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Preference of pear psyllid (*Cacopsylla pyri*) for specific colour inspires new application in plant protection

Bruna Czarnobai De Jorge[1,2] | **Rainer Meyhöfer**[3] | **Andreas Jürgens**[2] | **Jürgen Gross**[1]

[1]Institute for Plant Protection in Fruit Crops and Viticulture, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Dossenheim, Germany

[2]Plant Chemical Ecology, Technical University of Darmstadt, Darmstadt, Germany

[3]Institute of Horticultural Production Systems, Section Phytomedicine, Gottfried Wilhelm Leibniz Universität Hannover, Hannover, Germany

Correspondence
Jürgen Gross, Institute for Plant Protection in Fruit Crops and Viticulture, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Schwabenheimer Straße 101, Dossenheim 69221, Germany.
Email: juergen.gross@julius-kuehn.de

Abstract

Understanding the cues used by insects to select their hosts is essential for developing sustainable control strategies, particularly for plant disease vectors. Pear psyllids (*Cacopsylla pyri*) are vectors of the bacterial disease pear decline caused by '*Candidatus* Phytoplasma pyri'. Yellow sticky traps are typically used to monitor pest insects, but they are non-specific and capture many beneficial insects, too. Against the background of improving visual traps, this research aimed to investigate the colour choice behaviour of *C. pyri*. Our first approach was to screen insect colour preferences by performing choice assays with different LED colour wavelengths in a small-scale choice arena under controlled conditions. Over six LEDs tested, there was a strong significant preference of *C. pyri* for green 1 (532 nm) followed by green 2 (549 nm). Yellow (576 nm), orange (593 nm), red 1 (619 nm) and red 2 (633 nm) were less attractive than green. Subsequently, the trapping of pear psyllids was tested in the field using newly developed traps covered with transparent-coloured PVC sheets with a similar wavelength of the preferred LEDs. Red and completely transparent traps have been used as control and combined in randomized blocks in a pear orchard. Field trials revealed that green traps ranging from 525 to 537 nm attracted significantly more pear psyllids than longer wavelength green (543 nm), red and transparent ones. The construction of specific green-coloured sticky traps seems promising for improving psyllid trapping, especially during an early infestation at low population densities. Thus, these visual traps should be considered in future and integrated into psyllid monitoring as part of integrated pest management.

KEYWORDS
colour sticky traps, colour vision, IPM, monitoring, pear decline, psyllids

1 | INTRODUCTION

Pear psyllids are monophagous insects. Seven species of pear (*Pyrus*)-feeding psyllids (Homoptera: Psyllidae) in the genus *Cacopsylla* spp. are present in North America and Europe (Burckhardt & Hodkinson, 1986). Several of these species are important pests of commercial pear, most notably *Cacopsylla pyricola* (Förster), *C. pyri* (L.) and *C. bidens* (Šulc) (Jarausch et al., 2019). These species are specialist pests of commercial pear, reproducing only on certain species of *Pyrus* spp. The factors that affect psyllid distribution and movement

in orchards are still unknown (Gross et al., 2021; Horton, 1999). Numerous cues are likely to affect the behaviour of the adult insects in their environment, including olfactory cues from the host plant (kairomones) (Gallinger & Gross, 2020; Görg et al., 2021; Mayer et al., 2008a, 2008b) and conspecific mates (pheromones) (Soroker et al., 2004).

Pear decline is a severe and often lethal disease, limiting pear production in Europe and America. The causal agent is 'Candidatus phytoplasma pyri', a phloem-limited bacterium transmitted by pear suckers (psyllids) (Bangels et al., 2008; Garcia-Chapa et al., 2005; Jarausch et al., 2019). The most easily recognizable symptoms occur in late summer with premature autumn leaf colour (yellow and red) on affected trees. There may be some leaf cupping or curling, and there is usually premature leaf fall (EPPO/CABI, 1996). According to the Belgian research centre for fruit cultivation (pcfruit npo), monitoring data of the past decades, the mean date for the start of activity is February 3rd, soon followed by egg-laying. The first nymphal stages emerge in the field at the beginning of April. Nymphs develop through 5 instars secreting honeydew. This cycle repeats several times, resulting in 3 to 5 or even more generations per year (Belien et al., 2013).

Intensive and area-wide application of broad-spectrum chemical insecticides has been the primary means of controlling the vector and the disease. Although it is impossible to eliminate pear psylla from the environment, well-managed control strategies can significantly reduce the chance of an epidemic outbreak (Belien et al., 2013). Nevertheless, chemical pesticides should be restricted to the lowest level necessary to keep the pest under the economic threshold, that is, the pest density at which the value of resulting damage exceeds the cost of applying control measures (Zalom, 2010). To ensure this, accurate monitoring of the pest population to identify exceedance of the economic threshold is one of the most critical aspects of IPM, especially as for insect vectors the threshold is always very low (Gross & Gündermann, 2016).

It is essential to understand the role of different plant cues used by respective herbivorous insects to develop sustainable control strategies, especially for plant disease vectors (Gross & Franco, 2022; Gross & Gündermann, 2016). Phytophagous insects rely on visual and olfactory cues for orientation and successful host selection. Host selection by herbivorous insects follows frequently successive steps, from random foraging to location of the host habitat, location of the host within the habitat, host recognition, assessment and eventually acceptance or rejection of a plant as a host for feeding and oviposition (Schoonhoven et al., 1998). All steps involve different senses and occur at varying distances from the plant. Olfactory and visual cues can be perceived at larger distances and therefore they determine host plant location by foraging insects (Schoonhoven et al., 1998).

In contrast, the diversity of visual cues reflected by plants has limitations to the narrower variety of foliar pigments, which cause them to vary essentially in the 'green-yellow-orange' part of the light spectrum visible to insects. Three optical characteristics of plants may influence host selection behaviour: spectral quality, dimensions (size) and pattern (shape) (Prokopy & Owens, 1983). The spectral sensitivity of the insect compound eye ranges from 350 to 650 nm (near-ultraviolet to red) and thus includes shorter wavelengths than that of the human eye (Schoonhoven et al., 2005). Generally, species belonging to the Hemiptera are not attracted to visual targets that reflect or emit light <400 nm or >600 nm (Döring, 2014; Döring & Chittka, 2007). However, attraction to longer wavelengths is known for eucalyptus psyllids, *Anoeconeossa bundoorensis* Taylor & Burckhardt and *Glycaspis brimblecombei* Moore, which specialize in red anthocyanic leaves (Farnier et al., 2014). Prokopy and Owens (1978) speculated that monophagous-oligophagous insects are more likely to be visual specialists than polyphagous insects. They further suggested that visual traps which mimic specific host-plant visual stimuli should help monitor monophagous-oligophagous insect pest populations on crops. If so, the monophagous pear psyllids should be well suited for monitoring with a visual trap.

Methods for capturing psyllid populations are described by Weintraub and Gross (2013). Most common monitoring methods include jarring adults from a limb onto a framed cloth (beating tray method) or examining terminals or spurs for eggs and nymphs (Jenser et al., 2010; Müther & Vogt, 2003). However, there are drawbacks to these methods because weather conditions and characteristics of the limbs sampled affect the number of adults collected (Horton, 1999). Examinations of terminals and spurs are also tedious and time-consuming. In addition, these methods sample only a single point in time. The attraction of phytophagous hemipterans to yellow and green targets with a strong preference for yellow visual targets has been documented for aphids (Döring & Chittka, 2007), whiteflies (Coombe, 1981, 1982) and psyllids (Farnier et al., 2014; George et al., 2020; Hall, 2009; Hall et al., 2010; Horton, 1999; Krysan & Horton, 1991; Wenninger et al., 2009). Yellow-coloured sticky traps are widely used to monitor pear psyllids, but they become ineffective after brief deployment periods as they become fouled with other insects that are also attracted and require frequent replacement. Additionally, the numbers of bycatches of beneficial insects are very high on yellow-coloured sticky traps, especially for pollinators such as bees and hoverflies and some natural enemies (Beers, 2012; Roubos & Liburd, 2008). Until now, there are some improvements in the attractivity of yellow sticky traps, especially regarding the Asian citrus psyllid (Allan et al., 2020; Hall, 2009) with baits such as plant volatile lures for targeted psyllids (George et al., 2016, 2020; Patt & Setamou, 2010; Wenninger et al., 2009). Visual cues may play a dominant role in host location by adult psyllids and therefore may be more important than olfactory cues in designing effective traps or attract-and-kill devices (Hall, 2009; Patt & Setamou, 2010; Wenninger et al., 2009). More detailed insight into visually driven behaviours of *C. pyri* may facilitate the improvement of surveillance and management for this species.

The main objective of this research was to investigate the colour preference of *C. pyri* in detail for the development of an appropriate and specific trap. Thus, we started by selecting several wavelengths of the measured reflectance spectrum for investigation in a lab choice test. Subsequently, we selected respective coloured foils

and tested their attraction for *C. pyri* adults in sticky traps, which we want to use for monitoring and mass trapping. Also, we quantified the influence of several trap colours in capturing non-target insects in the field.

2 | MATERIALS AND METHODS

2.1 | Insect collection and rearing

In March and April 2019, *C. pyri* was collected from an extensively managed pear orchard of the Julius Kühn-Institut (JKI) in Dossenheim, Germany, using the beating tray method (Muther & Vogt, 2003). Species identification was carried out using the key of Burckhardt and Hodkinson (1986) and Ossiannilsson (1992) (Burckhardt & Hodkinson, 1986; Ossiannilsson, 1992). Rearing took place on healthy potted *Pyrus communis* cv. Willians Christ plants in a 47.5×47.5×93cm Bug Dorm rearing cages (NHBS, Devon, UK), located in a climate chamber at 20°C day and 15°C night temperature under long-day conditions (L16:D8) and 55% relative humidity (Görg et al., 2021).

2.2 | Construction of light traps

The constructed light traps consist of housings (13×10×10cm) made of polyvinyl chloride plates (4mm), as already used in previous studies (Otieno et al., 2018; Stukenberg et al., 2018, 2020; Stukenberg & Meyhöfer, 2019). Briefly, a colourless translucent acrylic glass plate with light scattering properties (Plexiglas®, LED 0M200 SC, Evonik Industries AG, Essen, Germany) is located at the front. At the back of the housing, aluminium panels (10×10cm) hold the respective high-power LEDs (Figure 1a). In order to trap psyllids, a wrapping film (polyethylene terephthalate) was stretched on the

translucent screen and coated with insect glue (TEMMEN GmbH, Hattersheim, Germany).

2.2.1 | Experimental design

All choice tests were done under controlled climatic conditions (23°C; 65% relative humidity) in a climate chamber (Johnson Controls, Essen, Germany). A flight cage with a base area of 1×1m and a height of 0.8m served as arena (Figure 1b). While the side walls were covered with black plastic foil, the upper side of the cage consisted of a translucent foil. The LED traps were placed in quadratic openings of a black cardboard box. Traps were positioned 7.5cm above the ground with a 5cm distance between each other. The order of LED traps was randomized for each experimental replicate on the six positions. LEDs were switched on prior to insect release for a minimum of 10min to ensure a constant operating temperature. Psyllids were always released at the same release point with a 0.7m distance to each of the traps from not more than three tubes containing approximately 50 adults (males or females groups). Insects were released in the flight cage under visible light (45μmol m^{-2} s^{-1} on average) and UVA radiation (2μmol m^{-2} s^{-1} on average) emitted by the fluorescent tubes to ensure that they moved from the releasing tubes. After 1h, the room lights were turned off, and the only light source was coming from LED lamps. Experiments were terminated after 6h, and all psyllids on traps, inside the cage or remaining in the tubes were counted. Ten consecutive replicates were performed.

2.2.2 | Measurement and adjustment of high-power LEDs

The experiment was conducted with six LED colours (green 1, green 2, yellow, orange, red 1, red 2). LEDs used in the choice tests were
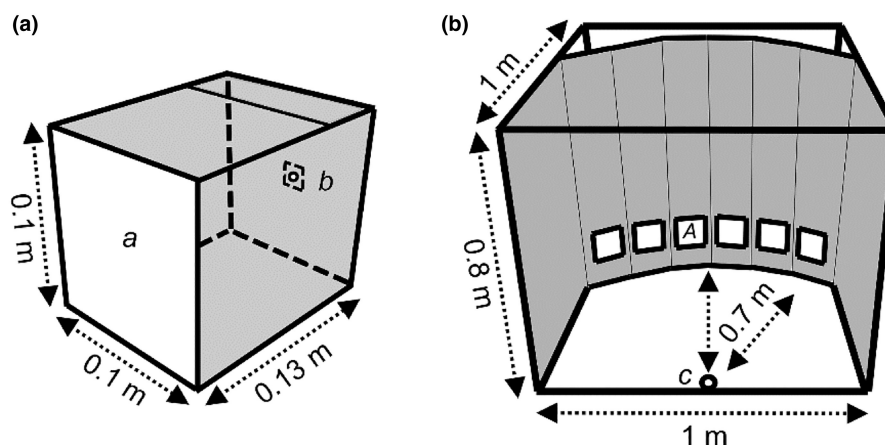

FIGURE 1 Schemes of LED trap and choice arena. (A) LED trap with acrylic glass screen front side (a) and LED panel backside (b). The inner side of the box was laminated with mirror film. (B) Choice arena with psyllid release point (c) and LED trap position (A). The background colour was black, and the bottom colour was black brown. Reprinted from 'Wavelength-specific behavior of the western flower thrips (*Frankliniella occidentalis*): Evidence for a blue-green chromatic mechanism' by Stukenberg et al. (2020). © 2020 Creative Commons Attribution (CC BY) licence (http://creativecommons.org/licenses/by/4.0/).

adjusted to equal intensities to obtain standardized, purely wavelength-dependent conditions. All intensities were adjusted using the same equipment and following the same protocols given by Stukenberg and Poehling (2019). After adjusting intensities, the spectra and peak wavelength of all LEDs were measured in complete darkness using the spectrometer AvaSpec-2048-2 (Avantes BV, Apeldoorn, The Netherlands, Figure 2). The specifications of all used LEDs are given in Table 1. Most LEDs were commercially available single-chip emitters, but, two specific multi-chip emitters were used, equipped with additional cooling and LEDs were operated as described in the previous study (Stukenberg et al., 2020), allowing individual intensity adjustment.

2.3 | Measurement of reflection and selection of colour films for building traps

Reflected light values of selected colour films used in the field experiment were measured in the laboratory with a Flame Miniature Spectrometer (Flame S-UV-VIS-ES, Ocean Optics Inc., Duiven, The Netherlands) using OceanView version 2.0.2.2 software (Ocean Optics Inc., Duiven, The Netherlands). Reflectance intensity readings from the near UV through the visible wavelengths (300–700 nm) were automatically scanned using an integration time of 4 ms. Film reflectance was measured with a bifurcated fibre optic cable (QR400-7-SR-BX, Ocean Optics Inc., Duiven, The Netherlands) with probe and illumination at a 45 angle to the film surface. Reflectance measurements were standardized to a white standard (WS-1 Diffuse Reflectance Standard, Ocean Optics Inc., Duiven, The Netherlands) consisting of PTFE (polytetrafluoroethylene powder) that reflected >98% from 300 to 15,000 nm and a dark standard (no light). Film illumination was provided through one side of the bifurcated fibre from a pulsed xenon lamp (PX-2, Ocean Optics Inc., Duiven, The Netherlands) in the spectrometer. Transparent PVC film (80 microns) with UV stabilizers and adhesive (Solvent polyacrylate, permanent) were purchased from ORAFOL Europe GmbH. The red colour was cut from high-density PVC sheeting (Art. Nr.: 0572020; Modulor, Berlin, Germany). The specifications of the films used are presented in Table 2.
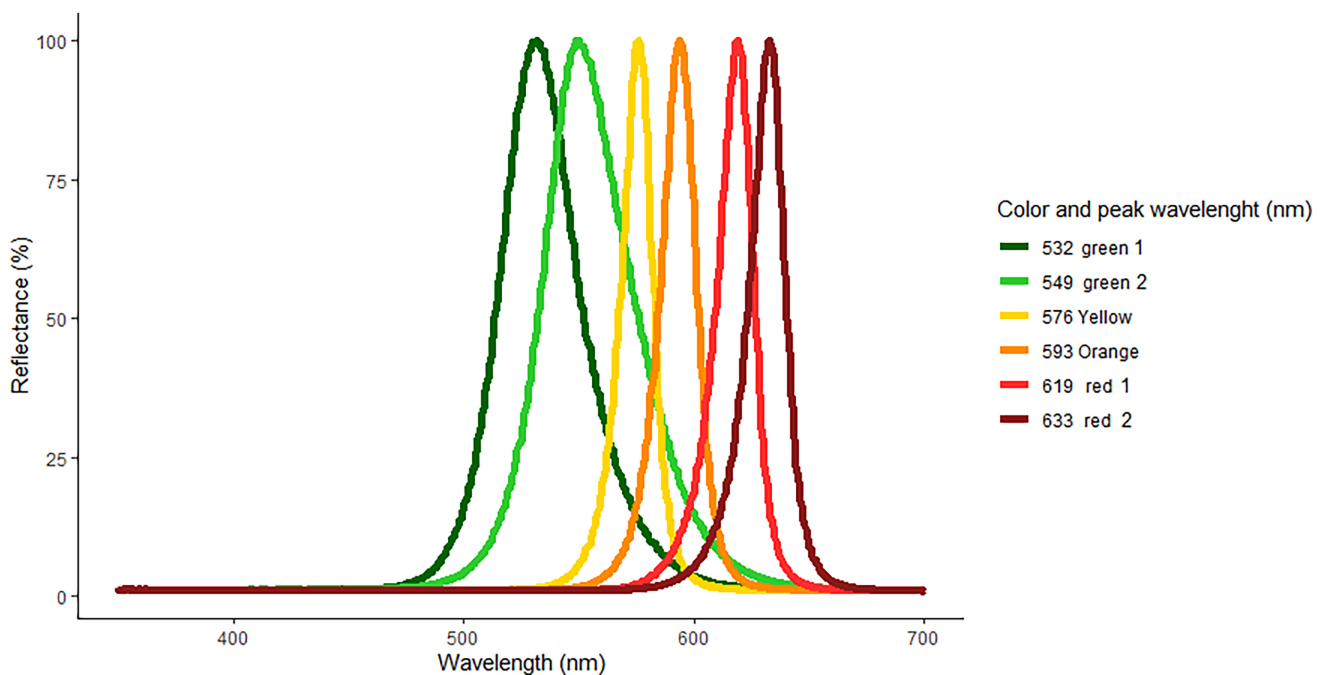

FIGURE 2 Spectra of high-power light-emitting diodes (LEDs) used. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Specifications of high-power light-emitting diodes (LEDs) and constructed panels used.

LED colour names	Peak wavelength (nm)	Manufacturer	Type (design)	LEDs/panel (cooling)
Green 1	532	Osram	Oslon SSL LT CP7P (sc)	1 (nc)
Green 2	549	Roithner	LED550-66-60 (mc)	1 (pc)
Yellow	576	Roithner	LED570-66-60 (mc)	4 (ac)
Orange	593	Osram	Oslon SSL LY CP7P (sc)	2 (nc)
Red 1	619	Osram	Oslon SSL LA CP7P (sc)	1 (nc)
Red 2	633	Nichia	NCSR119 T (sc)	1 (nc)

Abbreviations: ac, active cooling with fan; mc, multi chip emitter; nc, no additional cooling; pc, passive cooling with heat sink; sc, single chip emitter.

2.4 | Field trials

Transparent sticky traps used for studies were provided by Insect Services GmbH, Berlin. It is a prototype developed for specific trapping of psyllids (Figure 3a–e). The traps were produced from transparent 0.02 mm thick rigid vinyl plastic cylinders which provided 360 degrees of visibility to psyllids within their visual range, 9 cm in diameter × 25 cm in length. Traps were closed on the bottom and on the top to avoid insects entering, and the external surface was coated with a thin layer of insect glue, where insects were captured. The internal surface of test traps was covered with coloured transparent films or red sheets mentioned above (Table 2), and clear ones were used as control.

The field trial was conducted in an experimental orchard (ca. ¼ ha) at the Julius Kühn Institut, Dossenheim, Germany. The area was divided into five blocks, consisting of 192 m² per block

(Figure 3f). The first and last row and the first and last lines of the pear orchard were left free. Traps were hung between two pear trees, 10 m apart and 150 cm above the ground and at this height, the trap bottoms were always in the middle of the canopy of pear trees. Psyllids adults glued on the sticky traps were counted and removed from all visual traps weekly from May 5th to September 16th 2020 (20 weeks). Pollinators and other beneficial insects were also counted and removed from traps. Before the beginning of the experiment, the pear orchard was treated with sulfur against pear pox mite at the end of February. Then on the second week of March, the insecticide Calypso® was sprayed. At the end of April, pear trees were treated with fungicide Delan® and Systhane® against black spot disease and powdery mildew. One week after the experiment started and at the end of June, trees were treated with Delan® and Sercadis® for scab and powdery mildew.

Hue description	Colour number	Manufacturer/type	RGB
Green	#061	ORACAL® 8300	40, 208, 107
Grass green	#068	ORACAL® 8300	62, 194, 0
Lime-tree green	#063	ORACAL®	175, 229, 82
Signal red	#RAL 3001	8300Modulor	155, 32, 25

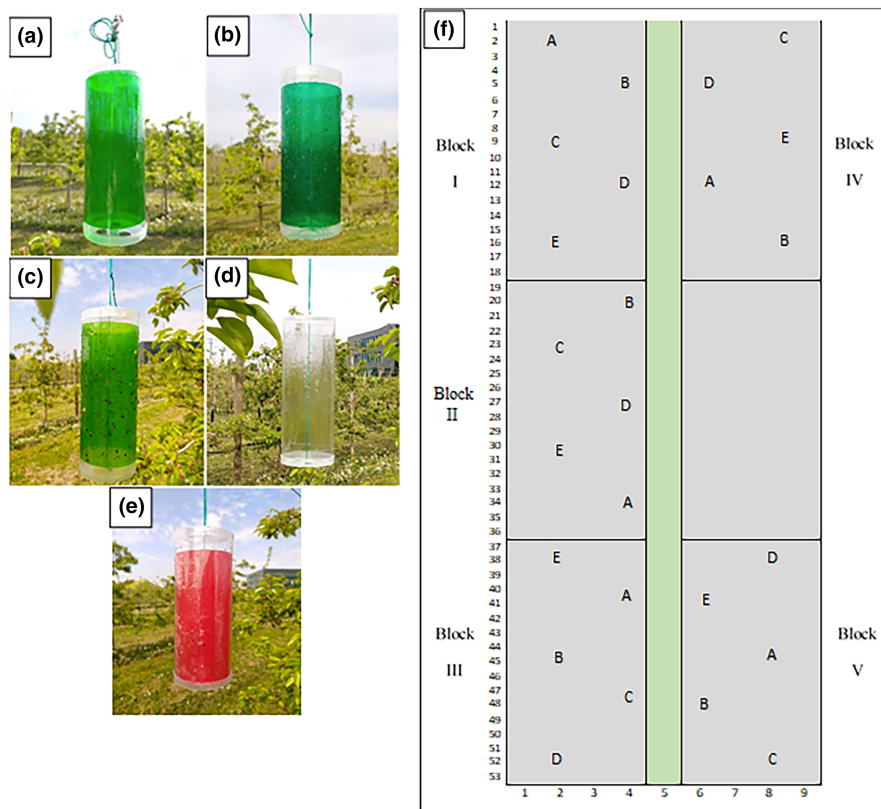**TABLE 2** Specifications of colour attributes of films used.

FIGURE 3 Photographs of different trap types: (a) green #061; (b) grass green #068; (c) lime-tree green #063; (d) Transparent; (e) signal red #RAL 3001; See Table 2 and Figure 5 for colour specifications; (f) Field setting: grey shading represents areas planted with pear cv. Williams-Christ. Green shading represents areas planted with cv. Conference; Rows: 1–9; Plants per row:1–53; Letters A–E: represent the position of traps correspondent to the photographs. [Colour figure can be viewed at wileyonlinelibrary.com]

2.5 | Data analysis and statistics

The statistical evaluation of the influence of wavelengths on *C. pyri* was conducted with the program R (Version 1.2.5033, Team, 2019). All figures were generated using the 'ggplot2' package (Wickham et al., 2019). The relative choice frequencies, that is, the preference of psyllids for different LED colours, were analysed using generalized linear models (GLMs), assuming a quasi-poisson distribution (count data with overdispersion). Using a deviation analysis (*F*-test, link function: 'log'), we investigated whether the factor 'colour' significantly influenced the number of insects oriented towards the traps. The temporal repetitions of experiments and the spatial dimensions were included in the models as block factors. Subsequent Tukey-type multiple comparisons at $\alpha = 0.05$ using the R-package 'lsmeans' (Lenth, 2016) were conducted to clarify which wavelength differs from another (mean value differences) regarding the relative choice frequencies. The field trial was fitted to the generalized linear model (GLM) package 'MASS', considering a negative binomial distribution (count data with overdispersion) (Demétrio et al., 2014). Using a deviation analysis (*F*-test, link function: 'log'), we analysed whether the factors 'colour' of traps, 'week' of evaluation, 'season' (mid-spring: week 1–4; late-spring: week 5–8; early-summer: week 9–12; mid-summer: week 13–16; late-summer: week 17–20) and 'block' significantly influenced the number of insects oriented towards the traps. Model tests were performed with a residual diagnostic for

hierarchical (multi-level/mixed) regression models 'DHARMA' package. Multiple pairwise comparisons were calculated with estimated marginal means and 95% confidence intervals with the function emmeans from the 'emmeans' package (Lenth et al., 2019) and *p* values adjustment by the method of Tukey.

3 | RESULTS

3.1 | The attractiveness of different wavelengths in the visible spectral range (532–633 nm)

The mean recapture rate during the experiment was 67%. Both the factors block and sex were not significant. Consequently, colour preference data for males and females were pooled. However, a significant effect of the LED colour on the choice frequency was observed ($F_{5,9} = 9.7712$, $p < 0.0001$, Figure 4). The results showed a significant preference of *C. pyri* for green 1 (40.6%) compared to the other colours tested ($F_{5,9} = 9.7712$, $p = 0.0005$, Figure 4). All other wavelengths attracted fewer psyllids. Green 2 (19.9%) did not differ in its attractiveness over yellow (11.4%), orange (14.2%) and red 1 (8%). Red 2 (5.9%) was of similar attractiveness as red 1, orange and yellow and attracted significantly fewer psyllids than green 2 ($F_{5,9} = 9.7712$, $p = 0.0387$) or green 1 ($F_{5,9} = 9.7712$, $p < 0.0001$ Figure 4).
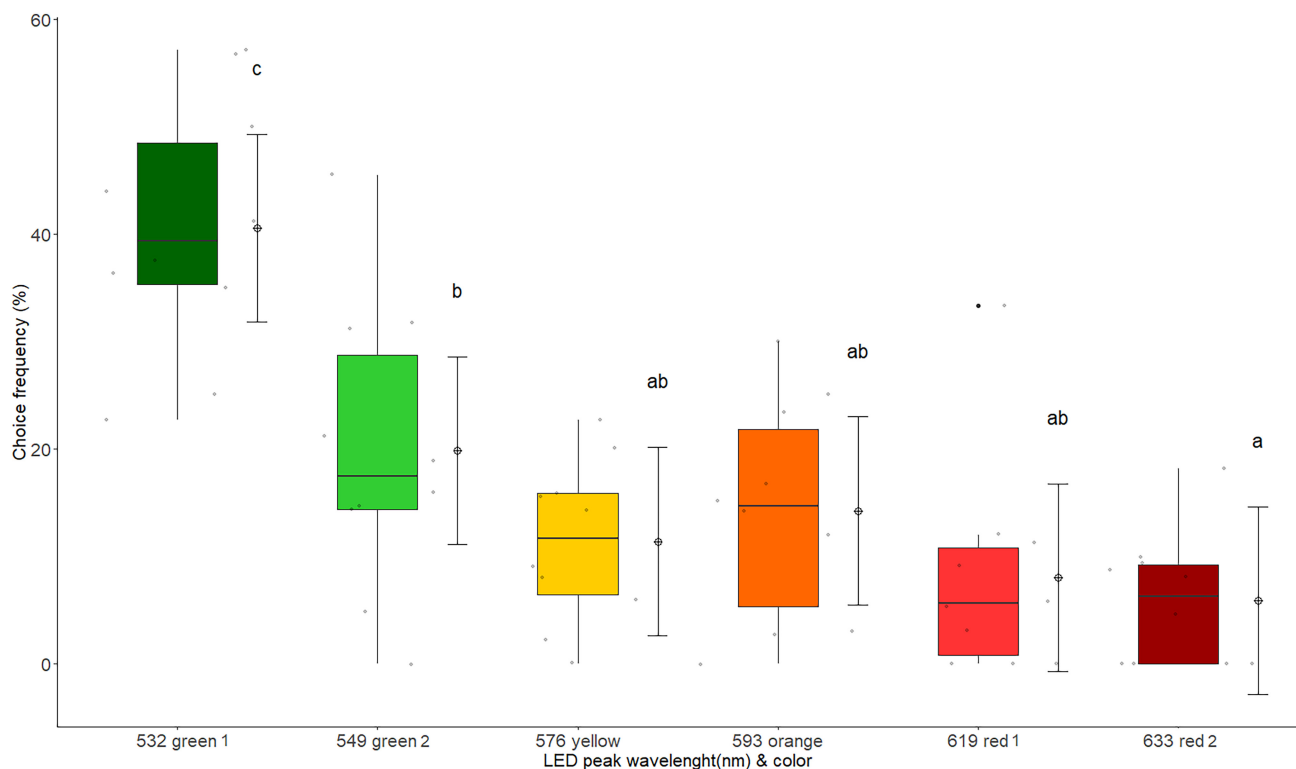

FIGURE 4 Wavelength preferences of *Cacopsylla pyri* in a multiple-choice experiment with light-emitting diodes (LEDs) in the spectral range of visible light. See Table 1 and Figure 2 for LED colour specifications. Significant differences are indicated by different letters (generalized linear model, multiple pairwise comparisons, Tukey test, *p* < 0.05, *n* = 10). Boxes correspond to the 25th and 75th percentiles, medians are shown as lines and whiskers extend to 1.5 times of the interquartile ranges. Dots represent raw values. Corresponding means and confidence intervals predicted from the generalized linear model are shown to the right of each box. [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Measurement of colour attributes of coloured sheets

The reflectance spectra of the coloured films were recorded in order to investigate whether pure colours, similar to wavelengths tested in the arena, could produce comparable results under field conditions. The wavelengths of the colour films are not exactly the same as of the peak wavelengths of the LED traps, as it was not possible to find films with the exact same wavelength specification on the market. Thus, we used colour films which reflected light very close to the peak, but still within the top of the spectrum (Figure 5). Therefore, sheets with a similar peak wavelengths to the green LEDs 532nm green 1 and 549nm green 2 were selected. For comparison, sheets similar to the least preferred colour in the arena test (633nm red 2) were also selected for the field trial. Since no perfect match between films and the LEDs tested was found, films spectral measurements within the range 525–549nm peak wavelength were selected (Table 2, Figure 5): #068 grass green film (537nm) and the #061 green film (525nm) were approximated to the green 1 LED (532nm); #063 lime-tree green film (543nm) to the green 2 LED (549nm).

3.3 | Field trials

The model revealed statistical significance interactions between psyllids catches and trap colour, week of evaluation, block and season. Trap colour significantly affected psyllid captures ($F_{4,499} = 145.87$; $p < 0.001$). We have also evidenced differences in the psyllid's distribution over the blocks in the orchard ($F_{4,499} = 75.4$, $p < 0.0001$), but in all blocks 537 green and 525 green traps captured more pear psyllids than all other traps ($p < 0.0001$, Figure 6a–e).

When observing the psyllids captures over the complete season (Figure 6f) we evidenced that 537 green and 525 green sticky traps caught similar numbers of psyllids ($F_{4,499} = 145.87$; $p = 0.5061$) and both caught significantly more psyllids than 543 green, 673 red and clear (control) ($p = 0.003$; $p < 0.0001$ and, $p < 0.0001$ respectively). In comparison, 543 green caught more psyllids than 673 red and clear traps ($p < 0.0001$). In total, 673 red and clear traps caught less psyllids than all the other colours tested (Figure 6f). Seasonal distribution of psyllids in the tested area were also observed. Psyllids captures were affected by the week of evaluation, showing variations in psyllids population in the field over the period of experiment from 5 May 2020 to 16 September 2020 ($F_{15,499} = 20.21$; $p < 0.001$, Figure 7). Season, has also influenced psyllids population, and trap captures ($F_{4,499} = 213.43$; $p < 0.0001$, Figure 7). Interactions between season and trap colour were also evaluated ($F_{4,499} = 2.7166$; $p < 0.00024$). In mid-spring (week 1 to 4), all green traps captured similar numbers of psyllids per day. However, statistically significant differences were observed when green traps 525 and 537 were compared to 673 red and clear traps ($p < 0.02$ and $p < 0.01$, respectively). In late-spring (week 5 to 8) 525 green and 537 green traps captures were higher than 543 green traps, red and transparent ($p < 0.0001$).

Bycatches of beneficial insects such as pollinators (Apidae, adult Syrphidae) and insect predators (Chrysopidae, Coccinellidae, Forficulidae) were also observed. The statistical model revealed differences between bycatches and trap colour ($F_{4,2499} = 112.926$, $p < 0.0001$) and insect's family ($F_{4,2499} = 208.479$, $p < 0.0001$, Figure 8). No Apidae captures were recorded on 673 red and 543 green traps. Captures of Apidae in clear traps were similar to all other traps. Clear traps captured 10 times more Syrphidae than all other colours ($p < 0.0001$), and there was no difference in captures between the different coloured traps. In total, 673 red and
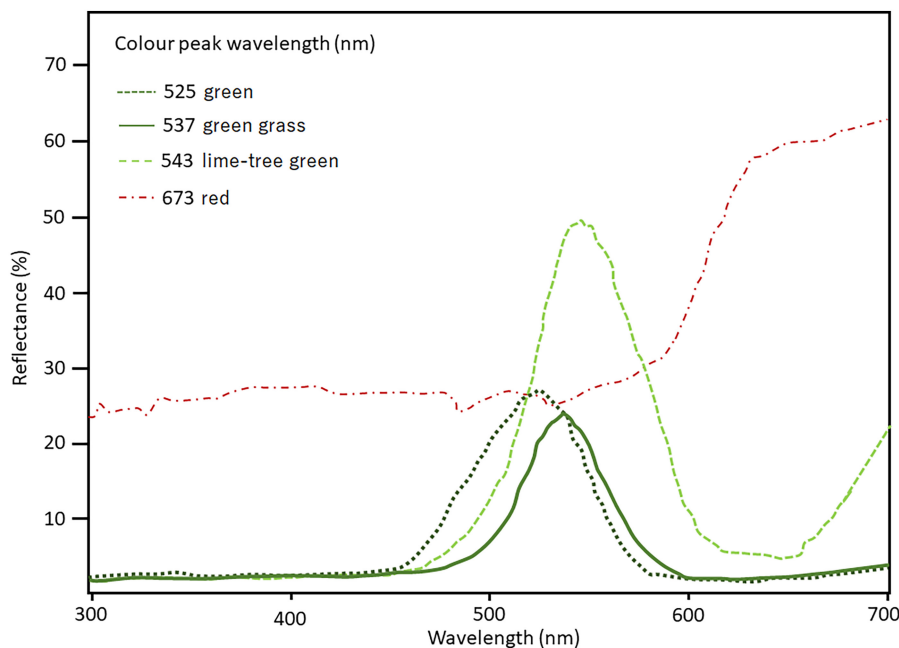

FIGURE 5 Spectral reflectance curves of coloured films. See Table 2 for colour specifications. [Colour figure can be viewed at wileyonlinelibrary.com]
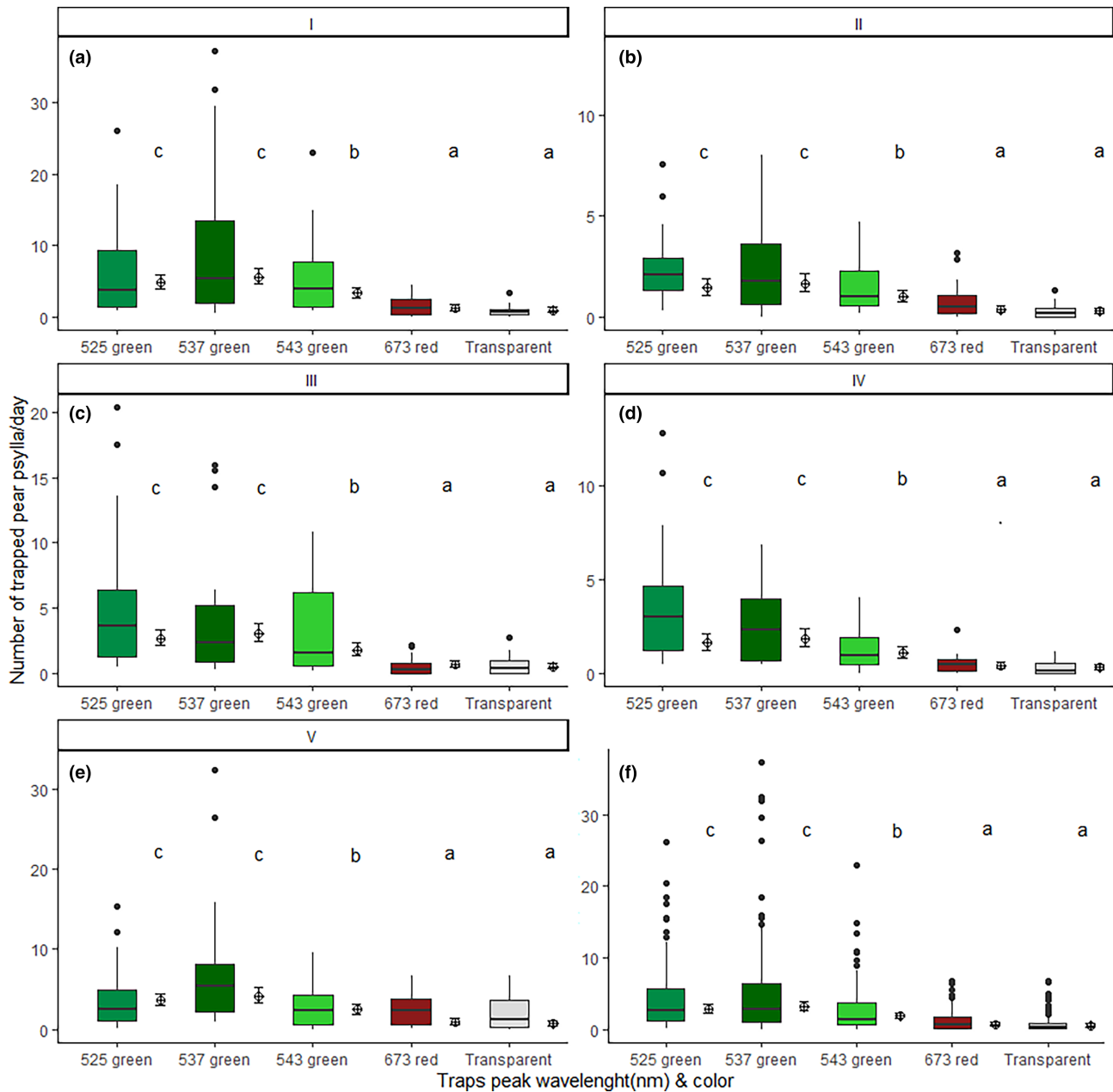
FIGURE 6 Wavelength preferences of *Cacopsylla pyri* in field experiment with coloured traps. Number of individuals captured per trap per day (*n*=25) over 20weeks from May 2020 to September 2020. (a–e) within each of the five blocks (I, II, II, IV, V); (f) overall captures in each colour. See Table 2 and Figure 5 for colour specifications. Significant differences are indicated by different letters (generalized linear model, multiple pairwise comparisons, Tukey test, *p*<0.05). Boxes correspond to the 25th and 75th percentiles, medians appear as lines and whiskers extend to 1.5 times of the interquartile ranges. Dots represent outliers. Dots with crosses represent the means. [Colour figure can be viewed at wileyonlinelibrary.com]

clear traps captures of Coccinellidae were similar and smaller than on green hues, 543 green, 537 green and 525 green sticky traps (*p*<0.0001; *p*=0.0329 and, *p*=0.0024, respectively) and there was no difference between captures on green hues. For Chrysopidae captures, the model revealed no statistical significance between traps, as they were extremely low in all traps over the whole season. Forficulidae were only caught in 537 green and 673 red traps, and there were no significant differences between them. In general, the total number of bycatches of beneficial insects in the green traps was very low compared to the number of psyllids

(*p*<0.0001, Figure 8; Table S1). However, clear traps captured as many psyllids as Syrphidae.

4 | DISCUSSION

Colour vision in some insect species has been extensively studied, especially for pollinators (Chittka & Raine, 2006; Lunau & Maier, 1995), for which colour is a cue for a specific flower's location, and *Drosophila melanogaster* (Meigen), where the
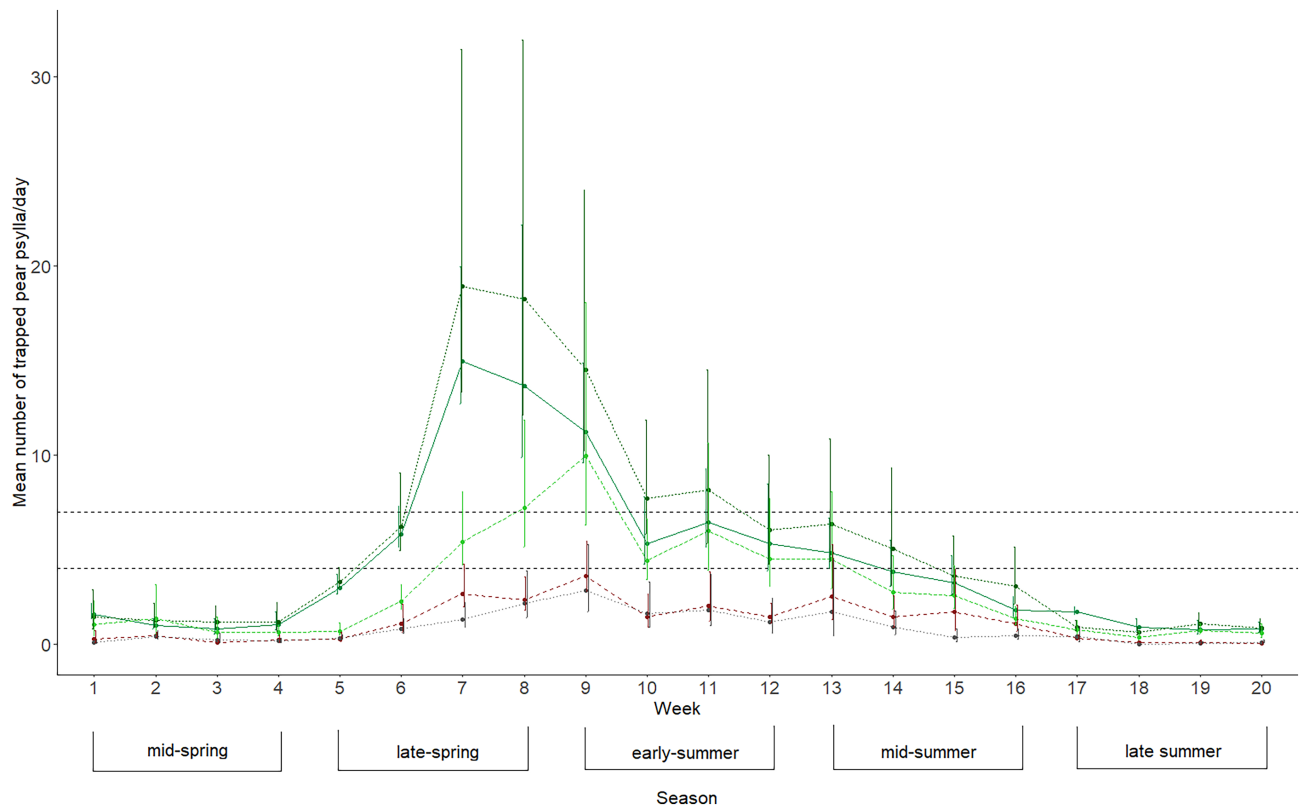
FIGURE 7 Implications for chemical plant protection. Mean±SE of *Cacopsylla pyri* adults collected per day on coloured traps (*n*=25) from May 2020 to September 2020. See Table 2 and Figure 5 for colour specifications. Dotted grey lines indicate the threshold of chemical control of 4–7 adults/trap/day (Adams & Los, 1989). [Colour figure can be viewed at wileyonlinelibrary.com]

well-characterized genome has enabled exploration of the genetic and developmental basis of colour vision (Morante & Desplan, 2008). However, for most insects, particularly those of economic or agricultural significance, colour vision studies are limited to outcome-driven experiments focusing only on relative responses to (often unquantified) colours without considering causative physiological or behavioural mechanisms, as has been highlighted by Döring and Chittka (2007). The design of experiments should consider existing knowledge of insect colour vision, as this can be used to refine control strategies (Lindh et al., 2012). Quantification of visual stimuli using a spectrophotometer provides spectral reflectance curves for coloured surfaces, facilitating the investigation of underlying mechanisms of colour-mediated behaviours (Doering et al., 2012).

In addition to 'true color vision', many animals, including insects, also show 'wavelength-specific behaviour'. These include photo-taxis towards a specific wavelength of light and wavelength-specific behaviour directed towards objects (Kelber & Osorio, 2010; Menzel, 1979). In contrast to 'true colour vision', such behaviour is highly dependent on intensity. The presented study is, to our knowledge, the first that comprehensively addresses *C. pyri* responses to colours under controlled conditions and using stimuli of known reflectance. It was demonstrated that adult pear psyllid *C. pyri* could distinguish between peak wavelengths and significantly preferred wavelengths in a specific and narrow range between 525 and 537 nm.

Detection of cues relies on various senses, that is, vision, olfaction, mechanoreception. Surface colours are known to affect the behaviour of psyllids, for example, yellow traps serving as attractants are used for monitoring of several psyllid species, including pear psyllids (Adams et al., 1983; Adams & Los, 1989; Horton, 1993; Krysan & Horton, 1991). Our results show that visual cues play an important part in pear psyllids movement and are consistent with other field studies, in which higher catches of psyllids were realized using green and yellow sticky traps (Al-Jabr & Cranshaw, 2007; Brennan & Weinbaum, 2001; Gross et al., 2019; Hall et al., 2010).

The peak reflectance of the LED most effective in capturing pear psyllids adults in the lab was 532 nm and in the field 537 nm wavelength. Similar results describe *C. pyricola* captures within the range of 520–600 nm wavelength, as hues and shades with a peak reflectance above 600 nm captured significantly fewer pear psyllid adults (Adams et al., 1983). In that publication, authors compared several fluorescent paints for monitoring summer generation pear psylla and found that hues having reflectance peaks that mimic pear foliage were most attractive. Green pear leaves (young and old) reflect most of their energy in the visible spectrum between 520 and 600 nm, with peak reflectance between 540 and 560 nm (Adams et al., 1983). Krysan and Horton (1991) showed also that the foliage-like colours were attractive to pear psylla during the time of year trees were fully foliated, but were not attractive in late winter when the foliage was absent, and the winter form of pear psylla was recolonizing the
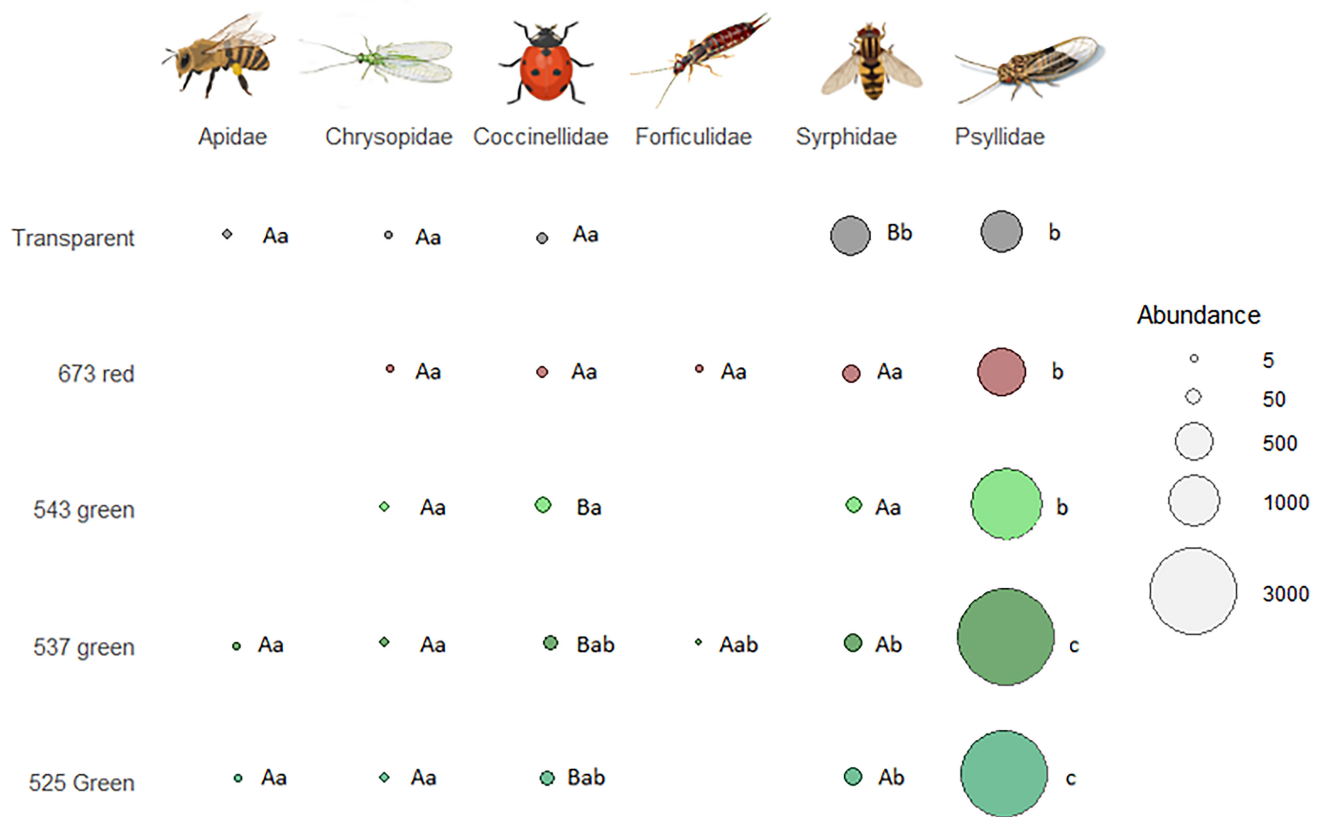
FIGURE 8 Bubble plot showing the absolute number of insects captured/family at each coloured trap over the whole season from May 2020 to September 2020 ($n = 25$). See Table 2 and Figure 5 for colour specifications. The size of the bubbles represents the number of insects captured. Scaling according to legend. Blank spots represent no catches. Different letters indicate significant differences in the mean of captures. Different uppercase letters represent the statistical differences between captures of different insect families in each trap colour and different lowercase letters represent the statistical difference between trap colour captures of each family (generalized linear model, multiple pairwise comparisons, Tukey test, $p < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

orchard. Interestingly, LEDs with longer wavelengths ranging from 576 to 633 nm captured statistically fewer psyllids than 532 green LED (Figure 4). That reveals an interesting aspect of pear psyllid's behaviour, as phytoplasma infected plants present red coloured leaves earlier in the season (late summer) than healthy pear trees (autumn). We conclude that symptomatic phytoplasma infected trees showing early reddening of leaves are less attractive for healthy pear psyllids during this period.

Horton (1994) sampled pear shoots to estimate absolute densities (numbers per leaf) of adult pear psylla. He used these data in regression analyses to determine whether beating trays or sticky traps were equally effective in predicting absolute densities of the summer form pear psylla; results indicate that beating tray samples were more effective at predicting absolute density than sticky traps. However, Adams et al. (1983) showed that psyllids adult counts were significantly higher on coloured sticky traps than counts obtained by beating tray. According to the author, the beating trays sampling method can only sample adult populations at one point of time and can be significantly affected by external factors, such as weather conditions (particularly wind, rain and dew) and time of day. In contrast, coloured sticky traps sample continuously for several days and therefore are less severely affected by conditions at the moment of sampling.

Our results suggest considerable potential for developing effective visual traps for monitoring adults of pear psyllids over the whole vegetation period (Figure 7). Still, experiments should be conducted in the future to compare the efficiency of green sticky traps in relation to other sampling methods to predict pear psyllid population dynamics. Additionally, our field observations indicate significant differences in psyllid catches among experimental blocks, which means psyllids are not evenly distributed in the orchard, revealing patterns of insect behaviour associated with host plant location and selection that remains unclear. Dispersion and distribution of pear psyllids in orchards are examples of aspects that should be further investigated with optimized 537 nm green traps. Only five traps in the experimental area (ca. ¼ ha) were able to characterize the population fluctuations and distribution patterns of psyllids over the season (Figure 7).

Vision in other psyllids as *D. citri* is mediated by photoreceptors sensitive to ultraviolet (UV), blue and green/yellow portions of the visual spectrum (Allan, 2015). Generally, Hemiptera are not attracted to visual targets that reflect or emit light <400 nm or >600 nm (Döring, 2014; Döring & Chittka, 2007). However, attraction of *D. citri* adults to transparent visual targets comprised of green or yellow filters can be enhanced with addition of UV light (<400 nm) (Paris et al., 2017).

Colour is an essential factor influencing flower-foraging pollinators and other beneficial insects (Frisch, 1967). Variable degrees of contamination of monitoring traps by honeybees and bumblebees *Bombus* spp., due to differences in trap colour, have been reported (Gross & Carpenter, 1991; Meagher, 2001). In general, white and yellow traps are attractive, and green traps are unattractive to Apoidea species due to their differences in spectral reflectance between 380 and 550 nm (Mitchell et al., 1989). Besides, the combination of UV and yellow was demonstrated to act as an attractant for bees and it was termed 'bee purple' by Daumer (1958) and was also demonstrated as important in the attraction of *Heliconius erato* scales (Gandía-Herrero et al., 2005).

Yellow was especially attractive to parasitic Hymenoptera (Thomson et al., 2004). Mondor (1995) reported that sticky red spheres in an apple orchard in Bremden (Manitoba, Canada) caught high numbers of hoverflies (Syrphidae) and Hymenopteran species as bycatch of apple maggot fly, *Rhagoletis pomonella* (Walsh) (Dolphin et al., 1970). In our field trials, massive Syrphidae catches were observed on transparent traps (Figure 8). Despite sticky traps for capturing harmful pest insect species, catches of non-targeted insects, such as pollinators and predators, have been mostly ignored. Mass trapping of beneficial or other non-targeted insects by sticky traps may have adverse effects, such as reducing the numbers of pollinators or increasing the pest numbers by reducing the numbers of their natural enemies (Mondor, 1995). According to our results, green traps would be an alternative to mitigate the captures of non-target species since the overall captures of beneficial insects were significantly lower than captures with other red and transparent (Figure 8). The 637 red trap was compared in a former study to commercially available yellow sticky traps in field experiments in Germany and Austria (Gross et al., 2019). Numbers of beneficial insects were significantly reduced in red traps compared to yellow traps, especially pollinators such as bees and hoverflies. As in our experiment, the 637 red trap caught similar numbers of hoverflies as the green traps, we conclude that the number of beneficial insects is lower in green traps compared to yellow ones. Aside from Coccinellidae, which prefer green trap over transparent and red ones, there were no statistical differences over the other beneficial insects captures on all tested traps (Figure 8). The presented results confirm that the peak wavelength of 537 green is very selective for pear psyllids. That rigour in specialization is desirable when developing monitoring tools, helping farmers rapidly to identify the pest in the orchard while avoiding negative side effects on beneficial insects.

The interaction of different cues (colour, leaf shape, smell, taste) in host selection by pear psylla has not been thoroughly studied. Some Psyllidae use olfaction to locate their host plants (Gross et al., 2021; Gross & Franco, 2022) or avoid non-hosts (Nehlin et al., 1994). Several studies reported the importance of olfactory and gustatory cues in combination with visual cues in the host selection behaviour of psyllid (George et al., 2016; Lapointe et al., 2016; Wenninger et al., 2009; Zanardi et al., 2018). Host odour volatiles were less attractive to psyllids in the absence of visual cues (Mann et al., 2011; Wenninger et al., 2009). Sétamou et al. (2012), reported

that *D. citri* did not differentiate between host and non-host plants under dark conditions and showed a stronger response to illuminated vs. darkened host plants. For identifying particular host plants for feeding and reproduction, volatile signals are used in many psyllid species during migration (Mayer et al., 2008a, 2008b; Soroker et al., 2004; Weintraub & Gross, 2013). Also, the taste of non-volatile phloem/xylem components influences host choice and oviposition behaviour of the summer apple psyllid *C. picta* (Adams et al., 1983; Görg et al., 2021; Mayer et al., 2011). However, there is still little information on the effect of constitutive or induced plant volatiles on the pear psylla behaviour. Still, the long-distance orientation to the host plant is not fully understood. Scutareanu et al. (2003) indicated that the volatile profile of the wild pear is like that of the commercial cultivars. During the coevolution of pear psylla and its host, the insect was exposed to cues like those emitted by commercial varieties of pear, and that strengthens the hypothesis that the psylla specialized in pear are adapted to detect these host characteristics. The current findings also complement lab studies showing that olfactory responses to host plant volatiles are enhanced when experienced in conjunction with green or yellow stimuli (Patt et al., 2011; Wenninger et al., 2009). Behaviour modifying compounds could further be combined with specific semiochemicals such as β-caryophyllene, which is known to attract *C. picta* emigrants (Mayer et al., 2008a, 2008b), for developing innovative approaches such as specific lures (Eben & Gross, 2013; J. Gross et al., 2019) or attract-and-kill strategies minimizing vector populations in the future (Gross, 2013; Gross & Gündermann, 2016).

5 | CONCLUSION

Accurately monitoring the pest population is one of the most critical aspects of IPM to reduce crop losses caused by pear psylla feeding or transmission of pear decline disease in pear orchards. Our findings reveal that green traps having peak wavelengths between 525 and 537 nm can efficiently capture pear psyllids. The 537 green trap developed and investigated in this study is very specific to capture pear psyllids and will be commercially available shortly on the market. Using this alternative trap can represent a significant step towards a more sustainable pear production by reducing also the bycatches of pollinators and other beneficial insects and by providing a tool for precise decision-making of the necessity and number of pesticide application in IPM.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw sequence read files are deposited in the https://doi.org/10.5281/zenodo.8389442.

ORCID

Bruna Czarnobai De Jorge https://orcid.org/0000-0003-3033-4282

Rainer Meyhöfer https://orcid.org/0000-0001-5624-001X

Andreas Jürgens https://orcid.org/0000-0002-5288-0498

Jürgen Gross https://orcid.org/0000-0003-2614-9658

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.