control beetles were allowed to walk on a net without insecticide to assess the natural mortality. Tests were carried out between 10:00 h and 14:00 h on a sunny day at a site within the infested area of Cameri (N 45° 31′ 58″, E 8° 41′ 59″). Treated and untreated insects were transferred to the same laboratory described above, kept in plastic cages, fed with fresh hazelnut leaves, and monitored for 13 days. Chi-square tests ($P < 0.05$) were performed and effectiveness was evaluated by Henderson–Tilton's formula.

2.4 Evaluation of different forms of A&K and residence time of *P. japonica* on different A&Ks

After the results of the proof of concept (see Section 2.1), three different closed A&Ks were conceived to determine whether the form of the A&K could play a role in increasing landings by *P. japonica*. Because Umbrella A&Ks were no longer available at the time of this experiment, we decided to replace this form with a similar one (pyramid) after a preliminary experiment that showed comparable effectiveness (See Supporting Information S1). Therefore, three different forms of A&K were tested: a pyramidal, an octahedral and an ellipsoidal form.

The pyramidal A&K was made of iron rods (base 80 cm × 80 cm, height 35 cm) welded together and the lure was tied to a small rope dangling ≈15 cm away from the apex [Fig. 2(A)]. The surface area of the LLIN covering the A&K was ≈10 000 cm^2. The octahedral A&K was built by coupling the bases of two pyramids [Fig. 2(B)]. The upper part was made of iron rods (base 80 cm × 80 cm, height 35 cm) welded together. The lower inverted part was made of polycarbonate and was not covered by LLIN. A layer of perforated polycarbonate was inserted between the upper and

Figure 2. Different forms of *attract-and-kill devices* (A&Ks). A: pyramidal A&K. B: octahedral A&K. C: ellipsoidal A&K.

lower parts. In this way, the odour of the lure, lying on the bottom of the inverted pyramid, could diffuse homogenously on the LLIN passing through the aeration holes. The surface of the LLIN covering the A&K was ≈8500 cm^2. The ellipsoidal A&K was modified from a commercial lampshade (26 cm × 50 cm) whose covering paper was replaced with a LLIN [Fig. 2(C)]. The surface of the LLIN covering the A&K was ≈4000 cm^2. The lure was hung from the apex.

Each of the different A&Ks was elevated to ≈1.8 m bottom height by an iron pole fixed in the ground. The A&Ks were tested on three different days, 29 and 30 June 2021, and 22 July 2021, in three different uncultivated fields, ≈1 km distant from each other, with a large presence of Poaceae, located in the municipality of Cameri (Province of Novara: field 1: N 45° 30′ 44″, E 8° 41′ 27″; field 2: N 45° 30′ 51″, E 8° 40′ 30″; field 3: N 45° 31′ 59″, E 8° 41′ 57″). Field 1 was a little bit closer to preferred host plants (e.g. *Prunus avium* or *Corylus avellana*), than fields 2 and 3. In each field the three A&Ks with different form were placed and shifted every hour from 10:00 h to 16:00 h, through three different positions (≈50 m distant from each other). In this way, each A&K occupied each position at every hour during the 3-day observations. Every 30 min the number of *P. japonica* walking (tarsal contact) on the A&K was counted.

Data were pooled for every A&K and analyzed by Kruskal–Wallis using the software PAST 4.05. Data also were pooled and analyzed per field by Kruskal–Wallis and a *post hoc* Mann–Whitney pairwise U-test. Bonferroni correction was applied. Mean residence time was calculated by recording duration of contact (walking or grasping) with the LLINs for ≈12 beetles per hour ($n = 1931$). Mean residence time was calculated both on cumulative and hourly bases for each A&K. Statistical comparison between residence times on the different A&Ks was carried out by Kruskal–Wallis H-test and a *post hoc* Mann–Whitney pairwise U-test for both cumulative and hourly data. Bonferroni correction was applied on data sorted by hour for each A&K device. A similar analysis on the mean residence time for within-field pooled data also was performed. Data were analyzed using the software PAST 4.05.

2.5 Activity rhythms of *P. japonica*

Temporal patterns of beetles on the A&Ks were calculated through the R package overlap.[24] For all *P. japonica* counts, we reported the date and the solar hour on a dataset. The use of solar hour allows a better evaluation of temporal patterns, as being defined by the position of the sun in the sky. The Hermans–Rasson r-test was computed to assess whether *P. japonica* showed a random activity pattern over the hours it is active.[25] We measured the coefficient of overlap between temporal patterns of beetles on the different A&K trap types; this coefficient ranges between 0 for no overlap and 1 for total overlap.[24] We calculated the Δ4 estimator because the smallest sample of pairwise comparison was >75 records (see[26]). The 95% confidence intervals (hereafter, CIs) were computed by using 10 000 bootstrap replicates.[27] Bootstrap tests were used to obtain a probability test in which two sets of circular observations belonged to the same distribution, for all species pairs, with the function compareCkern of R:activity.[28] The Mardia–Watson–Wheeler W-test (MWW test, U2) was used to compare temporal overlap between different A&K trap types, and it was calculated through R:circular.[29]

General linear models (GLMs)[30] were used to analyze the effects of average temperature, relative humidity, wind speed (km h^{-1}), sun radiation (W mq^{-1}), atmospheric pressure (hPa) and A&K trap type on the total number of beetles on the respective traps. Meteorological data were taken from Arpa Piemonte (Regional Agency for the Protection of the Environment). After the verification of lack of intercollinearity amongst variables, all variables were included in a total model. Nonsignificant variables were removed one at a time, until the elimination of terms caused a significant increase in the residual deviance ('stepwise-method'[30]).

3 RESULTS

3.1 Proof of concept of A&K set-up

Over the 7-day trial, significantly more beetles were counted on the Umbrella A&K than on the Trinet® A&K (4378 *versus* 2351 beetles, respectively; n = 63 observations; U = 1273; $P < 0.001$). These data led us to plan the subsequent experiments with LLINs conceived with a closed setup.

3.2 Effectiveness of field exposed LLINs and related pyrethroid content decay

Contact for 90 s with the LLINs tested at T0, early in the beetles' seasonal flight period, resulted in 100% mortality of treated adults and no mortality in the control group during the 13 days post-exposure holding period (Fig. 3). The overall percentage of mortality at T1 (July; after 1 month of LLINs exposure, just 1 week before the peak flight) was 68.8%, with a significant difference against the control ($\chi^2 = 16.8$; df = 1; $P < 0.001$), in which 15% mortality occurred. At T2 (August; after 2 months' field exposure of nets) the mortality of individuals on treated nets was 73.3%, however mortality in the control rose to 50% with no statistical difference between treated and control groups ($\chi^2 = 3.18$; df = 1; $P > 0.05$). At T3 (after 3 months of LLINs exposure, late in the flight period, which ended by the end of September) the mortality of treated individuals was 81.3% while mortality of the control individuals was 70%, with no difference between groups ($\chi^2 = 0.64$; df = 1; $P > 0.05$). Adjusted percentage mortality from exposure to treated nets, calculated through Henderson-Tilton's formula to account for control mortality, was 100%, 65.3%, 47.5%, and 37.5% at T0, T1, T2, and T3, respectively.

The α-cypermethrin content of LLINs declined with increasing duration of field exposure ($F = 1321$, df = 3; $P < 0.0001$). New netting (time T0) had content significantly higher than the field exposed LLINs collected at T1, T2, and T3 (Tamhane post-hoc test, $P < 0.05$) confirming that the pyrethroid degrades over time in the field. The LLINs placed in the field in June had lost nearly 80% of their pyrethroid active ingredient by August (Fig. 4). Furthermore, the curve of the pyrethroid decay was correlated with decrease of effectiveness against *P. japonica* adults (R = 0.979).

3.3 Effectiveness of new LLINs upon storage

LLINs stored in the dark for 3 months remained as effective as fresh nets. Indeed, 100% of the beetles exposed for 90 s to newly-opened LLINs in June 2019 or to stored LLINs in September 2019 had died in <1 week. However, when unused LLINs were stored over the winter and tested the following June, they gave just 71.4% mortality as opposed to 100% when the nets were new ($\chi^2 = 19.6$; df = 1; $P < 0.001$).

3.4 Evaluation of different forms of A&K and residence time of *P. japonica* on these A&Ks

In the 3-d observation trial we counted 8466 beetles on the ellipsoidal A&Ks, 8398 on the pyramidal A&Ks, and 8378 on the octahedral A&Ks. No significant difference was found in the cumulative attractiveness of the different types of A&Ks deployed in the three fields. There was, however, a significant field effect,

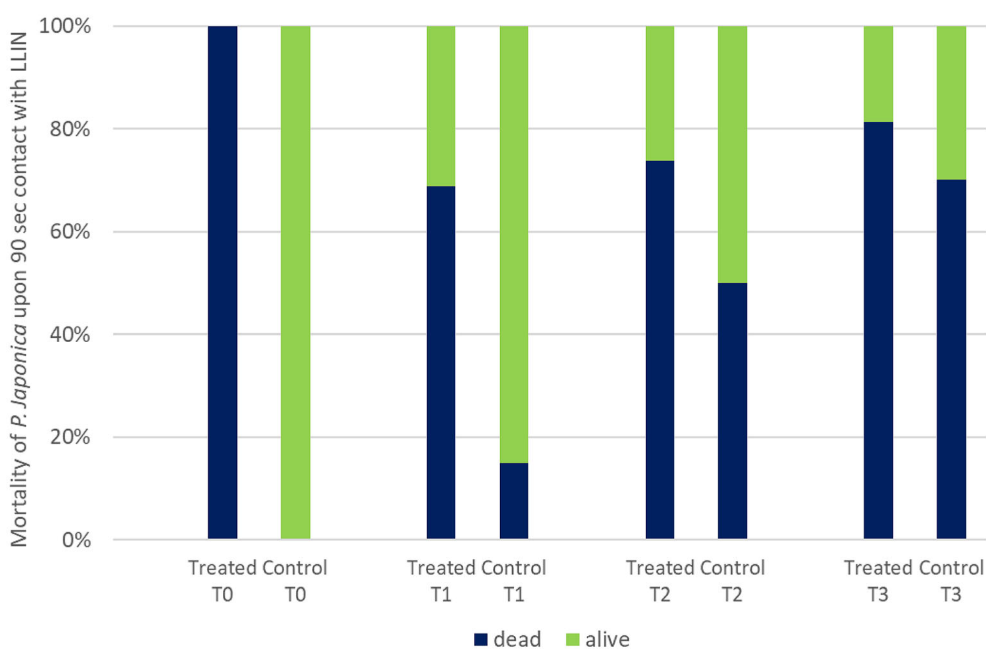

Figure 3. Percentage of dead (blue) and alive (green) field-collected *Popillia japonica* adults 2 weeks after treatment with 90 s tarsal contact with field-exposed Long-Lasting Insecticide-treated Nets (LLINs) at different times during the flight season. The T0 challenge was on 3rd June with a fresh (new) LLIN. The T1, T2, and T3 challenges were done on 2nd July, 2nd August, and 2nd September using LLINs that had been field-exposed for 1, 2, or 3 months, respectively, and beetles collected progressively later in the seasonal flight period.

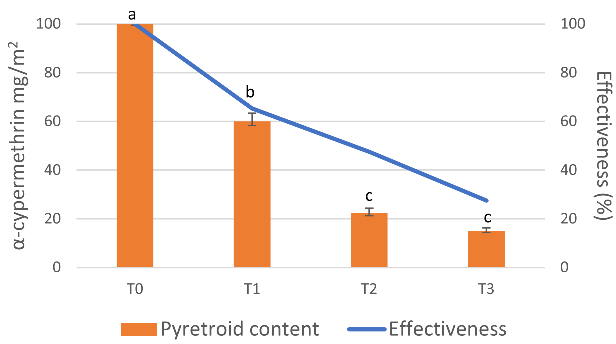

Figure 4. Mean (SE) α-cypermethrin content (mg/m^{-2}) of Long-Lasting Insecticide-treated Nets (LLINs) field exposed over different times (T0-T3). Different letters over the bars indicate significant differences in pyrethroid content among different exposure times of LLINs in the field. The blue line shows % effectiveness in mortality of beetles resulting from 90-s tarsal contact with the LLINs.

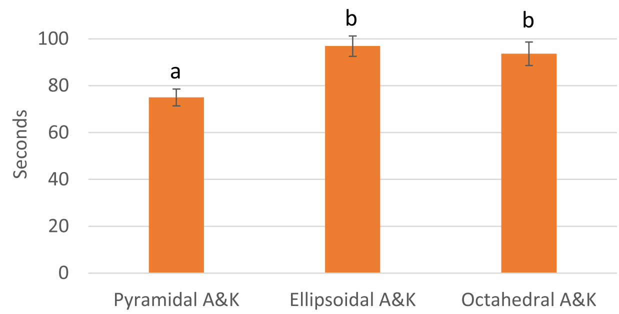

Figure 5. Mean (± SE) residence time of *Popillia japonica* on each *attract-and-kill device* (A&K), with different letters indicating a significant difference (Kruskal-Wallis test, $P < 0.05$; see text).

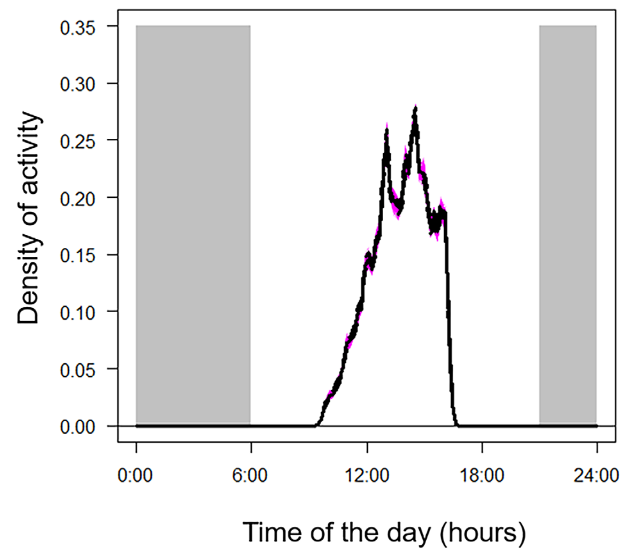

Figure 6. Kernel density estimate of activity of *Popillia japonica* (N = 25 245 records) throughout the study period (solid black line). Purple lines represent bootstrap estimates.

probably related to infestation level, with 13668, 6953, 4621 beetles counted on traps in fields 1, 2, and 3, respectively (Welch F test for unequal variances: $F = 10.9$, df = 13,69, $P < 0.01$).

Cumulative mean residence time was 75 ± 4 s, 97 ± 4 s, and 94 ± 5 s for the pyramidal, the ellipsoidal, and the octahedral A&Ks, respectively (Fig. 5), with overall statistical difference between A&K forms (H = 22.4; df = 2; $P < 0.0001$). Compared to the pyramidal form, there was longer residence on both ellipsoidal (U = 180164; $P < 0.001$) and octahedral (U = 180765; $P < 0.001$) A&Ks. Residence times were also plotted hourly for each A&K form which showed significance differences between A&K forms during the intervals of 1:00 to 2:00 p.m. (H = 8.08; df = 2; $P = 0.017$) and 3:00 to 4:00 p.m. (H = 6.08; df = 2; $P = 0.048$). Residence time on traps also differed between the three fields ($F = 31.45$; df = 2, $P < 0.001$) averaging 122 ± 6 s for field 1, 59 ± 3 s for field 2, and 83 ± 3 s for field 3.

3.5 Activity rhythms of *P. japonica*

We obtained a total of 25 245 records of *P. japonica* (pyramid, N = 8389; ellipsoid, N = 8466; octahedron, N = 8378), in a total sampling effort of 3 trapping days. Throughout the sampling period, a non-random diel activity pattern was exhibited, peaking at 2:30 p.m. (Hermans–Rasson test: r = 182.8; $P < 0.001$: Fig. 6).

Overlap of temporal patterns of *P. japonica* visitation according to numbers on the different traps was very high (D4 = 0.77–0.93 Fig. 7), with curves belonging to the same frequency distribution ($P = 0.42$ for test of probability that two sets of circular observations come from the same distribution). There was no significant difference in temporal overlap in comparisons between each trap-type pair (Mardia–Watson–Wheeler tests, U2 = 0.052–0.085, all $P > 0.10$).

In the final model, number of beetles on A&Ks was significantly influenced only by relative humidity ($R^2 = 0.58$; B = −4.30, Standard Error = 0.90; $P < 0.005$); those counts decreased significantly with increasing humidity. Effects of the other variables (temperature, wind speed, sun radiation, atmospheric pressure and A&K type) were statistically nonsignificant.

4 DISCUSSION

The use of LLINs for an *attract-and-kill* strategy is an emerging approach that can help to protect crops or stored products from insect pests.[31–36] It was derived from the use of insecticide-treated bed nets, which have been the most effective tool for reducing malaria morbidity and mortality in sub-Saharan Africa in recent decades.[37] Use of A&K devices is considered a low-impact pest control method because it does not require direct application of chemical pesticides over crops or orchards. Here we tested A&Ks with LLINs impregnated with α-cypermethrin whose use is being phased out in Europe but for which a possible replacement with similar effectiveness can be found in LLINs impregnated with deltamethrin.[16] Results of previous laboratory experiments evaluating control of adult *P. japonica* by means of LLINs were encouraging[16] and the current contribution extended the validity of those outcomes to field applications. In this regard, we highlighted strengths and weaknesses of this approach, shedding light on how A&Ks need to be managed to remain effective outdoors throughout seasonal use.

The color of the used LLINs was dark green in all the deployed devices. Although yellow-colored traps may be intrinsically more attractive to *P. japonica*, Hamilton *et al.*[38] and Sipolski *et al.*[39] warned that they might also increase bycatches of beneficial insects such as bumblebees. Moreover, Ladd and Klein[40] reported that there is no difference in effectiveness between yellow and green colored traps. Therefore, the green color of the LLIN, which

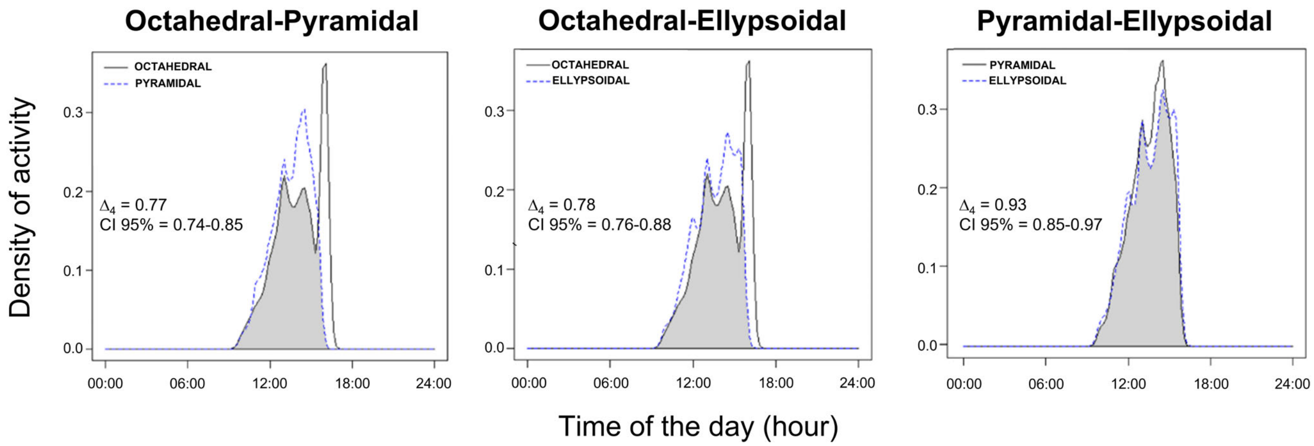

Figure 7. Overlap of temporal patterns of capture intensity (Kernel density estimates) of *Popillia japonica* using different trap types (all $P < 0.01$).

does not attract pollinators and does not impair the effectiveness of the A&K, was considered the best option to be used.

The first trial was a proof of concept aiming to evaluate whether open or closed A&Ks were equally effective in luring beetles to land and walk on the LLINs. The closed setup was more effective, having a number of walking beetles almost double in comparison to the open setup. With the latter, we observed some incoming beetles to walk on the lure without contacting the LLIN, therefore making the A&K partially ineffective. This indication was taken into account for the subsequent experiments.

During the experiments of field effectiveness of A&Ks, we observed that new nets (T0) had the same killing effectiveness as previously reported in laboratory trials.[16] By early July, however, after 1 month of field exposure (T1) the traps' effectiveness had decreased by about 30%, although mortality of beetles walking on them was still statistically higher than the control. The traps continued to kill some beetles after 2 or 3 months of field exposure (T2 and T3), but there also was increasingly high mortality of the controls, presumably due to the aging of the population. By August (T2), there was no difference in the mortality of beetles exposed to treated or non-treated nets, and it was impossible to determine whether the former died due to the treatment or from natural senescence. To overcome the problem of the mortality of the control, the effectiveness of A&Ks was calculated by the Henderson-Tilton's index which estimated the treatment-induced mortality to be 47.5% and 37.5% at T2 and T3, respectively.

There was strong correlation between decreasing mortality over time in the beetle exposure trials and degradation of α-cypermethrin residues on the field-exposed LLINs. In fact, the pyrethroid content had declined by about 40, 80, and 85% after 1, 2, or 3 months of field exposure, respectively, making the LLINs practically ineffective against *P. japonica* during the latter half of the seasonal flight period.

Our pilot study on the effectiveness of stored LLINs suggested that despite proper storage, new LLINs used in the second year after purchase will be less effective than if used the previous year. This suggests that degradation of the pyrethroid active ingredient occurs even when the LLIN material is stored in compliance with the label, in the dark at room temperature. We noted in this experiment that females had a lower rate of mortality than males. Females usually are larger than males,[41] so they may have needed a higher dose of insecticide to die, and this became evident when the active ingredient decreased from its initial concentration.

Our evaluation of different forms of A&Ks showed no statistical difference in number of beetles among pyramidal, octahedral, and ellipsoidal A&Ks. This means that, at least for the closed setup, a key role was played by the attractiveness of the lure itself, whatever the form. From the application point of view, however, the ellipsoidal A&K reached the same number of beetles as the other forms with a LLIN surface that was less than half of that of the pyramidal and octahedral A&Ks.

The overall mean residence time of *P. japonica* on the LLINs ranged from 75 to 97 s with the time on the ellipsoidal and octahedral A&Ks slightly longer than the pyramidal A&Ks. Interestingly, a statistical difference also arose when we compared the mean residence time of *P. japonica* by pooling data of A&Ks per field.

In this latter case we saw that the residence time also depended on the place where the A&Ks were installed. Whether or not food availability in nearby fields influences residence time of beetles on the LLINs warrants further study.

Finally, we investigated the diel activity rhythms of *P. japonica* adults on the A&K devices, comparing the results obtained from the trial on the three different forms. Flight activity had a normal distribution during the day with major activity between midday and 3 p.m., peaking around 2.30 p.m. Activity was inversely correlated with relative humidity; however, there was no correlation with average temperature, wind speed, solar radiation, and atmospheric pressure. This datum is somewhat consistent with Fleming[41] who stated that beetles fly with a relative humidity below 60%, and could explain why adults are less mobile early in the morning and late in the evening when the relative humidity is higher. Conversely, the data analysis did not report any correlation between flight activity rhythms and wind nor solar radiation, unlike reported in Lacey *et al.*,[42] presumably because our data were collected in sunny days with a relatively constant high solar radiation and moderate wind and so extreme conditions were not included in the model.

5 CONCLUSION

In conclusion, the use of A&K s appears to be a useful tool for the Integrated Pest Management of *P. japonica*.

The use of A&Ks could be proposed to eradicate initial outbreaks of *P. japonica*, or to protect high-value crops from infestation in pest-free areas, or to contain *P. japonica* population in areas with severe infestation or to tackle the natural spread in non-infested territories. In this regard, a field trial is currently running to evaluate the best ratio of A&Ks per hectare.

Our data suggest that A&Ks made of Storanet® and a standard *P. japonica* dual lure have a lasting effectiveness of about 1 month in the field. Afterwards it is necessary to replace their LLINs with new ones to maintain high efficacy. Because the duration of attractiveness of the commercial lure is fully efficient for about 40 days (pers. observation), we suggest that field operators replace the LLIN at the same time as the lure.

ACKNOWLEDGEMENTS

We thank Gian Luca Tabanelli from BASF™ for providing LLINs and Volker Frenz and Silke Strahberger from BASF™ for the chemical analysis on the pyrethroid decay. We thank Sara Boschi, from the University of Siena for her help during the field experiments, and D.A. Potter (University of Kentucky) for critically reviewing an earlier draft.

This work has received funding from the Piedmont Region Grant/Award number D. n. 1161—29/11/2016, and the European Union's Horizon 2020 research and innovation programme 'IPM POPILLIA' Integrated pest Management of the invasive Japanese Beetle, *Popillia japonica*, under grant agreement No. 861852.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Potter DA and Held DW, Biology and management of the Japanese beetle. *Annu Rev Entomol* **47**:175–205 (2002). https://doi.org/10.1146/annurev.ento.47.091201.145153.
2 Althoff ER and Rice KB, Japanese beetle (Coleoptera: Scarabaeidae) invasion of North America: history, ecology, and management. *J Integr Pest Manage* **13**:1–11 (2022). https://doi.org/10.1093/jipm/pmab043.
3 Commission Delegated Regulation (EU) 2019/1702 of 1 August 2019 supplementing Regulation (EU) 2016/2031 of the European Parliament and of the Council by establishing the list of priority pests. *Off J Eur Union* **260**:8 (2019).
4 Mazza G, Paoli F, Strangi A, Torrini G, Marianelli L, Peverieri GS *et al.*, Hexamermis popilliae n. sp. (Nematoda: Mermithidae) parasitizing the Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) in Italy. *Syst Parasitol* **94**:915–926 (2017). https://doi.org/10.1007/s11230-017-9746-0.
5 Benvenuti C, Barzanti GP, Marianelli L, Peverieri GS, Paoli F, Bosio G *et al.*, A new device for auto-disseminating entomopathogenic fungi against *Popillia japonica*: a study case. *Bull Insectology* **72**:219–225 (2019).
6 Barzanti GP, Enkerli J, Benvenuti C, Marianelli L, Paoli F, Sabbatini Peverieri G *et al.*, Preliminary analysis of genetic variability of *Metarhizium* isolates from Parco del Ticino (Northern Italy). *IOBC WPRS Bull* **150**:6–8 (2020).
7 Paoli F, Marianelli L, Torrini G, Mazza G, Benvenuti C, Bosio G *et al.*, Differential susceptibility of *Popillia japonica* 3rd instars to *Heterorhabditis bacteriophora* (Italian strain) at three different seasons. *Biocontrol Sci Technol* **27**:439–444 (2017). https://doi.org/10.1080/09583157.2017.1285866.
8 Torrini G, Paoli F, Mazza G, Simoncini S, Benvenuti C, Strangi A *et al.*, Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against *Popillia japonica* (Coleoptera: Scarabaeidae) in northern Italy. *Insects* **11**:804 (2020). https://doi.org/10.3390/insects11110804.
9 Barzanti GP, Enkerli J, Benvenuti C, Strangi A, Mazza G, Torrini G *et al.*, Genetic variability of *Metarhizium* isolates from northern Italy as a possible resource for *Popillia japonica* natural control. *J Invert Pathol* **197**:107891 (2023).
10 Marianelli L, Paoli F, Torrini G, Mazza G, Benvenuti C, Binazzi F *et al.*, Entomopathogenic nematodes as potential biological control agents of *Popillia japonica* (Coleoptera, Scarabaeidae) in Piedmont region (Italy). *J Appl Entomol* **142**:311–318 (2018). https://doi.org/10.1111/jen.12470.
11 Paoli F, Marianelli L, Binazzi F, Mazza G, Benvenuti C, Sabbatini Peverieri G *et al.*, Effectiveness of different doses of *Heterorhabditis bacteriophora* against *Popillia japonica* 3rd instars: laboratory evaluation and field application. *Redia* **100**:135–138 (2017). https://doi.org/10.19263/REDIA-100.17.17.
12 Regione Piemonte 2017 – *Popillia japonica*, Risultati Piano Azione 2017 [online] URL: https://www.regione.piemonte.it/web/sites/default/files/media/documenti/2018-12/risultati_piano_azione_2017.pdf
13 Bosio G, Venanzio D and Giacometto E, *Popillia japonica* Preferenze alimentari e danni riscontrati. https://www.ersaf.lombardia.it/it/file/2552/2cb99a45/Popillia+japonica+Presentazione+Giornate+Fito_7_3_2018_784_36235.pdf
14 Regione Piemonte 2020 – *Popillia japonica*, Risultati Piano Azione 2020 [online] URL: https://www.regione.piemonte.it/web/sites/default/files/media/documenti/2021-08/risultati_piano_dazione_2020.pdf
15 Ebbenga D, Burkness EC and Hutchison WD, Optimizing the use of semiochemical-based traps for efficient monitoring of *Popillia japonica* (Coleoptera: Scarabaeidae): validation of a volumetric approach. *J Econ Entomol* **115**:869–876 (2022). https://doi.org/10.1093/jee/toac049.
16 Marianelli L, Paoli F, Sabbatini Peverieri G, Benvenuti C, Barzanti GP, Bosio G *et al.*, Long-lasting insecticide-treated nets: a new integrated pest management approach for *Popillia japonica* (Coleoptera: Scarabaeidae). *Integr Environ Assess* **15**:259–265 (2019). https://doi.org/10.1002/ieam.4107.
17 Wawrzynski RP and Ascerno ME, Mass trapping for Japanese beetle (Coleoptera: Scarabaeidae) suppression in isolated areas. *J Arboric* **24**:303–307 (1998).
18 Alm SR, Yeh T, Dawson CG and Klein MG, Evaluation of trapped beetle repellency, trap height, and string pheromone dispensers on Japanese beetle captures (Coleoptera: Scarabaeidae). *Environ Entomol* **25**:1274–1278 (1996). https://doi.org/10.1093/ee/25.6.1274.
19 Dave RS and Patel AR, Photochemical and photocatalytic studies of cypermethrin under UV irradiation. *Der Pharma Chem* **2**:152–158 (2010).
20 Kreuger B and Potter DA, Diel feeding activity and thermoregulation by Japanese beetles (Coleoptera: Scarabaeidae) within host plant canopies. *Environ Entomol* **30**:172–180 (2001). https://doi.org/10.1603/0046-225X-30.2.172.
21 Henderson CF and Tilton EW, Tests with acaricides against the brown wheat mite. *J Econ Entomol* **48**:157–161 (1955). https://doi.org/10.1093/jee/48.2.157.
22 Rolf F, Volker F and Susanne S, Long Lasting Insecticidal Net (LN) Interceptor G2. Quantitative determination of the total content of alpha- cypermethrin and chlorfenapyr. Report BASF™ Doc ID 2014/1217856 (2014).
23 CIPAC, Collaborative International Pesticides Analytical Council, in CIPAC Handbook M, Page 40, (2009) https://www.cipac.org/index.php/methods-publications/handbooks/handbook-m.
24 Meredith M and Ridout M, Overview of the Overlap Package (2014) Available: http://cran.cs.wwu.edu/web/packages/overlap/vignettes/overlap.pdf [19 December 2020].
25 Landler L, Ruxton GD and Malkemper EP, The Hermans-Rasson test as a powerful alternative to the Rayleigh test for circular statistics in biology. *BMC Ecol* **19**:1–8 (2019). https://doi.org/10.1186/s12898-019-0246-8.
26 Linkie M and Ridout MS, Assessing tiger–prey interactions in Sumatran rainforests. *J Zool* **284**:224–229 (2011). https://doi.org/10.1111/j.1469-7998.2011.00801.x.

27 Monterroso P, Alves PC and Ferreras P, Plasticity in circadian activity patterns of mesocarnivores in southwestern Europe: implications for species coexistence. *Behav Ecol Sociobiol* **68**:1403–1417 (2014). https://doi.org/10.1007/s00265-014-1748-1.
28 Rowcliffe JM, Field J, Turvey ST and Carbone C, Estimating animal density using camera traps without the need for individual recognition. *J Appl Ecol* **45**:1228–1236 (2008). https://doi.org/10.1111/j.1365-2664.2008.01473.x.
29 Lund U, Agostinelli C, Arai H, Gagliardi A, Portugues EG, Giunchi D, Irisson JO, Pocernich M and Rotolo F, Package circular (2017). https://mirrors.ucr.ac.cr/CRAN. [19 Nov 2021].
30 Crawley M, *The R Book*. Wiley, Chichester, UK (2007).
31 Skrzecz I, Grodzki W, Kosibowicz M and Tumialis D, The alpha-cypermethrin coated net for protecting Norway spruce wood against bark beetles (Curculionidae, Scolytinae). *J Plant Prot Res* **55**: 156–161 (2015). https://doi.org/10.1515/jppr-2015-0020.
32 Kuhar TP and Kamminga K, Review of the chemical control research on *Halyomorpha halys* in the USA. *J Pest Sci* **90**:1021–1031 (2017). https://doi.org/10.1007/s10340-017-0859-7.
33 Gökçe A, Bingham GV and Whalon ME, Impact of long-lasting insecticide incorporated screens on Colorado potato beetle and plum curculio. *Turk J Agric For* **42**:38–44 (2018). https://doi.org/10.3906/tar-1709-55.
34 Sabbatini Peverieri G, Bortolotti PP, Nannini R, Marianelli L and Roversi PF, Efficacy of long-lasting insecticide nets in killing *Halyomorpha halys* in pear orchards. *Outlooks Pest Manage* **29**:70–74 (2018). https://doi.org/10.1564/v29_apr_05.
35 Ranger CM, Werle CT, Scultz PB, Addesso KM, Oliver JB and Reding ME, Long-lasting insecticide netting for protecting tree stems from attack by ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). *Insects* **11**:8 (2020). https://doi.org/10.3390/insects11010008.
36 Wilkins RV, Campbell JF, Zhu KY, Starkus LA, McKay T and Morrison WR III, Long-lasting insecticide-incorporated netting and interception traps at pilot-scale warehouses and commercial facilities prevents infestation by stored product beetles. *Front Sustainable Food Syst* **4**:561820 (2021). https://doi.org/10.3389/fsufs.2020.561820.
37 Lindsay SW, Thomas MB and Kleinschmidt I, Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. *Lancet Glob Health* **9**:e1325–e1331 (2021). https://doi.org/10.1016/S2214-109X(21)00216-3.
38 Hamilton DW, Schwartz PH, Townshend BG and Jester CW, Effect of color and design of traps on captures of Japanese beetles and bumblebees. *J Econ Entomol* **64**:430–432 (1971). https://doi.org/10.1093/jee/64.2.430.
39 Sipolski SJ, Datson SW, Reding M, Oliver JB, Alm SR and Pitts-Singer T, Minimizing bee (Hymenoptera: Apoidea) bycatch in Japanese beetle traps. *Environ Entomol* **48**:1203–1213 (2019). https://doi.org/10.1093/ee/nvz098.
40 Ladd TL Jr and Klein MG, Japanese beetle (Coleoptera: Scarabaeidae) response to color traps baited with phenethyl propionate+ eugenol + geraniol (3: 7: 3) and japonilure. *J Econ Entomol* **79**:84–86 (1986).
41 Fleming WE, *Biology of the Japanese Beetle*. United States Department of Agriculture Technical Bulletin No. 1449, Washington, D.C., p. 129 (1972).
42 Lacey LA, Amaral JJ, Coupland J and Klein MG, The influence of climatic factors on the flight activity of the Japanese beetle (Coleoptera: Scarabaeidae): implications for use of a microbial control agent. *Biol Control* **4**:298–303 (1994). https://doi.org/10.1006/bcon.1994.1038.