IUCN Tokyo Forum on Systemic Pesticides   IUCN Task Force on Systemic Pesticides (TFSP) Presents

Influence of Dinotefuran and Clothianidin on Honeybee Colony during Long-Term Field Experiment in Apiary

養蜂場における長期野外実験での
ジノテフランとクロチアニジンの蜂群への影響

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Background　背　景

We are now faced with a serious problem called CCD that worker bees suddenly disappear, leaving a queen with small numbers of bees. A variety of theories on the occurrence of CCD are proposed as follows:

1) A theory that systemic pesticides such as neonicotinoids are a cause of CCD

2) A theory that mites such as varroa and acarine are a cause of CCD

3) A theory that viruses such as an Israel acute paralysis virus are a cause of CCD

4) A theory that genetically modified crops such as a triploid maize are a cause of CCD

5) A theory that a decrease in immunity due to stresses is a cause of CCD

6) A theory that dystrophy due to the unbalanced feeding is a cause of CCD

7）A theory that immune deficiency due to excess antibiotics is a cause of CCD

8) A theory that electromagnetic waves from a cellular phone and so on are a cause of CCD

and so on.

我々は今、女王蜂、蜂児、食料を残して突然消滅する「蜂群崩壊症候群（**CCD**）」という深刻な問題に直面している。その原因として様々な説が提案されている。
例えば、ネオニコチノイドのような浸透性農薬説、ダニ説、ウィルス説など。

Objectives of this Work 本研究の目的

Systemic (neonicotinoid) pesticides have been already banned on circumstantial evidence in Europe. Not only beekeepers begin to fear their pernicious effects on bees but also medical authorities do them on human beings in Japan. ***We will focus our research on a scientifically unproven theory about systemic pesticides in this work and will clarify if the pesticides have any effects on the occurrence of CCD by field testing.***

浸透性（ネオニコチノイド）農薬は**EU**では既に禁止されている。日本においては、その農薬の蜂への有害な影響のみならず人類への影響を恐れ始めている。我々は、ここでは科学的に未解決な浸透性農薬説に焦点を合わせて、その農薬が**CCD**発生を及ぼすかどうかを野外実験で明らかにする。

It is difficult to reproduce CCD in a laboratory where the behavior of bees limited in number is observed because CCD is a phenomenon seen in a colony of bees which ***are eusocial insects****. In this work,* ***colonies which consist of enough honeybees*** *(Apis mellifera)* ***to behave as eusocial insects are prepared under a natural environment in an apiary.***

Dinotefuran and Clothianidin are widely used and well-known as a neonicotinoid pesticide in Japan mainly sprayed on rice field to exterminate stinkbugs that mark with spots on grains of rice and degrade them. We cannot find any reports about the influence of dinotefuran and clothianidin on a honeybee colony through a long-term field experiment in an apiary. ***In order to elucidate the long-term effect of these pesticides on a colony and the relationship between these pesticides and CCD, field experiments are carried out in an apiary.***

ジノテフランやクロチアニジンは広く用いられており、農薬斑点米の原因となっているカメムシ駆除用として水田で散布されるネオニコチノイド系農薬として日本ではよく知られている。これらの農薬のコロニーに及ぼす長期間の影響とこれらの農薬とCCDの関連性をあきらかにするために、養蜂場での野外実験を実施した。

Selection of Pesticides for an experiment
実験用農薬の選定

The following pesticides were prepared for experimental use.

Neonicotinoid pesticides　ネオニコチノイド系農薬

1) Starcklemate solution 10 ® :　スタークルメイト水溶液　１０　（ジノテフラン１０％含有）

with dinotefuran content of 10%　Mitsui Chemicals　(hereafter called "Starcklemate™")

2) Dantotsu water soluble powders ® :　ダントツ水溶剤　（クロチアニジン１６％含有）

with clothianidin content of 16%　Sumitomo Chemical Takeda Agro Company　(hereafter called "Dantotsu™")

Organophosphorus pesticide　有機リン系農薬

In order to compare the influence of neonicotinoid and that of organo-phosphoric pesticide on a honeybee colony, Sumithion® was used.

3) Sumithion Emulsion® :　スミチオン乳剤　（フェニトロチオン（**MEP**）５０％含有）

with fenitrothion (MEP) content of 50%　Sumitomo Chemical Company

Long-term field experiments (2010, 2011,1012)

長期野外実験（2010年、2011年、2012年）

Today, I will focus on the First Experiment in 2010. *今日は第1回の実験結果を中心に紹介する。*

1) First long-term field experiment from July 18 in 2010 to November 21 in 2010

Pesticide: dinotefuran (Starcklemate) & clothianidin (Dantotsu)

Objective: To clarify the influences of two pesticides with different concentrations

Exptl. Method: Pesticide administration from both sugar syrup & pollen paste with three concentrations of 10-, 50- &100-fold dilutions *against the solution of commercial pesticide with a dilution factor recommended for exterminating stinkbugs in practical use*

１） 第1回長期野外実験 （**2010**年**7**月**18**日～**2010**年**11**月**21**日）

実験農薬： ジノテフラン（スタークルメイト） およびクロチアニジン（ダントツ）

実験目的： 上記2種のネオニコチノイド系農薬の蜂群への影響の把握

3) Third long-term field experiment from July 21 in 2012 to the present (ongoing)

Pesticide: dinotefuran (Starcklemate) & fenitrothion (Sumithion)

Objective: **To clarify the difference between the influence of neonicotinoid pesticide and that of organophosphorus one**

Exptl. Method: Pesticide administration from sugar syrup only with a concentration of 50-fold dilution *against the solution of commercial pesticide with a dilution factor recommended for exterminating stinkbugs in practical use*

3） 第3回長期野外実験 （**2012**年**7**月**21**日～現在）

実験農薬： ジノテフラン（スタークルメイト） およびフェニトロチオン（スミチオン）

実験目的： ネオニコチノイド系農薬と有機リン系農薬の蜂群への影響の把握

First long-term field experiments (2010)

from July 18 to November 21

第1回長期野外実験（2010年7月18日～11月21日）

1. Reference solution of pesticide 農薬の基準溶液

The solution of commercial pesticide with a dilution factor recommended for exterminating stinkbugs is defined as "reference solution" of pesticide.

The reference solutions of pesticides are as follows; a solution with a 1,000-fold dilution factor of a commercial concentration in sugar syrup for Starcklemate^{TM} (dinotefuran of 100ppm in solution) and that with a 4,000-fold dilution factor for Dantotsu^{TM} (clothianidin of 40 ppm in solution).

Sugar syrup was made of an equal amount of sugar and water.

カメムシ駆除のための希釈倍数で販売農薬を希釈された農薬濃度の溶液を基準溶液と定義する。
即ち、スタークルメイト（ジノテフラン）は**1000**倍希釈、ダントツ（クロチアニジン）は**4000**倍希釈となる。

2. Experimental runs 実験

Administration concentrations of pesticide to each experimental run are diluted 10-, 50- and 100-fold against the reference solution. Now, we call the concentrations of 10-fold, 50-fold and 100-fold dilution "high", "middle" and "low", respectively. For example, S-high means the high concentration pesticide of Starcklemate (dinotefuran 10ppm), D-low means the low one of Dantotsu (clothianidin 0.4ppm), etc.

ここでは、基準溶液の**10**倍希釈を高濃度（**high**）、**50**倍希釈を中濃度（**middle**）、**100**倍希釈を低濃度（**low**）と呼ぶことにする。 また、スタークルメイトは **"S-"**、ダントツは**"D-"**を付けて表現する。

Table 2 Change in number of total adult bees with elapsed days for each run 成蜂数の変化

Start of the experiment after the adjustment on initial number of total adult bees

Date in 2010	Elapsed days	RUN 1 *control*	RUN 2 *S-high*	RUN 3 *S-middle*	RUN 4 *S-low*	RUN 5 *D-high*	RUN 6 *D-middle*	RUN 7 *D-low*	RUN 8 *control*	Average of Blanks
(Pesticide)		Blank 1	Starcklemate™	Starcklemate™	Starcklemate™	Dantotsu™	Dantotsu™	Dantotsu™	Blank 2	Blank 1 & 2
(Dilution[1)])		No pesticide	10,000-fold[1)]	50,000-fold[1)]	100,000-fold[1)]	40,000-fold[1)]	200,000-fold[1)]	400,000-fold [1)]	No pesticide	No pesticide
July 18	0	8950	11700	12720	10400	12880	11600	13400	10560	9755
July 23	5	11700	5450	5240	7900	5100	7800	11900	11400	11550
July 30	12	11850	(3900)	7250	8750	(1770)	8900	12100	11800	11825
August 8	21	11100	(2550)	1235	9500	(1775)	4060	10100	12400	11750
August 13	26	11400	(1450)	940	8500	(1530)	[70]	9900	11800	11600
August 21	34	8900	(861)	325	4750	(640)	[275]	6300	10700	9800
August 26	39	9800	(980	200	5150	(890)	[36]	4340	9400	9600
September 5	49	9650	(760)	[178]	4590	(830)	[0]	[1840]	6370	8010
September 11	55	10600	(666)	[110]	3550	(810)		[1180]	7450	9025
September 17	61	11150	(264)	[0]	3740	(730)		[975]	6150	8650
September 24	68	12300[2)]	(470)		1395	(895)		[150]	7680[2)]	9990
October 10	84	12300[2)]	(415)		0	(740)		[0]	7680[2)]	9990
October 30	104	12300[2)]	(0)			(285)			7680[2)]	9990
November 21	126	12300[2)]				[(0)]			7680[2)]	9990

S-high (dinotefuran 10ppm) and D-high (clothianidin 4ppm) were administered in the colony only at the start of experiment and afterwards no pesticides were done.

高濃度農薬（S-high; D-high）実験の場合は、農薬投与は実験開始時の1回だけで、以後は無農薬の糖液・花粉を投与。

Sugar syrup and pollen paste without pesticide were administered. *無農薬の糖液・花粉を投与*

A queen has been lost. *女王蜂消失*

Definition of normalized number of adult bees

規格化成蜂数の定義

It is difficult to equalize the initial numbers of adult bees at the start of experiment among runs. Therefore, in order to compensate for a difference in initial population among runs and that in seasonal fluctuation of bee population, a relative change in the number of adult bees is newly defined by the following equation (1)

各コロニー間の実験開始時の初期成蜂数を同じにすることは難しく、かつ、実験期間中に成蜂数が変動するので、これらを補正するために成蜂数のコントロール群との相対的変化である下式の規格化成蜂数を新たに定義した。

Normalized number of adult bees = $(n_{ij} / n_{i0}) / (n_{Bj} / n_{B0})$ (1)

n_{ij}=number of adult bees in RUN *i after the elapse of j days*

n_{i0}=initial number of adult bees in RUN *i at the start of experiment*

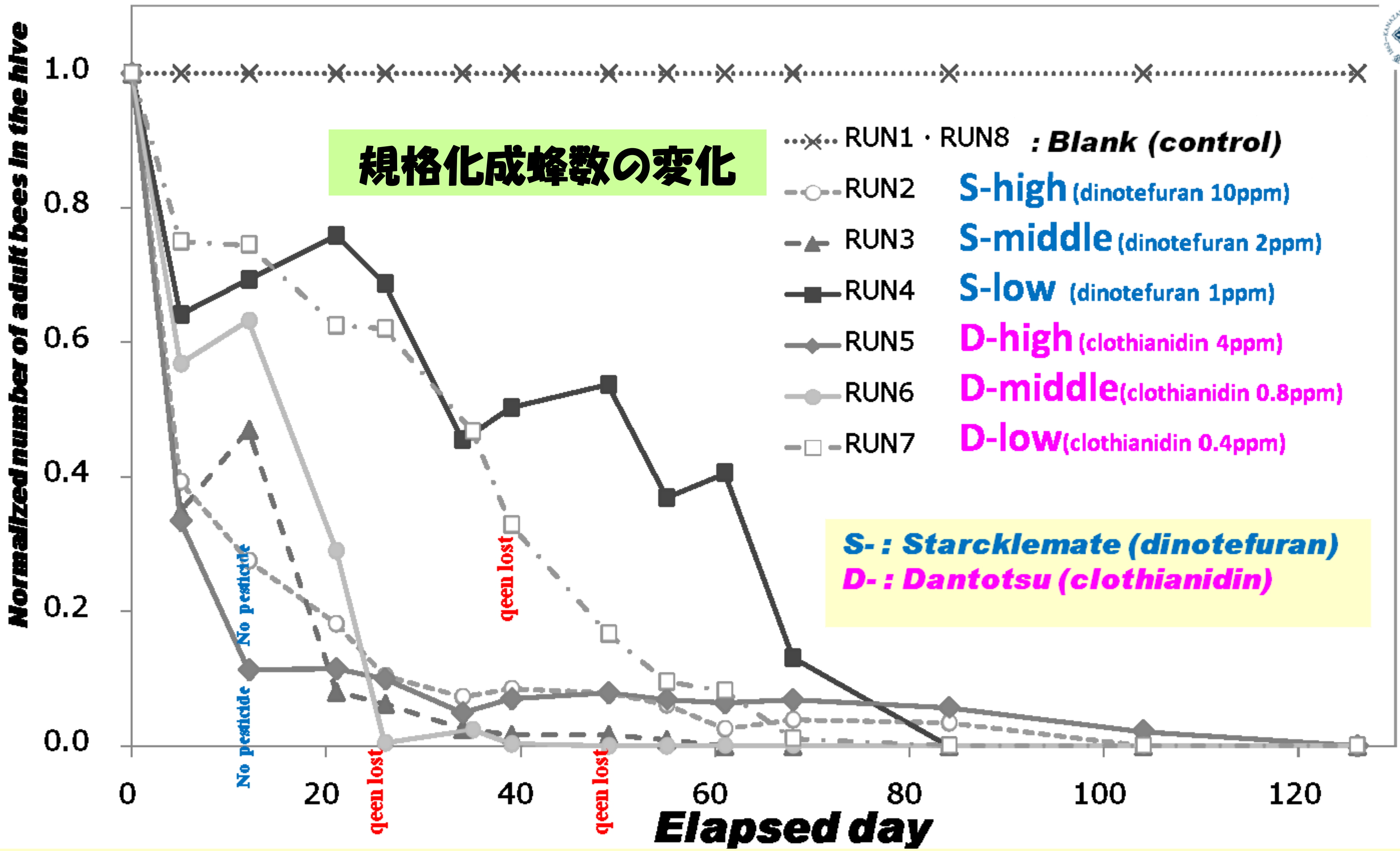
n_{Bj} =number of adult bees in blank run *after the elapse of j days*

n_{B0} =initial number of adult bees in blank run *at the start of experiment,*

where the arithmetic mean number of RUN01 and RUN08 is used as the number of blank run in Eq. (1).

A period of brood is considerably shorter than that of an adult bee and not always contemporary with each other colony. Therefore, the change in the number of brood was evaluated without normalization.

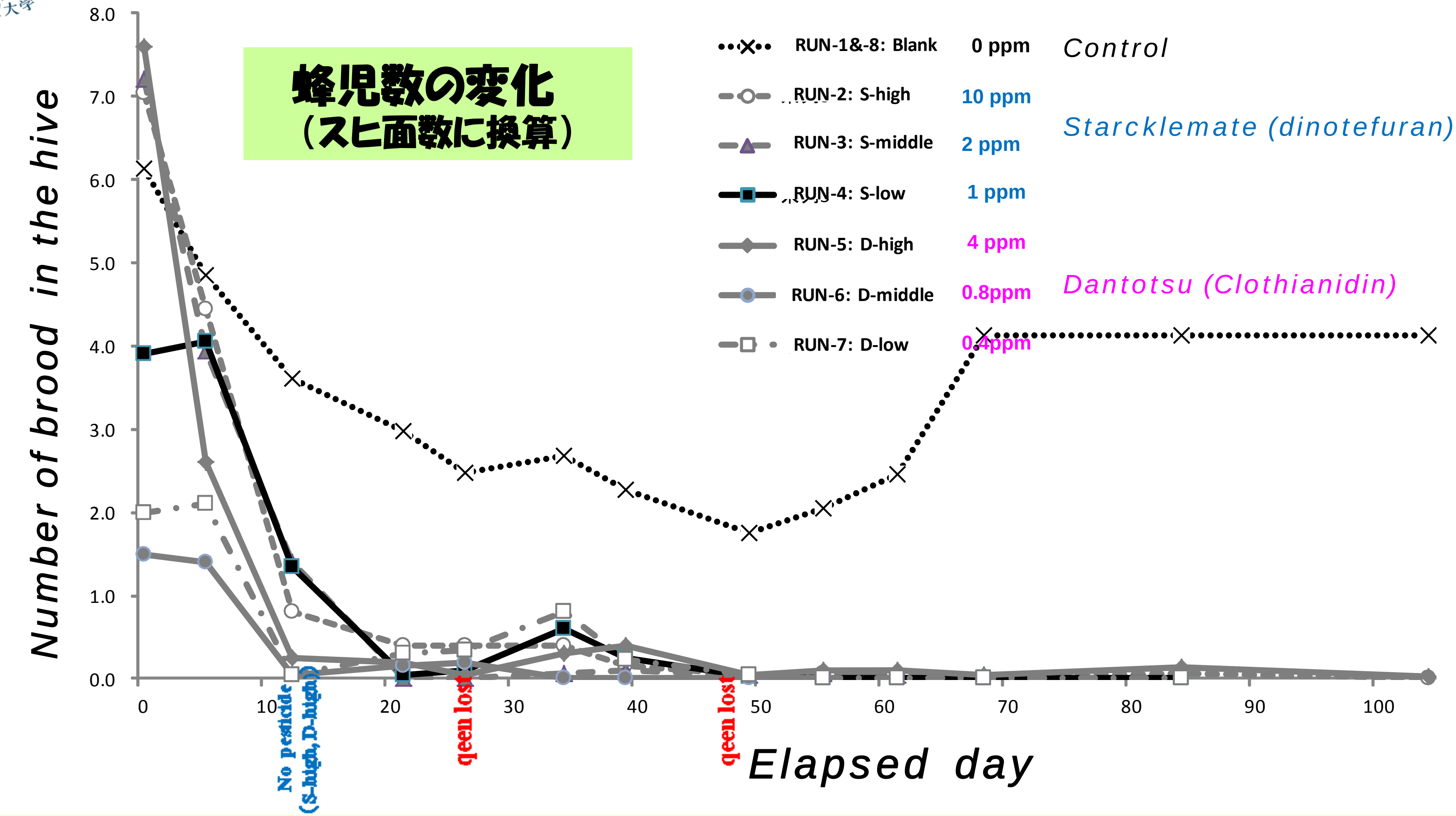
蜂児については、成蜂に比べてその期間がかなり短く、また各コロニー間でばらつきがあるので、蜂児数は規格化せずに直接評価した。



In S-middle and D-middle, some dead bees occurred only in the early period after the first administration but they almost never occurred in S-middle and D-middle afterwards.

中濃度群（S-middle, D-middle）では、死蜂は最初の農薬投与ではいくらか発生したが、その後殆ど発生しなかった。

In S-low and D-low, dead bees almost never occurred just after the first administration and afterwards.



This suggests that a pesticide has some effect on egg-laying and hardly any effect on eggs and larvae. The decrement in brood roughly suggests that the higher concentration of pesticide leads to the more serious egg-laying impediment of a queen.

このことから、大ざっぱにいえば、農薬は女王の産卵に何らかの影響を及ぼし、卵や幼虫に殆ど影響を与えないであろうことを暗示している。蜂児数の減少度合から、農薬濃度が高いほど女王の深刻な産卵障害を引き起こすと言えよう。

Comparison between adult bees on comb of D-high (clothianidin 4ppm) at the start of experiment and those at the elapse of 104 days in 2010

高濃度のダントツ1回投与群（D-high）の実験開始時と104日後の比較

In D-high (only one administration of pesticide) we can find that the colony rapidly dwindled and become small with a queen existing.

高濃度のダントツ1回投与（D-high）では、蜂群が急激に減少して女王がいる状態でわずかな蜂数となることが判る。

Comparison between adult bees on comb of D-low (clothianidin 0.4ppm) at the start of experiment and those at the elapse of 34 days in 2010

低濃度のダントツ連続投与群（D-low）の実験開始時と34日後の比較

Start of experiment

Elapse of 34 days

In D-low we can find the colony gradually dwindled with a queen existing.

低濃度のダントツ連続投与（D-low）では、蜂群は女王が存在しながら、徐々に減少してゆくことが判る

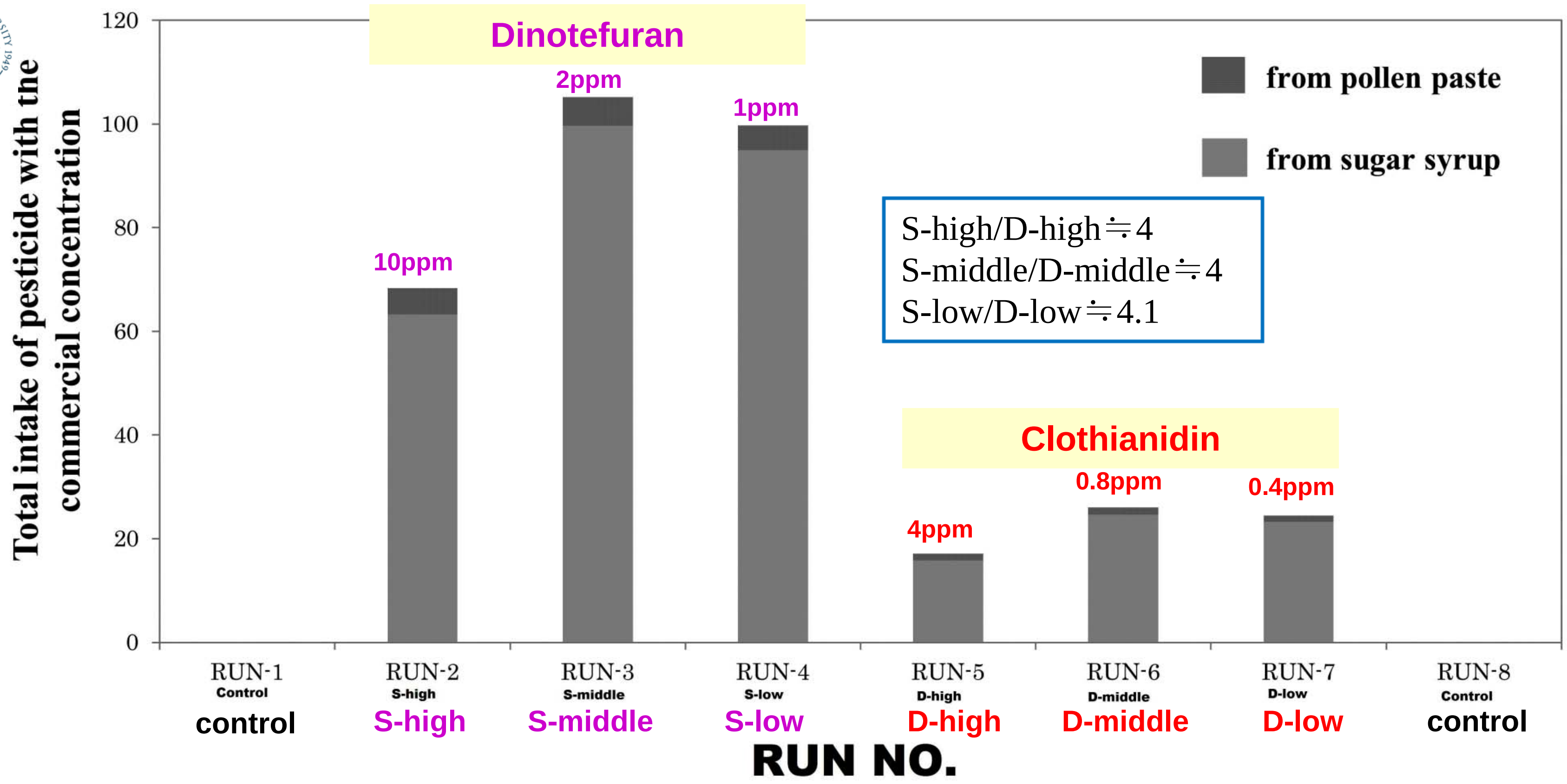


Figure 3 Total intake of pesticide with a converted concentration into that of commercial product for each run till a colony collapses 蜂群崩壊までの農薬摂取量（販売農薬濃度に換算）

The total intake of dinotefuran (Starcklemate™) leading to the collapse of a colony is almost four times as much as that of clothianidin (Dantotsu™) in the concentration of commercial product, independent of the pesticide concentration. The ratio between the dilution factor to make the solution to exterminate stinkbugs of clothianidin and that of dinotefuran is 4000:1000=4:1. Considering that each of them has the same insecticidal activity against a stinkbug, Starcklemate™ seems to have almost the same insecticidal activity against a honeybee as Dantotsu™

蜂群崩壊に至るまでに摂取した農薬の総量は、ジノテフラン（スタークルメイト）はクロチアニジン（ダントツ）の約4倍である。一方、カメムシ駆除のための販売農薬に対する希釈倍数が１：４であることから、これらの農薬のミツバチに対する殺虫能力はほぼ同じであるといえよう

Photolytic and pyrolytic properties of dinotefuran and clothianidin

On the assumption that an aqueous solution of pesticide is exposed to sunlight

ジノテフランとクロチアニジンの光分解特性および熱分解特性

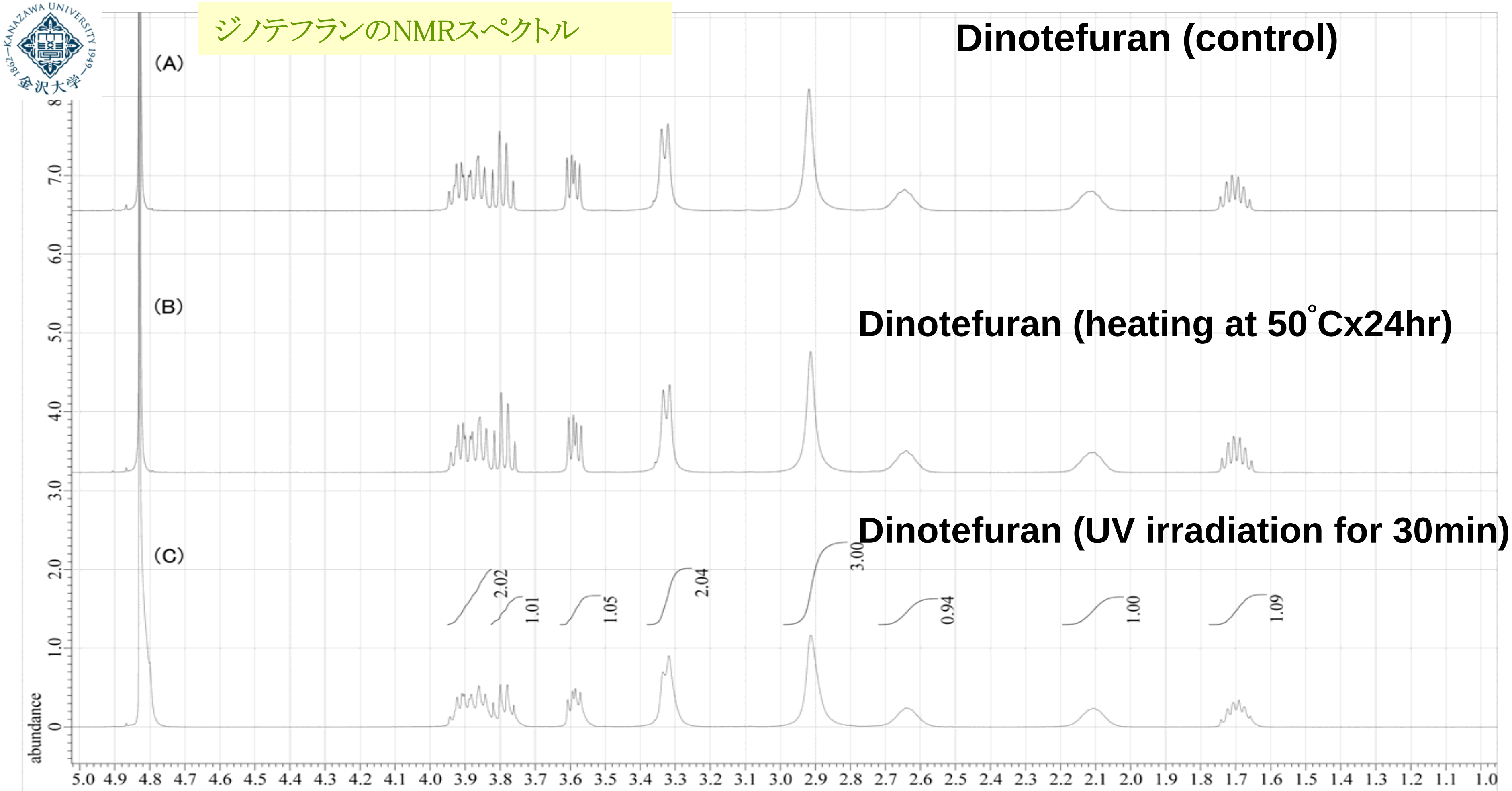
太陽光下で農薬水溶液が暴露された場合

Experiments were performed under the assumptions that dinotefuran and clothianidin were dissolved in water outdoors and the aqueous solutions were heated and irradiated with ultraviolet rays by the exposure to sunlight.

散布されたジノテフランやクロチアニジンが水中に溶解し、その水溶液が太陽光による加熱やUV光照射されるという仮定のもと実験を行った。

The aqueous solutions heated at 50 ºC and irradiated with ultraviolet rays were analyzed by the measurement of the proton NMR spectrum. The NMR spectral analyses give the following speculations:

50℃でUV光照射された水溶液を1H－NMRスペクトル解析して、次のようなスペキュレーションをした。

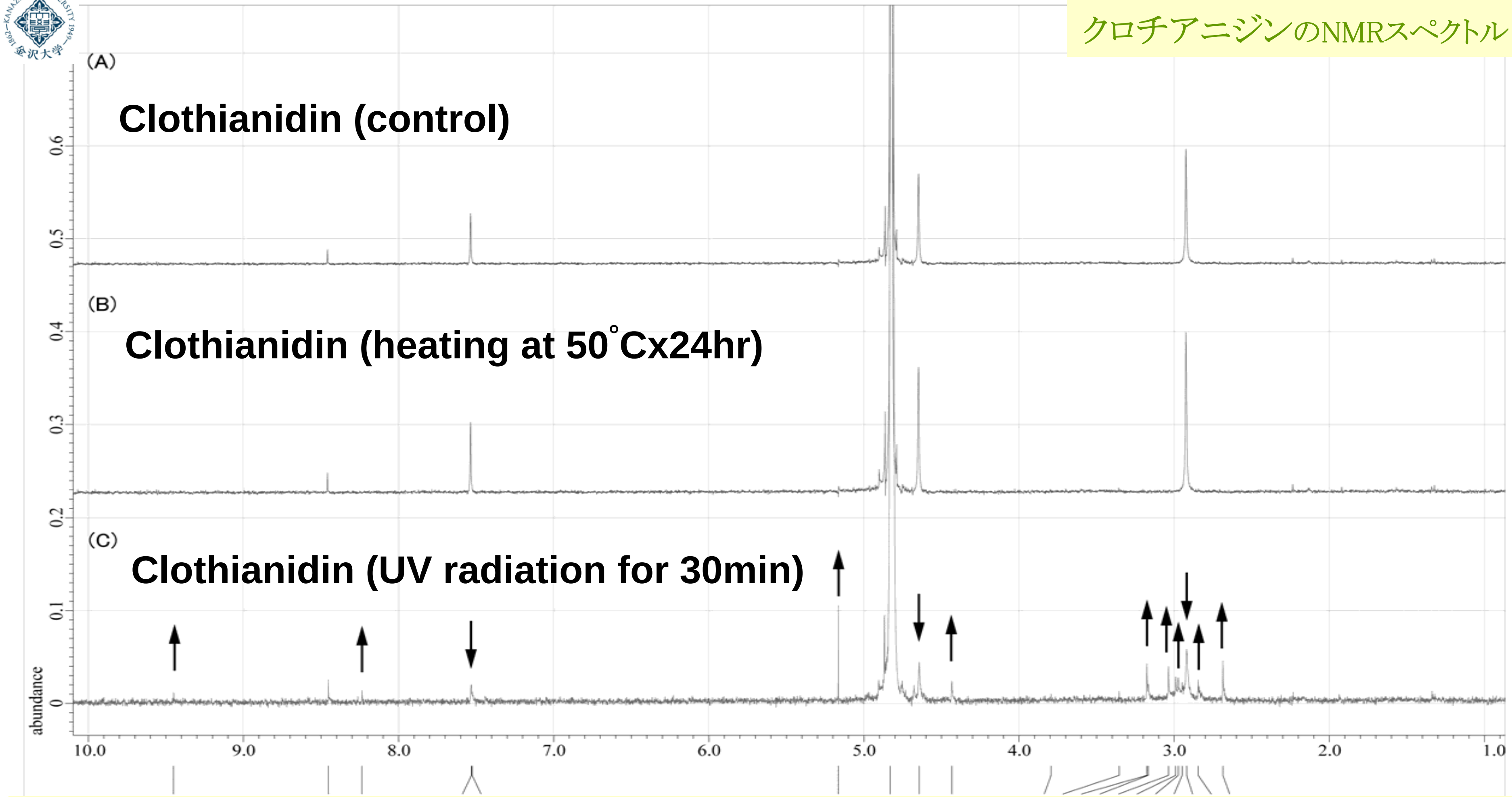


Dinotefuran seems to be stable under the exposure to sunlight because it is stable by heating at 50˚C for 24 hours and irradiating UV light for 30 min.

ジノテフランは50℃加熱時やUV光照射時で安定であることから、太陽光下では安定であると思われる。

Figure 4　NMR spectra of dinotefuran in D_2O (A) without any treatment, (B) after heating at 50 °C for 24 hours, and (C) after UV light irradiation for 30 min.

クロチアニジンのNMRスペクトル



Clothianidin seems to be unstable under the exposure to sunlight because it is unstable by irradiating UV light for 30 min though it is stable at 50°C for 24 hours.

クロチアニジン は５０℃の加熱時では安定であるものの、UV光照射時には不安定となるので、太陽光下では安定であると思われる。

Figure 5 NMR spectra of clothianidin in D_2O (A) without any treatment, (B) after heating at 50 °C for 24 hours, and (C) after UV light irradiation for 30 min. The increased and decreased signals were shown in the figure as up and downward arrows.

Second long-term field experiment (2011)
第2回長期野外実験（2011年）

Here, I will introduce a brief sumary of the second long-term field experiment in 2011.
第2回の実験概要を簡単に紹介する。

Period: from July 9 in 2011 to April 2 in 2012

Pesticide: dinotefuran (Starcklemate) only

Objective: To clarify the difference between the influence of pesticide from sugar syrup and that from pollen paste

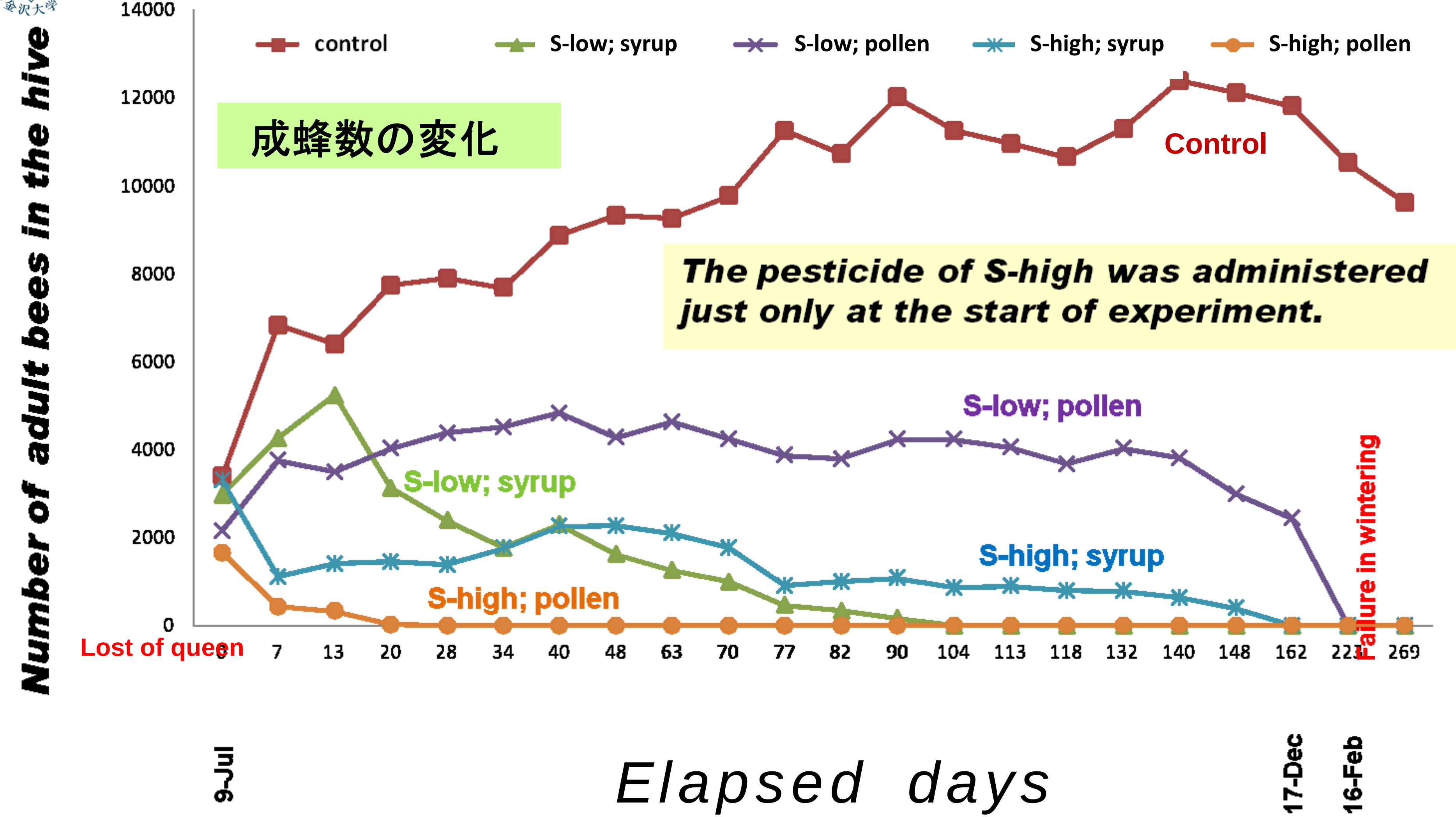
Exptl. Method: Pesticide administration from either sugar syrup or pollen paste with two concentrations of 10- & 100-fold dilutions *against the solution of commercial pesticide with a dilution factor recommended for exterminating stinkbugs in practical use*

第2回長期野外実験　（**2011**年**7**月**9**日～**2012**年**4**月**2**日）

実験農薬：　ジノテフラン（スタークルメイト）

実験目的：　農薬投与する食料（糖液と花粉ペースト）の違いによる蜂群への影響の把握

Second long-term field experiment: July 9 in 2011 to April 2 in 2012



2)Only pollen paste with 10-fold dilution (high conc.) pesticide brought about the death of many adult bees and a queen bee just after the first administration and led to the rapid collapse of a colony.

高濃度農薬含有の花粉ペーストだけが最初の農薬投与直後に多数の成蜂と女王を死に至らしめ、蜂群を崩壊させる。

Third long-term field experiment (2012)
第3回長期野外実験（2012年）

And furthermore, I will introduce a brief sumary of the third long-term field experiment in 2012.
第3回の実験概要を簡単に紹介する。

Period: from July 21 in 2012 to the present (ongoing)

Pesticide: dinotefuran (Starcklemate) & fenitrothion (Sumithion)

Objective: To clarify the difference between the influence of neonicotinoid pesticide and that of organophosphorus one

Exptl. Method: Pesticide administration from sugar syrup only with a concentration of 50-fold dilution *against the solution of commercial pesticide with a dilution factor recommended for exterminating stinkbugs in practical use*

第3回長期野外実験　（**2012**年**7**月**21**日～現在）

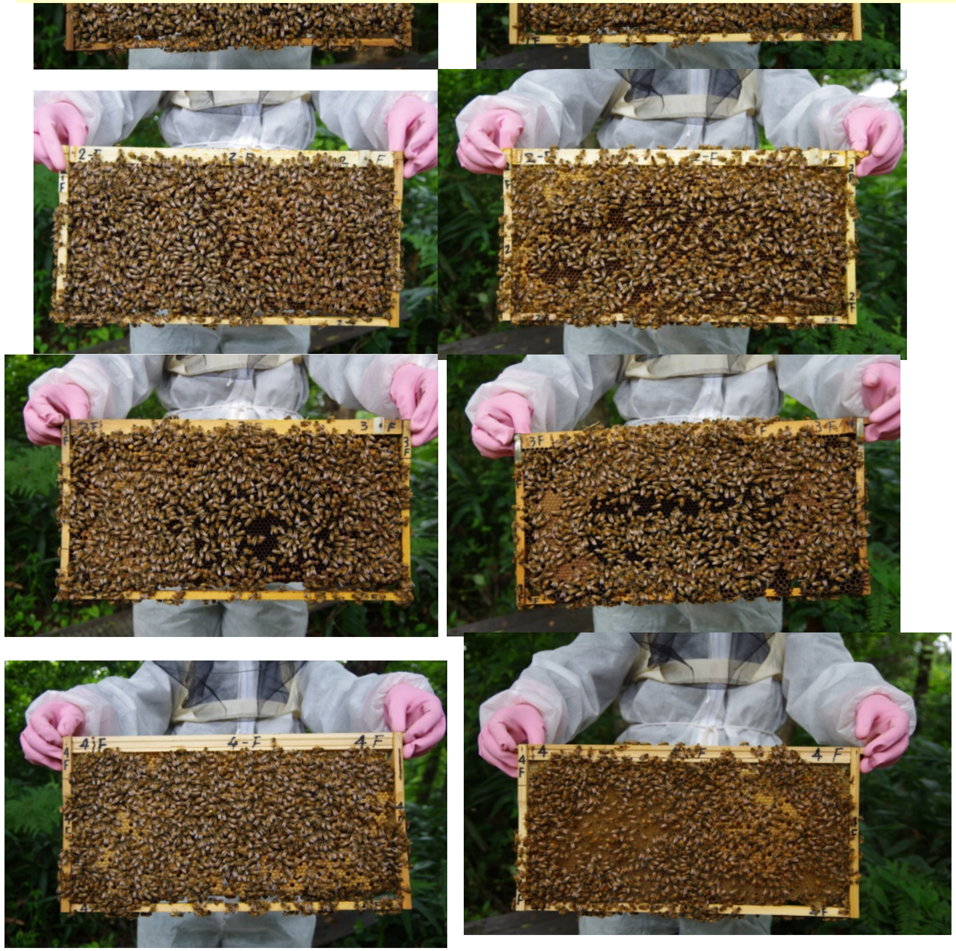
実験農薬：　ジノテフラン（スタークルメイト）　およびフェニトロチオン（スミチオン）

実験目的：　ネオニコチノイド系農薬と有機リン系農薬の蜂群への影響の把握

Comparison of Starcklemate (dinotefuran) & Sumithion

The colony with organophosphorus pesticide administered do not collapse after the colony with neonicotinoid pesticide administered has collapsed (August 8) via CCD phenomenon.

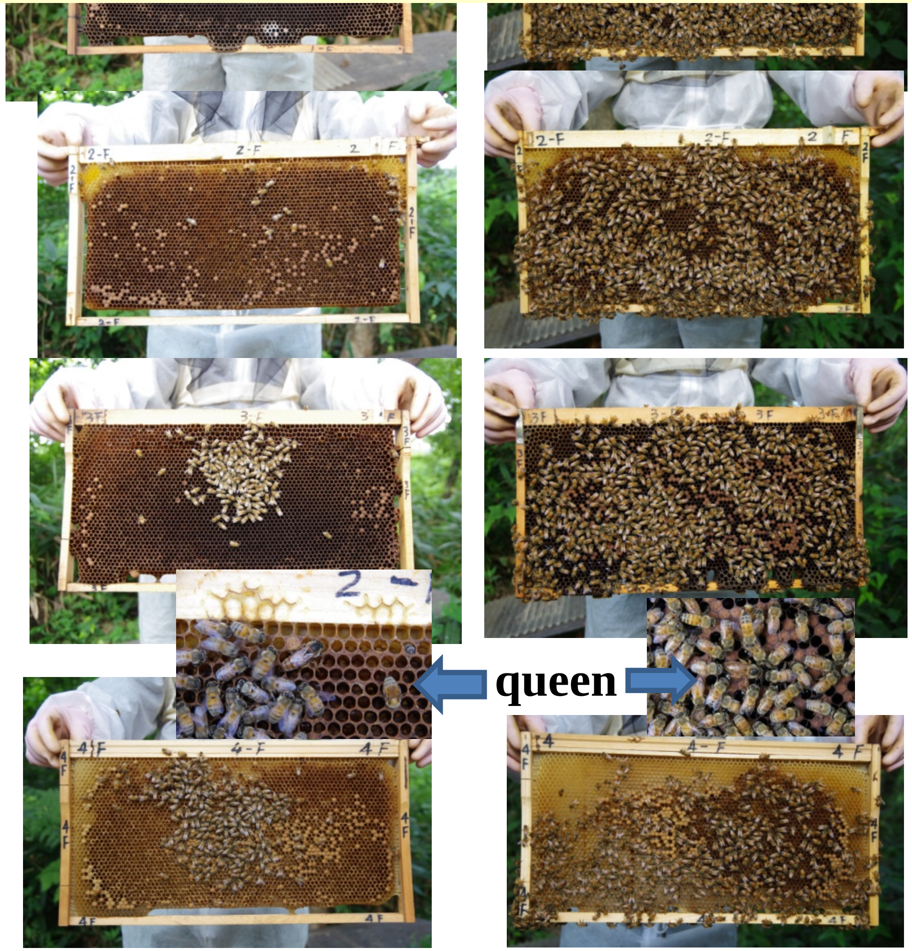
ネオニコイノイド系農薬が投与された蜂群は**CCD**現象を経由して崩壊した（８月８日）後でも、有機リン系農薬が投与された蜂群は崩壊していない。



Starcklemate (dinotefuran) | **Sumithion (fenitrothion)**

Neonicotinoid | ***Organophosphorous***

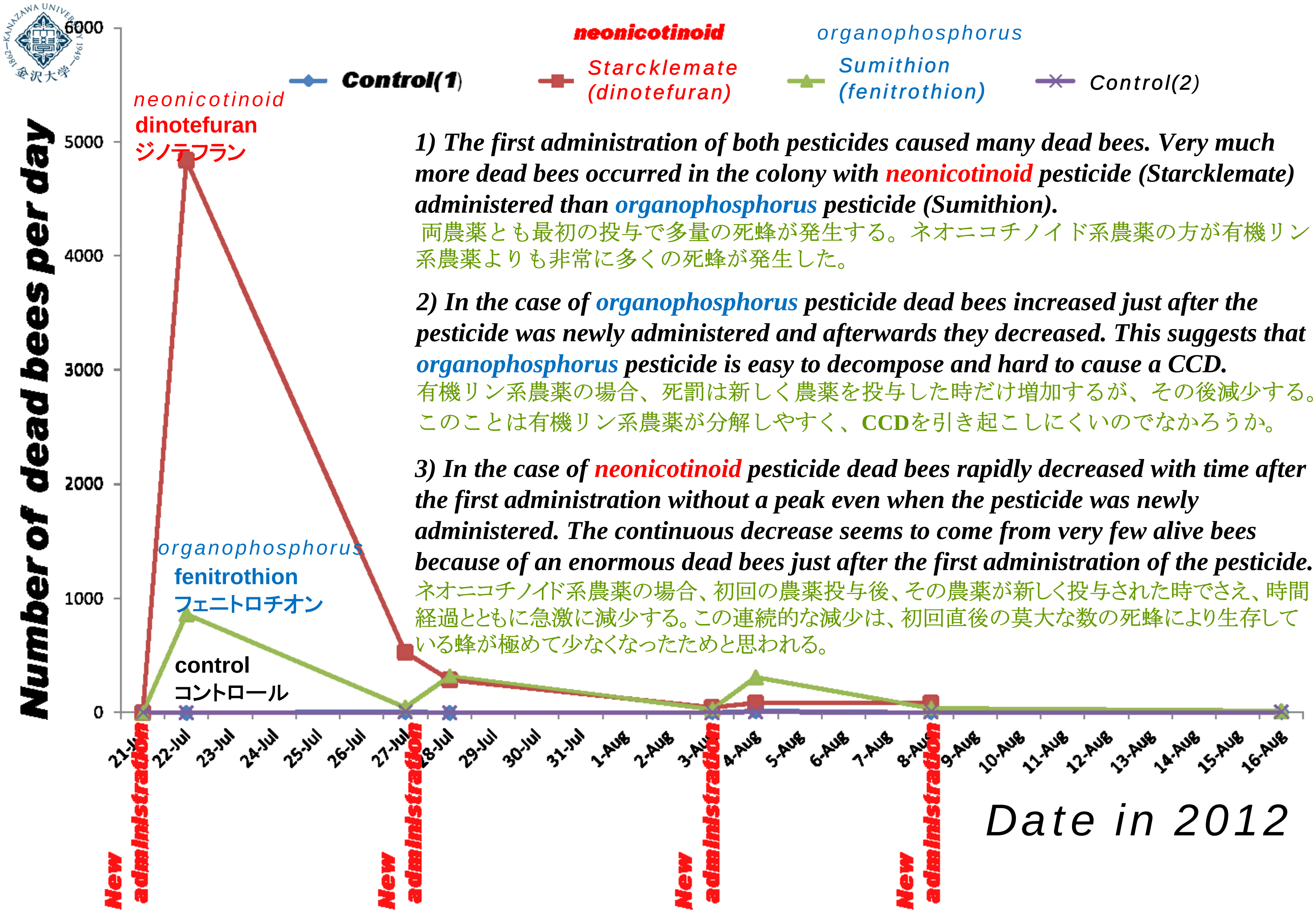
Start of experiment : July 21 in 2012



Starcklemate (dinotefuran) | **Sumithion (fenitrothion)**

Neonicotinoid | ***Organophosphorous***

After 14 days elapsed : August 4 in 2012



1) The first administration of both pesticides caused many dead bees. Very much more dead bees occurred in the colony with neonicotinoid pesticide (Starcklemate) administered than organophosphorus pesticide (Sumithion).

両農薬とも最初の投与で多量の死蜂が発生する。ネオニコチノイド系農薬の方が有機リン系農薬よりも非常に多くの死蜂が発生した。

2) In the case of organophosphorus pesticide dead bees increased just after the pesticide was newly administered and afterwards they decreased. This suggests that organophosphorus pesticide is easy to decompose and hard to cause a CCD.

有機リン系農薬の場合、死罰は新しく農薬を投与した時だけ増加するが、その後減少する。このことは有機リン系農薬が分解しやすく、CCDを引き起こしにくいのでなかろうか。

3) In the case of neonicotinoid pesticide dead bees rapidly decreased with time after the first administration without a peak even when the pesticide was newly administered. The continuous decrease seems to come from very few alive bees because of an enormous dead bees just after the first administration of the pesticide.

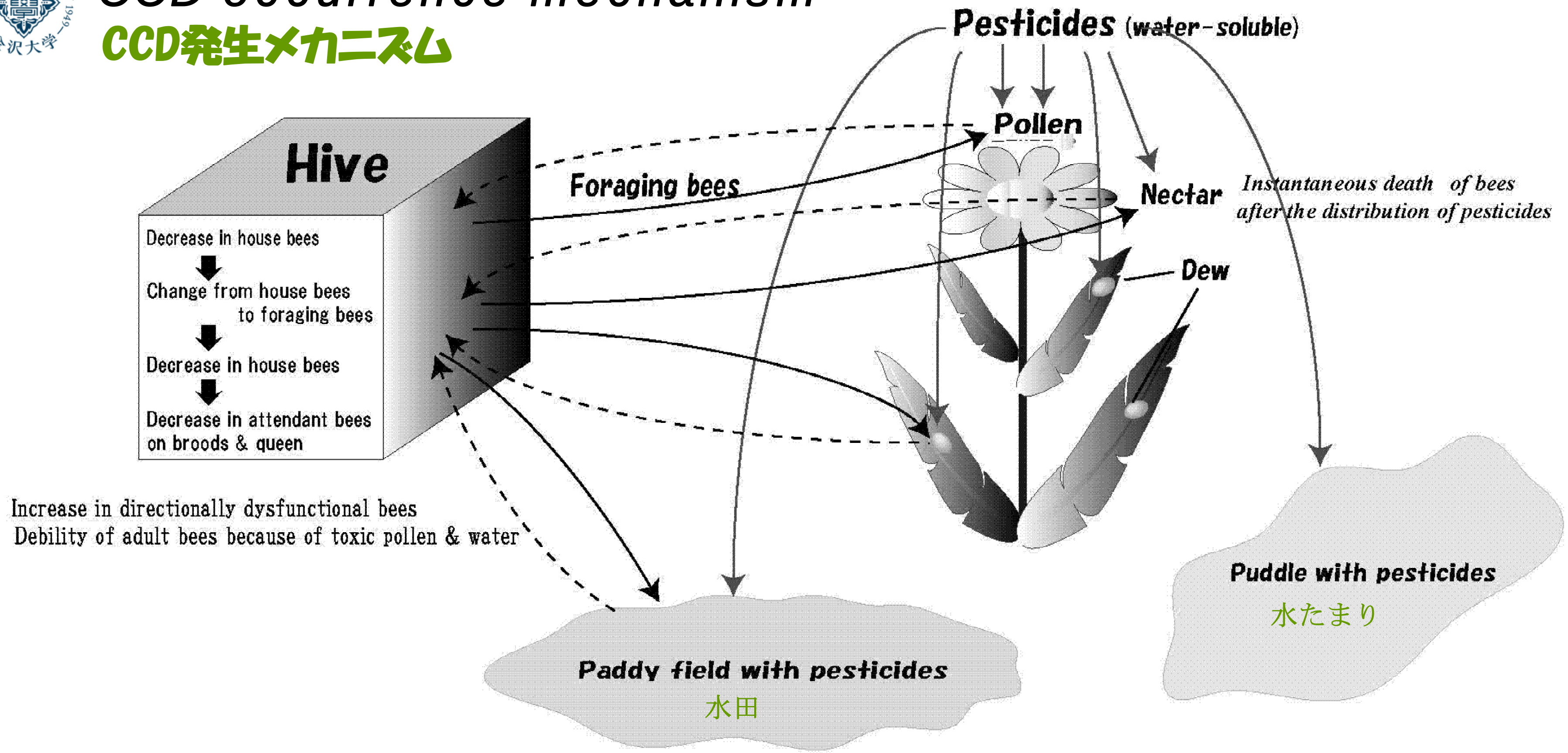
ネオニコチノイド系農薬の場合、初回の農薬投与後、その農薬が新しく投与された時でさえ、時間経過とともに急激に減少する。この連続的な減少は、初回直後の莫大な数の死蜂により生存している蜂が極めて少なくなったためと思われる。

Figure　Number of dead bees per day around and in the hive (July 21 to August 16 in 2012)

Neonicotinoids seem to be approximate to poisons rather than pesticides because of the hard-degradability.

CCD occurrence mechanism

CCD発生メカニズム



For concentrated pesticides, bees are killed on the spot.
For dilute pesticides, toxic water, pollen (& nectar) are carried back to hive, fed and stored.
Usually nectar with pesticides is washed out by welled-up nectar without pesticides .
When pesticides are continuously distributed, toxic nectar is carried back to hive, fed and stored.

Figure 6 Schematic diagram of CCD occurrence mechanisms due to neonicotinoid pesticides

4) The imbalance of colony composition causes the decrease in the egg-laying activity of a queen and finally causes a collapse of the colony with a queen remaining.

蜂群の構成(卵、幼虫、内役蜂、外役蜂)が不均衡になると、女王の産卵能力の低下を引き起こし、最終的には女王を残したまま蜂群が崩壊する。

Are the neonicotinoids the Trojan horse in the global ecosystem ?

Conclusion　結論

1) *A colony rapidly dwindled after the administration of dinotefuran or clothianidin and finally became extinct after taking on an aspect of CCD. This means that the CCD is just one of situations where a colony dwindles away to nothing although it may look mysterious.*

1）蜂群は、ジノテフラン又はクロチアニジン投与後、急激に小さくなり、CCDの様相を呈した後、最終的には消滅する。

2) *The mechanism of CCD occurrence can be proposed as follows:*

In supposing that a pesticide is sprayed and diluted in water of a rice paddy or an orchard and its concentration becomes low, the low-concentration pesticide carried by foraging bees continues to affect a colony for a long time and finally leads to a collapse of a colony or the failure in wintering. Even if a colony does not collapse and looks active, it causes an egg-laying impediment of a queen and a decrease in immune strength of bees leading to the infestation of mites in a colony.

2）下記のようなCCD発生メカニズムを提案する。

農薬が散布され、水田や果樹園内の水で薄められる場合を想定すると、外役蜂によって運ばれた低濃度の農薬は長期間に亘って蜂群に影響を及ぼし続け、ついには崩壊するか越冬に失敗する。たとえ、蜂群が崩壊しなくて元気なように見えたとしても、その低濃度の農薬は女王の産卵障害を引き起こしたり、蜂の免疫力の低下が蜂群中でのダニを蔓延させたりする。

3) *Organophosphorus pesticide is easy to decompose and hard to cause a CCD in comparison with neonicotinoid pesticides.*

3）ネオニコチノイド系農薬に比べて、有機リン系農薬は分解しやすく、CCDを引き起こしにくい。

Neonicotinoids are feared to become secondary asbestos.　When in doubt, take the safe route!

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Harm to honeybees are probably applicable to human beings. "The gate is wide and the way is easy, that leads to destruction."

Thank you for

your attention !