Comparison of long-term changes in size and longevity of bee colonies in mid-west Japan and Maui with and without exposure to pesticide, cold winters, and mites

[Short Title]
Bee longevity comparison in two regions

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Supplementary Data

Table S1. Experimental conditions of five long-term field experiments.

| Experiment name | 2010 experiment | 2011/2012 experiment | 2012/2013 experiment | 2013/2014 experiment | 2014/2015 experiment (This work) |
|-----------------|-----------------|---------------------|---------------------|---------------------|----------------------------------|
| **Experiment period** | Abe (Oct. 16 to November 23, 2010) (93 days) | From July 9, 2011 to April 28, 2012 (269 days) | From July 20, 2012 to July 26, 2013 (385 days) | From August 13, 2013 to February 19, 2014 (99 days) | From October 22, 2014 to July 20, 2015 (271 days) |
| **Experimental site (altitude and longitude)** | Abe (mid-west Japan, 37º16’10" N, 138º39’10" E) | Same as left | Same as left | Same as left | Abe (mid-west Japan, 37º16’10" N, 138º39’10" E) |
| **Object of Study** | To investigate the possibility for a scenario that ascribes to a CCD | To investigate the possibility for a scenario that ascribes to a CCD | To investigate the possibility for a scenario that ascribes to a CCD | To investigate the possibility for a scenario that ascribes to a CCD | To investigate the possibility for a scenario that ascribes to a CCD |
| **Circumstances around Experimental Site** | Limitation of honey bee activities | No limitation | No limitation | No limitation | No limitation |
| **Impact of other pesticide than the administered on the environment** | None | None | None | None | None |
| **Aerial crop-dusting farmland near exptl. Site** | None | None | None | None | None |
| **Starting time of each observation** | Till colony extinction or from July 18, 2010 to December 21 (213 days) | Till colony extinction or from July 8, 2011 to December 31 (177 days) | Till colony extinction or from July 21, 2012 to August 19, 2013 (343 days) | Till colony extinction or from September 5, 2013 to December 10 (117 days) | Till colony extinction or from October 22, 2014 to April 19, 2015 (163 days) |
| **Vehicle to administrate a pesticide** | Both sugar syrup and pollen paste | Both sugar syrup and pollen paste | Both sugar syrup and pollen paste | Both sugar syrup and pollen paste | Both sugar syrup and pollen paste |
| **Administration method of pesticide** | A pesticide was dissolved in sugar syrup and pollen paste was mixed with toxic sugar syrup containing the pesticide. Bees took toxic sugar syrup and toxic pollen paste was fed into a hive. | A pesticide was dissolved in sugar syrup and pollen paste was mixed with toxic sugar syrup containing the pesticide. Bees took toxic sugar syrup and toxic pollen paste was fed into a hive. | A pesticide was dissolved in sugar syrup and pollen paste was mixed with toxic sugar syrup containing the pesticide. Only toxic sugar syrup was fed into a hive. | A pesticide was dissolved in sugar syrup and pollen paste was mixed with toxic sugar syrup containing the pesticide. Only toxic sugar syrup was fed into a hive. | A pesticide was dissolved in sugar syrup and pollen paste was mixed with toxic sugar syrup containing the pesticide. Only toxic sugar syrup was fed into a hive. |

**Publications of research results**

- T. Yamada et al. (2012). *J. Biol. Ser.* 1(3): 084-107.
- T. Yamada et al. (2013). *J. Biol. Ser.* 1(4): 187-207.
- T. Yamada et al. (2018). *J. Biol. Ser.* 1(4): 99-109.
- T. Yamada et al. (2019). *J. Biol. Ser.* 2(1): 094-101.
- T. Yamada et al. (2019a). *J. Biol. Ser.* 2(2): 106-119.
- T. Yamada et al. (2019b). *J. Biol. Ser.* 2(3): 120-137.
- T. Yamada et al. (2019c). *J. Biol. Ser.* 2(4): 138-151.
- T. Yamada et al. (2020). *J. Biol. Ser.* 3(1): 104-117.
- T. Yamada et al. (2020a). *J. Biol. Ser.* 3(2): 118-129.
- T. Yamada et al. (2020b). *J. Biol. Ser.* 3(3): 130-141.
- T. Yamada et al. (2020c). *J. Biol. Ser.* 3(4): 142-153.
- T. Yamada et al. (2021). *J. Biol. Ser.* 4(1): 154-165.
Table S2. Field experimental procedures.

It is generally difficult to replicate a field experiment including a variety of extraneous disturbance factors. Someone may enable to replicate and our field experiment and check the outcomes, minimizing the uncertainties in the conduction of experiment according to the following procedures.

1) Select an experimental site where there are no crop-dusting area nearby and no animals to cause a great damage to a honeybee colony such as a bear to reduce uncontrollable disturbances.

2) Reduce or compensate the effect of the hive arrangement, for example, by placing control colonies at both sides of experimental colonies.

3) Start a field experiment after the initial size of each colony such as the numbers of adult bees and capped brood becomes almost the same.

4) Conduct a field experiment on a fine or cloudy day because bad weather makes honeybees nervous.

5) Avoid swarming season of honeybees because swarming disturbs the unity of experimental conditions among bee colonies.

6) Begin conducting an experiment just after dawn before foraging bees go out anywhere.

7) Conduct an experiment every two weeks or so if possible because capping period of brood is 12 days.

8) Leave observation results on photographic record as much as possible because the results can be checked again afterwards.

9) Attach a name plate by which prime conditions are described at the front of each hive.

10) Number both sides of each comb and to set each comb at a fixed position to keep the experimental conditions constant.

11) Take a photograph of the whole view of the experimental site.

12) Take a photograph of the front of a hive.

13) Take photographs of both sides of a comb with honeybees while pulling out a comb in comb-number order and afterwards to put in another empty hive which is prepared in advance.

14) Take an enlarged photograph of a queen bee when the queen is found on a comb or inside a hive.

15) Take a photographs the inside of the hive in which honeybees are left (four walls and the bottom) after pulling out every comb from the hive.

16) Take a photograph of the comb without honeybees after shaking them off the comb in comb-number order and to put every comb back where it belonged.

17) Measure the amount of food (sugar syrup, pollen paste) consumed by honeybees by a balance accurate to about 0.1 g.

18) Feed a fixed weight of a new food (sugar syrup only in Maui) in the hive after remove the old food.

19) Take a photograph of the irregularity such as queen cells, hive-beetles, mites (no exists in Maui), wax worm larvae, attacks of Japanese giant hornet (no exists in Maui) and so on during experimenting while recording the results on a datasheet.

20) Record the observational results in the research note after discussing them with experimenters.

21) Add a caption of a photograph of each side of every comb and each wall of the hive-inside to understand the condition on a computer, for example, “20150130 DF3-1B” denotes a photograph of the back side of the first comb of DF-3 (the third colony of dinotefuran-exposed colonies) which was taken on January 30, 2015.
Figure S1. Seasonal change apparent longevity of control colony and pesticide-exposed colony in Shika (midwest Japan).

CR-1, CR-2: Control (pesticide-free) colony, DF: Dinotefuran-exposed colony, CN: Clothianidin-exposed colony, FT: Fenitrothion-exposed colony, MT: Malathion-exposed colony, DF & CN are neonicotinoids, FT & MT are organophosphates. Using the numbers of adult bees and capped brood obtained from 2011/2012, 2012/2013 and 2013/2014 field experiments, apparent longevity in each colony was estimated from a mathematical model proposed by Y. Yamada et al. (2019). A pesticide (DF, CN, FT, MT) is administered to each colony through sugar syrup. Details of the long-term field experiments are described in previous papers (T. Yamada et al., 2018a, 2018b, 2018d). The apparent longevity begin to increase from the end of September with the approach of winter and it drops rapidly just after it reaches its maximum at the end of overwintering. Strong colonies succeed in overwintering through such a course, but weak colonies become extinct during overwintering. Until extinct, the apparent longevity of pesticide-exposed colony (DF, CN, FT, MT) changes in the same way as that of control colony.
Figure S2. Atmospheric temperature (a) and monthly precipitation (b) in Maui and Shika (mid-west Japan). Tmax= Daily maximum temperature; Tmin= Daily minimum temperature; Rainfall= Monthly precipitation. Data are available at https://shortvacation.jp/beach/hawaii/maui/ (Maui) and http://www.data.jma.go.jp/obd/stats/etrn/view/nml_amd_ym.php?prec_no=56&block_no=0564&year=2014&month=06&day=&view=p1, (Shika, mid-west Japan)