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RESEARCH

‘Flying agents’: hoverflies as a multitool for pollination, vectoring of beneficial microbes and biological control of grey mould disease in strawberries

Guillermo Rehermann[1] · Christine Petig[1,2] · Samar Khalil[3] · Johan A. Stenberg[1] · Rainer Meyhöfer[2] · Paul G. Becher[1]

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Abstract

Sustainable strategies are needed to manage plant pathogens and pests without disrupting ecological functions provided by beneficial organisms. Hoverflies (Diptera: Syrphidae), such as *Eupeodes corollae*, provide ecosystem services and are applied especially in cultivations of horticultural crops: adults serve as pollinators, while larvae prey on pests like aphids. Here, we investigated whether *E. corollae* can also function as an entomovector for delivering microbial biocontrol agents into flowering crops, similar to systems developed for bees. Targeting the strawberry (*Fragaria*×*ananassa*)–grey mould (*Botrytis cinerea*) pathosystem, we tested the yeast *Metschnikowia fructicola* (isolate UDA10) for its suitability in hoverfly entomovectoring and suppression of grey mould. Dual culture assays confirmed that *M. fructicola* inhibits *B. cinerea* growth. We further demonstrated that *E. corollae* effectively vectors *M. fructicola* to strawberry flowers. In a greenhouse experiment, we tested whether hoverflies and yeast, alone or combined, can suppress grey mould in postharvest strawberries from flowers artificially inoculated with *B. cinerea*. Hoverfly activity significantly reduced the fungal infection (lesion and mycelial coverage) on cold stored fruit by 50–70% after two weeks, especially in combination with the yeast. Additionally, fruits from hoverfly-pollinated flowers were of higher shape quality, indicating improved pollination. Our findings add value to *E. corollae* as a multifunctional ‘*flying agent*’ for integrated pest and pollination management, capable of enhancing pollination, entomovectoring for targeted plant pathogen suppression and controlling pests via larval predation. The ‘*flying agent*’ multitool can potentially be extended to other horticultural systems, contributing to both quality and yield improvements, while reducing reliance on chemical inputs for pest and disease control.

Keywords Entomovectoring technology · Flying doctors · Postharvest diseases · Plant protection · Precision agriculture · Integrated pest and pollination management (IPPM)

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✉ Guillermo Rehermann
guillermo.rehermann@slu.se

Christine Petig
christine@petig.eu

Samar Khalil
sammar.khalil@slu.se

Johan A. Stenberg
johan.stenberg@slu.se

Rainer Meyhöfer
meyhoefer@ipp.uni-hannover.de

Paul G. Becher
paul.becher@slu.se

[1] Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden

[2] Institute for Horticulture and Production Systems, Gottfried Wilhelm Leibniz University Hannover, Hannover, Germany

[3] Department of Biosystem and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden

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Key message

- Entomovectoring technology uses insects as vectors to deliver control agents for plant protection.
- This is the first study to assess hoverflies for entomovectoring-based biocontrol.
- *Eupeodes corollae* on strawberry flowers acts as both pollinator and vector of a beneficial yeast.
- Hoverfly-yeast entomovectoring greatly reduced fungal infection and improved fruit shape quality.
- We demonstrate a novel hoverfly integration into crop production, enhancing fruit marketability.

Introduction

A global decline of pollinating insects and an increase of pesticide resistance in pests and plant pathogens are major factors impairing ecosystem resilience and food security (Potts et al. 2010; Bras et al. 2022; Yin et al. 2023). To mitigate this threat, modern crop production systems increasingly integrate plant protection with pollination management (i.e. integrated pest and pollinator management, IPPM) (Biddinger and Rajotte 2015; Egan et al. 2020; Lundin et al. 2021).

The grey mould *Botrytis cinerea* (Ascomycota: Sclerotiniaceae) is a major destructive fungal pathogen in many agricultural crops including fruits, vegetables, ornamentals, legumes and postharvest commodities (Jarvis 1962; Williamson et al. 2007; Elad et al. 2016). In crops like strawberries, raspberries, grapes, tomatoes or peppers, flower infection is a primary pathway for *B. cinerea*, leading to latent infections during early development and fruit or tissue decay during ripening, harvest or postharvest (Bristow et al. 1986; Simpson 1991; Mertely et al. 2002).

Grey mould disease causes significant losses of both flowers and fruits if not managed (Ries 1995). Sprays of fungicides on open flowers are the most common practice to prevent or control floral infection (Mertely et al. 2002; Petrasch et al. 2019). This has negative effects in multiple ways. For instance, *B. cinerea* is classified as a high-risk pathogen for resistance development to many common fungicides (Myresiotis et al. 2007; Weber and Hahn 2019). In addition, fungicides may have detrimental effects on non-target organisms, including pollinators and natural enemies (Mertely et al. 2002; Rondeau and Raine 2022). Moreover, fungicides can negatively affect plant metabolism and symbiotic microbes (Wei et al. 2021; Voß et al. 2023). Therefore, over the past three decades, considerable research has focussed on developing sustainable and effective alternatives to reduce reliance on chemical inputs for controlling *B. cinerea* infections at both pre- and postharvest stages (e.g. Peng et al. 1992; Romanazzi and Droby 2016; Wisniewski et al. 2016; Ullah et al. 2024 and refrences therein).

Biological control is an important strategy in organic and integrated plant management to replace or reduce chemical inputs (Stenberg et al. 2021). Microbial antagonists against *B. cinerea* are recognized as a promising and effective alternative to chemical fungicides (Iqbal et al. 2023; Vero et al. 2023). Commercial microbial bioproducts range in their ingredients from fungi and fungus-like eukaryotes such as oomycetes, to bacteria (Abbey et al. 2019). Yeasts, in particular, possess multiple modes of action to antagonize *B. cinerea* such as competition for space and nutrients, production of lytic enzymes and volatile organic compounds, as well as induction of plant resistance (Spadaro and Droby 2016; Kowalska et al. 2022; Oztekin et al. 2023).

The genus *Metschnikowia* (Ascomycota: Saccharomycetes) contains yeast species that are mostly associated with angiosperms, and occur naturally on fruit surfaces (Guzmán et al. 2013). Different strains of *Metschnikowia fructicola* were found effective against *B. cinerea* (Karabulut et al. 2004; Zhimo et al. 2021). Strain NRRL Y-27328 is commercialized to target *Penicillium, Rhizopus* and *Aspergillus* pathogens, in addition to *Botrytis* (Wisniewski et al. 2016), and many wild strains probably have similar antifungal properties (Haniffadli et al. 2024).

The application of biological control agents against *B. cinerea* can be challenging because *B. cinerea* infects open flowers, and flowering occurs sequentially over an extended period. Therefore, repeated applications are required throughout the flowering period, leading to increased labour and operational costs. An alternative to conventional spraying is the targeted dissemination of control agents to flowers by pollinators. In particular, bees and bumblebees have been successfully used as vectors to deliver microbiological control agents directly to flowers, a technology known as entomovectoring or the 'flying doctors' approach (Peng et al. 1992; Hokkanen et al. 2015; Pozo et al. 2020; Smagghe et al. 2020).

Strawberry (*Fragaria x ananassa*) is a crop of high economic value globally, with both wild and managed pollinators playing a fundamental role in enhancing yield and fruit quality (Klatt et al. 2014; Wietzke et al. 2018; Gudowska et al. 2024). While *B. cinerea* remains one of the most significant threats to strawberry production (Ries 1995; Petrasch et al. 2019), the pathogen can be managed using entomovectoring-based biocontrol technology (e.g.Hokkanen et al. 2015; Iqbal et al. 2022), which so far has been limited to the use of bees and bumblebees.

Non-bee pollinators, particularly hoverflies (Diptera: Syrphidae), have been recently accounted as valuable

alternative pollinators that significantly enhance strawberry yield (Hodgkiss et al. 2018; Rader et al. 2020; James et al. 2024). Moreover, some hoverfly species provide dual ecosystem services, as adults contribute to pollination, while larvae prey on key pests such as aphids and thrips (Rodríguez-Gasol et al. 2020). This dual role supports their integration into sustainable crop production systems (Dunn et al. 2020; Van Oystaeyen et al. 2022). *Eupeodes corollae* is one of the most abundant hoverfly species in European, North African and Asian agroecosystems (Speight 2011), and it has recently been commercialized for pollination and biological pest control (Eupeodes system, Biobest Group NV, Westerlo, Belgium).

To date, hoverflies have not been developed as vectors for entomovectoring-based biocontrol. The objective of this study was to investigate the potential of the hoverfly *E. corollae* to improve strawberry fruit quality by pollination and entomovectoring of a grey mould-suppressing biocontrol agent. We speculated that *M. fructicola* strain UDA 10 could efficiently suppress *B. cinerea* growth. Moreover, we hypothesized that *E. corollae* could vector *M. fructicola* to strawberry flowers, leading to a significant reduction in fungal incidence on fruit compared to control treatments. Furthermore, we expected that strawberry flowers exposed to *E. corollae* would develop into berries of higher shape quality compared to control. We therefore assayed *M. fructicola* UDA 10 for its ability to inhibit *B. cinerea* and examined whether *E. corollae* could carry and deliver the yeast to strawberry flowers and suppress grey mould in fruit after harvest. Moreover, we measured the effect of *E. corollae* on fruit shape quality.

Materials and methods

Hoverflies

Eupeodes corollae were provided as pupae by Biobest Group NV (Eupeodes system, Westerlo, Belgium). Adults that emerged from the pupae were maintained at the Swedish University of Agricultural Sciences (SLU), Alnarp. Females and males were kept together from emergence in insect cages (W30 × D30 × H30 cm, BugDorm—1, MegaView Science Co., Ltd., Taichung, Taiwan) on an ad libitum supply of water and 5% sugar solution and multifloral pollen ('Natupol' Koppert, Starlen, Germany) at 22–24 °C and 50–65% R.H., under a 16:8 h L:D (light:dark) photoperiod. Flies were starved for 24 h before the start of the experiments and were provided only with water-soaked cotton balls. Four-day-old flies were used in all experiments.

Yeast and pathogenic fungi formulation

A strain of the yeast (UDA 10) was isolated from undamaged apples at Alnarp (SLU) and identified as *Metschnikowia fructicola* (unpublished). A stock culture of UDA 10 (subsequently referred to as *M. fructicola*) preserved in 20% (wt/vol) glycerol at − 80 °C was used for generating fresh cultures. Cultures of *M. fructicola* were grown on PDA (Difco, Potato Dextrose Agar: 39 g L^{-1}) plates. For liquid cultures, PDB (Difco, Potato Dextrose Broth: 24 g L^{-1}) was inoculated with single colonies and incubated at 25–30 °C for 24 h on an incubator shaker (Stuart Scientific). Liquid yeast culture for use in experiments was in an exponential growth phase ($OD_{595} = 4.0$; 10^{14} CFUs/mL).

Botrytis cinerea (B05.10) was isolated from strawberry plants at Alnarp (Khalil et al. 2024), and cultivated on V8-agar medium (70 mL V8-juice, 10.5 g agar, 1.05 g $CaCO_3$, filled to a volume of 700 mL with distilled water) under dark conditions. Fresh mycelium was obtained by transferring an agar disc (ø = 1 cm) with dense *B. cinerea* culture into on a new V8-media plate. The conidia produced by the fungus were harvested by adding 5 mL of sterile distilled water to the fungal culture, followed by scraping the surface of the mycelium with a spreader. The conidial suspension concentration was determined using a Fuchs-Rosenthal haemocytometer (Glaswarenfabrik Karl Hecht GmbH & Co KG, Sondheim vor der Rhön, Germany) under a Laborlux 12 light microscope (Leitz, Wetzlar, Germany). A conidial concentration of 10^5 conidia/mL was maintained for use in the experiments.

Plant material

Strawberry plantlets (*Fragaria* × *ananassa*, cultivar 'Sonata') obtained as A + Frigo-plants from Kraege Beerenpflanzen GmbH&Co.KG (Telgte, Germany) were potted in 1.5 L pots with peat and soil substrate (50:50, Gröna linjen). Fertilization with SUPERBA™ (8.2% N + 11.5% P + 36.1% K + 2,8% MgO + TE) and CALCINIT (15.5% N + 26.3% CaO) took place twice a week since flowering started.

Biocontrol activity assay of *M. fructicola* against *B. cinerea*

The in vitro inhibitory ability of *M. fructicola* against *B. cinerea* was determined by performing a dual-culture confrontation assay. A 9 cm PDA Petri plate was inoculated in the centre with a 5 mm diameter agar disc of a four-day-grown *B. cinerea* culture. On two opposite sides of the plate, 10 µL of *M. fructicola* suspension was streaked out at 3 cm distance to the agar disc in 2 cm long stripes (for details see supp. Figure S1). Petri plates were sealed with Parafilm and stored at 22–23 °C (room temperature). A control treatment

in which *B. cinerea* remained unconfronted by *M. fructicola* was performed concomitantly. After seven days of incubation, the growth of *B. cinerea* was quantified by measuring the radii of the mycelium growth from the centre of the plate. Two measurements were taken perpendicular to each yeast stripe (*R*1'; *R*1''), and the two parallel to the yeast stripes (*R*2'; *R*2''). The values for *R*1 and *R*2 were calculated as the averages of *R*1' and *R*1'', and *R*2' and *R*2'', respectively. The inhibition rate of *B. cinerea* confronted by the antagonistic *M. fructicola* was calculated for *R*1 as $[(R1\text{control} - R1\text{antagonist})/R1\text{control}] * 100\%$ and for *R*2 accordingly as $[(R2\text{control} - R2\text{antagonist})/R2\text{control}] * 100\%$. The experiment was replicated ten times.

Vectoring of biological control agent *M. fructicola* by *E. corollae*

To determine the capability of *E. corollae* adults to transport and transfer *M. fructicola* from one surface to another, single 3–5-day-old flies were exposed to four-day-old cultures of *M. fructicola* grown on PDA plates. After 1 h the flies were removed and transferred into a sterile PDA plate and allowed to move around. The flies were removed after 1 h and the plates that were exposed to the flies were incubated at room temperature and CFUs counted after four days ($n = 5$). A control treatment in which flies were exposed to sterile PDA plates instead of *M. fructicola* was conducted ($n = 3$). To determine whether yeast cells were still attached to the flies, each fly was in addition washed three times in series by bathing their bodies in 1 mL PDB in an Eppendorf Tube® for 30 s. Washed-off cells were concentrated by centrifuging the tube for 1 min at 10.000 rpm and removing the upper 900 µL. The remaining 100 µL was spread evenly on a 5 cm PDA plate (for details, see supp. Figure S2). Plates were stored at room temperature for four days, and yeast CFUs per plate were counted for all three washing steps.

Vectoring of the biological control agent *M. fructicola* to strawberry flowers by *E. corollae*

To investigate whether *E. corollae* transfers *M. fructicola* when visiting strawberry flowers, a polytunnel experiment was performed. Opening flowers were marked with a black pen on the stem and plants were completely covered with a 6 L net bag (Veggiebags, Veggio), including the surface of the potted substrate. The experiment consisted of four treatments ($n = 10$) that were applied to separate plants inside the bags: 1.) release of *E. corollae* previously exposed to *M. fructicola* ('Ec + Mf'); 2.) spray application of *M. fructicola* ('Mf') onto the flowers; 3.) a spray control with the yeast growth medium PDB ('PDB') onto the flowers and 4.) a spray control with H_2O ('H_2O') onto the flowers. For preparation of the 'Ec + Mf' treatment, a four-day-old male and a female were placed for 1 h in a 9 cm PDA Petri plate containing a four-day-old *M. fructicola* culture. To visually increase the attraction of the flies to the yeast, the cultures were provided with a yellow background by attaching yellow cardboard on the outside of the Petri plate bottom, visible through the partly transparent PDA (Sutherland et al. 1999; An et al. 2018). After release of the flies into the bags, the open *M. fructicola* culture plates were placed onto the plant substrate to allow flies to revisit the yeast culture. Flies were able to move inside the bags either by walking or flying. Three cotton balls soaked with water were added to provide water for the flies. For the sprayed 'Mf' treatment, 50 mL yeast liquid culture grown in PDB was mixed with 20 µL Tween®. Similarly, the control treatments formulations consisted of 50 mL of PDB medium or water mixed with 20 µL Tween® 20. For each of the three liquid formulations, 0.1 mL was applied to individual flowers using a 30 mL spray bottle (Ion Silver, Löddeköpinge, Sweden). After 48 h, flies were removed from the 'Ec + Mf' treatment, and the flowers (1–2 per plant) from all the treatments were cut with sterile scissors and kept individually in Falcon tubes at 4 °C for ca. 2 h prior to laboratory analysis. Each flower was washed two times in series in 2.5 cm Petri plates with 2 mL PDB. From each washing, an aliquot of 0.1 mL of the suspension was pipetted onto a 9 cm PDA Petri plate and spread evenly. Plates were incubated at room temperature for three days, and yeast CFUs were quantified per treatment.

Fruit quality: pollination and biological control of *B. cinerea* in strawberries

To assess the effectiveness of *E. corollae* and fly-vectored *M. fructicola* on strawberry pollination and grey mould control, an experiment was performed at a greenhouse biotron (3250 × 4390 × 2500 width × depth × height mm; Biotronen, SLU Alnarp), under controlled conditions (21 °C, 60% R.H., 360 ppm CO_2, 16:8 h (L:D) photoperiod). Six treatments were included in the experiment of which three served as controls: 1.) untreated control flowers; 2) a spray control application of H_2O; 3) *B. cinerea* spray application (positive flower infection control); 4) *E. corollae* and subsequent *B. cinerea* spray application; 5) *E. corollae* exposed to *M. fructicola* and *B. cinerea* spray application; and 6.) spray application of *M. fructicola* and *B. cinerea* (Table 1). Each treatment comprised 10–14 plants. Three to four freshly open flowers from each plant were marked with a colour pen on the stem, all remaining flowers were removed, and plants were covered with a mesh bag during the experiment as described before. For preparation of the treatments (4) and (5), a male and a female fly were enclosed for 1 h either in a sterile 9 cm Petri plate (treatment 4) or a PDA Petri plate containing a four-day-old *M. fructicola* culture (treatment 5), respectively. Individual couples were subsequently released

Table 1 Strawberry (*Fragaria* × *ananassa*, cultivar 'Sonata') flower treatments and comparison of strawberry fruit weight and shape at harvest

Flower treatment	Fruit (*n*)	Weight at harvest (g) Mean (SEM)	Shape quality at harvest Mean (SEM)
1. F	51	6.60 (0.56)	3.02 (0.15) c
2. F + H_2O	43	6.89 (0.52)	3.09 (0.13) c
3. F + Bc	51	6.60 (0.58)	3.31 (0.12) bc
4. F + Ec + Bc	59	7.28 (0.54)	3.61 (0.09) ab
5. F + (Ec/Mf) + Bc	58	6.38 (0.42)	3.71 (0.07) a
6. F + Mf + Bc	50	5.58 (0.46)	3.06 (0.14) c
		MLM, Res. df = 5	KW, Res. df = 5
		$X^2 = 6.226$, $p = 0.285$	$X^2 = 31.254$, $p < 0.001$***

Weight data was analysed using a mixed linear regression model (MLM). Shape quality was graded from 1 to 4, where 1 = un-marketable fruit both small and irregular, 2 = good marketable berry size but irregular shape, 3 = good marketable size of suboptimal shape and 4 = excellent marketable size and shape. These data were analysed by a Kruskal–Wallis rank test (KW-test). Column means followed by a different letter indicate significant differences in fruit shape between treatments after pairwise comparison ($p < 0.05$)

F: untreated control flowers; F + H_2O: a control sprayed with H_2O; F + Bc: a positive control sprayed with *Botrytis cinerea* for fungal infection; F + Ec + Bc: *Eupeodes corollae* alone and subsequent *B. cinerea* infection; F + (Ec/Mf) + Bc: *E. corollae* exposed to *Metschnikowia fructicola* and *B. cinerea* infection; F + Mf + Bc: spray application with *M. fructicola* and *B. cinerea* infection

on plants inside a net bag. Two water-soaked cotton balls were added to prevent desiccation of the flies. After 20 h the flies were removed from the plants, and to assess survival after treatment, maintained under the same conditions as pupae and newly emerged flies. To enhance the treatment effect, new fly couples were released inside the bags and removed 4 h later. Thus, flowers of treatment (4) and (5) were in total 24 h exposed to hoverflies, without or with *M. fructicola*, respectively. For the *M. fructicola* spray treatment (6), 0.1 mL yeast liquid formulation was applied onto individual flowers similar as described previously. To induce grey mould infection, 24 h after the experimental start, *B. cinerea* was sprayed onto the marked flowers (0.1 mL; 10^5 conidia mL^{-1}) of the treatments (3), (4), (5) and (6). For treatment (2), flowers were instead sprayed with 0.1 mL of water, and for treatment (1), flowers remained untreated during the experiment. The strawberry plants were kept in the greenhouse until fruits of the treated flowers had fully developed. Fruits were harvested at full ripeness characterized by a uniform red colour (Supp. Figure S3).

To assess the impact of the abovementioned treatments (1–6) on fruit quality traits, individual fruits were weighed, and graded based on marketable fruit size and shape. The grading ranged from 1 to 4, where 1 = un-marketable fruit both small and irregular, 2 = good marketable berry size but irregular shape, 3 = good marketable size of suboptimal shape and 4 = excellent marketable size and shape, respectively (adapted from Cheng et al. 2016; Yuan et al. 2023) (for details, see Table 1 and Fig. 1A).

Subsequently, to determine the effect of the treatments on grey mould development, each fruit was individually placed in a plastic sales box and kept in a cold room at 4–8 °C, simulating storage conditions of fresh fruit markets. Strawberry shelf life and fungal growth were measured after 1 days, 7 days, 14 days and 21 days of cold storage by dual inspection consisting of direct visual assessment and image analysis of digital photographs: areas of the disease lesions were assessed using a scale from 0 to 4; where 0 = healthy fruit (no disease symptoms), and 1 = ≤ 25% of the fruit surface affected, 2 = 25.1–50%, 3 = 50.1–75% and 4 = 75.1–100% of the total fruit area rotted (following Huang et al. 2011). External mycelium growth was scored on a scale from 0 to 5; where 0 = absence of mycelium; 1 = punctiform growth of mycelium, 2 = mycelium growth on less than ¼ of the fruit, 3 = mycelium growth on less than 1/2 of the fruit, 4 = mycelium growth on more than 1/2 of the fruit and 5 = mycelium growth all over (adapted from Adikaram et al. 2002; Iqbal et al. 2022) (for details see Supp. Figure S4).

Statistical analyses

Data analyses were performed using R statistical software (R Core Team 2024; RStudio Team 2024). Linear and generalized linear mixed models (MLM and GLMM, respectively), were performed using the R package 'lme4' (Bates et al. 2015). Model residuals were analysed by diagnostic plots with R package 'ggResidpanel' (Goode and Rey 2019), and figures were drawn using the package 'tidyverse' (Wickham et al. 2019).

In the dual-culture confrontation assay, *B. cinerea* growth was evaluated by a nonparametric Kruskal–Wallis rank sum tests. To determine the faculty of the flies to get yeast cells attached to the body a GLMM fitted with a negative binomial distribution was performed, where 'body washes' was used as explanatory variable and 'fly' as random effect (random intercepts). The capacity of the flies to vector the yeast

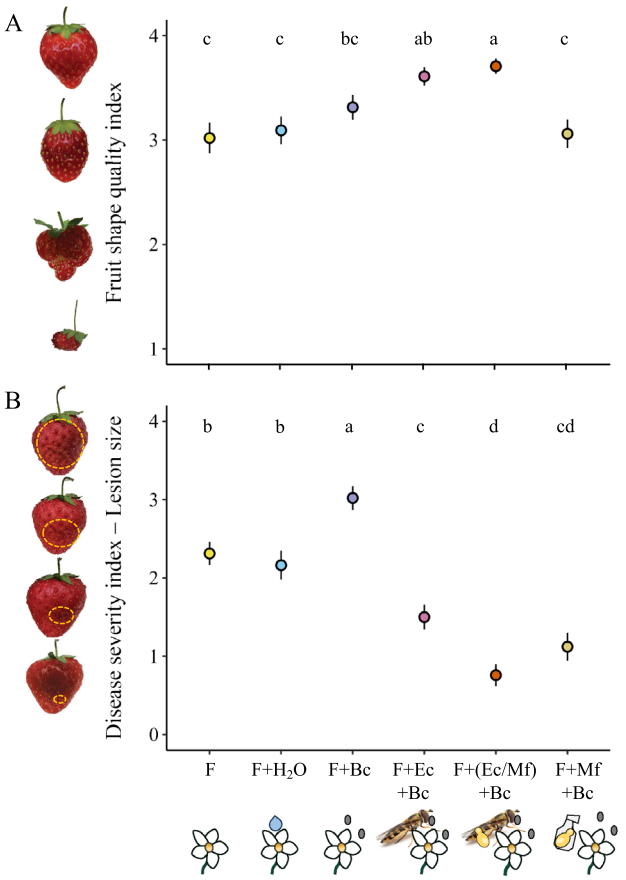

Fig. 1 Effects of pollination and biological control of fungal infection in the quality of strawberries by the hoverfly *Eupeodes corollae*-based system. Filled coloured points correspond to treatment groups (mean ± sem). **A** Fruit shape quality index at harvest. Fruit quality was graded from 1 to 4, where 1 = un-marketable fruit both small and irregular, 2 = good marketable berry size but irregular shape, 3 = good marketable size with suboptimal shape and 4 = excellent marketable size and shape. **B** Fungal disease severity index of the fruit stored in a cold room after 14 days of incubation. The areas of the disease lesions were assessed using a scale from 0 to 4; where 0 = healthy fruit (no disease symptoms), and 1 = ≤25% of the fruit surface affected, 2 = 25.1–50%, 3 = 50.1–75% and 4 = 75.1–100% of the total fruit area rotted. Strawberry (*Fragaria* × *ananassa*, cultivar 'Sonata') flowers were treated as following. F: untreated control flowers; F + H_2O: a spray control with H_2O; F + Bc: a spray positive control with *Botrytis cinerea* for fungal infection; F + Ec + Bc: *Eupeodes corollae* alone and *B. cinerea* infection; F + (Ec/Mf) + Bc: *E. corollae* exposed to *Metschnikowia fructicola* and *B. cinerea* infection; F + Mf + Bc: spray application with *M. fructicola* and *B. cinerea* infection. Different letters above plots indicate significant differences between treatments after pairwise comparison ($p < 0.05$). Hoverfly photo credits: A. Berg

onto strawberry flowers was analysed with a GLMM fitted with a negative binomial distribution where 'treatment' and 'flower developmental stage' were used as explanatory variable and 'plant' as random effect (random intercepts). *Eupeodes* survival analysis after treatment was performed using a Cox proportional hazard regression model (R package 'survival', Therneau 2024), using a survival object ('day of death and survival status') as the dependent variable, and 'treatment' as explanatory variable. To evaluate the strawberry weight of the respective treatment categories, a MLM fitted with a Gaussian error distribution was performed, where 'treatment' was used as explanatory variable and 'plant' was modelled as a random effect. In all models, a post hoc Tukey's contrast test was used for pairwise comparison between treatments (R package 'multcomp', Hothorn et al. 2008). Fruit shape quality at harvest, and mould severity for days 1, 7, 14 and 21 after harvest were evaluated by nonparametric Kruskal–Wallis rank sum tests, followed by a pairwise comparisons between treatments using the Wilcoxon rank sum (p-values were adjusted using the Benjamini–Hochberg method).

Results

Biocontrol activity of *Metschnikowia fructicola* against *Botrytis cinerea*

Metschnikowia fructicola antagonized the growth of *B. cinerea* in a dual-culture confrontation assay. The inhibition of *B. cinerea* mycelium growth was observed in both the radius perpendicular to the yeast stripe ($R1 = 54.65 \pm 1.12\%$ growth inhibition; $W = 16.351$, $p < 001$) and the radius parallel to yeast stripe ($R2 = 24.30 \pm 7.25\%$ growth inhibition, $W = 7.826$, $p = 0.005$) relative to those radii of *B. cinerea* culture in the absence of the yeast. The inhibition of *R*1 growth was systemically higher than the inhibition of *R*2 ($W = 12.757$, $p < 0.001$) (for details see Supp. Figure S1).

Vectoring of biological control agent *M. fructicola* by *E. corollae*

Eupeodes corollae flies that were exposed to a culture of *M. fructicola* for 1 h vectored the yeast to a sterile plate in the laboratory assay (CFU ± SE = 390.0 ± 140.0). Subsequent body washes of the flies showed that yeast cells were still attached to the flies, and the number of *M. fructicola* CFU maintained similar within the three washing steps ($p = 0.675$). In contrast, untreated flies exposed to a PDA sterile plate did not spread any yeast cell into a new sterile plate.

Vectoring of the biological control agent *M. fructicola* to strawberry flowers by *E. corollae*

Two stages of flower development were observed when flowers were harvested in all the treatments: 'fresh' and 'senescence'. Strawberry flowers were considered 'fresh' when

they had anthers filled with pollen and active reproductive organs, within 2–3 days after opening, or 'senescence' when they started wilting and declined in reproductive function, 4–5 days after opening. No significant effect was observed in the interaction between flower 'stage' and 'treatments' in the number of CFU counted ($p = 0.097$), neither for flower stage ($p = 0.077$). Therefore, for each treatment, fresh and senescence flowers were pooled before analyses.

The number of yeast-like CFUs counted three days after inoculation of PDA plates was significantly higher in flowers visited by *E. corollae* exposed to *M. fructicola* (CFU/0.1 mL ± SE = 491.0 ± 163,3) compared to flowers sprayed for control with PDB medium (46.7 ± 19) or sprayed with H_2O (36.4 ± 14.2) (post hoc test, $p = 0.004$ and $p < 0.001$, respectively). No significant difference was observed between the 'PDB' and 'H_2O' treatments ($p = 0.914$). Plates inoculated with a suspension of flowers sprayed with *M. fructicola* were not countable due to high number and density CFU; estimates were not included in the statistical analysis (Supp. Table S1).

Fruit quality: pollination and biological control of *B. cinerea* in strawberries

Exposure of *Eupeodes* flies to *M. fructicola* yeasts did not affect the survival of the flies (mean ± SD, 14.57 ± 1.24 days) relative to control, i.e. unexposed flies (14.77 ± 1.31) after treatment ($p = 0.997$).

At harvest, fruit weight did not differ significantly between treatments ($p = 0.285$) (Table 1). However, fruit shape quality was significantly higher in flowers exposed to hoverflies in combination with *M. fructicola* yeast compared with fruit from flowers that were untreated or sprayed with water or *B. cinerea* alone ($p < 0.001$). Moreover, vectoring of the yeast onto flowers by hoverflies significantly improved fruit shape quality compared with yeast inoculation in the absence of the pollinator ($p < 0.001$) (Table 1, Fig. 1A).

Fruits were stored in a cold chamber, and mould severity was evaluated once a week for 21 days. After 7 days, the areas of disease lesions were significantly smaller in fruit from flowers either exposed to hoverflies (regardless of the presence or absence of *M. fructicola* yeast), or treated with *M. fructicola* sprayed, compared to all other treatments ($\chi^2 = 36.18$; $p < 0.001$). These differences became more pronounced by day 14 postharvest, when hoverfly activity—especially in combination with the yeast—significantly reduced the fungal growth on the fruit ($\chi^2 = 95.86$; $p < 0.001$) (Fig. 1B). Similarly, by day 21 postharvest, external mycelium growth was significantly reduced by hoverfly presence compared to the positive control *B. cinerea* spray ($p < 0.001$). Moreover, hoverflies vectoring the yeast onto flowers significantly reduced the mycelium growth on fruit to the same extent as the sprayed *M. fructicola* treatment ($p = 0.795$) (for details, see Supp. Figure S5).

Discussion

Our study demonstrates a novel integrated strategy for biological control, combining the yeast *M. fructicola* and the hoverfly *E. corollae* to suppress the causal pathogen of grey mould *B. cinerea* and improve fruit quality in strawberries. Hoverflies simultaneously pollinate flowers and disseminate the yeast through flower visitation, resulting in a significant reduction in fungal growth and an improvement in fruit quality. We propose this multifunctional role as a novel proof of concept, which we term '*Flying agents*'.

Metschnikowia fructicola is a yeast species with well-documented antagonistic activity against several fungal pathogens, primarily through mechanisms such as space and nutrient competition, and the production of inhibitory metabolites (Spadaro and Droby 2016; Wisniewski et al. 2016; Kowalska et al. 2022). In our study, we showed antifungal bioactivity for a new strain of *M. fructicola* (UDA 10) with suppressive effect on *B. cinerea* in vitro and on strawberries. The incorporation of *M. fructicola* into a hoverfly-vectored system introduces a novel precision delivery method for this biocontrol agent, potentially overcoming challenges associated with repetitively needed spray application throughout the whole flowering period, or inconsistent spray coverage. Recently, Voß et al. (2023) showed that chemical fungicide application negatively affects the flower microbial community and pollen attributes in strawberries, with consequences on floral scent and reduction of flower visits by pollinators. Unlike chemical fungicides, *M. fructicola* is naturally occurring in fruit production systems and in association with insects (Guzmán et al. 2013). Furthermore, risk assessment of *M. fructicola* indicates minimal risk to human health, non-target organisms and the environment (European Food Safety Authority 2017; Freimoser et al. 2019). Its compatibility with *E. corollae* further highlights its potential as a component of sustainable disease management systems. Future studies should explore formulation stability, persistence on floral tissues and interactions with the native phyllosphere microbiota to optimize its performance in flowering crops (Aleklett et al. 2014; He et al. 2024).

Our results show that flower visitation by *E. corollae*, especially in combination with the yeast, positively influenced fruit shape and shelf life, indicating a potential improvement in overall fruit quality. This effect may be attributed to the reduction in fungal infection, which is known to compromise tissue development and leads to deformed fruits (Petrasch et al. 2019). By limiting early fungal growth through targeted yeast delivery, the flower is likely protected during critical stages of fruit initiation

and development. Additionally, hoverfly-mediated pollination may have contributed to better fruit set and symmetry, as successful insect pollination is linked to more complete fertilization of achenes, triggering hormone production that promotes uniform growth and firmness, factors directly associated with fruit quality in strawberries (Roussos et al. 2009; Klatt et al. 2014; Hodgkiss et al. 2018). Notably, in horticultural crops, traits related to the external appearance of fruits such as size, colour and shape are key factors for consumers and significantly influence the commercial quality of the fruits (e.g. Normann et al. 2019). Our findings highlight the potential of integrating microbial biocontrol and insect-mediated dissemination systems not only for plant protection, but also for enhancing fruit marketable traits. The effect of PDB medium alone on fruit quality was not tested in this study. Such an assessment could provide insights into the potential contributions of both the yeast and the growth medium, and therefore warrants further investigation. Further studies could also investigate whether these improvements extend to other qualitative parameters such as firmness, shelf life or taste and sugar content (e.g. Hodgkiss et al. 2018; Wietzke et al. 2018; Dung et al. 2021). The use of plants covered with net bags and prolonged hoverfly exposure (24 h) showed the efficacy of the system under controlled conditions, but may not fully represent field conditions. Studies with free-flying hoverflies in polytunnels could help to demonstrate the potential in practice of this multifunctional system at a larger scale, including assessment of floral attractiveness and floral visitation and revisitation frequencies, factors that are crucial for successful pollination (Pekas et al. 2020).

The role of pollinator-borne microbes in structuring floral microbial communities and its ecological significance has until recently been poorly understood (Beck et al. 2018; De Vega et al. 2021; Quevedo-Caraballo et al. 2025). As pollinators move between flowers, they transmit, acquire and redistribute microorganisms, significantly influencing microbial diversity and composition within floral environments (e.g. Herrera et al. 2009; Adler et al. 2018; Vannette 2020). Thereby, pollinators can affect plant health, nectar chemistry, floral scent and subsequent pollination behaviour and fitness, which influence plant–animal–microbe interactions in the broader ecological context (Pozo et al. 2009; Dharampal et al. 2019; Liu et al. 2019; Prado et al. 2020; Rering et al. 2020; Jacquemyn et al. 2021; Martin et al. 2022; Voß et al. 2023). While bees have been mainly studied in the context of pollinator ecology, the role of other pollinators, including hoverflies, remains understudied (but see Rader et al. 2020; Raguso 2020; Wei et al. 2021). Investigating the microbial contributions of diverse pollinator taxa, including managed and wild insects, is essential to understand the complex interplay between microorganisms, floral traits and pollination dynamics, especially in the context of global environmental change and pollinator decline. Furthermore, it may provide novel opportunities to develop new sustainable IPPM strategies (Álvarez-Pérez et al. 2024).

While in bee-vectoring technologies the bioagent is delivered by an integrated dispenser system connected to the enter or exit of the hive (i.e. bees pick up the biocontrol formulation when entering or exiting the hive), most hoverflies are solitary. Therefore, alternatives to hive-integrated dispenser systems are necessary for luring hoverflies. For example, mass-reared hoverfly pupae (similar to the Eupeodes system) could be released into the fields, using emergence cages designed to direct newly emerged adults through inoculum dispensers at the exit. In addition, flowers and yeasts share a significant overlap in volatile organic compounds, which can attract insects (Becher et al. 2018). In field trapping experiments conducted in an organic apple orchard, hoverflies were the most frequently attracted insects to live yeast cultures (Andreadis et al. 2015). Yeasts are rich in protein and often part of insect diets. In particular, *Metschnikowia* yeasts found in floral nectar contribute to insect attraction and serve as food source for pollinators (Schaeffer and Irwin 2014; Colda et al. 2021). Consequently, *Metschnikowia* might enhance both attraction and nutritional value in the diet of *E. corollae*, potentially improving reproductive output. This could support pollinator-mediated biocontrol, by higher production of aphidophagous larvae, and promoting yeast entomovectoring through flower visitation. A recent study has shown that providing sugar and pollen to hoverflies enhances aphid control in strawberry (Leman et al. 2023). In the pollination-entomovectoring experiment, we found that exposure of *Eupeodes* flies to *M. fructicola* yeast did not affect adult longevity compared to unexposed control flies. Jiang et al. (2022) reported a similar life time of the adult stage in *E. corollae* under controlled experiments as seen in our studies. However, Lillo et al. (2021) observed a longer adult lifespan. These similarities and discrepancies should be carefully considered in the design of future experiments. Moreover, other syrphid species currently applied for pest control and pollination services (e.g. Dunn et al. 2020; Pekas et al. 2020) could be evaluated to further test the 'flying agent' principle.

Overall, future studies could investigate the use of 'yeast dispensers' to attract hoverflies and to possibly provide them with a high-quality yeast diet, thereby improving their performance, with positive effects on the conservation of beneficial insects and pest control. Moreover, these dispensers could serve as a yeast source for targeted dispersal by 'flying agents' to specific flowering crops. In addition to *M. fructicola*, other yeast species and biocontrol agents, as well as alternative insect vectors, could be evaluated for their potential use in such system.

Conclusion and perspectives

This study introduces a novel strategy for IPPM by combining the hoverfly *E. corollae* with the biocontrol yeast *M. fructicola* for pollination and to control *B. cinerea* in strawberries. This strategy supports the use of beneficial insect and microorganism species simultaneously in agroecosystems (Hokkanen et al. 2015). Moreover, this approach offers a sustainable alternative to application of chemical fungicides. This adds to the well-documented service provided by hoverfly species in controlling agriculturally important pests such as aphids (e.g. Dunn et al. 2020; Pekas et al. 2020; Van Oystaeyen et al. 2022; Rodríguez-Gasol et al. 2020 and references therein). Furthermore, we speculate that the '*Flying Agents*' has the potential to coexist with, and complement existing vectoring systems and biocontrol products (e.g. 'Flying Doctors'). Future research may focus on evaluating this principle in combination with the hoverfly predation effects on pests. In addition, future studies may evaluate the effects on vector fitness and on the floral microbiota, or focus on optimizing biocontrol formulations and dispensers. The applicability of entomovectoring systems for other pollinators and agroecosystems should be considered.

Author contribution

GR and PGB conceived the idea and designed the study. GR and CP conducted the experiments and analyzed the data. SK contributed to the investigation of grey mould. JAS supervised quality assessment of the fruit. PGB, SK and RM supervised the study. GR wrote the manuscript with input from PGB. All authors contributed to the revision of the manuscript and approved the final version for submission.

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Declarations

Conflict of interests The authors declare no conflict of interests.

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Guillermo Rehermann is a chemical ecologist interested in how chemical substances mediate interactions among insects, microbes, and plants, with an emphasis on integrated pest and pollinator management. Guillermo completed his Ph.D. in 2022 at the Swedish University of Agricultural Sciences (SLU). He studied an invasive fruit fly in collaboration with University of the Republic (UDELAR, Uruguay), the Free University of Bozen-Bolzano, and the Laimburg Research Centre (Italy). As a postdoctoral researcher at SLU he collaborated with Uppsala University (Sweden), and investiaged chemical communication and biological control between insects and microbes in horticultural systems. Since 2023, he has also served as a councillor for the Latin American Association of Chemical Ecology and he is currently establishing himself at UDELAR.