






# Synthetic Nasonov gland pheromone enhances abundance and visitation of honeybee, *Apis mellifera*, in Korla fragrant pear, *Pyrus sinkiangensis*

Qian Li<sup>1,2,3</sup>  | Mengxiao Sun<sup>1,2,3</sup>  | Yangtian Liu<sup>1</sup> | Bing Liu<sup>1</sup> |  
Wopke van der Werf<sup>2</sup>  | Felix J. J. A. Bianchi<sup>3</sup>  | Yanhui Lu<sup>1</sup> 

<sup>1</sup>State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

<sup>2</sup>Centre for Crop Systems Analysis, Wageningen University & Research, Wageningen, The Netherlands

<sup>3</sup>Farming Systems Ecology, Wageningen University & Research, Wageningen, The Netherlands

## Correspondence

Yanhui Lu, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China.

Email: [luyanhui@caas.cn](mailto:luyanhui@caas.cn)

Felix J. J. A. Bianchi, Farming Systems Ecology, Wageningen University & Research, Wageningen, The Netherlands.

Email: [felix.bianchi@wur.nl](mailto:felix.bianchi@wur.nl)

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## Abstract

1. Korla fragrant pear (*Pyrus sinkiangensis* Yü) depends on cross-pollination by honeybees (*Apis mellifera*) but may suffer from low honeybee visitation.
2. We assessed whether honeybee abundance and visitation frequency are enhanced by using synthetic Nasonov gland pheromone (NGP), which is naturally produced by worker bees to stimulate the aggregation of bees to food resources or nesting sites.
3. The response of honeybees to synthetic NGP was firstly assessed using Y-tube olfactometer tests in the laboratory, and subsequently in the field, by placing NGP lures on Korla fragrant pear trees in orchards with and without beehives. Honeybee abundance was assessed using coloured pan traps while honeybee visits were assessed by visual observations on pear flowers.
4. Y-tube olfactometer tests showed a significant preference of honeybees for NGP. In pear orchards with beehives, honeybee abundance was 2.5-fold higher on trees with NGP lures than on trees without NGP, and 2.2-fold higher in orchards in which all trees contained NGP lures than in orchards without NGP lures. Such positive effects were not observed in orchards without beehives.
5. Flower visitation by honeybees was significantly higher in trees with NGP lures than without NGP lures, irrespective of the presence (5.7-fold higher) or absence of beehives (27.6-fold higher).
6. In mixed pear-apricot orchards, honeybee abundance was higher in pear trees with NGP lures than without lures.
7. Our results show that NGP lures attract honeybees to flowering pear trees in monoculture pear and mixed pear-apricot orchards, and that this effect is greatest in orchards with beehives.

## KEYWORDS

attractant, behaviour manipulation, floral resource, pheromone, pollination, pollinator

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## INTRODUCTION

The production of many kinds of fruit depends on insects for pollination (Hünicken et al., 2021; Kleijn et al., 2015; Sawe et al., 2020), but there are growing concerns about the decline of the abundance and diversity of wild insect pollinators in many parts in the world (LeBuhn & Vargas Luna, 2021; Outhwaite et al., 2022; Powney et al., 2019; Wagner et al., 2021). Some fruit crops, such as pear, depend heavily on managed European honeybees (*Apis mellifera* L.) because the flowers are less attractive for wild bees because of the low sugar content of the nectar (Delaplane & Mayer, 2000). Yet, even with honeybees fruit production can be constrained by pollination limitation (Aizen et al., 2019; Aizen & Harder, 2009; Mashilingi et al., 2022; Osterman et al., 2021). Therefore, there is a need for approaches that can further enhance the activity of honeybees for crop pollination, particularly in regions where there is a shortage of pollinators.

Pheromones are chemicals that are released by individuals for communication within the same species (Shorey, 1976). The Nasonov gland pheromone (hereafter 'NGP') is released by worker honeybees from their abdominal glands to stimulate aggregation and orient other bees to food resources or nest sites (Free et al., 1981, 1984; Pickett et al., 1980; Williams et al., 1981). NGP has been chemically characterized and synthesized. It consists of geraniol, (E)-citral, nerolic acid, (Z)-citral, nerol, geranic acid, and (E,E)-farnesol (Pickett et al., 1980). Synthetic NGP triggers a similar response in honeybees as the naturally produced pheromone (Williams et al., 1981) and it attracts honeybees at a distance of approximately 10 cm (Butler, 1970). Despite this relatively small range, attraction of honeybees to NGP has been observed under field conditions (Schmidt, 1994, 2001; Williams et al., 1981) and honeybee attractants based on NGP have been used to enhance fruit crop pollination in apple (Mayer, Britt, et al., 1989), sweet orange (Malerbo-Souza et al., 2004), guava (Anita et al., 2012) and kiwifruit (Jailiang et al., 2022).

Korla fragrant pear (*Pyrus sinkiangensis* Yü) is a local variety of pear which is usually intensively managed with high pesticide inputs and is typically grown in landscapes dominated by Korla fragrant pear orchards in Xinjiang, China. Like most rosaceous plants, Korla fragrant pear is a self-incompatible species that requires cross-pollination by insects with a compatible cultivar to set fruit (De Franceschi et al., 2012). There is a large pollination deficit in Korla fragrant pear and this deficit can be mitigated by using beehives with managed honeybees, *Apis mellifera* (Li et al., 2022). While Korla fragrant pear is usually grown in monocultures, it is also grown in mixed pear-apricot orchards. These mixed orchards usually have a low density of apricot trees to produce apricots for self-consumption. Apricot, *Prunus armeniaca* L., is an early flowering tree species which flowers 1 week before pear. The apricot flowers are more attractive to honeybees than pear flowers (Lan et al., 2021) and therefore the presence of apricot trees can increase honeybee abundance in pear orchards (Li et al., unpublished data). It is unclear to what extent NGP lures can increase honeybee abundance and pear flower visitation in pear trees in monoculture pear and mixed pear-apricot orchards.

Here, we assessed the effect of NGP on honeybee aggregation and flower visitation in Korla fragrant pear orchards, and assessed how this was influenced by placing honeybee hives in the orchard. These assessments were conducted in four complementary experiments. First, we assessed the attractiveness of NGP lures to honeybees under controlled conditions to ascertain the biological activity of the used source of NGP. Second, we assessed how NGP lures deployed on individual pear trees influenced honeybee abundance and visitation in orchards with or without beehives. Third, we assessed how NGP lures deployed on all pear trees in an orchard influenced the abundance of honeybees in orchards with or without beehives. Fourth, we assessed how NGP lures influence honeybee abundance in mixed apricot-pear orchards. We hypothesized that (1) honeybees should show a preference for the NGP under controlled conditions, (2) honeybee abundance and pear flower visitation rate should be higher on pear trees with NGP lures and in orchards with honeybee hives, (3) the aggregation effect of NGP on honeybee abundance in orchards where all pear trees have NGP lures should be consistent with the effect where NGP lures are deployed on individual pear trees, and (4) honeybee abundance should be higher on pear trees with NGP lures than pear trees without NGP lures in mixed pear-apricot orchards.

## MATERIALS AND METHODS

### Study site and NGP source

The field study was conducted in Korla fragrant pear orchards in four counties around the city Korla, Xinjiang, northwest China (85.48°E, 41.45°N) in 2021 (Tables S1–S3). The region has an average annual temperature of 13.4°C and average annual precipitation of 87 mm. The criteria of experimental orchards selection were (1) orchards had an in-row spacing of approximately 5 m and approximately 6 m between rows; (2) pear trees were 15–20 years old; (3) no chemical pesticides were applied from 1 week before flowering until the end of flowering. Most orchards consisted of Korla fragrant pear trees with some interspersed Dangshan pear trees (*Pyrus communis* L.) used as pollinizer to ensure cross-pollination. All orchards had conventional management with regular pesticide applications after the pear flowering period. We recorded whether orchards contained beehives or not.

We used the commercial product Polynat<sup>®</sup> as NGP lures (i.e., yellow plastic 'rings' in Figure 1), which were obtained from Bioglobal Co. in Shenzhen, China (<http://www.bioglobal.com.cn/product/detail/99.html>). Following product recommendation, three lures were established per Korla fragrant pear tree (which is equivalent to one lure per 10 m<sup>2</sup>).

### Experiment 1: Y-tube olfactometer trials

To verify the honeybee preference to NGP released from Polynat, we studied the behavioural responses of honeybees to NGP using a



**FIGURE 1** Korla fragrant pear tree with three ‘Polynate’ rings containing the Nasonov gland pheromone and yellow, blue and white pan traps to sample the pollinator community around the tree

Y-tube olfactometer. The olfactometer consisted of a 3 cm diameter, clear glass tube, made of a 15 cm long central tube that branched into two 15 cm lateral arms with a 60° angle between the arms. The Y-tube was placed in a 100 × 100 × 60 cm chamber, illuminated with two 40 W fluorescent lamps (light intensity 2000 lx) and maintained at  $25 \pm 1^\circ\text{C}$  and  $60 \pm 5\%$  RH. A vacuum pressure pump (Beijing Institute of Labor Instrument, Beijing, China) pushed air through activated charcoal and an Erlenmeyer flask filled with distilled water. The airflow through each of the olfactometer arms was maintained at 300 ml/min and entered the apparatus via a Teflon tube. One arm was connected with a glass conical flask place with the NGP source and other arm was connected with a glass conical flask place without NGP. Honeybees were obtained from a colony of a local beekeeper on the day of the bioassay, using bees that were approximately 20 days old. Before the behavioural bioassay, honeybees were starved for 4 h individually in a transparent glass container (1.5 cm diameter, 5 height).

Individual honeybees were introduced at the base of the main arm of the olfactometer via a 10 cm long glass vial and given 5 min to respond. A choice for the NGP or control treatment was recorded when honeybees passed the Y-junction by 3 cm for at least 5 s. If a honeybee did not make a choice within 5 min, it was recorded as ‘no choice’. Each honeybee was used only once. After each trial the Y-tube was replaced with a clean one, and the used Y-tube was cleaned with acetone and then air dried overnight at room temperature. The NGP source was changed every 4 h. In total, 100 individual honeybees were tested. All bioassays were conducted between 08:00 and 18:00.

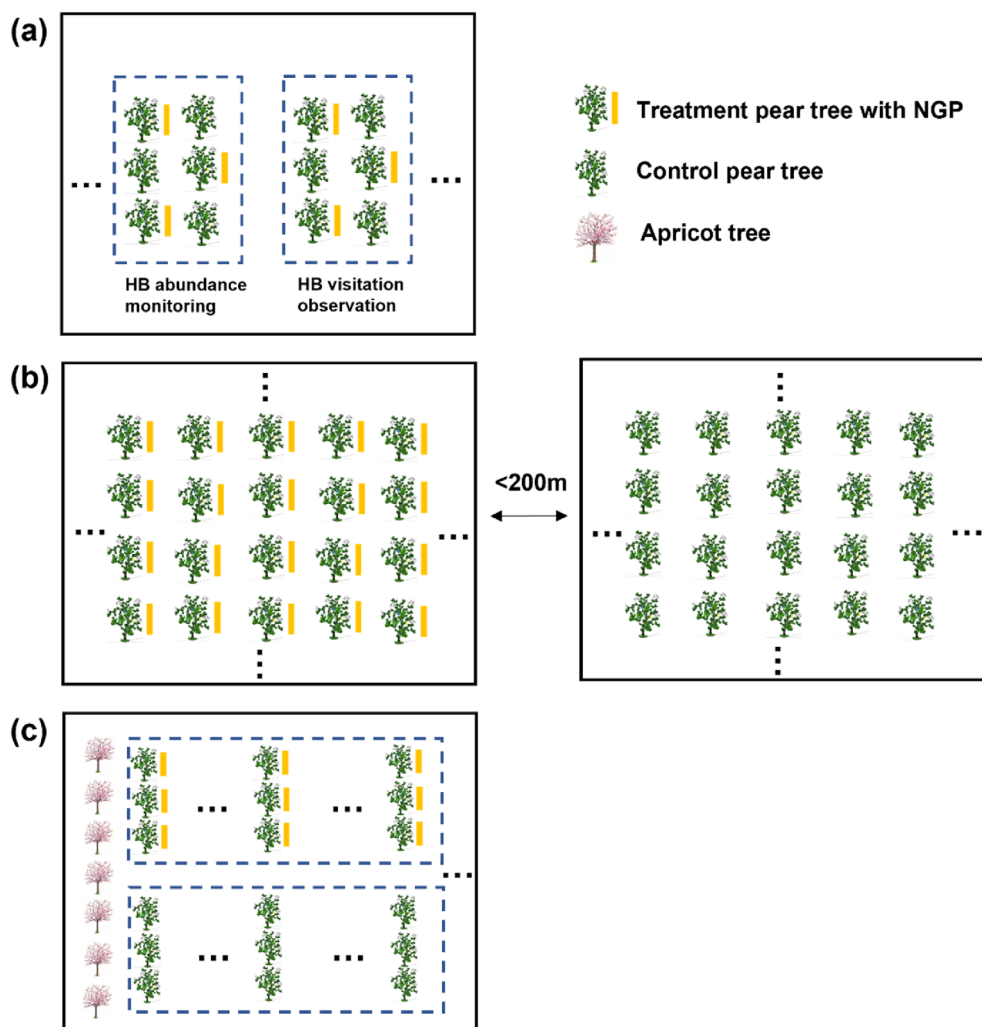
## Experiment 2: Tree-level effects of NGP on honeybee abundance and pear flower visitation

To assess the effect of NGP on honeybee abundance at the tree level, we selected nine pear monoculture orchards, of which two orchards

contained beehives and seven orchards did not (Figure 2a). The minimum distance between two focal orchards was 0.92 km. In each orchard, two blocks with same size (approximately 48 m × 20 m) were selected and each block contained around 45 trees (9 rows × 5 trees per row). One block was selected to assess honeybee abundance and another block to assess honeybee visitation rates. The two blocks were at least 20 m apart. Within each block, six trees were selected out of 45 trees, of which three pear trees received NGP lures (three lures per tree at 1.5–2.0 m height following product recommendation) and three trees did not (Figure 2a). The distance between the selected trees was 24 m between rows and 20 m within rows.

The abundance of honeybees was monitored using pan trap stations, which were placed in the six trees of one of the blocks (Figure 2a). The pan traps stations and NGP lures were installed at the same time. Each pan trap station consisted of three cups (12.1 cm diameter, 13 cm height) that were painted with ultraviolet yellow (SANO, type No. 1005), ultraviolet blue (SANO, type No. 1004) or ultraviolet white (SANO, type No. 1010) on the in and outside. Pans were placed on three different branches of the same tree. Cups were filled with 600 ml water and a few drops of detergent. Cups were emptied and refilled three times, at approximately 3-day intervals, for a total sampling period of 9 days. The abundance of honeybees per tree (three rounds and three cups) were pooled together for the analysis.

Honeybee visits to pear flowers were assessed on the three trees with and three trees without NGP lures in the other block (Figure 2a). Of each tree, a 1-cm diameter branch was selected at 1.5 m height and a group of 100 open flowers was marked for observation of flower visitation. The number of honeybees that visited the marked branch during a 10-min interval was recorded, and this was replicated four times on newly selected flower areas between 10:00 and 11:30, 12:00 and 13:30, 14:00 and 15:30 and 16:00 and 17:30 on different days (Table S1). A visit was recorded when a honeybee touched the stigma of a pear flower, and when the same honeybee visited another new flower it was recorded as another visit. All observations were conducted during dry weather conditions with temperature ranging



**FIGURE 2** Experimental design of three field experiments in Korla fragrant pear orchards. The green trees with/without yellow bars indicate pear trees with/without NGP dispenser, the pink tree line in panel c indicates the apricot trees in a mix pear-apricot orchard. The different panels show: (a) Experiment 2: tree-level effects of NGP on honeybee abundance and pear flower visitation in pear orchards; (b) Experiment 3: Orchard-level effects of NGP on honeybee abundance in pear orchards; and (c) Experiment 4: Effect of NGP on honeybee abundance in mixed pear-apricot orchards. For each experimental design, orchards with and without beehives were selected (not shown).

between 10 and 22°C and wind speeds below 29 km/h. The number of honeybee visits during the four observation periods per tree were pooled for the analysis.

### Experiment 3: Orchard level effects of NGP on honeybee abundance

To assess the effect of NGP on honeybee abundance at the orchard level, we selected 18 new orchards, in nine pairs of two orchards. Paired orchards were located less than 200 m apart, and the minimum distance between two focal orchard pairs was 1.03 km. Two of these orchard pairs consisted of orchards with beehives (4 orchards) while seven pairs (14 orchards) did not have beehives (Figure 2b). Of each orchard pair, one was randomly selected to have NGP lures in all the trees as described previously, while the trees in other orchard did not receive NGP lures and served as a control.

Honeybee abundance was monitored by placing five pan trap stations in a 'X' pattern in five trees in an approximately 54 × 45 m block in the middle of the orchard. The block contained around 100 trees (10 rows × 10 trees per row). Five trees were selected in the four corners and centre of the block, respectively. Pan trap stations were always located two trees away from the edge of the orchard (around 10–12 m). The methodology for NGP lures and pan trap installation and honeybee collection were similar as described in Section 2.3, and honeybees were sampled over a period of 9 days (Table S2).

### Experiment 4: Effect of NGP on honeybee abundance in mixed pear-apricot orchards

To assess the effect of NGP on honeybee abundance in mixed pear-apricot orchards, we selected four pear orchards with a row of apricot trees at the edge of the orchard, and pear trees in the rest



of the orchard (Figure 2c). One orchard contained beehives, and the other three orchards did not. The minimum distance between two focal orchards was 1.45 km. In each apricot-pear orchard, two blocks with same size (approximately  $12 \times 50$  m) were established at a distance of 50 m. Trees in one block received NGP lures and the other block served as a control (Figure 2c). Each block contained around 30 pear trees (3 rows  $\times$  10 trees per row), of which nine trees were selected at distances of 10, 30 and 50 m from the apricot tree row, three adjacent trees at each distance (Figure 2c). In the treatment block, the pan trap stations and NGP lures were established in the nine selected trees, while in the control block only the pan trap stations were set up in nine selected trees. The methodology for NGP lures and pan trap installation and collection were similar as described in Section 2.3, and honeybees were sampled for 9 days (Table S3).

## Data analysis

We conducted five analyses. In the first analysis (Expt 1), honeybee responses to NGP and control in the Y-tube olfactometer experiment was analysed with a Chi-square goodness-of-fit test. In the second analysis (Expt 2), we explored how the abundance of honeybees in pan traps and the number of honeybee flower visits in pear trees (response variables) were influenced by 'NGP' (NGP lures present or absent), 'beehive' (beehives present or absent) and their interaction at single tree level using generalized linear mixed effects models with a negative binomial error distribution. 'Orchard' was included as a random effect. We further explored the influence of NGP lures in subsets of data for orchards with and without beehives using the same model, but without the explanatory variable 'beehive'. For orchards without beehives ( $n = 7$ ) we used a GLMM and for orchards with beehives ( $n = 2$ ) we used a GLM because the inclusion of random effects is not recommended for a low number of sites (Zuur et al., 2009). In the third analysis (Expt 2), we explored the relationship between the total honeybee flower visits per site (response variable) and total number of honeybees in pan traps per site (explanatory variable) using a linear

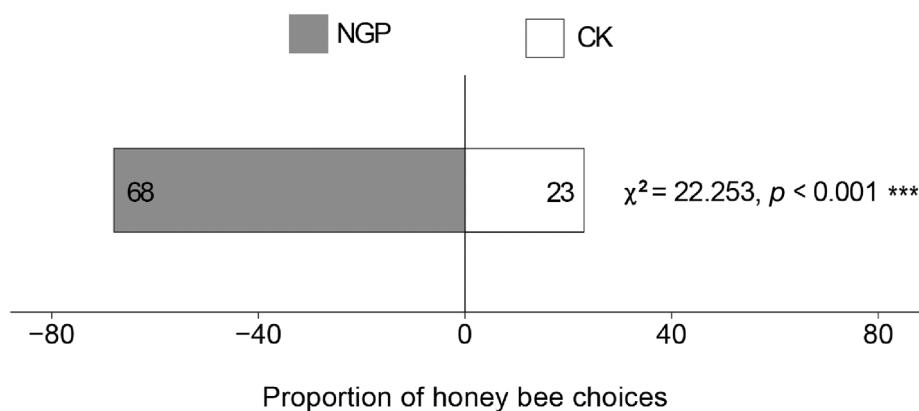
mixed effect model. 'Orchard pair' was included as a random effect. In the fourth analysis (Expt 3), we explored how the abundance of honeybee in pan traps (response variable) was influenced by 'NGP' (orchards with or without NGP lures), 'beehive' and their interaction at orchard level in the same way as in the second analysis. In the fifth analysis (Expt 4) we explored how honeybee abundance in mixed pear-apricot orchards (response variable) were influenced by 'NGP', 'beehive' and 'distance' (distance of sampled pear trees from the apricot tree row), and their interactions in the same way as in the second analysis. We used a model selection procedure using the 'dredge' function to select the most parsimonious model based on the smallest AIC value. The honeybee abundance and honeybee flower visits were all analysed at single tree level, and we assumed the individual trees in each block were independent. In addition, a data-analysis of experiment 4 was conducted using the total count for the nine trees per block in each orchard (i.e., aggregating over the distances and replicate trees per distance). In this case, the datafile comprised eight data records, with for each orchard one record for the total count of bees on the nine trees with NGP and one record for the total count of bees on the nine trees without NGP.

All models were validated using histograms of normalized residuals and plots of residuals against fitted values (Zuur et al., 2009). All calculations and analyses were conducted using R version 3.5.1 (R Core Team, 2018). We used the glmer function of the 'lme4' package (Bates et al., 2015) and the dredge function of the 'MuMIn' package (Bartón, 2017). Means and standard errors of the mean are reported throughout the text.

## RESULTS

### Y-tube olfactometer trial

Of 91 honeybees making a choice between NGP and the control arm of the Y-tube olfactometer, 68 chose NGP and 23 control, indicating significant preference for NGP ( $\chi^2 = 22.253$ ,  $p < 0.001$ ; Figure 3).



**FIGURE 3** Preference of honeybees for the synthetic Nasonov gland pheromone (NGP) treatment and the blank control (CK) in a Y-tube olfactometer

## Experiment 2: Tree-level effects of NGP and beehives on honeybee abundance and pear flower visitation

There was a significant interaction between the NGP treatment and 'beehives' ( $p = 0.033$ ; Table S5). In pear orchards with beehives, the honeybee abundance in pear trees with NGP lures was 2.50-fold higher than in pear trees without NGP lures ( $9.17 \pm 2.18$  vs.  $3.67 \pm 1.43$  individuals per tree,  $p = 0.024$ ), while in pear orchards without beehives, honeybee abundance was not significantly different between trees that had NGP lures or not ( $0.62 \pm 0.20$  vs.  $0.67 \pm 0.21$  individuals per tree,  $p = 0.876$ ; Tables S4 and S6; Figure 4a).

The number of pear flowers visited by honeybees was significantly higher in trees with NGP lures than control trees ( $p = 0.018$ ), and significantly higher in orchards with beehives than without beehives ( $p = 0.014$ ). The interaction between NGP lures and 'beehive' was not significant (Table S5). In pear orchards with beehives, the number of pear flower visits in trees with NGP lures was 5.7-fold higher than in control trees ( $15.17 \pm 5.55$  vs.  $2.67 \pm 1.71$  visits per 40 min,  $p = 0.028$ ), while in pear orchards without beehives, the number of pear flower visits in trees with NGP lures was 27.6-fold higher than in control trees ( $1.38 \pm 0.96$  vs.  $0.05 \pm 0.05$  visits per 40 min,  $p = 0.016$ ; Figure 4b; Tables S4 and S6).

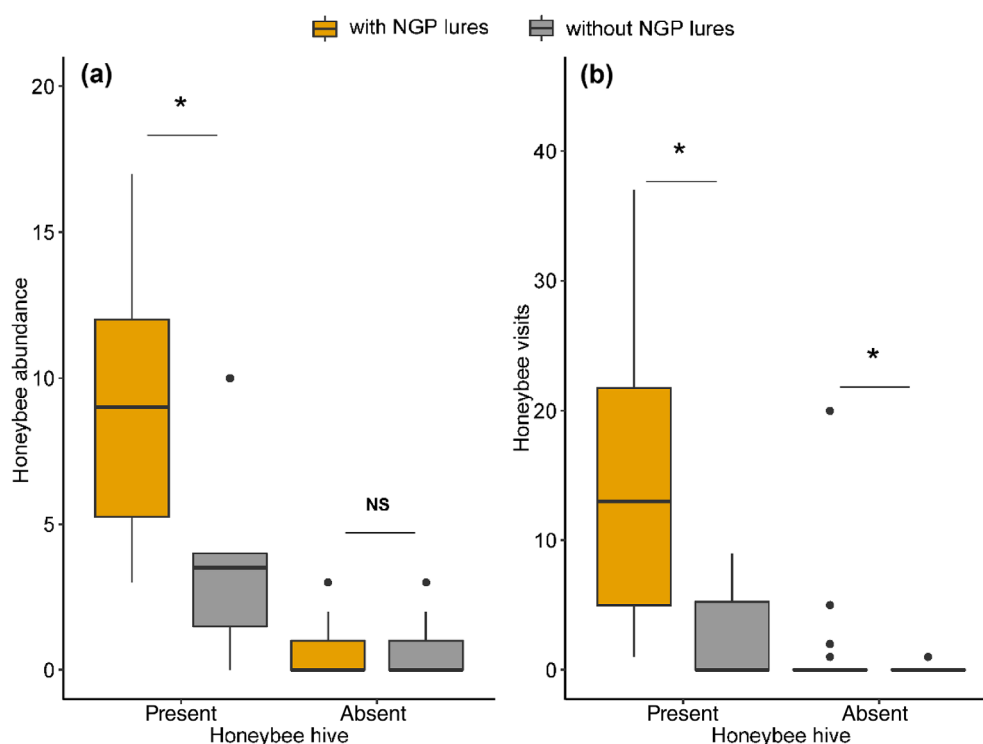
The honeybee flower visits were significantly positively associated with the honeybee abundance in pan traps ( $p < 0.001$ ; Figure 5; Table S7).

## Experiment 3: Orchard-level effects of NGP and beehives on honeybee abundance

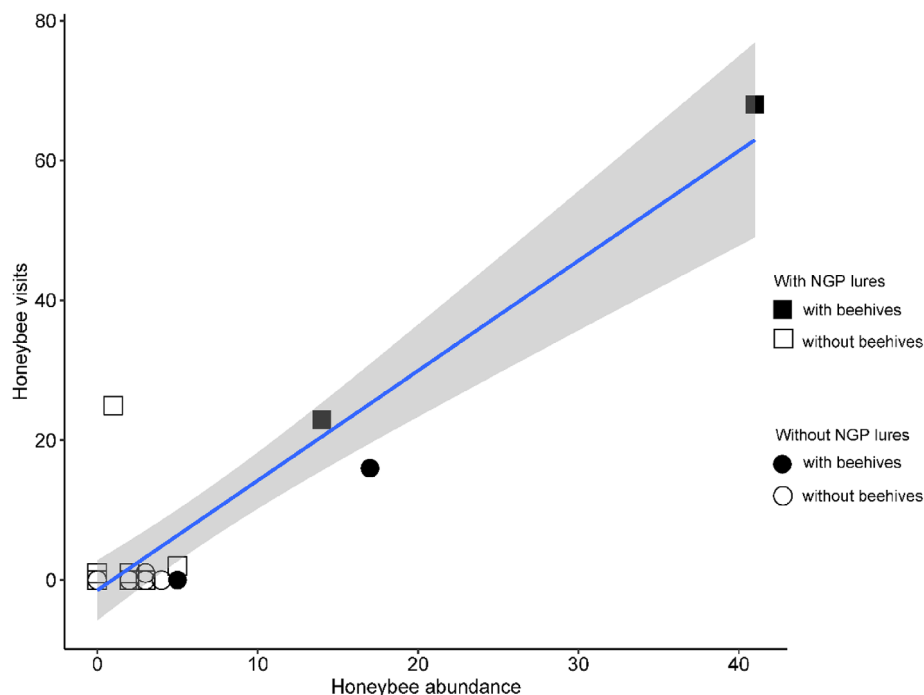
The abundance of honeybees was not significantly influenced by the presence of NGP lures, but was significantly higher in orchards with beehives than without beehives ( $p < 0.001$ ). The interaction between NGP and beehives was not significant (Table S9). However, when the abundance of honeybees was analysed separately for the orchard pairs that contained beehives, the abundance of honeybees in orchards with NGP lures was significantly higher than in orchards without NGP lures ( $8.40 \pm 1.51$  vs.  $3.80 \pm 0.70$  individuals per tree,  $p = 0.003$ ; Tables S8 and S10; Figure 6). In orchards without beehives, honeybee abundance was more than an order of magnitude lower, and the presence of NGP lures did not have a significant influence on the honeybee abundance ( $0.34 \pm 0.11$  [NGP] vs.  $0.31 \pm 0.10$  [control] individuals per tree;  $p = 0.835$ ; Tables S8 and S10; Figure 6).

## Experiment 4: Effects of NGP and beehives on honeybee abundance in mixed pear-apricot orchards

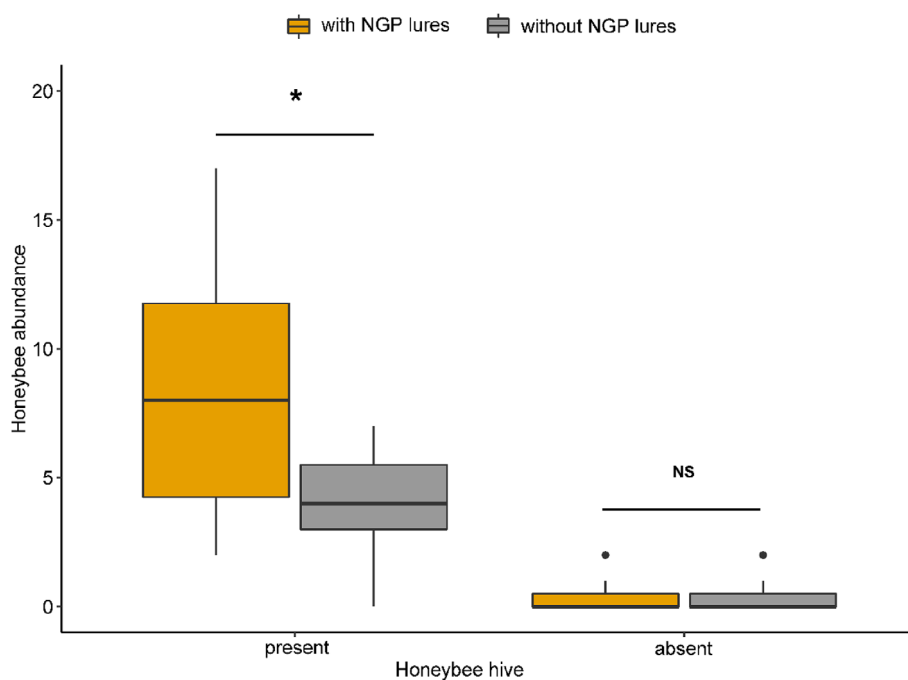
Model selection indicated that the most parsimonious model contained the main effects 'NGP' and 'beehive', without 'distance' and the interaction between NGP and beehive. Honeybee abundance in the block with NGP lures in mixed pear-apricot orchards was significantly higher



**FIGURE 4** Honeybee abundance in pan traps in pear trees (a) and number of pear flower visits by honeybees in 40 min (b) in pear trees that contained three Nasonov gland pheromone (NGP) lures or not, and have beehives (2 orchards) or not (7 orchards) (Expt 2). Asterisks (\* $p < 0.05$ ) and NS ( $p > 0.05$ ) indicate significance levels of the effect of NGP for orchards with and without beehives, respectively (Table S6).



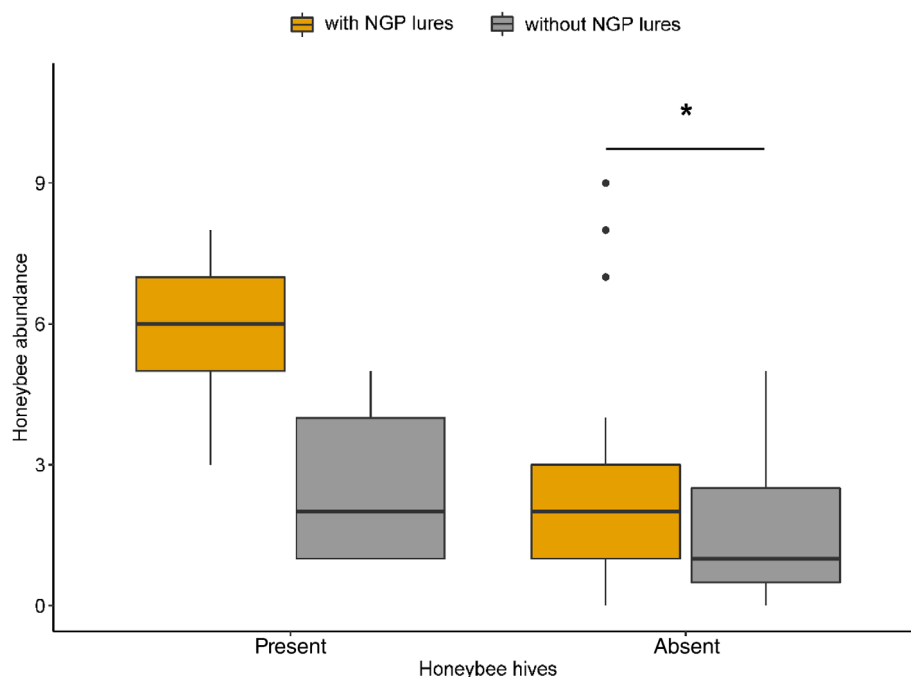
**FIGURE 5** Relationship between the honeybee abundance in pan traps and the number of honeybee visits on pear flowers per orchard in Korla fragrant pear orchards (Expt 2). The regression line indicates a significant relationship ( $p < 0.05$ ), and is based on a linear mixed effect model (Table S7)



**FIGURE 6** Honeybee abundance in pan traps per tree in pear orchards with or without beehives in which all trees have three Nasonov gland pheromone (NGP) lures or not, and have beehives or not (Expt 3). Asterisks ( $*p < 0.05$ ) and NS ( $p > 0.05$ ) indicate significance levels for the effect of NGP for orchards with and without beehives, respectively (Table S10)

than in the blocks without NGP ( $p = 0.019$ ), but not significantly different between orchards with and without beehives (Table S12). The analysis using the total count of bees per block of nine trees in each

orchard confirmed the results of the analysis using single tree data: there was a significant effect of NGP ( $p = 0.007$ ), no significant effect of presence of beehives ( $p = 0.143$ ) and no significant interaction



**FIGURE 7** Honeybee abundance in pan traps per tree in pear orchards with an edge row of apricot trees in which pear trees contained three Nasonov gland pheromone (NGP) lures or not and have beehives or not (Expt 4). Asterisks (\* $p < 0.05$ ) indicate significance levels for the effect of NGP for orchards without beehives (Table S13). The effect of NGP on honeybee abundance in the single orchard with beehives was not tested because of the lack of replication at the orchard level

between NGP and beehives ( $p = 0.288$ ) (Table S13). In the one mixed pear-apricot orchard with beehives, the honeybee abundance in the block with NGP lures was 2.2-fold higher than in the block without NGP lures ( $5.78 \pm 0.55$  vs.  $2.67 \pm 0.55$  individuals per tree, Figure 7). In the three mixed pear-apricot orchards without beehives, the honeybee abundance in the block with NGP lures was 1.6-fold higher than in the block without NGP lures ( $2.56 \pm 0.43$  vs.  $1.59 \pm 0.30$  individuals per tree;  $p = 0.049$ ; Tables S11 and S14; Figure 7).

## DISCUSSION

This is the first study to assess the attractiveness of Nasonov gland pheromone lures to honeybees in Korla fragrant pear orchards in Xinjiang. We found that NGP in pear trees enhanced honeybee abundance in pan traps and pear flower visitation rates, and that this effect was most pronounced in pear orchards that contained beehives. NGP also resulted in higher honeybee abundance in mixed pear-apricot orchards. As insect pollination is a limiting factor in Korla fragrant pear (Li et al., 2022), these findings suggest that NGP has a potential to improve crop pollination by honeybees in this crop.

The Y-tube olfactometer trial confirmed the attractiveness of NGP lures for honeybees, and this was further confirmed in our field experiments in Korla fragrant pear orchards. Placing NGP lures in individual pear trees increased honeybee abundance by 2.5-fold and pear flower visitation by 5.2-fold, but only in orchards with beehives. This aligns with the findings of other studies. For instance, Schmidt (2001) showed that beehives marked with synthetic Nasonov pheromone

were more attractive to honeybees and Mayer, Britt et al., 1989, Mayer, Johansen et al. (1989) found that NGP increased honeybee visitation and fruit set in apple, cherry and pear in the USA. In China, the application of NGP lures increased the honeybee visitation frequency and fruit set in blueberry (Liu et al., 2016) and sweet cheery (Wang et al., 2021). However, in orchards without beehives pear flower visitation rates were low, despite the 27.6-fold higher flower visitation rates in trees with NGP lures than without NGP lures ( $1.38 \pm 0.96$  vs.  $0.05 \pm 0.05$  visits per 40 min). Our findings at the individual tree level were consistent with our findings at the orchard level. When NGP lures were applied to all trees in pear orchards this lead to a 2.2-fold higher honeybee abundance in pan traps compared to orchards without NGP lures, but only when there were beehives in the orchard. When no beehives were present in the orchards the honeybee abundance was low, and most likely constrained fruit set (Li et al., 2022). Obviously, if there are no or only few honeybees present in the orchard, the use of NGP will not be meaningful (Delaplane & Mayer, 2000). Therefore, NGP should be used in combination with the establishment of beehives.

In mixed pear-apricot orchards, honeybee abundance was significantly higher in pear trees with NGP lures than without lures, and this was not influenced by the distance from the apricot tree row. While honeybees aggregate at apricot trees, honeybee densities quickly decline with increasing distance from apricot trees, such that only pear trees in the direct vicinity of apricot trees can benefit from increased honeybee visitation (Li et al, unpublished data). Here, we show that the use of NGP in combination with the presence of early flowering apricot trees leads to a higher honeybee abundance in pan



traps. We did not find a decrease of honeybee abundance at further distance from apricot trees. Possibly, the NGP lures functioned as stepping stones for honeybees, or arrested honeybees that happened to be nearby pear trees with NGP lures. While our experiment does not allow us to draw conclusions about the underlying mechanisms, our findings suggest that the use of NGP in combination with early flowering plant resources may be a promising approach to attract and retain honeybees in Korla fragrant pear orchards.

Here, we did not assess the influence of NGP lures on fruit set, yield and the quality of pears. However, our results show that honeybee abundance in pan traps was strongly correlated with pear flower visitation rate, indicating that honeybee abundance in pan traps is a useful indicator for pear flower visitation. Furthermore, in a 2-year study we showed that honeybee visitation rates were positively associated with initial fruit set and sugar content, but not fruit weight (Li et al., 2022). In addition, the effectiveness of NGP on fruit set and quality has also been reported in Guava and kiwifruit in India (Anita et al., 2012; Jailyang et al., 2022). Therefore, we expect that the use of NGP can improve Korla fragrant pear fruit set and quality.

While the use of NGP can increase the honeybee abundance and pear flower visitation, we cannot exclude the possibility that the use of NGP has side effects on the pollination system in and around the orchards where it is used. There may be at least two mechanisms. First, an enhanced aggregation of honeybees in pear orchards with NGP lures could possibly negatively affect the pollination of plants that flower at the same time as pear, that is, competition among plant species for pollination by honeybees. In the study region, there are relatively few co-flowering plant species around pear orchards, besides apricot, peach and plum. The low flower cover in early spring can be explained by weed management practices in pear orchards, and that the study region is an oasis area in an arid environment, where vegetation in absence of irrigation is sparse. Second, the use of NGP in pear also could increase competition for floral resources of pear among different pollinator groups. The increased visitation of honeybees to pear flowers could result in depletion of nectar in pear and/or increase the interference of different pollinator species (Weekers et al., 2022). However, even though wild pollinator species, such as wild bees, hoverflies and other flies, are common in pear orchards, their pear visitation rates are relatively low as compared to honeybees (Li et al., 2022). Based on this preliminary evaluation, the risks of using NGP in pear orchards are likely to be limited as compared to the risks of, for instance, the high agrochemical input in these orchards. The use of NGP in combination with insecticide applications could potentially be very harmful for pear pollinators, and therefore, NPG lures need to be removed after pear flowering before insecticide applications take place. Further study is needed for a more conclusive risk assessment for the use of NGP in orchards.

A limitation of the study in mixed pear-apricot orchards (Expt 4) was that these types of orchards are not so common in the study area and that we could only include a single pear-apricot orchard with beehives. While the lack of replication at the orchard level prevents drawing statistically underpinned conclusions, the observations in this

orchard provided circumstantial evidence that the use of NGP in mixed pear-apricot orchards with honeybee hives can attract honeybees to pear trees even though honeybees prefer apricot flowers to pear flowers (Lan et al., 2021). However, a replicated study is needed to ascertain whether this is a general pattern or not.

In conclusion, the honeybee attraction function of NGP has been shown in several crops and locations, and can therefore be considered robust. Therefore, NGP has the potential to attract honeybees and increase honeybee visitation, fruit set and quality in pollination-limited fruit crops (Jayaramappa et al., 2011; Ma et al., 2015; Sivaram et al., 2013). Our current results show that NGP is not effective in orchards without beehives, and therefore NGP can best be used in combination with placing honeybee hives in orchards.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Qian Li  <https://orcid.org/0000-0003-3548-647X>

Mengxiao Sun  <https://orcid.org/0000-0001-7411-8691>

Wopke van der Werf  <https://orcid.org/0000-0002-5506-4699>

Felix J. J. A. Bianchi  <https://orcid.org/0000-0001-5947-9405>

Yanhui Lu  <https://orcid.org/0000-0002-6552-0248>

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## SUPPORTING INFORMATION

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

### Data S1. Supporting Information.

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