Comparison of different attract-and-kill device densities to control the adult population of Popillia japonica (Coleoptera: Scarabaeidae)

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Research Article

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Comparison of different attract-and-kill device densities to control the adult population of *Popillia japonica* (Coleoptera: Scarabaeidae)

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Abstract

BACKGROUND: *Popillia japonica* Newman is a scarab beetle native to Japan that is considered a serious pest outside its native range. It can feed on more than 400 host plants and spread about 10 km per year in invaded territories, therefore it is considered the second most important quarantine pest in Europe. Both chemical and biological insecticides have been used for control, with variable results. Despite ongoing efforts, *P. japonica* remains a threat in invaded countries, and it is necessary to test more effective and sustainable solutions in the context of integrated pest management. Here we present a study on long-lasting insecticide-treated nets (LLINs) assembled in semiochemical-baited attract-and-kill devices (A&Ks) as a means to control adults of *P. japonica* with low environmental impact. This study complements previous ones in which we first evaluated the effectiveness of the LLINs in the laboratory and then tested both effectiveness and duration in field-exposed A&Ks against *P. japonica*. In the present work we compared the effectiveness of three different densities of A&Ks per hectare in areas where the population of *P. japonica* was numerically homogeneous.

RESULTS: The different densities of A&K (1, 2, 4 A&Ks per ha) resulted in an overall reduction of the population of *P. japonica* by about two thirds in comparison to the control area.

CONCLUSIONS: This study suggests that the use of one A&K per hectare, requiring minimal management effort, is an effective ratio for reducing local populations of *P. japonica*.

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

Keywords: A&K; alien insect; insecticide net; Japanese beetle; LLIN; pest management

1 INTRODUCTION

Popillia japonica Newman, commonly known as Japanese beetle, is an insect native to Japan. Since the beginning of the 20th century it has spread into North America (USA and Canada), where it has become a serious pest. Currently, *P. japonica* is also present in Europe, where it invaded the Azores (Portugal) in the 1970s, Italy in 2014, and Switzerland in 2017.[1–3]

Adults of this species are prolific and polyphagous, feeding on fruits and leaves of more than 400 plant species, including crop plants.[1,4,5]

In the USA, *P. japonica* management costs over 460 million dollars every year,[6] while in the European region, a recent study estimates potential economic damage ranging between 13 million and 7.8 billion euros per year if the pest is not managed and contained.[7] Thus, *P. japonica* has been classified as a quarantine pest[8] and listed as the second most important priority pest in Europe.[9] Due to its impact it is urgent to eradicate, or at least limit, the beetle's spread from infested to non-infested areas.[10] Significant

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control has been achieved in the USA through the use of chemical insecticides, which to date have been the most common strategy despite concerns about their environmental impact.[1,11] Biological agents, mainly entomopathogenic nematodes and fungi, can sometimes be effective larval control methods.[12–19] Unfortunately, thus far biological control agents have had a limited effect on adults.[1,11,20]

Funnel traps baited with a combination of floral attractant and synthetic pheromone lures, commonly used to monitor *P. japonica* adult activity,[20] have proved ineffective for control purposes: in heavily infested areas they rapidly reach trapping capacity, necessitating continuous management.[20]

To control *P. japonica* adults, a strategy combining the effectiveness of chemicals with minimal environmental impact involves long-lasting insecticide-treated nets (LLINs). These nets, made of polyester or polyethylene, are coated or impregnated with insecticides, thereby minimizing chemical dispersion in the environment.[10,21] LLINs are commonly used in tropical and subtropical areas to protect people from malaria or other vector-borne diseases by killing the insects responsible for pathogen transmission.[22,23] The insecticide effectiveness in the LLIN can last several weeks and is absorbed by the insect through tarsal contact. Within a few seconds of contact, insects are paralyzed or killed depending on the concentration of the active ingredient, the duration of exposure, and size of the species.[24] In laboratory tests, *P. japonica* adults showed high (89–100%) mortality when exposed for 5 s to LLINs activated with 1.57 mg/g fibre of alpha-cypermethrin.[21]

In recent years, the regional plant protection organisations (PPOs) of the Piedmont and Lombardy regions (northern Italy) have deployed a new type of device called attract-and-kill (A&K), which consists of LLINs mounted on a static frame, combined with a dual semiochemical lure for *P. japonica*.[10] These devices were used as measures of eradication and containment against the quarantine pest in the infested zones according to the Commission Implementing Regulation (EU) 2023/1581 of 1 August 2023.

The aim of this study was to evaluate the effectiveness of three different densities of A&Ks per hectare by comparing the overall reduction in beetle population sizes in areas with statistically homogeneous densities, as determined by historical trap data. This evaluation aimed to determine the optimal density of A&Ks for application in infested areas: if A&Ks are effective in reducing the pest population density in an area, then pest population density should be lower in the areas where A&Ks have been applied. In addition, the use of different ratios of A&Ks per hectare should reveal any variation in effectiveness.

2 MATERIALS AND METHODS

2.1 Analysis of trap data from invaded municipalities in Piedmont (northern Italy)

To ensure unbiased results despite natural fluctuations in beetle populations across the landscape, historical trap data (2015–2019) from the PPO of Piedmont Region (northern Italy) were first analysed. This analysis aimed to estimate the maximum distance at which population density can be considered spatially correlated, using spatial semi-variograms. Within that distance, beetle population density can be considered as uniform, hence possible differences in the beetle count under different A&K scenarios can be attributed to the killing effect of the devices. The analysis of the trap data and the methodology with which data were treated are reported in File S1.

2.2 Evaluation of the best ratio A&Ks/ha

2.2.1 A&K devices and mass trap description

The A&K devices were set up in an umbrella-like configuration as described by Paoli *et al*.[10] The LLINs were applied by wrapping them around the frame of the umbrella and tying the net to an iron pole. PermaNet® LLINs provided by Vestergaard™ (Losanne, Switzerland) were used in this trial. They consisted of black knitted netting fabric made of insecticide-incorporated polyethylene nanofilament yarn of ∼160 denier impregnated with deltamethrin 4 g/kg fibre ± 25% (product label).

A&K devices and mass traps were baited with a standard *P. japonica* dual semiochemical lure by Pherocon® (Trécé Inc.™, Adair, OK, USA). The dual lure was placed inside the umbrella cap so that it was unattainable by the insect.[10] Lures were replaced and nets were turned upside down after 45 days due to their loss of performance.[10]

2.2.2 Experimental areas

An infested zone with a large and homogeneous population of *P. japonica* (see section 2.1 and File S1) was selected in Vercelli province (Piedmont, Italy). Areas within rice fields with limited host plants were selected to avoid possible interactions between neighbouring attractants that may alter the efficacy of individual devices.[25] Four areas (area 1, located at CREA farm, 45°19′24.596″ N–8°22′25.418″ E; area 2, located at Cascina Dallodi, 45°20′13.145″ N–8°20′47.525″ E; area 3, located at Azienda Agricola Montonero, 45°18′30.319″ N–8°21′22.574″ E; area 4, located at Tenuta Muleggio, 45°19′56.161″ N–8°23′5.402″ E) of about 20 ha each were chosen within a 7 km^2 circle and located at least 500 m far from each other (Fig. 1).

2.2.3 Experimental setup

On 30 May, A&Ks were deployed at different densities across the four study areas, namely four A&Ks/ha in area 1 (total A&K = 80), two A&Ks/ha in area 2 (total = 40), one A&K/ha in area 3 (total = 20), and no A&K/ha in area 4, chosen as a control. To assess the effectiveness in population reduction through these devices, four large funnel traps were placed at the centre of each area, including the control area. A&Ks were activated with the lures from 13 June to 17 August. To estimate the impact of the A&Ks on beetle population density, the funnel traps were checked weekly throughout the experiment. To avoid interactions among different lures present in each area, A&K devices were deactivated the evening before the check by removing the dual lures from each device. The funnel traps were then activated for 24 h and the number of the captured *P. japonica* estimated. Afterwards, the funnel traps were deactivated by removing the lure and subsequently all the A&Ks were re-activated. The number of beetles in the traps was estimated by weight using a dynamometer Sauter FK 25 vers. 2.0 [capacity 25 N (≈2.5 kg), resolution 0.005 N (≈0.0005 kg)], or by direct counting when the numbers of the individuals were too low for the sensitivity of the dynamometer.

The total weight of the captured beetles was then converted to number of individuals by assuming an average weight of ∼0.0741 g per beetle.[26] All the live adults trapped were released in the field after each check to minimize any population reduction due to mass trapping captures. The days on which the trapping activity was carried out were mostly sunny, with moderate or no wind. Meteorological data, collected at the meteorological station of CREA DC in Vercelli, were recorded during sampling.

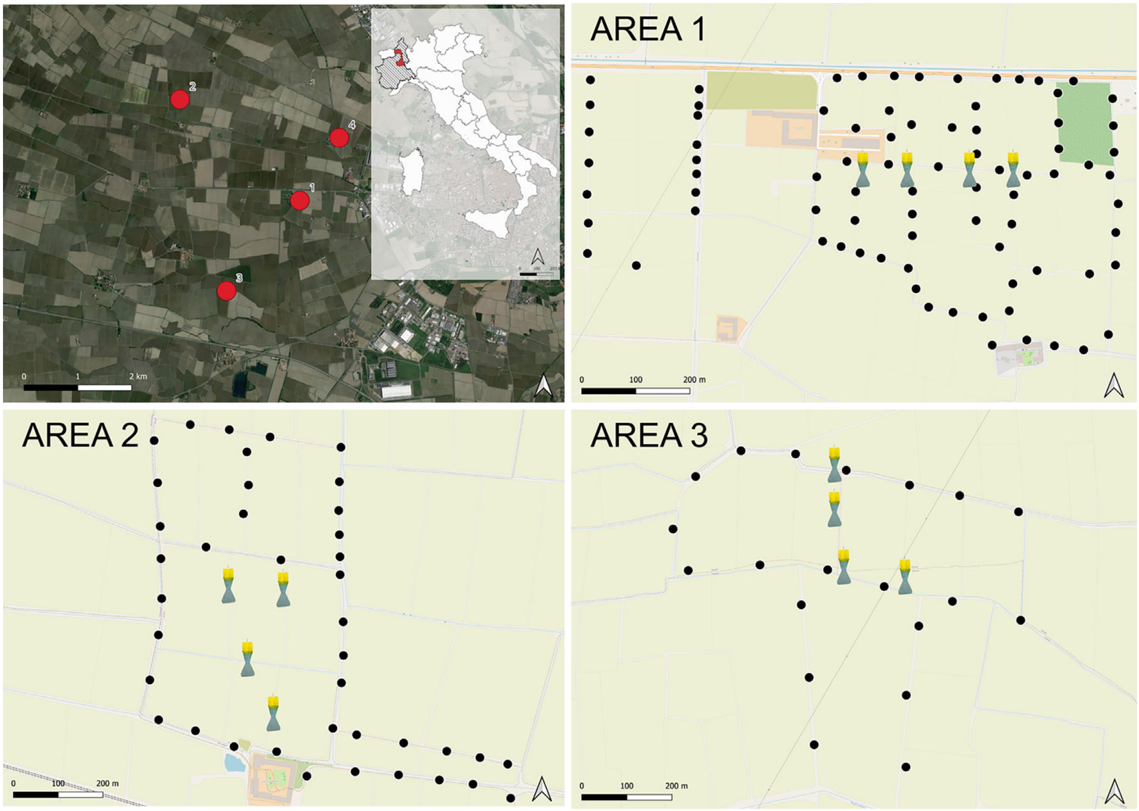

Figure 1. Infested zone in the municipality of Vercelli (Piedmont Region, Italy) where the four experimental areas were chosen (upper left). The A&Ks in each area (80 in area 1, 40 in area 2, 20 in area 3, 0 in area 4 – not shown) are represented by black dots and mass traps by yellow-green funnel trap symbols.

2.3 Data analysis

Two statistical analyses were conducted. The first estimated the range of the spatial semi-variogram of insect densities from historical mass captures in invaded municipalities in Piedmont region (2015–2019). This range represents the distance beyond which spatial correlation between trapped insect counts becomes negligible (File S1). The second analysis assessed the effect of the A&K experiment itself by using two approaches: a pairwise *t*-test to compare treatment effects and capture rates over time, as well as a generalized linear mixed model to handle repeated measures within areas. A negative binomial distribution (overdispersion in the data prevented the use of a Poisson distribution for count data) was used within a generalized linear mixed-effects model, where time and A&K densities were used as fixed effects and A&K densities as a random effect to control for within-area variability.

This test ensured that the observed variability between the four areas was due to the effect of A&K devices while accounting for within-area correlations.

All data were analysed using R 4.4.0. Spatial data were imported in R using the sf library,[27] semi-variograms were computed using the gstat library,[28] and data visualizations were produced using ggplot2 library.[29] Finally, the statistical analysis for the A&K experiment was conducted using the lme4 package[30] and the rstatix package.[31]

3 RESULTS

The spatial analysis of beetles trapped by PPOs from 2015 to 2019 gave a consistent value for the range of the empirical semi-variograms of ∼7 km (7.13 km in 2017, 7.88 km in 2018 and 7.42 km in 2019), in line with previous works that found spatial autocorrelation fading after 7.5–15 km.[32]

The evolution of beetle counts in the different areas of the A&K experiment is depicted in Fig. 2. A first peak of captured individuals was observed in the first check (T1 = 13 June), whereas in T2 (21 June) the counts decreased, likely due to cloudy weather. Then counts raised again in the following two checks (T3 = 29 June and T4 = 5 July), reaching their maximum at T4. Afterwards, the number of captured beetles steadily declined until reaching the lowest counts at the end of the experiment (T10 = 10 August).

The total number of captures per area was as follows: area 1, 3873.6 g (∼52 345 individuals); area 2, 3209.8 g (∼43 375 individuals); area 3, 2795.6 g (∼37 778 individuals); area 4, 9998.2 g (∼135 111 individuals). Statistical comparisons of beetle counts in the four areas obtained by combining all dates are reported in Table 1 (pairwise *t*-test with Bonferroni correction) and show statistical differences between the control group (area 4) and all other treatment groups (about two-thirds in weight of *P. japonica* captures in the areas treated with A&Ks compared to the control area), but no significant difference between the three treated areas.

A date-by-date comparison (pairwise *t*-test with Bonferroni correction) between the counts in the four areas showed that captures in area 4 were statistically different from those of all other areas from time T4 to T10, whilst no significant differences were found within the treated areas (see File S2). The most remarkable difference in population density among the four areas occurred at the flight peak (T4 = 5 July) in which the total number of captured

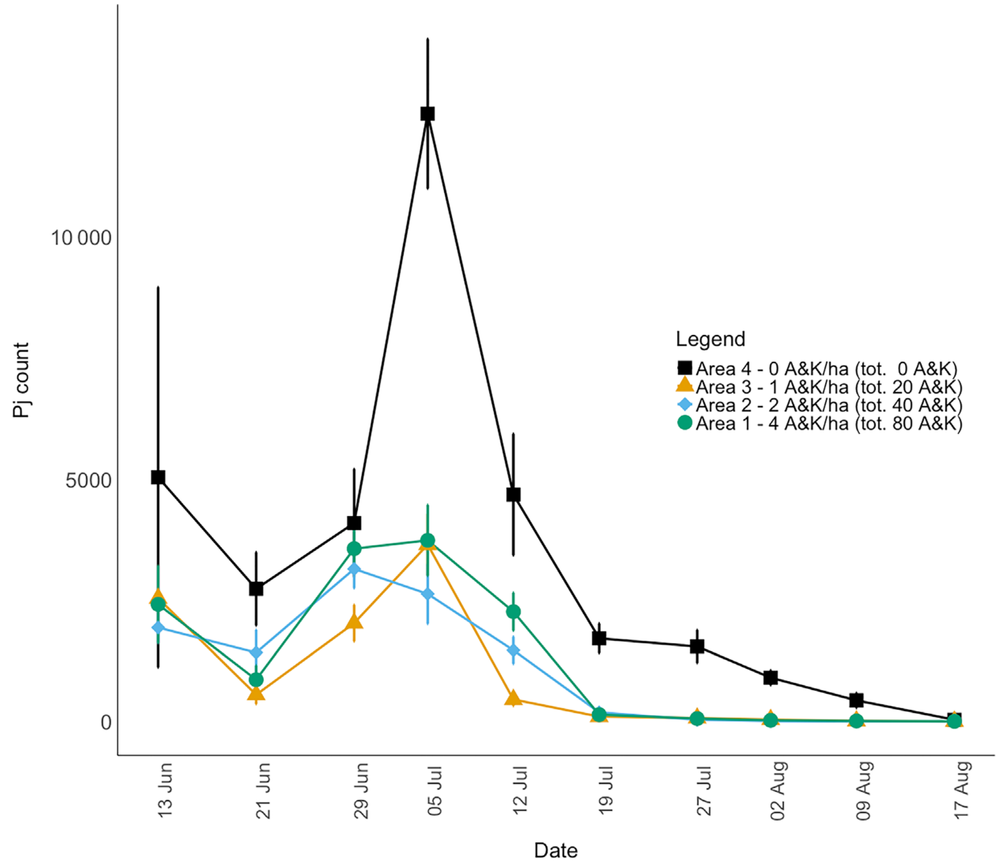

Figure 2. Mean (± standard error) of *Popillia japonica* captures in each of the four areas (four traps in each area for each time step) during 10 weeks from 13 June to 17 August.

Table 1. Test statistics of pairwise *t*-tests of *Popillia japonica* counts between the four different areas across all dates and their corresponding *P* values with Bonferroni correction in parenthesis (**P* < 0.05 and *P* > 0.01, ***P* < 0.01 and *P* > 0.001)

	Area 1 (4 A&K/ha)	Area 2 (2 A&K/ha)	Area 3 (1 A&K/ha)	Area 4 (0 A&K/ha)
Area 1 (4 A&K/ha)	–	−0.762 (1)	−1.01 (1)	2.95 (0.032*)
Area 2 (2 A&K/ha)	–	–	−0.433 (1)	3.17 (0.018*)
Area 3 (1 A&K/ha)	–	–	–	3.38 (0.01**)
Area 4 (0 A&K/ha)	–	–	–	–

Positive (respectively negative) values of the table indicate that the area in the column has, on average, more (respectively less) *P. japonica* counts than the area in the row. *P* values of 1 indicate no statistically significant difference in the average *P. japonica* counts.

adults in the control area (area 4) exceeded 3700 g (∼50 000 individuals) whereas captures in treated areas amounted to about 1000 g each (∼13 513 individuals). Regarding the areas with A&K devices (areas 1–3), the most notable drop in *P. japonica* population density was observed from 19 July onwards, with captures close to zero. This trend continued in subsequent samplings until the end of the experiments.

In the GLMM the estimates for the fixed effects showed that the coefficient for the time variable is negative and significant (−0.84635), suggesting a decrease in counts over time. The coefficients for the three treated areas were all negative, respectively −2.554 [standard deviation (SD) 0.29] for area 3, −2.488 (SD 0.30) for area 2 and −2.144 (SD 0.29) for area 1, indicating a decrease in the count compared to the control group (area 4). All treatment coefficients were statistically significant, suggesting that treatments have a significant effect compared to the control area, but there was no significant difference between them (overlapping confidence intervals). The estimated variance for the random intercept (1.292e^{-10}) was very close to zero, indicating little variability in intercepts among treatment groups, hence a consistent treatment effect across areas.

4 DISCUSSION

The spread of *P. japonica* in Europe is steady. The advancement of the invasion front in the Italian infested area moves at roughly 10 km/year,[33] thus reaching in 2023 a total infested area larger than 19 733 km^2, with incursions reported in other regions as a consequence of passive transport.[2,20] In addition, in 2023 another outbreak in northern Switzerland was observed.[3]

Moreover, according to the 2050 climatic and land-use change scenarios, together with the high dispersal capacity of *P. japonica*, in the next 30 years this pest could reach about half of its potential range in Europe.[34]

As already reported in Gotta *et al*.[20] for Italy, since it is not possible to eradicate the pest and completely stop its natural spread, it is necessary to continue developing more effective and sustainable solutions.

The monitoring activity in the Piedmont Region showed two distinct peaks of adult flight during the summer season of 2022.[35] A similar trend, starting from the tail end of the first seasonal peak, was observed in our study area.

The analysis of the spatial autocorrelation of the trapped beetles based on the presence of *P. japonica* during 2016–2019 showed that, in the infested territories of Piedmont, the local populations of *P. japonica* were numerically homogeneous within a radius of about 7 km. Although local variations may also occur, the population density within the area described by this radius could be considered statistically comparable. Similar results were obtained by Mondino *et al*.,[32] who suggested a range of spatial autocorrelation of *P. japonica* from 7.5 to 15 km by using a less conservative model.[32] Establishing such a radius was the basis for setting up this experiment, which aimed to evaluate the knockdown effect of different ratios of A&Ks.

Our results showed that in the areas where A&K devices were used the population density of *P. japonica* decreased by approximately two-thirds compared to the control area. In addition, the data showed a trend of increasing *P. japonica* population with more A&Ks deployed.

Our findings indicate that A&Ks are effective in decreasing *P. japonica* population in areas of about 20 ha. Since there was no statistical difference between the effectiveness of different numbers of A&Ks per hectare (area 1–3), the results showed that one device per hectare is the best trade-off between effectiveness and management effort to reduce the *P. japonica* population in a small area. However, it is worth stating that lower densities of A&Ks per hectare were not tested. Conversely, a trend towards higher population densities where more A&Ks were deployed was also observed. This suggests that if too many A&Ks are deployed in a given area, the luring effect may outweigh the killing effectiveness. It could be inferred that an effectiveness threshold of A&K density exists when the attractiveness is not balanced by the knockdown effect of the insecticidal net. Such a consideration suggests that the deployment of A&Ks must be done carefully and in agreement with the local competent authority.

The reduction of the population density of *P. japonica* by means of A&Ks had the advantage that the dual semiochemical lure is well targeted to *P. japonica*: in some personal observations, across 63 checks during a 2020 field trial, 6729 *P. japonica* adults walking onto the LLINs were counted compared to only 13 by-catches (mainly pollinators, such as hoverflies, bumblebees, and carpenter bees) (unpublished data), therefore from the environmental point of view we can consider that this technique minimizes harm to beneficial and non-pest insects, making it a minimally toxic tactic suitable for areas where chemical spraying is restricted.

5 CONCLUSIONS

This study suggests that one A&K/ha is the best ratio to reduce the number of *P. japonica* adults in an area of about 20 ha. However, if the aim of the activity is limiting the spread of *P. japonica* over larger territories, a higher number of A&Ks/ha may be effective as the more lures deployed the greater the total number of killed insects, even if attracted to that area from the neighborhood.

In conclusion, this new low-impact approach against the adults improves the control of *P. japonica* from both a strategic and an economic point of view since (i) the workload is manageable and (ii) the cost for the beetle control during the season (device and lure) is limited. Therefore, this tactic stands as a valuable tool to curtail *P. japonica* spread in the infested areas and could prevent its establishment in pest-free areas in the European Union within an integrated pest management strategy. Furthermore, this approach appears to be more environmentally friendly compared to the use of chemicals, which can adversely affect agriculturally beneficial insects, as already documented by Serrão *et al*.[36]

While no single control measure has proven completely effective against *P. japonica*, an integrated approach shows promise. In addition to the existing efforts of phytosanitary officers,[20] this approach could involve citizen science[37,38] for early detection of new infestations, engagement of farmers in adopting agronomic practices,[20] the implementation of biological control agents,[16,17,39] and targeted application of low-impact insecticides, such as the A&Ks.

AUTHOR CONTRIBUTIONS

FP and LM conceived and designed the study. FP, FB, II, CS, GPB, GT, GSP, GM, CB and LM conducted the field experiments and collected data. DS and PFR provided logistic support for field experiments. DM conducted the data analysis. The first draft of the manuscript was written by FP, FB and II. LM provided funding for the experiments. All authors reviewed and commented on first draft of the manuscript.

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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.

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