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Different responses of *Monochamus galloprovincialis* and three non-target species to trap type, colour, and lubricant treatment

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Abstract With the increasing threat to forests in Europe from the invasive pine wood nematode (PWN) *Bursaphelenchus xylophilus*, effective methods are needed to monitor and reduce populations of its insect vector, the pine sawyer beetle *Monochamus galloprovincialis*. In the present study, we tested the effectiveness of different trap types (multiple-funnel, cross-vane, and triangular), colours (black, white and clear), and lubricant (polytetrafluoroethylene, PTFE) treatments (different PTFE formulations and timing of trap treatment) on the catches of *M. galloprovincialis* and three most commonly captured non-target beetle species (the xylophagous *Spondylis buprestoides* and two predators, *Thanasimus formicarius* and *T. femoralis)* in Poland. Of the traps not treated with PTFE, the white and black 6-funnel traps were most effective in trapping *M. galloprovincialis* beetles, while the catches in the cross-vane traps (both white and clear) were low. Trap treatment with PTFE significantly increased trap effectiveness, regardless of PTFE type and time of application. The catches of *S. buprestoides* were affected by trap type, while those of *T. formicarius* depended on trap colour and size. Both species seem to respond positively to ethanol and/or α-pinene in the lure composition. PTFE treatment had a significant effect on the catches of *T. femoralis*. In conclusion, for the monitoring of *M. galloprovincialis*, we recommend the white cross-vane traps treated with dry PTFE. They are less but still effective in catching the target species, while their use, together with lures containing no ethanol and α-pinene, greatly reduces the catches of non-target insects *S. buprestoides* and *T. formicarius*.

Keywords: Cerambycidae; clerid beetles; monitoring; PTFE; *Spondylis buprestoides*; surfactant; *Thanasimus*; traps.

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Introduction

Invasive organisms are among the major threats to forests worldwide (Liebhold et al. 1995, Santini et al. 2013). Currently, one of the most destructive invasive species in forests is the pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Bührer) Nickle (hereafter PWN), a parasitic organism responsible for the dieback of pine trees called pine wilt disease (Mota & Vieira 2008, Vicente et al. 2012, and the literature therein).

PWN is vectored by cerambycid beetles of the genus *Monochamus* (Evans et al. 1996, Kobayashi et al. 2003). Tree-to-tree transmission occurs when dispersal larvae of PWN enter the tracheal tubes of the young beetles in pupal chambers in the wood and are then transferred to new trees during beetle maturation feeding on pine shoots and twigs and/or oviposition (Aikawa 2008, and the literature cited therein). In Europe, the only known vector of PWN is the pine sawyer beetle *M. galloprovincialis* (Olivier, 1795) (Sousa et al. 2001). However, a recent study has shown the possible transmission of PWN by the related cerambycid beetle *Monochamus saltuarius* (Gebler, 1830) (Li et al. 2020), which also occurs in many areas of Europe (Danilevsky 2020).

In the European Union, PWN is a quarantine species with obligatory annual surveys for its early detection (Commission Implementing Regulation (EU) 2019/2072). So far, the presence of PWN has not been confirmed in forests outside of Portugal and Spain, but an introduction into new areas is likely due to the increasing trade in timber and various wood-related commodities. In Central Europe, such a scenario could have devastating consequences considering that Scots pine (*Pinus sylvestris* L.) is one of the most important (economically and environmentally) tree species, but also most susceptible to PWN infestation (Evans et al. 1996, Menéndez-Gutiérrez et al. 2018). Currently, the risk of PWN establishment in Central Europe is favoured by observed climatic changes. In central Poland, for example, average temperatures in July and August often exceed 20°C (Sukovata et al. 2012), a climatic threshold considered favourable for PWN development (Rutherford & Webster 1987).

According to regulations in EU countries, surveys for the detection of PWN are based on the extraction and identification of nematodes from wood samples or from bodies of insect vectors (Commission Implementing Decision 2012/535/EU, EPPO 2013). The latter method requires the collection of *Monochamus* beetles, usually using traps baited with the lures consisting of 2-undecyloxy-1-ethanol (monochamol), an aggregation pheromone of *Monochamus* spp., and bark beetle- and host tree-derived kairomones (e.g. Pajares et al. 2010, Bonifácio et al. 2012, Álvarez et al. 2016). Traps are also considered a potential tool to reduce the population of PWN vectors (Sanchez-Husillos et al. 2015, but see Torres-Vila et al. 2015). Many studies have been conducted on trap optimization and black multiple-funnel traps and cross-vane traps were suggested as the most suitable for catching *M. galloprovincialis* (Rassati et al. 2012, Álvarez et al. 2015, Schroeder 2019). However, there are several other types and colours of commercially available traps whose trapping effectiveness has not been evaluated yet.

Moreover, there is ample evidence of the positive effect of trap treatment with polytetrafluoroethylene (hereafter referred to as PTFE), to increase surface slipperiness, and therefore trap effectiveness (Allison et al. 2014, Graham & Poland 2012, Álvarez et al. 2015). However, the importance of surfactant type and formulation (e.g. dry aerosol, oily spray, water solution) and timing of application for trap effectiveness, as well as the practical aspects and costs of lubricant treatment have received little attention so far.

Traps used for monitoring *M. galloprovincialis* often capture non-target insects (Pajares et al.

2004, Francardi et al. 2009, Jurc et al. 2012, 2016), because different species often respond similarly to volatiles released by host trees (Francardi et al. 2009, Boone et al. 2018) and by other insect taxa (Bakke & Kvamme 1981, Allison et al. 2001, Heber et al. 2021), and furthermore, these volatiles may act synergistically (Pajares et al. 2004). However, the capture of non-target species raises several issues related to trap functionality and, more importantly, could reduce populations of beneficial species such as predators, thus limiting their ability to regulate pest populations (Bracalini et al. 2021). Therefore, trap optimization should focus not only on trap effectiveness in capturing target species but also on minimizing captures of non-target taxa, particularly beneficial insects.

Considering all mentioned above, the main objective of our study was to test the effects of different trap types (multiple-funnel, cross-vane and triangular), colours (black, white, and clear), and PTFE treatments on the catches of *M. galloprovincialis* and three most commonly captured non-target beetle species, i.e. *Spondylis buprestoides* (Linnaeus, 1758) (Cerambycidae), *Thanasimus formicarius* (Linnaeus, 1758) and *T. femoralis* (Zetterstedt, 1828) (Cleridae) in Poland. We also compared the practical aspects of using different PTFE formulations and checked whether the timing of trap treatment with PTFE (i.e. before or after trap deployment in the field) affects trap effectiveness.

Materials and Methods

Experimental design and study area

This study comprised three experiments.

In experiment 1, seven trap type, colour, and PTFE treatment combinations (see pictures in Supporting Information, Fig. S1) were tested:

- •black 12-funnel traps, without PTFE treatment (thereafter called black_12-funnel),
- •black 6-funnel IBL-3 traps, without PTFE treatment (black_6-funnel),
- •white 6-funnel IBL-3 traps, without PTFE treatment (white_6-funnel),
- •white 6-funnel IBL-3 traps, with PTFE treatment (white_6-funnel_PTFE),
- •white cross-vane IBL-5 traps, without PTFE treatment (white_cross-vane),
- •white cross-vane IBL-5 traps, with PTFE treatment (white_cross-vane_PTFE),
- •clear cross-vane traps, without PTFE treatment (clear_cross-vane).

Vanes in the white and clear cross-vane traps were made of white corrugated polypropylene hollow sheets (hereafter coroplast) and clear polycarbonate sheets, respectively. In the vane traps, the 33 × 20 cm vanes were inserted into a 17-cm diameter funnel. Lids and funnels in black and white traps were made of hard plastic of respective colours (traps were not painted). PTFE was a 60 wt.% dispersion in water (hereafter called liquid PTFE) purchased from Sigma-Aldrich in Poland. Liquid PTFE was applied using paint brushes of different widths.

The beetles were captured in plastic collecting bottles without any capture liquid (dry cups). The base of the collectors had a hole covered with fine metal mesh for water drainage. Each collector contained a 3.5 × 3 cm strip saturated with an insecticide (7% transfluthrin) (Bros sp. z o.o., Poznań, Poland) to kill the trapped insects.

All traps were baited with the lures (cardboards in polyethylene bags) loaded with 2 ml of mixture of ethanol, ipsenol, 2-methyl-3-buten-2-ol, monochamol and 2,6-Di-tert-butyl-4-methylphenol used as a polymerization inhibitor, and additional lure (10 ml polyethylene container with capillary closure of 0.2 mm in diameter) containing 4 ml of α-pinene. All chemicals (98% chemical purity), except ipsenol, were purchased from Sigma-Aldrich (the branch in Poland), while ipsenol was obtained from Bedoukian Research Inc. (USA). All traps and lures were prepared and delivered by Chemipan R&D Laboratories (Poland).

The experiment was conducted in the Wronki forest district (FD) (N 52.7544, E 16.1522), in two pure Scots pine stands, 27 and 30 years old with mosses in a vegetation cover. The traps were suspended between trees, approximately 2 m above the ground, using synthetic thread. The traps were set up in a randomized complete block design in 8 blocks (replicates) along line transects, with 4 blocks in each stand, and with each block containing one of each trap type. A distance of at least 20 m was maintained between the traps within and between blocks. The traps were deployed on 16 June 2011, and checked and emptied on 4 and 14 July 2011 (2 inspections), without trap rotation.

In experiment 2, we tested six trap combinations used in experiment 1, while the clear cross-vane trap was replaced by a new type – a triangular IBL-2 trap not treated with PTFE (thereafter called triangle trap) (the picture is available in Supporting Information, Fig. S1). This trap has a triangular shape, with relatively clear polyethylene foil (with 80, 75, and 75 cm long sides) in the middle and 25 cm wide (on the top) and 17 cm wide (on the sides) white coroplast boards. Each trap collector contained a strip saturated with an insecticide (see above). All traps were delivered by Chemipan R&D Laboratories (Poland). They were baited with the Galloprotect 2D lures (SEDQ, Spain), consisting of ipsenol, 2-methyl-3-buten-1-ol and monochamol (without α-pinene).

This experiment was conducted in two FDs: Parciaki (N 53.1607, E 21.2786) and Ostrołęka (N 53.1636, E 21.5270), in the Scots pine-dominated stands (90%) with 10% of *Betula pendula* Roth in main tree composition. In the Parciaki FD, the stand was 37-year old, with *Juniperus communis* L. in undergrowth and mosses in a vegetation cover. In the Ostrołęka FD, the stand was 85-year old, with *J. communis*, *Frangula alnus* Mill. and *Prunus serotina* Ehrh. in undergrowth and mosses and *Vaccinium myrtillus* L. in a vegetation cover.

In the younger forest, the traps were suspended between trees using synthetic thread, while in the older forest they were hung on metal hooks from dead branches on the trees, 4-6 m above the ground, using an adapted telescopic pole (folded length – 1.1 m, extended length – 5 m) (Paradox Company, Krakow, Poland).

The traps were set up in a randomized complete block design in 10 blocks (replicates) along line transects, with 5 blocks in each stand, and with each block containing one of each trap type. The traps were deployed on 19 and 20 June 2012 and subsequently checked and emptied at 2-4 week intervals until 27 August 2012 (4 inspections), without trap rotation.

In experiment 3, we tested the type and timing of PTFE treatment. Prior to the field tests, four types of lubricants available on the market: dry PTFE in aerosol (Motip Dupli Group B.V., The Netherlands), liquid PTFE (Chemours, USA), PTFE grease (Boll, Poland) and silicone spray (K2, Melle, Poland), were tested under laboratory conditions for their slipperiness and type of film (wet or dry) they create on the surface. Each lubricant was applied on coroplast plates (approx. 15 × 20 cm) in the laboratory and left for drying up for about an hour. Later, *M. galloprovincialis* beetles (which emerged from infested wood in rearing cages) were placed on the plates, which were then positioned vertically. We found that each of the four lubricants tested increased the slipperiness of the surface – the insects slipped off the coroplast plates. However, PTFE grease and silicone spray formed a non-drying coating on the surface that would lead to inconvenience (messy, adherence of various debris, etc.) in trap handling and storage. Therefore, only dry PTFE and liquid PTFE were tested in the field. The treatments included:

- •untreated white cross-vane traps (thereafter called untreated),
- •white cross-vane traps treated with a dry PTFE before transporting, assembling, and deploying in a forest (dry PTFE_before),
- •white cross-vane traps treated with a dry PTFE after deployment (dry PTFE_after),
- •white cross-vane traps treated with a liquid PTFE before trap assembling and deployment (liquid PTFE_before).

A comparison of the traps treated with

either dry or liquid PTFE aimed at checking whether covering traps with a dry PTFE, which is more accessible, less messy, and seems to be less expensive, would affect their effectiveness. Testing the date of treatment aimed to check whether lubricating and antistatic characteristics of a PTFE film on treated surfaces would be affected during transportation, assembling, and deployment of traps. PTFE was applied on both sides of the vanes and an internal part and the bottom of a funnel. Liquid PTFE was applied using a 1 l hand pressure sprayer. It is worth mentioning that the use of the pressure sprayer was handy, however later on it appeared that PTFE did not adhere to the vane surface as well as when it was applied by paint brushes in our earlier experiments.

Collecting bottles contained approximately 400 ml of propylen glycol. The traps were delivered by Chemipan R&D Laboratories (Poland). They were baited with the Galloprotect Pack lures (SEDQ, Spain), consisting of ipsenol, 2-methyl-3-buten-1-ol, monochamol and α-pinene.

The experiment was conducted in the Międzychód FD (N 52.6982, E 15.7673), in four 86-91 years old pure Scots pine stands, with mosses alone or with *V. myrtillus* in a vegetation cover. The traps were suspended between trees, 1.5-2 m above the ground, using synthetic thread. The traps were set up in a randomized complete block design in 8 blocks (replicates), with 1-4 blocks in a stand, and with each block containing one of each treatment. A distance of approximately 70 m was maintained between the traps within and between blocks. The traps were deployed on 17 June 2021 and checked and emptied once, on 20 July 2021.

In all experiments, study sites were selected based on the availability of tree tops left on the ground after thinning in the previous year, with signs of *M. galloprovincialis* infestation. Captured insects (*M. galloprovincialis*, *S. buprestoides*, *T. formicarius* and *T. femoralis*) were identified and counted in the laboratory.

Statistical analyses

The catches of both *Thanasimus* species in the 12-funnel traps in experiment 1 were excluded from the analyses because extremely numerous *S. buprestoides* damaged those small beetles to such an extent that it was not possible to accurately count them. In addition, the catches of *T. femoralis* in experiment 2 were excluded from the analyses due to the very low frequency in the traps.

Before the analyses, the data from all trap inspections in the same experiment were pooled for each trap to obtain the total number of beetles. When trapping periods differed among blocks, the catches were unified by dividing the number of beetles by the actual number of days of exposure and then multiplying by the largest number of days in the period.

The effect of trap type, PTFE treatment, and their interaction in the field experiments 1 and 2 and the effect of type and date of PTFE treatment in experiment 3 on the total number of beetles of different species captured in each experiment were estimated using a generalized linear mixed model with either a Poisson, Conway-Maxwell Poisson, generalized Poisson or negative binomial distribution of the dependent variable. The block was considered a random factor. This was followed by a post hoc test with a Holm correction for multiple mean comparisons. The significance of fixed variables and their interactions was tested with a Wald χ^2 test (Bolker et al. 2009).

All analyses were performed using the R environment, version 4.0.3 (R Core Team, 2020) with RStudio, version 1.1.463 (R Studio Team, 2016). The following R packages were used: glmmTMB (Brooks et al. 2017) for GLMM, car (Fox & Weisberg 2019) for the Wald χ^2 test, and emmeans (Lenth 2020) for multiple mean comparisons. The goodness of fit of each model was estimated by checking for overdispersion and residual diagnostics (Zuur et al. 2009, Mangiafico 2016). The significance level was set at $\alpha = 0.05$ for all analyses.

Results

M. galloprovincialis

The numbers of *M. galloprovincialis* captured in the traps of different types and colours (without PTFE treatment) in both experiments 1 and 2 did not exceed 3 beetles/trap (Fig. 1). The lowest catches were in the clear cross-vane traps (0.4±0.23 beetle/trap, i.e. mean±SE) in experiment 1 and in the white cross-vane traps (0.1±0.09 beetles/trap) in experiment 2. The highest catches were in the white 6-funnel traps (2.6±0.74 beetles/trap) and the triangle traps (0.7±0.29 beetles/trap), respectively. The effect of trap type/colour had a significant effect on *M. galloprovincialis* catches only in experiment 1 ($\chi^2 = 15.0$, df = 4, P = 0.0046), however pairwise comparisons with the Holm correction did not reveal a significant difference between any pair of compared trap types; the difference between the lowest and highest catches in experiment 1 was nearly significant (Fig. 1).

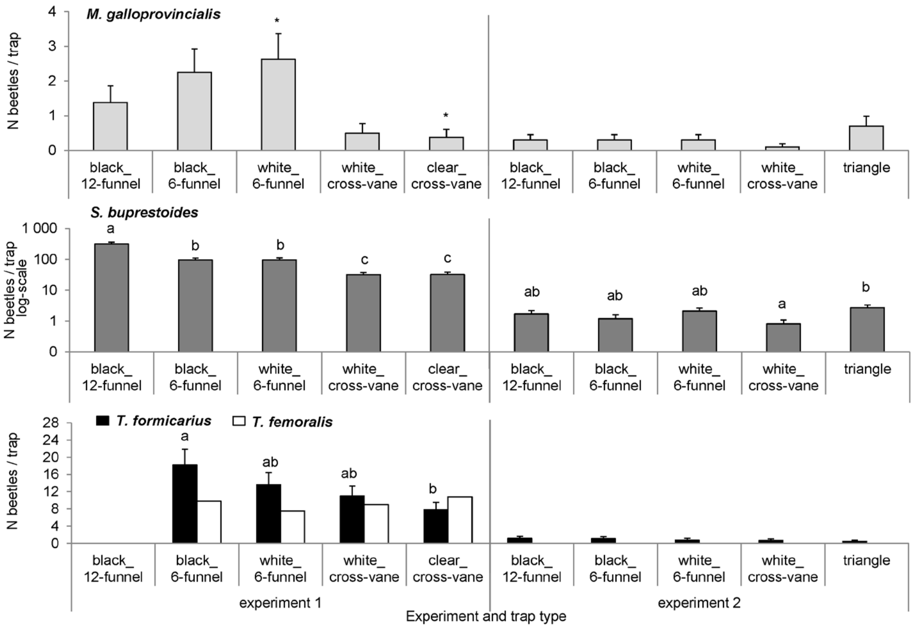

Figure 1 The number (mean and SE) of beetles of *M. galloprovincialis* and three most common insect species captured in the traps of different types and colours: in experiment 1, 15 June-14 July – the lures with α-pinene (N = 8 traps/type), in experiment 2, 19 June-27 August – the lures without α-pinene (N = 10 traps/type); data for both *Thanasimus* spp. catches in the 12-funnel traps in experiment 1 and for *T. femoralis* in all traps in experiment 2 were discarded (see Methods); different letters above bars indicate significant differences between the traps of different types/colours at α = 0.05 for each year separately (only significant differences are presented).

The effect of the PTFE treatment on the catches of *M. galloprovincialis* beetles was significant in both experiment 1 ($\chi^2 = 50.2$, df = 1, P < 0.0001) and experiment 2 ($\chi^2 = 17.2$, df = 1, P < 0.0001), with the treated traps of both types capturing significantly more beetles than untreated traps (Fig. 2). The effect of trap type was significant only in experiment 1 ($\chi^2 = 9.3$, df = 1, P = 0.0023) with the higher catches in the PTFE treated 6-funnel traps (20.7±3.51 beetles/trap) than in the PTFE treated cross-vane traps (11.4±2.34 beetles/trap). The interaction between the trap type and PTFE treatment was not significant in any of the experiments.

Experiment 3 confirmed the significant effect of the PTFE treatment on the total catches of *M. galloprovincialis* ($\chi^2 = 118.9$, df = 3, P < 0.0001). Untreated traps captured the lowest numbers of the beetles (34.9±6.07 beetles/trap) and the catches were significantly lower than in the traps treated with PTFE, regardless of the type of treatment (Fig. 3).

Neither the type of PTFE (dry or liquid) nor the date of treatment (before or after assembling and deploying in a forest) had any significant effect on the catches of *M. galloprovincialis*.

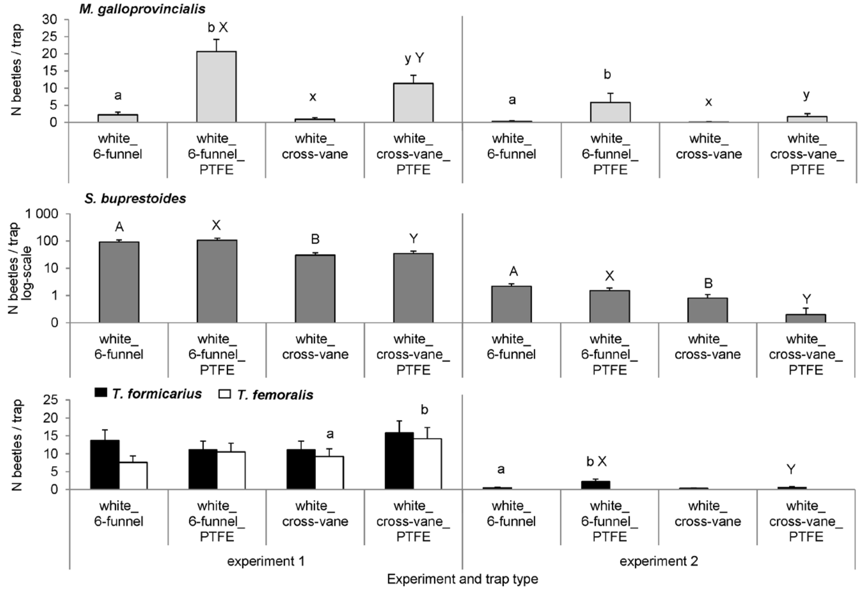

Figure 2 The number (mean and SE) of beetles of *M. galloprovincialis* and three most common insect species captured in the funnel and cross-vane traps either treated or untreated with PTFE in experiments 1 and 2 (see the caption of Fig. 1 for details on dates, lures, and replications); different letters above bars indicate significant differences at $\alpha = 0.05$ for each year separately: small letters – between the PTFE treatments within trap type, capital letters – between trap types within PTFE treatment (only significant differences are presented).

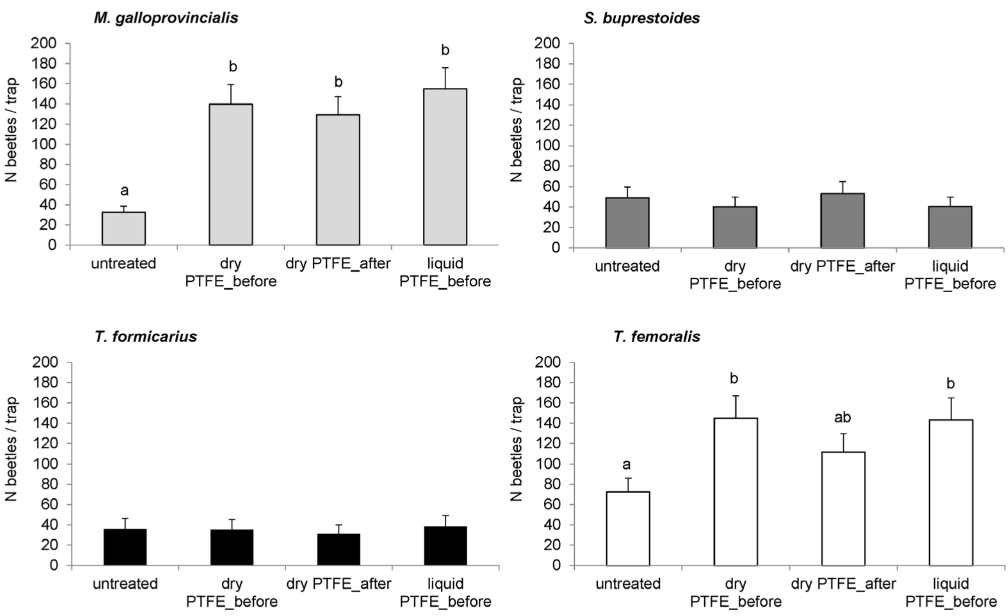

Figure 3 The number (mean and SE) of beetles of *M. galloprovincialis* and the three most common insect species captured in cross-vane traps either untreated or treated with dry PTFE or liquid PTFE before and after trap assembling and deployment; different letters above bars indicate significant differences at $\alpha = 0.05$

S. buprestoides

The non-target cerambycid beetle *S. buprestoides* was the most numerous species captured in the traps, particularly in experiment 1, when α-pinene was present in the lure. The trap type/colour had a significant effect on the catches of this insect in both experiments (experiment 1 – $\chi^2 = 669.6$, df = 4, P < 0.0001; experiment 2 – $\chi^2 = 11.3$, df = 4, P = 0.0238). In experiment 1, the highest numbers of the beetles were found in the black 12-funnel traps (313.6±44.69 beetles/trap), while the lowest catches were in the clear and white cross-vane traps (32.1±6.01 beetles/trap and 31.5±5.91 beetles/trap, respectively) and the difference in comparison to the 12-funnel traps was significant in both cases (Fig. 1). The effectiveness of the 6-funnel traps of both colours in capturing *S. buprestoides* was similar (95.8±14.88 beetles/trap in the white traps and 95.1±14.84 beetles/trap in the black traps) and intermediate in comparison to the traps mentioned above. They captured significantly more beetles than the clear and white cross-vane traps, but significantly fewer than the black 12-funnel traps (Fig. 1). In experiment 2, when the catches were generally lower than in experiment 1, the numbers of the beetles in traps of different types/colours were comparable (Fig. 1), with the only significant difference between the highest catches in the triangle traps (2.7±0.61 beetles/trap) and the lowest catches in the white cross-vane traps (0.8±0.29 beetles/trap).

Testing the effect of trap type (the white 6-funnel and cross-vane traps), PTFE treatment (treated and untreated) and their interactions on the catches of *S. buprestoides* revealed that in both, experiment 1 and experiment 2 it was significant only for the trap type ($\chi^2 = 95.1$, df = 1, P < 0.0001 and $\chi^2 = 11.8$, df = 1, P = 0.0006, respectively). The 6-funnel traps, either PTFE-treated or not, captured more beetles than the respective cross-vane traps (Fig. 2). Further, experiment 3 confirmed that PTFE treatment did not have any significant effect on the catches of *S. buprestoides* (Fig. 3).

Thanasimus spp.

In experiment 1, the trap type/colour had a significant effect only on the catches of *T. formicarius* ($\chi^2 = 11.7$, df = 3, P = 0.0085). The catches in the clear cross-vane traps were the lowest (7.8±1.74 beetles/trap) and differed significantly from the catches in the black 6-funnel traps (18.2±3.70 beetles/trap) (Fig. 1). The numbers of *T. femoralis* in the traps of different types/colours in experiment 1 did not vary much – from 7.5±1.79 to 10.8±2.48 beetles/trap. In experiment 2, the catches of both *Thanasimus* species were very low. The number of *T. formicarius* beetles exceeded 1 beetle/trap only in the black 12-funnel and 6-funnel traps (1.2±0.48 and 1.1±0.46 beetles/trap, respectively). *T. femoralis* was found only in 8 of 50 traps and was consequently even less numerous than *T. formicarius* (up to 0.4 beetles/trap).

In the test of the effect of trap type (the white 6-funnel and cross-vane traps), PTFE treatment (treated and untreated) and their interactions, no variable had a significant effect on the catches of *T. formicarius* in experiment 1, while in experiment 2 both single variables (without interaction) were significant (trap type – $\chi^2 = 15.4$, df = 1, P < 0.0001, PTFE treatment – $\chi^2 = 16.6$, df = 1, P < 0.0001). Contrast tests revealed that the number of beetles in the PTFE-treated traps (2.2±0.74 beetles/trap) was significantly higher than in the untreated traps (0.5±0.22 beetles/trap) only in the 6-funnel traps, while the difference between trap types was significant only in the PTFE-treated traps, with the 6-funnel traps capturing more beetles than the cross-vane traps (0.6±0.24 beetles/trap) (Fig. 2). Experiment 3 confirmed no effect of PTFE treatment on the catches of *T. formicarius* in the cross-vane traps (Fig. 3).

The analysis of *T. femoralis* catches in experiment 1 revealed that they were significantly influenced only by the PTFE treatment ($\chi^2 = 6.2$, df = 1, P = 0.0127). In addition, this effect was observed only in the cross-vane traps; the PTFE-treated traps captured significantly more beetles (14.2±3.17

beetles/trap) than untreated traps (9.2±2.15 beetles/trap). Experiment 3 confirmed the significant effect of PTFE treatment on the catches of *T. femoralis* in the cross-vane traps (χ^2 = 16.1, df = 3, P = 0.0011). The number of the beetles was the lowest in the untreated traps (72.4±13.5 beetles/trap) and differed significantly from the catches in the traps treated with either dry PTFE or liquid PTFE (145.1±22.0 beetles/trap and 143.3±21.6 beetles/trap, respectively) before trap assembling and deployment (Fig. 3).

Discussion

Effect of trap type, colour, and PTFE treatment on catches of *M. galloprovincialis*

Our tests of different combinations of trap types and colours did not reveal any significant effect on the catches of *M. galloprovincialis*. This might be explained by the overall low catches of the beetles (Bonifácio et al. 2012) probably resulting from the use of dry traps (with dry cups), not treated with surfactants. It has already been shown that some of the captured beetles can escape from the traps even though insecticides were added to the cups (Morewood et al. 2002, De Groot & Nott 2003, Miller & Duerr 2008), and the catches are significantly reduced if traps are not treated with any lubricants (this study, Graham et al. 2010, Graham & Poland 2012, Álvarez et al. 2015). Moreover, the overall catches in experiment 2 were even lower than in experiment 1, which could have resulted from the difference in the lure compositions, i.e. lack of ethanol and α-pinene in the lure used in experiment 2. These compounds, particularly α-pinene, often positively influence catches of *M. galloprovincialis* (Ibeas et al. 2007, Hoch et al. 2020, unpublished own data, but see Schroeder 2019).

Nevertheless, our research suggests that trap design may be an important factor influencing trapping efficiency. Namely, *M. galloprovincialis* beetles were most frequently caught in the 6-funnel traps, regardless of their colour (white or black), while the cross-vane traps (both white and clear) had an overall low trapping efficiency. These results initially contrast with previous studies that have shown cross-vane traps to be more effective in catching several North American *Monochamus* species than multiple-funnel traps (McIntosh et al. 2001, Morewood et al. 2002, Miller & Crowe 2011, Graham et al. 2012). However, as speculated by some authors (Morewood et al. 2002, De Groot & Nott 2003), this could be due to differences in the silhouette area of the two types of traps used in these studies, namely a much larger silhouette width in cross-vane traps than in multiple-funnel traps. In addition to chemical signals, *Monochamus* beetles likely use visual cues to locate trees suitable for colonisation (De Groot & Nott 2001), therefore traps with a larger silhouette area may be more visible to them. Indeed, other studies have shown that modifying multiple-funnel and cross-vane traps to standardize their silhouette area resulted in comparable or even greater effectiveness of the former one in capturing *Monochamus clamator* (LeConte, 1852) in North America (Costello et al. 2008).

In the present study, the multiple-funnel and cross-vane traps were similar in width (i.e. the diameter of funnels and the width of vanes were comparable), but the length of the funnel traps was greater, so their silhouettes were more conspicuous for *M. galloprovincialis*, perhaps leading to increased catches of this beetle. The above conjecture could also be confirmed by our results of testing the effect of the PTFE treatment, where the 6-funnel traps, characterized by a relatively long silhouette, caught more *M. galloprovincialis* beetles than rather short and therefore less conspicuous cross-vane traps. Another reason for the observed differences may be related to the physical properties of the materials used to manufacture the trap components (Graham & Poland 2012, Rassati et al. 2012). This may be supported by the fact that in our study overall more *M. galloprovincialis* was trapped in the multiple-funnel traps than in the cross-vane traps. The former traps were made of hard plastic and might therefore have been too slippery for the attracted beetles to walk on and escape from the trap surface. In contrast, the cross-vane traps were made of rather rough polypropylene/polycarbonate sheets, which could provide more grip for the beetles, allowing them to walk on and fly off.

The triangular trap developed for monitoring bark beetles in Poland (Skrzecz 2021) seems to deserve more attention, as it appeared slightly more effective in catching *M. galloprovincialis* than the other trap types tested in experiment 2. This could be related to the large surface (silhouette) of the trap. Trap treatment with a lubricant, e.g. PTFE, might largely increase the trap effectiveness, although it is difficult to predict how soft polyethylene foil would perform in comparison to coroplast in cross-vane traps or hard plastic in funnel traps. The potentially negative aspect of using triangular traps is their higher vulnerability to wind due to the large flat surface.

Trap colour is among the most important factors influencing the effectiveness of traps in capturing different insect species, e.g. wood-boring beetles (e.g. Rassati et al. 2019, Cavaletto et al. 2020, 2021). Previous studies have shown that *Monochamus* beetles were more often caught in black traps (De Groot & Nott 2001, Rassati et al. 2012), suggesting that the dark silhouette is an important cue for tree finding by these beetles. Therefore, we could expect more *M. galloprovincialis* to be captured in the black 6- or 12-funnel traps than in the white 6-funnel and both cross-vane traps, but the differences were not evident. Moreover, the white funnel traps seem to be even more efficient than the black traps (see experiment 1), although the difference was not significant, probably due to too low overall catches. Black and white colours, although represent absolute contrasts in total reflectance intensity, have relatively similar patterns of light reflectance across almost the entire visible light spectrum (Campbell & Borden 2005, Beresford & Sutcliffe 2006, Kerr et al. 2017). Therefore, our results suggest that *M. galloprovincialis* might prefer colours of a higher reflectance intensity if their reflectance patterns are similar. However, further studies are needed for a better understanding of the preference/response of *M. galloprovincialis* to different colours.

Several studies have been conducted to test different lubricants and showed that they increase the efficiency of traps in catching wood-boring beetles. For example, De Groot & Nott (2003) observed increased catches of three *Monochamus* species and several other cerambycid beetles in cross-vane traps coated with a silicone-based surfactant. Subsequently, similar results were obtained with the use of lubricants based on PTFE (Allison et al. 2011, 2014, 2016, Francese et al. 2013, Álvarez et al. 2015, Allison & Redak 2017), which furthermore proved durable and weather resistant, allowing the use of PTFE-covered traps for up to two years (Graham et al. 2010, Graham & Poland 2012). Finally, PTFE concentration has been shown to have little or no effect on trap efficacy, reducing the costs associated with its purchase (Allison et al. 2016). Our results confirm the positive effect of trap treatment with PTFE on the catches of *M. galloprovincialis* beetles and highlight the need to use this lubricant in both ecological studies focusing on this species and programs to monitor/reduce its population as a PWN vector. Furthermore, our study indicates that neither the PTFE formulation (dry or liquid) nor the timing of PTFE application affects trap effectiveness, thus allowing end-users for a more convenient and less messy application of dry PTFE (e.g. applying PTFE prior to trap deployment in the field, accurately applying PTFE to the trap surface etc.). The aerosol formulation is more readily available, easy to apply, and less expensive than liquid PTFE (Allison et al. 2011). Silicon-based and greasy formulations are less suitable as they do not dry up and may thus cause some inconvenience in their use. In addition, small insects might stick to the surface instead of slipping into a collection cup (Allison et al. 2011).

Effect of trap type, colour, and PTFE treatment on catches of non-target species

In the present study, *S. buprestoides* was much more abundant in the funnel traps (12-funnel traps followed by both black and white 6-funnel traps) than in the cross-vane traps. These results confirm the findings of Rassati et al. (2012) and suggest that trap type rather than colour is more important for attracting *S. buprestoides.* Moreover, the effect of trap type was significant only when the traps were baited with lures containing ethanol and α-pinene (experiment 1). A similar positive effect of these chemicals on the catches of *S. buprestoides* in the black traps was observed also by other researchers (Shibata et al. 1996, Sweeney et al. 2004, Jurc et al. 2012, 2016; Hoch et al. 2020). This tendency can be explained by the biology of this insect, i.e. development in conifer stumps that produce and release high amounts of ethanol (Kelsey & Joseph 1999).

In contrast to *M. galloprovincialis*, the trap treatment with PTFE had no significant effect on the catches of *S. buprestoides* in any of our experiments. Based on our field observations, *S. buprestoides* fly relatively efficiently but often fall to the ground when they encounter an obstacle. The same effect can occur when *S. buprestoides* collides with a trap, which could explain the similar catches of this species in PTFE-treated and untreated traps.

In our study, we encountered some problems related to catching high numbers of *S. buprestoides*, which was the most common non-target beetle also in other studies (Jurc et al. 2012, 2016; Rassati et al. 2012). Adult *S. buprestoides* are relatively large beetles with strong mandibles and often destroy other insects in traps, making their identification and counting impossible. In addition, some difficulties in extracting nematodes from insect vectors can be expected due to the more rapid desiccation of the dismembered *M. galloprovincialis* bodies. An obvious solution to this obstacle would be the use of an insecticide or liquid preservative in traps. However, as *S. buprestoides* is a common wood decomposer and thus play an important role in the chemical cycling of micronutrients in forest ecosystems, it is advisable to look for solutions to minimise the capture of this insect in traps (see Conclusions).

The effect of trap type on the catches of *Thanasimus* spp. was species-specific. The number of *T. formicarius* was increasing from the clear cross-vane traps followed by the white cross-vane traps to the black 6-funnel traps, while the catches of *T. femoralis* were comparable among trap types. Our results confirmed some preference of *T. formicarius* towards darker colours observed in other studies (Cavaletto et al. 2020, Akkuzu et al. 2021). Black traps appeared more attractive over white traps also for *Thanasimus dubius* (Fabricius, 1776) (Strom et al. 1999). In addition, Heber et al. (2021) found that the length of black funnel traps has a positive effect on the number of captured *Thanasimus* spp.; the catches were increasing with increasing number of funnels in the traps. If we consider that *T. formicarius* and *T. femoralis* are sympatric species, with relatively similar morphology and biology (Thomaes et al. 2017), a reason for no effect of trap type/colour on the catches of *T. femoralis* is not clear. It might be explained by the difference in beetle ecology and phenology. In Scots pine stands, *T. formicarius* predates on bark beetles developing under thick bark (e.g. *Tomicus piniperda* (Linnaeus, 1758), *Ips sexdentatus* (Börner, 1776) and *Hylurgops palliatus* (Gyllenhal, 1813)), i.e. in a lower and darker part of tree stems (Schroeder 1999). In contrast, *T. femoralis*, smaller than *T. formicarius*, seems to inhabit tree crowns (Thomaes et al. 2017), where small, thus more suitable bark beetles (e.g. *Pityogenes bidentatus* (Herbst, 1874), *P. quadridens* (Hartig, 1834) or *Ips acuminatus* (Gyllenhal, 1827) develop under thin bark. This hypothesis may partially be confirmed by the difference in kairomone compositions attractive to these species (Bakke & Kvamme 1981, Schroeder 2003, Wehnert & Müller 2012), but more studies are needed.

Likewise the effect of trap type, the effect of trap treatment with PTFE on the catches of *Thanasimus* spp. was species-specific, but inverse. The catches of *T. formicarius* were rather comparable in the PTFE-treated and untreated traps; the significant difference was observed only in experiment 2, with very low overall catches of this species. In contrast, the numbers of *T. femoralis* were much higher in the treated vs untreated traps, but the difference was significant only in the cross-vane traps. Similar dependence of the effect of PTFE treatment on the trap type was observed in the catches of *T. dubius* (Allison et al. 2011). *Thanasimus* beetles are agile fliers and can move efficiently on smooth surfaces. The material used for making vanes (the coroplast in our studies) might be more suitable (easier to attach to) for these beetles than the hard plastic of funnels, thus the PTFE treatment had a stronger effect on the insect catches in the cross-vane traps. However, it is not clear why a similar effect was not observed in *T. formicarius.*

The capture of two *Thanasimus* species was a frequent phenomenon in this and other studies (Francardi et al. 2009, Jurc et al. 2012, 2016; Foit et al. 2019). Both species are considered beneficial in forest ecosystems because they are antagonists of bark beetles (e.g. Hagen et al. 1999), therefore trapping them out may have negative consequences for the natural resistance of forest ecosystems. The use of traps equipped with perforated cups from which *Thanasimus* beetles can escape might be a reasonable solution. This would preclude the use of capture liquid in the cups but may be replaced by an extension of collector cups and PTFE treatment of their interior to prevent *M. galloprovincialis* beetles from escaping (Álvarez et al. 2015, Bonifácio et al. 2021). A modification of lure composition that would be less attractive to these non-target species is another possibility, but it requires further studies.

Conclusions

Based on our studies, the white 6-funnel traps treated with PTFE were the most effective in catching *M. galloprovincialis*. However, for the monitoring of *M. galloprovincialis*, we recommend less, but still highly effective white cross-vane traps, also treated with PTFE. Their use with lures without ethanol and α-pinene greatly reduces the catches of non-target *S. buprestoides* valuable in wood decomposition, and predatory *T. formicarius* (particularly in comparison to black traps). The cross-vane traps are relatively cheap and easy to use and can be conveniently stored after disassembly. More efficient white 6-funnel traps are recommended for the mass-trapping of *M. galloprovincialis* to reduce its population.

Dry PTFE in aerosol was shown to be as efficient as liquid PTFE in increasing the trap effectiveness but is less messy, easier to apply, more accessible, and much cheaper. It may be applied prior to or after trap deployment in the field, thus making it more convenient for end-user.

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Supporting information

Figure S1. Traps tested in the study; a) black 12-funnel, b) black 6-funnel IBL-3, c) white 6-funnel IBL-3, d) white cross-vane IBL-5, e) clear cross-vane, f) triangular IBL-2

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