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# Honeybee Colony Losses during 2008~2010 Caused by Pesticide Application in Japan

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## Abstract

Recent declining numbers of honeybees, *Apis mellifera*, in colonies threatens crop production as well as feral plant community biodiversity. Chemicals applied in agroecosystems are regarded as one of primary causes of the decline. In Japan, the heavy loss of honeybee colonies associated with pesticide use has been reported from beekeepers recently, leading to lessened use of honeybee colonies to facilitate pollination of cultivates. Nevertheless, no study has quantitatively examined the damage to honeybee colonies caused by the pesticide application in Japanese agricultural fields to date. To explore actual honeybee damage caused by the pesticide use, we performed an extensive survey of honeybee colony loss putatively caused by pesticide application by asking Japanese beekeepers to identify bee losses for three years: 2008~2010. The respective numbers of damaged honeybee hives were 11,659, 11,533 and 8,328. The estimated amounts of damage were ￥201.1 million, ￥253.8 million, and ￥178.0 million for 2008, 2009, and 2010. Heavy losses were concentrated in particular prefectures (Hokkaido, Iwate, and Wakayama) although losses occurred throughout Japan. In Hokkaido, the area of damage was concentrated exclusively in central areas. More than 90% of damage occurred in mid-summer. Pesticide-sprayed crops, suspected of causing the loss, amounted to 20 crops, but 75.0~83.3% of damaged hives, and 86.0~92.3% of damage amounts were of only two crops: rice and orange. Overwhelmingly, neonicotinoids were identified as responsible for the losses, accounting for 91.4% in 2009 and 81.7% in 2010 for damaged hives, and 93.2% in 2009, 92.4% in 2010 for damage amounts.

Key words: Honeybee, *Apis mellifera*, Neonicotinoid, Rice, Pesticide, Insecticide

## INTRODUCTION

Pollination is an important ecological function, contributing to the stability of the feral plant community (Ashman *et al.*, 2004) and facilitating crop production (Klein *et al.*, 2007). Honeybee, *Apis mellifera*, is an efficient natural pollinator of a wide range of flora and staple crops throughout the world (Watanabe 1994; Moritz *et al.*, 2010). Their role in modern agriculture is steadily growing as cultivated areas of pollinator-dependent crops are increasing steadily (Aizen *et al.*, 2008).

Heavy and sudden losses of managed honeybee colonies have been observed in Europe and the USA during the last decade. They have been gaining great attention (Potts *et al.*, 2010). Pesticides, especially new systemic insecticides, neonicotinoids, are suspected of contributing to the losses (Girolami *et al.*, 2009; Bacandritsos *et al.*, 2010; Johnson *et al.*, 2010; Maini *et al.*, 2010), although some studies have contradicted the relation between neonicotinoid application and honeybee loss (Schmuck *et al.*, 2001; Chauzat *et al.*, 2009; Nguyen *et al.*, 2009). Controversy about the role of neonicotinoids is continuing.

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In Japan also, many beekeepers have reported heavy losses of honeybee colonies located near crops to which neonicotinoids have been applied. Characteristics of Japanese honeybee loss, recognized by beekeepers as neonicotinoid-driven, differ in their exposure mechanisms from those which occurred in Europe and the USA. Honeybee losses in Europe and the USA that were putatively attributable to neonicotinoids are inferred to have resulted from neonicotinoid-coated seeds. However, direct exposure to neonicotinoids is reported by beekeepers as a factor of sudden and mass death of bees in Japan. Therefore, investigations of actual damage to Japanese honeybee colonies by neonicotinoid application contribute not merely the addition of one case study but also contribute to elucidation of the adverse effects of neonicotinoids on bee colonies. Although numerous studies of the possible adverse effects by neonicotinoid on honeybee have been conducted throughout the world, and although Japanese beekeepers have repeatedly reported the loss of honeybee colonies, no quantitative study to date has assessed damage by neonicotinoid application. Such a study is crucial for recognizing the problem, tracking trends, and suggesting causes of mortality. It would also facilitate development of future plans to prevent bee loss caused by pesticide application. In this study, we conducted an extensive survey of honeybee loss caused by pesticide application by asking beekeepers to identify the numbers of damaged honeybee colonies, amounts of damage, pesticide-applied crops, applied pesticides, dates, and locations for three years during 2008~2010.

## MATERIAL AND METHODS

To conduct an extensive survey of honeybee damage caused by pesticide application throughout Japan, we sent the following questionnaires to the presidents of beekeeping associations in all prefectures asking beekeepers for the following information: 1) name of branch (some prefectures include multiple branches), 2) numbers of beekeepers reporting pesticide damage, 3) numbers of damaged hives, 4) estimated amounts of damage, 5) the name of pesticides believed to have caused the damage, and 6) pesticide application to crops in the current year. The presidents asked members of beekeeping associations in each prefecture questions 1), 3) ~ 6) above through interviews, phone conversations, or distributed paper questionnaires and summed up the number of beekeepers reporting damage caused by pesticide application for question 2). We sent questionnaires on 15 January 2008, 8 January 2009, and 6 January 2010, and collected responses through 6 February 2008, 29 January 2009, and 31 January 2010, respectively. Total numbers of the association members and member-managed hives were 2516, 2592, and 2687, and 112075, 112433, and 11634, respectively, on 1 January 2008, 2009, and 2010, constituting approximately 50% and 65% of all legally registered beekeepers and managed bee hives in Japan. The number of respondents in each prefecture was unclear. All presidents responded to our inquiries by the deadline except three prefectures (Yamagata, Yamanashi, and Chiba) for the survey of 2008. When only the number of frames with pesticide damage was described, we estimated the number of damaged hives by dividing them by eight because an eight-frame hive is standard for Japanese beekeeping. The estimated amount of damage was not described in an answer sheet of Iwate Pref. in 2009. Therefore, we calculated it by multiplying the number of damaged hives described on the sheet by standard prices given by the Japan Beekeeping Association for the report of bee damage.

To clarify the honeybee damage caused by pesticide application instantly and in greater detail, additional surveys were conducted from 2009. We asked members of beekeeping associations in respective prefectures through their presidents to report, giving as much detail as possible, damage that had been putatively caused by pesticide application at any time a honeybee loss occurred. The format of the bee loss report is as follows:

1. Damage classification a) Varroa mite, b) pesticide, c) wild birds and animals, d) natural disaster, e) other.
2. Date when the loss occurred.

**Table 1.** Standard market prices used to estimate damage amount determined by Japan Beekeeping Association

| Item | Unit | Standard price (yen) |
|---|---|---|
| Big hive (10-frame) | One | 8,000 |
| Small hive (6-frame) | One | 6,000 |
| Junction hive box | One | 5,000 |
| Bee | One frame | 7,500 |
| Frame with full honey, no bees | One | 3,000 |
| Frame with moderate honey, no bees | One | 2,000 |
| Frame with no honey, bees | One | 1,000 |
| Feeder | One | 1,500 |

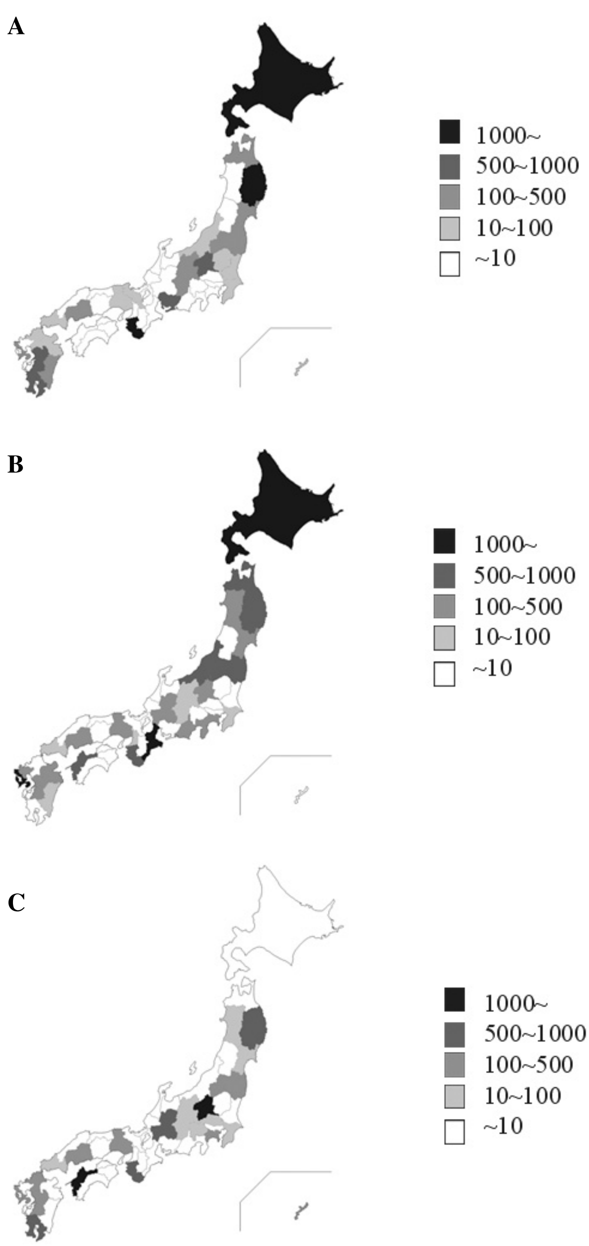


**Fig. 1.** Hives damaged by pesticide application by prefecture in Japan: A, 2008; B, 2009; C, 2010.

**Table 2-a.** Damaged hives and damage amounts associated with pesticide spraying of crops in 2008

| Crop | No. hives | Amount of damage (10,000 yen) |
|---|---|---|
| Rice | 7492.9 | 13964.27 |
| Orange | 1483.6 | 3132.1 |
| Apple | 801.3 | 148.17 |
| Soybean | 598.1 | 895.9 |
| Buckwheat | 225 | 256.7 |
| Asparagus | 101.3 | 1.67 |
| Other* | 76.5 | 128.5 |
| Total | 10778.7 | 18527.31 |

*Crops with number of damaged hives fewer than 100 are grouped together

**Table 2-b.** Damaged hives and damage amounts associated with pesticide spraying of crops in 2009

| Crop | No. damaged hives | Amount of damage (10,000 yen) |
|---|---|---|
| Rice | 6,487 | 15,295 |
| Orange | 1,716 | 3,351 |
| Apple | 520.5 | |
| Leek | 500 | |
| Pine | 350 | 350 |
| Golf course | 250 | 500 |
| Corn | 236.5 | 226.425 |
| Cucumber | 236.5 | 226.425 |
| Strawberry | 160.5 | 6 |
| Asparagus | 131 | 284.5 |
| Fruit | 131 | 284.5 |
| Vegetable | 131 | 284.5 |
| Other* | 82 | |
| Total | 10,931 | 20,808 |

*Crops with number of damaged hives fewer than 100 are grouped together

**Table 2-c.** Damaged hives and damage amounts associated with pesticide spraying of crops in 2010

| Crop | No. hives | Amount of damage (10,000 yen) |
|---|---|---|
| Orange | 3351.5 | 1995 |
| Rice | 3208.5 | 13317.2 |
| Tea plant | 497.5 | 356.5 |
| Bean | 158.5 | 288.5 |
| Strawberry | 158.5 | 288.5 |
| Apple | 129.7 | 623.7 |
| Buckwheat | 122.7 | 114.2 |
| Corn | 122.7 | 114.2 |
| Cucumber | 122.7 | 114.2 |
| Soybean | 122.7 | 114.2 |
| Other* | 86.3 | 469 |
| Total | 8081.3 | 17795.2 |

*Crops with number of damaged hives fewer than 100 are grouped together

**Table 3-a.** Damaged hives and damage amounts by beekeeper-identified applied pesticides causing honeybee losses in 2009

| Pesticide | Chemical family | No. damaged hives | Amount of damage (10,000 yen) |
|---|---|---|---|
| Clothianidin | Neonicotinoid | 4,902 | 13,635 |
| Dinotefuran | Neonicotinoid | 995.75 | 1620.5 |
| Neonicotinoid | Neonicotinoid | 568 | 356 |
| Acephate | Organophosphorus | 231 | 584.5 |
| Silafluofen | Pyrethroid | 131 | 384.5 |
| Acetamiprid | Neonicotinoid | 100 | 200 |
| Imidacloprid | Neonicotinoid | 100 | 200 |
| Spinosad | Organophosphorus | 100 | 200 |
| DBEDC | Organocopper | 80.75 | 3 |

**Table 3-b.** Damaged hives and damage amounts by beekeeper-identified applied pesticides causing honeybee losses in 2010

| Pesticide | Chemical family | No. damaged hives | Amount of damage (10,000 yen) |
|---|---|---|---|
| Clothianidin | Neonicotinoid | 2234.3 | 2207.3 |
| Dinotefuran | Neonicotinoid | 1105.3 | 645.8 |
| Herbicide | Herbicide | 585 | |
| Imidacloprid | Neonicotinoid | 277 | 518 |
| Fipronil | Phenylpyrazole | 147 | 288 |
| Neonicotinoid | Neonicotinoid | 147 | 288 |
| Ferimzine fthalide | Germicide | 110 | 13.5 |
| Total | | 4605.6 | 3960.6 |

**Table 4.** Damaged hives and damage amounts from pesticide application by month as reported in additional surveys in 2009 and 2010

| Month | No. damaged hives | | Amount of damage (yen) | |
|---|---|---|---|---|
| | Year | | Year | |
| | 2009 | 2010 | 2009 | 2010 |
| Jan. | 0 | 16 | 0 | 1,216,000 |
| Feb. | 0 | 29 | 0 | 2,609,333 |
| Mar. | 0 | 16 | 0 | 1,216,000 |
| Apr. | 0 | 0 | 0 | 0 |
| May | 0 | 0 | 0 | 0 |
| Jun. | 0 | 0 | 0 | 0 |
| Jul. | 2 | 417 | 237,500 | 14,100,000 |
| Aug. | 1,908 | 1,198 | 58,045,500 | 78,021,250 |
| Sep. | 0 | 0 | 0 | 0 |
| Oct. | 0 | 0 | 0 | 0 |
| Nov. | 0 | 0 | 0 | 0 |
| Dec. | 0 | 0 | 0 | 0 |

3. Place.
4. Outline of the damage (free description).
5. Details of the damage (number of damaged elements, amount of damage) to a) hives, b) bees, c) frames, d) feeders, e) lost revenues or other quantifiable damage (honey, bees for pollination, etc.).

The standard price used to estimate the amount of damage was determined according to the standard market prices for materials or products (Table 1).

When beekeepers gave multiple answers for one section, we divided the corresponding numbers of hives or amounts of damage equally by the number of answers and assigned them to each element on summation by month, applied pesticide, and pesticide-sprayed crops for all surveys.

Outline maps for Fig. 1 and 2 were downloaded at CraftMAP (http://www.craftmap.box-i.net/).

## RESULTS

The survey revealed common definite characteristics for the three years. Honeybee damage caused by pesticide application was concentrated geographically in


**Fig. 2.** Local area where beekeeper reported a honeybee hive damaged by pesticide application in Hokkaido: A, 2009; B, 2010.

particular prefectures. Of damaged hives, 58.6, 27.4, and 15.1% were in three prefectures (Hokkaido, Iwate, and Wakayama) in 2008, 2009, and 2010, respectively, accounting for 66.6, 33.1, and 59.7% of the respective amounts of damage in those years, although losses occurred throughout Japan (Fig. 1). In addition, even in Hokkaido, which reported the most severe bee damage in Japan, the damage-reporting areas are clustered in the central region (Fig. 2). Rice topped the list of crops suspected by beekeepers to have had pesticides applied, in terms of number of damaged hives and amount of damage, except for the amount of damage reported in 2010 (Table 2). Bee damage caused by pesticides applied to rice fields accounted for more than 70% of all pesticide damage for the amounts of damage in the three survey years (Table 2). Orange was the second most common crop reported as causing the bee loss (Table 2). Of pesticides reported by beekeepers as causing the bee loss, neonicotinoids were reported overwhelmingly as the chemicals, accounting for 91.4 and 81.7% of damaged hives in 2009 and 2010, respectively, and 93.2 and 92.4% for amounts of damage in 2009 and 2010 (Table 3). Almost all losses were reported in July and August in respective years (Table 4).

## DISCUSSION

Pollinators play an important ecological and economic role in wild plant communities and in agriculture. Recent declines in pollinator populations therefore constitute an important concern worldwide, possibly engendering severe ecological and economical impacts. In fact, parallel declines of pollinator-dependent plant species with pollinators have been reported (Biesmeijer *et al.*, 2006). Of pollinators, *A. mellifera* are efficient pollinators, facilitating crop production in a wide range of agricultural fields and also pollinating many feral plant species (Watanabe 1994; Moritz *et al.*, 2010). Similarly to those of other wild pollinators, numbers of managed honeybee colonies have dropped recently (Potts *et al.*, 2010). Agrochemicals are regarded as a primary factor contributing to the decline (Bacandritsos *et al.*, 2010; Johnson *et al.*, 2010; Maini *et al.*, 2010), although many studies have rejected agrochemical effects on honeybee loss (Schmuck *et al.*, 2001; Chauzat *et al.*, 2009; Nguyen *et al.*, 2009).

Of pesticides applied in agricultural fields, neonicotinoids, new systemic insecticides, have garnered particular attention because pollen and nectar of crops contain neonicotinoid chemicals (Laurent and Rathahao, 2003; Chauzat *et al.*, 2006), potentially threatening bees, and heavy losses of honeybee colonies located near crops treated with neonicotinoids (imidacloprid) have been reported (Rortais *et al.*, 2005). Concerns about possible side effect of some neonicotinoids on bees have led to government restrictions in France (Bonmatin *et al.*, 2005).

In Japan, no extensive survey of bee loss caused by pesticide application has been reported in the literature to date, although some beekeepers have implied that bee loss attributed to pesticide application increased rapidly along with the expanded use of neonicotinoids. This study quantitatively revealed actual conditions of bee loss putatively caused by pesticide application in Japan by soliciting information from beekeepers throughout the country. Three-year survey results show the following. 1) Bee loss putatively caused by pesticides occurred primarily in northern Japan, especially in central parts of Hokkaido. 2) Neonicotinoids sprayed on rice fields in mid-summer exclusively account for Japanese bee loss. 3) The degree of the loss did not vary much among years. These results quantitatively support beekeepers' contention and demonstrate that the losses caused by pesticides have not abated during these three years.

This study was conducted by asking beekeepers to identify factors, degrees of damage, pesticide-applied

**Appendix 1-a.** Honeybee damage caused by pesticide application reported in the 2008 survey

| Prefecture | Branches | Beekeepers | Hives | Amount of damage (10,000yen) | Pesticide-applied crop |
|---|---|---|---|---|---|
| Hokkaido | All | 47 | 4547 | 5428 | Rice |
| Aomori | 2 | 4 | 390 | 200 | Rice, Apple |
| Iwate | 3 | 19 | 1010 | 93 | Apple, Rice |
| Miyagi | 1 | 4 | 153 | 82 | Rice |
| Akita | 1 | a few | Unclear | Unclear | Unclear |
| Yamagata | Not done | | | | |
| Fukushima | 1 | 2 | 304 | 5+$\alpha$ | Rice, Apple, Asparagus |
| Ibaragi | 1 | 2 | 60 | Unclear | Rice |
| Tochigi | 2 | 11 | 850 | 1700 | Unclear |
| Gunma | 2 | 3 | 45 | 150+$\alpha$ | Fruit, Rice, Konjak |
| Saitama | 0 | 0 | 0 | 0 | |
| Chiba | 1 | 1 | 23 | Unclear | Lettuce |
| Tokyo | 1 | 1 | Unclear | Unclear | |
| Kanagawa | 0 | 0 | 0 | 0 | |
| Niigata | 1 | 1 | 20 | 20 | Various |
| Toyama | 0 | 0 | 0 | 0 | |
| Ishikawa | 0 | 0 | 0 | 0 | |
| Fukui | 0 | 0 | 0 | 0 | |
| Yamanashi | Not done | | | 0 | |
| Nagano | 3 | 3 | 178 | 270 | Rice |
| Gifu | 0 | 0 | 0 | 0 | |
| Shizuoka | 2 | 2 | 2 | Unclear | Strawberry, Pea, Rice, Orange |
| Aichi | 3 | 13 | 862 | 1220 | Soybean, Orange, Rice |
| Mie | 0 | 0 | 0 | 0 | |
| Shiga | Not done | | | 0 | |
| Kyoto | 1 | 1 | 10 | 15 | |
| Osaka | 0 | 0 | 0 | 0 | Rice |
| Hyogo | 1 | 1 | 20 | 60 | Rice |
| Nara | 0 | 0 | 0 | 0 | |
| Wakayama | 3 | 27 | 1300 | 4168 | Rice, Orange |
| Tottori | 0 | 0 | 0 | 0 | |
| Shimane | 0 | 0 | 0 | 0 | |
| Okayama | 0 | 0 | 0 | 0 | |
| Hiroshima | 2 | 9 | 290 | 710 | Rice |
| Yamaguchi | 2 | 3 | 70 | 330 | Rice |
| Tokushima | 0 | 0 | 0 | 0 | |
| Kagawa | Unclear | 0 | 0 | 0 | |
| Ehime | All | Unclear | Unclear | Unclear | Rice, Orange |
| Kohchi | Unclear | 0 | 0 | 0 | |
| Fukuoka | 2 | 2 | 90 | 110 | Rice, Soybean, Orange, Potato |
| Saga | 0 | 0 | 0 | 0 | |
| Nagasaki | 2 | 5 | 105 | Unclear | Orange |
| Kumamoto | 3 | 10 | 675 | 770 | Rice, Orange, Buckwheat |
| Oita | 2 | 4 | 30 | 60 | Rice |
| Miyazaki | 2 | 2 | 115 | 115 | Soybean |
| Kagoshima | 2 | 6 | 520 | 1040 | Rice, Soybean, Orange |
| Okinawa | 1 | 1 | 20 | 10 | Orange |
| Total | 47 | 183 | 11659 | 16401 | |

crops, and applied pesticides. Neither chemical analysis nor epidemic research was conducted. Honeybee loss caused by pesticide application, however, is characterized by a mass of dead individuals piled in front of the hive entrance. Such a large number of corpses are only rarely observed without pesticide damage. In addition, mass death occurred within 24hr following pesticide spraying (Matsumoto *et al.* submitted). Consequently, identification by beekeepers can be regarded as trustworthy.

**Appendix 1-b.** Honeybee damage caused by pesticide application reported in the 2009 survey

| Prefecture | Branches | Beekeepers | Hives | Amount of damag (10,000 yen) | Applied pesticide | Pesticide-applied crop |
|---|---|---|---|---|---|---|
| Hokkaido | 1 | 9 | 1,451 | 3,681 | Clothianidin | Rice |
| Aomori | 2 | 6 | 568 | 356 | Neonicotinoid | Rice, Apple |
| Iwate | 5 | 19 | 946 | 906 | Dinotefuran, Clothianidin | Corn, Cucumber, Apple, Rice |
| Miyagi | 3 | 5 | 323 | 12 | Ethiprole, Clothianidin, Dinotefuran, DBEDC | Rice, Strawberry |
| Akita | 3 | 5 | 230 | 674 | Dinotefuran | Rice |
| Yamagata | 6 | 6 | 450 | 30-100% | Unclear | Rice |
| Fukushima | 4 | 11 | 524 | 1,138 | Dinotefuran, Clothianidin, Silafluofen, Acephate | Rice, Asparagus, Vegetable, Fruit |
| Ibaragi | | | | | | |
| Tochigi | 4 | 8 | 450 | 1,250 | Unclear | Rice |
| Gunma | unclear | | | | | |
| Saitama | 1 | 1 | 3 | Unclear | Unclear | Rice |
| Chiba | 1 | 1 | 70 | Unclear | Unclear | Rice |
| Tokyo | 1 | | | | | |
| Kanagawa | 1 | 3 | 120 | 420 | Unclear | Unclear |
| Niigata | 1 | Many | 500 | Unclear | Unclear | Unclear (leek) |
| Toyama | 1 | 1 | 4 | 14 | Unclear | |
| Ishikawa | Unclear | | | | | |
| Fukui | 0 | | | | | |
| Yamanashi | 0 | | | | | |
| Nagano | 1 | 1 | 81 | 106 | Dinotefuran | Rice |
| Gifu | 1 | 3 | 250 | 500 | Clothianidin | Golf course |
| Shizuoka | 1 | 12 | 300 | Unclear | Unclear | Orange |
| Aichi | 0 | | | | | |
| Mie | 3 | 18 | 1,050 | 1,050 | Unclear | Orange, Rice, Pine |
| Shiga | Unclear | | | | | |
| Kyoto | 0 | | | | | |
| Osaka | 1 | 1 | 30 | Unclear | | |
| Hyogo | 3 | 7 | 116 | 232 | | |
| Nara | 0 | | | | | |
| Wakayama | 3 | 20 | 768 | 3,801 | Clothianidin, Unclear | Orange, Rice |
| Tottori | 0 | | | | | |
| Shimane | Unclear | | | | | |
| Okayama | Unclear | | | | | |
| Hiroshima | 1 | 5 | 350 | 3,500 | | |
| Yamaguchi | 1 | 2 | 30 | 60 | Clothianidin | Rice |
| Tokushima | 0 | | | | | |
| Kagawa | 0 | | | | | |
| Ehime | 1 | 60 | 500 | 1,000 | Clothianidin, Acephate, Spinosad, Imidacloprid, Acetamiprid | Orange |
| Kohchi | 0 | | | | | |
| Fukuoka | 0 | | | | | |
| Saga | 1 | 4 | 100 | 100 | Unclear | Orange |
| Nagasaki | 1 | 78 | 1,910 | 6,327 | Clothianidin | Rice |
| Kumamoto | 2 | 2 | 110 | 110 | Unclear, Clothianidin | Rice |
| Oita | 5 | 8 | 245 | | Unclear | Rice, Orange, Plum, Japanese Plum |
| Miyazaki | 3 | 4 | 74 | 142 | Unclear, Clothianidin | Unclear, Rice |
| Kagoshima | 1 | 1 | Unclear | Uunclear | | |
| Okinawa | 0 | | | | | |
| Total | 63 | 301 | 11,553 | 25,379 | | |

Direct exposure to neonicotinoids is primary factor causing bee loss in Japan, although neonicotinoids are implicated because of their systemic use in Europe and the United States (Laurent and Rathathao, 2003; Chauzat *et al.*, 2006). However, neonicotinoid concentrations in pollen and nectar observed in fields are insufficiently high to affect honeybee viability (Schmuck *et al.*, 2001; Bailey *et al.*, 2005; Cutler and Scott-Dupree

**Appendix 1-c.** Honeybee damage caused by pesticide application reported in the 2010 survey

| Prefecture | Branches | Beekeepers | Hives | Amount of damage (10,000 yen) | Applied pesticide | Pesticide-applied crop |
|---|---|---|---|---|---|---|
| Hokkaido | 1 | 18 | | 9,186 | Unclear | Rice |
| Aomori | 0 | 0 | 0 | 0 | | |
| Iwate | 5 | 16 | 736 | 685 | Clothianidin, Dinotefuran, Unclear | Rice, Soybean, Corn, Buckwheat, Apple, Cucumber |
| Miyagi | 2 | 2 | 22 | 66 | Clothianidin | Rice |
| Akita | 1 | 1 | 20 | | Clothianidin, Dinotefuran | Rice |
| Yamagata | 0 | 0 | 0 | | | |
| Fukushima | 2 | 5 | 220 | 27 | Unclear, Clothianidin, Ferimzine fthalide | Rice, Unclear, Asparagus |
| Ibaragi | 0 | 0 | 0 | 0 | | |
| Tochigi | 3 | 16 | 1,170 | Unclear | Dinotefuran, Herbicide | Rice |
| Gunma | Unclear | Unclear | Unclear | Unclear | Unclear | |
| Saitama | 1 | 5 | 15 | Unclear | Clothianidin | Rice |
| Chiba | 3 | 6 | 95 | Unclear | Unclear | |
| Tokyo | 0 | 0 | | 0 | | |
| Kanagawa | 0 | 0 | 120 | 0 | | |
| Niigata | All | Some | Some | Unclear | Unclear | Vegetable |
| Toyama | 0 | 0 | 0 | 0 | | |
| Ishikawa | 0 | 0 | 0 | 0 | | |
| Fukui | 0 | 0 | 0 | 0 | | |
| Yamanashi | 3 | 3 | 13 | 460 | Unclear | Peach, Grape |
| Nagano | 2 | 3 | 14 | 119 | Unclear | Apple, Rice |
| Gifu | 4 | 11 | 735 | 1,440 | Neonicotinoid, Fipronil, Clothianidin, Imidacloprid, Unclear | Rice, Unclear |
| Shizuoka | 1 | 1 | 6 | 68 | Unclear | |
| Aichi | 0 | 0 | 0 | 0 | | |
| Mie | 0 | 0 | 0 | 0 | | |
| Shiga | Unclear | | 0 | | | |
| Kyoto | 0 | 0 | 0 | 0 | | |
| Osaka | 0 | 0 | 0 | 0 | | |
| Hyogo | 3 | 7 | 260 | 460 | Imidacloprid, Dinotefuran | Rice |
| Nara | 0 | 0 | 0 | 0 | | |
| Wakayama | 5 | 37 | 634 | 1,154 | Unclear, Clothianidin | Strawberry, Orange, Rice, Bean |
| Tottori | 0 | 0 | 0 | 0 | | |
| Shimane | 0 | 0 | 0 | 0 | | |
| Okayama | 0 | 0 | 0 | 0 | | |
| Hiroshima | 2 | 3 | 100 | 260 | Clothianidin | Rice |
| Yamaguchi | 1 | 40 | 68 | 102 | Unclear | Unclear |
| Tokushima | 0 | 0 | 0 | 0 | | |
| Kagawa | 0 | 0 | 0 | 0 | | |
| Ehime | all | 70 | 3,000 | 1,500 | Unclear | Orange |
| Kohchi | 0 | 0 | 0 | 0 | | |
| Fukuoka | 1 | 8 | 270 | 375 | Clothianidin, Dinotefuran | Rice, Orange |
| Saga | 1 | 1 | 5 | 10 | Unclear | Orange |
| Nagasaki | 1 | 1 | 280 | 1,190 | Unclear | Rice |
| Kumamoto | 2 | 6 | 106 | 18 | Unclear | Orange, Unclear |
| Oita | 0 | 0 | 0 | 0 | | |
| Miyazaki | 1 | 1 | 20 | 30 | Unclear | Tea plant |
| Kagoshima | 4 | 20 | 955 | 653 | Clothianidin | Tea plant, Rice |
| Okinawa | 1 | 1 | 200 | | Clothianidin | |
| Total | 50 | 282 | 8,328 | 17,802 | | |

2007; Chauzat *et al.*, 2009; Bernal *et al.*, 2010; Chauzat *et al.*, 2010; Mullin *et al.*, 2010) although concentrations in guttation fluid are above the lethal threshold (Girolami *et al.*, 2009): a fact which calls for future research. In addition, direct exposure to neonicotinoids through aerial powder during sowing in seed-coated maize fields led to lethal effects in honeybees in Italy (Marzaro *et al.*, 2011). Consequently, as in the Japanese case, neonicotinoids used in Europe and the United States might impose severe harmful effects on honey-

**Appendix 2-a.** Details of damage from pesticide application reported in an additional survey in 2009

| Prefecture | Date | Place | Item | Amount | Amount of damage (yen) | Outline of damage (free description) |
|---|---|---|---|---|---|---|
| Hokkaido | Aug. 20 | Biei | Bees | 280 frames | 2,100,000 | |
| | Aug. 20 | Higashikaguraoka | Bees | 960 frames | 7,200,000 | |
| | Aug. 20 | Higashikaguraoka | Bees | 900 frames | 6,750,000 | |
| | Aug. 16-24 | Wassamu, Asahikawa | Bees | 1,050 frames | 7,875,000 | 350 hives |
| | Aug. 16-25 | Shibetsu | Bees, frames | 15 hives | 340,000 | |
| | Mid-Aug. | Wassamu, Shibetsu, Kenbuchi | Bees | 280 frames | 210,000 | |
| | Aug. 21 | Wassamu | Bees | 180 frames | 1,350,000 | |
| | | | Frames | 60 frames | 120,000 | |
| | | | Cost for feeding | 60 hives | 48,000 | |
| | Aug. 22-26 | Shibetsu | Bees | 450 frames | 3,375,000 | 90 hives |
| | Mid, late Aug. | Wassamu, Shibetsu | Honey | 450 kg | 562,500 | 400 hives |
| | | | Bees | 1,200 frames | 5,400,000 | |
| | Aug. 14-16, 20 | Nayoro | Honey | 160 hives | 6,400,000 | |
| | | | Bees | 320 hives | 4,160,000 | |
| | | | Bees | 1200 frames | 9,000,000 | |
| Iwate | Aug. 10-18 | Tohno | Bees | 48 hives | 1,080,000 | Approximately 40% of workers in each hive died A damaged colony, howe ver, can recover because young workers tend to survive. |
| | Aug. 12 | Shiwa | Bees | 5 frames | 37,500 | Beekeeping site is about 3.5km distant from the closest rice field. |
| | Aug. 14 | Hachimandaira | Bees | 900 frames | | Insecticide was probably sprayed on 12 Aug. Sixty hives suffered fatal damage. |
| | Aug. 15 | Waga | Bees | 150 frames | | Approximately 40% of workers in each of 50 hives suffered from pesticide poisoning. |
| | Aug. 15 | Kanegasaki | Frames | 60 frames | | Two frames of each hive were damaged. |
| | Aug. 16 | Kamaishi | Hives | 81 hives | | Approximately 50% of workers in each of 37 hives died. |
| | | | Bees | 729 frames | | |
| | | | Frames | 729 frames | | |
| | Aug. 18, 19 | Kanegasaki | | unclear | unclear | Two frames (approximately 4000 workers) in each hive died because of pesticide application. |
| | Aug. 21 | Hanamaki | Hives | 23 hives | | Loss caused by insecticide application for stinkbug control |
| | | | Frames | 92 frames | | |
| | Aug. 17 | Ninohe | Bees | 100 frames | 500,000 | Ten to 20% of workers died in 50 hives of 70 managed hives. |
| Miyagi | Aug. 9-18 | Ohsaki | Bees | 20 hives | 400,000 | Mass death began from 9 Sep. 2009. The number of dead workers varied by date with the maximum of more than 300. Insecticide was sprayed on 16 Aug. |
| Fukushima | Aug. 17 | Minamiaizu | Bees | 120 frames | 900,000 | |
| Nagano | late-Jul.-Aug. 10 | Kiso | Bees | 30 frames | 225,000 | Old workers likely died. |

**Appendix 2-b.** Details of damage suffered from pesticide application reported in an additional survey in 2010.

| Prefecture | Date | Place | Item | Amount | Amount of damage (yen) | Outline of damage (free description) |
|---|---|---|---|---|---|---|
| Hokkaido | Aug. 1 | Sapporo | Bees | 120 frames | 600,000 | Dead workers were observed in front of hive entrances of 30 of 40 hives |
| | | | Honey | 312kg | 455,000 | |
| | Jul. 30 | Sapporo | Bees | 120 frames | 600,000 | |
| | Aug. 9 | Sapporo | Hives | 20 hives | 0 | |
| | | | Bees | 40 frames | 200,000 | Colony size dropped |
| | | | Frames | 40 frames | 0 | |
| | | | Honey | 720kg | 700,000 | |
| | Aug. 6 | Ishikari | Bees | 150 frames | 750,000 | Worker numbers dropped sharply |
| | Aug. 10 | Sapporo | Bees | 12 frames | 60,000 | Many dead workers were observed in front of the hive entrance |
| | Aug. 18 | Ishikari | Hives | 15 hives | | |
| | | | Bees | 80 frames | 400,000 | Bee loss was probably caused by stink-bug control in rice fields |
| | | | Frames | 80 frames | | |
| | | | Honey | 54 liters | 120,000 | |
| | Aug. 1-5 | Shibetsu | Bees | 800 frames | 6,000,000 | |
| | | | Honey | | 4,000,000 | |
| | Aug. 12 | Kamikawa | Bees | 180 frames | 2,250,000 | |
| | | | Frames | 120 frames | | |
| | | | Honey | 360kg | 700,000 | |
| | Aug. 1-19 | Kamikawa | Bees | 1,200 frames | 9,000,000 | |
| | | | Honey | 360kg | 6,000,000 | Colony sizes reduced 30,000 to 8000 |
| | | | Royal jelly | 15kg | 1,800,000 | |
| | Aug. 13-16 | Wassamu | Bees | 140 frames | 1,050,000 | Approximately 2000-4000 workers died in each hive |
| | | | Bees for pollination | 40 hives | 800,000 | |
| | Jul. 25-Aug. 7 | Kamikawa, Shibetsu | Bees | 1,300 frames | 9,750,000 | |
| | | | Honey | 3,600kg | 7,000,000 | |
| | Aug. 14 | Shibetsu | Bees | 1,400 frames | 10,500,000 | |
| | | | Honey | 1440kg | 2,100,000 | |
| | | | Royal jelly | 15kg | 1,800,000 | |
| | Aug. 3-17 | Nayoro | Bees | 1,560 frames | 11,700,000 | Colony size dropped |
| | | | Honey | | 3,885,000 | |
| | | | Bees | | 2,520,000 | |
| | Jul. 20 | Nayoro | Hives | 120 hives | | Colony growth rate was reduced |
| | | | Frames | 360 frames | 1,800,000 | |
| | Jul. 20 | Nayoro | Hives | 160 hives | | No honey was harvested |
| | | | Honey | 450kg | 925,000 | |
| | Jul. 28 | Hiratori | Bees | 320 frames | 2,400,000 | |
| | Aug. 20 | Urakawa | Bees | 50 frames | 375,000 | 10 hives |
| | Aug. 12 | Abira | Bees | 200 frames | 1,500,000 | |
| | | | Frames | 60 frames | 120,000 | |
| Nagano | Aug. 29 | Nagano | Bees | 1,415 frames | 1,061,250 | Dead workers were observed in front of every hive, although the number varied among the hives. Bees have died one after another since the first discovery of dead workers |
| Fukuoka | Jan. 10- Mar. 24 | Chikujo | Bees | 384 frames | 2,880,000 | Bees have been killed gradually |
| | | | Frames | 384 frames | 768,000 | |
| Miyazaki | Feb. | Miyakonojo | Hives | 14 hives | 123,333 | |
| | | | Bees | 114 frames | 850,000 | |
| | | | Frames | 67 frames | 66,667 | |
| | | | Feeder | 14 | 20,000 | |
| | | | Honey | 120kg | 333,333 | |

bees not by systemic features but by direct exposure.

Low concentrations of neonicotinoids within pollen and nectar observed in field do not always indicate a lack of effects on bees. Sublethal effects must be considered. Sublethal effects are apparent in either dosed colonies or in individuals by modified performance in aspects of growth, fecundity, longevity, or behavior. They have been overlooked to date (Desneux *et al.*, 2007). Sublethal effects on bees caused by neonicotinoid exposure below the lethal threshold have been reported frequently, affecting longevity and development (Wu *et al.*, 2011), learning ability (Decourtye *et al.*, 2004; 2005; Aliouane *et al.*, 2009), homing flight (Yang *et al.*, 2008), and foraging activity (Ramirez-Romero *et al.*, 2005). Prevention of colony growth and degraded honey and royal jelly production caused by putative sublethal effects of pesticides must also be examined, although beekeepers are most likely to notice mass death of bees as bee loss attributable to pesticide use. Particularly, interaction of Nosema disease with neonicotinoid nonlethal exposure, as described by Alaux *et al.* (2010) and Vidau *et al.* (2011), should be given greater attention.

Japanese bee loss attributed to pesticide application is concentrated geographically in the central part of Hokkaido. Moreover, it can be attributed to pesticides applied to a particular crop, rice, for several reasons. First, many Japanese beekeepers transferred their hives to Hokkaido for its flowers (ex. *Tilia japonica*, *Robinia pseudoacacia* and *Trifolium repens*), and cool, rainy-season-free weather in summer. Secondly almost all pest control during crop flowering period within agricultural fields in summer is applied within rice fields in the middle part of Hokkaido (Matsumoto, personal observation), although rice fields occupy less than 20% of the total agricultural area in Hokkaido (MAFF, 2011a). Furthermore, the central part of Hokkaido is a prominent rice production area, with rice planted acreage accounting for more than 77% of all rice planted acreage there (MAFF, 2011b). Lastly, bee colonies located near rice fields prefer rice flowers as a pollen source during the rice flowering period (Matsumoto *et al.*, submitted), which is precisely when neonicotinoids (imidacloprid or clothianidin) are sprayed for stinkbug control. The factors cited above contribute synergically to the observed concentrations of the Japanese bee losses there.

Heavy bee loss is apparently synchronized with the expansion of neonicotinoid use (Japan Beekeeping Association, personal communication). For this reason, beekeepers blame neonicotinoids for bee loss. Toxicity levels of neonicotinoid insecticides against honeybees vary (Iwasa *et al.*, 2004; Laurino *et al.*, 2011). Most insecticides sprayed for stinkbug control during rice flowering and post-flowering periods, when bee loss occurs, are neonicotinoid insecticides (JA Kitahibiki, 2011; Matsumoto, personal communication). Consequently, whether neonicotinoids damage bees more severely than other insecticides remains unclear. Future studies including chemical analyses of dead individuals, field experiments, and epidemic research to investigate the sort of insecticide sprayed and occurrence of mass death are required.

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