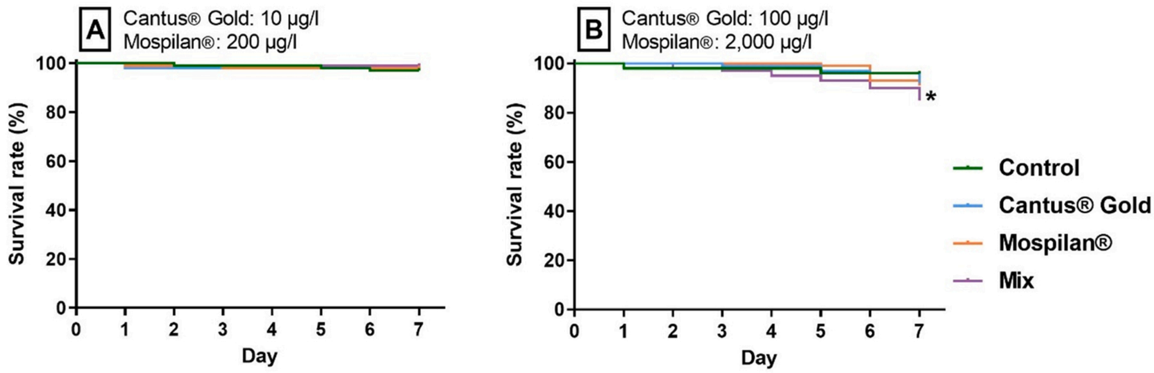

Fig. 3. Survival curves. (A) Kaplan Meier survival curves of the honeybees that were treated with the low sublethal concentrations of Cantus® Gold (blue; n = 100; 10 µg/l), Mospilan® (orange; n = 100; 200 µg/l) or the mixture of both (purple; n = 100). The control bees received a sucrose solution (green; n = 100). There was no treatment effect on the survival rate of the different groups (mortality rate: control: 3 %, Cantus® Gold: 2 %, Mospilan®: 2 %, mix: 1 %) (Log-rank test with Bonferroni correction; $p_{\text{Control vs Cantus® Gold}} = 1.000$, $p_{\text{Control vs Mospilan®}} = 1.000$, $p_{\text{Control vs Mix}} = 0.952$). (B) Kaplan Meier survival curves of the honeybees that were treated with the high sublethal concentrations of Cantus® Gold (blue; n = 100; 100 µg/l), Mospilan® (orange; n = 100; 2000 µg/l) or the mixture of both (purple; n = 100). The control bees received a sucrose solution (green; n = 100). There was a treatment effect as significantly more mix animal died compared to control bees (mortality rate: control: 4 %, Cantus® Gold: 8 %, Mospilan®: 9 %, mix: 15 %) (Log-rank test with Bonferroni correction; $p_{\text{Control vs Cantus® Gold}} = 0.749$, $p_{\text{Control vs Mospilan®}} = 0.509$, $p_{\text{Control vs Mix}} = 0.028$ (*)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

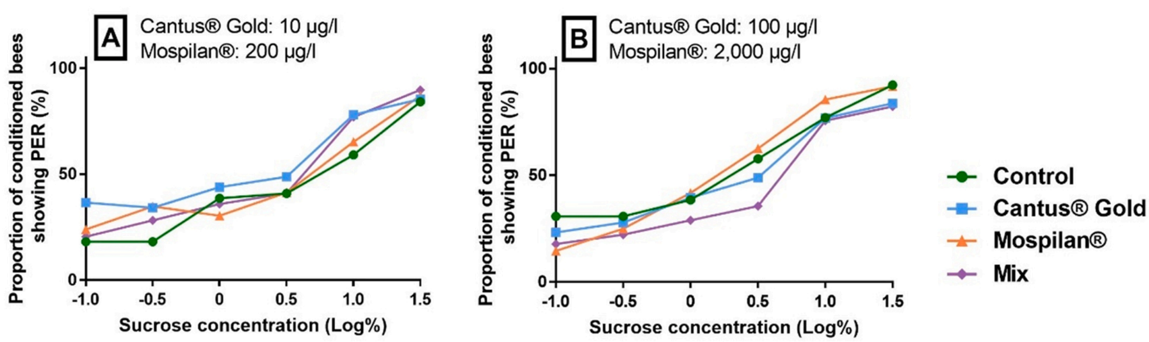

Fig. 4. PER curves. (A) Proportion of conditioned honeybees showing a proboscis extension response (PER) to increasing sucrose concentrations after oral treatment with a control solution (green circle; n = 44) or a low sublethal concentration of Cantus® Gold (blue square; n = 41; 10 µg/l), Mospilan® (orange triangle; n = 46; 200 µg/l) or the mixture of both (purple rhombus; n = 39). There were no treatment effects on sucrose responses (proportion PER after low treatment: control: 84 %, Cantus® Gold: 85 %, Mospilan®: 87 %, mix: 90 %) (GLM: treatment effect on sucrose responsiveness, $p_{\text{low dose}} = 0.505$). (B) Proportion of conditioned honeybees showing a PER to increasing sucrose concentrations after oral treatment with a control solution (green circle; n = 52) or with a high sublethal concentration of Cantus® Gold (blue square; n = 43; 100 µg/l), Mospilan® (orange triangle; n = 48; 2000 µg/l) or the mixture of both (purple rhombus; n = 45). There was no treatment effect on sucrose responses (proportion PER after high treatment: control: 92 %, Cantus® Gold: 84 %, Mospilan®: 92 %, mix: 82 %) (GLM: treatment effect on sucrose responsiveness, $p_{\text{high dose}} = 0.355$).

3.3. Olfactory learning

The learning experiments were performed following the same sublethal oral treatments as the experiments investigating sucrose responsiveness (Cantus® Gold; low: 10 µg/l, high: 100 µg/l; Mospilan®; low: 200 µg/l, high: 2000 µg/l). After oral treatment, bees in all groups learned to respond to the CS+ during acquisition (proportion of responses to CS+ after low treatment: control: 66 %, Cantus® Gold: 63 %, Mospilan®: 48 %, mix: 49 %; proportion of responses to CS+ after high treatment: control: 46 %, Cantus® Gold: 44 %, Mospilan®: 52 %, mix: 38 %) (GLM: effect of trial; CS+: $p_{\text{low dose}} < 0.001$; $p_{\text{high dose}} < 0.001$). Only a maximum of 2 % of the bees showed a response to the CS-. Treatment with the fungicide, the insecticide, or the combination of both did not affect learning performance (GLM: treatment effect on learning; CS+: $p_{\text{low dose}} = 0.165$; $p_{\text{high dose}} = 0.612$). (Fig. 5 A and Fig. 6 A). During the reversal learning phase, responses to the new CS+ became more frequent (proportion of responses to CS+ after low treatment: control: 28 %, Cantus® Gold: 27 %, Mospilan®: 18%, mix: 21 %; proportion of responses to CS+ after high treatment: control: 13 %, Cantus® Gold: 21 %, Mospilan®: 24 %, mix: 18 %), while the responses to the former CS+ , which now represented the punished CS-, became less frequent (proportion of responses to CS- after low treatment: control: 21 %, Cantus® Gold: 38 %, Mospilan®: 18 %, mix: 16 %; proportion of responses to CS- after high treatment: control: 21 %, Cantus® Gold: 11 %, Mospilan®: 28 %, mix: 18 %) (GLM: effect of trial; CS+: $p_{\text{low dose}} < 0.001$; $p_{\text{high dose}} < 0.001$; CS-: $p_{\text{low dose}} < 0.001$; $p_{\text{high dose}} < 0.001$). There was no treatment effect on the reversal learning performance independent of the concentration used (GLM: treatment effect on reversal learning; CS+: $p_{\text{low dose}} = 0.500$; $p_{\text{high dose}} = 0.748$; CS-: $p_{\text{low dose}} = 0.197$; $p_{\text{high dose}} = 0.484$) (Fig. 5B and Fig. 6B).

4. Discussion

4.1. Effects of PPP mixtures

The results of our study show that the responsiveness to sugar water and the learning behavior of honeybees were not affected by the combination of Cantus® Gold and Mospilan®. However, the mortality studies showed a synergistic effect, as the mix group (100 µg/l Cantus® Gold + 2000 µg/l Mospilan®) differed significantly from the other groups.

Different studies on PPP mixtures already showed similar synergistic

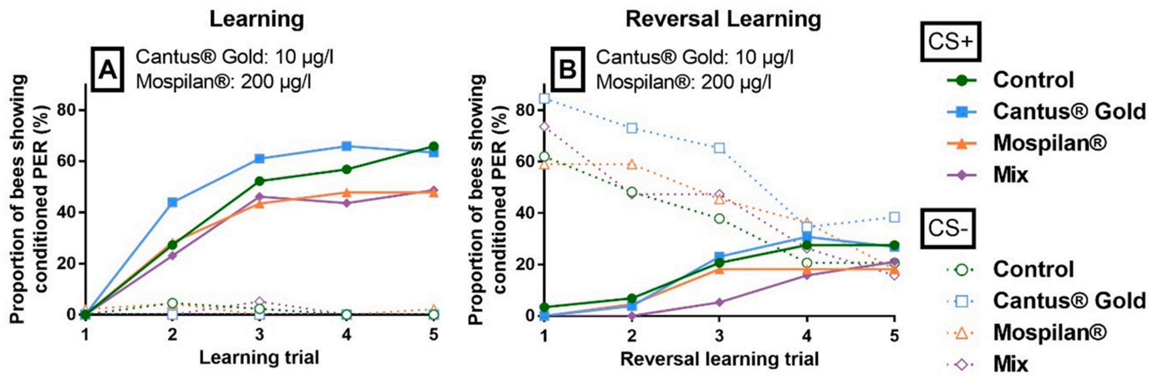

Fig. 5. Learning and reversal learning curves of bees treated with a low field realistic dose of PPPs. (A) Learning curves of honeybees treated with a control solution (green circle; n = 44) or with a low sublethal dose of Cantus® Gold (blue square; n = 41; 10 µg/l), Mospilan® (orange triangle; n = 46; 200 µg/l) or the mixture of both (purple rhombus; n = 39). The proportion of bees showing a conditioned proboscis extension response (PER) is shown for each group. The honeybees learned well to respond to the CS+ (solid lines) (GLM: effect of trial, CS+: $p_{low\ dose} < 0.001$) and not to react to the CS- (dotted lines) (proportion of responses to CS+ after low treatment: control: 66 %, Cantus® Gold: 63 %, Mospilan®: 48 %, mix: 49 %; proportion of responses to CS- after low treatment: control: 0%, Cantus® Gold: 0 %, Mospilan®: 2 %, mix: 0 %). Treatment with the different PPPs had no significant effect on learning performance (GLM: treatment effect on learning, CS+: $p_{low\ dose} = 0.165$). (B) Reversal learning curves of honeybees treated with a control solution (green circle; n = 27) or with a low sublethal dose of Cantus® Gold (blue square; n = 28; 10 µg/l), Mospilan® (orange triangle; n = 18; 200 µg/l) or the mixture of both (purple rhombus; n = 16). The proportion of bees showing a conditioned proboscis extension response (PER) is shown for each group. The proportion of responses to the new CS+ increased slowly with progressive trial (solid lines), while the responses to the former CS+ decreased (dotted lines) (proportion of responses to CS+ after low treatment: control: 28%, Cantus® Gold: 27 %, Mospilan®: 18 %, mix: 21 %; proportion of responses to CS- after low treatment: control: 21 %, Cantus® Gold: 38 %, Mospilan®: 18 %, mix: 16 %) (GLM: effect of trial, CS+: $p_{low\ dose} < 0.001$, CS-: $p_{low\ dose} < 0.001$). There was no effect of treatment on the reversal learning performance (GLM: treatment effect on reversal learning, CS+: $p_{low\ dose} = 0.500$, CS-: $p_{low\ dose} = 0.197$).

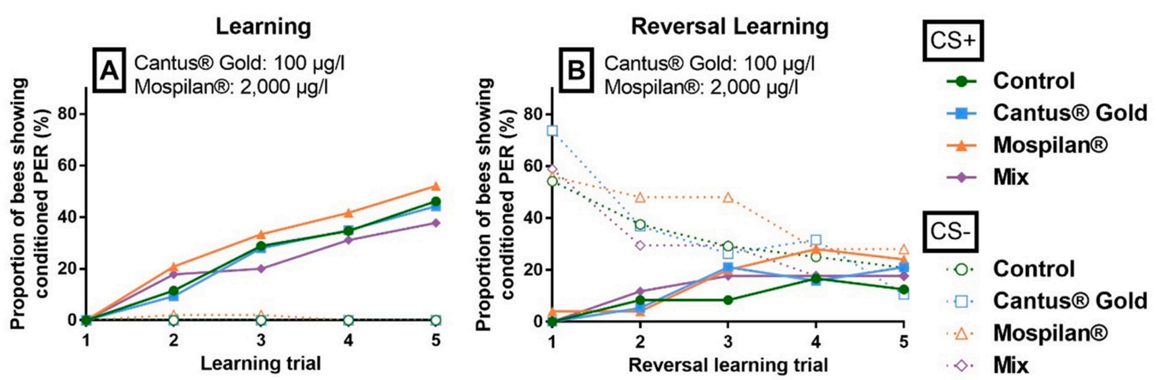

Fig. 6. Learning and reversal learning curves of bees treated with a high field realistic dose of PPPs. (A) Learning curves of the honeybees that were treated with a control solution (green circle; n = 52) or with a high sublethal dose of Cantus® Gold (blue square; n = 43; 100 µg/l), Mospilan® (orange triangle; n = 48; 2000 µg/l) or the mixture of both (purple rhombus; n = 45). The proportion of bees showing a conditioned proboscis extension response (PER) is shown for each group. The honeybees learned well to respond to the CS+ (solid lines) (GLM: effect of trial, CS+: $p_{high\ dose} < 0.001$) and not to react to the CS- (dotted lines) (proportion of responses to CS+ after high treatment: control: 46 %, Cantus® Gold: 44 %, Mospilan®: 52 %, mix: 38 %; proportion of responses to CS- after high treatment: control: 0 %, Cantus® Gold: 0 %, Mospilan®: 0 %, mix: 0 %). Treatment with the different PPPs had no significant effect on learning performance (GLM: treatment effect on learning, CS+: $p_{high\ dose} = 0.612$). (B) Reversal learning curves of the honeybees treated with a control solution (green circle; n = 21) or with a high sublethal dose of Cantus® Gold (blue square; n = 19; 100 µg/l), Mospilan® (orange triangle; n = 23; 2000 µg/l) or the mixture of both (purple rhombus; n = 19). The proportion of bees showing a conditioned proboscis extension response (PER) is shown for each group. The proportion of responses to the new CS+ increased slowly with progressive trial (solid lines), while the responses to the former CS+ decreased (dotted lines) (proportion of responses to CS+ after high treatment: control: 13 %, Cantus® Gold: 21 %, Mospilan®: 24 %, mix: 18 %; proportion of responses to CS- after high treatment: control: 21 %, Cantus® Gold: 11 %, Mospilan®: 28 %, mix: 18 %) (GLM: effect of trial, CS+: $p_{high\ dose} < 0.001$, CS-: $p_{high\ dose} < 0.001$). There was no effect of treatment on the reversal learning performance (GLM: treatment effect on reversal learning, CS+: $p_{high\ dose} = 0.748$, CS-: $p_{high\ dose} = 0.484$).

effects. The combination of the SBI fungicide propiconazole and the neonicotinoid clothianidin led to synergistic effects on mortality in *A. mellifera* and different wild bee species (Sgolastra et al., 2017). The SBI fungicides triflumizole and propiconazole significantly increased the toxicity on honeybees when applied in combination with the neonicotinoids thiacloprid or acetamiprid (Iwasa et al., 2004; Manning et al., 2017). The toxicity on honeybees was also synergistically increased when, among others, the SBI fungicide tetraconazole and the neonicotinoid imidacloprid were applied in combination (Zhu et al., 2017). The SBI fungicide difenoconazole and the neonicotinoid imidacloprid also reduced the survival rate of honeybees significantly (Almasri et al., 2020). Small synergistic effects were observed when SBI fungicides (myclobutanil, propiconazole, flusilazole, tebuconazole) were applied together with neonicotinoids (thiamethoxam, clothianidin, imidacloprid, thiacloprid) via oral or contact exposure to honeybees (Thompson et al., 2014). The combined application of different SBI fungicides with the pyrethroid lamda-cyhalothrin to the thorax of honeybees increased the toxicity as part of a synergistic effect (Pilling and Jepson, 1993).

Synergistic effects have also been discovered in other bee species, wild bees and other beneficial insects. The combined action of the SBI