learning, a test was performed between the learning and reversal learning trials (Fig. 2) (For details on test trial see Supplementary Information, Fig. S2).

2.7. Statistics

Kaplan Meier curves with Log-rank tests were used for comparing the mortality rates as there was no replicate effect. For pairwise comparisons Bonferroni-Dunn method was used (GraphPad Prism® version 7.03 for Windows, GraphPad Software, La Jolla, CA USA).

To compare the PER performance in the sucrose responsiveness tests of the different treatment groups a generalized linear model (GLM[10]) was applied with sucrose concentration as within-subject factor and treatment as between-subject factor (Šidák test for pairwise comparisons). Only bees displaying the PER to 50 % sucrose which did not show any spontaneous response to either odor were analyzed (SPSS® Statistics 26 (Version 26, IBM®, Armonk, NY USA), GraphPad Prism® version 7.03 for Windows, GraphPad Software, La Jolla, CA USA)).

Learning performance and reversal learning performance are shown by learning and reversal learning curves. For the learning trials all conditioned bees were included in the GLM, while for the reversal learning performance only the bees that had learned before (i.e. honeybees that showed a response to the CS+ and no response to the CS- during the test (see Supplementary Information)) were analyzed. The learning or reversal learning trials were used as within-subject factor while the treatment was used as between-subject factor. During the learning trials, there were almost no responses to the CS-. Therefore, we did not perform a GLM (SPSS® Statistics and GraphPad Prism®).

3. Results

3.1. Mortality

The concentrations of 10 μg/l Cantus® Gold and 200 μg/l Mospilan® or their mixture did not increase the mortality of the bees (mortality rate: control: 3 %, Cantus® Gold: 2 %, Mospilan®: 2 %, mix: 1 %) (Log-rank test with Bonferroni correction; $p_{\text{Control vs Cantus® Gold}} = 1.000$, $p_{\text{Control vs Mospilan®}} = 1.000$, $p_{\text{Control vs Mix}} = 0.952$). The tenfold higher concentrations of 100 μg/l Cantus® Gold and 2000 μg/l Mospilan® also did not increase the mortality, but the mixture of both led to a higher mortality rate compared to the control group (mortality rate: control: 4 %, Cantus® Gold: 8 %, Mospilan®: 9 %, mix: 15 %) (Log-rank test with Bonferroni correction; $p_{\text{Control vs Cantus® Gold}} = 0.749$, $p_{\text{Control vs Mospilan®}} = 0.509$, $p_{\text{Control vs Mix}} = 0.028(*)$) (Fig. 3 A and B).

3.2. Responsiveness to sucrose

For testing responsiveness to sucrose, we used two sublethal concentrations of Cantus® Gold (low: 10 μg/l and high: 100 μg/l) and Mospilan® (low: 200 μg/l and high: 2000 μg/l). The proportion of bees showing PERs increased with increasing sucrose concentration in all groups (proportion PER after low treatment: control: 84 %, Cantus® Gold: 85 %, Mospilan®: 87 %, mix: 90 %; proportion PER after high treatment: control: 92 %, Cantus® Gold: 84 %, Mospilan®: 92 %, mix: 82 %) (GLM: effect of trial; $p_{\text{low dose}} < 0.001$; $p_{\text{high dose}} < 0.001$). Response of trained bees to sucrose was unaffected by treatment with PPPs (GLM: treatment effect on sucrose responsiveness; $p_{\text{low dose}} = 0.505$; $p_{\text{high dose}} = 0.355$). Bees treated with the different concentrations of Cantus® Gold and Mospilan® did not differ from control bees in their responses to increasing sucrose concentrations (Fig. 4 A and B).

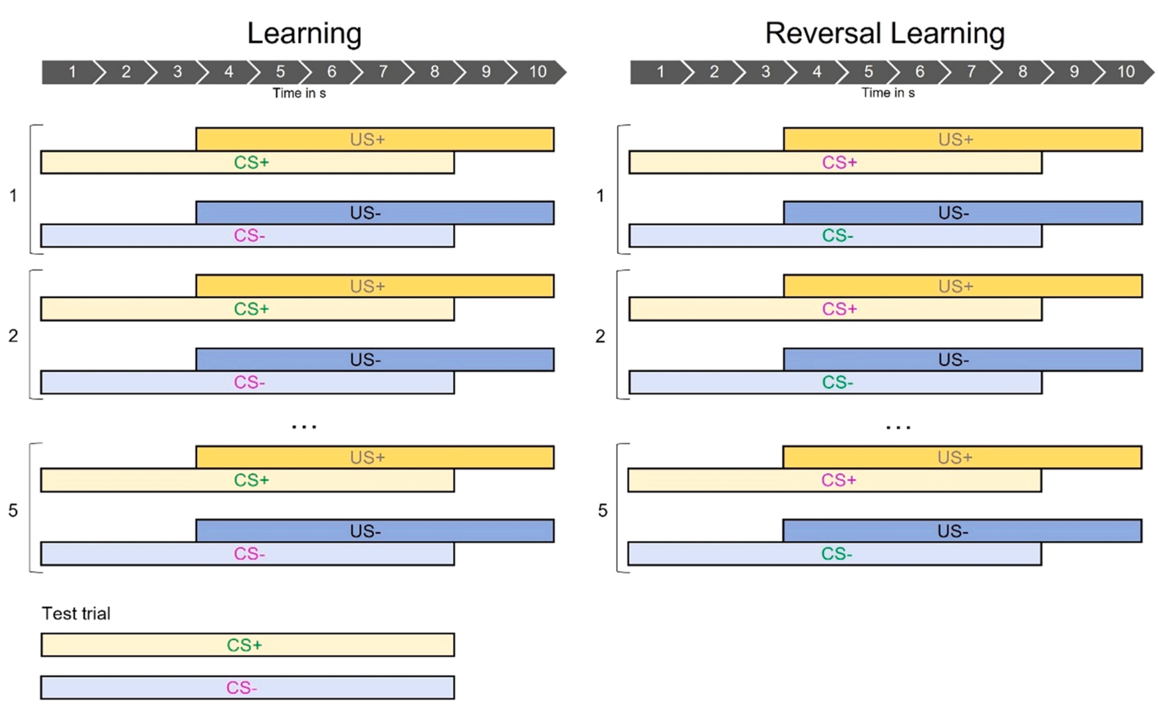

Fig. 2. Procedure of the learning (left) and reversal learning (right) paradigm. First, the odor (CS+/CS-) was presented for three seconds. During the following five seconds the odor was presented in combination with the reward or the punishment (US+/US-). When the bee showed a proboscis extension response (PER), the US+ /US- was presented for another two seconds, while the CS+ /CS- was removed. Learning and reversal learning consisted of 5 trials. CS+ and CS- were always shown in alteration. The green color represents one odor, while the magenta color represents the other odor. The odors in their function as CS+ and CS- were switched between the learning and the reversal learning paradigm. After the five learning trials a test was performed. The CS+ and CS- were shown without any reward our punishment for eight seconds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

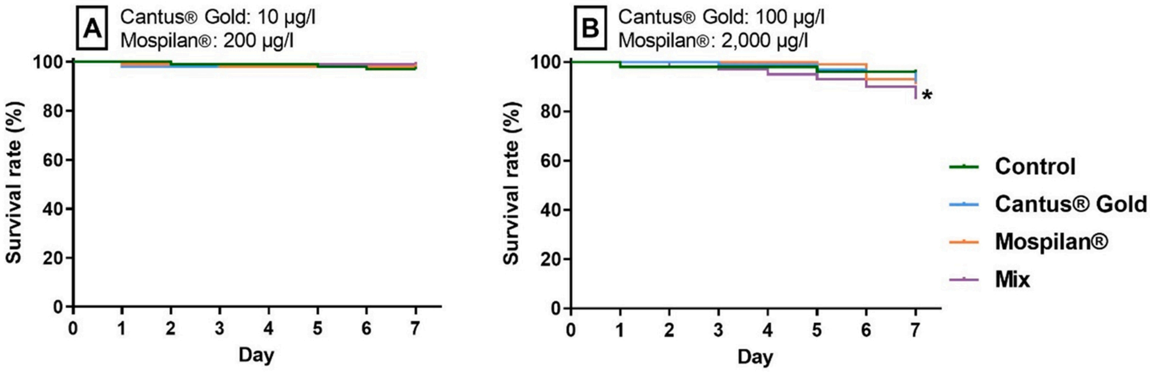

Fig. 3. Survival curves. (A) Kaplan Meier survival curves of the honeybees that were treated with the low sublethal concentrations of Cantus® Gold (blue; n = 100; 10 μg/l), Mospilan® (orange; n = 100; 200 μg/l) or the mixture of both (purple; n = 100). The control bees received a sucrose solution (green; 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.)

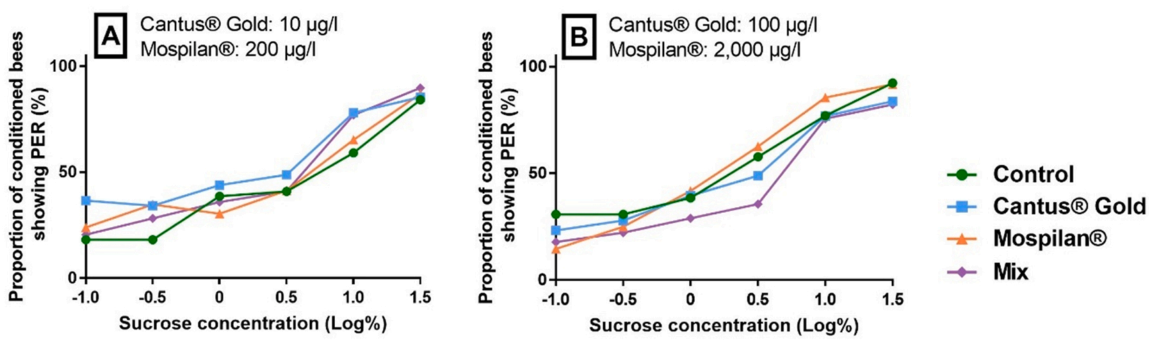

Fig. 4. PER curves. (A) Proportion of conditioned honeybees showing a proboscis extension response (PER) to increasing sucrose concentrations after oral treatment with a control solution (green circle; n = 44) or a low sublethal concentration of Cantus® Gold (blue square; n = 41; 10 μg/l), Mospilan® (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